



## Article

# Chromium Poisoning in Buffaloes in the Vicinity of Contaminated Pastureland, Punjab, Pakistan

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**Abstract:** This article focuses on the toxic element chromium (Cr) in wastewater, its incorporation into soil plant systems, and its relevant toxicity in the food chain as assessed by a health risk assessment from dietary intake. The Nili Ravi buffalo is an important cattle inhabiting Sargodha, Punjab, Pakistan, and forage crops grown on soils contaminated with Cr might cause toxicity in the food chain by local inhabitants eating meat. The soil, forage and animal blood samples were collected from five different locations in Tehsil Sahiwal (Chak Dhool, Bagabalocha, Chandia, Dhool Bala and Kakrani) twice at six-month intervals. A total of 30 samples from each ecological zone were collected from the soil and forage crops (*Zea mays*, *Sorghum bicolor*, *Trifolium alexandrinum*). The samples from zone-V and zone-IV showed the maximum concentration of Cr because these areas receive highly contaminated water for irrigation. The Cr was greater than the permissible limits. Environmental indices for all samples ranged below 1. The bioaccumulation and pollution load of Cr in soil and forage crops due to wastewater irrigation can contaminate the whole food chain via the soil, forages and animals. The health risk index (HRI) and a high value of enrichment factor were found for Cr in some sites. The Cr concentration was higher during the summer season than winter. Fodder crops with different concentrations and an elevated level of Cr were observed in maize. Attention should be paid when wastewater is used for fodder crop irrigation and its potential risks to human health following dairy product (milk, meat) entry into the food chain.

**Keywords:** heavy metals; bioaccumulation; chromium; health risk index



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## 1. Introduction

By 2050, the universal food system will need to accommodate an increase in the number of people on the planet, while also enhancing their quality of life [1]. This intensification of agricultural systems and farming practices needs to look for alternative non-conventional water resources and to save the fresh water resources for other high-value tasks [2]. Low-quality salt water, treated wastewater and marginal, degraded lands could be employed to meet food, feed and fiber requirements for the growing population in Asia and Africa, but this has not been adequately addressed in the past [3]. Organic pollutants such as petroleum aromatic hydrocarbons, explosives and pesticides have posed harmful effects on human health and soil microbiota. Essential and poisonous metals are two categories of metals. Essential metals are required for the growth of crops (Zn, Fe, Mn, Cu) and maintenance of

microbial cells (Mo, Co, Ni), but in limited amounts. Several metabolic problems such as cancer, kidney failure and bone problems are caused due to different trace metals [4]. These heavy metals originate in the soil through two major processes: anthropogenic (human activities) and geogenic (weathering of rocks). Anthropogenic processes include mining and smelting of ores, and other industrial and domestic sources. Emissions of Pb, Cd and Zn have increased 100-fold more due to anthropogenic activities than those from natural sources. The geogenic process is the destruction and weathering of rocks due to different factors: climate, living organisms, parent material, time and topography. These factors cause the dissemination of respective materials from parent rocks and deposit them in the environment.

Chromium (Cr) is an important toxic trace element widely used in the chemical and metal industry and is naturally released from the earth's crust in trivalent chromium [Cr(III)] and [Cr(VI)] form [5]. Cr (VI) is highly toxic and soluble and, hence, dangerous to plants, animals and humans [6]. There are several reports in the literature that demonstrate that chromium is a highly toxic metal that significantly impedes the growth, productivity and yield of terrestrial plants, vegetables and crops [6–8], even at lower concentrations of 0.5–5.0 mg L<sup>-1</sup> in plant nutrient solution and 5–100 mg g<sup>-1</sup> in soil [9]. Cr causes heavy damage to crops through chlorosis, necrosis, protein degradation and enzyme activity retardation [10]. The toxic impacts of Cr accumulation in plants are demonstrated by the retardation in biochemical and physiological attributes. The leaf water relation, nutrient uptake and photosynthetic processes decrease due to a higher Cr accumulation in the plant organs in rice [11]. Long-term exposure to agriculture soils leads to Cr accumulation in the soil that reduces the plant growth and productivity [12].

Livestock are an important source of milk, meat, leather and manure and play an important part in the economic development of Pakistan [13]. Forage crops are an important source of minerals, nutrients and fodder for farm animals. Fodder analysis shows the presence of trace element accumulation in water, the soil rhizosphere and tissues of animals (liver and kidneys, blood samples), which are vital bio-indicators highlighting heavy metal toxicity in the food chain [14]. Heavy metals in plants may be absorbed, transported and bioaccumulated when fodder crops are irrigated with wastewater. Therefore, feeding farm ruminants this polluted fodder can have a negative influence on human health through the ingestion of milk and meat. Trace metals are dangerous to the ecology and terrestrial environment [15].

Domestic animals serve as key biological indicators of various environmental pollutants that are used to assess the level of contamination in the terrestrial environment. The tissues of domestic animals act as bioindicators for the occurrence of contagious substances. Compared to Sargodha and Bhakkar, the neighboring district of Mianwali had a higher proportion of chromium [16]. The mean chromium concentrations in the forages used for feeding ranged from 0.672 to 1.631 mg/kg in Sargodha, 1.493 to 2.612 mg/kg in Mianwali, and 0.7804 to 1.047 mg/kg in Bhakkar, respectively. In Sargodha, Mianwali and Bhakkar, goat blood plasma displays Cr concentrations that range from 0.666 to 1.269 mg/L, 0.657 to 0.752 mg/L, and 1.39 to 2.37 mg/L, respectively [16]. As animals grew older, their amounts of fat and ash increased along with their protein and water levels, increasing the likelihood that heavy metals would accumulate in the body. This is due to the fact that animal tissues, such as meat and its derivatives, contribute to human nutrition by offering necessary components [17]. Small amounts of the mineral chromium are needed by humans, although it must be obtained by diet [18]. For people with diabetes or poor glucose tolerance, more Cr is useful. Chromium improves the body's capacity to metabolize fat, aiding in weight loss. Chromium enhances mental performance, disintegrates fats and carbs, and regulates blood sugar—all of which are critical for overall health [19]. Overconsumption of chromium through food can result in hypoglycemia and abdominal pain (hypoglycemia). A heartbeat that is erratic may result from the negative effects of excessive chromium supplementation on kidneys, nerves and the liver [20].

