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Toxicological implications of grazing on forage grasses in Dareta Village, Zamfara, Nigeria

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ABSTRACT

The increasing level of pollution in the environment with which some animals that forms part of the human diet are constantly in contact has been a global concern. In Nigeria as well as several other countries, meat and milk from cattle, goats and sheep are the most common sources of animal protein, the need to assess the level of contaminants in forage grazed by these animals is absolutely necessary, especially where grazing by privately owned domestic livestock is the most extensive economic use of the public lands. A field study was conducted on forage grasses in Dareta village, Nigeria, to assess the intake of heavy metals by grazing animals, following the identification of mass acute lead poisoning situation in the area. Levels of Lead, Nickel and Zinc were determined in five different natural grazing pastures around the village. The samples were analyzed using Atomic Absorption Spectrophotometer (AAS) model AA 6800 after wet digestion. The concentration of lead ranged between 5.09 - 1312.73mg/kg, nickel between 1.12 – 8.62mg/kg and zinc between 5.00 – 289.68mg/kg. The implications of these findings to public health are fully discussed.

Keywords: Contaminants forage grasses, grazing animals, public health

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INTRODUCTION

Environmental pollution is a major global problem. Industrial, agricultural, mining and natural processes have resulted to the release of many toxic substances into the environment. These substances are readily transferred through food chains. Studies on the impacts of contaminants on livestock have largely focused on animals with relatively high levels of exposure [1]. 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 toxic substances. Among environmental pollutants, heavy metals affect biological functions and are potentially dangerous, particularly due to bio-accumulation along the food chain [2]. Heavy metals can be transported, dispersed to and accumulated in plants and animals and can be passed across the food chain to humans [3, 4]. Since metals concentrations may consistently biomagnify from one trophic level to the next, animals higher in the food chain may accumulate more toxins than their food contains [5]. Whereas some metals, such as sodium chloride are excreted with ease, hence non-toxic to the body, the excretion rate of others is extremely low, resulting in the accumulation of some minerals, metals and metalloids, in biological tissue. These may eventually reach toxic levels [4, 6]. Food chain contamination is one of the major routes for entry of metals into the animal system and therefore, monitoring metals in contaminated soil, food stuff and water are of a paramount concern. 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 [4, 7].

Metal intoxication is one of the most frequent diseases in farm animals, particularly in those grazed on pasture in the vicinity of metallurgic/other industrial complexes, mining communities and busy roads. Specy-specific susceptibility to lead has been described in cattle, particularly the young ones. Grazing animals are directly affected by the consumption of forage and feed contaminated by airborne lead and somewhat indirectly by the up-take of lead or other metals through plant roots [8]. The risk of metal poisoning through the food chain increases as the soil metal level rises above the permissible concentration for a given metal. Even at soil levels above permissible limit, most of the risk is from metal contaminated soil or dust deposits on the plants rather than from uptake of metal by the plant [9, 10, 11, 12]. This implies that grazing animals are more at risk as forage grasses are consumed directly without being washed. In the evaluation of metal accumulation in cattle raised in a serpentine-soil area, it has been observed that tissue accumulation in animals was related to concentrations of the metals in soils and forage- concentrations of some heavy metals in animal tissues were correlated positively with the heavy metal content in the soil [13].

The US EPA report generalizes that a regular diet of 2-8 mg of lead per kilogram of body weight per day, over an extended period of time, will cause death in most animals [8]. Lead and Cadmium have been labeled as major environmental pollutants since they are easily transferred into the food chain and they are not known with any significant biological functions. They rather produce varied harmful effects in animals and man on exposure, which may result in undesirable biochemical and physiological alterations. Plasma hormonal changes and abnormal liver functions have been observed in cows that were exposed to Lead and Cadmium in industrial areas [14, 15]. Some essential elements such as Copper and Zinc, though necessary for life and are particularly involved in some metabolic processes at certain concentrations, could be toxic when ingested in excess. Concentration of a metal may affect the level of other metals in animal tissues; elevated levels of lead for instance, interfered with normal copper and Zinc absorption [13, 14].

