BLOOD LEAD CONCENTRATIONS OF HORSES AND DONKEYS IN THE VICINITY OF HEAVILY POLLUTED RIVER BY INTENSIVE INDUSTRY IN SOUTHEASTERN TURKEY

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The aim of the present study was to determine the concentration of Pb were assessed in the blood of horses and donkeys which were living in the vicinity of Nizip River where is the discharging area of intensive industry of Gaziantep City. A total of 66 (< 15 years) male horses and donkeys (41 horses and 25 donkeys) were sampled during 2005-2006. The concentration of the blood Pb concentrations were determined by the atomic absorption spectrometry (AAS) method. Mean concentrations of Pb in horses blood serum ranged from 0.06 to 1.88 ppm, and in donkeys from 0.20 to 2,23 ppm. The study allows concluding that the levels of Pb in both horses and donkey are significantly high depending on the feeding or grazing by the products that are grown in the agricultural area where irrigated with pollutioned river water or using it as a drinking sources.

Keywords: blood serum, donkey, horse, lead, pollution

INTRODUCTION

Environmental pollution due to the heavy metals is an increasingly important problem (Arslan *et al.*, 2011; Yarsan *et al.*, 2014; Yipel and Yarsan, 2014). The accumulation of metals in soil and water is of great concern because has adverse effects on environmental health, crop growth, and potential health risk to the local inhabitants by ingested or transferred through food (Dounay *et al.*, 2013). Toxic metal levels such as lead (Pb) in biological samples like blood in donkeys and horses are used to assess diseases (Asano *et al.*, 2002). Because of contact with organs and tissues where chemicals are stored, blood is the ideal matrix for most chemicals (Esteban and Castano, 2009). The most commonly used biomarker of Pb exposure is the Pb concentration in whole blood because of %60-90 of absorbed Pb found in erythrocytes (Nordberg *et al.*, 2007). It has been known by current studies that Pb exposure connected to cardiovascular (Weisskopf *et al.*, 2009), gastrointestinal, renal, nervous, musculoskeletal and haematopoietic diseases (Puschner and Aleman, 2010), lower body mass index, obesity (Scinicariello *et al.*, 2013), infertility (Rahman *et al.*, 2013) and crosses the placental barrier (Puschner and Aleman, 2010). Additionally Pb has a special concern about its neurotoxical effects (Zubero *et al.*, 2010) on animals like muscle twitching, convulsive seizures, depression, eyelid snapping, teeth grinding and blindness (Arslan *et al.*, 2011; Puschner and Aleman, 2010). Besides it leads to anaemia and renal damage (Zubero *et al.*, 2010). The toxicity is due a to the disruption of the prooxidant and antioxidant balance of the cells by Pb (Arslan *et al.*, 2011). In horses 0.2-0.5 ppm of blood Pb levels associated with chronic intoxication while blood <0.2 ppm is acceptable (Palacios *et al.*, 2002; Puschner and Aleman, 2010). Donkeys and horses are exposed to environmental effects of metals and at risk of chronic Pb poisoning feeding or grazing on contaminated pastures by polluted river or using it as a

drinking sources due to their long life span (Donkeys 40 years, horses 50 years) (Janiszewska and Cieśla, 2002; Puschner and Aleman, 2010). The main entranceway of Pb to the food chain of animals are Pb accumulated plants. Thus the pastures near industrial sites or polluted rivers must be evaluated for potentially toxic concentrations of Pb and other pollutants (Puschner and Aleman, 2010).