The uptake of contaminated forages has caused serious threats to animal health. The approximation of the daily intake of metal does not tell us only about the metabolic discharge of metal but it also tells us about the intake rate of metal. The hazardous effects of heavy metals occur after many years of contact, while a lower level of metal ingestion does not cause any serious disorder [18,19]. There are many factors which tend to increase the concentrations of heavy metals, some of which are direction of precipitation, wind, emission of factories and unprocessed wastewater irrigation [20,21]. Wastewater is contaminated with metals and metalloids such as chromium, lead, manganese, zinc, arsenic, cobalt, molybdenum, boron and copper. Heavy metals are absorbed by the roots of forage crops and grasses from the soil. Furthermore, they are transported to the stem and aerial parts of the plants. Some of these elements are non-essential and create oxidative stress and deadly indications on plants, animals and humans [16,18,20]. The objective of this study was to measure the levels of Cr in soil and several forage crops that were irrigated using wastewater. The current work also aimed to check the chromium levels in dissimilar tissues of buffaloes consuming those forage crops. In this study, we set up a multidisciplinary experiment (soil–plant–livestock) to evaluate the relationship between Cr impact, toxicity, pollution load in forage crops and bioassimilation in buffalo body tissues. Our main objectives were: (1) to investigate how wastewater affects the bioaccumulation of Cr in different forage crops (*Zea mays*, *Sorghum bicolor*, *Trifolium alexandrinum* biomass), (2) to surveil animals following contaminated pasture consumption (3) to evaluate food chain contamination, (4) to determine health risk indices, (5) to assess ecological risk through the determination of trace metal levels in diversified fodder crops that were ingested by buffalo.

## 2. Materials and Methods

### 2.1. Study Area

Sargodha division's Tehsil Sahiwal was chosen for investigation (Figure 1) (Table 1). Although there are some hills in Sargodha, it is primarily made up of lush flat plains. Small and forward-thinking farmers in this region primarily use wastewater to irrigate the field crops, forages and vegetable production. Sahiwal and Sargodha experience cold winters (16 to 22 °C) and moderately hot summers (32 to 43 °C).

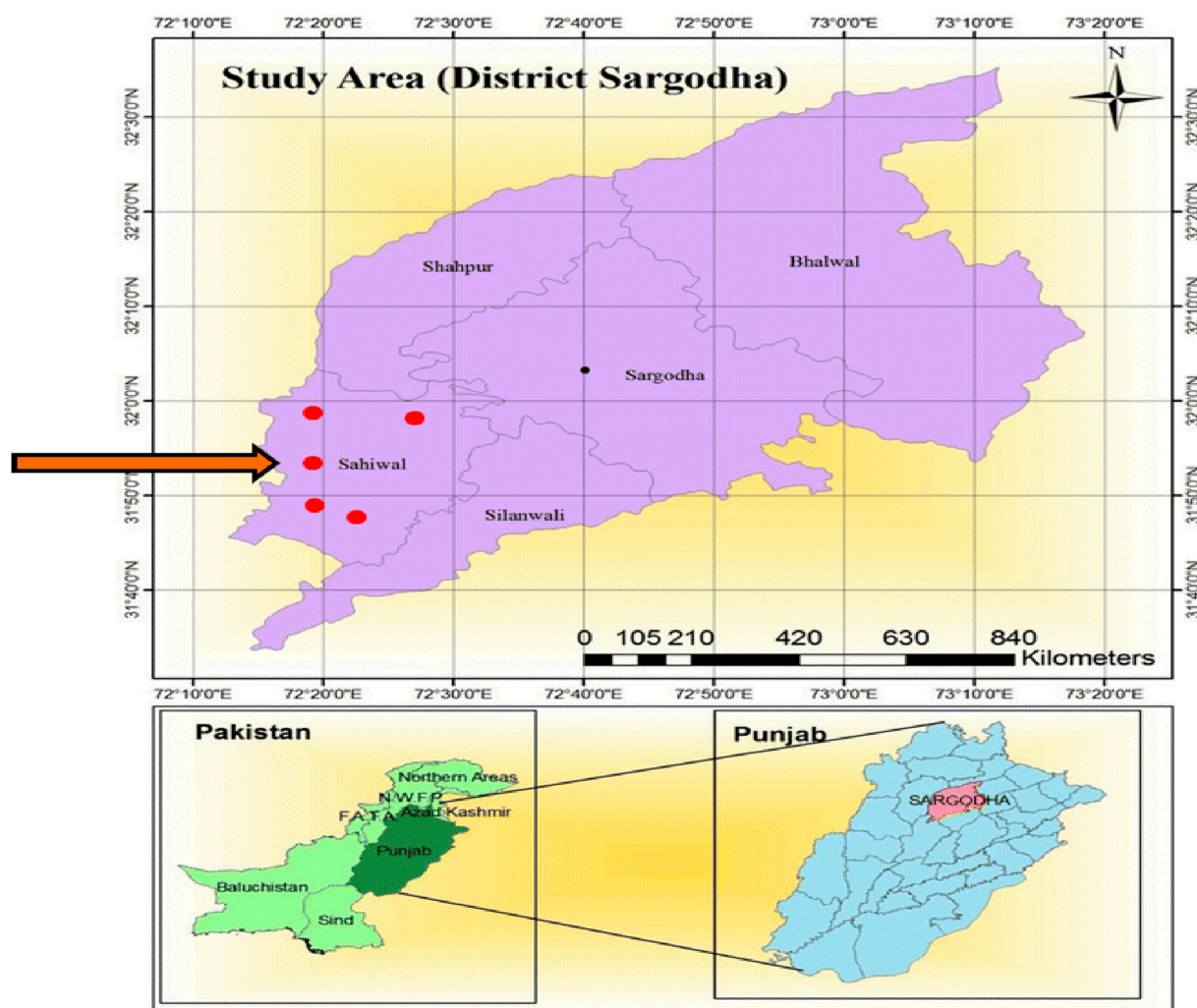
**Table 1.** Sampling Coordinates.

Zone Name	Coordinates
Chak Dhool	31.940529, 72.322270
Bagabalochan	31.931772, 72.306762
Chandia	31.935719, 72.339797
Dhool Bala	31.938038, 72.329641
Kakrani	31.911043, 72.351819

### 2.2. Soil, Forage Crops and Farm Ruminant Sampling (Time and Concentration-Dependent Cr Accumulation in Soil Rhizosphere and Fodder Crops)

Sahiwal Town is a small peri-urban region in the Sargodha District. Five irrigation zones for wastewater were selected for this study and given the designations zone-1, zone-2, zone-3, zone-4 and zone-5. Vegetables, fruits, milk, wheat, fodder and meat are the main agricultural products produced in this region and are sold to the general public in the study area and its surrounding regions. These five ecological zones were the source of all soil, plant and animal samples.





