Bioconcentration of some macrominerals in soil, forage and buffalo hair continuum: A case study on pasture irrigated with sewage water

Zafar Iqbal Khan a,*, Kafeel Ahmad a, Iqra Ashraf a, Sumaira Gondal a, Muhammad Sher b, Zafar Hayat c, Vito Laudadio d, Vincenzo Tufarelli d

a Department of Biological Sciences, University of Sargodha, Pakistan
b Department of Chemistry, University of Sargodha, Pakistan
c Department of Animal Sciences, University of Sargodha, Pakistan
d Department of Emergency and Organ Transplantation, Section of Veterinary Science and Animal Production, University of Bari ‘Aldo Moro’, Bari, Italy

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Abstract The present study aimed to evaluate the bioaccumulation of some macrominerals in grazing buffaloes fed forage irrigated with sewage water or canal water. In particular, the transfer of sodium (Na), magnesium (Mg), potassium (K) and calcium (Ca) from soil to plant and in turn to animals was evaluated under sub-tropical environmental conditions. Samples of soil, forage and buffalo hair were collected and digested by wet method. Sodium and K concentrations were significantly higher in the soil but lower in the forages; however, Mg and Ca concentrations in both soil and forages were higher. The correlation between soil, forage and hair showed an imbalanced flow of Na, Mg and K and a balanced flow of Ca from soil to forage and then to animals. Based on the findings, the highest rates of transfer of minerals were found for sewage water treatment, whereas lowest rates were found for canal water treatment, except for Na. As the transfer of minerals depends on their bioavailability, the highest values may be due to the high rates of mineral uptake by plants. Thus, the high transfer rate of some elements by plants could become toxic in future causing detrimental effect to grazing livestock.

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1. Introduction
The most important mineral elements for the ruminants are represented by macrominerals such as Ca, Na, K, Cl, Mg and S (Underwood, 1981; Masters et al., 1993). The treated waste water is widely used for unrestricted irrigation all over the world (Khan et al., 2010). In particular, treated waste
water enhances crop growth and yield providing a wide variety of nutrients to plants. It also serves as a water source for agriculture practices (Bouwer and Idelovitch, 1987; Feigin et al., 1991; Al-Jaloud et al., 1995).

Potassium is essential in making of sugars, carbohydrates, starches, protein synthesis and cell division in roots and other portions of the plant. It maintains the water equilibrium; stabilize stem hardiness and cold tightness, increase the taste of fruit and vegetables (Khan et al., 2005). By its deficiency the forage yield decreases, become marked or spiraled eaves, mottled, burned show to leaves (Khan et al., 2006). Magnesium is a significant portion of the chlorophyll molecule and is essential for active performance of forage enzymes responsible for carbohydrates, sugars and fats production. If it becomes deficient, Mg leads to chlorosis, veins turned yellow of matured leaves and leaves droop. Calcium also helps the plant in the absorption of other mineral like nitrogen (N). Its deficiency causes stunting of new growth in stems, flowers and roots, resulting also in a reduction of plant growth (Goswami et al., 2005; Khan et al., 2005). Sodium deficiency in legumes is well documented in semi-arid regions and it can be overcome by regular supplementation throughout the year (Khan et al., 2005, 2006). With the time, the mineral status in legumes and grasses decreased (Gonzalez et al., 2006).

Therefore, the current study was conducted to evaluate the content of some macrominerals and their bioaccumulation in soil, forage and grazing buffaloes fed forage irrigated using sewage water or canal water.

2. Materials and methods

2.1. Study area

The research was conducted to evaluate the minerals composition of forages in city the Bhalwal, a subdivision of Sargodha District in the Punjab province of Pakistan. It is located within 30.05°N and 72.67°E having a sub-tropical to temperate environment and it is characterized by a mean altitude of 187 m above sea level. Its temperature range in summer is 25–48 °C and temperature range during winter is 7–25 °C, respectively.

