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Appraisal of Trace Metal Elements in Soil, Forage and Animal Continuum: A Case Study on Pasture Irrigated with Sewage Water

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The study determined the mineral excesses and deficiencies for grazing livestock, and analyzed the heavy metal content and bioaccumulation of certain trace elements to assess the health risk in ruminants from consumption of forage irrigated with either sewage or canal water. The transfer of chromium (Cr), nickel (Ni), cadmium (Cd) and lead (Pb) from soil to forage and to animals was evaluated. Samples of soil, forage and buffalo hair were collected and analyzed. Data showed that heavy metal concentrations were higher in forage irrigated with sewage water compared with forage irrigated with canal water. Among the heavy metals investigated, Cr had the highest concentration followed by Pb whereas Ni and Cd were found in lower concentrations. The highest mineral transfer rates were detected in sewage irrigated forage, and the lowest rates in canal water irrigated forage. There was a positive correlation between Pb and Cr in soil and forage, and a negative correlation for Ni and Cd. The transfer of minerals depends on bioavailability; the highest values may be due to the high rates of mineral uptake by forage. Therefore, the high transfer rate of trace elements by forage could become toxic, resulting in unfavorable consequences to grazing animals.

Key Words: forage, minerals, sewage water, soil

INTRODUCTION

In most regions of Pakistan, irrigation makes use of sewage water, a rich source of metals. This practice is quite common in some parts of Sargodha, Pakistan, so it is likely that forage species grown on sewage-irrigated soils would accumulate high amounts of metal. Among the heavy metals, Cd, Pb, As and Hg are of interest due to their rapid transmision via the food chain and the threat they pose to animal and human health (Friberg et al. 1979). From industrial sites, increased concentrations of toxic trace elements have been identified in farms (Kottferova and Korenekova 1995). Lead (Pb) is transferred in animals through soil, water, feed and forage. The transfer of Pb from blood to milk was investigated in rats and mice, showing that the Pb concentration found in milk is about 100 times more than that found in blood (Swarup et al. 2005; Peralta-Videa et al. 2009).

Nickel (Ni) affects the function of rumen, and animals are particularly sensitive to its toxicity. The level of Ni found in the liver can be directly correlated with the level of Ni in the soil (Miranda et al. 2005). Chromium (Cr) is categorized as an essential nutrient for animals. However, its results are toxic for many plants even at low concentrations (Danish et al. 2014). Many plant parts have changeable level of Cr (Anderson et al. 1990). Cr was found to influence growth, lipid metabolism, and immune response and interacts with nucleic acids. Enhanced growth has been reported in ruminant species because Cr is usually supplemented to their diets (Stoecker 1996). This study was conducted to determine the mineral excesses and deficiencies for grazing livestock and to analyze their heavy metal content and their bioaccumulation so as to assess the health risk in ruminants from consumption of forage irrigated using either sewage water or canal water.

MATERIALS AND METHODS

Study Area

The study was conducted during the winter and summer seasons in Bhalwal, Pakistan, located between 30.05° N and 72.67° E. The area has a subtropical to temperate environment and a mean altitude of 187 m. The temperature ranges from 25 to 48 °C in summer and from 7 to 25 °C during winter.

Sample Collection

Two fields were selected for the soil, forage and animal (buffalo) hair sampling. One field was irrigated with sewage water (T_1 treatment) and the other with canal water (T_0 treatment). The soil samples were collected from each field based on the method used by Sanchez (1976) and placed in sealed polythene bags. Samples from four different forage species were collected: berseem clover (F1, *Trifolium alexandrinum*), oat (F2, *Avena sativa*), guar (F3, *Cyamopsis tetragonoloba*) and sorghum (F4, *Sorghum bicolor*). Three replicates of each variety of forage were taken during the sampling period. The forage samples were oven dried at 60 °C for 48 h to remove all moisture.

Twenty-four buffaloes (males and dry females with an average weight of 400–450 kg and 3–6 yr of age; twelve from each field) were selected for the collection of hair samples. The buffaloes were grazed on the same fields where the forage and soil samples were collected. The samples of soil, forage and buffalo hair were digested by using the wet digestion method according to Vukadinović and Bertić (1988). A total of 1 g of soil, forage and buffalo hair were digested with H_2SO_4 and H_2O_2 in a flask by placing the digesting material in a digestion chamber, based on the wet digestion method (Richards 1968). The samples were filtered and the volume was made up to 50 mL with distilled water. Then, the samples were placed in plastic bottles in the laboratory for further analyses.

