Pakistan J. Zool., vol. 45(6), pp. 1629-1637, 2013.

Assessment of Heavy Metals and Metalloids Toxicity in Buffaloes Fed on Forages Irrigated With Domestic Wastewater in Bhalwal, Sargodha, Pakistan

Kafeel Ahmad,[1] Zafar Iqbal Khan,[1] Hina Jabeen,[1] Muhammad Ashraf,[2] Muneeba Shaheen,[1] and Syed Hammad Raza[3]

[1]*Department of Biological Sciences, University of Sargodha, Pakistan*
[2]*University College of Agriculture, University of Sargodha, Pakistan*
[3]*Department of Botany, Government College University, Faisalabad-38040, Pakistan.*

Abstract.- The main purpose of the present investigation was to examine if heavy metal and metalloid toxicity had occurred in grazing livestock fed with forages irrigated with sewage water. In particular the transfer of arsenic (As), selenium (Se) and molybdenum (Mo) from soil to plant and in turn to animal (buffaloes) was conducted in Bhalwal, Sargodha, Pakistan which falls under sub-tropical environmental conditions. Arsenic and Se concentrations were significantly lower in the soil but higher in the forages; however, Mo concentration in both soil and forages was higher. The correlation between soil, forage, hair and feces showed an imbalanced flow of As and Mo and a balanced flow of Se from soil to, forage and then to buffaloes. Based on the findings of the present study, the highest rates of transfer of metals were found in case of sewage water treatment and lowest rates were found in canal water treatment. As the transfer of metals depends on the bioavailability of metals, so the highest values may be due to the high rates of metals uptake by the plants. The lowest values of transfer showed that the plants may be resistant to these three metals or it may be due to the antagonistic effect of other metals present in the sewage water or soil. The high transfer rates show the plants are prone to become toxicant with time in future, which may cause hazardous effects to livestock rearing therein.

Key words: Forage, soil, livestock, metal toxicity, metalloid toxicity, domestic wastewater.

INTRODUCTION

Industrial sites are usually a rich source of a variety of heavy metals and the animals rearing there in may usually accumulate high levels of these metals which can prove to be highly toxic for them (Spierenburg *et al.*, 1980; Kottferova and Korekenova, 1995). Of various heavy metals, arsenic and selenium are of great concern because they cause severe toxicity in organisms as compared to most of the other metals known in nature (Friberg *et al.*, 1979; Yusuf *et al.*, 2003; McDowell and Arthington, 2005). Selenium is a significant ingredient of glutathione peroxidase, a key enzyme of oxidative resistance system (Murray *et al.*, 2000). Due to Se deficiency animals frequently turn out to be lame or atypical. For example, Se deficiency in animals in elevated rate of death. Similarly, the major cause of mulberry heart disease in boar has been recognized to be due to Se deficiency (Hays and Swenson, 1985; McDowell and Arthington, 2005). A sufficient quantity of Se is indispensable to avoid oxidative injuries to various membranous structures caused by different free radicals in animal body (Judson and McFarlane). Deficiency of Se in the ruminants hastens the deterioration of different organs of the domestic animal (McDowell and Arthington, 2005). It can also result in a variety of problems in ewes especially in reproduction and development (Masters *et al.*, 1993; Langlands *et al.*, 1986). Selenium is a necessary fraction for a variety of enzymes, particularly those involved in defence mechanism against oxidative damage to different biological membrane systems in the animal body (McDowell, 2003).

Most of the arsenic poisoning is during the utilization of water. As-polluted groundwater is used for irrigation as well as for food preparation, it is probable that the irrigation in leads to disseminate pollution of land relying on As-contaminated groundwater. Thus top soil–produce–

* Corresponding author: zikhan11@gmail.com; kafeeluaf@yahoo.com
0030-9923/2013/0006-1629 $ 8.00/0

foodstuff transport, as well as food preparation and undeviating intake of consumption water may be amid the main contact pathways of As transport (Huq *et al*., 2003).

Molybdenum is considered as an important micronutrient both for plants and animals (Deosthale and Belavady, 1978; Anke, 2004a). This element is considered essential for functioning of nitrate reductase, a key enzyme for nitrogen obsession in leguminous plants (Anke and Seifert, 2007).

The purpose of this study is to estimate the risk to health in Buffaloes from certain elements in forage and animals consuming that forage plants as a major source of mineral requirements. The present study was conducted to evaluate the heavy metal and metaloids contents and their bioaccumulation (As, Se and Mo) in soil, forages and buffaloes being reared on agricultural lands in Bhalwal, Sargodha irrigated frequently with industrial sewage water.

MATERIALS AND METHODS

Study area

Study was conducted to appraise mineral composition of forages at a Rural livestock farm in the peri-urban areas of Bhalwal city, Sargodha, Pakistan. Two fields were selected for the soil and forage samplings. One field was irrigated with sewage water and other with canal water.

