BUKTI SEBAGAI REVIEWER
ARTIKEL JURNAL INTERNASIONAL BEREPUTASI

Judul artikel : Chromium poisoning in buffaloes in the vicinity of contaminated pastureland, Punjab, Pakistan

Jurnal : Environmental Science and Pollution Research (Q1, SJR 0.94)

Penulis : Ghazzal, Maria ; Hussain, Muhammad Iftikhar; Khan, Zafar Iqbal; Rahman, M. Habib ur; El-Habeeb, Abeer A.; Yang, Hsi-Hsien

No	Perihal
1	Undangan mereview
2	Persetujuan mereview
3	Submitt hasil review
4	Keputusan editor
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asep ali <asepali76@gmail.com>

ESPR: Reviewer Invitation for Chromium poisoning in buffaloes in the vicinity of contaminated pastureland, Punjab, Pakistan

Environmental Science and Pollution Research <em@editorialmanager.com> Tue, Sep 21, 2021 at 3:49 AM
Reply-To: Environmental Science and Pollution Research <carmina.cayago@springernature.com>
To: asep indra munawar ali <asepali76@gmail.com>

Dear Dr. ali,

Given your expertise in the field, I would like to ask you on behalf of Environmental Science and Pollution Research if you would be willing to review the above manuscript "Chromium poisoning in buffaloes in the vicinity of contaminated pastureland, Punjab, Pakistan" for a possible publication in our journal.

This is the abstract:
Food chain is the main channel for Cr to enter human body. Excessive accumulation of Cr can lead to a variety of diseases and threaten human health. Therefore, it is urgent to evaluate Cr poising in the soil-plant-livestock environment. The present article highlights the trace element, chromium (Cr) in the wastewater, its uptake in the soil-plant-livestock system, and its associated toxicity in food chain through consumption of meat by humans for health risk assessment. In this study, Cr poising was determined in soil, water, forages and blood of selected cattles (Nili Ravi buffalo), under trial from five different experimental sites (chak dhool, bagabalocha, Chandia, Dhool bala, Kakrani) in the vicinity of the Sahiwal town area, Sargodha (Punjab), Pakistan. Forage crops (Zea mays, Sorghum bicolor, Trifolium alaxandrium), soil and blood (30 samples each) were collected twice with the interval of 6 months, from all the experimental sites, dried and digested by using wet
digestion method. The forage and soil from the site-V and site-IV exhibited the maximum concentration of heavy metals because these areas receive highly contaminated water for irrigation. The Cr was highest among all the metals and their values are greater as compared to the permissible limits of WHO. Heavy metals tend to bioaccumulation in the food chain and can cause serious problems in humans and animals. The accumulation of Cr in collected samples could be accredited to anthropogenic activities. Pollution load index values for all samples fell in the range below 1. Health risk index indicated the probability of health damage caused by ingestion of contaminated fodder. Metals can penetrate in food chain from soil, forage to animal. The health risk index (HRI) and high value of enrichment factor was found for Cr in some sites. The Cr concentration was higher during summer season than winter. Fodder crops different in the concentration of Cr and elevated level was observed
in maize. An increase of heavy metal concentration in soil, fodder and blood owing to waste water irrigation to crops was indicated as an outcome of this investigation.

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With kind regards,

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Philippe Garrigues <em@editorialmanager.com> Tue, Sep 21, 2021 at 12:04 PM
Reply-To: Philippe Garrigues <philippe.garrigues@u-bordeaux.fr>
To: asep indra munawar ali <asepali76@gmail.com>

Dear Dr. ali,

Thank you for agreeing to review manuscript ESPR-D-21-12375, Chromium poisoning in buffaloes in the vicinity of contaminated pastureland, Punjab, Pakistan for Environmental Science and Pollution Research.

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any time. (Use the following URL: https://www.editorialmanager.com/espr/login.asp?a=r). Please contact the publication office if you have any questions.

The study titled “Chromium poisoning in buffaloes in the vicinity of contaminated pastureland, Punjab, Pakistan” has been reviewed. In my opinion, the study is an interesting one but poorly presented. The number of samples was limited, the data in tables and figures is not presented in a scientific way. Potential accumulation of Cr in animal organs such as milk, liver, meat, hair, and other deposit organs might serve better indicator for potential accumulation of the heavy metal in the animal. So, I recommend rejection.

For an improvement of the manuscript, my correction and suggestion might useful for the author.

Thank you

Environmental Science and Pollution Research

Chromium poisoning in buffaloes in the vicinity of contaminated pastureland, Punjab, Pakistan

--Manuscript Draft--

Manuscript Number:	ESPR-D-21-12375
Full Title:	Chromium poisoning in buffaloes in the vicinity of contaminated pastureland, Punjab, Pakistan
Article Type:	Research Article
Abstract:	Food chain is the main channel for Cr to enter human body. Excessive accumulation of Cr can lead to a variety of diseases and threaten human health. Therefore, it is urgent to evaluate Cr poising in the soil-plant-livestock environment. The present article highlights the trace element, chromium (Cr) in the wastewater, its uptake in the soil-plant-livestock system, and its associated toxicity in food chain through consumption of meat by humans for health risk assessment. In this study, Cr poising was determined in soil, water, forages and blood of selected cattles (Nili Ravi buffalo), under trial from five different experimental sites (chak dhool, bagabalocha, Chandia, Dhool bala, Kakrani) in the vicinity of the Sahiwal town area, Sargodha (Punjab), Pakistan. Forage crops (Zea mays, Sorghum bicolor, Trifolium alaxandrium), soil and blood (30 samples each) were collected twice with the interval of 6 months, from all the experimental sites, dried and digested by using wet digestion method. The forage and soil from the site-V and site-IV exhibited the maximum concentration of heavy metals because these areas receive highly contaminated water for irrigation. The Cr was highest among all the metals and their values are greater as compared to the permissible limits of WHO. Heavy metals tend to bioaccumulation in the food chain and can cause serious problems in humans and animals. The accumulation of Cr in collected samples could be accredited to anthropogenic activities. Pollution load index values for all samples fell in the range below 1. Health risk index indicated the probability of health damage caused by ingestion of contaminated fodder. Metals can penetrate in food chain from soil, forage to animal. The health risk index (HRI) and high value of enrichment factor was found for Cr in some sites. The Cr concentration was higher during summer season than winter. Fodder crops different in the concentration of Cr and elevated level was observed in maize. An increase of heavy metal concentration in soil, fodder and blood owing to waste water irrigation to crops was indicated as an outcome of this investigation.
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Editor

Environmental Science & Pollution Research

Dear Editor

I am submitting the a research article **"Chromium poisoning in buffaloes in the vicinity of contaminated pastureland, Punjab, Pakistan"** for your kind evaluation, suggestions for improvement, and subsequent possible publication in this prestigious journal. This is an original paper, neither published nor being considered for publication and has not been submitted to a preprint server**.**

The world is facing an increasing challenge of severe fresh water scarcity in most of the arid and semi arid regions of the world. The use of wastewater (WW) and marginal land for forage crops has been proposed as an option to augment limited freshwater resources particularly for these regions. The present study attempts to quantify the potential agricultural use of WW in the soil-plant-cattle system and to alleviate impact on forage crops production for animal feed, and its potential health risk via food consumption. The study provides new clues to understand the morphological traits, pollution load and potential heal risks associated with consuming forages by cattle's grown in the soil irrigated with sewage water.