In general, Plants do not take up large quantities of metals in soil; however, in soils testing high in a given metal, it is possible for some to be taken up. Heavy metal transfer from soil to plant is dependent on many factors, such as soil properties, plant species and metals bioavailability for uptake in the soil-plant system [2]. The mobility and phyto-availability of metals depend on their chemical nature. Most of the metals absorbed by plants on land are retained in their roots. There is some evidence that plant foliage may also take up lead (and it is possible that this lead is moved to other parts of the plant). Some species of plant have the capacity to accumulate high concentrations of lead. Lead is relatively unavailable to plants when the soil pH is above 6.5 [8].

Dareta village in Zamfara state is highly influenced by metal pollution sourced from mining activities. Although there have been a long history of illegal mining in Zamfara, little or no study was undertaken to assess the extent of contamination and by implication the pollution status of the environment until the Lead pollution crisis of 2010 in which over 10,000 people were estimated to have been affected [16, 17]. The source of this crisis was traced to environmental exposure to Lead from the processing of lead-rich ore mined by artisans for gold extraction. Grinding of the rocks into find particles in the mills scattered round the villages resulted to the dispersal of Lead dust (figure 1). Inhalation, accidental ingestion of contaminated soil, ingestion of contaminated water and absorption through the skin were also identified as the exposure route of this contaminant. [17, 18, 19, 20]. The joint UN Office for the Coordination of Humanitarian Affairs (OCHA) and UN Environment Programme (UNEP) Environment unit, led the investigation of lead pollution emergency in Zamfara state in mid 2010. The mission focused on determining quantities of lead in the environmental media, building on investigations already conducted by the CDC, the World Health Organization (WHO), and the National Water Resources Institute of Nigeria (NWRI), and a team from TerraGraphics Environmental Engineering/The Blacksmith Institute. High concentrations of lead were found in soil, ponds, rivers and lakes sampled. It was not in the mission's Terms of Reference to assess the risk of consuming plant and animal products from the contaminated areas. Sickness and deaths among livestock was reported in the villages, it is therefore reasonable to suspect that the consumption of such animal products (meat and milk) might also be an important exposure route for humans [, 16, 19, 21, 22]. Studies during the remediation exercise implicated a few other metals. Levels of Cadmium, Chromium, Nickel and Cobalt in human blood were found to be sufficiently high to cause health problems [23, 24]. Chromium, Nickel and magnesium were seriously implicated in hand dug wells in Dareta [17, 25]. Soil Lead and Copper levels in many areas, including residential compounds, around drinking water sources and grinding mills exceeded the acceptable limit for residential areas [18, 26]. This study is focused on the evaluation of lead, Nickel and zinc levels in forage grasses and other growing plants that are used as food for livestock in Dareta village. The implications of the findings to public health are fully discussed.

Figure 1. Artisanal miners in Dareta, Zamfara, Nigeria, use a flour mill to grind ores to liberate gold. The ores are rich in lead [20].

The anthropogenic sources of heavy metals in the soil are either as a consequence of mining, smelting and aerosol deposition, agriculture as well as industries [27]. Mining and smelting of ores have thus increased the prevalence and occurrence of toxic elements through dust emissions, mine tailing and waste water [3, 28]. Mining is a major source of contamination of land surfaces as well as surface- and groundwater. There is a significant association between the presence of heavy metals and the incidence of some human diseases [4].

MATERIALS AND METHODS

Sampling

Native pastures are the major sources of feed for different ruminants in Dareta 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, [29].

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, Lead, Nickel, and Zinc.

Data analysis
Data collected were subjected to statistical tests of significance using the one way 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).

Table1. 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

A Value = Analyzed value, R value = Reference value

The mean levels, range and standard deviation of Lead, nickel and zinc in forage grasses across the five sampling stations are presented in table 2. The distribution of metal across the five sampling stations presented in figures 1,3, and 4. The trend of the metals was as follows: Pb > Zn > Ni.