Reagents and material

Hydrocholoric acid (37%, E Merck, Grade-AR) Sulphuric acid (98%, E Merck, Grade-AR) and Hydrogen peroxide (30% E Merck ,Grade-AR,) were used for wet digestion of soil, forages, hair and fecal samples

Sample collection

Three composite samples of soil were collected from each field for elemental analysis. Samples of soil were collected and sealed in label paper bags. Forage samples contained four plant species, wheat (*Triticum aestivum*), lucern (*Medicago sativa*), oat (*Avena sativa*) and sarson (*Brassica campestris*). The forage samples were collected from the same place from where soil samples were collected. Three composite samples of each forage type were taken for elemental determination. The collected samples were washed well with a dilute solution of HCl followed by distilled water so as to remove surface contaminants. All the forage samples were air-dried and placed in an oven at 72^{o}C for 48 hr to attain constant dry weight.

Twenty four buffaloes (12 from each field) were selected of which hair and fecal samples were collected. These buffaloes grazed on the same fields from where the forage and soil samples were collected. The hairs from the neck and tail region were cut using a sterilized scissors. The hair samples were washed with distilled water to remove impurities. Then the collected hair and fecal samples were air-dried and packed into labeled paper bags. Then all samples were in an oven at 90 ^{o}C for 48 hr to attain constant dry weight.

Sample preparation for analysis

The samples were prepared for analysis of inorganic elements using the following methods:

The digestion of the soil samples were done with Mehlich-1 (M_1) (0.05 mol L^{-1} HCl + 0.0125 mol $L^{-1}H_2SO_4$) in a soil; solution ratio of 1:10 and shaken for 5 min on a reciprocating shaker at 120 oscillations min^{-1} and standing over-night (16 h).

The forage, hair and fecal samples were washed with dilute HCl, air dried and sealed in paper bags. Then the samples were oven dried and digested by the wet digestion method. Each sample (1.0 g) was digested with the help of sulphuric acid (2 ml) and hydrogen peroxide (4 ml). The samples were heated in a digestion chamber for 30 min and then cooled them. To the cooled samples, 2 ml of hydrogen peroxide per sample were added. The mixture was heated again until a colorless solution appeared. Distilled water was used to attain the final volume of each sample up to 50 ml.

Determination of Mo concentration was performed with the help of an atomic absorption spectrophotometer (Model # AA-6300, Shimadzu, Japan) equipped with a graphite furnace (AA-6300&GFAEXi7i, Shimadzu, Japan) while Arsenic and selenium concentrations were determined with the help of a hydride technique (Lopez *et al*., 2000; Pohl, 1987).

Statistical analysis

Data for various attributes were statistically analysed with the help of the SPSS software using correlation and one-way analysis of variance. Statistical significance among the mean values was tested at 0.05, 0.01 and 0.001 levels of probability as suggested by (Steel and Torrie, 1986).

Transfer factor and bio concentration factor

The transfer factor from soil to forage was calculated using Microsoft excel by the following formula:

$$TF = \frac{\text{Mean concentration of forage}}{\text{Mean concentration of soil}}$$

and from forage to hairs by following formula:

$$TF = \frac{\text{Mean concentration of hairs}}{\text{Mean concentration of forage}}$$

Similarly from forage to feces by the following formula:

$$TF = \frac{\text{Mean concentration of feces}}{\text{Mean concentration of forage}}$$

RESULTS AND DISCUSSION

Molybdenum (Mo)

Both water treatments had a non-significant effect ($P>0.05$) on Mo concentration of soil and forage as showed in Table I. Soil Mo under sewage water irrigation ranged between 1.45 to 1.64 mg/kg while in canal water irrigation it was between 1.24 to 1.34 mg/kg. Average soil Mo concentration was greater than the permissible level of 1 mg/kg (Hornick *et al.,* 1977). In most agricultural soils 50 mg Mo/kg was suggested by O'Connor *et al.* (2001) which is greater than the concentrations determined in the present investigation. However, higher levels of Mo are also reported in literature to cause multiple disorders in animals being reared on such pasture soils. It can cause, high incidence of uretic diathesis as reported at a site in Armenia where the soil contained Mo 77 mg/kg and Cu 39 mg/kg (NRC, 1996). However, in view of the soil Mo levels reported in the present study there is no potential hazards of Mo toxicity for ruminants being reared at the study area.