Best regards

Iftikhar

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Chromium poisoning in buffaloes in the vicinity of contaminated pastureland, Punjab, Pakistan

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Abstract

Food chain is the main channel for Cr to enter human body. Excessive accumulation of Cr can lead to a variety of diseases and threaten human health. Therefore, it is urgent to evaluate Cr poising in the soil-plant-livestock environment. The present article highlights the trace element, chromium (Cr) in the wastewater, its uptake in the soil-plant-livestock system, and its associated toxicity in food chain through consumption of meat by humans for health risk assessment. In this study, Cr poising was determined in soil, water, forages and blood of selected cattles (Nili Ravi buffalo), under trial from five different experimental sites (chak dhool, bagabalocha, Chandia, Dhool bala, Kakrani) in the vicinity of the Sahiwal town area, Sargodha (Punjab), Pakistan. Forage crops (*Zea mays, Sorghum bicolor, Trifolium alaxandrium*), soil and blood (30 samples each) were collected twice with the interval of 6 months, from all the experimental sites, dried and digested by using wet digestion method. The forage and soil from the site-V and site-IV exhibited the maximum concentration of heavy metals because these areas receive highly contaminated water for irrigation. The Cr was highest among all the metals and their values are greater as compared to the permissible limits of WHO. Heavy metals tend to bioaccumulation in the food chain and can cause serious problems in humans and animals. The accumulation of Cr in collected samples could be accredited to anthropogenic activities. Pollution load index values for all samples fell in the range below 1. Health risk index indicated the probability of health damage caused by ingestion of contaminated fodder. Metals can penetrate in food chain from soil, forage to animal. The health risk index (HRI) and high value of enrichment factor was found for Cr in some sites. The Cr concentration was higher during summer season than winter. Fodder crops different in the concentration of Cr and elevated level was observed in maize. An increase of heavy metal concentration in soil, fodder and blood owing to waste water irrigation to crops was indicated as an outcome of this investigation.

Key words: Heavy metals; Bioaccumulation; Chromium; Health Risk Index;

Introduction

Anticipating of the global population growth will rise, therefore, universal food system will have to cope with increasing human population and improve the quality of life by 2050 (Qadir et al. 2010; Hussain and Qureshi 2020). 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 (Hussain et al., 2019). The current knowledge about the impact of different non-conventional water resources on the ecosystem, plant biology, pollution load index and terrestrial environment should be shared with all stakeholders in order to determine the prospects for using wastewater for land, soil and ecosystem.

The annual wastewater discharge in Pakistan is approx. 4.3 billion m^3, of which 1.37 billion m^3 is industrial wastewater and 3.06 billion m^3 is urban wastewater (PCRWR 2006). Around 10% of the world population consumes products made from wastewater (Qureshi et al. 2018). All major cities in Pakistan discharge their wastewater untreated into rivers and canals; this polluted water is then used to irrigate agricultural land (PCRWR 2006). About 26% of Pakistani vegetables are grown in urban slums using wastewater, and this practice will double in West Asia and North Africa due to increasing population and will double by 2050 (Hussain and Qureshi 2020). Pakistan, which had abundant water resources in 1947, has become a water scarce country and the use of wastewater in agriculture will increase in the future (CCD 2013).

Organic pollutants like petroleum aromatic hydrocarbons, explosive and pesticides have posed harmful effects on human health and microbiota. Inorganic contaminants include heavy metals like (Al, Zn, Cd, Se, Pb, Hg, As, Cr, Bi) are major cause of concern because metals could not be degraded but can be changed from one form to other through oxidation reduction reactions. ~~These introduce into soil through mining, nuclear processing and industrial manufacturing (Hussain et al. 2020).~~ Several authors described the harmful effects of heavy metals on soil biogeochemistry, plant growth and ecotoxicology via wastewater irrigation (Lu et al., 2016; Turner et al., 2016; Khan and Bano, 2016; Meng et al. et al., 2016). Toxic elements presence in the plant-soil-water ecosystem can lead to the environmental degradation, and human health risks (Cao et al. 2016; Afzal et al., 2020; Yadav, 2021).

~~Heavy metals are categorized into essential and toxic metals~~. Essential heavy metals are required for the growth of crops (Zn, Fe, Mn, Cu) and maintenance of microbial cell (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~~ (WHO, 2007). These heavy metals are originated in soil through two major processes; anthropogenic (human activities) and geogenic (weathering of rocks). ~~Anthropogenic processes include mining and smelting of ores, industrial and domestic sources. Emissions of Pb, Cd and Zn has exceeded 100-fold due to anthropogenic activities than those from natural sources. Geogenicprocess means the destruction and weathering of rocks due to different factors; climate, living organisms, parent material, time and topography. These factors caused dissemination of respective materials from parent rocks and deposited in the environment.~~

~~Chromium (Cr) is an important toxic trace element widely used in chemical and metal industry and naturally released from earth's crust as trivalent chromium [Cr(III)] and [Cr(VI)] form (Stambulska et al., 2018). The Cr (VI) is highly toxic and soluble and hence dangerous to plants, animals and humans (Nakkeeran et al., 2018)~~. There are several reports in literature whom demonstrate that Cr is an highly toxic metal that significantly impede the growth, productivity and yield of terrestrial plants, vegetables and crops (Hou et al. 2020). It was noted that even at lower concentration of 0.5–5.0 mg L^{-1} in plant nutrient solution and 5–100 mg g^{-1} in soil (Oliveira, 2012). Cr causes heavy damage to crops through chlorosis, necrosis, protein degradation and enzymes activity retardation (Damera et al. 2015). Toxic impacts of Cr accumulation in plants showed the retardation in biochemical and physiological attributes. According to reports of Davis et al. (2002), leaf water relation, nutrient uptake and photosynthetic process was highly decreased due to higher Cr accumulation in the plant organs. In bean plants, root length was decreased following exposure of plants to elevated Cr level (0, 50, 100, 150, 200, and 250 mg kg^{-1}) (Azmat and Khanum, 2005). Long term exposure of agriculture soils will led to Cr accumulation in the soil that reduces the plant growth and productivity (Wakeel and Ming, 2020).