**Figure 1.** Map of study area, Sahiwal, Sargodha (Punjab), Pakistan.

### 2.3. Sampling of Soil and Forages from Five Ecological Zones

Five agroecological zones (Bagabalocha, Chandia, Chak Dhool, Kakrani and Dhool Bala) provided soil samples, which were then transferred immediately to plastic bags (ziplocked). Before beginning the soil sampling, all of the sampling equipment, including the stainless-steel auger, was thoroughly cleaned. A total of five samples (0–30 cm soil depth) were collected from each ecological zone. Target forage crops were also sampled from the same plot [14,15]. To completely remove all moisture, soil samples were dried in a forced air oven (72 °C) before being placed in plastic bags for additional biochemical examination.

The feedstock for farm ruminants, such as *Zea mays*, *Sorghum bicolor* and *Trifolium alexandrinum*, was picked from each ecological zone, rinsed under running water and finely chopped using a machine. All three target sample types had their sampling campaigns finished in the summer and winter, in the months of August/September and January/February, respectively. The fodder samples were cleaned with distilled water, baked in an oven for three days at 72 degrees Celsius and then crushed into a fine powder. For additional biochemical analysis, soil and forage crop samples were both baked in an oven (60–75 °C) for a week. After drying, the samples were then placed in airtight bags.

### 2.4. Collection of Blood Samples from Nili Ravi Buffaloes at Five Ecological Areas

All three target sample types had their sampling campaigns finished in the summer and winter, in the months of August/September and January/February, respectively. Chak Dhool, Bagabalocha, Chandia, Dhool Bala and Kakrani are among the sampling locations.

The wastewater from each of these locations was used to irrigate the fodder crops. Since they are well-acclimated to warm and dry environments, the Sahiwal breed of buffaloes was chosen for this investigation. Chosen forage crops were cultivated on soil that was irrigated with wastewater in order to meet the needs of these animals for feed. To provide the animals with the nutrition they needed, the forage crops were gathered and fed to them.

### 2.5. Sample Digestion

The sample processing method was wet digestion. The following equipment and chemicals were used for digestion: 70%  $H_2SO_4$ , 50%  $H_2O_2$ , 100 mL of distilled water, a measuring cylinder of 50 mL, a digestion flask of 100 mL, a pipette, 42 Whatman filter papers and a stirrer.

#### 2.5.1. Water Digestion

The  $H_2SO_4$  (few drops) were added to a beaker holding 5 mL of water, which was then boiled until smoke appeared. 2 mL hydrogen peroxide was then added. The procedure was continued until the water became clear. Water was purified and put into a bottle using filter paper.

#### 2.5.2. Soil Digestion

Soil sample of 1 g was kept in digestion flask with 8 mL  $H_2SO_4$  on top. After that, sulfuric acid and the mixture of soil were heated for approximately 30 min. With the addition of 10 mL  $H_2SO_4$ , the flame deposits become more brilliant. Then, 4 mL  $H_2O_2$  were added and heated again. For dilution of solution, distilled water was used, and the volume exceeded up to (50 mL), and was saved until metal analysis.

#### 2.5.3. Digestion of Forages

The  $H_2SO_4$  and  $H_2O_2$  were used in a 4:2 ratio to digest one gram of forage sample at 250 c for 3–4 h until colorless solution formed and colorless white vapors emerged in the flask. To wash the remaining flask, distilled water was used, filtered by using Whatman paper. The solution was filtered to remove impurities so there should be no problem using the Atomic Absorption Spectrophotometer, while determining the metal. By adding distilled water to the remaining flask, 50 (mL) diluted solution was formed.

#### 2.5.4. Digestion of Blood Samples

All collected blood samples that were stored in a freezer at  $-20\text{ }^\circ\text{C}$  were digested with dry digestion. Approximately 2 mL of blood plasma was placed in a digestion tube, and 50 mL of sulfuric acid was added to the tube. When the solution was heated for 15–20 min, yellow granules were formed. Approximately 2 mL of hydrogen peroxide was added to the digestion tube, and the solution was heated until yellow granules in the solution vanished completely. After cooling, the digested materials were filtered with Whatman filter paper. For the evaluation of heavy metals, the solution was saved in plastic bottles.

#### 2.5.5. Filtration

Wet digestion methods for elemental analysis involve the chemical degradation of sample matrices in solution, usually with a combination of acids which dissolve the metals as solutions form. Dissolving the sample in this manner enables researchers to carry out full chemical quantifications via different forms of elemental analysis. The metal is dissolved in the solution. The purpose of filtration is to remove undissolved materials that contain contamination [16].

#### 2.5.6. Quality Accuracy and Quality Control

To ensure the accuracy of the analysis, a well-washed apparatus was utilized during the research, and the samples were analyzed many times. Quality control measures were modified for determination of accuracy. To validate the results, chemicals from the best

known company were utilized in the process of digestion and atomic absorption spectrophotometry. The results were compared with the International Standard for insurance of precision of analysis.

## 2.6. Chromium Assessment

Digested samples were put through an atomic absorption spectrophotometer to measure the amount of Cr (PerkinElmer Corp. 1980). In order to obtain precise results, the Cr levels in three replicates of each specimen were analyzed and contrasted to an international standard. A standard unit is used for all liquid samples (mg/L) and solid samples (mg/kg). The heavy metal analysis of forage crops, soil and animal tissues were carried out at the Pakistan Council for Scientific and Industrial Research (PCSIR) laboratories, Lahore, Pakistan.

## 2.7. Environmental Pollution and Health Risk Indices

### 2.7.1. Bio-Concentration Factor

Using Equation (1), as previously reported by Akhtar et al. [4], the BCF was determined using Equation (1):

$$BCF = \frac{\text{Metal value in forage crop}}{\text{Metal in selected Soil}} \quad (1)$$

### 2.7.2. Pollution Load Index

The PLI was determined using Equation (2), as stated by Akhtar et al. [4] (2):

$$PLI = M \left( \frac{IS}{RS} \right) \quad (2)$$

### 2.7.3. Enrichment Factor

The Buat-Menard and Chesselet [7] formula was used to compute the enrichment factor:

$$EF = \frac{\text{Metal} \left( \frac{\text{Forage crop}}{\text{Soil}} \right) \text{Sample}}{\text{Metal} \left( \frac{\text{Forage crop}}{\text{Soil}} \right) \text{Reference value}} \quad (3)$$

FAO/WHO advised the chromium values for soil (9.07 mg/kg) and crops (2.3 mg/kg) [17].

