

## Article

# Effects of Mining Activities on *Gerbillus nanus* in Saudi Arabia; A Biochemical and Histological Study

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**Simple Summary:** Mining is the extraction of materials of economic importance from the earth. It is an important source of employment and economic development. However, mining activities can impact the biodiversity, environment and human health, mainly through the emission of large quantities of heavy metals. Our objective was to investigate the impact of gold mining on the Balochistan gerbil, a rodent occurs in natural environments in Saudi Arabia. Our results demonstrated the accumulation of heavy metals in the soil, the plant Arabian boxthorn and different tissue of the gerbils. In addition, the gerbils exhibited liver, kidney and lung injury associated with declined antioxidants. These data could be of public interest and may call the attention to evaluate the impact of gold mining on the environment and nearby communities.

**Abstract:** Mining can impact the environment, biodiversity and human health through direct and indirect practices. This study investigated the effects of gold mining on *Gerbillus nanus*, pointing to organ dysfunction and redox imbalance. Soil samples, *Lycium shawii* and *G. nanus* were collected from a site near a mining planet and a control site. Soil and *L. shawii* samples from the mining site showed a significant increase cadmium (Cd), copper (Cu), mercury (Hg), arsenic (As), zinc (Zn), lead (Pb) and vanadium (V). Hepatic, renal and pulmonary Cd, Pb, Hg, Zn, Cu, Fe, As and V concentrations were increased significantly in *G. nanus* at the mining site. Markers of liver and kidney function were elevated in serum, and several histological manifestations were demonstrated in liver, kidney and lung of *G. nanus* at the mining site. Malondialdehyde and nitric oxide were increased, and glutathione and antioxidant enzyme were declined in the liver and kidney of *G. nanus*. In conclusion, mining practices triggered tissue damage and oxidative stress in *G. nanus* living close to the mining site. These findings can represent the scientific basis for evaluating the environmental and health impact of mining in the on the nearby communities.

**Keywords:** Heavy metals; Mining; Balochistan gerbil; liver injury; Kidney damage.

## 1. Introduction

Mining refers to the extraction of geological materials, such as minerals, coal, limestone and other materials of economic interest from the earth. The mining activities include both small- and large-scale activities and have a significant contribution to the economic growth and development. However, mining activities can impact the environmental and social systems, living standards, health and traditional practices of the nearby communities [1-3]. Erosion, altered soil profile, contamination of soil and local streams and emissions are among the environmental impacts occur during the construction, operation and maintenance of mines [4,5]. Despite its importance as a source of employment and the progress in occupational health and safety, the mining sector remains a high-risk environment where workers are highly susceptible to occupational injuries [6].

The widespread contamination of the environment with heavy metals (HMs) is one of main consequences of mining [7]. Owing to their non-degradative nature, increased HMs concentrations can exert long-term negative impact on the ecosystem and human health [8]. HMs possess a high tendency for bioaccumulation and biomagnification and hence trigger serious health problems [9,10]. Accordingly, several reports have investigated the *in vitro* and *in vivo* toxic effects of HMs [11,12]. Lead (Pb), cadmium (Cd), arsenic (As), mercury (Hg) zinc (Zn) and copper (Cu) have been determined in the mining waste. Some of these HMs function as essential micronutrients and play central roles in redox processes, biochemical reactions and electron transport chain, whereas the non-essential HMs have no biological importance and are very toxic to the living organisms [13]. Cd is a potent HM pollutant with high tendency for bioaccumulation in living organisms [14]. Exposure to Cd is common in mining industry and hence pose a health threat to humans [15]. Pb is a well-documented HM pollutant with hazardous health effects and its worldwide emission rate is high [9,14]. The long-term exposure to Pb and Cd is linked to the development of liver and neurological disorders, cancer, osteoporosis [11,16], and cardiovascular disease [17]. In rats chronically exposed to Pb and Cd, liver and heart tissue injury and oxidative damage have been observed [12]. As is a very dangerous pollutant that attracted significant worldwide attention due to its potential toxic effects [18]. Lung, kidney and skin cancers, cardiovascular and neural alterations, hepatomegaly, peripheral vascular disease and others might be triggered by exposure to As (reviewed by [19]). Hg is another serious HM that can induce cell death *via* binding cysteine residues of proteins and depletion of cellular antioxidants [20].

Given the hazardous impact of mining and its HMs waste on the environment, society and health, we investigated the impact of mining on the Balochistan gerbil (*Gerbillus nanus*) in Riyadh (Saudi Arabia). *G. nanus* belongs to the murid subfamily Gerbillinae [21] and occurs in natural environments in Saudi Arabia [22], and is therefore selected to be investigated.

