

## A STUDY ON SOIL, FORAGE AND PLASMA LEAD LEVELS IN LACTATING COWS REARED IN SUBURB OF SARGODHA: TRANSPORT OF LEAD INTO MILK

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### ABSTRACT

To assess the transport of lead (Pb) in soil-plant-animal continuum, a study was conducted in a semi arid region of Punjab, Pakistan. Samples of soil, forage species, blood, and milk of lactating cows were taken four times during the study period and analyzed after wet digestion with atomic absorption spectrophotometry. The data were statistically analyzed and results showed that soil, forage, and plasma Pb remained unaffected ( $P < 0.05$ ) at different sampling periods. The maximum level of Pb in soil was at the 2<sup>nd</sup> sampling period (November) while, minimum concentration was observed at 4<sup>th</sup> sampling period during this study. Highest level of forage Pb was found at sampling period 1<sup>st</sup> (October) and lowest was at 3<sup>rd</sup> sampling period (December). Mean plasma Pb level was higher at 4<sup>th</sup> sampling time (January) and lower value at 3<sup>rd</sup> sampling period (December). The maximum level of milk Pb was observed during 4<sup>th</sup> interval while minimum level was at 3<sup>rd</sup> sampling period. Overall, results of the present study showed that accumulation of Pb in soil forage, plasma, and milk is very low that was non toxic for plants and animals and there is no warranted need of supplementation of animals with mineral mixture antagonistic or synergistic to Pb in this studied area.

**Keywords:** Lead, Soil, Forage, Plasma, Milk, Critical level.

### INTRODUCTION

Minerals are inorganic in nature and are present in all the body tissues and fluids as a vital substance. Minerals are needed for the proper functioning and fundamental chemical processes of life in many ways. Even though minerals generate no energy but they play vital roles in the functions of the body (Malhotra, 1998; Eruvbetine, 2003). Uptake and accumulation of lead (Pb) in animals from water has been reported by (Ahmed and Bibi, 2010) which is indirectly reaches in human through food chain. In this century, biological evaluation methodologies elucidated the importance of minerals for livestock nourishment and current investigative methods lead to the revealing of trace minerals as necessary elements and this is still a vigorous area of recent exploration. Trace elements deficiency is one of the important health problems facing new born and pregnant animals particularly in under developed countries (Batra and Seth, 2002). Lead is a ubiquitous industrial as well as environmental pollutant that has been found in almost every biological systems and its excessive usage causes lead contamination in soil, dust and air (Kiran *et al.*, 2008). The continuous exposure of lead induces serious health problems (Pirkle *et al.*, 1998). It has been observed that animal's reproductive health is severely affected by various environmental pollutants

including lead (Bonde *et al.*, 2002). Information about the toxic and trace metal concentrations in soil, water, food and grazing animals is significant to check the consequences of pollutants on domestic animals and the intake of contaminants by the human beings. The lead induces most common problems in male and female reproductive dysfunction includes disorganized epithelia, reduced androgen level and considerable alteration in sperm quality, number and its morphology. In addition, Pb has a lethal consequence on the hypothalamic-pituitary unit, testes and reproductive axis (Sokol *et al.*, 2002). It is well known that water, food and sediment are the main sources for heavy metal uptake (Beldi *et al.*, 2006). Though, efficacy of minerals absorption from these sources varied significantly depending on ecological needs, animal's metabolism, amounts of heavy metals and some additional abiotic features (Roesijadi and Robinson, 1994). It has been found that composition of feed resources is more important as compared to that of their deficiency (Aletor and Omodara, 1994). Little information is available on the status of minerals in the plants that are consumed as food by animals and human beings in Pakistan, particularly the latest varieties of food and less common legumes. Some of the previous information was based on the hypo-sensitive techniques for the analysis of minerals. The purpose of present the level of lead in soil, forage, and animals. Information on mineral amounts of soil, plant and livestock diets are

important for preparation of supplementing strategies and foodstuff dispensation methodology.

## MATERIALS AND METHODS

The present study was carried-out at a livestock farm in suburb of Sargodha city during 2010- 2011). This livestock farm falls under semi-arid environment situated in central Punjab, (32° 8' O'' N 73° 7' O'' E; altitude 187 m). The pH of the soil ranging from 7.6-8.2 and soil texture is clay to clay loam. Annual temperature varied from 5-51°C and precipitation 180- 200 mm.

**Pastures and Animals Description:** During the present study, dominant forage species were *Avena sativa*, *Cichorium intybus*, *Medicago sativa*, and *Trifolium alexandrinum*. These forage species were being consumed by the animals during the investigation period. The main sources of irrigation were tube well and canal waters. Twenty healthy “Desi” lactating cows (6 years old) were selected and their average body weight recorded was about 350-440 kg. The selected class of “Desi cows” was basically allowed to graze on the pastures of the rural during different seasons.