Livestock are an important source of milk, meat, leather and manure and play an important part in the economic development of Pakistan (Chandio et al., 2016). Forage crops are an important source of minerals, nutrients and fodder for farm animals. Fodder analysis shows the presence of trace element accumulation in soil rhizosphere, water while, animal tissues (liver and kidneys, blood samples), are vital bioindices to highlight heavy metal toxicity in the food chain (Ahmad et al. 2021; Boudebbouz et al. 2020; Christophoridis et al. 2019). The presence, absorption, and translocation of heavy metals plants and feed to the ruminants can adversely impact ecological and human health concern (Ali et al. 2021;

Boudebbouz et al. 2020; Christophoridis et al. 2019). Trace metals are dangerous to ecology and terrestrial environment (Dogan et al. 2010; Ugulu 2015b).

The cities of developing countries are facing huge problems of waste-management which mainly include heaps of garbage, improper disposal of toxic waste, unchecked disposal sites etc. These unchecked disposal sites are creating the hazardous health issues in the residential areas (Kibwage 2002; Ebong et al. 2008). Treated and untreated wastewater is used for irrigating the forage crops and perennial forage grasses in urban and peri-urban areas in arid and semi-arid countries. Trace metals presents in the waste water can cause several contaminations to these forages through absorption, translocation and remaining in the different plant organs. When these forages are ingested by animals they can enter into the body of animals and in this way, they tend to bioaccumulation in the environment. The presence of metals in the rhizosphere and within the plants that are present along the roadside are transported by the channel of food chain, then they are transported to the body of animals and cause extreme toxicity in animals. These heavy metals also have leading cause different diseases in developing countries (Muchuweti et al. 2006).

The uptake of contaminated forages has caused serious threats to animal's health (Manyiwa et al. 2021; Ali et al. 2021). Approximation of daily intake of metal did not tell only about the metabolic discharge of metal but it also tells about the intake rate of metal. Hazardous effects of heavy metals occurred after many years of exposure with metal its low-level intake did not cause any serious disorder (Ahmad et al. 2021; Oluyemi et al. 2008; Orisakwe et al. 2012). ~~There are many factors which tend to increase the concentrations of heavy metals, some of which are direction of wind, precipitation, seeded strip, emission of factories and wastewater irrigation~~ (Carrero et al. 2009; Bai et al. 2010). Wastewater is contaminated with metals and metalloids such as chromium, lead, manganese, zinc, arsenic, cobalt, molybdenum, boron and copper. Heavy metals are being absorbed by the roots of grasses from the soil (Prasad et al. 2000). Further they are being transported to the stem and aerial parts of the plants. Some of these elements are non-essential and cause various toxic effects on plants, animals and humans (Kanwar and Sandha 2000). The present study aimed to assess Cr and Co concentration in soil and forages of different varieties which was irrigated with wastewater. Current work also aimed to check the heavy metals level in buffaloes consuming those forage crops. The present study was planned with the following objectives; (1) animal surveillance following pasture consumption (1) Human health risks assessment, (2) evaluation of food chain contamination, (3) determination of health risk indices, (4) ecological risk assessment through determination of Cr levels in the pasture crops (5) evaluation the fodder species from *Zea mays, Sorghum bicolor, Trifolium alaxandrium*) to screen and select more suitable pasture for ruminant feed.

The study highlights the importance of determining the availability of heavy metal, Cr, in soils, the uptake of heavy metals by the forage crops in the irrigated area, and the potential risk associated with heavy metal accumulation in diversified fodder crops and soils to ruminants (buffaloes) grazing in the area.

MATERIALS AND METHODS

Study area and experimental design

Tehsil Sahiwal of Sargodha division was selected for study. Sargodha mainly comprises of flat fertile plains but it contains some hills. Waste water of this area is used for forages irrigation on which animals are fed. The Cr-polluted soil and pastures were located in Sahiwal town area, Sargodha District, Punjab province, Pakistan. For testing and evaluation the soil, samples were collected from the polluted area. Plants samples were collected from commonly browsed forages by the buffalo at the selected sites. Aerial parts of plants were collected, as these are the parts consumed by the animals. Samples were taken from where soil samples were collected. Collection of plants was done in transparent paper bags. All plants samples were rinsed with distilled water. Cleaned samples were air dried before placing them in oven at 72ºC for drying. The desiccated samples were weighed, ground to powder and passed through 1mm sieve.

Soil sampling

Time and concentration dependent Cr accumulation in soil rhizosphere

Samples were collected in zipped lock plastic bags from five sites at each of the sites from where the plants were sampled. Stainless steel auger was used to dig soil 12 inches deep and all stratum of soil were equally included in all samples (Dey et al., 1999). Samples were dried by placing them in oven at 72ºC until the complete removal of moisture. Dried samples were stored in tagged plastic bag for analyses.

Sampling procedure

Selection of animals

~~The breed of buffalo reared in Sahiwal, Sargodha district has adapted to hot dry climate with low rainfall patterns. It has coat color completely black. The horns are of large size and curved backwards.~~ The studied buffaloes were not supplemented; they depended on naturally occurring browse plants and grass for their nutritional requirements. The selected buffaloes were identified using numbered ear tags.

Sample Collection

Soil, plant and blood samples were collected in triplicates during summer (August and September) and winter seasons (January and February) from five sites i.e. site-1 (Chak dhool), 25 km from main city site-2 (Baga balocha) 20 km, site-3 (Chandia), 27 km from sahiwal site-4 (Dhol bala) 15 km from sahiwal and site-5 (kakrani) 30 km from Sahiwal. All these sites receive wastewater irrigation.

Sampling of blood

Blood samples were collected from selected sites twice in summer as well as in winter season. Animals were given ear tags for collection from same animals. Blood samples were collected in three replicates and total fifteen samples were collected. Blood samples were taken by 10cc disposable syringe and the collected in ~~ADTA~~-K3 tubes from jugular vein. Blood samples were centrifuged for 2 minutes at 2500 rpm for separation of plasma, which was then stored at -20°C.

Digestion of samples

Wet digestion was adopted to digest the samples of soils, plants and blood. 1g sample was soaked overnight in 10 mL nitric acid. Digestion was carried out using hot plate and H_2O_2, which was added drop by drop until colorless solution was appeared. The final volume of this solution was made upto 50 mL by addition of distilled water. After filtration, the samples were stored in labeled bottles until further analysis.

Preparation of standard

S~~tandard solution was prepared by washing the glassware carefully. Funnel and flask were rinsed with distilled water~~. Sample was weighted with electrical balance and then in water. Hot plate was used for quick suspension. Sample was dissolved completely and contamination was avoided by washing the beakers again and again. Made the volume up to1000mL and distilled water was added so the lower meniscus touches the line.100 mg/L solution should be prepared, from this seven-standard solution of 2,5,10,15,20,25 were made. Then samples were examined by Atomic Absorption Spectrophotometer.

Analysis of metals:

Digested samples were subjected to metal analysis by using AAS (Atomic Absorption Spectrophotometer). The heavy metals determined was Cr.