Table 2. Mean ± S.D, and Range of lead, nickel and zinc in forage grasses across the sampling stations, Dareta village, Nigeria

Element	Sampling stations	Mean ± S.D	Range
Lead	1	1222.43 ± 89.32	1143.16-1312.73
	2	31.69 ± 10.43	21.69-42.38
	3	17.31 ± 7.110	10.21-24.42
	4	10.65 ± 2.12	8.55-12.80
	5	5.59 ± 0.55	5.09-5.50
Nickel	1	8.08 ± 0.54	7.54-8.62
	2	2.40 ± 0.15	2.10-2.50
	3	6.05 ± 0.97	5.05-7.00
	4	1.88 ± 0.27	1.62-2.16
	5	1.15 ± 0.05	1.12-1.21
Zinc	1	257.33 ± 32.16	225.32-289.68
	2	103.89 ± 3,35	100.56-107.27
	3	100.50 ± 0.26	100.30-100.80
	4	103.61 ± 3.11	100.50-106.73
	5	7.45 ± 2.42	5.00-9.85

Lead was detected according to the order below; station 1 > station 2 > station 3 > station 4 > station 5. The concentration ranged from 5.59mg/kg – 1312.73.mg/kg (table 2, figure 1). Statistically significant difference in forage lead levels across the sampling stations (Anova, $P < 0.05$) was observed. Forage lead level in station 1 was significantly higher than station 2, station 3, station 4 and station 5. Stations 2, staion3, station4 and station5 did not show any statistically significant difference with each other (Anova, $P > 0.05$). The mean lead levels of 1222.43 ± 89.32, 31.69 ± 10.43, 17.31 ± 7.110 and 10.65 ± 2.12 for stations 1, station 2, station 3, and station 4 recorded were higher than the recommended limits of 0.5-10 mg/kg in normal plant [14, 30]. The Maximum tolerable level of lead in complete feed is 10 ppm [31]. The concentrations of lead recorded in the present study were several folds higher than the maximum tolerable limit, especially in the forage from station 1. A range of 209-899mg/kg was reported for forage grasses around lead slag contaminated sites, Ibadan, Nigeria [14]. Ahmad recorded 0.034 mg/g to 0.069 mg/g in the leaves and 0.040 mg/g to 0.065 mg/g in pods of different forage species in Pakistan [32]. Higher forage lead content can result in higher levels of intake by grazing animals and subsequently accumulation along the food chain. The mean Lead level at station 5 was 5.59 ± 0.55 which is within the permissible limits. With respect to lead toxicity, the findings of this study implies that, only sampling station 5 is fit for grazing and that lead in forage grasses poses serious toxicological risk as transfer from forage to grazing animals and subsequently to humans cannot be completely ruled out.

