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Assessing Zinc Amassing in Forages, Buffalo Blood and Topsoil Collected from Sargodha City, Pakistan

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Abstract: *The article is focused on estimation of Zinc in the roadside forages, and blood of buffaloes feeding on these forages. This study was carried out in Sargodha during December 2015 to January 2016 (winter) and May 2016 to June 2016 (summer). Five road sites (Mateela, Faisalabad roadside, Shaheenabad roadside, Bhalwal roadside and 50 chak) were selected from sampling of forages, soil and buffalo blood sample. Heavy metal analysis of all digested samples was done with atomic absorption spectrophotometer. Analysis of variance and correlation was done with two way ANOVA. This study regarding the accumulation of zinc in forages, soil and the buffalo blood would help the authorities to exactly determine the agents which are responsible for increasing pollution in the environment.*

Keywords: *forage,tTransfer factor, environment, toxic, zinc*

1.Introduction

The increased urbanization and industrialization due to high increase in population have led to increase in the use of machines and toxic chemicals in various products in order to facilitate mankind [1-2]. These agents add up toxic compounds to the environment in the form of toxic smoke, toxic chemicals and toxic gases etc. These all dangerous compounds are enriched with the heavy metals which are defined as “A heavy metal is one density or specific gravity of 3.5-5 g/dm^3 to 4.5-7g/dm^3”. These heavy metals set entered into the environment which is air, soil and the organism living in the surroundings [3-4]. From soil, these heavy metals are absorbed by plants of all types and in our concern are the forages [5]. Great men and Scientists have been scratching their heads since long in order to find methods which could help in eradicating heavy metals from the environment [6]. Among the techniques used, the phytoremediation technique is a well-known technique in which the heavy metals are rendered harmless or are removed by using plants and their associated microbiota. It is quite interesting to note that the phytoremediation is dependent on the complex interactions between roots, metals, soil and microorganisms so it is called as site-specific technology [5].

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Animal health and plant production have been widely affected by the heavy metals as these can easily enter the food chain via soil, air or any other source. As these heavy metals are difficult to be converted into non-toxic agents in the bodies of plants and other organisms and they are not easy to remove out of the living bodies so these heavy metals get hoarded in their bodies [7]. The extent upto which the heavy metals get amassed in the animal bodies can be estimated by examining the contents of heavy metals in the bodies of plants and animals [8].

This study aims at determining of zinc concentrations in the automobile contaminated soil, forage and blood of buffalo.

2.Materials and methods

2.1. Study site

The investigation territory was Sargodha, Punjab, Pakistan. The study area has busy roads which are replete with the heavy traffic which emits toxic smoke in the environment. This study was done to analyse heavy metal concentrations in the forages and soil found on the roadside and the blood of buffalos which consumed the heavy metal contaminated forage.

2.2. Collection of samples

This study sample of buffalo blood forages and soil were collected from the six sites of Sargodha. Sampling was done in the months of December 2015 to January 2016 in winter while in the month May 2016 to June 2016 in summer.

Total 120 samples of forage were collected from which 60 samples were collected in winter seasons (Gandum and Sorghum) and 60 samples of Berseem and Millet were collected in the summer time. The sampling of soil and forage was done from each site of Sargodha, as four replicates of each forage. Six road sites (Mateela, Faisalabad roadside, Shaheenabad roadside, Bhalwal roadside, 50 chak and Dera Saudi) were selected for sampling of forages and soil sample. Buffalo blood samples were collected as 60 samples in the summer and winter seasons. 12 samples of buffalo blood was collected in 16×150 mm sealed test tubes and saved in Na-citrate vials to prevent them from clotting.

2.3. Preparation of Samples

The samples of forage and soil were air-dried after their collection followed by drying in the oven at 70-75^{o}C for 7 days. After collection, the serum was isolated by centrifugation at 3000 rpm at 15-20 min. The centrifugation of blood samples was followed by their preservation in small labelled vials at 20^{o}C. Preparation of samples includes four sub-steps which include the acid digestion of the samples (forage, soil and buffalo blood serum), dilution of the digested samples, and filtration of the samples and their heavy metal analysis using atomic absorption spectrophotometer (AA-6300 Shimadzu Japan).