2.2. Sample collection

Two fields were selected for the soil, forage and buffalo hair sampling. One field was irrigated with T1 (sewage water treatment) and other was with T0 (canal water treatment). The soil samples were collected from each field according to the method of Sanchez (1976). The collected soil samples were packed in labeled sealed paper bags. Forage samples contained mainly four species as follow: berseem (Trifolium alexandrinum), oat (Avena sativa), guar (Cyanopsis tetragonoloba) and sorghum (Sorghum bicolor). Three replicates of each variety of forage were taken during sampling period. These samples were dried and placed in oven at 60 °C temperature for 48 h to remove the moisture content. Twenty-four buffalo (twelve from each field) were selected to collect the hair samples from head (shaving area: 4 × 4 cm), and also these samples were dried and placed in oven at 60 °C temperature for 24 h to remove the moisture.

2.3. Sample preparation for analysis

Samples from soil, forage and buffalos’ hair were collected and digested by wet digestion method (Vukadinović and Bertić, 1988; Gabryszuk et al., 2010; Ahmad et al., 2013). A total of 1 g of soil, forage and buffalo hair samples was taken and digested with H2SO4 and H2O2 in a flask by placing the digesting material in a digestion chamber by following wet digestion method (Richards, 1968). The samples of soil, forage and hair were filtered using up to 50 ml of distilled water. Filtered samples were stored in labeled clean plastic bottles and placed in laboratory.

2.4. Mineral analysis and bioconcentration transfer factor

After wet digestion, soil, forage and hair samples were subjected to the determination of minerals content (Na, Mg, K and Ca) by using atomic absorption spectrophotometer Perkin-Elmer AAS-5000 (Perkin-Elmer Corp). The bioconcentration transfer factor (TF) from soil–forage–hair was calculated using the following formula according to Khan et al. (2007):

\[ TF = \frac{\text{Mean concentration of Forage/Hair}}{\text{Mean concentration of Soil/Forage}} \]

2.5. Statistical analysis

Data were subjected to the statistical analysis using the SPSS software (SPSS, Chicago, IL, USA) for correlation and one-way analysis of variance (ANOVA) worked out. Statistical significance between the means was tested at 0.05, 0.01 and 0.001 level of probability as suggested by Steel and Torrie (1980).

3. Results and discussion

3.1. Sodium (Na)

There was no significant (P > 0.05) effect of T1 treatment on Na concentration of soil, but its opposite results were noticed in case of T0 treatment (Table 1). Soil mean Na concentration during T1 treatment ranged from 54.28 to 55.72 mg/kg, while it differed from 49.46 to 59.61 in T0 treatment. The Na concentrations found were higher during T0 treatment as compare to T1 (Fig. 1). From the present research it is resulted that the mean Na concentration was higher during canal water treatment than in sewage water. All the soil samples of both treatments were below the critical level 62 mg/kg for Na concentration (Rhue and Kidder, 1983). Soil Na mean values found during present investigation was in agree with the findings of Khan et al. (2005). High soil Na concentrations were found working in another ranch of south western Punjab, Pakistan. Similar values for Na content were reported by different researchers at different places (Prabowo et al., 1990; Espinoza et al., 1991). From our findings, this study suggested that there is need of fertilizers because of these soils have severe deficiency of Na concentration in the studied area during both the treatments.

There was no significant (P > 0.05) effect of T1 treatment on forage Na concentration (Table 1). The forage Na concentration varied from 0.065% to 0.100% in case of T1 treatment.
The analysis of variance showed no significant ($P > 0.05$) effect of T0 treatment on forage Na concentration (Table 1). The forage Na concentration differed from 0.065% to 0.075% in case of T0 treatment. The values of forage Na during T0 treatment were lower in F1 and higher in F3 (Fig. 2).

