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## Determination of contaminants levels in forage grasses, Dareta Village, Nigeria

\*<sup>1</sup>Udiba Udiba U., <sup>1</sup>Hassan Diya'uddeen B., <sup>1</sup>Mahmud Abdullahi., <sup>2</sup>Odey Michael. O.,  
<sup>1</sup>Gauje Balli, <sup>1</sup>Umar Shittu M., <sup>1</sup>Bashir Inuwa and <sup>1</sup>Babakura Alhaji M.

<sup>1</sup>Environmental Technology Division, National Research Institute for Chemical Technology, Zaria Nigeria

<sup>2</sup>Cross River State University of Technology, Calabar, Cross River State, Nigeria

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### ABSTRACT

*At present, domestic and wild animals are being exposed to various substances and energies which are foreign to the habitat in which they live. Pollutants from anthropogenic activities enter into the livestock production systems and then ultimately into the food chain. Many heavy metals accumulate in one or more of the body organs in food animals and are transmitted through food chain, causing serious public health hazard. Heavy metal pollution in Zamfara has become a serious health concern following the identification of acute mass lead poisoning crises in north-western Nigerian state, in which over 10,000 people were estimated to have been affected. Studies in the area have implicated a few other metals in the environmental media. Current levels of Cadmium, Chromium, and Copper in forage grasses grazed freely by livestock in five natural pastures in Dareta village were examined in this study. A range of 0.37 - 0.96mg/kg, 3.48 -22.75mg/kg and 1.89 - 240.30mg/kg was recorded for Cadmium, Chromium and Copper respectively. Statistical analysis revealed a positive correlation between metals under study suggesting same source is responsible for the present of these metals at the concentrations determined. Except cadmium, there was statistically significant difference in metal concentrations across the sampling stations (Anova,  $P < 0.05$ ). The implications of these findings to livestock and public health are fully discussed.*

**Key words:** Anthropogenic activities, heavy metals, forage grasses, livestock and public health hazard

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### INTRODUCTION

That our environment is under increasing pressure from human activities world over is by now beyond debate. Various anthropogenic activities such as burning of fossil fuel, mining and metallurgy, industries and transportation have led to the production and/or introduction of substances or energies into the environment resulting in deleterious effects. Pollution of the environment has significant impact on living organisms. Adverse impact of pollution on domestic and wild animals in the form of specific chemical toxicities, behavioural changes and population decline are well documented in developed countries [1]. Many surveys involving human population in industrial, mining and urban areas in developing countries have also indicated toxicities due to toxicants [1, 2, 3, 4]. Heavy metals, pesticides, effluents and other agro-chemicals are some of the major causes of environmental toxicity in livestock [1]. The wide spread heavy metals contamination in the last decades has raised public and scientific interest hence, special attention is given to them throughout the world due to their toxic effects even at very low concentrations [5]. The high levels of heavy metals found in water, soil and fodder have significant impact on livestock particularly when the given metal is in the form that is bioavailable. Animals and humans get exposed to non permissible levels

of these heavy metals mainly through ingestion of contaminated food and water. Inhalation and absorption through the skin are other important exposure routes. An important feature of heavy metals is that, the chemical form in which they are present may change during passage through the intestine or storage in animal tissue, but they are not metabolized. Heavy metal toxicity is one of the major current environment health problems and is potentially dangerous because of bio-accumulation along the food chain. In general, toxicity depends on animal species, and dose and length of their action upon the organism [1].