### 2.7.4. Daily Intake of Metals

Equation (4) was used to calculate the DIM (Akhtar et al. [4]):

$$DIM = \frac{\text{Cr in forage crops} * \text{Daily food intake} * \text{conversion factor}}{\text{Average animal body weight}} \quad (4)$$

For buffalo, the daily intake values were 12.5 kg. However, the acceptable body weight for these animals was 550 kg, with a reported conversion factor value of 0.085.

The Table 2 showed the chromium metal daily intake limit and Oral Reference Dose (RfD).

**Table 2.** The chromium reference values in soil and fodder were as follows.

Category	Metal	Daily Intake Limit	Oral Reference Dose (RfD)	References
	Cr	Cr	Cr	
Reference soil	9.07	1.05	1.4	[13–15]
Reference in fodder	1.3			[17,18]

### 2.7.5. Health Risk Index

The US Environmental Protection Agency (USEPA) [18] ascertained the formula for this index as:

$$\text{HRI} = \text{Daily intake of metal} / \text{Oral reference dose} \quad (5)$$

The US Environmental Protection Agency (USEPA) [19] ascertained the oral reference dose of Cr as 1.5 mg/kg.

### 2.8. Statistical Analysis

SPSS (17.0 version) software was used to statistically analyze the data from each attribute. A three-factor factorial design was used for Cr soils, fodder crop samples and animal data to detect significant variations between mean values; a *p*-value of less than 0.05 was regarded as statistically significant.

## 3. Results

### 3.1. Chromium (Cr) Analysis in Soil and Fodder Crops (*Zea mays*, *Sorghum bicolor*, *Trifolium alexandrinum*) from Different Zones across Five Ecological Zones

To assess the toxicity of chromium and its effects on forage species and livestock consuming these forages, the average concentrations in water samples and the content of Cr in soil (across five zones), root and leaf samples from three forage crops were evaluated.

### 3.2. Cr Accumulation in the Soil

The Cr values of the soil samples collected in the summer ranged from 1.23 to 1.10 and 1.04 to 2.15 at zone-1 and zone-5, respectively (Table 3; Figure 2). The Cr values of the winter samples ranged from 1.13 to 1.10 and 1.49 to 5.36 at zone-1 and zone-5, respectively (Tables 3 and 4). The higher Cr values of the collected soil samples in the study area ranged from 1.23 to 1.10 mg/kg and 1.04 to 2.15 mg/kg at zone-1 and zone-5, respectively. In this experiment, we found that soil exhibited a lower range of Cr values in the soil samples than the permissible limits.

**Table 3.** Mean concentrations of heavy metal, Cr (mg/kg), in soil from five ecological zones in the peri-urban areas of Sahiwal town.

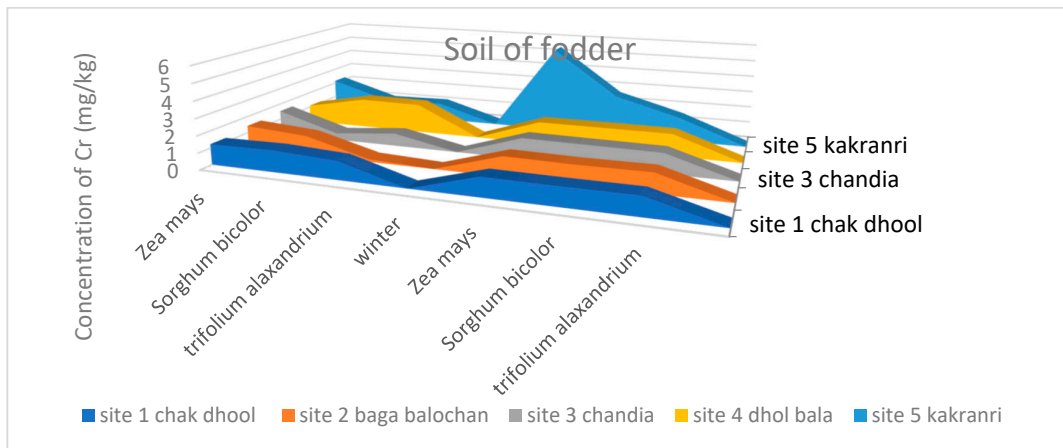
	Zone-1	Zone-2	Zone-3	Zone-4	Zone-5
Soil sample collection in field plots with summer forage crops					
<i>Zea mays</i>	1.23 ± 0.14	1.14 ± 0.14	1.53 ± 0.16	1.09 ± 0.220	2.15 ± 0.20
<i>Sorghum bicolor</i>	1.28 ± 0.14	1.16 ± 0.10	0.42 ± 0.14	1.84 ± 0.29	1.04 ± 0.22
<i>Trifolium alexandrinum</i>	1.10 ± 0.28	1.15 ± 0.22	0.18 ± 0.30	1.80 ± 0.36	1.12 ± 0.22
Soil sample collection in field plots with winter forage crops					
<i>Zea mays</i>	1.13 ± 0.22	1.19 ± 0.22	1.28 ± 0.22	1.36 ± 0.29	5.36 ± 0.22
<i>Sorghum bicolor</i>	1.11 ± 0.22	1.14 ± 0.29	1.24 ± 0.14	1.36 ± 0.22	2.58 ± 0.22
<i>Trifolium alexandrinum</i>	1.11 ± 0.14	1.17 ± 0.14	1.23 ± 0.1	1.36 ± 0.22	1.49 ± 0.29

**Table 4.** Analysis of variance for Cr content in soil, fodder and blood samples from five ecological zones in the peri-urban areas of Sahiwal town.

	Zone-1	Zone-2	Zone-3	Zone-4	Zone-5
Summer Soil	3.365 ***	0.754 ***	0.053 ***	0.000 ns	0.003 ***
Winter soil	50.929 ***	0.000 ns	0.004 ***	0.005 ***	0.009 ***
Summer Fodder	22.876 *	0.098 ***	0.092 ***	0.870 ***	0.011 *
Winter Fodder	0.001 *	0.126 ***	0.033 *	1.260 ***	0.519 ***
Summer blood	0.007 ***	0.003 ***	0.011 ***	0.000 ***	0.000 ns
Winter blood	0.004 ***	13.238 ***	0.007 ***	0.005 ***	0.782 *

\*, \*\*\* = Significant at 0.05 and 0.001.