Mean forage Mo concentration ranged from 6.88 to 8.23 mg/kg due to sewage water treatment, with lower values being in *Brassica campestris* and higher in *Avena sativa.* Forage Mo concentration ranged from 5.64 to 7.52 mg/kg under canal water treatment, with lower values being in *Avena sativa* and higher values in *Triticum aestivum.* In present study, the concentration of Mo in forage was much higher than 6 mg/kg which is established as critical level by McDowell (1997) for forage crops. Both hair and fecal Mo levels were not affected significantly by either of the water treatments (Table I). The hair Mo concentrations varied from 0.72 to 0.98 mg/kg and 0.59 to 1.35 mg/kg by due to application of sewage and control water, respectively. In buffalo's feces the Mo values fluctuated from 0.51 to 1.84 mg/kg under sewage water treatment and 0.669 to 0.89 under canal water treatment. The correlation of Mo content was non-significant and negative between soil and hair, soil and feces, forage and hair and hair and feces, but it was positive between soil and forage and forage and faeces. However, under canal water treatment, the correlation between soil and forage, forage and feces and hair and feces was positive. In contrast, a negative correlation between soil and hair, soil and feces and forage and hair (Table II). Under both treatments, the transfer factor for Mo in soil-forage varied from 4.5 to 5.6 and for forage-hair from 0.08 to 0.23, while for forage-feces, it varied from 0.06 to 0.24 (Figs. 1, 2).

Selenium (Se)

Analysis of variance for Se showed a significant ($P<0.05$) effect of sewage water treatment while the reverse was true for canal water treatment (Table I). The soil Se concentration varied from 0.13 to 0.17 mg/kg in sewage water treatment and from 0.14 to 0.24 mg/kg in soil treated with canal water. The mean concentration of soil Se was higher under canal water treatment and lower under sewage water treatment (Fig. 3). The values found during the present findings were lower than the critical value of 0.5 mg/kg established by McDowell (1985). Similar low levels of soil Se have already

Table I.- **Analysis of variance of data for Mo, Se and As concentrations in soil, forage, hairs and feces treated with canal or sewage water.**

Source of variation	Degree of freedom (df)	Soil		Forage		Hair		Feces	
		Sewerage water	Canal water	Sewerage water	Canal water	Sewerage water	Canal water	Sewerage water	Canal water
Molybdenum									
Treatment	3	.005ns	.022ns	1.961ns	.999ns	.334ns	.041ns	.024ns	1.185ns
Error	8	.011	.016	.969	.422	.138	.113	.027	1.390
Mean square									
Selenium									
Treatment	3	.003ns	.001**	.026ns	.069**	.002*	.003*	.002ns	.001ns
Error	8	.002	.000	.008	.008	.000	.000	.000	.000
Mean square									
Arsenic									
Treatment	3	0.145^{ns}	6.302**	0.021^{ns}	0.016^{ns}	0.001^{ns}	0.003^{ns}	0.002^{ns}	0.005**
Error	8	0.435	0.505	0.027	0.076	0.000	0.000	0.000	0.000
Mean square									

SWT=Sewage water treatment, CWT=Canal water treatment
*=significant at 0.05 levels, **=significant at 0.01 levels
***=significant at 0.001 levels, ns= non-significant

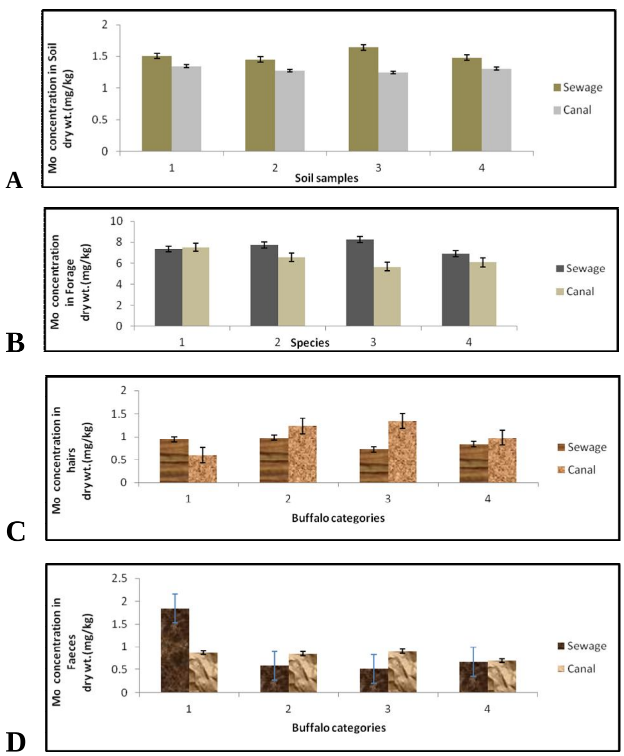


Fig. 1. Fluctuation in Mo concentration soil (A), forage (B), hair (C) and feces (D) after treatment with sewage water and canal water.

