



## **A study on wool arsenic concentration and some blood parameters in sheep flocks grazing around tailing dams of gold mines in Takab, Iran**

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### **Abstract**

Arsenic is an environmental toxicant with a worldwide distribution that causes neuropathy, skin and vascular lesions and leads to cancer in chronic exposure. While arsenic concentration is extremely high in drinking water due to its high level in the earth's crust in some areas, major causes of arsenic contamination are mining and smelting industries. The aim of this study was to determine the amount of arsenic and some blood parameters including total protein, AST (Aspartate Aminotransferase) and ALT (Alanine Aminotransferase) activity in sheep flocks grazing around two neighbour smelting centres of gold mines and their tailing dams located in Takab, West Azerbaijan province, Iran. The samples of wool and blood were collected randomly from 54 (46 female and 8 male) sheep in 4 villages surrounding the area. Results showed that arsenic concentration in the wool taken from exposed animals in this area was apparently higher than normal range (Mean values are more than 40 mg/kg comparing to the normal amount that is 5-10 mg/kg). The mean values measured for AST activity in the present study indicated the potential liver damage caused by chronic arsenic poisoning. However, there were no significant changes in ALT activity and total protein. Further large scale studies are suggested to confirm chronic arsenic toxicosis in sheep and other domestic animals.

**Keywords:** arsenic, blood, sheep, gold mining

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### **Introduction**

Arsenic (As) occurs naturally as an element, ranks as the 20th most occurring trace element in the earth's crust and is widely distributed in the environment (Duker et al., 2005). This solid, brittle metalloid element exists in 200 different forms, with approximately 60 percent as arsenate, and binds covalently with most metals and nonmetals, forming stable organic compounds (Council of National Research, 2005). Arsenic, being a normal component of human body is transported by the blood to different organs, mainly in the form of MMA (monomethylarsonic acid) after ingestion (Mandal and Suzuki, 2002). It is excreted rapidly after absorption, chiefly in the urine, and after the ingestion of non-toxic

amounts by the cow there is no detectable excretion in the milk. However, when large doses are taken arsenic may be excreted in the milk, as well as in urine and faeces, the concentration is still low (Radostits et al., 2007). Although large number of responses to apparent deprivation (e.g., <12 µg/kg diet for rats and chicks; <35 µg/kg diet for goats) has been reported for a variety of animal species (Council of National Research, 2005), arsenic is known as a general tissue poison (Radostits et al., 2007). Its compounds exert their toxic effects by combining with and inactivating the sulfhydryl groups in tissue enzymes (Radostits et al., 2007). Chronic exposure to arsenic can result in skin and organ cancer, impaired nerve function, as well as liver and kidney damage (Sarkar et al., 2007).

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Arsenic pollution in the environment has gained importance owing to its widespread toxic effects on human, animals, birds, aquatic life and plants through polluted ground water and food chains. Various livestock animals are also the likely victims of such catastrophes arising from arsenic pollution (Biswas et al., 2000). Although microorganisms play an essential role in the environmental fate of arsenic in relation to mechanisms of arsenic transformations (e.g. soluble and insoluble forms, toxic and nontoxic forms), human activities have exacerbated arsenic contamination in the environment. One of the examples of human activities that have adversely affected environment is mining (Duker et al., 2005). Some metal-bearing ore deposits including iron, arsenic pyrites in volcanic soils, gold and copper ores contain large quantities of arsenic which may be licked in situ, or carried off in the fumes from smelters and contaminate surrounding pastures and drinking water supplies (Radostits et al., 2007). Jahan et al. (2002) reported that in the state of Victoria (Australia), mining of gold had caused an estimated 30,000 tone of arsenic to be redistributed to the surface across the landscape through erosion into streams and rivers. Many incidents of arsenic contamination of the environment have been reported in several countries (Duker et al., 2005). The situation can have significant adverse influence on health due to arsenic uptake in water and food especially by developing and rural population who depend on local sources of food and water. Therefore, any arsenic geochemical anomaly may impact negatively on health (Duker et al., 2005; Ono et al., 2012).

Arsenic deposition in the hair occurs, and it persists there until the hair is shed, making possible the detection of prior arsenic ingestion in the absence of arsenic from the blood and faeces (Radostits et al., 2007). The hair of animals not exposed to arsenic should contain less than 0.5 mg/kg, but that of normal exposed animals may contain as much as 5-10 mg/kg (Radostits et al., 2007).

The aim of this study was to measure wool concentration of arsenic and some blood parameters such as total protein, ALT and AST in sheep herds that are grazing in suburb of two neighbour gold mines tailing dams (Takab) in West Azerbaijan province, Iran, which could affect the health of livestock and human.