## 2. Materials and Methods

### 2.1. Study site and collection of samples

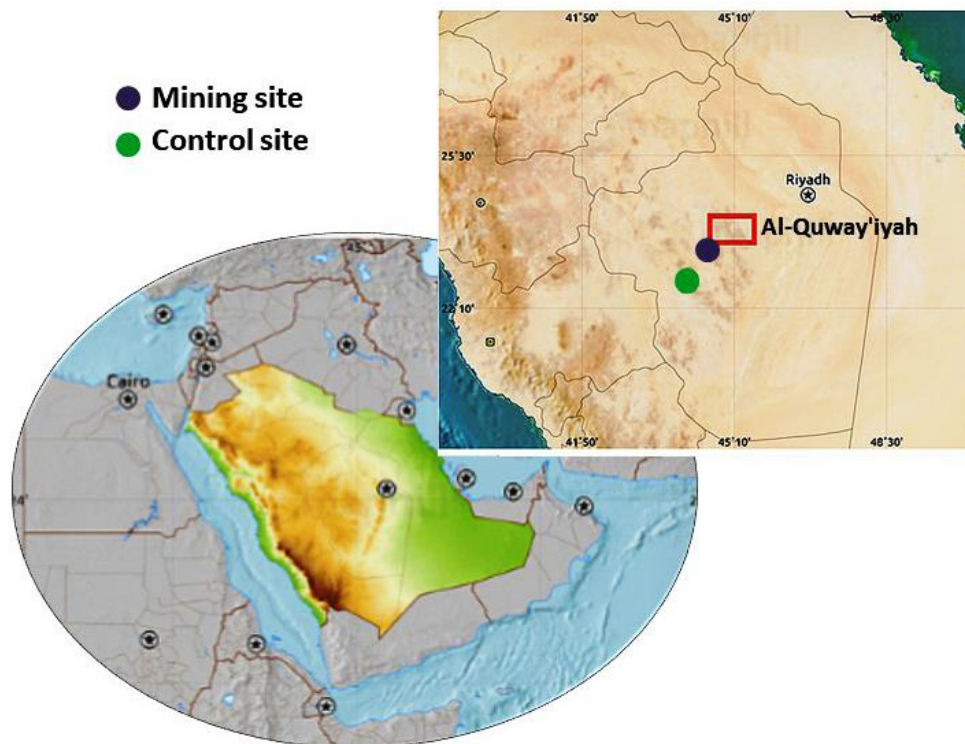
Samples were collected from the area of mining activities in Al-Quway'iyah, a city located 165 km west to Riyadh Province (Saudi Arabia). This city is one of the large governorates in Saudi Arabia and hence selected for the study.

The investigated gold mine site is located southwestern to Al-Quway'iyah city. The samples were collected from two sites as following (Fig. 1):

Site 1 (Mining): Located at 500 m from the gold mine between E45 05' and N23 47'.

Site 2 (Control): Located at 20000 m away from the gold mine between E45 06' and N23 36 '.

Samples from the soil at 20 cm depth (N = 8) and *Lycium shawii* (Arabian boxthorn) (N = 8) were collected from both sites for the determination of HMs. Eight *G. nanus* were collected from each site with the help of specific rodent traps and were transferred to the laboratory. The animals were sacrificed under anesthesia and blood and tissue samples were collected for analysis. The protocol and procedures were approved by the institutional animal ethics committee of King Saud University (No. KSU-20165).



**Figure 1.** Location map of the mining and control sites.

## 2.2. Determination of HMs

The concentrations of HMs were determined in soil, *L. shawii*, and *G. nanus* kidney, liver, and lung using ELAN 9000 ICP/MS (Perkin Elmer Sciex Instruments, Canada).

## 2.3. Assay of liver and kidney function markers

Serum alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), creatinine, urea and uric acid were measured using Biomerieux (France) reagent kits, following the manufacturer's instructions.

## 2.4. Assay of oxidative stress markers and antioxidants

Samples from the liver and kidney were homogenized in phosphate buffered saline (10% w/v), centrifuged and the supernatant was used for the assays. Malondialdehyde (MDA) [23], nitric oxide (NO) [24], reduced glutathione (GSH) [25], superoxide dismutase (SOD) [26] and catalase (CAT) [27] were assayed in the liver and kidney homogenate samples. Protein content was determined using Bradford assay.

## 2.5. Histological study

Samples from the kidneys, liver and lungs of *G. nanus* collected from the mining and control sites were fixed in 10% neutral buffered formalin and processed for paraffin embedding. 5  $\mu$ m sections were cut, stained with hematoxylin and eosin (H&E) and examined using a light microscope.

## 2.6. Statistical analysis

Data were analyzed using GraphPad Prism 7 (La Jolla, CA, USA) and expressed as means  $\pm$  standard error of means (SEM). All statistical comparisons were performed using T. test and a P value  $< 0.05$  was considered significant.

## 3. Results

### 3.1. HMs concentration in soil and *L. shawii* samples

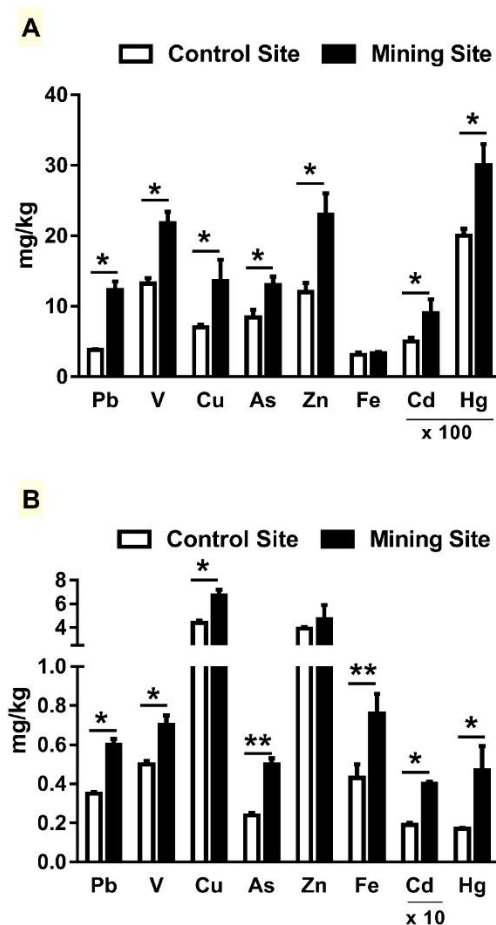
The concentration of HMs has been determined in soil samples collected from the control and mining site. Pb, V, Cu, As, Zn, Cd and Hg were increased significantly ( $P < 0.05$ ) at the mining site when compared with the control site (Fig. 2A). The results revealed non-significant difference in Fe concentration between both sites. *L. shawii* samples at the mining site showed higher concentrations of Pb, V, Cu, As, Fe, Cd and Hg, whereas Zn concentration wasn't significantly different when compared with the control site (Fig. 2B).