Heavy metals are generally present in agricultural soils at low levels. The input of heavy metals to soil from various sources may prove detrimental to plant through its uptake to toxic limit, thereby facilitating its entry into the food chain. There is possibility of biomagnification of toxins as it travels up in the food web. The transfer factor of heavy metals from soil-plant may be an indicator of the plant accumulation behaviour [5]. Forage plants under specific conditions may absorb toxic metals from soil as well as from metal deposits on the surfaces of plant parts exposed to a polluted environment. Ruminants such as goats, sheep and cattle feed on grasses which have absorbed and accumulated elements from the soil over time. A number of reports have confirmed the transference of trace metals from contaminated soil to plants and from plants to livestock [6, 7]. Regardless of all this, trace amounts of some metals like copper, cobalt, zinc, manganese and chromium are essentially required for a normal animal and plant growth [8, 9, 10]. Excessive amounts of metals in animal feed and feedstuffs are most often due to human actions. Contamination of animal feeds by toxic metals cannot be completely eliminated considering their levels of distribution in the environment, although can be minimized with the aim of reducing both direct effects on animals and indirect effects on human health. Metal content in tissues and blood of animals that live in polluted areas is usually elevated and risk of acute or chronic poisoning is increased [2]. Excessively higher levels of these metals in blood and tissues of animals suggest an exposure either from the air, soil, water or feeds or all these sources [3].

An unusually high number of deaths (400 -500), primarily among children under age 5 in several villages of Zamfara State, northern Nigeria, was reported in March 2010. The deaths were traced to massive environmental contamination from artisanal mining and processing of gold found in lead-rich ore. The grinding of the ore into fine particles resulted to extensive dispersal of lead dust in the villages concerned [11]. Investigations by a coalition of National and international organizations confirmed the report with over 10,000 people estimated to have been affected. High soil lead levels some time exceeding 600,000mg/kg were observed. An immediate remediation of the villages was carried out. Daretta village was remediated between June and July 2010. Studies after the remediation exercise have implicated a few other metals. This study was carried out to assess Cadmium, Chromium, and Copper levels in forage grasses graze freely by cow, goats and sheep in the area.

Mining is arguably the largest anthropogenic source of mineral contamination. Geochemical processes acting upon mining wastes initiates the process of transporting heavy metals from contaminated areas and redistributing them to the surrounding soils, streams, groundwater, thus endangering the health of the surrounding ecosystem and human population. The presence of some metals in almost all places facilitates the increasing possibility of exposure to man and animals [6, 12].

Humans, as the final consumers in the food chain, are thus the likely recipients of high levels of minerals from 'contaminated' food and may accumulate high concentrations of some minerals in their tissues [7]. Accumulation of the investigated heavy metals (Cd, Cr and Cu) in forage grasses could be useful indicators of possible toxic effects on livestock and the consumers. Numerous studies have linked excessive accumulation of heavy metals to development of health abnormalities which include: cardiovascular, kidney, nervous and bone diseases, impairment of reproductive function of males, spontaneous abortion, still birth, blue line on gum, mottling of teeth, low birth weight and abnormal pregnancy, gastrointestinal morbidity and language delay [2, 3, 5]. Although metals are essential nutrients, they have a variety of biochemical functions in all living organisms, and important industrial uses. Their potential toxicity to humans and animals is a source of concern. It is therefore necessary to monitor and control their levels in consumed food. The measurement of metal levels is helpful not only in ascertaining risk to human health but also in the assessment of environmental quality [13]

## MATERIALS AND METHODS

### Sampling

Native pastures are the major sources of feed for different ruminants in Daretta village. Five feeding sites or pastures where cattle, goats and sheep are grazed freely around the village were selected for the study. The pastures or

feeding sites were designated as sampling stations; 1, 2, 3, 4, and 5 respectively. Grazing animals were followed and forage samples corresponding to those consumed by the ruminants were collected from each pasture. Forage grasses were collected from three different points per sampling station, stored in polyethylene bags and transported to the environmental technology division, National Research Institute for Chemical Technology, Zaria-Nigeria for analysis.

### Sample preparation

Samples from each point in the sampling stations were cut into small pieces, air dried for 5 days in the laboratory and thoroughly mixed together. The samples were pulverized and passed through 1 mm sieve. Digestion of these samples (1g each) was carried out using 5 ml of concentrated nitric acid, according to Awofolu, [14].