Statistical analysis

The data was analyzed by software SPSS17. One-way Analysis of Variance was used to determine differences between metal contents in blood, soil and fodder samples.

Bioconcentration factor (BCF)

Bio concentration factor is the mean value of metal in the fodder and the mean value of metal in the soil (Cui et al., 2004):

$BCF = [M]_{fodder} / [M]_{soil}$

Where BCF is for bioconcentration factor;

$[M]_{fodder}$ is metal concentration in fodder (mg/kg)

$[M]_{soil}$ is the metal concentration (mg/kg)

Enrichment Factor (EF)

EF was calculated by using following formula (Hwaiti and Khashman, 2015).

$EF = [Mean]_{fodder} / [Mean]_{Soil} / [Mean\ reference]_{fodder\ S} / [Mean\ reference]_{soil\ S}$

Where EF stands for enrichment factor;

$[Mean]_{Fodder}$ is the metal concentration in the fodder.

$[Mean]_{Soil}$ is the metal concentration in the soil.

$[Mean\ reference]_{Fodder\ S}$ represents the reference value of metal in fodder

$[Mean\ reference]_{soil\ S}$ represents the reference value of metal in soil.

Pollution load index (PLI)

PLI was calculated by using the equation of Liu et al. (Liu et al., 2005).

PLI= concentration of metal in tested soil/concentration of metal in reference soil.

Daily intake of metal (DIM)

Daily intake of metal was calculated by using following formula Chary et al. (2008):

$DIM = C_{metal} \times D_{food\ intake} / B_{average\ weight}$

C metal content (mg/kg)

D food intake (mg/kg)

B average body weight (kg)

Health risk index (HRI)

HRI was evaluated by using daily intake of metal (DIM) and oral reference dose (R_fD) (USEPA, 2002):

Health risk index (HRI) = DIM/ R_fD

Preparation of standard

Standard solution was prepared by washing the glassware carefully. Funnel and flask were rinsed with distilled water. Sample was weighted with electrical balance and then in water. Hot plate was used for quick suspension. Sample was dissolved completely and contamination was avoided by washing the beakers again and again. Made the volume up to1000mL and distilled water was added so the lower meniscus touches the line.100 mg/L solution should be prepared, from this seven-standard solution of 2,5,10,15,20,25 were made. Then samples were examined by Atomic Absorption Spectrophotometer.

Analysis of metals:

Digested samples were subjected to metal analysis by using AAS (Atomic Absorption Spectrophotometer). The heavy metals determined were Co and Cr.

Statistical analysis

The data was analyzed by software SPSS 20. One-way Analysis of Variance was used to determine differences between metal contents in blood, soil and fodder samples.

Results

Heavy metal Chromium (Cr) analysis in wastewater, soil, fodder crops (*Zea mays, Sorghum bicolor, Trifolium alaxandrium*) plants, and ground water across 5 sites

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 5 sites), root and leaf samples from three plant species were evaluated.

Cr content in soil samples

The Cr values of soil samples collected in summer ranged from 1.23335 to 1.1025 and 1.0417 to 2.1567 at site-1 and site-5, respectively (Table 2). The Cr values of winter samples ranged from 1.1336 to 1.1050 and 1.492 to 5.361at site-1 and site-5, respectively (Table 2, 3). The higher Cr values of collected soil samples in the study area ranged from 1.23335 to 1.1025 mg/kg and 1.0417 to 2.1567 mg/kg at site-1 and site-5, respectively. In this experiment, the permissible limit of Cr in the soil are significantly lower than WHO values.

Cr content in fodder samples

In summer season, Cr content of the collected plant samples ranged from 0.700 to 0.2592 at site-1 and 1.080 to 5.905 at site-5 (Table 4). In winter season, the Cr values of the plants at site-1 were between 2.1157-1.1550 and at site-5 were between 1.239-1.1825 (Fig. 4). Chromium induce toxicity to plants it may cause reduction in pigment content, germination in seed growth, early seedling development. In the present study, higher Cr values of the plants at site-1 were between 2.1157-1.1550 mg/kg and at site-5 were between 1.239-1.1825 mg/kg (Fig. 4, Fig. 3).

Cr content in blood samples

The maximum Cr content was found at site-5 (0.4617-0.480) and the minimum concentration was found at site-1(0.150-0.1683) in summer (Fig. 4). During the winter season, the highest level of trace metals was obtained from site-5 (0.2050-1.087) and the minimum concentration was found at site-1 (0.2950-0.3425) (Table 5). The concentration of Cr was highest at site-5 (0.4617-0.480mg/kg) from the blood samples while it was lowest at site-1 (0.150-0.1683 mg/kg) in summer.

Correlation among forage, soil and toxicity indices attributes

Significant correlation was found between soil-fodder and positive non-significant correlation between fodders-blood plasma in summer for *Z. mays*. Negative and non-significant coefficient was observed between soil-fodder and positive non-significant correlation was found between fodders-blood plasma in winter (Table 6).

Negative and non-significant correlation was found between soil-fodder and significant correlation between fodders-blood plasma in summer for *S. bicolor*. Positive and non-significant coefficient was observed between soil-fodder and negative non-significant correlation was found between fodders-blood plasma in winter (Table 7).

Positive and non-significant correlation was found between soil-fodder and positive non-significant correlation was found between fodders-blood plasma in summer for *T. alaxandrium*. Negative and non-significant coefficient was observed for soil-fodder and fodder-blood plasma in winter (Table 8).

Pollution load index

Pollution load index value for Cr was found to be lower than 1 at all the sites except for site-5 which shows less contamination at all other sites than site-5 (Table 9). Pollution load index of metals was determined by ratio of concentration of metal in examined soil to the reference soil. Value of PLI> 1 indicate that metal can show harmful effects on environmental.

Monitoring of soil quality and suitability for cultivation can be determined by pollution load index. All PLI values for heavy metals except for Cr at site-5 are lower than 1 indicating that soil is not contaminated.

Bio concentration factor

Bio concentration factor of Cr in fodder was lower at site-1 and higher at site-5 (Table 10). Bioconcentration factor for cobalt was at different sites were in range site-5>site-4>site-3>site-2>site-1 in both seasons. Bioconcentration factor determines the level of toxicity in animals. It was found in the order site-5>site-4>site-3>site-2>site-1in both seasons. Higher value was observed in *T. alaxandrium* and lower for *Z. mays.*

Daily intake of metal (DIM)

Daily intake of metal values for Cr was found lower at site-1 and site-2 and higher at site-4 and site-5. This showed that animals grazing at site-1 and site-2 were at lower risk than site-4 and site-5 (Table 11). Tolerable daily intake of metal for chromium is 1.05. It was significantly smaller than tolerable limit. Order of sites were site-5>site-4>site-3>site-2>site-1. All the values were within range. DIM value was higher for *Z. mays* at site-5 ad lower for *T. alaxandrium* at site-1. Order of sites were site-5>site-4>site-3>site-2>site-1.