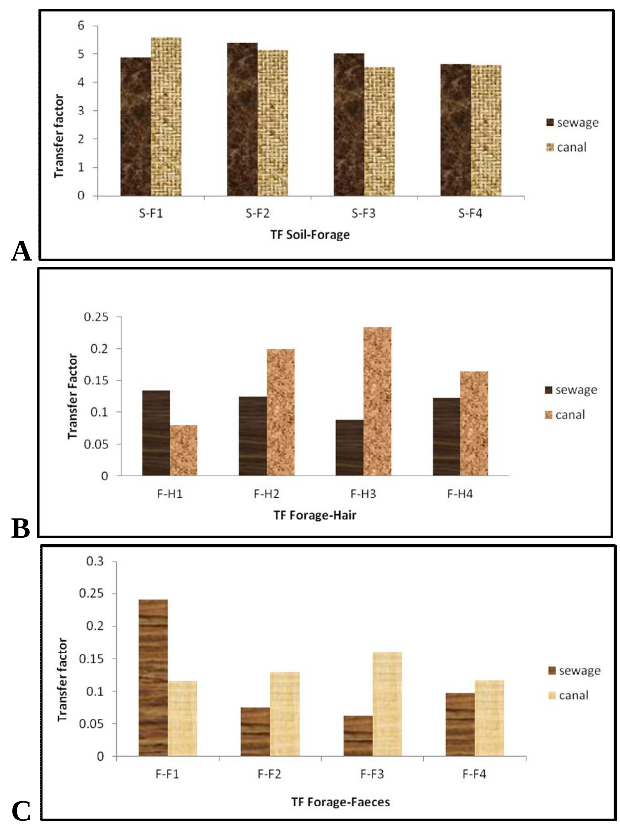


Fig. 2. Transfer factor for Mo soil-forage (A), forage-hair (B) and forage-feces (C).

been reported by Khan *et al*. (2005, 2008) from a similar animal ranch in another area of Punjab, Pakistan. There was a significant (P<0.05) effect sewage water treatment on forage Se concentration but a non-significant effect of canal water on this variable (Table I). The mean forage Se values ranged from 0.983 to 1.313 mg/kg (Fig. 3). The Se status was found to be higher in *Medicago sativa* and lower in *Brassica campestris*. The mean forage Se values ranged from 0.593 to 0.808 mg/kg under canal water treatment (Fig. 3). The Se status was found to be higher in *Brassica campestris* and lower in *Medicago sativa*. The mean forage Se concentrations observed during the present study were much higher than the critical level 0.1 mg/kg established by Anonymous (1985). Our results for forage Se were also greater than those reported elsewhere (McDowell *et al.*, 1983; Khan *et al*., 2005). However, forage Se concentrations found in this study were similar to those reported for Guatemala (Tejada *et al.*, 1987; Pastrana *et al.*, 1991), Pakistan (Khan, 2003) and Indonesia (Prabowo *et al.*, 1990).

Effect of sewage and control water treatment on buffalo fecal Se concentration was found to be non-significant while significant on hair Se levels (Table I). The Se concentration in hairs varied from 0.002 to 0.005 mg/kg and 0.003 to 0.006 mg/kg by applying sewage and control water treatments, respectively. In buffalo's feces, the Se values fluctuated from 0.0025 to 0.0028 mg/kg under sewage water treatment and 0.0016 to 0.003 under canal water treatment. The Environment Protection Agency (EPA) and the International Atomic Energy Agency (IAEA) have recommended the use of hair as an important biological indicator for worldwide environmental monitoring.

The positive correlation was found between soil and hair, soil and feces and hair and-feces, which reveals a strong relation among them. However, in contrast, a negative correlation was found between forage and feces, while a significant negative correlation between soil and forage and forage and hair (Table II) under sewage water treatment. However, under canal water treatment, Se showed a negative correlation only between soil and hair, while for others it showed a positive correlation. This revealed a balance flow of Se from soil and forage to buffaloes.

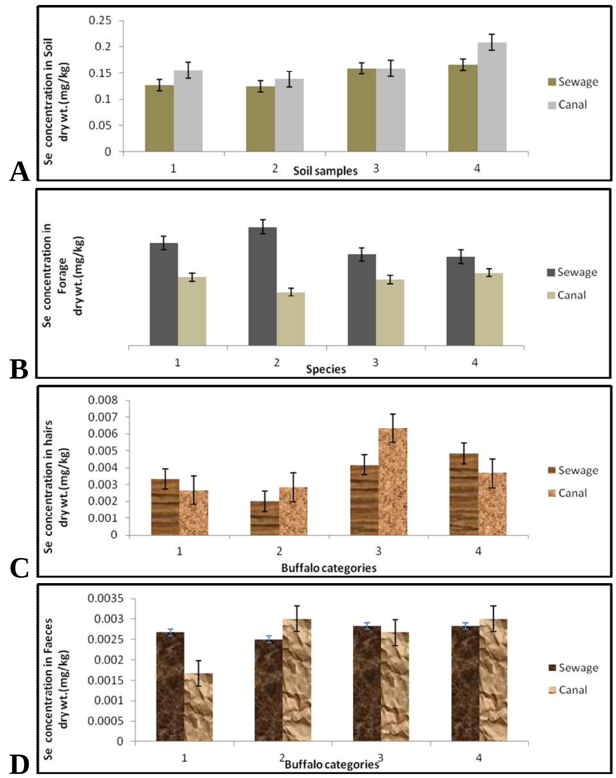


Fig. 3. Fluctuation in Se concentration in soil (A), forage (B), hair (C) and feces (D) after treatment with sewage water and canal water.