### Metal analysis

Metal analysis was carried out using flame atomic absorption spectrophotometer AA-6800 (Shimadzu, Japan) at National Research Institute for Chemical Technology (NARICT), Zaria-Nigeria. The calibration curves were prepared separately for all the metals by running different concentrations of standard solutions. The instrument was set to zero by running the respective reagent blanks. Average values of three replicates were taken for each determination and were subjected to statistical analysis. The metals determined includes, Cadmium, Chromium, and Copper.

### Data analysis

Data collected were subjected to statistical tests of significance using the analysis of variance (ANOVA) to assess significant variation in the concentration levels of the heavy metals in forage grasses across the five sampling stations. Probabilities less than 0.05 ( $p < 0.05$ ) were considered statistically significant. Correlation coefficient was used to determine the association between the heavy metals in the samples at  $\alpha = 0.05$ . All statistical analyses were done by SPSS software 17.0 for windows.

### Analytical Quality Assurance

In order to check the reliability of the analytical methods employed for heavy metals determination, Lichens coded IAEA-336 was also digested and then analyzed following the same procedure.

## RESULTS AND DISCUSSION

To evaluate the accuracy and precision of our analytical procedure, a standard reference material of lichen coded IAEA-336 was analyzed in like manner to our samples. The values determined and the certified values of the three (3) elements determined were very close suggesting the reliability of the method employed (table 1).

**Table 1. Shows the results of analysis of reference material (Lichen IAEA -336) compare to the reference value**

Element (Mg/l)	Pb	Cd	Cu	Mn	Zn
A Value	5.25	0.140	4.00	55.78	29.18
R value	4.2-5.5	0.1-2.34	3.1- 4.1	56-70	37-33.8

Five feeding sites or natural pastures were selected for this study. Three sampling points were established in each pasture area. The mean levels, range and standard deviation of cadmium, chromium, and copper in forage grasses across the five sampling stations are presented in table 2. The distributions of each metal across the five sampling stations are presented in figures 1-4. The trend of the metals was as follows:  $Cu > Cr > Cd$ .

Cadmium has been labeled as a major environmental pollutant since it is easily transferred into the food chain and it is not known with any significant biological functions. Rather it produces varied harmful effects in animals and man on exposure, which may result to undesirable biochemical and physiological alterations. Plasma hormonal changes and abnormal liver function have been observed in cows that were exposed to Pb and Cd in industrial areas [15, 16]. Cadmium occurs naturally in soils as a result of the weathering of parent rocks. It has a relatively low crustal abundance. Anthropogenic sources are much more significant than natural emissions and account for its ubiquitous presence in soil [17].

Table 2. Mean  $\pm$  S.D, and Range of Cadmium, Chromium and Copper in forage grasses across the sampling stations, Dareta village, Nigeria

Element	Sampling stations	Mean $\pm$ S.D	Range
Cadmium	1	0.84 $\pm$ 0.07	0.77-0.91
	2	0.66 $\pm$ 0.20	0.47-0.88
	3	0.81 $\pm$ 0.21	0.64-0.96
	4	0.71 $\pm$ 0.07	0.65-0.78
	5	0.58 $\pm$ 0.22	0.37-0.82
Chromium	1	19.5 $\pm$ 2.29	16.25-22.75
	2	10.44 $\pm$ 1.61	8.84-12.06
	3	4.65 $\pm$ 1.19	3.48-5.86
	4	6.82 $\pm$ 2.00	4.89-8.88
	5	3.80 $\pm$ 0.38	3.56-3.98
Copper	1	218.27 $\pm$ 22.03	196.24-240.30
	2	11.12 $\pm$ 2.56	8.56-13.68
	3	11.8 $\pm$ 0.79	11.00-12.58
	4	14.40 $\pm$ 1.6	12.80-16.00
	5	2.53 $\pm$ 0.64	1.89-3.18