Health risk index (HRI)

Health risk index for Cr were found lower except for summer sample of *Z. mays* at site-5. High value of HRI showed that animals grazing at that fodder were at greater risk at site-5 (Table 12). Health risk index values for Cr were lower than 1 in the present study.

Enrichment factor

Enrichment factor were higher at site-4 and site-5, and lower at site-1, site-2 and site-3. These values indicated that sites that were highly enriched with Cr were at higher risk (Table 13). Higher value of EF indicates less retention lower EF value indicates high retention of metal in soil. The values in present investigation were mostly lower than 1 except for some samples of site-5 and site-4.

Discussion

Heavy metals are originated in soil through two major processes; anthropogenic (human activities) and geogenic (weathering of rocks). Anthropogenic processes include mining and smelting of ores, industrial and domestic sources. Emissions of Pb, Cd and Zn has exceeded 100-fold due to anthropogenic activities than those from natural sources. Mining operations are liable for the contamination of heavy metals (Navarro et al., 2008). Chromium can be lethal and harmful to fruit trees, vegetables and food grain crops; it causes many health issues to the general public (Wang et al. 2014). Therefore, it is dire need to investigate the critical toxic level for fodder (*Zea mays, Sorghum bicolor, Trifolium alaxandrium*) growth and biomass yield. There were significant differences observed in various fodder crops such as *Zea mays, Sorghum bicolor,* and *Trifolium alaxandrium* when these were exposed to different Cr contaminated wastewater. The environmental areas (sites) and season also showed significant impact on plant growth and Cr accumulation that varies from one to other plant (Table 4; Fig. 3).

Chromium cause physiological growth retardation, germination, early seedling growth and photosynthetic pigments. Higher Cr values of the plants at site-1 were between 2.1157-1.1550 mg/kg and at site-5 were between 1.239-1.1825 mg/kg. The soil contaminated with Cr was the main cause in the reduction in the plant height in sunflowers (Fozia et al. 2008). Several ecophysiological attributes such as gas exchange, photosystem II photochemistry, CO_2 fixation, antioxidant enzymes activity, respirations, cell division and plant growth and yield attributes were highly decreased following exposure of plants to Cr (Shanker et al. 2005; Anjum et al., 2016). Transpiration, stomatal conductance and CO_2 uptake levels were significantly inhibited following plants exposure to chromium stress (Schiavon et al. 2009). The changes in stomatal conductance were mainly due to change in the stomata size and cell morphology of the spongy parenchyma following treatment with Cr.

The different fodder crops showed different growth behavior and Cr accumulation. In this context, Similarly, *Zea mays* showed highest Cr concentration on exposure to Cr followed by *S. bicolor* at site 5 during summer. Except at site 5, the Cr level was lower in all fodder crops during summer and was higher during winter season. Amongst fodder crops, *S. bicolor* better executed in Cr stressed conditions while *Zea mays* relatively most sensitive one, especially during summer season. The Cr transfer from soil to plant also depends upon plant species, genotypes and different soil attributes like pH, soil Cation exchange capacity and soil texture (Zeng et al., 2011b, Santos and Rodríguez, 2012). Crops irrigated with waste water shows high yields. However, proper strategies for the management and irrigation through municipal wastewater in Pakistan have not yet been developed. Some farmers started to irrigate their crops with municipal wastewater. Application of wastewater in agriculture requires especial management (Asgari *et al*., 2007).

Chromium is mostly entered in the soil-plant ecosystem via municipal wastewater irrigation for agriculture crop and hence poses a serious threat to environment, public health and livestock via food chain contamination (Broadway et al., 2010, Ahmed et al., 2016). The soil collection showed lowest Cr accumulation at site-4 and highest at site-5 from the maize field while levels of Cr in the soil samples were highest at the site-4 and site-4 from Sorghum fields and Trifolium field (summer crop season). Highest level of Cr was obtained from site-5 from all the three tested field crops during the winter crop season). The highest values of Cr metal was lower than international standards (Chiroma et al. 2014) and USEPA (2002). Chromium levels in the soil can increase mainly due to anthropogenic deposition, such as atmospheric deposition and also through the discharge of chromium-containing liquids and solid wastes in the form of chromium by-products, ferrochrome slags or chrome plating (Guo et al. 2020; Bashir et al. 2020). In this experiment, the permissible limit of Cr in the soil are significantly lower than WHO values. The Cr contents in the soil samples were lower than reported by other researchers in the literature. The soil Cr was lowest (11.12 mg / kg and 12.05 mg / kg) during June and September, respectively (Banks et al. 2006). At higher pH, Cr (III) forms complex structures with water and hence its mobiloity is enhanced several times. The samples obtained from *Triticum aestivum*, *Brassica juncea* and *Hordeum vulgare* demonstrate 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 (Kumar and Chopra, 2015).

The maximum Cr content was found at site-5 (0.4617-0.480) and the minimum concentration was found at site-1(0.150-0.1683) in summer (Fig. 4). In winter, the maximum concentration was found at site-5 (0.2050-1.087) and the minimum concentration was found at site-1 (0.2950-0.3425) (Table 5). In blood samples, the maximum concentration of Cr was found at site-5 (0.4617-0.480mg/kg) and the minimum concentration was found at site-1 (0.150-0.1683 mg/kg) in summer.

Significant correlation was found between soil-fodder and positive non-significant correlation between fodders-blood plasma in summer for *Z. mays.* Negative and non-significant coefficient was observed between soil-fodder and positive non-significant correlation was found between fodders-blood plasma in winter (Table 6). Negative and non-significant correlation was found between soil-fodder and significant correlation between fodders-blood plasma in summer for *S. bicolor*. Positive and non-significant coefficient was observed between soil-fodder and negative non-significant correlation was found between fodders-blood plasma in winter (Table 7). Positive and non-significant correlation was found between soil-fodder and positive non-significant correlation was found between fodders-blood plasma in summer for *T. alaxandrium.* Negative and non-significant coefficient was observed for soil-fodder and fodder-blood plasma in winter (Table 8).

Pollution load index value for Cr was found to be lower than 1 at all the sites except for site-5 which shows less contamination at all other sites than site-5 (Table 9). Pollution load index of metals was determined by ratio of concentration of metal in examined soil to the reference soil. Value of PLI> 1 indicates that metal can show harmful effects on environmental. Monitoring of soil quality and suitability for cultivation can be determined by pollution load index. All PLI values for heavy metals except for Cr at site-5 are lower than 1 indicating that soil is not contaminated.

