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The effect of pollution on the livestock management, microbial evaluation, health risks, and HPLC analysis of aflatoxins in animal meat and organs

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Free-range animal rearing system is a practical approach to monitor terrestrial pollution in livestock management and public health. This research evaluated the potential health hazards, linked with heavy metals (HMs) and microbial pollution of forage and soil, ingested by free-range domestic animals (cattle, and goats) and wild animals, as well as their subsequent impact on human health. Eighty vegetation samples, 40 soil samples, and a total of 240 animal samples (120 muscle tissues and 120 livers) were extensively collected from the Guinea savannah and rainforest regions. The specimens' heavy metals (Cd, Pb, As, Cr, Ni, Zn, and Cu) concentrations and microbial contamination were determined, in accordance with ASTM and ISO approved guidelines. Remarkably, the HPLC analysis was used to detect the aflatoxins in the animal samples. The results revealed that the vegetation, soil and animal's tissues specimens contained significant amount of HMs and pathogenic microorganisms. Notably, Pb had the maximum concentration, with levels ranging from 1.515 to 1.919 mg/kg and 1.558 to 2.107 mg/kg, respectively, in the animal's muscle and liver samples; while arsenic had the least concentration, which varied from 0.021 to 0.027 mg/kg and 0.022 to 0.037 mg/kg respectively, in the animal's muscle and liver specimens. Though, the values of some of the HMs were quite high, their average concentrations were less than the maximum limits approved by the World Health Organization, for edible food items. Similarly, the results highlighted that the animal specimens exhibited a considerable pathogenic bacteria (< 3,760 cfu/g), fungi (\leq 2,940 cfu/g), and aflatoxins (\leq 8.04 ppb) loads. The HMs content and microbial loads were higher in the liver than in the muscle; and the cow tissues recorded the optimal levels of the HMs and pathogens investigated. Although, the health risk indices (hazard index and cancer risk) indicated that the consumption of the animal samples posed inconsequential

non-carcinogenic or carcinogenic risk; but the elevated HMs and pathogenic microorganisms' levels documented, depicted the necessity of consistent environmental control and monitoring. This is to prevent the bioaccumulation of toxic HMs and pathogens in the vegetation and animal bodies, along with the associated risks in animal production and the food supply chain.

KEYWORDS

biosecurity, heavy metals, nutritional value, toxicology, zoonotic potential

Introduction

The livestock management, a critical constituent of the world economy, is currently facing some challenges. These problems arise from the dependency of animal production mainly on the nutritional and environmental conditions, both of which are immensely affected by environmental pollution (Sánchez-Casanova et al., 2020; Wang et al., 2023; Afzal and Mahreen, 2024). Environmental pollution subject animal to severe health risk, as it interrupts the fragile equilibrium between livestock nutrition and environmental conditions. Contaminated environments often contributes to rapid buildup of toxic remnants in animal products; hence, affecting their public safety, nutritional quality and market value. Environmental pollutants are the major causes of communicable diseases and non-communicable diseases (NCDs), which have adverse effect on livestock management and meat production (Espinosa et al., 2020; Khmaissa et al., 2024). The free-range and semi-free-range systems are the most common animal production systems globally, attributed to their economic feasibility and ecological flexibility. Healthy and well-nourished animals typically yield meat products with significant levels of protein, antioxidants, vitamins, and minerals (Espinales et al., 2024; Stadnik, 2024).

Vegetation plays a major role in animal production, as livestock nutrition and environments have either direct or indirect affiliations to the vegetation. Vegetation supports biodiversity which creates effective balanced ecosystems, helping to prevent pests and diseases buildup with the animal environment (Barry and Huntsinger, 2021; Prospero et al., 2021). Wang et al. (2024) stated that plants materials contain a lot of natural additives beneficial for animal production, which helps to decrease reliance on inorganic antibiotics and feed supplements. Some plants contain potent bioactive compounds with antimicrobial properties, which help to improve the animal health status the animal products quality. It has been scientifically proven that flavonoids and polyphenols help to strengthen the animal's immune system, as well as inhibiting gastrointestinal parasites survival and performance (Niderkorn and Jayanegara, 2021); thereby, contributing immensely to improve productivity. Plants rich in natural Flavonoids, polyphenols, antioxidants and other bioactive compounds include: legumes, vegetables, herbs and spices; though, agricultural by-products such as pineapple, plantain, ginger, turmeric and onion waste materials contains substantial amount of bioactive compounds (Aqilah et al., 2023; Zaky et al., 2024).

Environmental pollution tends to increase the contaminants concentration in the environment; subsequently, leading to rapid build-up these deadly toxins and pathogens in plant and animal tissues. Bioconcentration of lethal materials and microbial in animals' body, compromises the animal safety and its meat quality; consequently, resulting in serious health challenges to the endusers, which are mostly human beings (Atikpo et al., 2021; Ali and Alsayeqh, 2022; Uguru et al., 2024). Basically, animal assimilate most contaminants (particularly heavy metals "HMs") from the environment through the oral pathway, which are deposited in the animal's tissues, resulting in the formation of more complex toxins (Afzal and Mahreen, 2024). Based on their toxicity degree, these elements are classified into potential toxic elements (PTEs) and non-potential toxic elements (non-PTEs). Common PTEs are: lead (Pb), cadmium (Cd), nickel (Ni), chromium (Cr) and arsenic (As); PTEs include: zinc (Zn), magnesium (Mg), copper (Cu), iron (Fe) and zinc (Zn; Atikpo et al., 2021; Uguru et al., 2023). Scientific studies have revealed that non-PTEs play some crucial biological roles in the body functionality, though they are toxic to the body at high concentrations; whereas, the PTEs are poisonous to animals, retarding their productivity even in trace quantities (Wang et al., 2022; Hossain et al., 2023; Kia et al., 2024).