Mineral Analysis and Bioconcentration Transfer Factor

After acidic digestion, the forage, soil and buffalo hair samples were analyzed for mineral determination (Cr, Ni, Cd and Pb) by atomic absorption spectrophotometry using Perkin-Elmer AAS-5000 spectrophotometer according to Ajasa et al. (2004). The bioconcentration transfer factor (TF) from soil to forage and forage to hairs was calculated according to the formula of Khan et al. (2016).

Statistical Analysis

Data were statistically analyzed using the SPSS software for correlation and one-way ANOVA was used. The significant differences between mean data were determined at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively (Steel and Torrie 1980).

RESULTS AND DISCUSSION

Chromium (Cr)

There was no noteworthy ($P>0.05$) effect of both sewage (T_1) and canal water (T_0) irrigated treatment on Cr concentration in the soil (Table 1). Cr concentrations in the soil were found to be higher in T_1 compared with T_0 (Fig. 1). The Cr levels were higher than the critical level (0.02 mg kg^{-1}) as stated by Anderson et al. (1972). The Cr contents found in our trial were higher than those found in preceding investigations by Hodgson (1990). In the present study, the Cr values were less than those in the study by Kabata-Pendias and Pendias (1992) who found 75–100 mg kg^{-1} to be the decisive level for soil Cr.

Dumping of Cr contained in marketable goods and petroleum residues from industries are major sources of Cr release in the soil (Nriagu and Pacyna 1988). Concrete slag formed during industrialized chromate processes, when disposed of improperly in landfills, can be probable sources of Cr contact as well (Barceloux 1999; Kimbrough et al. 1999). Data showed no noteworthy ($P>0.05$) consequence on forage Cr level in the case of T_1 treatment. Forage Cr concentrations varied from 3.66 to 4.005 mg kg^{-1} (Fig. 2). The significant elevated levels were found in F4 and the lower levels in F2. T_0 treatment had no significant ($P>0.05$) effect on forage Cr concentration (Table 1). The mean concentration of Cr in the different forages varied from 3.20 to 3.28 mg kg^{-1} in T_0 (Fig. 2). The higher Cr status was found in F2, and the lower in F4.

The Cr levels in our trial are higher than the critical level of 3 mg kg^{-1} stated by McDowell (1985); in particular, the values were higher during winter compared with the summer season. Cr levels in all the soil samples were below the critical level. The mean concentrations of Cr in shoots of forages irrigated with the different water treatments were less than the Cr levels reported by Ahmad et al. (2009). Cr has been suggested to be extremely hazardous for the health of livestock species, especially if their diet contained higher levels of Cr than the tolerable levels (Khan et al. 2014).

For both T_1 and T_0 treatments, there was no important ($P>0.05$) effect on Cr concentration in the hair of buffaloes (Table 1). Cr concentration in buffalo hair varied from 0.218 to 0.876 mg kg^{-1} in T_1 and from 0.308 to 0.333 mg kg^{-1} with application of T_0 treatment. Cr concentration in the hair of buffaloes was higher in T_1 compared with T_0 (Fig. 3). The Protection Agency (PA) and the International Atomic Energy Agency (IAEA) have recommended the use of hair as an important biological material for worldwide environmental monitoring (IAEA 1994).

The Cr correlation was positive between soil-forage ($r = 0.292$), but non-significant results were observed among soil-hair ($r = -0.075$) and forage-hair ($r = -0.071$) in T_1 (Table 3). This result indicates a weak relationship among soil forage and hair and improper flow of mineral from soil to forage and forage to buffalo. In T_0 treatment, the Cr correlation was negative, non-significant between soil-forage ($r = -0.399$), forage-hair ($r = -0.034$) and soil-

Table 1. Analysis of variance for concentrations of trace metal elements (Cr, Ni, Cd and Pb) in soil, forage and buffalo hair in T_1 and T_0 treatments.