Transfer factor for Se in soil-forage varied from 3.9 to 10.5, in forage-hair it varied from 0.002 to 0.009 and in forage-feces it varied from 0.002 to 0.005in both sewage and canal water treatments. The highest transfer rate was found in sewage water treatment and lowest in canal water treatment (Figs.3, 4).

Arsenic (As)

The soil arsenic concentration was affected significantly (P<0.05) by sewage water treatment but affected non-significantly by canal water treatment (Table I). The mean concentration of arsenic in soil irrigated with sewage water ranged from 13.53 to 16.83 mg/kg while it ranged from 11.63 to 12.12 mg/kg in canal water treatment (Fig. 3). The values of As in soil is lower than the critical range of soil As 20 mg/kg as reported by O'Neill (1995). The values found in the present study were lower than those reported by Bhumbla and Keefer (1994) for other regions. Arsenic pollution in soils may have alarming effects on vegetation as well as animals being reared therein. However, the soil As levels found in the present study were safe for growing different types of forages.

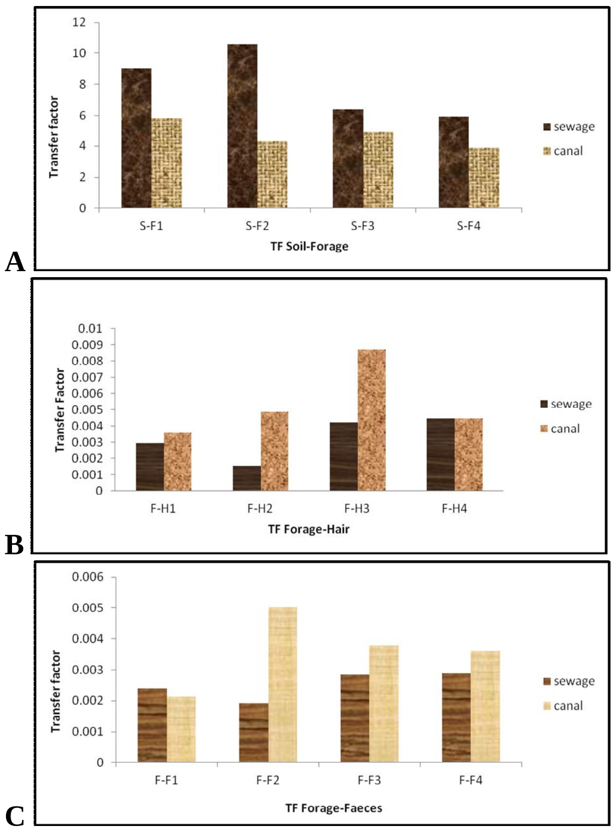


Fig. 4. Transfer factor for Se from soil-forage (A), forage hair (B) and forage feces (C).

Analysis of variance for data for forage As shows that sewage water or canal water treatment had a non-significant effect (P>0.05) on forage As levels (Table I). The forage As values ranged from 3.07 to 3.25 mg/kg in forages treated with sewage water (Fig. 5). However, As levels were higher in *Medicago sativa* and lower in *Brassica campestris* supplied with sewage water. The mean forage As values ranged from 3.001 to 3.19 mg/kg when the forages were treated with canal water (Fig. 5). The maximum values were found in *Triticum aestivum* and minimum values in *Brassica campestris* treated with canal water.