Public health and food security are under serious threat from contaminated environment - vegetation and soils. According to Sangkachai et al. (2024) findings, contaminated vegetation is one of the key sources that diseases are transmitted to animals, mainly through oral pathway (grazing) or other exposure routes. Anthropogenic activities-mostly waste materials are significant causes of pathogenic, toxic compounds and HMs pollutions; hence, posing substantial health complications to free-range animals, and adversely affecting their productivity (Siddiqua et al., 2022; Uguru et al., 2023; Oliva-Vidal et al., 2022). Microorganisms such as: E. coli, Salmonella spp, Leptospira spp, Listeria ssp, Monocytogenes ssp, Brucella ssp, Mycobacterium Bovis, Aspergillus (producing aflatoxins), Fusarium (producing mycotoxins), Toxoplasma ssp, Eimeria spp, and noroviruses, tend to have high predominance in contaminated environments (Kostoglou et al., 2023; Sharma et al., 2024). These pathogens have serious consequences on livestock production, as they can result in diseases outbreak, which can retards reproductive and growth rates, eventually threatening human nutrition and the farmers' livelihoods.

Recently, researchers have highlighted the relevance of adopting the semi and free-ranged systems, in animal production due the versatility in their diet (Miclean et al., 2019; Leroy et al., 2022; Ponnampalam et al., 2022; Afzal and Mahreen, 2024). Cuchillo-Hilario et al. (2024) noted that animals produced under the in free-range conditions frequently benefit from a varied natural essential nutrients, antimicrobial and antioxidants,

leading to production of meat with better nutritional value. Significant attention has been directed toward the adverse impacts of environmental contamination on soil, vegetation, water, air and animal productivity (Birnin-Yauri et al., 2018; Wang et al., 2023; Uguru et al., 2023; Chowdhury and Alam, 2024). However, the dual focus of the impact of environmental pollution (contaminated soil and vegetation), on both nutritional quality and microbial contamination of meat from free-range animals, has not been thoroughly investigated. Therefore, the paramount goal of this research is to comprehensively explore the influence of ecological pollution on animal productivity, which is critical for updating food safety standards. This research incorporates special features of environmental science, public health, and animal husbandry, to comprehensively investigate the impact of environmental pollution on livestock production.

Materials and methods

Study area depiction

This research was carried out within the North central region of Nigeria, with landmass of about 296,898 km². The north central area of Nigeria supports free and semi-free ranged animal production, resulting from its diverse vegetative cover and moderate climatic conditions. This area has networks of fresh rivers, streams and lakes; coupled with ample savanna, forests and agricultural byproducts. The region has an yearly mean temperature of 30°C and rainfall of about 1,600 mm per annual, with the rainy season occurring between April and October (Uguru et al., 2024). The majority of the region, particularly in the southern areas, is characterized by substantial precipitations and elevated relative humidity. These provide abundant grazing areas, natural water and food sources for both ruminant and non-ruminant areas. Apart from the domesticated animals, the North central zone of Nigeria supports wide varieties of wild animals commonly referred to as "bush meat or bush animals," primarily due to its diverse ecosystems. The region is currently facing some environmental pollution challenges due to illegal mining and other anthropogenic activities (Atikpo et al., 2021; Uguru et al., 2024).

Chemical, reagents and quality control

The reagents utilized for the experimental work, were typically analytical-grade, procured from Merck KGaA Company (Darmstadt, Germany). Also, the Nutrient Agar (NA), Potato Dextrose Agar (PDA), digestion tube, and most of the other apparatus used to accomplish the laboratory analysis were produced by Fisher Scientific Inc., USA. The acids were of these qualities: HCl (37% W/V), HNO₃ (65% W/V), H₂SO₄ (98% W/V). Additionally, the containers and bottles used for the research were washed with tap water and liquid soap, immersed in acidified water (60% nitric acid) for 12 h, and rinsed with distilled water. Furthermore, fundamental steps were taken to prevent occurrences of cross-contamination of the samples; and all samples were measured in triplicate and their average taken, and the certified reference materials recovery percentages ranged from 92 to 103%.

Sample collection

To achieve the goals of this research—soil, grass leaves, tree leaves, animal muscles and animal liver were extensively sampled throughout the study area. Samples consisting of grass leaves (n=40), tree leaves (n=40), and topsoil obtained at a depth of 0–0.2 m (n=40), were randomly collected from areas susceptible to free-range livestock management. Additionally, goat meat (n=40), beef (n=40), bush meat (n=40), cow liver (n=40), goat liver (n=40) and bush animals liver (n=40) were collected from meat markets across the central region of Nigeria. All the sampled materials were coded, placed in ice-cold containers at $15\pm3^{\circ}\mathrm{C}$, and taken immediately to the laboratory for extraction and other relevant laboratory analyses. The research was conducted between January 2023 and August 2023, considering the versatility of the experimental design and the extensive area covered.

Samples preparation

Heavy metals (HMs) analysis

The HMs concentrations in the sampled specimens were evaluated through the American Society for Testing and Materials (ASTM) International accepted guidelines. The leaves and meat specimens were washed thoroughly using distilled water to eradicate all invasive objects. Thereafter, the soil and leaves were then sun-dried, while the meat was dried in a laboratory oven set to a temperature of $105 \pm 5^{\circ}$ C. Then the soil and leaves samples were thoroughly crushed using the ceramic mortar and pestle, while the meat was grind using an electric grinder. All the ground specimens were filtered with 1.00 mm sieve size, and the filtrate poured into an air tight plastic container at room temperature ($26 \pm 3^{\circ}$ C; Atikpo et al., 2021).