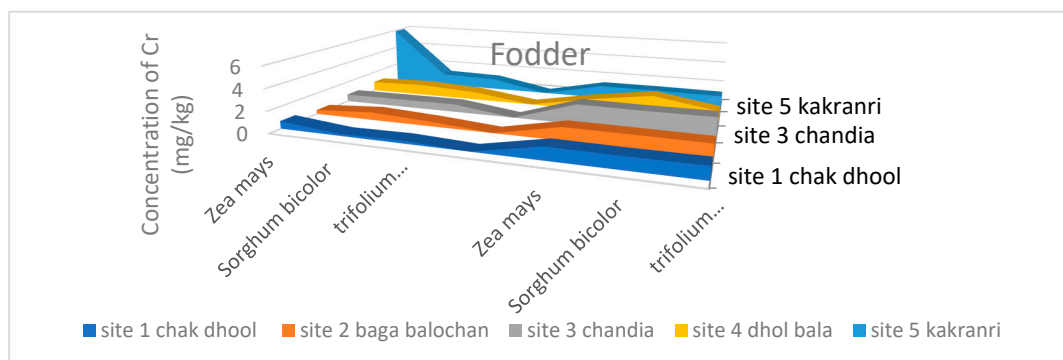
**Figure 2.** Fluctuations in mean values of chromium in soil from five ecological zones in the peri-urban areas of Sahiwal town during summer and winter seasons.

3.3. Cr Content in Fodder Samples

In summer, the Cr content of the forage crops ranged from 0.70 to 0.25 at zone-1 and from 1.08 to 5.90 at zone-5 (Table 5; Figure 3). In winter, the Cr values of the forage crops at zone-1 were between 2.11 and 1.15 and, at zone-5, were between 1.23 and 1.18 (Figure 3). Chromium-induced toxicity in plants may cause a reduction in pigment content, germination in seed growth and early seedling development. In the present study, the higher Cr values of the plants at zone-1 were between 2.12 and 1.15 mg/kg and, at zone-5, were between 1.23 and 1.18 mg/kg (Figure 3).

**Table 5.** Mean concentrations of heavy metal, Cr (mg/kg), in three fodder crops from five ecological zones in the peri-urban areas of Sahiwal town during summer and winter seasons.

Plants	Zone-1	Zone-2	Zone-3	Zone-4	Zone-5
Forage crop sample collection in summer					
<i>Zea mays</i>	0.70 ± 0.10	0.37 ± 0.14	0.70 ± 0.14	0.91 ± 0.22	5.90 ± 2.53
<i>Sorghum bicolor</i>	0.17 ± 0.28	0.07 ± 0.14	0.73 ± 0.22	1.04 ± 0.22	1.20 ± 0.14
<i>Trifolium alexandrinum</i>	0.26 ± 0.80	0.43 ± 0.22	0.75 ± 0.14	0.75 ± 0.14	1.08 ± 0.14
Forage crop sample collection in winter					
<i>Zea mays</i>	1.15 ± 0.00	1.19 ± 0.84	1.23 ± 0.36	1.08 ± 0.22	1.86 ± 0.14
<i>Sorghum bicolor</i>	1.10 ± 0.00	1.16 ± 0.14	1.81 ± 0.14	1.24 ± 0.14	1.93 ± 0.14
<i>Trifolium alexandrinum</i>	1.12 ± 0.00	1.12 ± 0.8	1.08 ± 0.30	1.97 ± 0.36	1.18 ± 0.46

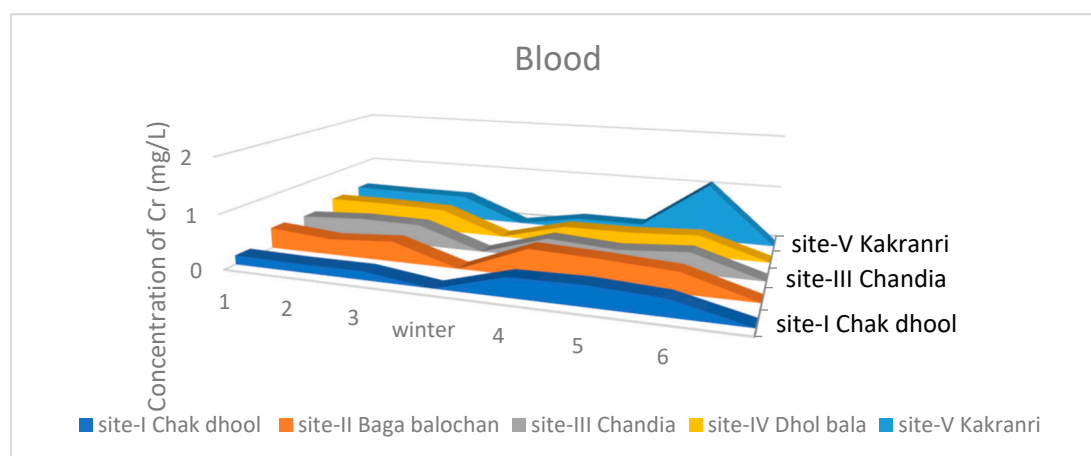


**Figure 3.** Fluctuations in mean values of Cr (mg/kg) in three fodder crops from five ecological zones in the peri-urban areas of Sahiwal town during summer and winter seasons.



### 3.4. Cr Content in Blood Samples

The maximum Cr content was found at zone-5 (0.46–0.48), and the minimum concentration was found at zone-1 (0.15–0.16), in summer (Figure 4). During the winter season, the highest level of trace metals was obtained from zone-5 (0.20–1.08), and the minimum concentration was found at zone-1 (0.29–0.34) (Table 6). The concentration of Cr was highest at zone-5 (0.46–0.48 mg/kg) from the blood samples, while it was lowest at zone-1 (0.15–0.17 mg/kg) in summer.



**Figure 4.** Fluctuations in mean concentration of Cr in blood from five ecological zones in the peri-urban areas of Sahiwal town during summer and winter seasons.

