Assessing Health Risk in Livestock through Quantification of Iron in Forages, Soil and Buffalo Blood from Sargodha, Pakistan

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Abstract: Air pollution is very important issue now-a-days. This has affected the components of environment to a great extent. Organisms like animals, plants and human beings are forced to live in such polluted environments. One of the pollution sources to the environment is vehicular traffic which has toxic effect on the roadside plants. In this study, roadside forages, soil and buffalo blood were studied to estimate the level of heavy metal pollution, iron in particular, in Sargodha from six chosen sites at perimeter of major roads. Collected samples were processed and digested for quantification of iron by atomic absorption spectrophotometer. Soil and buffalo samples from the site V showed high concentration of iron, whereas in forage, samples collected from site IV had greatest iron concentration. The bio concentration factor found for forage-soil was highest in the samples collected from site IV. On the other hand, bio-concentration factor for blood-forage and pollution load index was found highest in the samples collected from site V. Correlation for soil-forage and blood-forage was found negatively non-significant. The daily intake of metals for iron was found highest for site IV. The Health risk index was highest for the samples collected from site VI.

Keywords: forage, soil, iron, buffalo blood, bio-concentration factor

1.Introduction

Biota has been adversely affected by pollution caused by various agents in the environment. Most concerning factor causing pollution is the heavy traffic on roads. Biota has been adversely affected by pollution caused by various agents in the environment. Most concerning factor causing pollution is the heavy traffic on roads [1]. Rusting of body parts of automobiles, burning of lubricants and fuel, wear and tear of tires, removal of paint, and breakage of the batteries are the major operations of the automobiles from which heavy metals are added to the atmosphere. Cu, Fe, Pb, Zn and Cd are added to the environment via these pollution sources [2- 4]. Throughout the world the inorganic pollution caused by heavy metals has been a matter of grave concern [5- 9]. Intensity of the traffic on roads affects the extent of heavy metal pollution. Low fuel quality and fuel inefficient vehicles intensify the traffic associated pollution [10]. The elevated concentrations of heavy metals released from heavy traffic pose extremely toxic effects on the living organisms like roadside plants [11, 12]. Plant growth along with metabolic processes of plants is widely affected by heavy metal accumulation in soft plant parts [13-15]. Stomata openings are blocked by dust and heavy metals inhibiting gaseous exchange.

Heavy metals cause cellular damage, metabolic disorders, inhibit enzyme function, disturb biochemical and physiological processes and stunt shoot/root growth [16] decreasing the productivity of plants [17].

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Animal feeding areas close to the roads are mainly subjected to heavy metal contamination. This heavy metal toxicity is responsible for appearance of various diseases among animals. In the past and even now various studies have been carried out in order to find the exposure of toxic metals to the animals. Healthy diet is needed by animals so that they could remain safe from the direct hazardous effects of the toxic heavy metals on animal health and indirect effects on the human health [18]. Heavy metals after getting entry into food chain cause various disorders. Heavy metals pose dangerous effects on each and very trophic level of food chain [19]. As these heavy metals step up each trophic level, these become more and more toxic simultaneously. Some of these heavy metals are eliminated by the bodies of organisms but some heavy metals could not be eliminated. So they remain accumulating in the bodies of organism causing severe damage.

The removal of the toxic heavy metals from the bodies of the living organisms is a matter of concern [20]. For the management and monitoring of the heavy metals in the environment the technique named phyto-monitoring is gaining much importance. This technique utilizes a number of wild and cultivated plant species. In order to gain accurate knowledge about the heavy metals' existence in the environment, the selection of plant to be used as a phyto-monitor is quite important [21]. The most important species in this respect are *Rosa rugosa* [22], *Sagittaria sagittifolia* [23] and *Nerium oleander* [24].

The need of hour is that the heavy metals concentration of the road side forages, soil and the blood of animals like buffalo rearing on these forages should be analyzed. To assess the heavy metal pollution caused by roadside traffic this study was carried out. So that the remedies of eliminating heavy metals from the environment could by chalked out which is a very important and concerning issue now-a-days.

2.Materials and methods

2.1. Study area

Sargodha is an east central city of Punjab. The climate of Sargodha is very hot in summer especially in May, June and July. The summer temperature mostly reaches 50ºC in aforementioned months. River Chenab flows on the eastern side of the city while river Jhelum flows on the western and northern sides. The surrounding areas of Sargodha contain fertile and irrigated land having a variety of domestic plants and wild species.

Current study was carried out at six sites (major roads) in Sargodha city, five of them were at the road perimeters while one was away from main road. Five roadside sites (I-V) were: Mateela, Faisalabad, 50 Chak and Bhalwal road. Sixth site (VI) was the site away from the road; Dera Saudi. Sampling was done in summer and winter seasons respectively. Winter sampling was done in December and January 2016 and summer sampling was done in May and June 2016.