Two grams of each sample was poured into a digestion tube, and 10 mL of a mixture of HNO3, and $\rm H_2SO_4$, in a ratio of 4:1 was added to the specimen. This set-up was heated (digested) at 95 \pm 5°C on a modified laboratory hot plate equipped with thermostat, until a clear liquid was achieved. After digestion, the liquid was cooled at ambient environmental conditions (26 \pm 3°C and 83 \pm 9% RH), sieved into a volumetric beaker and its concentration weaken with distilled water to 100 mL volume. Then, the elemental content (Cd, Pb, Zn, Ni, Cr, As, and Cu) of the digested samples were accurately measured through the Atomic Absorption Spectrometry (AAS) technique, using the AAS system produced Fisher Scientific Inc. (Chowdhury and Alam, 2024).

Microbial analysis

The microbial population and isolation in the specimens were determined by adopting the procedures recommended by International Organization for Standardization (ISO). The soil was crushed with a sterilized mortar and pestle, while the leaves and meat samples were ground with a sterile blender. Ten gram of each specimen was treated (diluted) with 90 mL of sterile saline solution.

Bacterial population count

One mL of the diluted sample obtained from the above preparation, was spread evenly across the sterile NA plate, and

incubated at 32°C for 24 h. Then, the bacterial clusters produced on the NA plate, were appropriately acknowledged and counted.

Fungal count

The diluted specimen (1 mL) was spread uniformly on the sterile PDA plate, and subjected to incubation operation at 27°C for 96 h. At the conclusion of the incubation duration, fungal colonies formed on the PDA plates were counted and recorded.

The bacterium isolates (*S. aureus, E. coli, Pseudomonas aeruginosa, S. pyogenes, S. epidermidis, Bacillus subtilis, and Salmonella spp*), as well as the fungal isolates (*Aspergillus niger, Mucor spp., Rhizopus stolonifer, Aspergillus flavus, and Penicillium spp.*) were identified following the standard procedures outlined in ASTM D5465 and ISO 4833-1. Regardless of the bacteria to be isolated, the culture incubation was 35°C for 24 h; similarly, irrespective of the fungi isolate, the mycological incubation was 27°C for 5 days (Olise et al., 2020). Clusters formed after the incubating duration were classified and tallied, by utilizing an electronic colony counter (model FTDCC-4, produced by India).

Aflatoxins evaluation

The High-performance liquid chromatography (HPLC) method was employed, to identify and quantify the Aflatoxins (AFs) population in each sample. This test was conducted with the aid of HPLC machine (model LC-W100B, produced by Wincom Company Ltd, China).

Health risk (hazard) assessment

Prospective Health complications, which are associated with heavy metal's toxicity, were determined through these models: bioaccumulation factor, projected daily dose, hazard quotient, hazard index, and target cancer risk.

Bioaccumulation factor (BF)

Regardless of the HM investigated, the BF level of the vegetation was calculated using Equation 1.

Bioaccumulation factor =
$$\frac{C_{\nu}}{C_{\rm s}}$$
 (1)

Where C_v is the HM level in the plant leaves, and C_s is the HM level in the surrounding soil.

Estimated daily intake (EDI)

The EDI values of the HMs were calculated through Equation 2.

$$EDI = \frac{C \times QFC}{BW} \times 10^{-3} \tag{2}$$

Where C is the individual HM level, QFC = estimated meat quantity consumed daily, BW = body weight of the individual, $\times 10^{-3}$ is the conversion factor, BW is taken as: children ~ 35 kg and adults ~ 70 kg (Uguru et al., 2024). QFC is estimated as 19.09 g/person/day (Emurotu et al., 2024); hence, to convert this to mg/person/day, a conversion factor of 10^{-3} is needed.

Hazard quotient (HQ)

The heavy metals HQ values were computed through Equation 3.

$$HQ = \frac{EDI}{RfD} \tag{3}$$

RfD = Reference Dose of the HMs, and thevalues for Cd, Pb, Cr, As, Cu, Zn and Ni were 0.001, 0.0014, 0.003, 0.0003, 0.04, 0.3 and 0.02 mg/kg/day, respectively (Uguru et al., 2023; Kia et al., 2024).

Hazard index (HI)

This was achieved through summation of HMs' hazard quotient values and it is calculated through Equation 4 (Hossain et al., 2023).

$$HI = HQ_{Cd} + HQ_{Pb} + HQ_{Cr} + HQ_{As} + HQ_{Cu} + HQ_{Zn}$$

$$+ HQ_{Ni}$$
(4)

Cancer risk (CR)

Theoretically, each HM cancer risk worth as calculated by applying the formula shown in Equation 5.

$$CR = EDI \times SF$$
 (5)

Where: SF is the cancer slope factor. From literatures, the accepted SF values for these PTEs—Cd, Pb, Cr, As and Ni were 0.38, 0.0085, 0.5, 1.5 and 0.84 mg/kg/day, respectively (Uguru et al., 2023; Kia et al., 2024). Additionally, the computation of the total cancer risk (TCR) prediction was done, by summarizing cumulative HMs' CR outcomes.

Statistical analysis

The consequences of the ecological pollution on the meat quality and health surveillance, was evaluated using Analysis of Variance (ANOVA) model, with the aid of the SPSS (version 20.0). Also, the means values obtained from the laboratory screening, were compared by applying Duncan's Multiple Range Test (DMRT) sub-model, to identify their significant at 5% probability level ($p \le 0.05$).