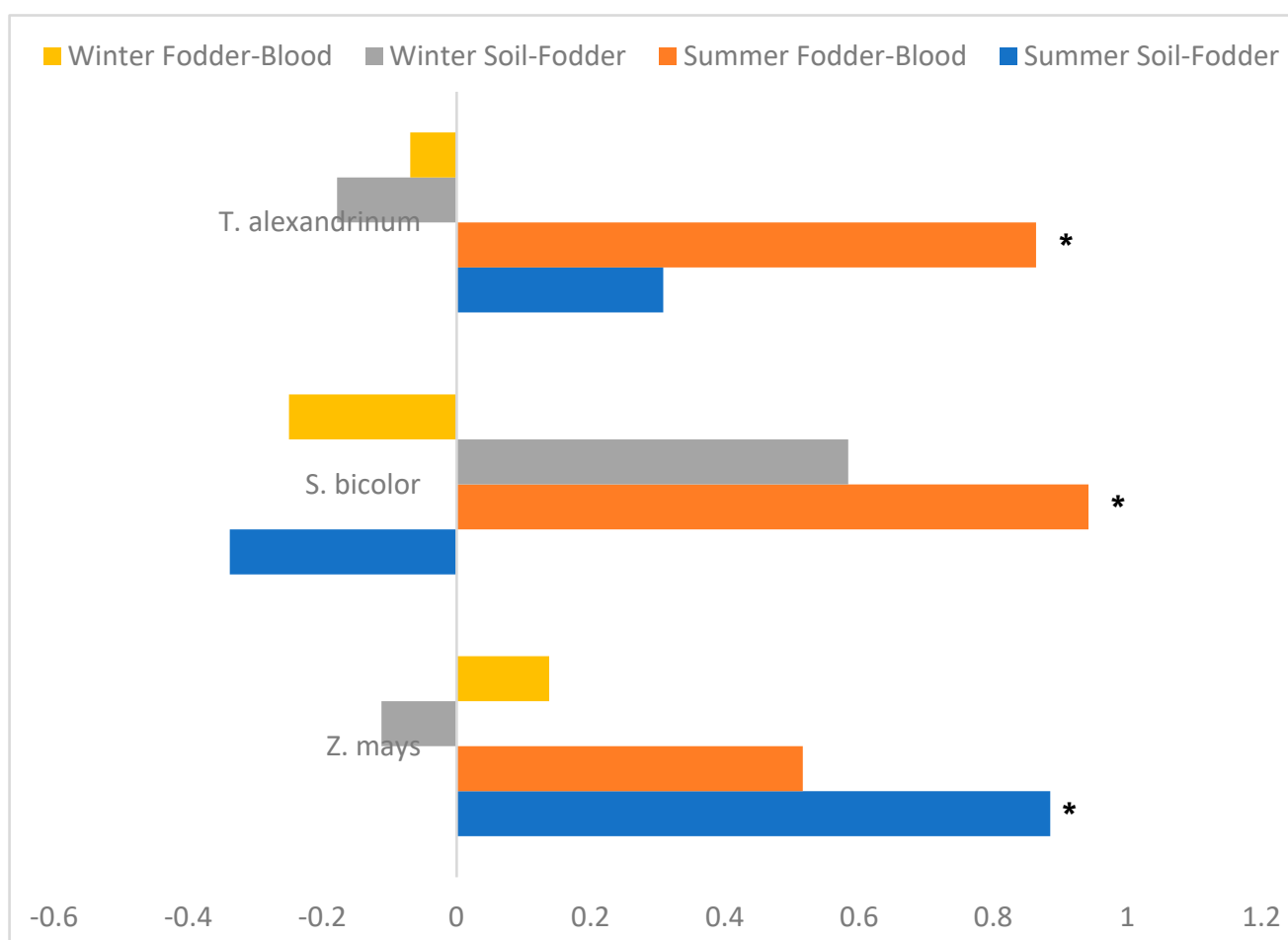
**Table 6.** Mean values of Cr in blood samples (mg/l) from Nili Ravi buffaloes from five ecological zones in the peri-urban areas of Sahiwal town during summer and winter seasons.

	Zone-1	Zone-2	Zone-3	Zone-4	Zone-5
Sample collection of blood plasma in summer					
1	0.17 ± 0.22	0.38 ± 0.14	0.34 ± 0.14	0.45 ± 0.22	0.48 ± 0.14
2	0.15 ± 0.10	0.29 ± 0.22	0.41 ± 0.10	0.45 ± 0.22	0.46 ± 0.22
3	0.15 ± 0.14	0.36 ± 0.22	0.39 ± 0.14	0.42 ± 0.20	0.46 ± 0.22
Sample collection of blood plasma in winter					
4	0.34 ± 0.14	0.49 ± 0.10	0.37 ± 0.14	0.31 ± 0.22	0.21 ± 0.14
5	0.36 ± 0.10	0.44 ± 0.14	0.29 ± 0.20	0.31 ± 0.22	0.20 ± 0.14
6	0.29 ± 0.14	0.37 ± 0.10	0.38 ± 0.14	0.38 ± 0.20	1.08 ± 0.46

### 3.5. Correlation of Cr with Forage, Soil and Agro-Ecological Zones during Two Different Seasons

In the summer, there was a positive non-significant association between blood plasma and fodders and a significant link between soil and fodder for *Z. mays*. In the winter, there was a negative non-significant correlation between soil and fodder and a positive non-significant correlation between fodders and blood plasma (Figure 5).

Significant correlation was found between soil-fodder and positive non-significant correlation between fodders-blood plasma in summer for *Z. mays*. For *S. bicolor*, a negative and non-significant association between soil-fodder and a significant relation between fodder-blood was obtained during summer. In the winter, there was a positive, non-significant correlation between soil and fodder and a negative, non-significant correlation between fodder and blood plasma. For *T. alexandrinum*, a positive non-significant association between soil and fodder and a positive-significant correlation between fodder and blood plasma were identified in the summer. In the winter, a negative and insignificant coefficient for soil-fodder and fodder-blood was seen (Figure 5).



**Figure 5.** Correlation coefficient of Cr for different fodder crops during different seasons at different zones. (\* Correlation is significant at the 0.01 level (two-tailed).

### 3.6. Determination of Pollution Load Index (PLI) for Chromium

The soil quality of a particular zone should be determined to understand its suitability for forage crop cultivation and the possible impact of heavy metal toxicity and contamination and uptake of HMs in those plants. All PLI values for heavy metals, except for Cr at zone-5, are lower than 1, indicating that the soil is not contaminated. During the summer, we obtained Cr PLI values higher than 1 during the summer, when *Z. mays* was cultivated on that soil, showing Cr uptake during the summer from zone-5. (Table 7).

**Table 7.** Pollution load index (PLI) of heavy metal Cr from five ecological zones in the peri-urban areas of Sahiwal town during summer and winter seasons.