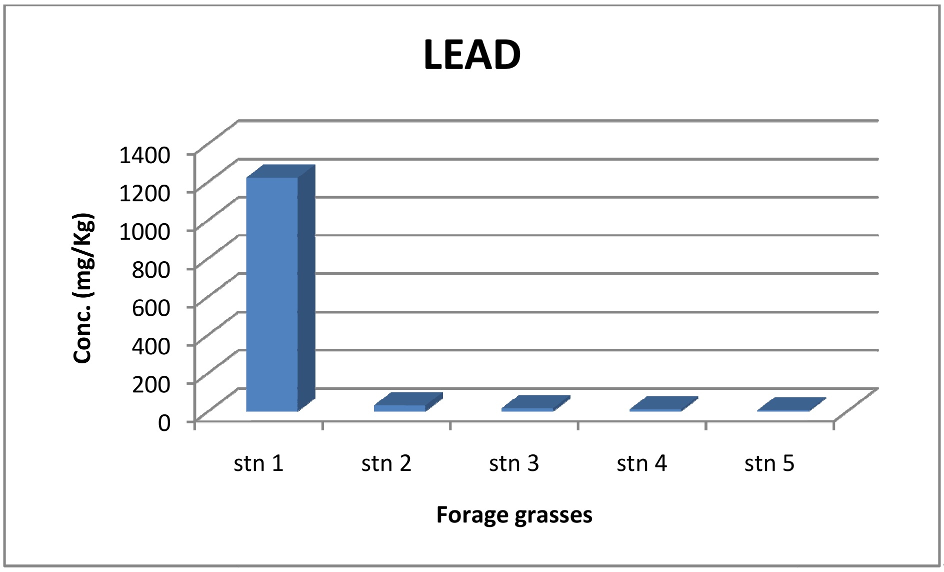

Fig 2, Distribution of Lead concentration in forage grasses across five sampling stations, Dareta village, Anka, Nigeria

The result of statistical analysis revealed positive correlation between the Lead levels in station 1 and station 2, station 3 and station4, station 3 and station 5, and between station 4 and station 5. Suggesting that same lead source is responsible for its presence at the concentrations recorded at station 1 and station 2. In the same vein, stations 3, station 4 and station 5 have same lead source. The correlations were not statistically significant at 95% confidence levels. A negative correlation was observed between station 1 and station 3, station 1 and station 4, station 1 and station 5, station 2 and station 3, station 2 and station 4, and between station 2 and station 5. Suggesting that different lead sources are responsible for the present of lead at this stations at the concentrations detected in the study. Only the correlation between station 1 and station 3 was statistically significant at 95% confidence level.

High soil lead levels between 60,000mg/kg – 100,000mg/kg was reported for Dareta village before the remediation exercise in June 2010 [22] and 81.65mg/kg - 684.27mg/kg after the remediation exercise [18]. The USEPA guideline for soil Lead levels in residential area is 400mg/kg. The risk of lead poisoning through the food chain increases as the soil lead level rises above this concentration. Even at soil levels above 400mg/kg, most of the risk is from lead contaminated soil or dust deposits on the plants rather than from uptake of lead by the plant [9, 10, 11, 12]. There is much more concern therefore, about lead contamination from external lead on unwashed produce than from actual uptake by the plant itself [11]. Forage grasses examined in this study were not washed to reflect intake by grazing animals. This could partly account for the high forage lead level especially in station 1 which is considered phytotoxic. Grazing animals are directly affected by the consumption of forage and feed contaminated by airborne lead and somewhat indirectly by the up-take of lead through plant roots. Some species of plant have the capacity to accumulate high concentrations of lead [8]. Lead levels which range from 30-300mg/ kg have been considered phytotoxic to plants. Transfer of Pb to cattle through consumption of contaminated plants was also corroborated in literature [33].

Even a small amount of lead can kill cattle and other livestock. When consumed, lead in forage settles in the stomachs of animals particularly cattle, where stomach acids gradually change the lead into poisonous salts. Lead causes anemia. It damages the blood vessels, causing bleeding, and deprives nerves, brain and other organs of oxygen. Lead severely damages kidney and liver. It also causes sterility, fetal death and abortion [34]. Consumption of lead contaminated animals constitutes serious risk to public health. There is no exposure limit below which lead is said to be safe. It induces reduced cognitive development and intellectual performance in children, increased blood pressure, and cardiovascular diseases in adult as well as liver and kidney dysfunction [18].

Nickel occurs naturally in soil as a result of the weathering of parent rock. The underlying rock and soil forming process strongly influence the amount of nickel in soil. Anthropogenic activities such as burning of oil and coal, smelting/plating works, mining and agricultural activities have resulted in wide spread atmospheric nickel [26].

