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Evaluation of Lead Concentration in Pasture and Milk: A Possible Risk for Livestock and Public Health

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Abstract.- This experiment was conducted to investigate the lead (Pb) status of soil, forage and milk of Sahiwal breed cows from October 2010 through January 2011. Soil, forage and milk were sampled on monthly basis, four times during the investigation. An atomic absorption spectrophotometer coupled with a graphite furnace technique was used to determine Pb concentrations. Concentrations of Pb in soil ranged from 1.2 to 3.5 mg/kg, forage 0.33 to 0.70 mg/kg and in mik 0.018 to 0.050 mg/L, respectively. Pb concentration decreased gradually in soil, forage and milk with sampling time. Pb concentrations in both soil and forage appeared to be lower than those of permissible limits reported in the literature. However, cow milk Pb concentration was slightly higher than the permissible limit which may be anticipated to be hazardous for public health in addition to the health of suckling neonate animals. This can be mainly due to greater pollution of the environment (air, water and soil). Further studies are therefore necessary to evaluate the Pb and probably other heavy metals status in the soil, water, forages and milk in larger sample numbers from the region.

Keywords: Lead, lead contaminated soil, lead concentration in forage, cow milk, grazing cow.

INTRODUCTION

Milk is a primary source of nutrients in diets all around the world (Buldini *et al.*, 2002). Technical progress, various industrial activities and increased roadway traffic resulted in significant increase of environmental contamination (Licata *et al.*, 2004). By their spread speed in biosphere and increasing concentrations, heavy metals are considered to be among the most hazardous pollutants (Staniškiene and Garalevičiene, 2004). The almost ubiquitous presence of some metal pollutants, especially cadmium and lead in the environment facilitates their entry into the food chain, thus increasing the hazard of human and animal health (Licata *et al.*, 2004).

Cow milk, in spite of its nutritions value, is not quantitatively comparable with either sheep or goat milk. Cow's milk being rich in fat, protein and minerals, belongs to an outstanding valuable human food (Antunovic *et al.*, 2001). Daily milk yield varies due to various factors such as lactation stage, feeding, breed, season of the year, etc. (Jelinek *et al.*, 1993). In general, cow milk contains very low concentrations of heavy metals (Lopez *et al.*, 2002), which however, increase at times, though their excretion through milk is proportionally very low (Miller, 1971; Houpert *et al.*, 1997). Accumulation of heavy metals in the ecosystem (water, soil, plant and animal) makes them very toxic and leads to undesirable consequences for live organisms (Bogut *et al.*, 2000). Free-living animals are important indicators of the environmental pollution with heavy metals (Kottferová and Koréneková, 1998) Sheep and cattle reared freely on pasture are also indicators of the environmental pollution (Gallo *et al.*, 1996). Increased concentrations of heavy metals in the body of domestic animals result in low fitness of animals and reproduction problems as well as in immunity decline and occurrence of cancerous and teratogenic diseases (Bires *et al.*, 1995). It is also known that heavy metals excretion such as cadmium (Cd), lead (Pb) and mercury (Hg) is significantly lower in the offspring (Oskarsson *et al.*, 1995). The assessment of dump site soils for the concentration of hazardous metals is imperative for healthy crop production.

The current study was therefore carried out to

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assess the concentration levels of some hazardous metals in soils and some plants in selected urban and rural areas in Pakistan. The objective of this investigation was to determine Pb concentrations in forage and cow milk and their fluctuations depending on periods of sampling~~.~~

MATERIALS AND METHODS

The present investigation was carried out at a rural livestock farm during 2010-2011 in central Punjab, Sargodha, Pakistan. This area experiences seasonal variation during a year, so these seasons have varying influences on both plants and various livestock herds. The animals were at the average age of four years, healthy and in good conditions. At the farm they were exposed to rotation pasture (*Trifolium alexandrium, Medicago sativa* and *Avena sativa, Brassica compestris, Cichorium intybus* and *Hordeum vulgare).* The cows had free access to salt block and fresh water. An average annual temperature of this region during the summer is 40.7 °C whereas a long-time average amount of total annual precipitation is 200 mm.

Sample collection

For soil and forage samples, three places in the pasture were selected randomly at a distance of an acre. The selected places were dug up to 12-15 cm deep partially containing all the soil layers by stainless steel auger. The sampling of both soil and forage was done for four consecutive months along with milk sampling, *i.e.* October, November, December during 2010 and January 2011, and five composite soil and forage samples were collected during each sampling. These collected samples of soil and forages were air-dried, stored in labeled sealed paper bags and placed for drying in an oven for 15 days at 60 ^{0}C. The samples of soil and forages were crushed and ground in a porcelain mortar to pass through a 2 mm mesh and placed in drug poly-bags of 7 cm × 10 cm size, labeled and sealed. These prepared samples were stored until analysis.