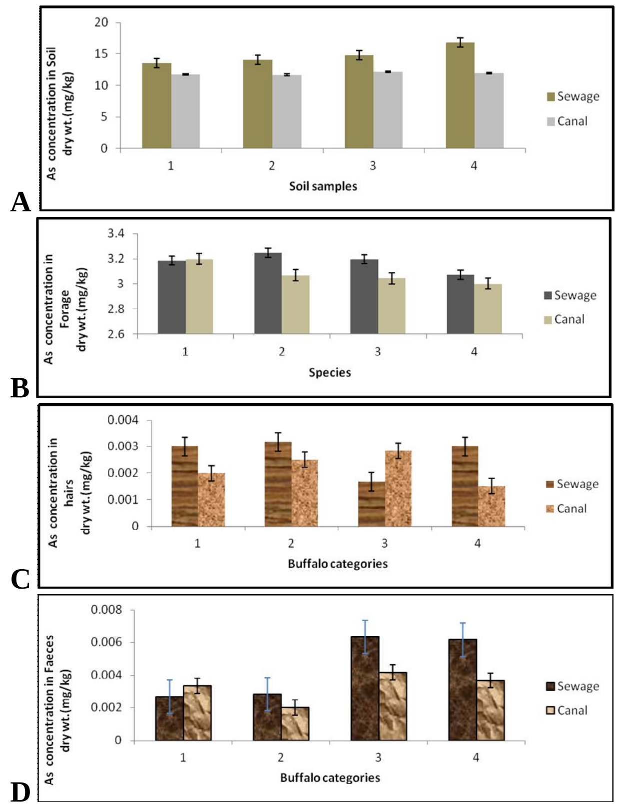


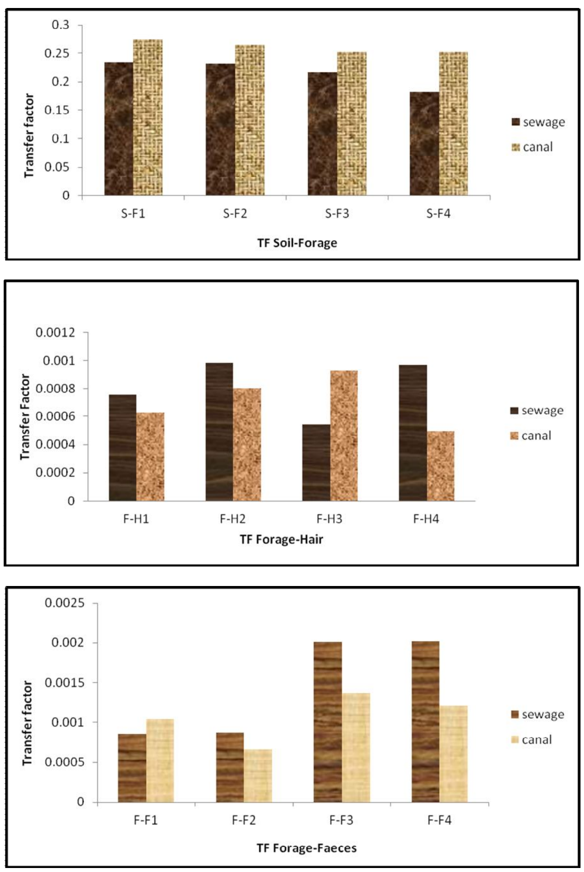
Fig. 5. Fluctuation in As concentration soil (A), forage (B), hair (C) and in feces (D) after treatment with sewage water and canal water.

Arsenic concentrations in forages were higher than the critical limit 3.0 mg/kg established by O'Neill (1995). Thus, methylation of arsenic in plants may markedly reduce the expected risk associated with intake of forage by the animals (Nissen and Benson, 1982; Humphreys, 1990; Lopez *et al.*, 2000). Analysis of variance showed non-significant (P>0.05) effect of canal water treatment on As concentration in both hairs and feces of buffalos whereas that on fecal As was significant and on hair As non-significant. The mean As concentration in hairs was found to be 0.001 to 0.003 mg/kg under sewage and canal water treatments, respectively. In buffalos' feces the As values fluctuated from 0.003 to 0.006 mg/kg under sewage water treatment and 0.002 to 0.004 mg/kg under canal water treatment.

Table II.- Correlations between soil-forage-hair and feces with respect to As, Se and Mo.

Mineral	Soil - Forage	Soil - Hair	Soil - Feces	Forage - Hair	Forage - Feces	Hair - Feces
Sewage water						
Mo	.137	-.255	-.371	-.108	.107	-.145
Se	-.640*	.445	.009	-.714**	-.226	.298
As	-.156	-.012	.700*	.108	-.275	-.421
Canal water						
Mo	.501	-.142	-.050	-.386	.338	.337
Se	.080	-.015	.000	.160	.128	.411
As	-.368	-.156	.304	.293	.134	.326

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).



A

B

C

Fig. 6. Transfer factor for As from soil-forage (A), forage-hair (B) and forage-hair (C).

The sewage water treatment showed a positive and significant correlation between soil and feces and between forage and hairs (Table II).The negative and non-significant correlation was observed between soil and forage, soil and hair, forage and feces and hairs and faeces. However, under canal water, soil-feces, forage-hair, forage-feces and hair-feces showed a positive correlation, but soil-forage and soil-hair showed a negative correlation. This shows mineral imbalance of As in the area under study. The transfer factor for As in soil-forage varied from 0.18 to 0.27 and in forage-hair from 0.0004 to 0.0009 while in forage-feces the transfer factor varied from 0.0006 to 0.002 under both treatments. The highest transfer rate was found in sewage water treatment and the lowest in that of canal water (Figs. 5, 6).