Results and discussion

Vegetation and soil

Heavy metals levels

The result of the metals concentration in the sampled vegetation and soil specimens are given in Table 1; and notably the foliage and soil contain significant amount of the HMs evaluated. In the grasses, the Pb, Cd, Cr, As, Cu, Zn and Ni levels were 0.108, 0.114, 0.101, 0.031, 4.320, 6.532 and 0.526 mg/kg dw, respectively; while in the plants leaves the Pb, Cd, Cr, As, Cu, Zn and Ni concentrations were 0.095, 0.105, 0.069, 0.010, 5.662, 6.208 and 0.538 mg/kg dw, respectively. Particularly, the HMs concentration in the plants and grasses took this decreasing pattern Zn Cu

TABLE 1 Heavy metals concentration in the vegetation and soil (mg/kg dw).

НМ	Vege	tation	Soil	WHO*
	Grasses	Trees		
Pb	$0.108^{b} \pm 0.122 (0-0.423)$	$0.095^{\rm b} \pm 0.104(0$ – $0.389)$	$9.998^a \pm 7.760 (0.065 - 38.453)$	0.3*, 10**
Cd	$0.114^{\mathrm{b}} \pm 0.126 (0 - 0.471)$	$0.105^{c} \pm 0.150 \ (0-0.631)$	$0.283^a \pm 0.413 (0 - 1.328)$	0.2*, 0.3**
Cr	$0.101^{\rm b}\pm0.140~(0$ – $0.521)$	$0.069^{c} \pm 0.090 \ (0-0.294)$	$2.444^{a} \pm 1.856 (0-7.024)$	2.3*, 2.3**
As	$0.031^{b} \pm 0.055 (0 - 0.283)$	$0.010^{c} \pm 0.011 (0-0.036)$	$0.064^{a} \pm 0.116 (0-0.461)$	0.1*, 0.2**
Cu	$4.320^{\circ} \pm 4.800 (0.286 15.831)$	$5.662^{b} \pm 5.670 \ (0.338-19.64)$	$8.397^a \pm 4.678 (1.899-26.352)$	10*, 40**
Zn	$6.532^{b} \pm 4.649 (0.049 - 18.113)$	$6.208^{b} \pm 6.088 \ (0.397-19.24)$	$11.547^{a} \pm 7.75 (1.934-29.609)$	50*, 60**
Ni	$0.526^{\rm b} \pm 0.813 \; (0 3.081)$	$0.538^{b} \pm 0.913 (0 3.094)$	$1.320^{a} \pm 1.367 (0-4.262)$	2.7*, 1.5**

Mean \pm standard deviation, n = 40, values that have different superscripts within the same rows are significantly different (P < 0.05) according to DMRT; parentheses values represent the minimum and maximum levels of the HMs; * maximum acceptable HM level in vegetation; ** maximum tolerable HM concentration in soil, WHO* = FAO/WHO (2011).

> Ni > Cd > Pb > Cr > As. This is an indication that the vegetation has large Cu and Zn concentrations, when compared to the lower arsenic and chromium concentrations obtained in the results. Likewise, the Pb, Cd, Cr, As, Cu, Zn and Ni content in the soil samples were 9.998, 0.283, 2.444, 0.064, 8.397, 11.547 and 1.320 mg/kg dw, respectively, and these HMs concentration declining pattern followed thus: Pb > Cu > Cr > Ni > Cd > As. The results depicted that the vegetation contains substantial amount of PTEs (Cd, Ni, Zn, Pb and As). The HMs contents in the vegetation and soil fell below the maximum limits recommended by FAO/WHO. Heavy metals are among the most dangerous environmental toxic substances, posing significant health risks, particularly in animal production (Souri et al., 2018), and anthropogenic activities contributed immensely to the boosting of HMs pollution of the environmental resources (Uguru et al., 2023).

Remarkably, the Investigation findings revealed that the vegetation's HMs concentrations were principally smaller, when compared to the magnitudes obtained in the soil specimens. These outcomes bolstered previous scientific reports Tasrina et al. (2015) and Uguru et al. (2023). This depicted that soil is a potent natural reservoir of HMs, and the plants root system were able to effectively inhibits the permeation of these heavy metals by the vegetation. Interestingly, the vegetation's (grasses and trees) Pb contents fell below the concentration documented for comestible plant materials (Sultana et al., 2022). Also the Cd, Zn and Pb contents documented in this investigation were relatively lower, than the amounts registered by these scholars (Rehman et al., 2017; Atikpo et al., 2021; Uguru et al., 2023). Additionally, the Cu amounts identified in the vegetation, were higher than those concentrations observed by these academicians (Souri et al., 2018; Miclean et al., 2019); though their verified Cd and Pb contents were comparatively smaller, to those achieved in this research.

Although the HMs levels in the vegetation and soil were relatively small, and fell beneath the maximum tolerable amounts approved by the WHO, they can still have an impact on livestock production. Herbivorous animals are vulnerable to accumulating heavy metals, primarily due to their feeding behavior. This accumulation tends to interfere with animals normal metabolic functions, leading to toxicity over time (Gall et al., 2015). Bioaccumulation of toxic elements in livestock greatly affects their meat and milk production, as well as their overall productivity

(Afzal and Mahreen, 2024); consequently, compounding the dangers associate with heavy metal exposure in the food chain (Hajipour, 2023). Extended exposure of animals to potentially toxic metals can weaken their immune systems, resulting in health complications such as gastrointestinal issues, liver problems, kidney failure, neurological disorders and various infectious diseases (Miclean et al., 2019; Chen et al., 2021).