Milk samples were obtained from cows four times with one month interval simultaneously with soil and forage sampling from 25 clinically healthy animals with age ranging from 6-8 years and these cows were in their 3$^{rd.}$lactation. The milking was carried out into special small bottles intended for field milk sampling and an amount of 100 ml milk per cow was collected during each sampling. Milk samples were stored at -20°C until analysis.

Sample preparation and analyses of metals

The sample preparation was carried out at the Department of Biological Sciences, University of Sargodha. Air and oven dried ground soil and forage samples were well mixed and 1.0 g of each sample was taken in 250 ml glass beakers covered with watch glass and digested with a mixture of nitric acid and perchloric acid in a ratio of 3:1 on hotplate for 2 hours to ensure complete digestion. After evaporation to near dryness, 5.0 ml of ultrapure water was added to the content of the beaker, filtered while hot into volumetric flask and then diluted to 50 ml with ultrapure water. Digestion of milk was carried out using 2 ml milk with 5 ml of concentrated nitric acid, according to Tsoumbaris and Papadopoulou (1994).

The final digest was diluted to 50 ml with demineralised water. The Pb concentrations in soil, forage and milk were determined using atomic absorption spectrophotometer coupled with a graphite furnace was used (AA-6300 and GFAEXi7i, Shimadzu, Japan). The calibration curves were prepared by running different concentrations of standard solutions. The instrument was set to zero by running the respective blank reagents. In order to check the reliability of the analytical methods employed for trace metals determination, Lichens coded IAEA-336 was also digested and then analyzed following the same procedure. Transfer factor (TF) was calculated for each metal according to the following formula:

$$TF = Ps / St,$$

Where *Ps* and *St* are plant and soil metal content (µg/g dry wt), respectively (Houpert *et al.,* 1997).

Statistical analysis

Average values of three replicates were taken for each determination and were subjected to statistical analysis using statistical software (Statistica, 2001) and the following model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where Y_{ij} is j^{th} observation in i^{th} sampling time, μ is the average, T_i is sampling time effect (I = 1 to 4) and e_{ij} is the residual error. Differences were considered significant at the level of 0.05 (P<0.05).

Table I.- Transfer factor of Pb from soil to plant and from plant to milk.

	Sampling time			
	October	**November**	**December**	**January**
Soil - forage	0.23	0.20	0.25	0.28
Forage – milk	0.72	0.75	0.65	0.54

RESULTS AND DISCUSSION

Soil Pb concentration

The sampling time affected (P<0.01) soil Pb concentration; the highest level of soil Pb concentration was found at the first sampling time (October) and the lowest value was observed at the last sampling time (January) (Fig. 1A). The mean soil Pb ranged from 1.20 to 0.35 mg/kg dry weight among various samplings. A consistent decrease pattern in soil Pb was observed by the time indicates the exhaustion of this metal by the forage crops. The soil Pb levels observed in our investigation were much lower than those reported by Hayashi *et al.* (1985), during his investigation which were in the range of 5 to 25 mg/kg in the studied pastures.

Forage Pb concentration

The sampling time had a significant effect (P<0.001) on forage Pb concentration; the highest level of forage Pb concentration was found at the first sampling time (October) and the lowest value was observed at the last sampling time (January, Fig. 1B). The mean forage Pb ranged from 0.33 to 0.70 mg/kg dry weight among various samplings. The forage Pb followed the similar gradual decrease as in the soil by time. The forage Pb levels detected in the present investigation were lower than the acceptable Pb limit of 3.0 ppm reported for plants (Allen, 1989). Forage Pb concentrations in our experiment are similar to the values found by Ross (1994) while the values are higher than those reported elsewhere (Oluokun *et al.*, 2007; Mlay and Mgumia, 2008).