Bio concentration factor of Cr in fodder was lower at site-1 and higher at site-5 and was in the order of site-5>site-4>site-3>site-2>site-1in both seasons. Higher value was observed in *T. alaxandrium* and lower for *Z. mays.* This factor indicates the mobility and transfer of elements from the soil to the plant. Plants generally have very low TF values. In Datura innoxia, TF tends to decrease in response to an increased Cr concentration (Vernay et al. 2008). In a study by Sauerbeck (1991), Cr showed the lowest TF values among the heavy metals tested (Cd, Zn, Cu, Ni). The Cr concentrations measured in the root tissue of some plant species (spinach, oats, carrots, peas, beans, radishes) were very low despite the presence of Cr in the treated soil (Sauerbeck 1991). Heavy metals transfer in the ecosystem (from soil to plants) significantly contributes to human exposure to heavy metals (Zeng et al. 2015; Hseu and Lai 2017). In this study, the BAF values for Cr and Ni in 3 soils were <1, indicating a relatively ineffective uptake potential for Cr and Ni in rice. According to the findings of Satpathy et al. (2014), rice plants demonstrate an exclusion mechanism of Cr to avoid toxicity and any damage to the physiological machinery. The activating antioxidant defenses and immobilizing the Cr ions on cell wall are an example of Cr tolerance as documented by Zeng et al. (2014).

Our results clearly indicate that grazing of buffaloes at site-1 and site-2 were at lower risk than other sites because of lower daily intake values. However, all of the values obtained during this experiment were lower than tolerable limit. All the values were within range except *Z. mays* (higher) at site-5 and lower for *T. alaxandrium* at site-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 (Prado et al., 2016a). Cr(VI) oxyanions are highly toxic and can cause cancer to humans. These Cr forms are toxic to flora and fauna in the agro- ecosystem (Singh et al., 2013; Prado et al., 2015a). Health risk index for Cr were found lower except for summer sample of *Z. mays* at site-5. High value of HRI showed that animals grazing at that fodder were at greater risk at site-5 (Table 12). Health risk index values for Cr were lower than 1 in the present study. Enrichment factor were higher at site-4 and site-5, and lower at site-1, site-2 and site-3. These values indicated that sites that were highly enriched with were at high risk of Cr pollution (Table 13). Higher value of EF indicates less retention lower EF value indicates high retention of metal in soil. The values in present investigation were mostly lower than 1 except for some samples of site-5 and site-4. The presence of trace metals in the soil-water environment ecosystem has shown severe threats to human health (Goix et al., 2014). Several authors documented that trace metals could enter the food chain and can cause severe health consequences for children and adult human beings (Ghaedi et al., 2008, Niu et al., 2013). The heavy metals accumulation in the human body might led to acute respiratory disorders, kidney failure, heart problems, urinary disorders and weak immunity (Johannes et al., 2006, Tong et al., 2000).

Previously, it was documented the presence of trace metals in cow milk from various geological regions of the world (Elsaim and Ali, 2018; Najarnezhad and Akbarabadi, 2013; Temiz and Soylu, 2012). However, the presence of trace metals depends upon various attributes such as breed of cattle, lactation stage, exposure pathway, animal nutrition (Bousbia et al., 2019; Safaei et al., 2020). The higher metal detected in the cattle's milk was attributed to the heavy metals presence in the plant-soil-water system and their entry into the animal's diet through these pathways. Trace elements presence in the forage crops will ultimately impact the cattle's milk and meat quality (Najarnezhad and Akbarabadi, 2013).

Health exposure of local population was at risk near the Hunan Province, China following a dietary study in association with Cr and concluded that local inhabitants were at high risks due to Cr exposure (Wang et al. 2011). Hussain and Qureshi (2021) reported that treated wastewater irrigated vegetables pose a serious potential risk to consumers' health due to their high EDI and target hazard quotient (THQ).

It was essential to think about the awareness of issues related to wastewater like present municipal wastewater disposal substructure; quality of wastewater reuse in agriculture and health problems (Rutkowski *et al.,* 2006). Scott *et al.*(2004) stated that according to the first ever global survey of waste water irrigation, untreated sewage water was used to irrigate round 10% of the crops of the world. Thwas was outlawed in many countries and a largely hidden practice. Sewage water was used by several farmers, particularly in urban areas, because it was abundant, rich in nitrates, phosphates and was free of cost and available to plants even in drought conditions. Municipal policy planners and makers should tackle the reality and face the challenges by using advanced ways.

Conclusions

We concluded that, significant difference was found among three forages (*Zea mays*, *Sorghum bicolor*, and *Trifolium alaxandrium*) for Cr accumulation and pollution load indices. We found that *Trifolium alaxandrium* seems to be better adapted to cope with Cr-induced stress than other two forage crop species. It was documented that fast growth rate and abundant biomass production might help a particular plant species to exclude the heavy metals and for phytoremediation strategies. It was recommended that caution should be taken in the screening, selection of a particular forage crop grown at a contaminated soil for ruminant feeding. In fact, *Zea mays*, and *Sorghum bicolor*, can be used in the phytoremediation programs because of 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, Cr transfer from soil to plant also depends upon plant species, genotypes and different soil attributes like pH, soil cation exchange capacity and soil texture. Hence, it is always recommended to make a screening of the local bacterial species to remove Cr(VI), and to avoid risk to ecosystem, environment and local inhabitants via food chain.

Ethical Approval: Departmental Ethical Review Committee provided ethical approval to conduct study.

Consent to Participate: Informed consent was taken from formers to conduct the study and to collect the samples. They were briefed about the research plan in details.

Consent to Publish: All authors gave written consent for the publication of this paper.

Authors Contributions

Zafar Iqbal Khan and Kafeel Ahmad conceived and designed the study. Maria Ghazzal, and M. Iftikhar Hussain drafted the Ms and critically revised the manuscript. Mudasra Munir, Ifra Saleem Malik, Sonaina Nazar, Muhammad Nadeem, Mubeen Akhtar, executed the experiment and compiled data. Pervaiz Akhter, Asma Ashfaq, Shahid Mahmood, statistically analyzed the data and help in chemical analysis. Zafar Iqbal Khan, Taimoor Hassan Farooq, Jawaher Alkahtani, Mohamed Soliman Elshikh critically edited and revised the manuscript.

Maria Ghazzal helped in sample collection and chemical analysis. All authors approved the final version.

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Fig. 1. Map of study area, Sargodha (Punjab), Pakistan.