Source of Variation		***df***	**Mean Squares**					
			Soil		**Forage**		**Hair**	
			T_1	T_0	T_1	T_0	T_1	T_0
Treatment	Cr	3	0.002^{ns}	0.003^{ns}	0.064^{ns}	0.003^{ns}	0.303^{ns}	0.003^{ns}
	Ni		0.023^{**}	0.003^{ns}	0.019^{ns}	0.041^{ns}	0.005^{ns}	0.008^{ns}
	Cd		1.988^{*}	0.047^{ns}	0.003^{ns}	0.001^{*}	0.020^{*}	0.007^{ns}
	Pb		1.477^{ns}	47.623^{***}	0.033^{*}	0.020^{ns}	0.002^{ns}	0.001^{*}
Error	Cr	8	0.001	0.004	0.078	0.005	0.288	0.001
	Ni		0.001	0.001	0.042	0.084	0.008	0.006
	Cd		0.239	0.085	0.001	0.000	0.002	0.003
	Pb		2.139	1.658	0.008	0.068	0.001	0.001

ns, not significant; * $P < 0.05$, $P < 0.01$, $P < 0.001$
T_1 sewage water treatment, T_0 canal water treatment

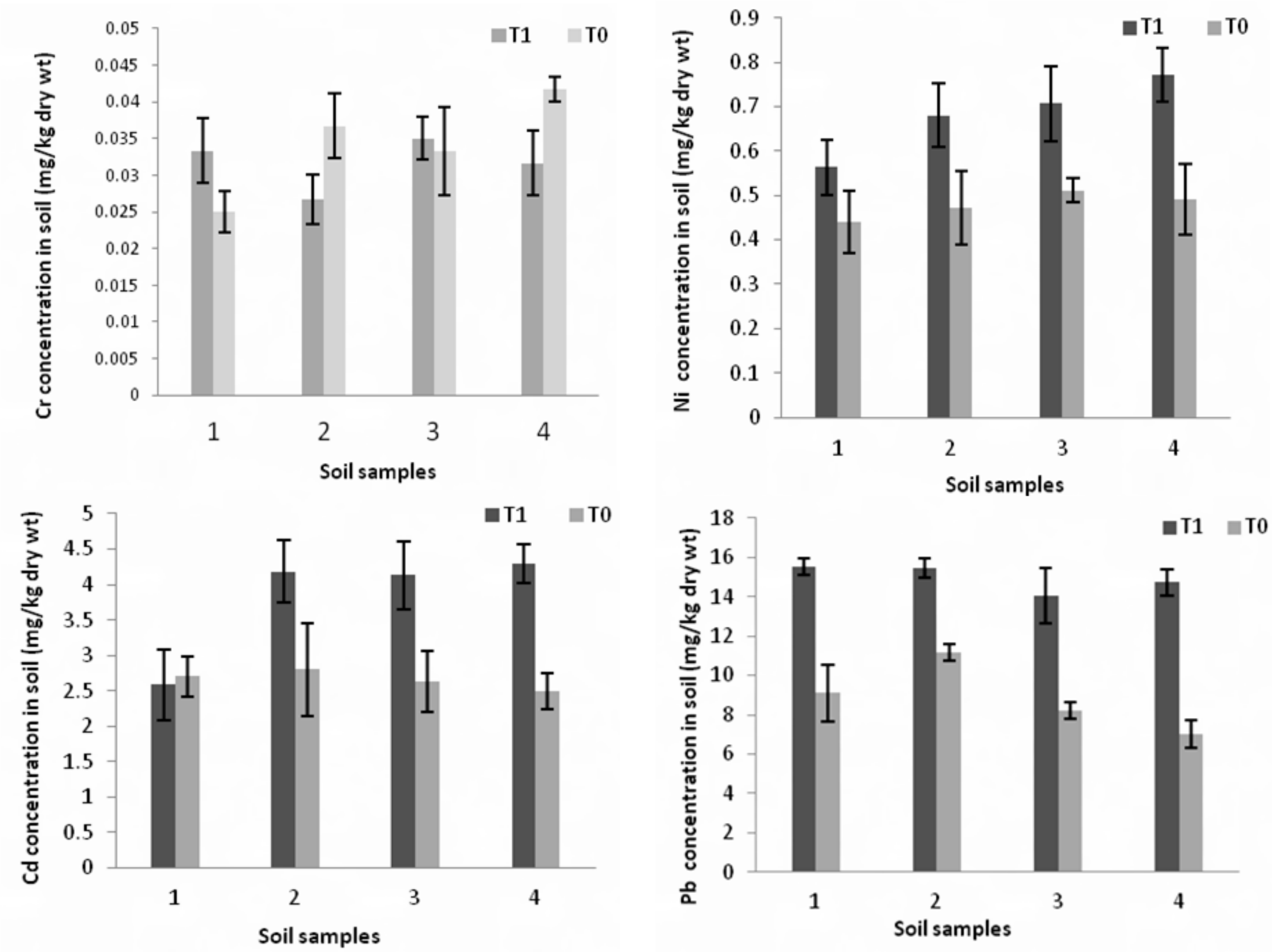

Fig. 1. Fluctuation of different metal concentrations in the soil at both treatments (T_1 – sewage water treatment, T_0 – canal water treatment).