Bioaccumulation factor (BF)

Results of the bioaccumulation factor of the various HMs are presented in Figure 1. It was observed that the BF values of Pb, Cd, Cr, As, Cu, Zn and Ni were 0.01, 0.387, 0.035, 0.32, 0.594, 0.552 and 0.403, respectively. Interestingly, the BF of all the metals investigated in this research were less than 1 (BF <1), signifying the low bioaccumulation of these HMs in the environment. The heavy metals' BF values followed this thread: Cu > Zn > Ni > Cd > As >Cr > Pb, signifying that this region's vegetation had greater affinity to accrue nickel, zinc, and copper compared to lead and chromium, and which can be attributed to the volume and concentration of metallic-based discharge in the area. This discrepancy in the BF results can be linked to the soil's chemistry and HMs' absorption efficiency. According to Atikpo et al. (2021) observations, some elements-Pb and Cr, tend to have higher potential in creating a prefect bond between the soil grains and organic matters; thereby decreasing their mobility in the soil, subsequently bioavailability to vegetation roots.

Remarkably, this research's BF results were smaller than those documented by Zhou et al. (2022), and were similar to those highlighted by Edogbo et al. (2020). Also, the zinc BF amount exceeded the range of values stated by Tasrina et al. (2015); while this study's Cd and Cr BF levels were smaller than the findings presented by Atikpo et al. (2021). Notably, high BF amounts tend to give insight of the plant's potency of assimilating metals from the soil, which can be linked to the deprived phytoremediation potential of the plant; whereas, smaller BF levels signify the plant enhanced phytoremediation competency (Atikpo et al., 2021). Lower BF results achieved in this research, can be associated with the region's high soil fertility level, moderate temperature, and high annual rainfall volume; factors that facilitate phytoremediation effectiveness (Uguru et al., 2023).

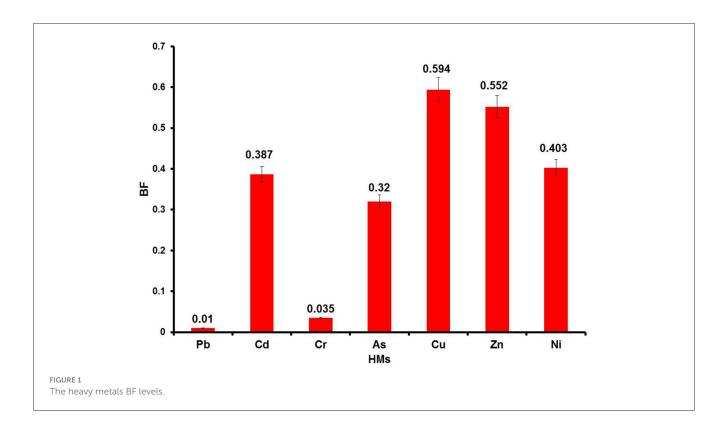


TABLE 2 The bacteria load in the vegetation and soil (cfu/g).

Parameter	Grass leaves	Tree leaves	Soil	
ТВС	$8.39 \times 10^4 \pm 2.71 \times 10^5$	$5.50 \times 10^3 \pm 2.07 \times 10^4$	$1.77 \times 10^7 \pm 7.64 \times 10^7$	
Bacteria Isola	ites			
S. aureus	+	+	+	
E. coli	++	+	++	
Pseudomonas aeruginosa	+++	+++	+++	
Streptococcus + pyogenes +		+	++	
S. epidermidis	+	++	+	
Bacillus subtilis	+++	++	+++	
Salmonella	++	+	++	

Mean \pm standard deviation, n = 40, + + + high prevalence, ++ moderate prevalence, + low prevalence.

Microbiology of the vegetation and soil

Bacteria load

Table 2 displayed the results of the bacteria load recorded in the vegetation and soil specimens. According to the results, the grass leaves, tree leaves and soil specimens TNC populations were 8.39×10^4 , 5.50×10^3 and 1.77×10^7 cfu/g, respectively. It was noted that the soil contained higher bacteria population compared to the grasses and trees. This could be linked to the

favorable conditions—humid conditions, organic residues, lower temperature and protection from UV radiation- provided by the soil, which help to promote microbial growth (Qiu et al., 2022). Some of these microorganisms are pathogenic, which can negatively affect the health of livestock grazing on the contaminated grasses or wallowing in the contaminated soil. The higher microbial recorded in the grass leaves compared to the tree leaves could be attributed to the proximity to the microbial vectors. Grasses are much closer to the ground (soil) compared to plants, which is the main natural microbial reservoir. This close proximity establishes favorable transmission routes for microorganisms to reach the leaves of the grass (Vincze et al., 2024), through plants leaves acquire substantial amount of microorganisms through atmospheric depositions (Uguru et al., 2024).

Furthermore, these bacteria S. aureus, E. coli, Pseudomonas aeruginosa, Streptococcus pyogenes, S. epidermidis, Bacillus subtilis and Salmonella spp were identified from the bacteria population recorded in the leaves and soil samples. Notably, the grass leaves, tree leaves and soil recorded high frequency of Pseudomonas aeruginosa, which is an indication of the bacterium to adapt and thrive in diverse environmental conditions. Similarly, Staphylococcus aureus recorded low frequencies in the leaves and soil samples, likely due to its sensitivity to non-host environments. Warm-blooded animals have been found to be the primary hosts of Staphylococcus aureus, and they highly survive outside their host environment, and sensitive to prolonged exposure to extended exposure to UV radiation and other abiotic stresses (Howden et al., 2023). Escherichia coli are clinically linked to diarrhea, colitis, and hemolytic uremic syndrome in both livestock and humans (Bae et al., 2006). Salmonella species which are transmitted through contaminated environment, feed and carrier animals have been

linked to salmonellosis condition in livestock management. This condition leads to diarrhea, dehydration and high mortality if not well managed (Hoelzer et al., 2011). *Pseudomonas aeruginosa* is an opportunistic pathogen associated with health conditions such as bovine mastitis, endometritis, dermatitis, pneumonia, and bronchopneumonia in animals. These infections can result in decreased reproductive efficiency, respiratory distress, and elevated mortality rates if not promptly treated (Qin et al., 2022). *Salmonella* is a zoonotic pathogen which originates mainly livestock and poultry, is responsible for most laboratory-confirmed foodborne illnesses globally (CDC, 2014).