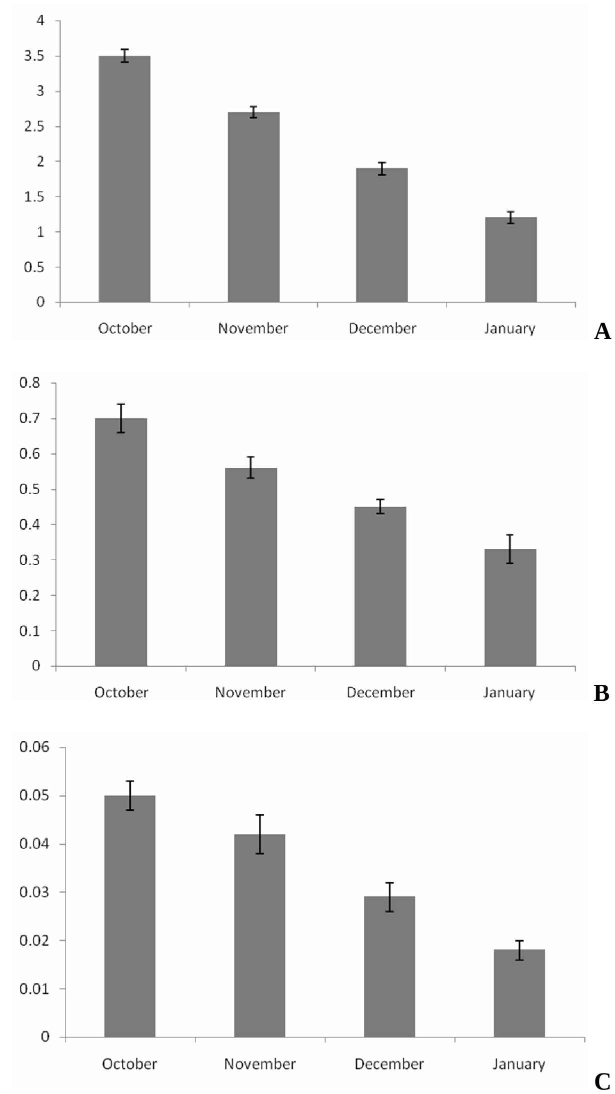

Fig. 1. Soil (mg/kg) (A), forage (mg/kg) (B) and Milk (C) Pb concentration (mg/L) at different sampling times.

Milk Pb concentration

There was a significant (P<0.01) effect of sampling time on the Pb concentration in milk. The values of Pb in milk ranged from 0.018 to 0.050 mg/L during all sampling periods. The maximum level of Pb was observed in the first sampling (October) and the minimum level was observed at last sampling time period (January, Fig. 1C). The milk Pb values followed the similar gradual decrease as in the soil and forage by time, showing probably no antagonistic effect of other metals in the forage and in the gastrointestinal tract of the animals.

Our observed values are higher than those reported by Gabryszuk *et al.* (2008). Concentrations of Pb in ewe milk was dependent on lactation stage. Pb concentrations were substantially high in milk collected during first month compared to milk on the 2nd, 3rd and 4th lactation months. It has been reported that ewes excrete greater proportion of Pb through milk relative to cows (Mehennaoui *et al.*, 1997). Rodriguez *et al.* (2001) detected lower concentrations of Pb in cow milk in evaluation in his investigations. They also discovered that Pb might have been a nutritional ingredient. Kottferová and Koréneková (1998) detected greater Pb concentrations (0.048 mg/kg) in milk of cows from the average value. A dwindle in goat milk Pb concentrations with progress of lactation stage calculated from goats fed meadow was reported Simsek *et al.* (2000) determined standard Pb absorption of 0.018 mg/L, while Okada *et al.* (1997) established Pb concentrations of 0.04 mg/l in cow milk. The results of the present study are consistent with the mentioned previous studies.

Our observed Pb values in soil, forage and milk are very low, so there are no hazards of any toxicity of lead for animals and public health. The Pb concentrations were higher in early sampling with consistent decreases over time as well as in forage and milk. The Pb levels in milk were parallel to forage Pb as the sole dietary source for the animal at the studied farm. Similar trend have already been reported (Mehennaoui *et al.*, 1997).

The lowest transfer factor of Pb from soil to forage was observed in November while the greatest was observed in January which did not follow the changes in soil or forage Pb concentrations. This indicates that some soil factors apart from the total soil content of the metal also affect the rate of metal uptake by plants. The application of some materials as dolomite, phosphates or organic matter into soils were found to reduce the concentration of metals by precipitation, adsorption or complex formation (Mench *et al.*, 1994; Chen and Lee, 1997) and thereby making them unavailable to plants, which might have had similar effects on the metals as observed by these researchers and therefore resulting in the lower transfer ratios of the metals. A crucial factor which affects metal availability in the soil is soil pH. According to Smith (1996), metal mobility decreases with increasing soil pH due to precipitation of hydroxides, carbonates or formation of insoluble organic complexes. However, soil pH was not measured in the current experiment.

Higher lead levels were detected in milk in Lithuania 20 years ago. The lead concentrations of 60–80 µg/kg were determined in some samples though concentrations <10 µg/kg were found as well (Urbiene and Čiučkinas, 1993). Lead levels of 20–30 µg/kg have also been previously detected by Čiučkinas and Urbiene (1994). Our results are similar or lower than those reported earlier from other regions of the world (Licata *et al.* (2004). Rodriguez *et al.* (1999) determined the heavy metals in samples of raw cow's milk and the concentration of lead reported to be 14.82 µg/L. It has been reported that in a monitoring study the mean concentration of lead at 7 µg/L was found by Oleszek *et al.* (2003). Based on findings of the present study, very low concentrations of Pb found in forage as well as in milk, anticipating no potential threat to livestock grazing in those pastures of the farm.

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