hair (r = -0.100) that showed a weak relationship and improper flow of minerals (Table 2). In both T_1 and T_0, the bioconcentration factor for Cr from soil-forage fluctuated from 76.999 to 137.248 and from forage-hair, it varied from 0.0545 to 0.239 (Table 3). The highest rate was found in the sewage water treatment and lowest in the canal water treatment. The transport of metal is dependent upon the availability of metals, so the maximum level may be due to elevated speed of metal absorption by the plant.

Nickel (Ni)

The Ni concentrations in the soil were significantly affected ($P<0.05$) in T_1 but not in T_0 (Table 1). Ni concentration in the soil varied from 0.563 to 0.771 mg kg^{-1} and from 0.441 to 0.511 mg kg^{-1} with application of

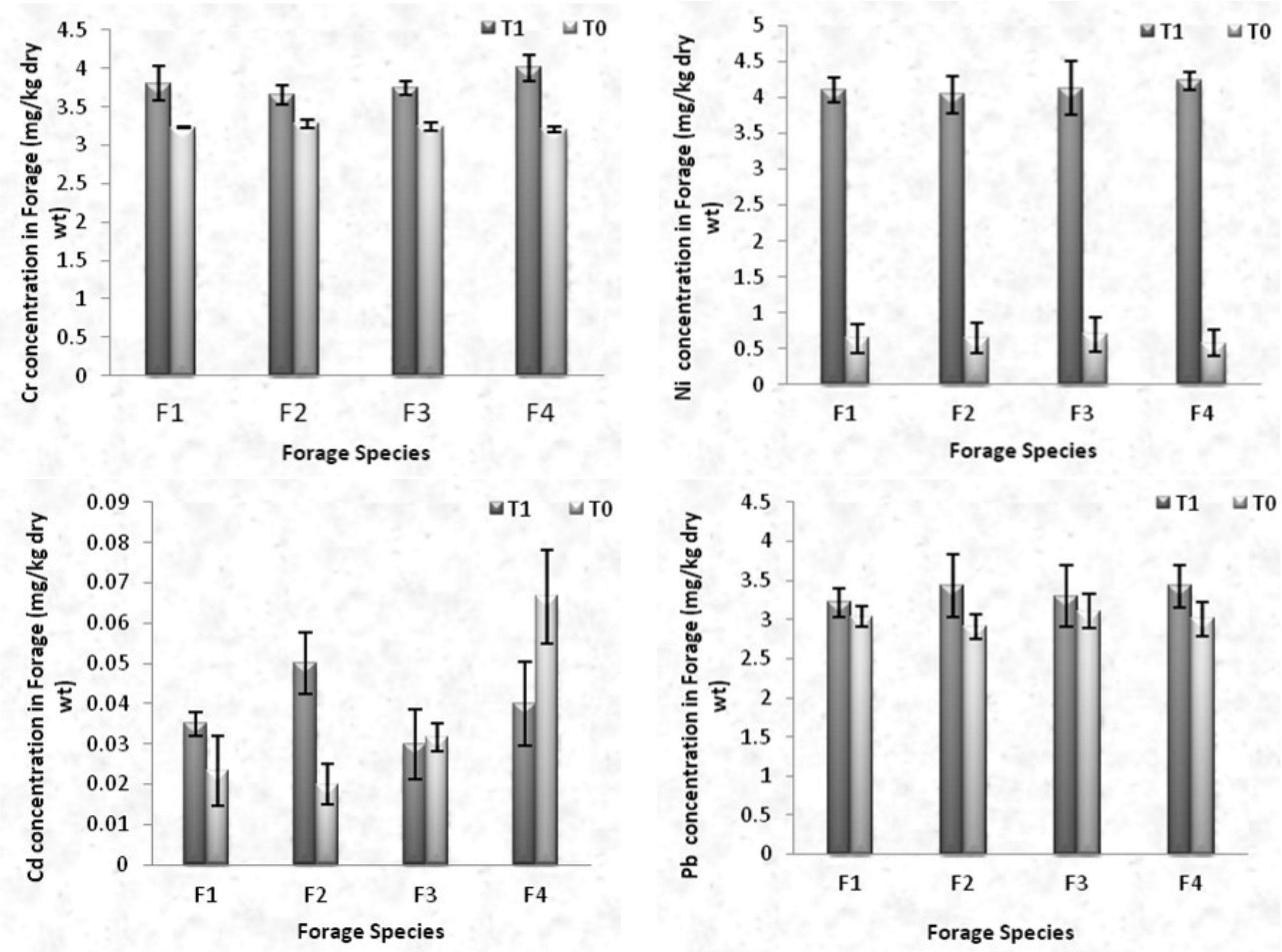

Fig. 2. Fluctuation of different metal concentrations in forage at both treatments. Forage species: F1 – berseem clover (*Trifolium alexandrinum*), F2 – oat (*Avena sativa*), F3 – guar (*Cyamopsis tetragonoloba*), and F4 – sorghum (*Sorghum bicolor*). T_1 – sewage water treatment, T_0 – canal water treatment.

Table 2. Heavy metals (Cr, Ni, Cd and Pb) correlation between soil-forage-hair in the different treatments.

Mineral	Soil-Forage		Soil-Hair		Forage-Hair	
	T_1	T_0	T_1	T_0	T_1	T_0
Cr	0.292	-0.399	-0.075	-0.034	-0.071	-0.100
Ni	0.106	0.242	0.354	-0.104	-0.438	-0.529
Cd	0.184	-0.358	0.233	-0.052	-0.193	-0.444
Pb	0.018	0.047	-0.056	-0.418	0.162	-0.013

T_1 – sewage water treatment, T_0 – canal water treatment

T_1 and T_0 treatments, correspondingly. Ni concentrations in the soil were higher in T_1 compared with T_0 (Fig. 1). In our study, the obtained levels were less than the decisive level of 0.85 mg kg^{-1} stated by Adriano (1986). Berrow and Reaves (1986) reported a numerical mean level of Ni in soil equal to 27 mg kg^{-1}. McGrath and Loveland (1992) found a numerical mean level of 20 mg kg^{-1}, which is higher than our obtained values. Therefore, there was no toxic hazard during our trial.