The Gaziantep City is placed in the in the southeast of Turkey which is a vital commercial, economic and industrial center with a population of over 1.5 million people. Although there are 67 large-scale industrial firms (27 textile manufacturing, 17 chemical, 6 cement construction, 3 energy, 3 machine manufacturing and 11 in other industrial fields) the city has only one urban wastewater treatment plant. Also a number of industrial activities (leather processing etc.) which are still not registered. The main source of the wastewater is industrial zone that discharge into the Nizip River (Avci, 2012). In a current study that aims to determine the heavy metal levels of wastewater of factories involved in various sectors within the province of Gaziantep City, has been emphasized that even after waste treatment a significantly high level of Pb (especially in motor oil industries, battery manufacturing industry wastes) has been determined which is classificated as ''can not be discharged'' (Yılmaz and Dinç, 2013). The Gaziantep City is placed in the southeast of Turkey and has the most developed industries in the region with over 1.5 million population. The river is 80 km length and a large land area (over 65,000 acres) where the samples collected surround of it is 45 km far away the Gaziantep City that uses this river water for agricultural applications which is contaminated with chemicals especially heavy metals. The Nizip Town and their urban environment has been exposed to the chemical pollution heavily by rivers and air due to intensive industrial activities in the Gaziantep City over the past two decades. Especially the Nizip River is the main sources of the inorganic pollution due to being main discharge area of urban and industrial wastewaters of Gaziantep City. The heavy metals enter the food chain of horses and donkeys primarily by using the river water in the growing process of plants that they grazing or feeding by (Avci, 2012). In a current research that aims to determine the metal levels in some plants which irrigated by river water such as maize (Zea mays L.) that is mostly consumed agricultural plant by local inhabitants and animals which has been sampled from Salkım village that is located on the area 1 (Fig.1). The researchers determined the increased Pb levels ($P< 0.05$) in all plant, soil and water samples to compare with control area by repeated investigations (Kafadar and Saygıdeger, 2010). On the other hand there is no data currently about use of Pb-containing pesticides or insecticide in the study area.

The present paper is concerned with the possible effects of polluted river by Pb intensively on human being and others by determine the blood serum levels of the donkeys and horses from southeastern Anatolia of Turkey.

MATERIALS AND METHODS

Study Area and Sampling

The blood samples were collected during the period between 2005-2006 from the areas where people were using water of Nizip River in agriculture for a long time depending on their living at a smaller distance (≤ 1 km) to the river (see in figure 1).

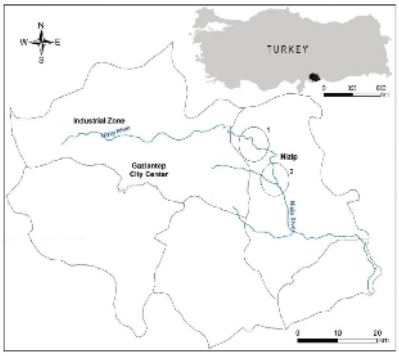

Figure 1. Sampling areas

The blood of 25 donkeys and 41 horses were used as the sample in this study because it has been considered as ideal matrix for biomonitoring of Pb (Esteban and Castano, 2009). All the animals that selected for the sampling were male and at the age between 1-15 years. The blood samples were taken 10 ml in to anticoagulant tubes from the vena jugularis after cleaned of the region by ethanol and deionized water by a sterile plastic syringe and needle and after immediately 20% trichloroacetic acid (TCA) was added was centrifuged (Arslan, 2011). The samples were frozen and stored at -20 until analysis.

Instrumental Analysis

Blood Pb levels were determined by atomic absorption spectrometry (ASS) (Unicam-929).

Statistical Analysis

The results were statistically analysed by using independent samples of t-test or Mann Whitney U-test. A p value less than 0.05 was considered to indicate statistical significance.

RESULTS AND DISCUSSION

Sample Profile

A total of 66 animals (Donkeys *n=25* and Horses *n=41*) that are members of farmer communities in the eastern traditional farming villages of Gaziantep City were studied. The ages of the included animals, who were divided in to two groups as under 5 years and over 5 years old, ranged from 1 to 15 years, with the mean age equalling 5.3 years in total *(n=66)*, 2.6 years in group 1 *(n=45)*, 9.2 years in group 2 *(n=21)*. 37.9 percent of the sample belongs to donkeys, and all the animals were feeding or grazing mainly by the agricultural plants which are grown in irrigated lands by Nizip River.

The majority part of the animals were healthy in terms of clinical findings (gastrointestinal, urinary, neurological, and dermatological vs.) outside of common intestinal parasite infestation.

Horses, Donkeys and Lead

Nizip River more than 8 mounts of a year. 38 percent of horses and 53 of donkeys use the river water for drinking if it is necessary.