| Source of variation | df | Mean squares |
|---------------------|----|--------------|
|                     |    | Soil         | Forage        | Hair          |
| Treatment           |    | T1           | T0           | T1           | T0           |
| Na                  | 3  | 1.381 ns     | 77.031 ***   | 0.001 ns     | 0.002 ns     | 2.790 ns     | 28.447 ns    |
| Mg                  | 3  | 1684.042 *   | 3.446 *      | 0.002 *      | 0.527 *      | 0.001 *      | 0.014 ns     |
| K                   | 3  | 4.752 *      | 225.605 ***  | 0.009 *      | 0.001 *      | 6.052 *      | 4.312 ns     |
| Ca                  | 3  | 2.942 *      | 39.825 *     | 0.001 *      | 0.001 *      | 0.019 *      | 0.017 ns     |
| Error               | 8  | 0.820        | 0.465        | 0.001        | 0.001        | 0.004        | 0.001        |

**T1, sewage water treatment; T0, canal water treatment.**

**ns Not significant.**

* $P < 0.05$.  
** $P < 0.01$.  
*** $P < 0.001$.  

Figure 1 Fluctuation in various metal concentrations in soil during both treatments.

The mean Na concentrations were similar to the optimal range determined by NRC (1984). Our research revealed that there is no need of mineral supplementations for grazing livestock. By comparing our findings with the earlier researches were not corroborate as they investigate lower forage Na, in fact same findings were also obtained by the Rehman et al.
(1998), Tiffany et al. (2001) and Khan et al. (2009) in different regions of the world. The analysis of variance showed significant \( P < 0.05 \) effect of \( T_1 \) treatment on Na concentration in buffalo hairs, while opposite results were noticed by applying \( T_0 \) treatment (Table 1). The Na concentration in hairs varied from 5.598 to 7.633 mg/kg and 6.32 to 13.166 mg/kg by applying \( T_1 \) and \( T_0 \) treatment, respectively. The Na concentrations found were higher during \( T_0 \) treatment as compare to \( T_1 \) (Fig. 3). The Protection Agency (EPA) 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 Na correlation was negative, no significant between soil and forage \( (r = -0.022) \), soil-hair \( (r = -0.177) \) and forage-hairs \( (r = -0.164) \) during \( T_1 \) treatment (Table 2). This result indicates a weak relationship among soil forage and hair and improper flow of mineral from soil to forage and hair to buffalo, while in case of \( T_0 \) treatment the Na correlation was positive, no significant between soil and forage \( (r = 0.311) \) and forage-hairs \( (r = 0.223) \) that showed strong relationship and proper flow of minerals (Table 2). While negative, no significant between soil-hair \( (r = -0.458) \).

3.2. Magnesium (Mg)

There was significant \( P < 0.05 \) effect of both \( T_1 \) and \( T_0 \) treatments on Mg concentration in soil (Table 1). Soil mean Mg concentration during \( T_1 \) treatment varied from 4.177 to 8.520 mg/kg, while it differed from 5.82 to 8.19 mg/kg during \( T_0 \) treatment. The Mg concentrations found were higher during \( T_1 \) treatment as compare to \( T_0 \) (Fig. 1).

All the Mg concentrations in soil samples analyzed were below then the critical level 9.10 mg/kg (McDowell et al., 1983). In the present study soil Mg concentration was similar to those reported earlier (Cuesta et al., 1993; Tiffany et al., 2001) and it was suitable for grazing ruminants. All the Mg concentrations in soil were higher in soil during \( T_1 \) than \( T_0 \).

The forage Mg levels were not significantly affected \( (P > 0.05) \) by treating with \( T_1 \) (Table 1). Forage Mg values varied from 0.231% to 0.281%, where the high levels were found in F1 and low levels were found in F4. The levels of Mg in forage treated with \( T_0 \) also showed no significant \( (P > 0.05) \) results that ranged from 0.750% to 1.616%, with the higher levels in F3 and lower levels in F4 (Fig. 2). From our data, it is concluded that the forage Mg level during sewage water treatment was high, but during canal water treatment it was low, however both treatments resulted higher than the critical value 0.12% (McDowell et al., 1983). Forage Mg concentrations observed in our present study are mostly similar then the concentrations observed by Prabowo et al. (1990). The level of forage Mg in the present study was lower than those reported by Fujihara et al. (1992) and Khan et al. (2009).