Pollution Load Index (PLI)						
Zone	<i>Z. mays</i>		<i>S. bicolor</i>		<i>T. alexandrinum</i>	
Season	Summer	Winter	Summer	Winter	Summer	Winter
Zone-1	0.82	0.75	0.85	0.73	0.73	0.73
Zone-2	0.95	0.79	0.77	0.76	0.10	0.78
Zone-3	0.38	0.85	0.28	0.83	0.52	0.81
Zone-4	0.72	0.90	0.56	0.91	0.53	0.91
Zone-5	1.43	3.57	0.69	0.24	0.74	0.99

### 3.7. Bio-Concentration Factor

The bio-concentration factor of Cr in fodder was lower at zone-1 and higher at zone-5 (Table 8). The bio-concentration factor for cobalt at different zones was in the order zone-5 > zone-4 > zone-3 > zone-2 > zone-1 in both seasons. The bio-concentration factor determines the level of toxicity in animals. It was found in the order zone-5 > zone-4 > zone-3 > zone-2 > zone-1 in both seasons (Table 8). A higher value was observed in *T. alexandrinum* and a lower value for *Z. mays*.

**Table 8.** Bio-concentration factor (BCF) of heavy metal Cr from five ecological zones in the peri-urban areas of Sahiwal town during summer and winter seasons.

Bio-Concentration Factor (BCF)						
Zone	<i>Z. mays</i>		<i>S. bicolor</i>		<i>T. alexandrinum</i>	
Season	Summer	Winter	Summer	Winter	Summer	Winter
Zone-1	0.56	0.83	0.13	0.99	0.22	0.93
Zone-2	0.26	0.79	0.62	0.62	0.23	0.95
Zone-3	0.45	0.79	0.96	1.45	0.96	1.50
Zone-4	1.01	1.00	1.23	1.41	1.01	0.71
Zone-5	2.73	12.74	1.15	2.14	1.74	1.45

### 3.8. Daily Intake of Metal (DIM)

The daily intake of metal values for Cr were found to be lower at zone-1 and zone-2 and higher at zone-4 and zone-5. This showed that animals grazing at zone-1 and zone-2 were at lower risk than those at zone-4 and zone-5 (Table 9). The tolerable daily intake of metal for chromium is 1.05. The DIM values recorded in several zones throughout this study were much lower than the permissible limit. The order of zones was zone-5 > zone-4 > zone-3 > zone-2 > zone-1. All the values were within range. The DIM value was higher for *Z. mays* at zone-5 and lower for *T. alexandrinum* at zone-1. The order of the zones was zone-5 > zone-4 > zone-3 > zone-2 > zone-1.

**Table 9.** Daily intake of metal (DIM) of heavy metal Cr from five ecological zones in the peri-urban areas of Sahiwal town during summer and winter seasons.

Daily Intake of Metal (DIM)						
Zone	<i>Z. mays</i>		<i>S. bicolor</i>		<i>T. alexandrinum</i>	
Season	Summer	Winter	Summer	Winter	Summer	Winter
Zone-1	0.025	0.04	0.0062	0.039	0.0090	0.039
Zone-2	0.013	0.042	0.0258	0.041	0.015	0.039
Zone-3	0.025	0.06	0.026	0.064	0.026	0.065
Zone-4	0.032	0.03	0.037	0.068	0.026	0.034
Zone-5	0.210	0.044	0.043	0.044	0.038	0.042

### 3.9. Health Risk Index (HRI)

The health risk index for Cr was found to be lower, except for summer sample of *Z. mays*, at zone-5. A high value of the HRI showed that animals grazing on that fodder were at greater risk at zone-5 (Table 10). The health risk index values for Cr were lower than 1 in the present study.

**Table 10.** Health risk index (HRI) of heavy metal Cr from five ecological zones in the peri-urban areas of Sahiwal town during summer and winter seasons.

Health Risk Index (HRI)						
Zone	<i>Z. mays</i>		<i>S. bicolor</i>		<i>T. alexandrinum</i>	
Season	Summer	Winter	Summer	Winter	Summer	Winter
Zone-1	1.25	2.06	0.31	1.96	0.45	1.99
Zone-2	0.66	2.1	1.29	2.08	0.77	1.99
Zone-3	1.25	3.32	1.30	3.23	1.33	3.27
Zone-4	1.63	1.93	1.33	3.44	1.33	1.73
Zone-5	10.54	2.21	2.1	2.22	1.92	2.11

### 3.10. Enrichment Factor

The enrichment factor was higher at zone-4 and zone-5, and lower at zone-1, zone-2 and zone-3. These values indicate that zones that were highly enriched with Cr were at a higher risk (Table 11). A higher EF value indicates less retention and a lower EF value indicates high retention of metal in the soil. The values in the present investigation were mostly lower than 1 except for some samples of zone-5 and zone-4.

**Table 11.** Enrichment factor (EF) of heavy metal Cr from five ecological zones in the peri-urban areas of Sahiwal town during summer and winter seasons.

Enrichment Factor (EF)						
Zone	<i>Z. mays</i>		<i>S. bicolor</i>		<i>T. alexandrinum</i>	
Season	Summer	Winter	Summer	Winter	Summer	Winter
Zone-1	0.49	0.88	0.12	0.86	0.19	0.87
Zone-2	0.22	0.87	0.53	0.88	0.68	0.8
Zone-3	0.39	0.72	0.20	0.83	0.83	0.80
Zone-4	1.25	0.68	1.07	1.22	1.30	0.61
Zone-5	2.37	1.51	1.00	1.86	1.26	2.37

## 4. Discussion

Chromium can be lethal and harmful to fruit crops, vegetables and forages; it causes many health issues to the general public [22–24]. In the present study, the ecological zones and seasons also showed a significant impact on the bioaccumulation of Cr that varies from one to another plant (Table 5). Chromium causes physiological growth retardation, germination, early seedling growth and photosynthetic pigments. Higher Cr values of the plants at zone-1 indicate that the soil might be contaminated with a higher Cr level and forage crops had a significant concentration of Cr. Several ecophysiological attributes such as gas exchange, photosystem II photochemistry, CO<sub>2</sub> fixation, antioxidant enzymes activity, respirations, cell division and plant growth and yield attributes were highly decreased following exposure of the plants to different levels of Cr stress [25]. Transpiration, stomatal conductance and CO<sub>2</sub> uptake levels were significantly inhibited following plant exposure to chromium stress [26]. They concluded that changes in stomatal conductance were mainly due to change in the stomata size and cell morphology of the spongy parenchyma following treatment with Cr [26].