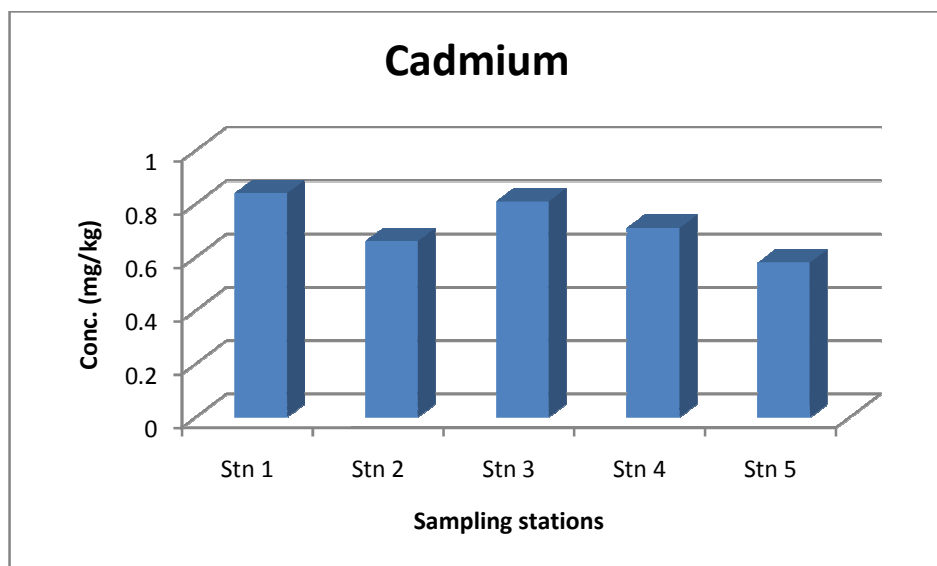


Fig 1, Distribution of Cadmium concentration in forage grasses across five sampling stations, Dareta village, Anka, Nigeria

Cadmium concentration in forage grasses ranged from 0.37mg/kg - 0.96mg/kg. The mean cadmium level across the sampling station showed the trend: station 1 > station 3 > station 4 > station 2 > station 5. The highest concentration of 0.96 mg/Kg was recorded in station 3, the lowest concentration of 0.37mg/kg at station 5. The mean values were as follows: 0.84 $\pm$ 0.07mg/kg, 0.81 $\pm$ 0.21mg/kg, 0.71 $\pm$ 0.07mg/kg, 0.66 $\pm$ 0.20mg/kg, 0.58 $\pm$ 0.22mg/kg for station 1, station 3, station 4, station 2 and station 5 respectively (table 2, figure 1). The difference in cadmium concentration in forage grasses across the sampling stations was not statistically significant (ANOVA  $P > 0.05$ ). The results of statistical analysis also reveal a positive correlation between station 1 and station 3, station 2 and station 4, station 2 and station 5, and between station 4 and station 5 suggesting same source is responsible for its presence at the concentration determined in the study. Only the correlation between station 4 and station 5 was statistically significant at 95% confidence level. The correlations between station 2 and station 4, and between station 2 and station 5 were statistically significant at 99% confidence level. A negative correlation was observed between station 1 and station 2, station 1 and station 4, station 1 and station 5, station 2 and station 3, station 3 and station 4 and between station 3 and station 5 suggesting different sources. The correlation between station 2 and station 3, station 3 and station 4 and between station 3 and station 5 was statistically significant at 95% confidence level.