2.2. Processing of samples

After the collection of soil and forage from the six sites of Sargodha, they were subjected to air drying. The collected forages were *Trifolium alexandrinum Triticum aestivum, Pennisetum glaucum and Sorghum bicolor.* 120 samples of forages and soil were collected in two seasons while the 60 samples of buffalo blood were collected in winter and summer seasons respectively. The buffalo blood serum was separated by centrifugation for further analysis. The soil and forage samples were oven dried at 70-75ºC for 7 days after their oven drying.

2.3. Acid digestion and mineral analysis

Soil samples (1g each) were digested according to Kilburn [25] in a digestion flask with 7.5mL HCL and 2.5 mL HNO_3. Forage samples (0.5g each) were digested using 1.5mL $HClO_4$ and 5 mL HNO_3 [26]. Blood serum was digested following the protocol devised by Richards [27]. All samples (soil, forage, blood) were obtained as clear solutions and their final volume was made up to 50 mL by adding distilled water. Mineral analysis was carried out in atomic absorption spectrophotometer (AA 6300 Shimadzu Japan).

2.4.Analysis of data using statistical tools

Analysis of variance and correlation was calculated by using two-way ANOVA. Statistical program for Social Sciences (SPSS) was aiding tool to find the above parameters. The levels of probability were 0.05, 0.01 and 0.001[28].

2.5.Bio-concentration factor

Bio-concentration factor (BCF) is defined as rate of the total metal concentration in the plant to that in the soil.

$$BCF = \frac{amount\ of\ trace\ metal\ in\ edible\ part}{amount\ of\ trace\ metal\ in\ soil}$$

and similarly for blood plasma

$$BCF = \frac{metal\ concentration\ in\ blood\ plasma}{metal\ concentration\ in\ forage}$$

2.6.Daily intake of metals

The daily intake of metals (DIM) was given by Chary and his collaborators [29].

$$DIM = \frac{C_{metal} \times C_{daily\ food\ intake}}{B_{average\ weight}}$$

In this equation:

$B_{average\ weight}$ – quotes for average body weight of a buffalo of that particular area where the study conducts.

The average daily forage consumption per animal taken as 12.5 Kg and average body weight referred as 550 Kg per cattle

C_{metal} – concentrations of metals,

$C_{daily\ food\ intake}$ – daily consumption of forage per cattle per day in Kg [30]

2.7.Health risk index and pollution load index

Daily intake of metals in food crops divided by the oral reference dose (Table 1) was referred to as Health risk index (HRI)[31].

Table 1. Oral reference dose and reference values of iron in soil

Metal	R_fD (mg/kg/day)	Reference value ($\mu g\ g^{-}$)
Fe	0.7[a]	56.90[b]

[a][31] [b][52]; [53]; [54]

$$HRI = \frac{DIM}{R_f D}$$

Pollution load index (PLI) was given by Liu and his collaborators [32].

$$PLI = \frac{C_{Soil}}{C_{reference\ values}}$$

where; C (reference) = metal concentration taken as reference (Table 1) and C_{soil} = metal concentration in soil.

3.Results and discussions

3.1.Concentration of metals in soil

The results from analysis of variance of the soil data showed non-significant effect ($P > 0.05$) of sites and seasons on concentration of iron in soil (Table 2). Iron concentration in soil samples in at six sampling sites was: Site V > Site VI > Site III > Site II > Site I > Site IV respectively (Table 3).

Table 2. Analysis of variance for iron in soil, forage and blood of buffaloes

Source of variation	Degree of freedom	Mean square		
		Soil	Forage	Blood
Sites	5	1.64^{ns}	0.751^{ns}	15.93^{ns}
Seasons	1	7.84^{ns}	0.055^{*}	1.261^{ns}
Sites*Seasons	5	3.411^{ns}	3.181^{ns}	0.681^{ns}
Error	36	1.393^{ns}	1.229^{ns}	1.742^{ns}

Table 3. Mean concentration of iron in soil, forage and buffalo blood

Sites	Soil	Forage	Buffalo blood
Site I	2.339	2.039	2.109
Site II	2.893	2.134	2.41
Site III	3.156	1.928	3.84
Site IV	2.170	2.624	3.838
Site V	3.337	1.888	5.465
Site VI	3.280	2.184	1.737

Nazir and his team [33] and Ravi and Singh [34] studied the heavy metals in forages and the concentration of Fe was found lower as compared to current study. Lesser the traffic on the roads lesser is the iron at the site from where samples were collected. Heavy metals in soil depend on the texture of soil as arid and semi-arid soils contain elevated concentrations of iron unlike the boreal regions and humid tropic zones. Considerably low industrialization and limited anthropogenic activities in the area of study could be responsible for less accumulation of Fe in the agricultural soils [35].

3.2.Concentration of metals in forages

Sites affected non-significantly on the concentration of iron whereas, seasons had significant effect on the concentration of iron (Table 2). The concentration of Fe in the samples of forages at the six sites of sampling was: Site IV > Site II > Site VI > site I > Site III > Site V respectively (Table 3). The study done by Cerrilo and his collaborators [36] on the forages reported lower concentration of Fe in the examined samples unlike the concentrations in current study. The concentration of Fe found by Bahadur [37] was the similar to current study. The differences in the concentration of the iron or other heavy metals in the forage depend on the type of forage, extent of soil being contaminated, and physical properties of soil e.g soil pH etc. Lesser heavy traffic in the one part of the examined area may be responsible for the lower concentration of Fe [38].