Data from ANOVA indicated no effect (P>0.05) of T_1 treatment on forage Ni levels (Table 1). The mean Ni level in forage ranged between 4.035 and 4.220 mg kg^{-1}. The mean Ni level in F4 was higher, whereas it was lower in F2 (Fig. 2). Moreover, from ANOVA, the T_0 treatment had no significant effect (P>0.05) on Ni forage level (Table 1). The mean Ni status in forage varied from 5.80 to 7.03 mg kg^{-1}, the maximum in F3 and the minimum in F4 (Fig. 2).

All the Ni forage levels were less than the critical value of 4.3 mg kg^{-1} suggested by Adriano (1986) in T_1, but the reverse was true in the case of T_0. In line with the findings of Painter et al. (1953), the Ni in forage ranging from 40 to 60 mg kg^{-1} may be toxic for plants. However, the values from our trial were lower, and therefore had no

Table 3. Bioconcentration transfer factor of heavy metals (Cr, Ni, Cd and Pb) from soil-forage and forage-hair.

Bioconcentration Transfer Factor				
	Soil-Forage		**Forage-Hair**	
Forage Species[a]	**T_1**	**T_0**	**T_1**	**T_0**
Chromium				
F1	114.1511	129.1333	0.062199	0.101188
F2	137.2483	89.4992	0.239527	0.101574
F3	107.0476	97.45096	0.072509	0.099025
F4	126.4724	76.99938	0.054515	0.096104
Nickel				
F1	7.278111	1.460376	0.119919	0.762274
F2	5.933824	1.363956	0.145395	0.911918
F3	5.83962	1.374591	0.125202	0.734598
F4	5.47732	1.181263	0.126972	0.925288
Cadmium				
F1	0.013548	0.008642	21.61906	34.85763
F2	0.011952	0.007143	15.03334	41.08335
F3	0.007258	0.012025	25.1111	26.4734
F4	0.009302	0.026667	22.91668	10.99994
Lead				
F1	0.206974	0.333516	0.003888	0.008237
F2	0.222114	0.260299	0.003643	0.005161
F3	0.234917	0.377688	0.002629	0.017723
F4	0.232466	0.428504	0.003747	0.018847

[a]Forage species: F1 – berseem clover (*Trifolium alexandrinum*), F2 – oat (*Avena sativa*), F3 – guar (*Cyamopsis tetragonoloba*), and F4 – sorghum (*Sorghum bicolor*).
T_1 – sewage water treatment, T_0 – canal water treatment.