Low concentrations of As and Se is present in soil and in plants, however the occurrence of such elements in soil does not mean their availability to plants, especially if they are insoluble in the soil solution. Most significance is the presence of these elements in appropriate concentration in the soil extract which simulates if a given element could be absorbed by plants. So, there is a need to opt an extraction process that would enable determination of the quantity of metals available to plants during growing season. This may propose that plant absorption is controlled by the composition of the soil solution. Determination of the composition of the solution-soluble soil fraction and knowledge of transfer factors enables prediction of whether plants cultivated on a given soil can be used as food or feed, on the basis of knowledge of soil extract composition. This could be useful for environmental monitoring and screening.

CONCLUSIONS

This is the first study to quantify metal contamination in cattle in Pakistan. Arsenic and Se soil concentrations were considerably poorer in the soil, however, elevated in the forages, though, Mo level was elevated in both soil and forages. The relationship involving soil, forage, hair and feces showed an unprovoked surge of As and Mo and a evenhanded run of Se from soil to, forage and followed by to buffaloes.

Based on the conclusion of the current study, the maximum rates of transport aspect were found in case of sewage water management and lowly rates were found in canal water management.

REFERENCES

ANKE, M., 2004a. Essential and toxic effects of macro, trace and ultratrace elements in the nutrition of animals. In: *Elements and their compounds in the environment* (eds. E. Merian, M. Anke, M. Ihnat and M. Stoeppler). 2nd ed., Wiley-VCH Verlag, Weinheim, pp. 305–341.

ANKE, M.,2004b. Molybdenum. In: *Elements and their compounds in the environment* (eds. E. Merian, M. Anke, M. Ihnat and M. Stoeppler). 2nd ed.,Wiley-VCH Verlag, Weinheim, pp. 1007–1037.

ANKE, M. and SEIFERT, M.,2007. The biological and toxicological importance of molybdenum in the environment and in the nutrition of plants, animals and man. Part 1: Molybdenum in plants. *Acta biol. Hung.*, **58**: 311–324.

ANONYMOUS.1985 *Nutrient requirements of domestic animals. nutrient requirements of sheep* (6^{th}. Revised ed.), National Academy of Science-National Research Council, Washington, D.C.

BHUMBLA, D. AND KEEFER, R., 1994. *Arsenic in the environment*. Part I, John Wiley and Sons, New York.

DEOSTHALE, Y.G., BELAVADY, B., 1978. Mineral and trace element composition of sorghum grain: effect of variety, location and application of the nitrogen fertilizer. *Ind. J. Nutr. Dietet.*, **15**: 302-308.

FRIBERG, L., KJELLSTROM, T., NORDBERG, G. AND PISCATOR, G., 1979. Cadmium. In: *Handbook on the toxicology of metals* (eds. R. Friberg, G.F. Nordberg and V.B. Vouk). Elsevier/North-Holland Biomedical Press, Amsterdam New York,Oxford, pp. 355-381.

HAYS, V.W. AND SWENSON, M.J., 1985. Minerals and bones. In: *Dukes? Physiology of domestic animals* (eds. H.H. Dukes and M.J. Swenson), 10^{th}Edn. Cornell University Press, Ithaca, NY., USA, pp. 449-466.

HORNICK, S.B., BAKER, D.E. AND GUSS, S.B., 1977. *Molybdenum in the environment* 2. Marcel Dekker, New York.

HUMPHREYS, D.J.,1990. *Toxicologıa veterinaria*. Mc-Graw-Hill-Interamericana, Buenos Aires, pp. 17:85.

HUQ, I,,SMITH, E, CORNELL R, SMITH, L, SMITH J, AHMAD M, ROY S, BARNES M, NAIDU R, M.G.M. ALAM, E.T. SNOW AND TANAKA, A., 2003. Arsenic transfer in water soil crop environments in Bangladesh. I: assessing potential arsenic exposure pathways in Bangladesh. In: *Arsenic in the Asia-Pacific Region workshop* (ed. R. Naidu), 'Managing. The Science of the Total Environment 308: 83–96

KHAN, Z.I. 2003. *Effect of seasonal variation on the availability of macro-and micro, Nutrients to animals (sheep and goats) through forage from soil*. Ph.D. thesis, University of Agriculture, Faisalabad, Pakistan, pp. 286.

KHAN, Z.I., AHMED, K., ASHRAF, M., VALEEM, E.E. AND JAVED, I., 2008. a comparative study on mineral status of blood plasma of small ruminants and pastures in Punjab, Pakistan. *Pak. J. Bot*., **40**: 1143-1151.

KHAN, Z.I., HUSSAIN, A., ASHRAF, M., VALEEM, E.E. AND JAVED, I., 2005. Evaluation of variation of soil and forage minerals in pasture in a semiarid region of Pakistan. *Pak. J. Bot*., **37**: 921-931.