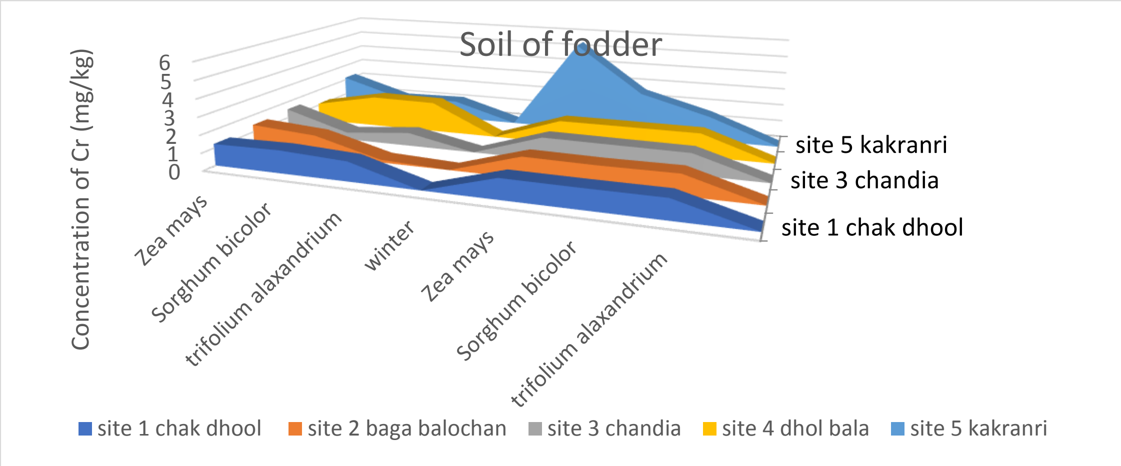

Fig. 2. Fluctuations in mean values of chromium in soil at different sites during summer and winter season

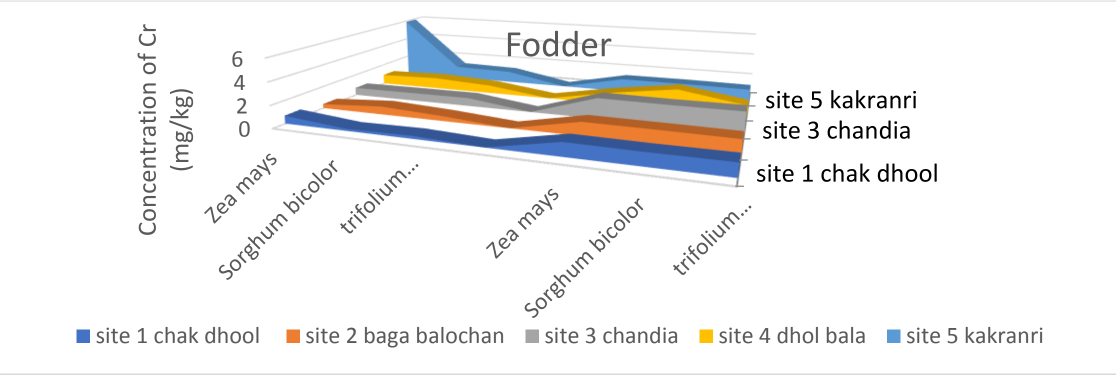

Fig. 3. Fluctuations in mean values of Cr in fodder at different sites during summer and winter season

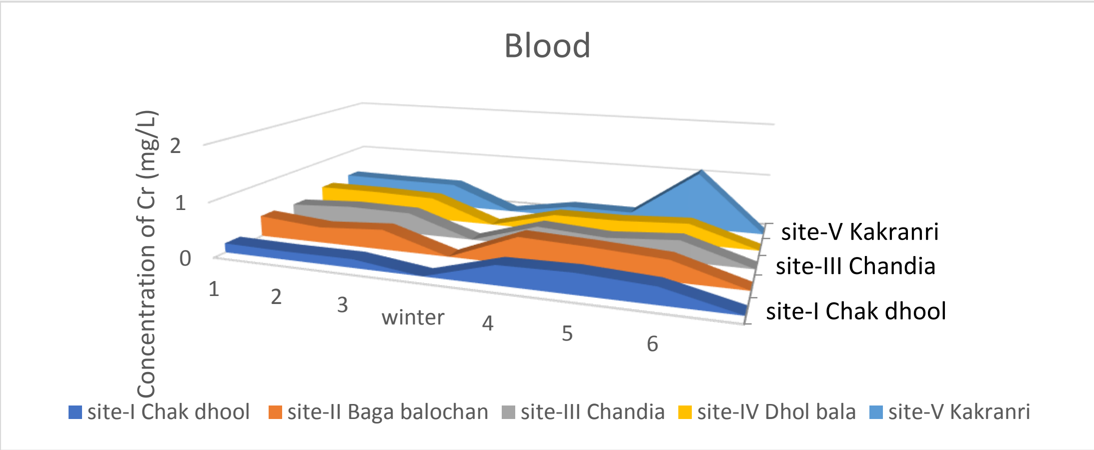

Fig. 4. Fluctuations in mean concentration of Cr in blood at different sites during summer and winter season

Table 1. Reference values of Cr 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	13,14,15
Reference in fodder	1.3			17, 18

TABLE 2. Mean values of Cr in soil samples (mg/kg)

	Site-1	**Site-2**	**Site-3**	**Site-4**	**Site-5**
Soil collected with summer fodder					
Zea mays	1.23±.0.144	1.14±0.144	1.53±0.167	1.094±0.220	2.15±0.2
Sorghum bicolor	1.28±0.144	1.165±0.10	0.420±0.14	1.842±0.29	1.041±0.22
Trifolium alaxandrium	1.102±0.289	1.15±0.22	0.180±0.3	1.803±0.36	1.115±0.22
Soil collected with winter fodder					
Zea mays	1.13±0.22	1.19±0.220	1.28±0.22	1.360±0.29	5.36±0.22
Sorghum bicolor	1.105±0.22	1.141±0.29	1.24±0.14	1.366±0.221	2.58±0.22
Trifolium alaxandrium	1.105±0.14	1.170±0.14	1.216±0.1	1.365±0.220	1.492±0.29

TABLE 3. Analysis of variance for Cr content in soil, fodder and blood samples at different sites

	Site-1	**Site-2**	**Site-3**	**Site-4**	**Site-5**
Summer Soil	3.365***	0.754***	0.053***	0.000ns	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, 0.01 and 0.001

TABLE 4. Mean values of Cr in fodder samples (mg/kg)

Plants	**Site-1**	**Site-2**	**Site-3**	**Site-4**	**Site-5**
Fodder samples collected in summer					
Zea mays	0.700±0.10	0.37±0.14	0.70±0.144	0.913±0.22	5.905±2.532
Sorghum bicolor	0.1750±0.289	0.07±0.14	0.733±0.22	1.043±0.22	1.205±0.144
Trifolium alaxandrium	0.2592±0.8	0.43±0.22	0.750±0.144	0.75±0.144	1.08±0.144
Fodder samples collected in winter					
Zea mays	1.1550±.001	1.19±0.84	1.23±0.36	1.080±0.22	1.860±0.144
Sorghum bicolor	1.1001±.0002	1.16±0.14	1.81±0.144	1.24±0.14	1.93±0.144
Trifolium alaxandrium	1.1157±.0002	1.117±0.8	1.08±0.30	1.97±0.36	1.18±0.463