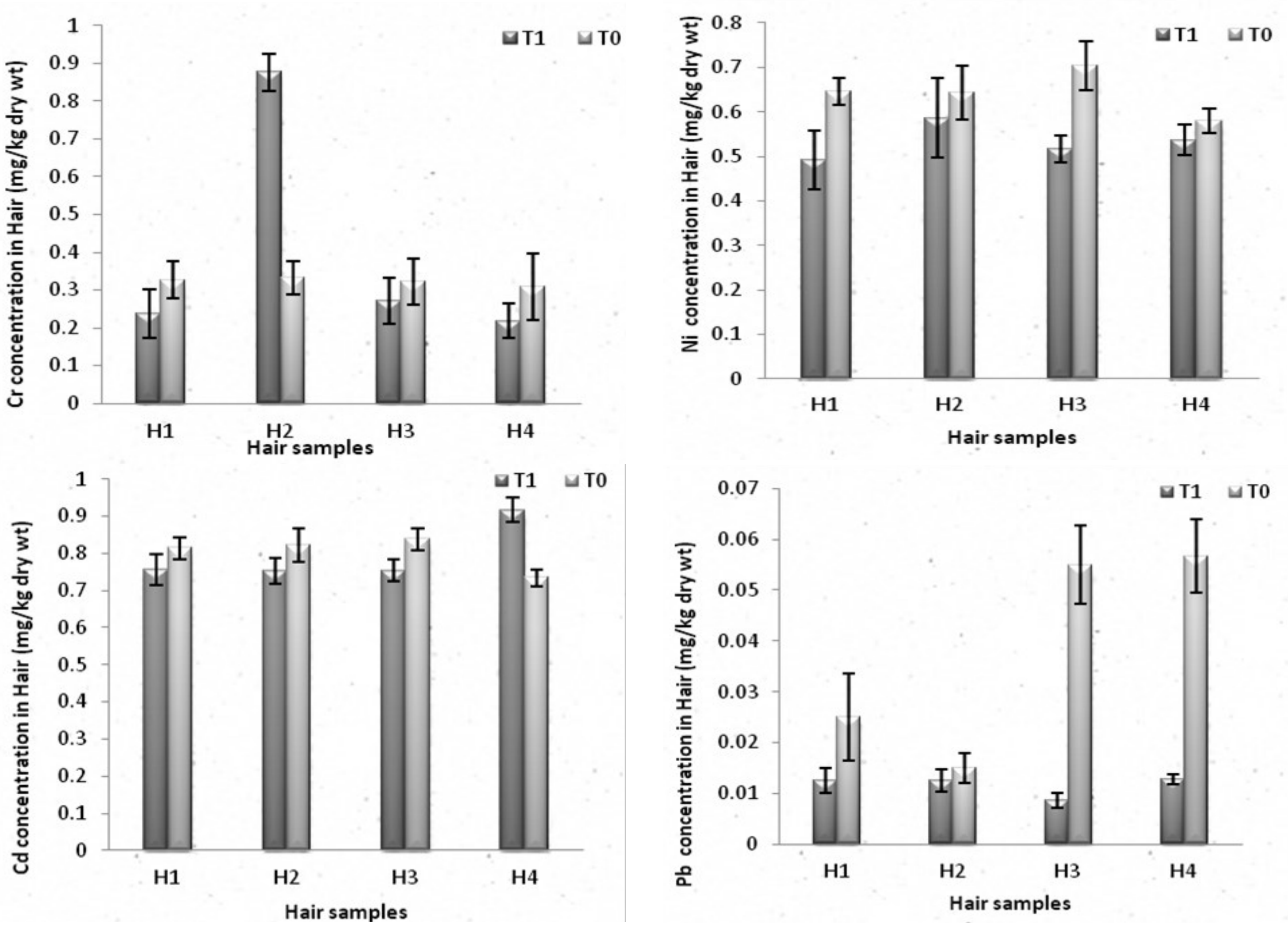

Fig. 3. Fluctuation in different metal concentrations in buffalo hair (H1–H4) at both treatments. T_1 – sewage water treatment, T_0 – canal water treatment.

potential risk for ruminant species. The ANOVA showed no significant ($P>0.05$) effect of both T_1 and T_0 treatments on Ni concentration in the hair of buffaloes (Table 1). The Ni concentration in buffalo hair varied from 0.491 to 0.586 mg kg^{-1} and from 0.580 to 0.703 mg kg^{-1} with application of T_1 and T_0 treatments, respectively. Ni concentration in buffalo hair was higher in T_0 compared with T_1 (Fig. 3). Livestock species suffering from Zn shortage have decreased tolerance to glucose, resulting in infections, hair loss or improper growth, with similar effects on wool or feathers. Toxicity of Ni is mostly associated with less Cu intake, however, Zn has low toxicity in livestock species (Medvedev 1999).