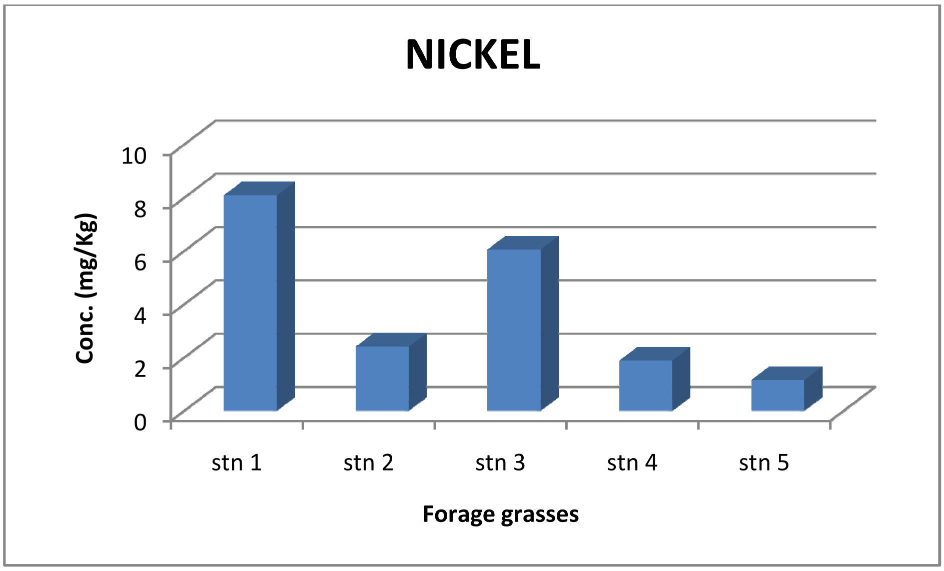

Fig 3, Distribution of Nickel concentration in forage grasses across five sampling stations, Dareta village, Anka, Nigeria

In this study, nickel was detected according to the order: station 1 > station 3 > station 2 > station 4 > station 5. The average concentrations of nickel in forage grasses across the sampling stations were 8.08 ± 0.54, 2.40 ± 0.15, 6.05 ± 0.97, 1.88 ± 0.27 and 1.15 ± 0.05 for station 1, station 2, station 3, station 4, and station 5 respectively (table 2, figure 3). The average concentrations recorded for station 1 and station 3 were found to be higher than the critical value (0.00005-0.005 mg/g) for nickel in typical plant [35]. No significant difference in forage nickel levels across the sampling station was observed (Anova, P > 0.05). EPA does not presently regulate nickel levels. Though it accumulates in aquatic life, its presence is not magnified along the food chain. Long term exposure to nickel causes decrease body weight, skin Irritation, heart and liver damage [36]. Statistical analysis revealed positive correlation in forage nickel levels between all the stations suggesting same source is responsible presence of nickel at the sampling stations at concentrations detected in the study. Only the correlation between station 1 and station 3, and station 1 and station 4 were statistically significant at 95% confidence level. A range of 0.030mg/g-0.069mg/g was reported for leaves collected from pastures in pakistan [32]. A minimum concentration of 0.041mg/kg dry weight and a maximum concentration of 66.21mg/kg dry weight were reported for Dareta soil (Udiba et al 2012b). Nickel was not implicated in the study except around grinding mills. Both the US EPA maximum permissible limits (MPL) and the Europian Union regulatory standards (EURS) for lead in soil is 50mg/mg [26].

Recorded data from the present study indicates that zinc was detected in forage grasses with an average concentration of 257.33 ± 32.16mg/kg, 103.89 ± 3.35mg/kg, 100.50 ± 0.26mg/kg, 103.61 ± 3.11mg/kg, and 7.45 ± 2.42mg/kg for station 1, station 2, station 3, station 4 and station 5 respectively (Table 2). The values recorded in this study across the sampling stations were found to be within the recommended limits of 10-150 mg/kg [30], except for station 1. Two out of three sampling points in station 1 recorded values that were almost twice the acceptable limit. Statistically significant difference in forage zinc concentration across the sampling stations was observed (Anova, $P < 0.05$). Forage zinc level in station 1 was significantly higher than station 2, station 3, station 4 and station 5 (figure 4). The result of statistical analysis also revealed positive correlation between the zinc levels in all the stations. Only the correlation between station 1 and station 2 was statistically significant at 99% confidence level. The correlation between station 1 and station 3, station 1 and station 4, station 2 and station 3, and between station 2 and station 4 were statistically significant at 95% confidence level.