## Materials and Methods

The samples were taken from 54 apparently healthy sheep in both sex and in two age groups. The area of sampling was located around two nearby gold mine smelting plants and their dumps in Takab, West Azerbaijan province, Iran. Based on the prior pilot study, topsoil samples had very high value (580.4 ppm) of arsenic in this mining regions and it was very high compared to previous study around one of the other gold mines of Iran (Keshavarzi et al., 2012). The samples of

wool and blood were taken from 46 female and 8 male sheep from 4 villages (Zarshouran 15, Alchalloo 13, Yaraziz 11, and Shirmard 15). Wool samples were collected from both costal sites of each animal with a stainless steel shear (Andreae, 1978; Ramirez-Perez et al., 2000). Then the samples were kept individually in clean, sealed plastic bags until analysis. Approximately 20 gram of wool per animal was used as representative sample for the measurement of arsenic concentration. In order to get ash from the wool samples, they were kept in 550°C furnace for 24 h. Two gram of ash was digested by adding 20 ml nitric acid (25%). After centrifugation, the supernatant was diluted in 1:10, 1:50, 1:100, 1:200, and 1:400 ratios and then prepared for arsenic measurement according to multiparameter photometer (HANNA, HI 83200, USA) instruction. The blood samples were taken from jugular vein by Venoject tube (Vacuette®, EDTAK3, Greiner Bio-one, Austria). Plasma was separated from whole blood by centrifugation at 3000 rpm for 5 min. Plasma samples were transferred to 1.5 ml microtubes and stored at -20 °C until subsequent analysis. The values of total protein, ALT and AST were measured with photometric method using diagnostic kit (ZiestChem Diagnostics, Tehran, Iran) in 54 samples. The SSPS (version 14) statistical tool was used for one-way ANOVA computation and  $P < 0.05$  was considered significant.

## Results

The values of wool arsenic concentration in four villages surrounding Takab gold mine dumps were all higher than normal range (Table 1). Although there was no significant difference between two age groups (under two years old versus over two years old), maximum level of arsenic amount was higher in older group (Table 2). There was no significant difference among sex groups (Table 3). The values measured for blood parameters had no significant difference between two age groups (Table 4). Unlike total protein content and ALT activity that were within their normal range in sheep (6-7.9 g/dl, 22-38 units/L, respectively), AST activity was higher than its normal range (60-280 units/L) in animal species.

## Discussion

Arsenic is a cumulative poison and its chronic exposure through contaminated drinking water has become an increasing global problem of public health concern (Jin et al., 2004). Except for localized areas where arsenic is extremely high in drinking water, major arsenic contamination has occurred by mining and smelting industries (Council of National Research, 2005). Natural soil concentration of arsenic typically ranges from 0.1 to 40 mg/kg with an average of 5 to 6 mg/kg (Girouard and Zagury, 2009). High arsenic concentrations,

**Table 1: Area and number of samples with maximum, minimum, and mean  $\pm$  SE values of arsenic in 54 wool samples**

| Number | Name of villages | Number of samples | Maximum (mg/kg) | Minimum (mg/kg) | Mean $\pm$ SE (mg/kg) |
|--------|------------------|-------------------|-----------------|-----------------|-----------------------|
| 1      | Zarshouran       | 15                | 104             | 7               | 43.000 $\pm$ 8.106    |
| 2      | Alchaloo         | 13                | 182             | 5               | 43.077 $\pm$ 13.013   |
| 3      | Yaraziz          | 11                | 107             | 13              | 46.091 $\pm$ 10.576   |
| 4      | Shirmard         | 15                | 364             | 5               | 59.533 $\pm$ 23.602   |

There was no significant difference between flocks ( $P>0.05$ )

**Table 2: The amount of arsenic in age groups (mg/kg)**

|                   | Number of sample | Maximum (mg/kg) | Minimum (mg/kg) | Mean $\pm$ SE       |
|-------------------|------------------|-----------------|-----------------|---------------------|
| Under 2 years old | 32               | 182             | 5               | 45.031 $\pm$ 6.889  |
| Over 2 years old  | 22               | 364             | 7               | 52.909 $\pm$ 16.405 |

There was no significant difference ( $P>0.05$ ) in amount of arsenic in both age groups

**Table 3: The amount of arsenic in Male and Female (mg/kg)**

|        | Number of sample | Maximum (mg/kg) | Minimum (mg/kg) | Mean $\pm$ SE      |
|--------|------------------|-----------------|-----------------|--------------------|
| Female | 46               | 364             | 5               | 50.957 $\pm$ 8.965 |
| Male   | 8                | 78              | 5               | 32.625 $\pm$ 7.971 |