**Sample collection:** Samples of soil forage and blood plasma were taken on monthly basis from October 2010-January 2011. For soil and forage samples collection five different sites known as “feeding sites” were selected within the pasture. Five composite soil samples were taken at a depth of 20 cm as described by Sánchez (1981). Similarly five composite forage samples each comprising of the dominant forage species commonly grazed by the animals were collected at the same time. To simulate the grazing behavior of the animals, the forage samples were clipped to a height of 3-6 cm from the ground. Air dried soil and forage samples were placed in cloth bags. The blood samples (15 mL) from cows were collected at the same time using jugular vein punctured with a sterilized plastic syringe containing heparin (anticoagulant). Plasma was separated by centrifugation and frozen at -20°C for mineral analysis (Fick *et al.*, 1979). The 20 lactating “Desi cows” were sampled for milk collection (100 mL/cow) concurrently with blood sampling. Samples of milk obtained were stored in special small bottles, four times after one month interval.

**Sample preparation and analysis:** Air and oven-dried soil samples (1g each) were transferred in digestion tubes and 5 mL of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) were added, incubated overnight at room temperature and H<sub>2</sub>O<sub>2</sub> (25 mL) was poured down through the sides of digestion tubes and these tubes placed on a hot plate to heat them until the samples became colorless. The extract was diluted up to 50 mL using de-ionized water, followed by filtration and used for the analysis of Pb concentration (Wolf, 1982). Samples of air-dried forage were further dried in an oven

at 60 °C for two days, crushed, ground and processed with nitric acid (HNO<sub>3</sub>) and perchloric acid (HClO<sub>4</sub>) (3:1) at 250 °C until the digestion material became colorless (Koh and Babidge, 1986; Anonymous, 1990). The contents were diluted up to 50 mL using distilled water and used for mineral analysis determination. The plasma and milk samples (1.0 mL) were pretreated with 50% HNO<sub>3</sub> to avoid swelling or spattering and then subjected to wet digestion with HNO<sub>3</sub> and HClO<sub>4</sub> as described earlier. Atomic absorption spectrophotometer was used for examination of Pb from soil and forage (flame method) and plasma and milk (graphite furnace method) samples.

**Statistical Analysis:** Analysis of variance technique (Steel *et al.*, 1997) was employed to test the significance level at 0.05, 0.01 and 0.001 with the help SPSS statistical software.

## RESULTS AND DISCUSSION

**Soil:** The data from analysis of variance for soil Pb showed a non-significant (P>0.5) effect of sampling periods on soil Pb concentration (Table 1). The maximum level of Pb in soil (0.0031mg/kg) was observed at 2<sup>nd</sup> sampling period (November) and minimum value (0.0016 mg/kg) was found at 4<sup>th</sup> sampling period (January; Fig. 1). The soil Pb levels were lower than the values reported previously by various researchers (Oluokun *et al.*, (2007), but above the values reported by Aksoy and Shahin (1999) in Turkey who investigated on biomonitoring of heavy metal pollution in that region. According to Ross (1994) the Pb levels in soil were below than the toxic level exposing no danger to life of plants and animals. Lead toxicity has no hazardous effects on animals in the premeditated areas where the lead levels of soil and pasture forage estimated was far less than the lethal amounts, and there is no threat of lead toxicity in the cows under investigation which could be due to grazing this uncontaminated pasture by any industrial wastes and effluents (Lemos *et al.*, 2004).

**Forage:** From the analysis of variance data it is evident that the non significant (P>0.05) effect of sampling periods on forage Pb concentration (Table 1). Highest level of forage Pb (0.0026 mg/kg) was found at sampling period 1<sup>st</sup> (October) and lowest (0.0019 mg/kg) was at 3<sup>rd</sup> sampling period (December; Fig. 1). Lead concentration in forage was similar to the values found by Ross (1994) in Turkey while evaluating forage for heavy metals. The mean lead value in forage samples was higher than those established earlier by Oluokun *et al.*(2007); Mlay and Mgumia,(2008) in Tanzania and below than the values described by Aksoy and Shahin (1999) in Turkey. It has been reported that plant varies in their ability to accumulate heavy metals and elevated level of these

metals related to those plants growing in soil with less amount of heavy metal Ross, 1994).