The different fodder crops showed different growth responses following Cr accumulation. In this context, *Zea mays* showed the highest Cr concentration on exposure to Cr, followed by *S. bicolor* during the summer. Our results also show that the Cr level was lower in different fodder crops during the summer and was higher during the winter season. Amongst fodder crops, *S. bicolor* performed better in Cr-stressed conditions, while

*Zea mays* was, relatively, the most sensitive one, especially during the summer season. The Cr transfer from soil to plant also depends upon plant species, genotypes and different soil attributes, such as pH, soil Cation exchange capacity and soil texture [27]. Therefore, proper strategies for the management and irrigation through municipal wastewater in Pakistan needs to be addressed. Some farmers have started to irrigate their crops with municipal wastewater in Pakistan. However, the application of wastewater in agriculture requires certain management strategies and mixing with canal water to avoid excessive accumulation of heavy metals in the soil rhizosphere [28].

Chromium mostly enters the soil–plant ecosystem via municipal and tannery wastewater irrigation for agriculture crops and, hence, poses a serious threat to the environment, public health and livestock [29]. The soil collection showed the lowest Cr accumulation at zone-4 and the highest at zone-5 from the maize field, while levels of Cr in the soil samples were highest at zone-4 in both Sorghum fields and Trifolium field (summer crop season). The highest level of Cr was obtained from zone-5 from all the three tested field crops during the winter crop season. Other researchers also reported that the highest value of Cr metal was lower than international standards [30,31]. Soil is a vital sink for Cr deposition and its level in the soil can be significantly elevated following discharge of chromium-containing liquids and solid wastes in the form of chromium by-products, ferrochrome slags or chrome plating [32]. The Cr contents in the soil samples were lower than reported by other researchers in the literature. The soil Cr was lowest during June and September, respectively [33]. At higher pH, Cr (III) forms complex structures with water and, hence, its mobility is enhanced several times. The samples obtained from *Triticum aestivum*, *Brassica juncea* and *Hordeum vulgare* demonstrate a significantly low level of Cr contents, and the soils had a Cr concentration in the range of 0.24–0.28 mg/kg following sewage water irrigation [34].

In the summer, there was a positive insignificant association between blood plasma and fodder and a significant link between soil and fodder for *Z. mays*. In the winter, a negative non-significant relationship between soil and fodder was found, and a positive non-significant association between fodder and blood plasma. For *S. bicolor*, there was a negative and non-significant association between soil and feed, but a significant correlation between feed and blood plasma. In the winter, there was a positive but insignificant association between soil and fodder and a negative but insignificant correlation between fodders and blood plasma. Similar results were documented by other researchers [35–38]. For *T. alexandrinum*, a positive non-significant association between soil and fodder and a positive non-significant correlation between fodder and blood plasma were identified in the summer. In the winter, a negative and insignificant correlation between soil and blood plasma and feed was found.

Our results clearly indicate that buffalo grazing at zone-1 and zone-2 were at a lower risk than other zones because of the lower daily intake values. However, all of the values obtained during this experiment were lower than the tolerable limit. All the values were within range, except *Z. mays* (higher) at zone-5 and *T. alexandrinum* (lower) at zone-1. The most stable forms of chromium in the environment include hexavalent chromate [Cr (VI)] and trivalent chromite [Cr (III)]. This is due to its high solubility and potential to transfer from one to another oxidation state [37]. Cr (VI) oxyanions are highly toxic and can cause cancer in humans. These Cr forms are toxic to flora and fauna in the agro-ecosystem [38]. The health risk index for Cr was lower except for the summer sample of *Z. mays* at zone-5. The high HRI value showed that animals grazing on that fodder were at greater risk at zone-5. Health risk index values for Cr were lower than 1 in the present study. The enrichment factor was higher at zone-4 and zone-5, and lower at zone-1, zone-2 and zone-3. The higher EF value indicates less retention, and a lower EF value indicates high retention of metal in soil. The values in the present investigation were mostly lower than 1, except for some samples of zone-5 and zone-4. The presence of trace metals in the soil–water environment ecosystem has shown severe threats to human health [39]. Several authors documented that trace metals could enter the food chain and can cause severe health consequences for



children and adult human beings [40]. The accumulation of heavy metals in the human body might lead to acute respiratory disorders, kidney failure, heart problems, urinary disorders and weak immunity [41].

Previously, the presence of trace metals was documented in cow's milk from various geological regions of the world [42]. However, the presence of trace metals depends upon various attributes such as breed of cattle, lactation stage, exposure pathway and animal nutrition [43]. The higher metal level detected in the cattle's milk was attributed to the presence of heavy metals in the plant–soil–water system and their entry into the animal's diet through these pathways. The presence of trace elements in the forage crops ultimately impacts the cattle's milk and meat quality [42].

The health of the local population was at risk near the Hunan Province, China, following a dietary study in association with Cr, which concluded that local inhabitants were at a high risk due to Cr exposure [24]. However, treated wastewater can be used as an alternative option for the irrigation of field crops, vegetables and fodder grasses under hyper-arid environments [43–46].

It is essential to think about the awareness of issues related to wastewater such as the present municipal wastewater disposal substructure and the quality of wastewater reuse in agriculture and health problems [47]. Similar results were documented by other researchers from the same region [48–57]. According to the first ever global survey of wastewater irrigation, untreated sewage water was used to irrigate around 10% of the crops in the world. Sewage water was used by several farmers, particularly in urban areas, because it was abundant, rich in nitrates and phosphates and was free-of-cost and available to plants even in drought conditions [53]. Municipal policy planners and makers should tackle the reality and face the challenges by using advanced methods.

## 5. Conclusions

We conclude that a significant difference was found among three forages (*Z. mays*, *S. bicolor* and *T. alexandrinum*) for Cr accumulation and pollution load indices. We found that *T. alexandrinum* seems to be better adapted to cope with Cr-induced stress than the other two forage crops. It was shown that a certain plant species' quick growth and extensive biomass production may aid phytoremediation and the exclusion of heavy metals. It was advised to exercise caution when screening and choosing a specific fodder crop cultivation on polluted soil for ruminant feeding. In fact, *S. bicolor* and *Z. mays* can be used in the phytoremediation programs because of the excessive bio-accumulation of Cr from the contaminated soils. The planting season can be taken into account for growing forage crops because, in the present research, excessive Cr was present in the plant samples during the summer season. Meanwhile, plant species, genotypes and other soil characteristics, such as pH, soil cation exchange capacity and soil texture, all play a role in how much Cr is transferred from the soil to the plant. To assess the prospects for using wastewater for land, soil and ecosystems, it is important to communicate current knowledge about the effects of various non-conventional water supplies on the ecosystem, plant biology, pollution load index and terrestrial environment contamination with all stakeholders. Therefore, it is always advised to screen the local bacterial species for Cr (VI) removal in order to reduce the risk to the ecosystem, environment, forages, ruminants and local population via the food chain.

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