The concentration of cadmium in this study was found to be within the recommended limit of 5.0- 20mg/kg for a normal plant [18]. The maximum tolerable level of cadmium in feed is 10ppm [19]. This implies that cadmium in

forage grasses does not pose any toxicological risk to livestock and humans in Dareta. A mean value of 0.94mg/kg was reported for forage grasses around lead slag contaminated sites in Ibadan, Nigeria [15]. Cadmium accumulates in the kidney and liver causing kidney dysfunction and liver failure, interferes with the metabolism of Calcium and Phosphorus, causing painful bone diseases, in addition to being a teratogenic and carcinogenic agent. Eating food or drinking water with high Cadmium concentration irritates the stomach causing vomiting and diarrhea. Chronic exposure can also cause irreversible damage to the lungs [17]

Chromium (Cr) is most commonly found in nature in two oxidation state- chromium (III) and chromium (VI). Chromium (III) is ubiquitous in nature, occurring in air, water, soil, and biological materials. It plays an important role in the metabolism of living organisms. Other function of Chromium relates to its effects on growth, lipid metabolism, immune response and interactions with nucleic acids. Increased growth rates have been recorded in various animals due to Chromium [20]. Chromium is an essential nutrient for animals but is toxic for plants even at very low concentration. Various plant parts have variable amount of Cr concentration. Legumes may contain more Cr than most other foods [21]. Intestinal absorption of trivalent Cr is low with estimates ranging < 0.522 to 3 % in fasted animals [21]. Although Chromium (III) is relatively harmless to most animal species, ingestion of a high dose of Cr (VI) has deleterious effects. Higher levels of chromium (VI) than the allowable limits are toxic to livestock and it badly affects the reproductive potential of ruminants [21]