Blood Pb Distributions of Horses and Donkeys

Among the study animals *(n=66)*, the median blood Pb level was 0.96 ppm in the range from the minimum 0.06 ppm, to the maximum 2.23 ppm 0.06-2.23 (Table1). Mean Pb concentration in the horse blood serum (*n=27*) was found as 0.82 ppm in the range from the minimum 0.06 ppm, to the maximum 1.43 ppm and in donkeys (*n=27*) 0.88 ppm in the range from the minimum 0.20 ppm, to the maximum 1.64 ppm for group 1 (<5 years old). While mean Pb concentrations in the horse blood serum (*n=14*) were found as 1.14 ppm in the range from the minimum 0.28 ppm, to the maximum 1.88 ppm and in donkeys (*n=17*) 1.37 ppm in the range from the minimum 0.54 ppm, to the maximum 2.23 ppm for group 2 (>5 years old). Blood Pb levels in the total horse samples ranged from 0.06 to 1.88 ppm, with the mean level 0.93 (SD=0.42) (*n=41*) and donkeys ranged from 0.20 to 2.23 ppm, with the mean level 1,01 (sd=0.46) (*n=25*). More than eighty percent of the horses and eighty four percent of the donkeys had blood Pb levels greater than or equal to 0.5 ppm that is recognized as a chronic Pb intoxication in references (Palacios *et al.*, 2002).

Table 1 gives the blood Pb distributions by groups of age. As can be seen, blood Pb levels were higher in 5 years old and younger group relative to 5 years old and older group (p=0.02). Horses also had the highest proportion (%54.5) of samples with blood Pb levels >5 ppm, compared to donkeys.

Table 1. Levels of Pb (ppm) in horses and donkeys

		< 5 years	> 5 years	Total	p[a]
Horse	N	27	14	41	0,02
	mean	0,82 ppm	1,14 ppm	0,93 ppm	
	Standard deviation	0,38	0,44	0,42	
	Min.–Max.	0,06-1,43	0,28-1,88	0,06-1,88	
Donkey	N	18	7	25	0,01
	mean	0,88 ppm	1,37 ppm	1,01 ppm	
	Standard deviation	0,37	0,51	0,47	
	Min.–Max.	0,20-1,64	0,54-2,23	0,20-2,23	
Total	N	45	21	66	0,02
	mean	0.84	1,22	0,96	
	Standard deviation	0,35	0,32	0,44	
	Min.–Max.	0,06-1,64	0,28-2,23	0,06-2,23	

[a]Mann–Whitney test

It has been demonstrated by findings of our investigation that higher blood Pb levels (>0.2 ppm) of healthy horses and donkeys were associated with Pb intake by ingestion over tolerable concentrations. As is well known feeding is one of the environmental factor that controls the concentrations of various elements in the animal body. Combined with results of current researches (Kafadar and Saygıdeğer, 2010; Yılmaz and Dinç, 2013) it has been understood that the source of the Pb is diet. The major part of their diets is forming by plants which accumulated Pb in high concentrations because of irrigation by Nizip River. The river has been heavily pollutioned by extensive

industrial activities of Gaziantep City which has an inadequate waste treatment process. Also one of the main way of Pb intake is inhalation but distance of city centre of Gaziantep (45 km) and industry area (50 km) and there is no road (closer than 15 km) with intensive traffic. Thus it can be considered as not necessary the Pb intake by inhalation. Statistical analysis showed that diet Pb levels has significant correlation with blood Pb level. Also, there was significant difference in Pb levels between group 1 (<5 years) and group 2 (>5 years).

The Joint FAO/WHO Expert Committee on Food Additives established a provisional tolerable weekly intake of 0.05 mg/kg b.w. for adult humans. However, increased blood Pb levels did occur when the dietary intakes of Pb were 8-9 µg/kg b.w./day (JECFA, 2013).

Horses from Gaziantep City exhibited relatively low blood Pb levels compared with horses from other cities, and higher Pb levels than donkeys. This may be related to leaded gasoline environmental pollution and children's hand-to-mouth activities.

CONCLUSIONS

Even after treatment of the Gaziantep City industrial wastewaters Pb level is high according to current studies. However, discharged wastewaters and an agricultural crops used for foodstuffs did not comply with the legislation of authorities. Due to discharging wastewater of intensive industrial and urban activities, the Nizip River and agricultural area that located around the river is strongly contaminated by Pb and create to risk on ecology. With this study the reflection of the ecological risks on some animals like donkeys and horses has been demonstrated. The elder participant's metal concentrations higher than young's associated with exposure time.

This observed results could have significant implications for assessing of health risks to local inhabitants and consumers of agricultural and livestock products of the region as well as investigations of ecological risk and monitoring studies.

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