Fungal load

The results presented in Table 3 showed that the total viable fungal load of the grass leaves, tree leaves and soil samples were 6.08×10^4 , 2.32×10^3 and 4.22×10^6 cfu/g of dry leaves and soil, respectively. This indicated that the maximum and minimum TFC were obtained in the soil and the tree leaves, respectively. This could be attributed to the high humidity, warm conditions and higher nutrient availability in the soil compared to the leaves. Fungi spores require moisture and warm conditions for germination and survival. These factors play essential roles in fungi lifecycle, and are the primary reasons why fungi are more prevalent in humid soils, decaying plant matter, and warm ecosystems (Bahram and Netherway, 2022). Though the concentrations of these fungi are quite, there is tendency of free range animals to contact fungal infections from them. Fungi in the environment pose serious health risks to animals reared under the free-range system, as prolonged exposure to contaminated materials or consumption of contaminated feeds can precipitate fungal infections (Davies et al., 2021; Simões et al., 2023).

Additionally, Aspergillus niger, Mucor spp., Rhizopus stolonifer, Aspergillus flavus and Penicillium spp. were segregated from the leaves and soil clinical specimens. It was noted that the soil samples have high occurrences of Aspergillus niger, Aspergillus flavus and Penicillium spp.; the grass leaves specimens had high frequencies of Mucor spp. and Rhizopus stolonifer; while the tree leaves had high

TABLE 3 Fungal population in the vegetation and soil (cfu/g).

Parameter	Grass leaves	Tree leaves	Soil	
TFC	$6.08 \times 10^4 \pm 2.20 \times 10^5$	$2.32 \times 10^{3} \pm 4.12 \\ \times 10^{3}$	$4.22 \times 10^6 \pm 2.08 \times 10^7$	
Isolates				
Aspergillus niger	++	+++	+++	
Mucor sp.	+++	+	+	
Rhizopus +++ stolonifer		++	++	
Aspergillus + flavus		+	+++	
Penicillium sp.	++	+++	+++	

Mean \pm standard deviation, n = 40, + + + high occurrence, ++ moderate occurrence, + low occurrence.

prevalence of Aspergillus niger and Penicillium spp. Specifically, the leaves show a low occurrence of Aspergillus flavus, while both the soil and tree leaves exhibit low frequencies of Mucor spp. These pathogens will substantially affect livestock productivity and meat quality; therefore, posing serious problems to public health and economic feasibility of livestock farming. Aspergillus niger causes aspergillosis and cutaneous aspergillosis in animals, as well as production secondary metabolites such as ochratoxins or fumonisins. These conditions can lead to respiratory diseases, immunosuppression, intestinal disturbances, liver and kidney complications in animals. Ruminants and swine are particularly vulnerable to these health risks (Seyedmousavi et al., 2015). Certain pathogenic species of Penicillium produce mycotoxins that can interrupt the functionality of the nervous system, liver, and kidneys. Additionally, Penicillium species are known for causing respiratory problems and reproductive system failure (Janik et al., 2020).

Furthermore, seepage from biomaterials, agricultural activities, and other poorly managed solid waste can be linked to the large microbial population documented in this research. Bizarrely, this studied region is known for its improper waste management approaches; and previous ecological investigations had identified ineffective waste handling, as a major contributor to pathogenic microorganisms' pollution (Uguru et al., 2024). Also Uguru et al. (2023) and Tasrina et al. (2015) reported that discharge from agricultural activities contains substantial amount of PTEs and microorganisms, which tends to create severe health challenges to animals; and subsequently, human beings within the food web.

Animals' body tissue analyses

HMs concentration

The result of the HMs level in the animals' products is presented in Table 4. It was noted that the beef recorded 0.053, 0.092, 0.303, 0.027, 0.528, 1.856 and 0.070 mg/kg dw for Cd, Pb, Cr, As, Cu, Zn and Ni, respectively; and the goat meat had Cd, Pb, Cr, As, Cu, Zn and Ni contents of 0.060, 0.098, 0.348, 0.032, 0.887, 1.919 and 0.079 mg/kg dw, respectively. Likewise, the amounts of Cd, Pb, Cr, As, Cu, Zn, and Ni in the bush meat were recorded to be 0.063, 0.082, 0.361, 0.021, 0.892, 1.515, and 0.058 mg/kg dry weight, respectively. With respect to the liver samples, the cow liver had Cd, Pb, Cr, As, Cu, Zn and Ni levels of 0.071, 0.098, 0.445, 0.037, 0.919, 1.856 and 0.080 mg/kg dw, respectively, the goat liver had Cd, Pb, Cr, As, Cu, Zn and Ni values of 0.078, 0.127, 0.463, 0.046, 1.006, 1.919 and 0.121 mg/kg dw, respectively; while the wild animals liver recorded Cd, Pb, Cr, As, Cu, Zn and Ni levels of 0.076, 0.105, 0.507, 0.022, 1.013, 1.558 and 0.075 mg/kg dw, respectively. The higher HMs concentration recorded in the cattle compared to the other animals could be attributed to the lifestyle and feeding habit of cattle. Cattle can consume diverse range of plants, which increases their risk of exposure to contaminants. Additionally, cattle have quicker higher feed intake rates, subsequently leading to greater accumulation of trace metals in their bodies.

Remarkably, it was noted that the liver had higher HMs contents compared to the meat (muscle) samples, which could be attributed to the liver's detoxification properties. Liver play a vital role in detoxifying and accumulating toxic substances, including

TABLE 4 The HM levels in the animals muscle and liver (mg/kg dw).