No significant \( P > 0.05 \) effect resulted by the analysis of variance on Mg concentration treated with \( T_1 \) and \( T_0 \) (Table 1).
The Mg concentration in hairs varied from 0.885 to 0.910 mg/kg and 0.616 to 0.775 mg/kg by applying T1 and T0 treatment, respectively (Fig. 3). The Mg concentrations found were higher during T1 treatment as compared to T0. Hair has the potential of being an excellent bio-monitor due to its historical representation of intake over prior weeks to years and can be utilized for investigating the exposure of individuals or populations to toxins and pollutants, such as heavy metals (Deanna et al., 2006). Hair samples from domesticated and wild species such as cattle, horse, goat, sheep, camel, European bison, moose, brown bear, wild boar, squirrel and seal have been used as a bio indicator of metal pollution (Medvedev, 1999).

The Mg correlation was negative, no significant between soil and forage (\(r = -0.090\)) and soil–hair (\(r = -0.030\)) and forage–hairs (\(r = 0.312\)) during T1 treatment (Table 2). This result indicates a weak relationship among soil forage and hair and improper flow of mineral from soil to forage and forage to buffalo while in case of T0 treatment the Mg correlation was negative, non significant between soil and forage (\(r = -0.417\)) that showed week relationship and proper flow of minerals, while positive non significant between soil–hair (\(r = 0.87\)) and forage–hairs (\(r = 0.506\)).

### 3.3 Potassium (K)

There was non-significant (\(P > 0.05\)) effect of T1 treatment on K concentration in soil, but its opposite results were noticed in case of T0 treatment (Table 1). Soil mean Na concentration during T1 treatment ranged from 93.53 to 96.58 mg/kg while it differed from 67.79 to 85.56 mg/kg in T0 treatment. The K concentrations were higher during T1 treatment as compared to T0. The mean K concentration examined during sewage water treatment was similar to the critical level (80 mg/kg) as suggested by Warncke and Robertson (1976), while the values found during canal water treatment resulted higher than the above mentioned critical level. The K concentration observed during present investigation was lower if compared to those reported by Espinoza et al. (1991) in central Florida and Khan et al. (2007) in Pakistan. The possible explanation for low soil K may be due to high K leaching in soil (Espinoza et al., 1991). The K concentration in soil observed during present findings is enough for the use of ruminants and no need of fertilizer in this ranch of Punjab, Pakistan.

Table 2 Macrominerals (Na, Mg, K and Ca) correlation between soil and forage–hair based on the applied treatment.

| Mineral | Soil–Forage | Soil–Hair | Forage–Hair |
|---------|-------------|-----------|-------------|
| Na      | T1 –0.022   | T0 0.311  | T1 –0.177   | T0 0.458 | T1 –0.164 | T0 0.223 |
| Mg      | T1 –0.090   | T0 0.074  | T1 –0.030   | T0 0.087 | T1 –0.312 | T0 0.506 |
| K       | T1 0.015    | T0 0.064  | T1 –0.203   | T0 –0.122| T1 –0.093 | T0 0.101 |
| Ca      | T1 0.373    | T0 0.304  | T1 0.166    | T0 0.126| T1 0.372  | T0 0.529 |

T1, sewage water treatment; T0, canal water treatment.

Figure 3 Fluctuation in various metal concentrations in hairs during both treatments.
ranging from 0.766% to 0.781% with higher levels in F3 and lower levels in F4, respectively. However, all mean forage K concentrations were in the range of critical value 0.8% (McDowell et al., 1983). The similar values of forage K was also reported by Espinoza et al. (1991) in Florida, but they were lower than data reported by from Florida by Kiatoko et al. (1982) and by Khan et al. (2009) in Pakistan. In many areas of the world, it is possible that K deficiency could results, as it is considered that the mineral contents decreased by increasing forage maturity (McDowell and Valle, 2000). High forage diets typically contain several times the amount of K contained in high grain diets. Moreover, K is not readily stored and it must be supplied daily in the diet.