### 3.2. Concentration of HMs in liver, kidney and lung of *G. nanus*

Pb, Cd and Hg levels were increased significantly in the liver, kidney and lung of *G. nanus* collected from the mining site when compared with the control site animals, as depicted in Figures 3A-3C. Similarly, V (Fig. 3D), Cu (Fig. 3E) and Zn (Fig. 3F) were significantly increased in the target tissues of *G. nanus* at the mining site. Fe (Fig. 3G) and As (Fig. 3H) were also increased remarkably in the liver, kidney and lung of *G. nanus* at the mining site.

### 3.3. Effect of mining on liver function and histology in *G. nanus*

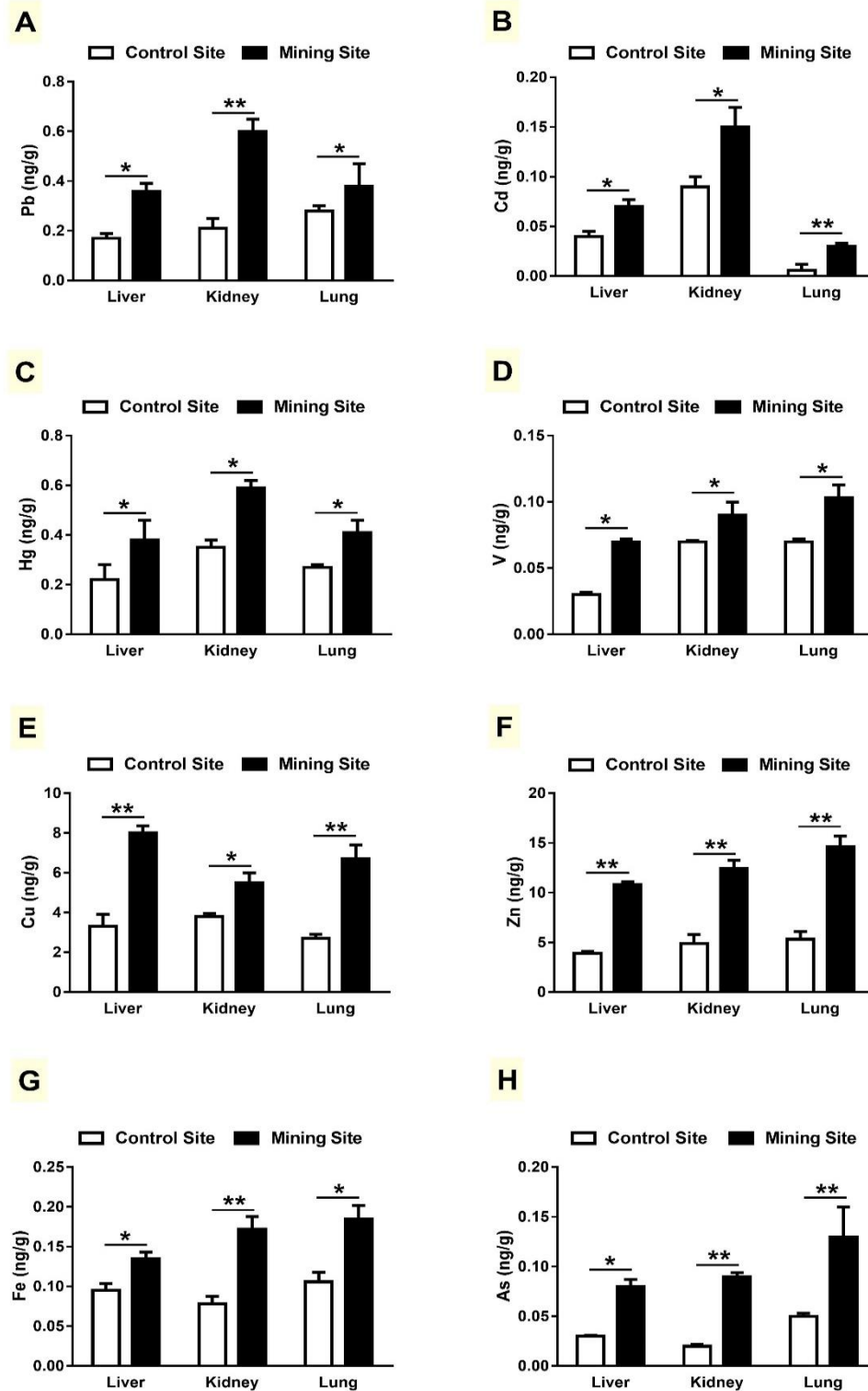
The liver function markers ALT, AST and ALP were elevated in serum of *G. nanus* at the mining site ( $P < 0.05$ ) when compared with the corresponding control site animals (Fig. 4A). Examination of the liver section showed normal structure of the hepatic lobules and hepatocytes in *G. nauns* at the control site (Fig. 4Ba and b). In contrast, *G. nanus* at the mining site showed hepatocyte vacuolations, hemorrhages and dilated central vein (Fig. 4Bc and d).