НМ	Part	Cow	Goat	Bush animal	WHO
Cd	Muscle	$0.053^{a} \pm 0.061 (0-0.279)$	$0.060^{b} \pm 0.067 (0-0.337)$	$0.063^{b} \pm 0.058 (0-0.213)$	0.5
	Liver	$0.071^a \pm 0.080 (0-0.291)$	$0.078^a \pm 0.092 (0$ – $0.482)$	$0.076^{a} \pm 0.083 (0-0.356)$	0.5
Pb	Muscle	$0.092^{b} \pm 0.090 (0-0.331)$	$0.098^{\rm b} \pm 0.098 (0-0.354)$	$0.082^a \pm 0.097 (0-0.339)$	0.1
	Liver	$0.098^a \pm 0.116 (0$ – $0.46)$	$0.127^{b} \pm 0.144 (0-0.538)$	$0.105^{a} \pm 0.129 (0-0.502)$	0.1
Cr	Muscle	$0.303^a \pm 0.627 (0-2.918)$	$0.348^{b} \pm 0.630 (0-2.801)$	$0.361^{\circ} \pm 0.663 \ (0-2.973)$	1.0
	Liver	$0.445^{b} \pm 0.876 (0-4.056)$	$0.463^{\mathrm{b}} \pm 0.911 (0-4.258)$	$0.507^{c} \pm 0.971 \ (0-4.204)$	1.0
As	Muscle	$0.027^{b} \pm 0.039 (0-0.167)$	$0.032^{\rm b} \pm 0.054 (0$ – $0.276)$	$0.021^a \pm 0.034 (0 - 0.174)$	0.5
	Liver	$0.037 \pm 0.051 \ (0-0.232)$	$0.046 \pm 0.084 (0 - 0.432)$	$0.022 \pm 0.034 (0 - 0.155)$	0.5
Cu	Muscle	$0.528^a \pm 0.959 (0.002 - 2.642)$	$0.887^{b} \pm 0.926 (0.005 - 3.633)$	$0.892^{c} \pm 1.149 (0.016-4.593)$	
	Liver	$0.919^a \pm 0.940 (0.003 - 3.223)$	$1.006^{\circ} \pm 1.003 (0.008 - 4.624)$	$1.013^{b} \pm 1.084 (0.038 - 4.549)$	
Zn	Muscle	$1.856^{a} \pm 1.707 (0.033-5.449)$	$1.919^{ab} \pm 2.012 (0.032-8.091)$	$1.515^{b} \pm 2.025 (0.024-9.338)$	20
	Liver	$2.107^{a} \pm 1.695 (0.037-6.867)$	$1.941^{\circ} \pm 1.858 (0.049 - 7.406)$	$1.558^{b} \pm 1.603 (0.017 - 6.064)$	20
Ni	Muscle	$0.070^{b} \pm 0.097 (0-0.353)$	$0.079^{b} \pm 0.100 (0-0.429)$	$0.058^{a} \pm 0.091 (0-0.356)$	
	Liver	$0.080^a \pm 0.129 (0-0.491)$	$0.121^{c} \pm 0.156 (0-0.652)$	$0.075^{a} \pm 0.128 (0-0.527)$	

Values are mean \pm standard deviation; n = 40; values with different superscript in the same rows are significantly different (P < 0.05) based DMRT; parentheses values are minimum-maximum levels of the HM; WHO = FAO/WHO (2011), FAO/WHO (2003).

trace elements; hence, the levels of HMs in animals' livers tend to be greater than the concentration in the muscles (Kia et al., 2024; Emurotu et al., 2024). Similar results pattern were reported by these authors (Korish and Attia, 2020; Edet et al., 2024), during their investigation on the HMs amassing situation in different animal tissues. The high concentrations of HMs detected in the animals' bodies could be linked to the high levels of HMs in the vegetation within the region, as reflected in Table 1. Plants' being the primary producer—in the food chain, is the main constituent of animals' diet, mainly herbivorous animals. Soil assimilation is common in animals mainly through feeding and wallowing operations (Wang et al., 2023), which is another major source of accumulation of toxic metals levels in animals' bodies (Zhou et al., 2022).

The cadmium contents detected in this study varied among all the animals, with values ranging from 0 to 0.361 mg/kg. Notably, the highest Cd level was found in the bush animals, while the lowest was noted in the beef. Remarkably, the Cd levels obtained in this research were lower than those reported by Birnin-Yauri et al. (2018), Chowdhury and Alam (2024) and Emurotu et al. (2024) for cow, goat, and game meats; while they were higher than the values documented for chicken offal (Korish and Attia, 2020; Hossain et al., 2023; Kia et al., 2024; Edet et al., 2024). The mean Cd, Cu, AS and Zn concentrations in the animals' muscles examined in this investigation were greater than those detected in sheep muscle by Korish and Attia (2020) and Wang et al. (2023); though the Wang et al. (2023) reported higher Cd and As contents for sheep's liver. Also, this research's Pb amounts (irrespective of the offal), were greater than the amounts obtained for poultry products (Korish and Attia, 2020; Aendo et al., 2022). Furthermore, the Cr concentrations recorded in this study were in harmony with previous findings of Kalu et al. (2021). Notably, the Cr content, regardless of the animal's part, were greater than the results reported for chicken (Hossain et al., 2023), but fell below the Cr amount reported for beef and chevon (Chowdhury and Alam, 2024). Also, the Pb concentrations reported in this study, were larger compared to the results reported for poultry (Korish and Attia, 2020; Aendo et al., 2022).