The Ni correlation was positive between soil-forage ($r = 0.106$) and soil-hair ($r = 0.354$) but negative between forage-hair ($r = -0.438$) in T_1 treatment (Table 2). This result indicates a strong relationship between soil forage and hair and improper flow of minerals from soil to forage and from forage to buffalo. In T_0, the Ni correlation was positive between soil-forage ($r = 0.242$), but negative among forage-hair ($r= -0.104$) and soil-hair ($r = -0.529$) that showed a weak relationship and improper flow of minerals (Table 2). The Ni transfer factor for soil-forage varied from 1.181 to 7.278 and for forage-hair from 0.7345 to 0.9252 in both treatments (Table 3). The highest rate was found in the treatment applied with sewage water and lowest in the one applied with canal water. The variation in plant absorption was probably due to the bioavailability of Ni in plants.

Cadmium (Cd)

Cd concentration in the soil was affected significantly ($P<0.05$) by T_1, while the reverse was true in T_0 (Table 1). Cd concentration in the soil ranged from 2.58 to 4.30 mg kg^{-1} and from 2.50 to 2.80 mg kg^{-1} in T_1 and T_0, respectively. Cd concentrations in the soil were higher in T_1 compared with T_0 (Fig. 1).

The Cd levels obtained in our study were lower than the critical level of 3 mg kg^{-1} reported by McDowell (1985). Ross (1994) obtained a Cd value in soil of 3 to 8 mg kg^{-1} that was considered toxic, therefore, the Cd level obtained in our study can be considered as safe. The levels of Cd in soils for agriculture are dependent on the levels present in the parent rock from which the soils are formed, the amount added in the form of fertilizers and amendment to the soil, and the levels deposited in the soil from the atmosphere. The low amounts of Cd obtained in our study may be due to harvested crops and frequent leakage in summer. T_1 had no significant ($P>0.05$) effect on forage Cd concentration (Table 1). The mean concentration of Cd in forage varied from 0.030 to 0.050 mg kg^{-1} in T_1 (Fig. 2). Cd status was higher in F2 but lower in F3. A significant ($P<0.05$) effect was observed in the concentration of Cd in forage in T_0. The mean values of Cd in forage varied from 0.020 to 0.066 mg kg^{-1}. Cd level was higher in F4 and lower in F2 (Fig. 2). The Cd level reported in our study was higher than the limit (0.03 mg kg^{-1}) found by Kloke (1980) in sewage water treatment. The adequate Cd level in plants was reportedly higher than 3.0 mg kg^{-1} (Cicek et al. 2004). The Cd level in our study was below the toxic level reported by Aksoy et al. (1999) and therefore had no potential threat for animals.

T_1 had a significant ($P<0.05$) effect on Cd level in buffalo hair while reverse results were observed in T_0 (Table 1). Cd level in buffalo hair ranged from 0.751 to 0.916 mg kg^{-1} and from 0.733 to 0.838 mg kg^{-1} in T_1 and T_0, respectively. Cd concentrations were higher in T_1 compared with T_0 (Fig. 3). The Cd correlation was positive between soil-forage ($r = 0.184$) and soil-hair ($r = 0.233$) and negative in forage-hair ($r = -0.193$) in T_1 (Table 3). This result indicates a strong relationship among soil, forage and hair, and improper flow of minerals from soil to forage and forage to buffalo. Whereas in T_0, the Cd correlation was negative between soil-forage ($r = -0.358$), forage-hair ($r = -0.052$) and soil-hair ($r = -0.444$), showing a weak relationship and improper flow of minerals (Table 2).

Transfer factor for Cd from soil-forage differed from 0.007143 to 0.02666 and from forage-hair, the transfer factor ranged from 0.2069 to 0.428 (Table 3) as a result of using both treatments. The highest value was observed in sewage water treatment which showed the highly sensitive nature of plants. The lowest level of transfer factor found in canal water treatment indicated the opposing plant type. Furthermore, the limited bioavailability may be due to the opposite effect of other trace metal elements.