**Figure 2.** Concentration of heavy metals in (A) soil and (B) *L. shawii* at the mining and control sites. Data are means  $\pm$  SEM, (N = 8). \* $P < 0.05$  and \*\* $P < 0.01$ .

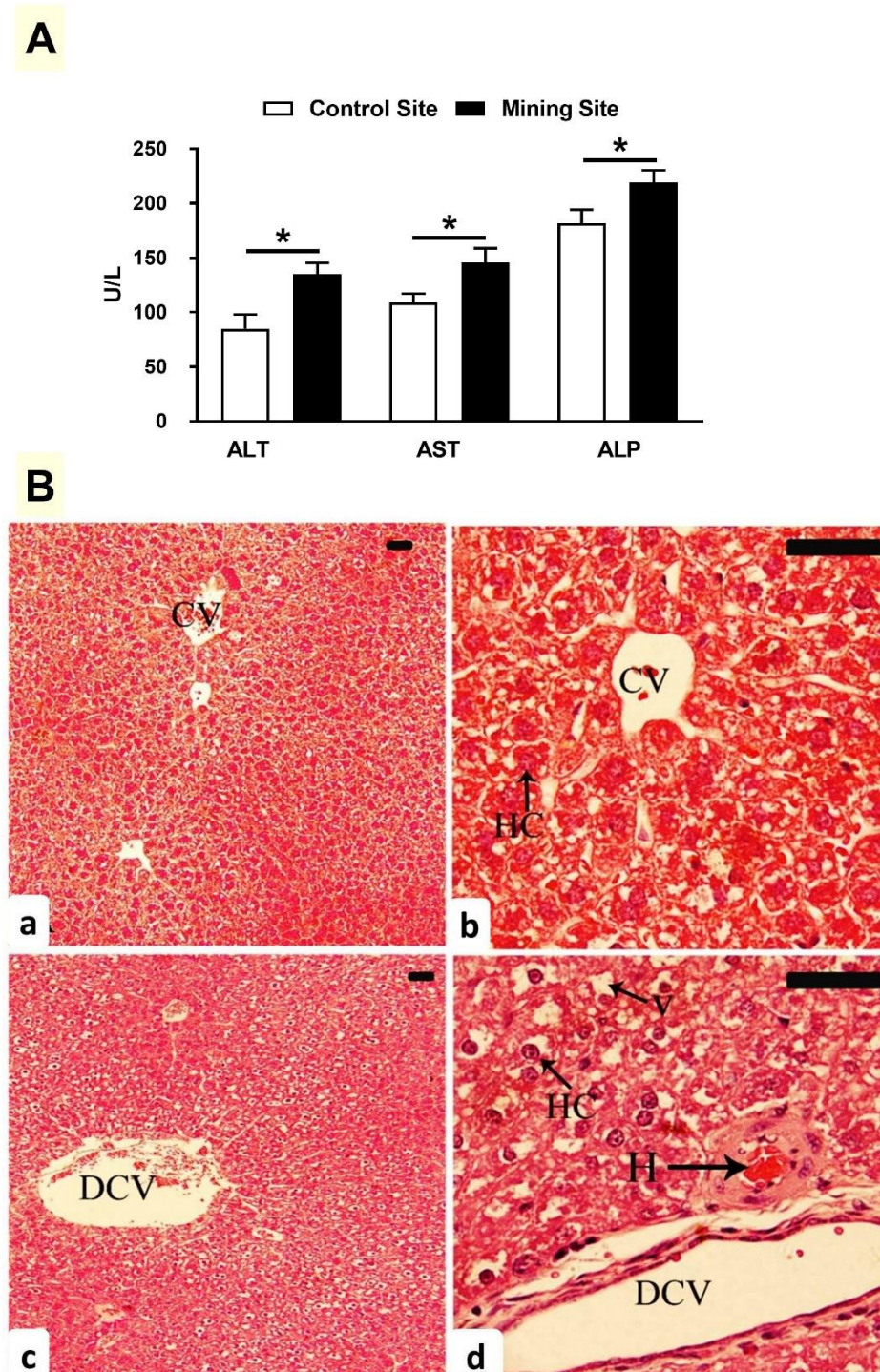
### 3.4. Effect of mining on kidney function and histology in *G. nanus*

Serum creatinine, urea and uric acid were elevated significantly ( $P<0.05$ ;  $P<0.01$ ;  $P<0.05$ ) in *G. nanus* at the mining site (Fig. 5A). While the control site *G. nanus* showed normal kidney (Fig. 5Ba and b), animals at the mining site exhibited glomerular degeneration (Fig. 5Bc and d).