From the analysis of variance it was found that the effect of T1 and T0 treatment was non significant (P > 0.05) on K concentration in hairs of buffaloes (Table 1). The K concentration in hairs ranged from 5.25 to 8.68 mg/kg and 8.36 to 11.16 mg/kg by the treatment of T1 and T0, respectively. The K concentrations found were higher during T1 treatment as compare to T0. Hair has the potential of being an excellent bio-monitor due to its historical representation of intake over prior weeks to years and can be utilized for investigating the exposure of individuals or populations to toxins and pollutants, such as heavy metals (Pereira et al., 2004).

The K correlation was positive and not significant between soil and forage (r = -0.015) but negative and non significant among soil–hair (r = -0.203) and forage–hairs (r = -0.093) during T1 treatment (Table 2). This result indicates a weak relationship among soil forage and hair and improper flow of mineral from soil to forage and forage to buffalo while in case of T0 treatment the K correlation was negative and non significant between soil and forage (r = -0.064) and soil–hair (r = -0.122) that showed week relationship and an improper flow of minerals (Table 2), while non significant positive it was found between forage and hair (r = 0.101).

3.4. Calcium (Ca)

Analysis of variance showed no significant (P > 0.05) effect for both T1 and T0 treatments on Ca concentration in soil (Table 1). The Ca concentration in soil varied from 75.79 to 78.19 mg/kg and 75.12 to 83.00 mg/kg by applying both T1 and T0 treatments, respectively (Fig. 1). The Ca concentrations in soil found were higher during T1 treatment as compare to T0. The mean concentration of Ca is higher than the critical values of 71 mg/kg as reported by McDowell in 1983. The values obtained during present findings are lower than the values reported by Li et al. (2004). Soil type, drainage, liming and cropping practices affect the levels of calcium found in the soil. Moreover, Ca is closely related to soil pH. From the analysis of variance it was revealed that there was no significant (P > 0.05) effect of T1 on forage Ca contents (Table 1). The forage Ca concentrations varied from 0.265% to 0.303%. The maximum levels of Ca were found in F1 and the minimum levels were found in F3 (Fig. 2). Further, the analysis of variance showed no significant (P > 0.05) effect of T0 on forage Ca concentration (Table 1). The forage Ca levels in T0 varied from 0.190% to 0.235%. The higher levels of Ca were found in F3 and the lower levels were found in F1 (Fig. 2). All the forage samples analyzed were below the critical level of 0.3% for Ca concentration. The average forage Ca concentration from this investigation was much lower than values previously reported in Pakistan (Khan et al., 2005, 2006) and in Columbia by Pastrana et al. (1991), and similar to those found by Rojas et al. (1993) in Venezuela. These low forage Ca values imply that the grazing livestock were not subjected to good quality forages, especially during the winter season.

The analysis of variance showed no significant (P > 0.05) effect of both T1 and T0 treatments on Ca concentration in buffalo hairs (Table 1). The Ca concentration in hairs varied from 0.483 to 0.665 mg/kg and 0.400 to 0.55 mg/kg by applying both T1 and T0 treatments, respectively (Fig. 3). The Ca concentrations in hairs of buffaloes found were higher during T1 treatment as compare to T0. Hair provides a better estimate of the total body intake of certain elements than blood or urine (Wilhelm et al., 1989). The Ca correlation was positive but no significant between soil and forage (r = 0.373), soil–hair (r = 0.166) and forage–hair (r = 0.372) during T1 treatment (Table 2). This result indicates a strong relationship among soil forage and hair and proper flow of mineral from soil to forage and forage to buffalo, while in the case of T0 treatment the Ca correlation was positive, but no significant between soil and forage (r = 0.304), forage–hair (r = 0.126) and soil–hair (r = 0.529) that showed a strong relationship and proper flow of minerals.

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