**Blood Plasma:** The analysis of variance of data showed non-significant ( $P>0.05$ ) effect of sampling periods (Table 1). Mean plasma lead level was higher (0.0007 mg/L) at 4<sup>th</sup> sampling time (January) and lower value (0.0004 mg/L) at 3<sup>rd</sup> sampling period (December; Fig. 1). Our values were above the values studied by Lopez *et al.* (2008). It has been demonstrated that when there is 0.01mg/L lead in the pasture it indicated that the grazing livestock were exposed to Pb contaminants. The long-term storage site for Pb in animals is the bones, and during parturition, the mobilization of bone can increase the cow's blood Pb concentrations. The effect of this mobilization is not dramatic in terms of inducing toxicosis or causing contamination of milk in the cows studied here. Nevertheless, this study points out the no potential hazards for more severe or more proximate cases of lead toxicosis in "Desi cows" investigated in this

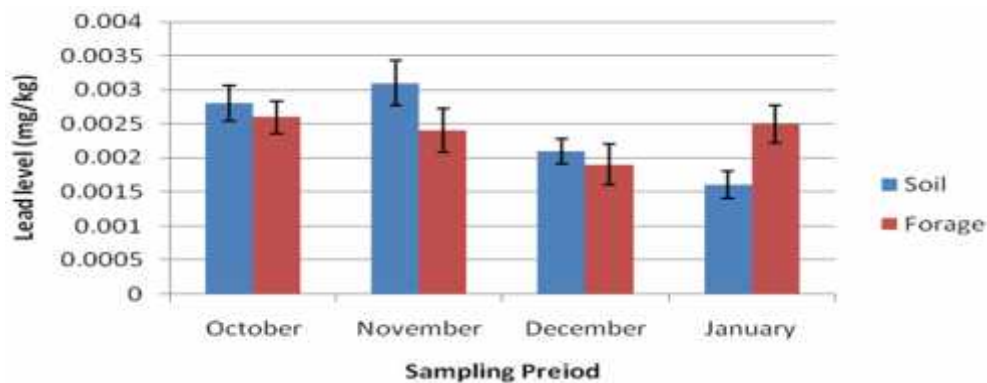
study. Similar variation in blood serum/plasma of animals has described by Akhtar *et al.*, (2009) in Pakistan, and Türkmen *et al.*, (2011) in Turkey as have been reported in our study.

**Milk:** There was a non significant ( $P>0.05$ ) effect of sampling periods on the Pb status in milk (Table 1). The values of Pb in milk ranged from 0.0003 to 0.0006 mg/L during all sampling periods. The maximum level of Pb was observed during 4<sup>th</sup> interval and minimum level was at 3<sup>rd</sup> sampling period (Fig. 1). Our studied values are Pb values in soil, forage, plasma, and milk are very low, so there are no hazards of any toxicity of Pb for forage plants from soil, animals blood plasma from forage, and consequently transfer of Pb from plasma to milk of cows pointing out to the safe and acceptable limits for cows and human health consuming meat and milk of these animals. Based on our findings, no potential threat of Pb toxicity can be anticipated in animals in this particular animal farm of the specific region.

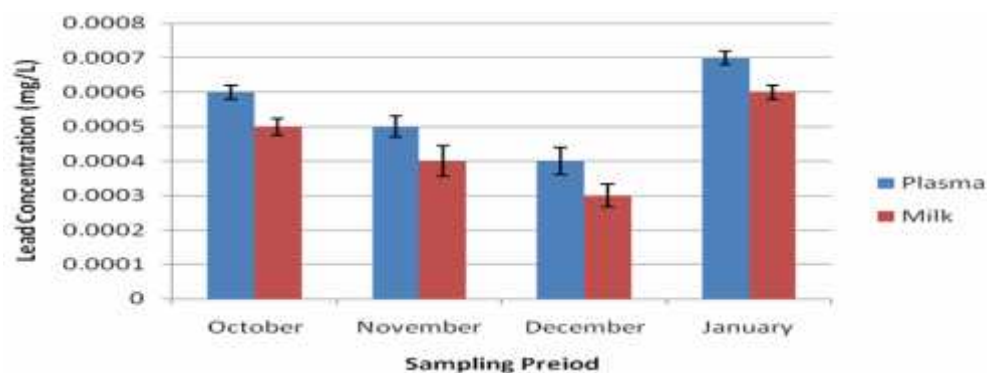
**Table 1: Analysis of variance of data for Lead concentration in soil, forage plants, blood plasma, and milk, at different sampling intervals**

Source of Variation	Degree of freedom	Mean Squares			
		Soil	Forage	Blood plasma	Milk
Sampling Period	3	0.0002 <sup>ns</sup>	0.0003 <sup>ns</sup>	0.0002 <sup>ns</sup>	0.0001 <sup>ns</sup>
Error	16	0.0001	0.0002	0.0001	0.0001

ns: non significant



**Figure 1. Fluctuation in levels of Lead in soil and forage at different sampling periods**



**Figure 2. Fluctuation in concentration of Lead in plasma and milk at different sampling periods.**

**Conclusion:** Regardless of the presence of potential supply of environmental contaminants containing Pb, the levels of this metal in the soil, pastures, milk and blood plasma of cows in the area in the suburb of Sargodha, Pakistan have not reached alarming level.

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