TABLE 5. Mean values of Cr in blood samples (mg/l)

	Site-1	Site-2	Site-3	Site-4	Site-5
Blood samples collected in summer					
1	0.1683±0.220	0.3875±0.144	0.3450±0.144	0.4558±0.22	0.4800±0.144
2	0.1500±0.1443	0.2908±0.22	0.4100±0.144	0.4558±0.22	0.4658±0.22
3	0.1550±0.144	0.3692±0.22	0.3950±0.144	0.4258±0.22	0.4617±0.22
Blood samples collected in winter					
4	0.3425±0.144	0.4950±0.144	0.3770±0.144	0.3133±0.22	0.2050±0.144
5	0.3625±0.144	0.44500±0.144	0.2965±0.2	0.3183±0.22	0.2000±0.144
6	0.2950±0.144	0.3700±0.144	0.3825±0.144	0.3833±0.22	1.087±0.4636

TABLE 6. Correlation coefficient of Cr for *Z. mays* during different seasons at different sites

Summer		Winter	
Soil-Fodder	Fodder-Blood	Soil-Fodder	Fodder-Blood
0.885^{*}	0.516	-0.112	0.138

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

TABLE 7. Correlation coefficient of Cr for *S. bicolor* during different seasons at different sites

Summer		Winter	
Soil-Fodder	Fodder-Blood	Soil-Fodder	Fodder-Blood
-0.338	0.942^{*}	0.584	-0.250

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

TABLE 8. Correlation coefficient of Cr for *T. alaxandrium* during different seasons at different sites

Summer		Winter	
Soil-Fodder	Fodder-Blood	Soil-Fodder	Fodder-Blood
0.308	0.864	-0.178	-0.069

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

TABLE 9. Pollution load index (PLI) for Chromium

Pollution load index (PLI)						
Site	***Z. mays***		***S. bicolor***		***T. alaxandrium***	
	Summer	**Winter**	**Summer**	**Winter**	**Summer**	**Winter**
Site-1	0.82	0.75	0.85	0.73	0.735	0.73
Site-2	0.95	0.797	0.77	0.76	0.105	0.78
Site-3	0.381	0.85	0.28	0.831	0.520	0.810
Site-4	0.72	0.907	0.56	0.911	0.53	0.91
Site-5	1.43	3.57	0.69	.024	0.74	0.994

TABLE 10. Bio concentration factor (BCF) for Chromium

Bio concentration factor (BCF)						
Site	***Z. mays***		***S. bicolor***		***T. alaxandrium***	
	Summer	**Winter**	**Summer**	**Winter**	**Summer**	**Winter**
Site-1	0.56	0.83	0.13	0.99	0.22	0.93
Site-2	0.26	0.79	0.62	0.62	0.23	0.95
Site-3	0.45	0.79	0.96	1.45	0.96	1.50
Site-4	1.018	1.005	1.23	1.41	1.009	0.71
Site-5	2.73	12.74	1.15	2.14	1.74	1.451

TABLE 11. Daily intake of metal (DIM) for Chromium

Daily intake of metal (DIM)						
Site	***Z. mays***		***S. bicolor***		***T. alaxandrium***	
Season	**Summer**	**Winter**	**Summer**	**Winter**	**Summer**	**Winter**
Site-1	0.025	0.04	0.0062	0.039	0.0090	0.039
Site-2	0.013	0.042	0.0258	0.041	0.015	0.039
Site-3	0.025	0.06	0.026	0.064	0.026	0.065
Site-4	0.032	0.03	0.037	0.068	0.026	0.034
Site-5	0.210	0.044	0.043	0.044	0.038	0.042

TABLE 12. Health risk index (HRI) for Chromium

Health risk index (HRI)						
Site	***Z. mays***		***S. bicolor***		***T. alaxandrium***	
	Summer	**Winter**	**Summer**	**Winter**	**Summer**	**Winter**
Site-1	1.25	2.06	0.31	1.96	0.45	1.99
Site-2	0.66	2.1	1.29	2.08	0.77	1.99
Site-3	1.25	3.32	1.30	3.23	1.33	3.27
Site-4	1.63	1.93	1.33	3.44	1.33	1.73
Site-5	10.54	2.21	2.1	2.22	1.92	2.11

TABLE 13. Enrichment factor (EF) for Chromium

Enrichment factor (EF)						
Site	***Z. mays***		***S. bicolor***		***T. alaxandrium***	
	Summer	**Winter**	**Summer**	**Winter**	**Summer**	**Winter**
Site-1	0.49	0.88	0.117	0.86	0.198	0.87
Site-2	0.22	0.87	0.53	0.88	0.68	0.8
Site-3	0.39	0.72	0.200	0.83	0.83	0.80
Site-4	1.25	0.688	1.073	1.223	1.30	0.61
Site-5	2.37	1.512	1.0025	1.860	1.26	2.37

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ESPR: Thank you for the review of ESPR-D-21-12375

Philippe Garrigues <em@editorialmanager.com> Sat, Oct 2, 2021 at 7:21 PM
Reply-To: Philippe Garrigues <philippe.garrigues@u-bordeaux.fr>
To: asep indra munawar ali <asepali76@gmail.com>

Ref.:
Ms. No. ESPR-D-21-12375
Chromium poisoning in buffaloes in the vicinity of contaminated pastureland, Punjab, Pakistan
Environmental Science and Pollution Research

Dear Dr. ali,

Thank you for your review of this manuscript.

You can access your review comments and the decision letter (when available) by logging onto the Editorial Manager site at:

https://www.editorialmanager.com/espr/
Your username is: asepali
If you forgot your password, you can click the 'Send Login Details' link on the EM Login page

Kind regards,

Dr. Philippe Garrigues
Managing Editor
Environmental Science and Pollution Research

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ESPR: Reviewer Notification - decision made on Chromium poisoning in buffaloes in the vicinity of contaminated pastureland, Punjab, Pakistan - [EMID:3ade61bf02863b4e]

Philippe Garrigues <em@editorialmanager.com> Wed, Jan 5, 2022 at 10:12 PM
Reply-To: Philippe Garrigues <philippe.garrigues@u-bordeaux.fr>
To: asep indra munawar ali <asepali76@gmail.com>

CC: philippe.garrigues@u-bordeaux.fr, edito-ism@u-bordeaux.fr

Dear Dr. ali,

Thank you very much for your evaluation of the manuscript Chromium poisoning in buffaloes in the vicinity of contaminated pastureland, Punjab, Pakistan for Environmental Science and Pollution Research.

Based on the reviewers' suggestions a decision about this paper has been reached: Reject with possible Resubmission.

The decision letter to the author can be read from within your Editorial Manager account by using the Reviewer Login.

Yours sincerely,
Dr. Philippe Garrigues
Managing Editor
Environmental Science and Pollution Research