2.4.Wet Digestion

Wet digestion is a method meant for the preparation of the samples free from organic matter of all types in a way that all organic matter was converted into CO_2. It was done for obtaining a clear solution of the samples of soil, forage and blood samples [9]. Soil and forage samples (each 1g) was digested by using acids ($HCLO_4$, H_2SO_4 and HNO_3). 15 mL mixture of above three acids was mixed in 1:1:5 at almost 80^{o}C until the sample solution become colourless [10]. Protocol for digestion of blood sample was given by Richards, by which the serum samples were wet digested by taking 0.5mL serum sample along with 10mL nitric acid, 5mL perchloric acid for almost 15-20 in a digestion flask. This mixture was heated until the solution become colourless.

2.5. Analysis of samples

Prior to doing heavy metal analysis by atomic absorption spectrophotometer needs to be first standardized to find precise value so for this purpose calibration solution for metal should be made.

The atomic absorption spectrophotometer (AA6300 Shimadzu Japan) was used for the heavy metal analysis of the digested samples. The standard solutions for zinc were prepared.

2.6. Statistical Analysis

Special Programme for Social Sciences (SPSS) was used to found variance and correlation analysis. Version no. 20 of the SPSS Software was used here. To find the variance among samples, two way ANOVA was used. Significance was at 0.05, 0.001 and 0.01 according to Steel and Torrie [11].

2.7. Transfer factor (TF)

The proportion of centralization of metal in scrounges is called as soil to plant transfer factor dry weight premise.

$$TF = [Metal]\ forage / [Metal]soil$$ [12]

2.8. Daily intake of metals (DIM)

$$DIM = C_{metal} \times C_{daily\ food\ intake} / B_{average\ weight}$$ [13]

C_{metal} stands for the concentration of heavy metal in forage, Cdaily food intake means the daily forage consumption, and $B_{average}$ weight means the buffalo (average weight) in kg (550kg) [14] in particular area. 12.5 kg was the daily consumption of forage by animals

2.9. Health risk index (HRI)

The extent of heavy metal threat is calculated by health risk index [15].

$$HRI = DIM / RfD$$

From the integrated risk information system, RfD values used for heavy metals in this study was for Zn-0.3 [16].

2.10. Pollution load index (PLI)

The pollution burden record was determined by dividing the convergence of metal in soil partitioned by the reference estimations of metal in soil (Table 1).

$$PLI = C_{soil} / C_{referance\ values}$$ [17]

Table 1. Reference values of zinc in soil

Metal	Reference values (µg/g)
Zinc	44.19

3.Results and discussions

3.1. Soil

According to the samples of soil collected from six sites of sampling the contamination by roadside smoke affected non-significantly on zinc concentration on sites, seasons, and sites×seasons (Table 2).

Table 2. Analysis of variance for zinc in the soil at six sites of sampling

Sources of variation	Degree of freedom	Mean squares
		Zinc
Sites	5	5.65^{ns}
Seasons	1	0.305^{ns}
Sites×seaosns	5	2.104^{ns}
Error	36	1.324

ns: non-significant

The order of the concentration of zinc in soil samples collected from six sites of Sargodha was of the order: Site-VI>Site-IV>Site-II>Site-V>Site-III>Site-I respectively (Table 3).

Site	Zinc
Site-I	0.636
Site-II	1.563
Site-III	1.074
Site-IV	2.096
Site-V	1.451
Site-VI	3.332

Table 3. Mean concentration of zinc in soil

As found in the present study, the mean values of zinc were higher (Zn-0.222mg/kg) as reported by Nazir et al. [18]. Studies done by Faridullah et al. [19] and Kannaujia and Singh [20] also showed higher concentration of zinc as compared to that found in this study. Higher levels of lesser traffic in the parts of the examined area led to the lower heavy metal concentrations during the elevated concentration, so the heavy metals were due to the more pollution caused by factories in the examined part of the study. The heavy metals concentration in the soil is affected by the type of soil in which they are present. For example, the soil of temperate and boreal regions along with humid regions contains a lesser concentration of heavy metals unlike the Arid and Semi-arid soils. Jimenez et al. [21] found that Zn and Cu concentrations increase in the soil due to the increased $CaCO_3$ content in the soil.