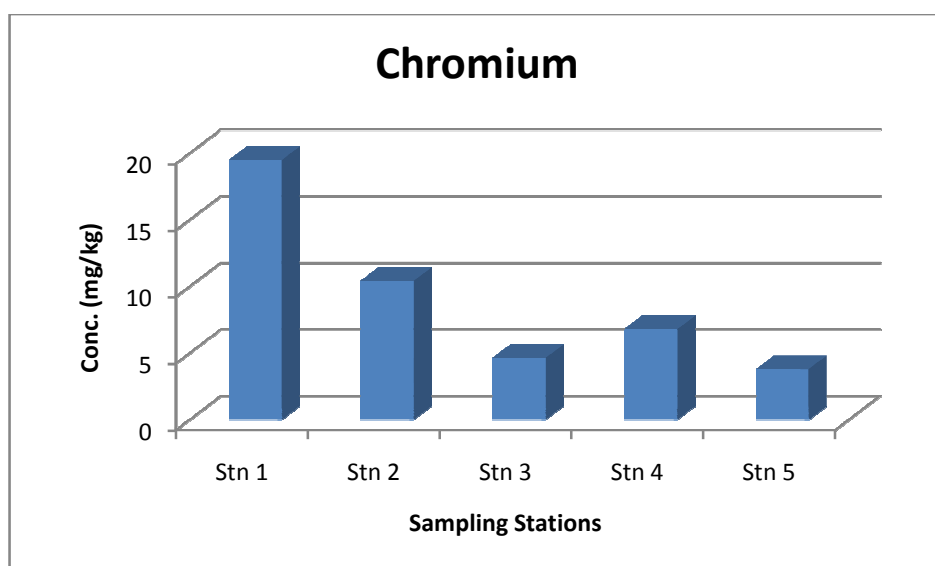


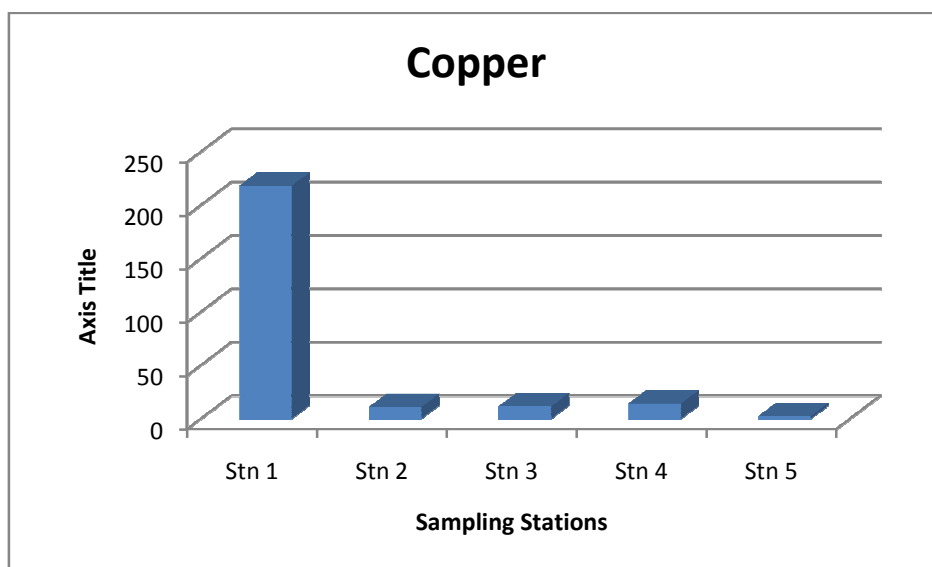
Fig 2, Distribution of Chromium concentration in forage grasses across five sampling stations, Dareta village, Anka, Nigeria

Chromium was detected in the following order across the sampling station: station 1 > station 2 > station 4 > station 3 > station 5. The concentration ranged between 3.48 and 22.75mg/kg. The highest concentration of 22.75 mg/Kg was recorded in station 1, the lowest concentration of 3.48mg/kg at station 3. The mean forage chromium levels were as follows:  $19.5 \pm 2.29$ mg/kg,  $10.44 \pm 1.61$ mg/kg,  $4.65 \pm 1.19$ mg/kg,  $6.82 \pm 2.00$ mg/kg,  $3.80 \pm 0.38$ mg/kg for station, station 2, station 3, station 4 and station 5 respectively (table 2, figure 2). The difference in chromium concentration in forage grasses across the sampling stations was statistically significant (ANOVA  $P < 0.05$ ). Station 1 was significantly higher than station 2, station 3, station 4, and station 5. Station 2 was significantly higher than station 3 and station 5. The result of statistical analysis also reveals a positive correlation between all the stations suggesting same source is responsible for the presence of chromium at the concentration determined in the study. The correlations between station 1 and station 2, and between station 2 and station 3 are statistically significant at 99% confidence level while the correlation between station 1 and station 3, station 1 and station 4, station 2 and station 4 and between station 3 and station 4 are significant at 95% confidence level.

Chromium has been considered as an essential trace element for man and laboratory animals, because it plays a variety of roles in the metabolisms of both animals and plants. The concentration of chromium in this study was several folds higher than the critical values for Chromium (0.00005-0.0005 mg/g) as suggested by Tokalioglu for a normal plant [9, 18]. The maximum chromium concentration has no threshold limit, but different reports suggested a

concentration between 0.03 to 1 mg/kg in samples [22, 23, 24]. Chromium concentration for livestock requirement ranges from 0.3 to 1.6 mg/kg. Chromium levels higher than these values are toxic to livestock and it badly affects the reproductive potential of ruminants [8]. Chromium was therefore implicated in this study. Grazing on forage grasses from these pastures may cause toxicosis. Long term exposure can cause kidney and liver damage as well as circulatory and nerve tissue problems [13]. A range of 0.156 – 0.285 mg/g was reported in the salt range Pakistan [25].