3.3.Concentration of metals in blood

Analysis of variance of buffalo blood data showed that sites and season had non-significant impact on the concentration of iron (Table 2). Iron concentration in blood samples was of the following order: Site V > Site IV Site > III > Sites II > Site I > Site VI (Table 3). Higher concentration of iron was found in the study coordinated by Malhat et al. [39].

3.4.Bio-concentration factor for forage and soil

The order of bio-concentration factor for iron at soil and forage was**:** Site IV > site II > Site VI > Site III > Site V > Site I respectively (Table 4). The bio-concentration factor obtained by Opulwa [40] was

higher as compared to current study. BCF of heavy metals depends upon their retention in soil. For instance, lower bio-concentration factor means that the heavy metals do not easily get transported to the forage as they are in tight bonding with the soil. Contrary to that, the higher BCF suggests that the heavy metals are not in tight bonding with the soil and they get readily transferred to the forages [41, 42].

Table 4. Bio concentration factor and correlation of iron in soil-forage and forage-buffalo blood mediums

Sites	Soil-forage	Forage-buffalo blood
Site I	0.26	0.79
Site II	0.73	1.16
Site III	0.610	1.98
Site IV	1.209	1.46
Site V	0.56	2.89
Site VI	0.65	0.78
Correlation	-0.434	-0.218

3.5.Bio concentration factor for blood and forage

The order of Bio-concentration factor for blood-forage medium was: Site V > site III > Site IV > Site II > Site I > Site VI respectively (Table 4). According to Liu [32] the BCF if found greater than 1 suggests that the plants can potentially accumulate heavy metals. Alloway and Ayres [43] analyzed that the extent of heavy metal uptake by forages depends upon their age, edaphic factors and the climatic factors. The soil properties like pH influence the mobility of heavy metals in soil. The movement of the heavy metals was restricted by high value of pH unlike in case of low pH [44]. It may be possible that the pH of examined soil affected the bio-cocnetration factor in current study.

3.6.Correlation

The correlation for soil-forage and blood-forage was non-significantly negative (Table 4). The correlation results as reported by Rattan [45] were lower for Fe unlike the analysis done in this study. Negative non-significant correlation was due to more convoluted relationships of heavy metals which mean there is imbalance between the concentrations of iron between soil and forage.

37.Daily intake of metals

The daily intake of metal for Fe at six sites of sampling was in the order: Site IV > Site VI > Site II > site V > site III > site I (Table 5). The values of iron concentrations as reported by Lente [46] were found lower contrary to the concentration of iron found in this study. Added by Radwan and Salama [47] that if daily intake of metal does not exceed from 1, it means that there is no health risk associated with the consumption of that diet.

Table 5. DIM, HRI and PLI via consumption of forage from six different sites of Sargodha District

Sites	DIM	HRI	PLI
Site I	0.033	0.025	0.040
Site II	0.046	0.065	0.05
Site III	0.042	0.06	0.055
Site IV	0.066	0.094	0.038
Site V	0.043	0.05	0.058
Site VI	0.063	1.09	0.057

3.8.Health risk index

The health risk index for iron was of the order: Site VI>site IV>site II>Site III>Site V>site I (Table 5). Levels of Fe found by Ashfaq [48] were quite lower unlike the values found in this study. According to Sajjad [49] if the health risk index is found greater than 1 it means that a serious health risk is associated with the consumption of roadside contaminated forages. Health risk index depends on the

chemical composition and the physical characteristics of soil, type of forage being consumed and rate of consumption of forages.

3.9.Pollution load index

Pollution load index for Fe was of the order: Site V > site VI > Site III > site II > site I > site IV (Table 5). Ahmad and his collaborators [50] found lower pollution load index for Fe compared to that found in this study. According to the criteria provided by Thomilson [51] the greater pollution load index by any metal, greater is the pollution in that area and vice versa. For example, values of PLI < 1 denote perfection, PLI = 1 presents that only baseline levels of pollutant present PLI > 1 would indicate deterioration of site quality. Pollution load index basically suggests the degree of soil pollution in any area.

4.Conclusions

Soil and buffalo blood, Pollution load index value and Bio-concentration factor for blood-forage contained highest Fe in the samples collected from site V while when the samples collected from site VI were analyzed the highest values of the Fe was found in samples of forages, Health risk Index value was obtained. While the values of Daily intake of metal and bio-concentration factor for forage-soil was found highest in the samples collected from site IV.

Ethics. All the study protocols were approved by Institutional Animal Ethics Committee, University of Sargodha (ApprovalNo.25-A18 IEC UOS). All the experiments performed complied with the rules of National Research Council [55] and all methods were performed in accordance with relevant guidelines and regulations.

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