3.2. Forage

Variance affected non-significantly to the zinc concentrations on sites, seasons, sites×seaosns, respectively (Table 4). The order of the concentration of zinc in forage at six sites of sampling in Sargodha was in the order: Site-IV>Site-III>Site-II>Site-VI>Site-V>Site-I respectively (Table 5). The heavy metal levels studied by Moreki et al. [22] showed a higher concentration of Cd, Cr, Cu and zinc as compared to that found in this study. The concentration of zinc and Ni was found lowest as found by Bahadur et al. [23]. The reasons behind the minor concentration of heavy metals in forages were due to lower automobile exhaust in Sargodha, unlike other big cities. The heavy metal concentrations in forage may differ due to soil *p*H, soil fertility and many such soil properties [24].

Table 4. Analysis of variance for zinc in forage at six sites of sampling

Sources of variation	Degree of freedom	Mean squares
		Zinc
Sites	5	1.217^{ns}
Seasons	1	0.819^{ns}
Sites×seaosns	5	4.228^{ns}
Error	36	0.508

ns: non-significant

Site	Zinc
Site-I	0.372
Site-II	0.845
Site-III	0.954
Site-IV	1.354
Site-V	0.485
Site-VI	0.505

Table 5. Mean concentration of the zinc in forage

3.3. Blood

The smoke of traffic non-significantly affected zinc on sites, seasons and sites×seasons (Table 6). The order of concentration of zinc in the samples of buffalo blood from the six areas of sampling was in the order: Site-IV>Site-VI>Site-II>Site-III>Site-I>Site-V respectively (Table 7). The trend of heavy metals in blood studied by Aslam et al. [25] was a bit different from that found in this study. The concentrations of heavy metals such as heavy metals retained in the sensitive organs of the body like liver and kidneys which further lead to poisoning by them [26]. Management of metallurgical waste and roadside smoke should be appropriately managed in order to be safe from their toxic effects

Table 6. Analysis of variance for zinc in buffalo blood at six localsof sampling

Sources of variation	Degree of freedom	Mean squares
		Zinc
Sites	5	3.94^{ns}
Seasons	1	43.47^{ns}
Sites×seaosns	5	5.583^{ns}
Error	36	5.583

ns: non-significant

Site	Zinc
Site-I	2.054
Site-II	2.623
Site-III	2.498
Site-IV	3.9000
Site-V	2.032
Site-VI	3.049

Table 7. Mean concentration of zinc in buffalo blood

3.4. Transfer factor for soil to forage

The order of transfer factor of zinc from soil to forage at six sites was found in following order: Site-III>Site-VI>Site-V>Site-IV>Site-I>Site-II respectively (Table 8).The dirt properties, like its *p*H, impact the versatility of overwhelming metals in soil. The movement of the heavy metals was restricted by the high value of *p*H unlike in case of low *p*H heavy metals mobility from soil to plants [27]. It may be possible that the *p*H of examined soil affect the transfer factor of heavy metal. It was reported by Pawan et al. [28] that the ions of zinc associated metal pollution is caused by the property of zinc ions to bind with the soil particles and the also get dissolved in the water found in soil.

Site	Zinc
Site-I	0.501
Site-II	0.290
Site-III	0.88
Site-IV	0.57
Site-V	0.67
Site-VI	0.68

Table 8. Transfer factor in forage and soil obtained from six sites of Sargodha District

3.5.Transfer factor for forage to blood

The order of bioconcentration factor for zinc at the six sites of sampling from forages to blood was in the order: Site-VI>Site-V>Site-II>Site-IV>Site-III>Site-I respectively (Table 9). Kamal et al. [29] found Transfer factor, which was found more for zinc (Zn-0.256). Tshibangu et al. [30] found that zinc was highest as compared to that found in this study. The Transfer factor depends on the soil properties [12, 31]. The transfer factor if found lower than 1 means that the plants store heavy metals.