Copper is an essential trace element required for both plant growth and animal nutrition. It is required at certain concentrations for normal metabolic processes including the regulation of the activities of a variety of enzymes and in the oxidation reduction process [26, 27]. Copper could be toxic when ingested in excess and when deficient can lead to impairment of biological activities [28]. The minimum requirement for animals is slightly higher than the minimum requirement for plant growth. Sheep have a lower requirement for copper than cattle. Sheep have adequate copper at levels between 6 and 10 ppm. Cattle require about 10 ppm. However, levels of up to 20 ppm are recommended for cows during late pregnancy and for rapidly growing yearlings [29]. Copper toxicosis occurs following the ingestion and accumulation of excessive amounts of copper in the liver. All species are susceptible to developing copper toxicity. Although chronic copper poisoning (CCP) is common with many animals, it is particularly common with sheep. Feed with 10-20ppm are likely to cause death within two days. Excess copper over time results in accumulation in liver which can be released into blood stream upon stress [30, 31].



**Fig 6, Distribution of Copper concentration in forage grasses across five sampling stations, Dareta village, Anka, Nigeria**

As indicated by data in table (2), the mean levels of copper recorded was  $218.27 \pm 22.03$ ,  $11.12 \pm 2.56$ ,  $11.8 \pm 0.79$ ,  $14.40 \pm 1.6$  and  $2.53 \pm 0.64$  for station 1, station 2, station 3, station 4, and station 5 respectively. The trend of copper across the stations followed the order station 1 > station 4 > station 3 > station 2 > station 5 (Table 2, figure 3). The highest concentration of copper in forage (218.25 mg/kg) was recorded at sampling station 1 and the lowest concentration (1.89mg/kg) at sampling station 5. Statistically significant difference in copper concentration was observed across the sampling stations (Anova  $P < 0.05$ ). Station 1 was significantly higher than station 2, station 3, station 4 and station 5. A strong positive correlation was observed between all the stations indicating same source is responsible for the presence of copper at the concentration determined. The correlation between station 1 and station 2, station 1 and station 4, station 1 and station 5, station 2 and station 4, and between station 4 and station 5 were statistically significant at 99% confidence level while the correlation between station 1 and station 3, station 2 and station 3, station 3 and station 4 and between station 3 and station 5 were statistically significant at 95 % confidence level.

All the samples analyzed from the three sampling points, sampling station 1 indicated higher level than the normal value of copper in plant, agreeing to gross contamination of the sites with copper. The minimum value recorded for



station 1 was 196.24mg/kg. Grazing animals in this pasture area has serious toxicological implications both to the animal health and human especially in Nigeria as well as several other countries where meat and milk of cattle, sheep and goats are the most common sources of proteins. Most animal species absorb copper through the small intestine. Sheep, however, absorb copper through the small and large intestine. Sheep also store more copper in the liver than other species thus uniquely susceptible to copper poisoning. Common clinical signs include: fever, dark red or brown urine, diarrhea, weakness, jaundice and difficulty breathing. Concentrations of Cu in forage grasses from sampling station 2, 3, 4 and 5 are within the recommended limits of 5.0-20.0 mg/ kg for most livestock [18]. Only pasture area 5 is fit for grazing sheep. A range of 4.01 – 8.78 mg/kg was reported for forage grasses around lead slag dumpsite in Ibadan, Nigeria was reported [15].

### CONCLUSION

Heavy metals, such as cadmium (Cd), chromium (Cr) and copper (Cu) are a group of potentially toxic compounds (PTC) that are of concern when dealing with the quality of animal feedstuffs. The supply of safe feed products to animals is crucial not only to safeguard animal health and welfare but also to reduce human exposure to potentially toxic compounds. The results of the present investigation clearly depict that cadmium level was within the tolerable level of cadmium in forage across the five natural pastures. This implies that cadmium in forage grasses does not pose any toxicological risk to livestock and humans in Dareta village. Chromium and copper were seriously implicated in the study especially at sampling station 1. Except sampling station five, the pastures are not fit for grazing sheep which are uniquely susceptible to chronic copper toxicity. This study concludes that transfer of chromium and copper contaminants from forage grasses to animal products cannot be completely ruled out and recommend that studies on chromium and copper levels in livestock grazed on natural pastures in Dareta be carried out to enable the authorities concern take appropriate decisions.

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