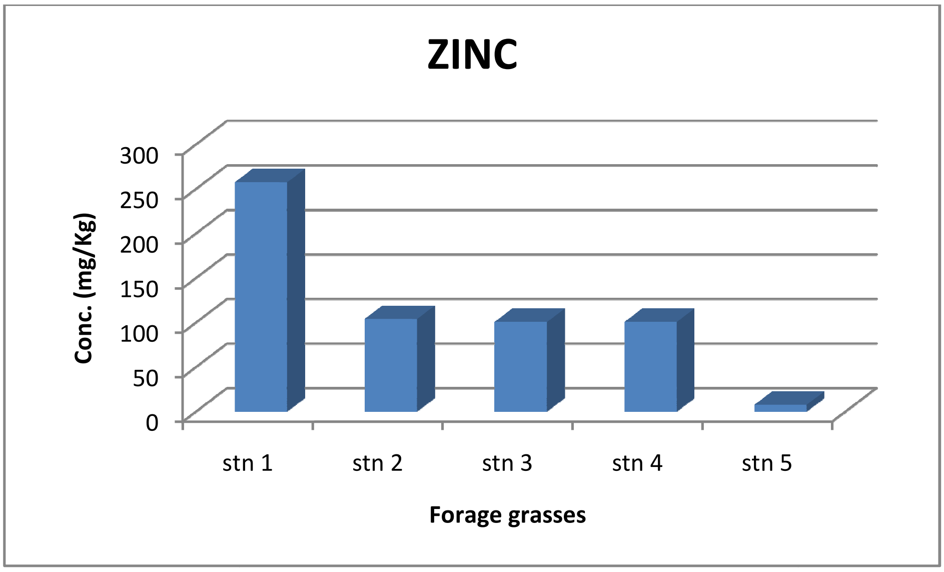

Fig 4, Distribution of Zinc concentration in forage grasses across five sampling stations, Dareta village, Anka, Nigeria

Zinc is essential trace minerals required for many biological processes, particularly enzyme functions, and have a positive influence on livestock growth and reproduction. Zinc is present in the body as a co-factor for enzymes such as arginase and diaminase. It takes parts in the synthesis of DNA, proteins and insulin. It is essential for the normal functioning of the cell including protein synthesis, carbohydrate metabolism, cell growth and cell division. Due to low zinc contents in some home grown animal feed, supplementation of this metal is necessary for most livestock specie, and is most commonly added as mineral supplements to the animal ration [37]. However, exposure to excess amount of Zinc can result to Zinc poisoning. When the soil is polluted with zinc, Plants often have zinc uptake that their system cannot handle. Plants take up zinc by absorbing them from contaminated soils, as well as from deposits on different parts of the plants exposed to the air from polluted environments. Animals that graze on zinc contaminated forage may absorb concentrations that are damaging to their health. Zinc is able to magnify along the food chain. Consumption of animals with elevated lead levels may lead to serious health risk. Soil zinc concentration ranging from 4.49mg/kg dry weight to 33.39mg/kg dry weight was recorded for Dareta soil [26]. US EPA maximum permissible limit (MPL) and the EU Regulation Standards for zinc in soil are 200mg/kg and 300mg/kg respectively. Zinc was therefore not implicated in Dareta soil. The high concentration of zinc in station 1 could be attributed to anthropogenic sources.

CONCLUSION

Nickel and zinc were not seriously implicated in the study except at sampling station 1. This study concludes that transfer of lead from forage grasses to animal products cannot be completely ruled out and emphasizes the essentiality of further investigations to determine the levels of heavy metals in different tissues and fluids of grazing animals in Dareta, to enable the authorities concern make appropriate decisions. The general possible eating of contaminated edible tissues of food animals like cattle, goat and sheep in the area under study may cause the excessive accumulation of these heavy metals particularly lead. This scenario thus will pose a threat to public health.

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