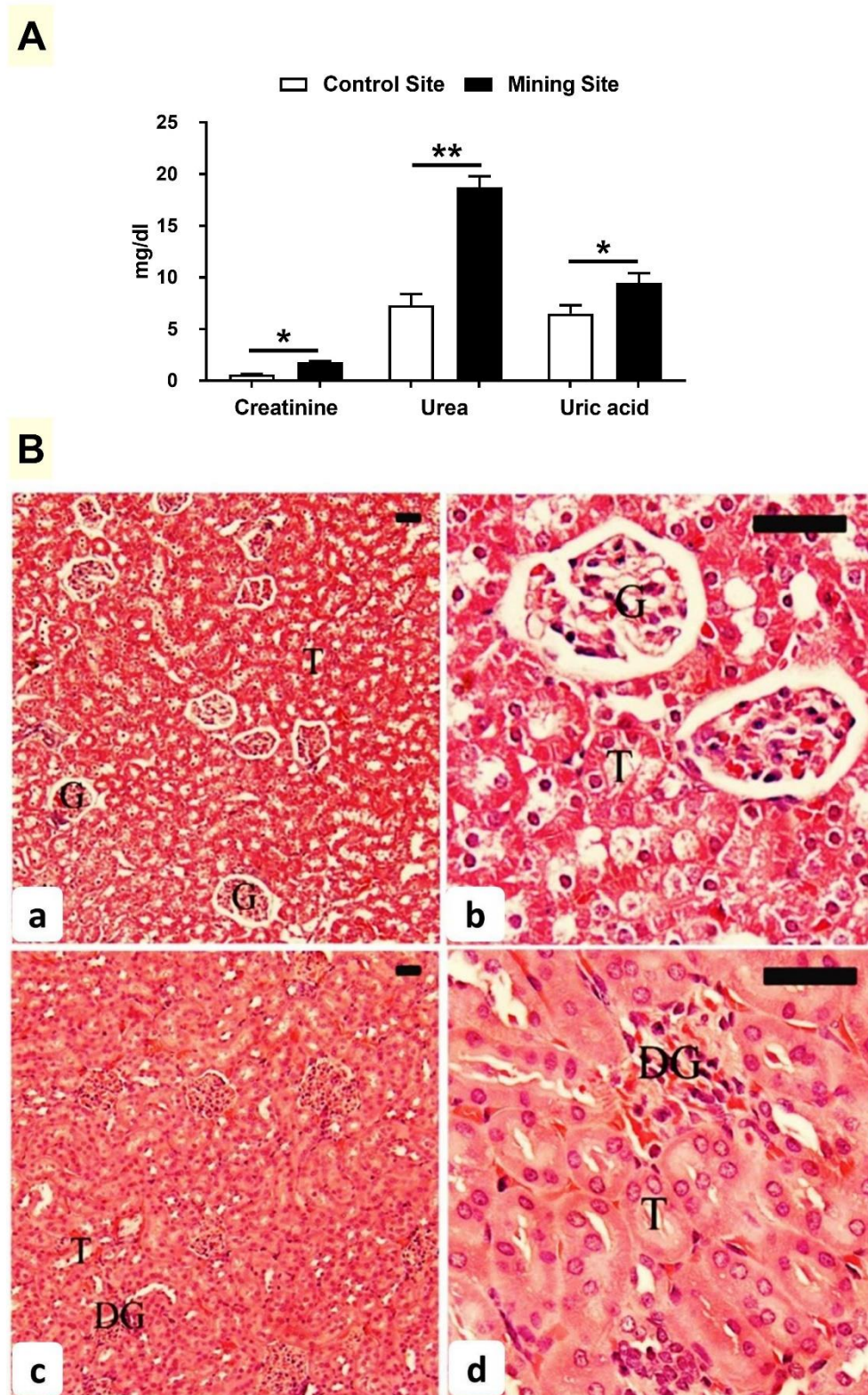


**Figure 3.** Concentration of heavy metals in liver, kidney and lung of *G. nanus* at the mining and control sites. Data are means  $\pm$  SEM, (N = 8). \*P<0.05 and \*\*P<0.01.





**Figure 4.** Effect of mining activities on liver function markers (A) of *G. nanus*. Data are means  $\pm$  SEM, (N = 8). \*P<0.05. (B) Photomicrographs of H&E-stained sections in liver of *G. nanus* at the control site (a and b) showing normal structure of hepatocytes (HC) and central vein (CV), and animals at the mining site (c and d) showing dilated central vein (DCV), hemorrhage (H) and vacuolations (V). [Scale bar = 100  $\mu$ m].