There was no significant difference ( $P>0.05$ ) in amount of arsenic in both sexes

**Table 4: Blood parameters with Maximum, Minimum, and Mean  $\pm$  SE in two age groups**

| Number | Parameter | Number of samples | Maximum | Minimum | Mean $\pm$ SE       | Age groups (Year) |
|--------|-----------|-------------------|---------|---------|---------------------|-------------------|
| 1      | TP (g/dl) | 32                | 8.7     | 4.2     | 5.988 $\pm$ 0.1923  | 2>Y               |
|        |           | 22                | 8.2     | 4.8     | 6.132 $\pm$ 0.2123  | 2<Y               |
| 2      | AST (U/l) | 32                | 617     | 207     | 333.97 $\pm$ 16.158 | 2>Y               |
|        |           | 22                | 694     | 206     | 344.76 $\pm$ 25.361 | 2<Y               |
| 3      | ALT (U/l) | 32                | 73      | 13      | 34.581 $\pm$ 2.680  | 2>Y               |
|        |           | 22                | 57      | 17      | 29.571 $\pm$ 2.635  | 2<Y               |

There was no statistically difference ( $P>0.05$ ) between blood parameters of age groups

typically caused by anthropogenic sources, ranging from 10 to >1000 mg/kg have been observed worldwide (Girouard and Zagury, 2009). Mining activities cause arsenic to be released in high concentrations from oxidized sulphide minerals. This has resulted in high concentrations of arsenic in surface water, groundwater, soil and vegetation (Duker et al., 2005).

To the best of the authors' knowledge, no previous published research on arsenic concentration has been held in Takab gold mining district, so that data from this study could be compared. However, data from researches in gold mining sites worldwide and in some parts of Iran have shown average values of arsenic concentration higher than normal range. Keshavarzi and his colleagues (2012) showed that the natural background value for arsenic in Muteh gold mining district (located in Isfahan, Iran) was higher than both average arsenic concentration in Earth's crust (1.8 mg/kg) (Mason and Moore, 1982) and the mean global concentration given for soils (4.7 mg/kg) (Kabata-Pendias and Mukherjee, 2007). Although, the main source of arsenic in such areas may be geogenic, there is very little doubt that soil arsenic content is greatly enhanced by anthropogenic activity, i.e., exploitation of gold deposit. Therefore, in present study, the samples were taken from around smelting centres and their dumps. Prior to the study, 5 samples were taken from topsoil of the pastures around the tailing dams and analyzed to determine the value of arsenic in this area. The results

showed high arsenic contamination with the mean of 580.4 ppm.

The wool and blood samples were taken from animals that were grazing around upstream of the plants and downstream of the dams. As shown in table 1 to 3, arsenic concentration in nearly all collected wool samples in the Takab mining zone is considerably higher than the normal ranges given for sheep wool by Radostits et al. (2007). The measured values were noticeably higher than those calculated by Keshavarzi et al. (2012) in Muteh gold mining district that they showed sheep wool contained 4.24 mg/kg of arsenic. This difference may be because of the sampling sites in these two studies, as the samples were collected from around the mining zone in the previous study other than around the smelting centre and dam that was used in present study. The results show no significant correlation between sex and concentration of arsenic in wool (Table 3). Similarly, no significant difference has shown between two age groups (Table 2). Armienta et al. (1997) have shown that arsenic levels in human organs vary with age. Keshavarzi et al. (2012) also indicated that there is a positive correlation between arsenic level in human hair and age. However, same results were not found in animal population tested in this study. It could be because of incongruity among herds that samples are collected from.

Since the liver tends to accumulate arsenic with repeated exposures, hepatic involvement is reported most

commonly as a complication of chronic exposures over periods of months or years. Chronic arsenic induced hepatic changes include cirrhosis, portal hypertension without cirrhosis, fatty degeneration and primary hepatic neoplasia. The analysis of blood sometimes has shown elevated levels of hepatic enzymes (Mandal and Suzuki, 2002). Guha Mazumder (2008) also showed evidence of elevation of ALT, AST and ALP by 25.8, 6.3 and 29 percent of cases respectively. As shown in Table 4, AST activity was apparently higher than normal range (60-280 units/l) in sheep (Radostitis et al., 2007), indicating the potential of liver injury caused by chronic arsenic poisoning. However, because elevation of AST activity is not specific for liver injury and the enzyme exists in many other tissues (Latimer et al., 2003). This high AST activity may be due to injury of kidney or heart. Further studies are suggested to confirm chronic arsenic toxicosis in sheep herds and other domestic animals around Takab gold mining district and the hazardous effect on public health.

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