KOTTFEROVA, J. AND KORENEKOVA, B., 1995. The effect of emissions on heavy metals concentrations in cattle from the area of an industrial plant in Slovakia. *Arch. environ. Contam. Toxicol.*, **29**:400–405

LANGLANDS, J.P., BOWLES, J.E., DONALD, G.E. AND SMITH, A.J., 1986., Selenium excretion in sheep. *Aust. J. agric. Res*., **37**: 201-209.

LOPEZ, M., ALONSOA,U, J.L., BENEDITOA, M., MIRANDAA, C., CASTILLOA, J., HEMANDEZA, R.F. AND SHOREB, R.F., 2000. Arsenic, cadmium, lead, copper and zinc in cattle from Galicia, NW Spain. *Sci. Total Environ.*, **246**: 237-248

MASTERS, D.G., PURSER, D.B., YU, S.X., WANG, Z.S., YANG, R.Z., LIU, N., WU, L.H., REN, J.K. AND LI., G.H., 1993. Mineral nutrition of grazing sheep in Northern China. II. Selenium, copper, molybdenum, iron and zinc in pasture, feed supplements and sheep. *Asian-Aust. J. Anim. Sci*., **6**: 107-113.

McDOWELL, L.R., 1985. *Nutrition of grazing ruminants in warm climates*. Academic Press, New York, pp. 443.

McDOWELL, J.K., 1997. *Minerals for grazing ruminants in tropical regions*. Extension Bulletin, third ed. Department of Animal Science, Center for Tropical Agriculture, University of Florida, Gainesville, Florida, USA. pp. 1–81.

McDOWELLl, L.R., 2003. *Minerals in animal and human nutrition*, 2^{nd} Ed., University of Florida, Gainesville, pp. 235-276.

MCDOWELLl, L.R. AND ARTHINGTON, J.D., 2005. *Minerals for grazing ruminants in tropical regimes*, Bulletin (4th ed.). University of Florida, Gainessville, pp. 1-86.

McDOWELL, L.R., CONRAD, J.H., ELLIS, G.L. AND LOOSLI, L.K., 1983. *Minerals for grazing ruminants intropical regions*. Extension Bulletin Animal Science Department, University of Florida.

MURRAY, R.K., GRANNER, D.K., MAYES, P.A. AND RADWELL, V.W., 2000. *Harper's Biochemistry*. 25thed., McGraw Hill Health Profession Division, USA.

NISSEN, P. AND BENSON, A.A., 1982. Arsenic metabolism in freshwater and terrestrial plants. *Physiol. Plant.*, **54**: 446 -450.

NRC, 1996. *Nutrient requirements of beef cattle*, 7th edn. National Academy Press, Washington.

O'CONNOR, G.A., BROBST, R.B., CHANEY, R.L., KINCAID, R.L., MCDOWELL, L.R., PIERZYNSKI, G.M., RUBIN, A. AND RIPER, G.G.V., 2001. A modified risk assessment to establish molybdenum standards for land application of biosolids. *J. environ. Qual.*, **30**:1490–1507.

O'NEILL, P., 1995. Arsenic. In: *Heavy metals in soils* (ed. B.J. Alloway), Blackie Academic and Professional, London.

PASTRANA, R., MCDOWELL, L.R., CONRAD, J.H. AND WILKINSON, N.S., 1991. Macromineral status of sheep in the Paramo region of Colombia. *Small Rumin. Res*., **5**: 9-21.

POHL, B., 1987. *Das kontinuierliche hydridsystem*, Laborpraxis 12, 11. Jahrgang, Vogel.

PRABOWO, A., MCDOWELL, L.R., WILKINSON, N.S., WILCOX, C. J. AND CORNAD, J.H., 1990. Mineral status of Grazzinmg cattle in South Sulawesi, Indonesia; I. Macrominerals. *Am. J. Anim. Sci.*, **4**:111-120.

SPIERENBURG, T.J., GRAAF, G.J., BAARS, A.J., BRUS, D.H.J., TIELEN, M.J.M. AND STEEL, R. G. D. AND TORRIE, J.H., 1980. *Principles and procedures of statistics. A biometrical approach* (2nd Ed.). McGraw Hill Book Co., New York.

STEEL, R. G. D., AND J. H. TORRIE. 1980. *Principles and Procedures of Statistics. A Biometrical Approach (2nd Ed.)*. New York: McGraw Hill Book Co.

TEJADA, R., MCDOWELL, L.R., MARTIN, F.G. AND CONRAD, J.H., 1987. Evaluations of cattle trace mineral status in specific region of Guetemala. *Tropic Agric*., **6**:55-60.

YUSUF, A.A., AROWOLA, T.A. AND BAMGESEBESE, O., 2003. Cadmium, copper and nickel levels invegetables from industrial and residential area of Lagos city, Nigeria. *Fd. Chem. Toxicol*., **41**: 375-378.

(Received 27 July 2013, revised 1 September 2013)