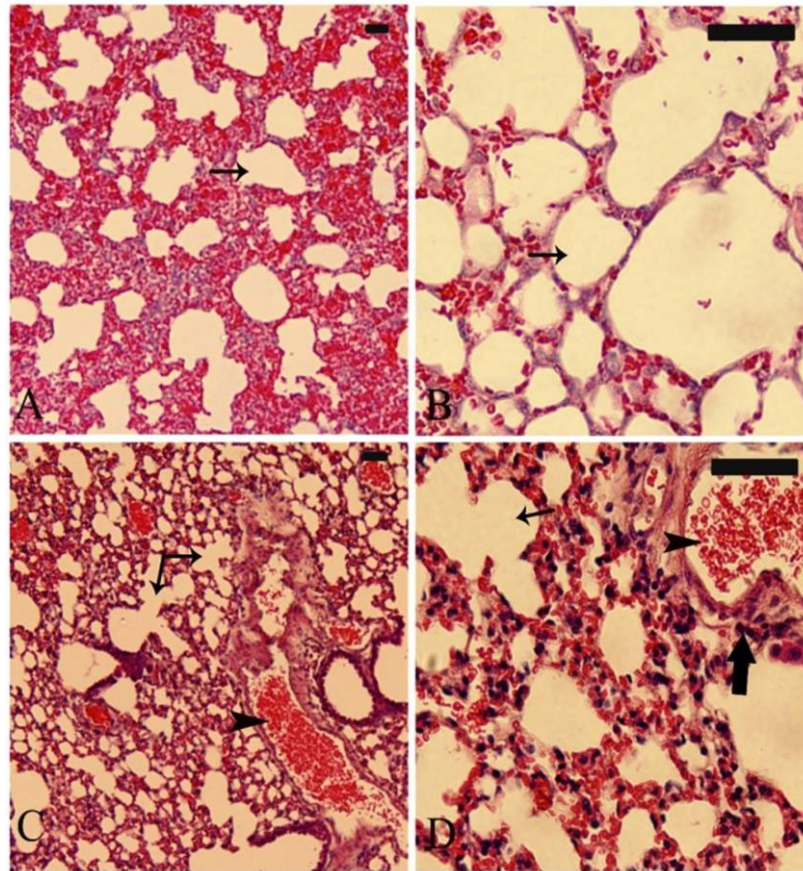


**Figure 5.** Effect of mining activities on renal function markers (A) of *G. nanus*. Data are means  $\pm$  SEM, (N = 8). \* $P < 0.05$  and \*\* $P < 0.01$ . (B) Photomicrographs of H&E-stained sections in kidney of *G. nanus* at the control site (a and b) showing normal structure of the glomeruli (G) and renal tubules (T), and animals at the mining site (c and d) showing degenerated glomeruli (DG). [Scale bar = 100  $\mu$ m].

### 3.5. Effect of mining on the lung of *G. nanus*



As represented in Figures 6A and 6B, the lung of *G. nanus* at the control site showed normal structure of the bronchioles and alveoli. In contrast, the lung of animals at the mining site showed dilated alveoli, inflammatory cells infiltration and congested blood vessels (Fig. 7C and D).