Site	Zinc
Site-I	1.49
Site-II	3.104
Site-III	2.62
Site-IV	2.882
Site-V	4.19
Site-VI	6.03

Table 9. Transfer factor in forage and blood obtained from six sites of Sargodha District

3.6. Correlation

The result showed negative non-significant correlation for zinc when soil-forage was correlated. On the other hand, the positive non-significant correlation was found for Zinc when blood-forage was correlated (Table 10). Rattan et al. [32] discovered lower zinc in searches when contrasted with that found in this investigation. The correlation for zinc found in the present study. In the current study, the soil-forage and blood-forage heavy metal concentrations were correlated separately. The trend of correlation found by Pathak et al. [33] was found lowest for correlation for zinc. The weak relationship between soil-forage leads to non-significant. Imbalance of heavy metals between soils led to negative non-significant correlation and this is a convoluted relationship. According to Fatoki [34] zinc concentrations in soil and rummage is legitimately related with the separation of the scrounges from streets the centralization of zinc increment with the expansion of searches from the street.

Table 10. Correlation of metal between soil-forage at three sites

Correlation	Soil-forage	Blood-forage
Zinc	-0.273	0.256

3.7.Daily intake of metal

The order of daily intake of metals in zinc was in the order: Site-III>Site-V>Site-VI>Site-VI>Site-IV>Site-I> Site-II respectively (Table 11). The results of the study done by Saskia et al. [35] were the lower zinc concentrations, unlike the zinc concentration found in this study. In the present outcomes, the estimations of day by day consumption of metals were lower than 1; it suggests that no risk of health is associated with the intake of such contaminated forages [36].

Site	Zinc
Site-I	0.041
Site-II	0.115
Site-III	0.094
Site-IV	0.053
Site-V	0.073
Site-VI	0.057

Table 11. Everyday consumption of metal by means of the utilization of forage from six distinct destinations of Sargodha District

3.8. Health risk index

The order of health risk for zinc at the six sites of sampling was: Site-VI>Site-III>Site-II>Site-IV>Site-I>Site-V, respectively (Table 12). Zahara et al. [37] observed the lowest zinc concentration in the forage samples when they found health risk index and it was unlike the zinc concentrations in our study. There was: Site-VI>Site-III>Site-II>Site-IV>Site-I>Site-V, respectively (Table 12). Zahara et al. [37] observed the lowest zinc concentration in the forage samples when they found health risk index and it was unlike the zinc concentrations in our study. There was no difference between the health risk index caused due to zinc in this study and in the study done by Ashfaq et al. [38]. Health risk index if greater than 1 means there is a considerable risk associated with examined diet while on the other hand health risk index lower than 1 means there is no considerable health risk [15]. Type of the soil, soil physical characteristics, rate of consumption of forages and the forage type being consumed are some of the factors which affect the possible health risk caused by contaminated forages.

Site	Zinc
Site-I	0.21
Site-II	0.313
Site-III	0.717
Site-IV	0.234
Site-V	0.19
Site-VI	0.72

Table 12. Health risk index, from six sites of Sargodha via consumption of forage

3.9. Pollution load index

The order of pollution load index due to zinc at six sites of sampling was in the order: Site-VI>Site-IV>Site-V>Site-II>Site-III>Site-I respectively (Table 13). Pollution load index was found lowest for zinc (Zn-1.528mg/kg) when studied by Ahmad et al. [39] which was unlike the value of pollution load index for zinc found in this study. The most minimal estimation of the contamination burden record proposes that there is lesser contamination in the concerned are while on the other hand, the high value of pollution load index suggests that there is more pollution in the studied area.

Site	Zinc
Site-I	0.059
Site-II	0.215
Site-III	0.36
Site-IV	0.343
Site-V	0.345
Site-VI	0.379

Table 13. Pollution load Index via consumption of forage from six sites of Sargodha

4.Conclusions

Heavy metal contamination is the major international concern in current era. Vehicular emissions also release large amount of heavy metals and these metals enter in the food chain and pose various health hazards in animals. This study conducted on the roadside affected forages, soil and buffalo blood of various areas of Sargodha city was an effort to estimate the extent upto which the zinc accumulates and is proved toxic to the living systems. Careful steps ought to be experienced so as to dispose of the dangerous impacts of the substantial metals. The need of the hour is that the vehicular smoke should be made less toxic and the fuel of the automobiles should be made with considerably less toxic agents so that the toxicity of smoke should be lessened.

Ethical Statement: All the study protocols were approved by Institutional Animal Ethics Committee, University of Sargodha (Approval No. 25-A18 IEC UOS). All the experiments performed compiled with the rules of National Research Council and all methods were performed in accordance with relevant guidelines and regulations.

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