Lead (Pb)

Pb level in the soil was also affected considerably ($P<0.05$) by T_0, while the reverse was true in the case of T_1 (Table 1). Pb concentration in the soil ranged from 14.03 to 15.53 mg kg^{-1} and from 7.01 to 11.16 mg kg^{-1} as a result of applying T_1 and T_0, respectively. Pb concentrations in the soil were higher in T_1 compared with T_0 (Fig. 1). Pb in the soil varied from 5 to 25 mg kg^{-1} as reported by Hayashi et al. (1985); the concentrations found in our study were similar in range as those reported, and did not exceed the toxic level. Conversely, our levels of soil Pb were higher than those found in the study of Aksoy et al. (1999) on the biomonitoring of metal pollution in Turkey. Similar to the results of Ross (1994), the Pb levels in the soil were less than the toxic level, posing no hazard to both plant and animal life. The smallest amount of soil lead may be due to the nonexistence of pollution from bio-solids, manure and slush. Moreover, the soil Pb levels observed in our study were safe for use in forage.

There was considerable ($P<0.05$) effect of T_1 treatment on forage Pb concentration (Table 1). Pb forage concentration ranged from 3.21 to 3.47 mg kg^{-1} in T_1. The maximum forage Pb level was reported in F2 and the minimum in F1 (Fig. 2). ANOVA showed no significant ($P>0.05$) effect of T_0 on forage Pb (Table 1). Pb concentration in forage varied from 2.90 to 3.10 mg kg^{-1} in T_0. The forage Pb status was higher in F3 and lower in F2 (Fig. 2). The forage lead concentration in our study was higher than the adequate Pb limit level (3.0 mg kg^{-1})

reported for plants by Allen (1989). The addition of metals by plant depends mostly on soil type, physio-chemical individuality, organic matter, soil reaction, redox potential, opposition or synergism between heavy metals, as well as interaction with other substances such as phosphates altering the toxicity of a metal for plant (Grigoryan et al. 1990).

The ANOVA showed no significant ($P>0.05$) effect of T_1 on Pb level in buffalo hair while opposite results were observed with application of T_0 (Table 1). Pb concentration in buffalo hair varied from 0.0086 to 0.128 mg kg^{-1} and from 0.015 to 0.0566 mg kg^{-1} with application of T_1 and T_0, correspondingly. The Pb concentrations found were higher in T_0 compared with T_1 (Fig. 3). Hair samples from both domestic and untamed species (such as cattle, horse, goat, sheep, camel, European bison, moose, brown bear, wild boar, squirrel and seal) have been used as a bioindicator of heavy metal pollution (Medvedev 1999).

The Pb correlation was positively non-significant between soil-forage ($r = 0.018$) and forage-hair ($r = 0.1692$) while negative and non-significant between soil-hair ($r = -056$) in T_1 (Table 3). This finding indicated a strong relationship between soil, forage and hair and an improper mineral flow from soil to forage and from forage to buffalo; conversely, in T_0, the Pb correlation was positive between soil-forage ($r = 0.047$) but negative between forage-hair ($r = -0.418$) and soil-hair ($r = -0.013$) that showed a weak relationship and improper flow of minerals (Table 2).

The Pb transfer factor of soil-forage varied from 0.2069 to 0.4285 and from 0.00263 to 0.01884 for forage-hair (Table 3). The highest rate was reported in sewage water treatment, and the lowest in canal water treatment. The overall rate of transfer was high, indicating that the plants became sensitive to specific metals, which may result in the death of plants. The lowest level of Pb showed that the plants may be resistant to Pb. Moreover, the low Pb level may be due to the occurrence of some other metals in soil suppressing Pb absorption by plants.

CONCLUSION

Heavy metal concentrations were higher in forage irrigated with sewage water compared with forage irrigated with canal water. Among the heavy metals investigated, Cr was found to be in higher concentration followed by Pb, and Ni and Cd in lower concentrations. Mineral transfer rates were highest in sewage-irrigated forage and lowest in canal water irrigated forage. The transfer of minerals depends on bioavailability, and the highest values may be due to the high rates of mineral uptake by forage. Therefore, the high transfer rate of trace elements by forage could become toxic, resulting in unfavorable consequences to grazing animals.

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