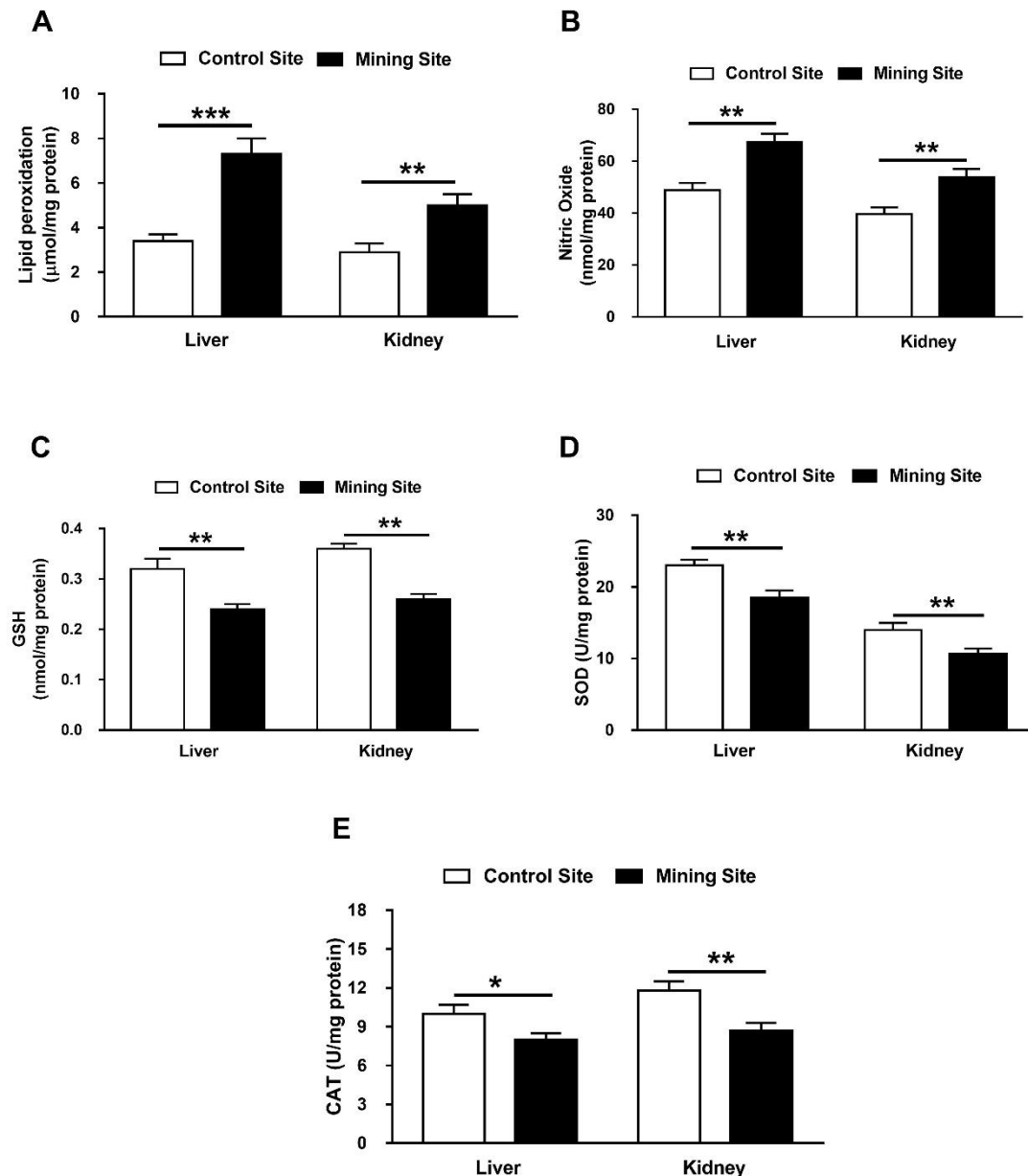


**Figure 6.** Photomicrographs of H&E-stained sections in lung of *G. nanus* at the control site (a and b) showing normal structure of the bronchioles and alveoli (arrow), and the lung of animals at the mining site showing dilated alveoli (thin arrow), inflammatory cells infiltration (thick arrow) and congested blood vessels (arrow head). [Scale bar = 100  $\mu$ m].

### 3.6. Effect of mining on hepatic and renal redox balance in *G. nanus*

To evaluate the impact of mining on the redox balance in *G. nanus*, we determined lipid peroxidation (LPO), NO, and cellular antioxidants in the liver and kidney. *G. nanus* at the mining site showed remarkable increase in hepatic ( $P<0.001$ ) and renal ( $P<0.01$ ) LPO (Fig. 7A). Similarly, hepatic and renal NO was increased significantly ( $P<0.01$ ) in *G. nanus* at the mining site (Fig. 7B). When compared with the control site, animals at the mining site showed a decrease in hepatic and renal GSH content ( $P<0.01$ ;  $P<0.01$ ; Fig. 7C), SOD ( $P<0.01$ ;  $P<0.01$ ; Fig. 7D) and CAT ( $P<0.05$ ;  $P<0.01$ ; Fig. 7E).





**Figure 7.** Effect of mining activities on oxidative stress and antioxidants in liver and kidney of *G. nanus*. Animals at the mining site showed an increase in (A) lipid peroxidation and (B) nitric oxide, and decreased (C) GSH, (D) SOD and (E) CAT in liver and kidney. Data are means  $\pm$  SEM, (N = 8). \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$ .

#### 4. Discussion

This study evaluated the impact of mining activities at Al-Quway'iyah, Riyadh (Saudi Arabia) on *G. nanus* with an emphasis on HMs accumulation and redox imbalance. To evaluate the emission and distribution of HMs in the mining site, we determined the concentrations of Pb, V, Cu, As, Zn, Cd, Fe and Hg in soil and *L. shawii* samples, and different organs of *G. nauns*. Pb, V, Cu, As, Cd and Hg were increased in both collected samples of soil and *L. shawii*. Fe and Zn concentrations were not significantly increased in soil and *L. shawii*, respectively. These findings showed the effect of mining on the soil profile as well as plants growing in the nearby areas.

Despite their negative impact on the ecosystem and adverse health effects, exposure to HMs continues to increase in many regions [28]. Here, Pb, V, Cu, As, Zn, Cd, Fe and Hg were increased in the liver, kidney and lung of *G. nanus*. Besides their environmental impact, these HMs have been

proven to be a major threat to the body systems and its proper functioning [28]. In accordance, liver and kidney dysfunction along with histopathological manifestations in the liver, kidney and lung of *G. nanus* were observed. These hazardous effects were directly connected to the accumulation of HMs in different tissues of *G. nanus*.

Pb, Cd, As and Hg have been demonstrated as the common HM pollutants produced through various human activities, including mining, and all of which are hazardous to the human health. Pb is a highly toxic HM whose widespread use resulted in health problems as well as environmental contamination. It has been estimated that 540000 deaths per year, particularly in developing countries occur due to Pb contamination [29] and that 26 million people are at the risk [30]. The most common depository of Pb within the body are the liver and kidneys [31], and this might explain the observed hepato- and nephrotoxicity in *G. nanus* at the mining site. The accumulation of Pb in liver and kidney, elevated transaminases, ALP, and renal function markers along with the histopathological changes indicated the injury of liver and kidney tissues. In this context, *in vivo* studies have demonstrated both hepato- and nephrotoxicity in rodents exposed to low concentrations of Pb [32-34]. The toxicity of Pb has been attributed mainly to its ionic properties and induction of redox imbalance [35]. It can disturb cellular signaling and metabolism, ionic transportation and enzyme activities through replacing mono- and bivalent cations [36]. In addition to its ionic activity, Pb triggers the generation of reactive oxygen species (ROS) which promote oxidative damage to lipids, DNA and proteins [37]. In accordance, LPO and NO were elevated accompanied with depletion of the cellular antioxidants in *G. nanus* at the mining site, demonstrating an oxidative stress status. Furthermore, Pb concentration was increased in the lung of *G. nanus* which showed dilation of alveoli, infiltration of inflammatory cells and congestions. The relationship between lung injury and Pb accumulation was supported by the findings of Li et al who demonstrated increased Pb concentration in the lungs of rats following exposure to atmospheric fine particulate matter [38].

Cd is a highly toxic nonessential HM and serious environmental pollutant. When released to the environment, Cd can remain for several decades in soils and sediments because of the lack of degradability [39,40]. It has a high rate of soil-to-plant transfer and is therefore accumulates in plants, predominantly in vegetables and fruits, and ultimately reach the human body [41]. The present study showed accumulation of Cd in the soil and *L. shawii* samples collected from the mining site. In addition, Cd concentrations were increased in different tissues of *G. nanus* and its accumulation was associated with oxidative stress, hepato-/nephrotoxicity and lung injury. Although the exact mechanisms of Cd toxicity are not fully elucidated, the role of oxidative stress has been well-acknowledged. Cd produces hydrogen peroxide which acts as a source of free radicals generated *via* Fenton reaction [42], and its toxicity has been reported to cause injury of liver and kidney [43,44]. Upon exposure, Cd binds to albumin and is taken up and deposits primarily in the liver [45]. Within the liver, Cd promotes ROS generation, LPO and membrane damage, GSH depletion and cell death [46]. Cd binds to metallothionein and the produced complexes induce liver injury and circulate to the kidney where they accumulate and cause renal tissue injury [47]. The activity of SOD and CAT was declined in the liver and kidney of *G. nanus* at the mining site and this results from the binding of Cd with the thiol groups of these enzymes [48]. In addition, Cd can inhibit complex III of the mitochondrial electron transport chain [48]. The observed nephrotoxicity in this study was associated with glomerular degeneration which has been shown in rats exposed to Cd [49]. Furthermore, the lungs represent one of the main routes through which Cd enters the body [40]. *G. nanus* in this study showed lung injury associated with elevated Cd concentration. The potential of Cd to provoke pulmonary toxicity has been supported by studies reported bronchial and pulmonary irritation, transient bronchial inflammation [50], diminished pulmonary function [51], and primary lung cancers [52] following exposure to Cd.

Mining is one of the major sources of As and Hg pollution. Accordingly, As and Hg were found in high concentrations in the soil, *L. shawii* and different tissue of *G. nanus* collected from the mining site. As is extensively available in different forms and possesses toxic and carcinogenic effects [53]. The toxicity of As includes the formation of inorganic arsenic species (iAs) monomethylarsonic acid and dimethylarsinic acid which produce methylated arsenicals via enzymatic conversion. Monomethylarsonic acid, an intermediate product produced during the transformation of iAs, is

highly toxic and carcinogenic [53]. The toxicity of As has been associated with its ability to increase ROS levels, activate apoptosis signaling, alter metabolism and energy generation, and replace metal ions in cellular biomolecules [54]. Therefore, the accumulation of As in *G. nanus* has a role in the observed oxidative stress, and hepato-/nephrotoxicity. Accordingly, recent reports have shown As-induced hepatic, renal and pulmonary manifestations in experimental animals [55-57].

Hg is a well-known HM pollutant and its toxicity is commonly responsible for acute HM poisoning. Although neurotoxicity is the most common effect of Hg and methylmercury which induced LPO and cell death [58], it can cause nephrotoxicity [59] and hepatotoxicity [60]. Hence, Hg accumulation in *G. nanus* participated, at least in part, to liver and kidney injury and redox imbalance.

V, Cu, Zn and Fe were the other HMs shown to accumulate in the soil, *L. shawii*, and different tissues of *G. nanus* at the mining site. V is considered as an environmental pollutant that possess hazardous effects, and inhalation of vanadium pentoxide dust has caused occupational toxicity [61]. Liver injury and other toxic effects have been demonstrated in rats exposed to V [62], and inhalation of V caused bronchopneumonia and chronic productive cough [63,64]. Cu exists in oxidized [Cu(II)] and reduced [Cu(I)] states and is therefore considered as potentially toxic. Generation of ROS during the conversion between the oxidized and reduced states represent the main reason of Cu toxicity [65]. Zn is an essential micronutrient needed for vital cell functions in microorganisms, plants and human; however, concentrations beyond the physiological value are toxic. At high concentrations, Zn interacts with sulfhydryl groups or replaces other essential metals in different cellular proteins [66]. Iron is a vital nutrient that acts as cofactor for multiple enzymes within the living cells. It mediates reactions that can catalyze the formation of free radicals, leading to cell damage. Hence, iron overload can induce toxicity particularly in children [67], hepatotoxicity [68] and nephrotoxicity [69]. Therefore, excess V, Cu, Zn and Fe are associated with ROS generation and participate in liver, kidney and lung injury in *G. nanus* living close to the mining site.

## 5. Conclusions

These findings highlight the hazardous effects of mining activities on the soil, plants and animals. HMs were increased in the soil and *L. shawii* at the mining site, demonstrating the negative environmental impact of mining. *G. nanus* living at the mining site exhibited hepato- and nephrotoxicity, and lung manifestations. The deleterious effects of mining activities on *G. nanus* were associated with oxidative stress and depletion of antioxidants. These results demonstrated the environmental and health impact of mining and might represent the scientific basis for evaluating the effects of mining on the nearby communities.

**Author Contributions:** Cconceptualization, A.M.M.; J.A. and A.A.A.; methodology, A.M.A.; A.M.M.; J.A.; A.A.A. and S.N.M.; validation, J.A.; A.A.A. and A.M.M.; formal analysis, A.M.M.; investigation, A.M.A.; A.M.M.; J.A.; A.A.A.; S.N.M.; F.S.A-O and N.A.; resources, F.S.A-O and N.A.; data curation, A.M.A. and A.M.M.; writing—original draft preparation, A.M.M.; writing—review and editing, A.M.M.; visualization, J.A.; A.A.A.; A.M.A.; supervision, A.M.M.; J.A. and A.A.A.; project administration, J.A. and A.A.A.; funding acquisition, J.A.”

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