Notably, the mean Cd, Pb, Cr and Zn amounts verified for the animal's parts (in this research), were lower than the FAO/WHO maximum allowable concentration for meat products (FAO/WHO, 2011; Zhou et al., 2016). HMs, toxicity affects the edible offal and muscle tissue of animals, posing significant concerns for the proper functioning of their organs and systems. Lead has the potential of creating neurological, gastrointestinal, anemia, and reproductive issues in animals; while extended exposure to Cr accumulation can leads enzymatic malfunctioning problems (Chowdhury and Alam, 2024). Arsenic toxicity include: gastroenterological condition, nervous tension, cardiovascular and respiratory distresses (Afzal and Mahreen, 2024). Though studies had shown that trace amount of Cu plays a vital role enzymatic performance and reproductive health, its overdose cancause stomach pains, anorexia and sialorrhea (Afzal and Mahreen, 2024). Additionally, Cd toxicity tends to cause health implications—liver impairment, renal problem and porous bones (Wang et al., 2021).

Toxic metals accumulation in animal bodies, presents a direct risk to humans who consume products derived from these animals (Năstăsescu et al., 2020; Afzal and Mahreen, 2024). Though the mean values of HMs in the sampled animal products were within tolerable limits set by WHO and FAO, it was observed that the range of HMs values shows inconsistency, with some individual specimens exceeding the internationally recommended standards. Since animal exposure to toxic metal poisoning mainly results from contaminated feeds and soils, it is critical to regularly monitor the ecosystems to prevent toxic metal contamination, improve animal productivity and public health. Additionally, maximum cooperation among veterinary,

TABLE 5 The bacteria population of the animal parts ($\times 10^2$ cfu/g).

Parameter Part		Cow	Goat	Bush animal	
TBC Muscle		$26.76 \pm 15.25 (4.2-61.90)$	$22.70 \pm 13.80 (3.70-55.10)$	$28.70 \pm 17.50 \ (4.7-69.30)$	
	Liver	$32.51 \pm 18.34 (5.10-73.40)$	$27.20 \pm 15.40 \ (4.50 - 64.30)$	33.34 ± 18.78 (5.40-76.20)	
Staphylococcus aureus	Muscle	$0.75 \pm 0.57 (0 - 1.89)$	$0.61 \pm 0.59 (0 1.89)$	$0.79 \pm 0.73 (0 - 2.42)$	
	Liver	$0.83 \pm 0.79 (0 - 2.34)$	$0.65 \pm 0.74 (0 - 2.39)$	$0.83 \pm 0.78 (0 - 2.47)$	
Escherichia coli	Muscle	$0.89 \pm 0.79 (0 - 2.45)$	$0.77 \pm 0.74 (0 - 2.18)$	$0.66 \pm 0.69 (0 – 2.29)$	
	Liver	$0.97 \pm 0.83 (0 - 20.35)$	$0.74 \pm 0.71 \; (0 - 2.06)$	$0.92 \pm 0.90 (0 – 2.91)$	
Pseudomonas aeruginosa	Muscle	$0.49 \pm 0.46 (0 - 1.37)$	$0.49 \pm 0.44 (0 1.28)$	$0.60 \pm 0.51 (0 1.45)$	
	Liver	$0.63 \pm 0.54 (0 1.93)$	$0.53 \pm 0.49 (0 1.32)$	$0.68 \pm 0.68 (0 – 2.13)$	

TBC, Total Viable Bacterial Counts.

environmentalist and public health agencies is crucial for a thorough approach to tackling heavy metal contamination of the environment. By implementing these remediation approaches, it will help to enhance livestock management and ensure food supply chain safety.

Microbial load

The results of the microbial population of the animal samples were presented in Tables 5 and 6. The Total Viable Bacterial Counts (TBC) for the cow, goat and bush animals muscle tissue samples were 2,676, 2,270 and 2,870 cfu/g, respectively. Similarly, their liver tissue samples recorded TBC population of 3,760, 3,251, 2,720, and 3,334 cfu/g, respectively. The bacterial isolates identified were S. aureus, E. coli and Pseudomonas aeruginosa. In beef, the counts were 74.50, 89.10, and 48.50 cfu/g, respectively. For goat muscle samples, the populations were 61.20, 77.00, and 49.20 cfu/g, respectively. In bush meat muscle specimens, their levels were 79.10, 65.50, and 60.20 cfu/g, respectively. In addition, the counts of Staphylococcus aureus, E coli, and Pseudomonas aeruginosa per gram of liver tissue were 82.9, 96.8, and 62.7 for cow liver samples, 65.3, 74.0, and 53.3 for goat liver samples, and 82.6, 92.1, and 68.3 for bush animal liver samples, respectively. These analysis uncovered that S. aureus was the most prevailing bacterium in the animals' bodies, similar to Nagase et al. (2002) and Olise et al. (2020) clinical observations. S. aureus is a predominant gram-positive bacterium, has potent zoonotic prospective causing substantial health risks to public health (Haag et al., 2019). Generally, the bacteria liver bacteria count was greater than the amounts present in the muscle tissue; a situation which can link to its higher exposure degree to pathogens and toxic substances (Zaefarian et al., 2019).

Furthermore, the clinical findings depicted that the cattle muscle and liver, had highest bacterial prevalence, when compared to the other animals investigated. This specifies that the cattle were more susceptible to bacterial invasion, largely resulting from their feeding habits and physiological responses. Also, the higher E. *Coli* quantities verified for the cattle can be attributed to their gastrointestinal tract, which provides ideal circumstances that enhances pathogenic E. coli survival and proliferation. Ruminant animals' digestive tract creates perfect environmental conditions, for E. coli bacteria

performance; thereby, cattle are prone to E. coli incursion (Sapountzis et al., 2020; Lange et al., 2022; Perez et al., 2024).

Fungi load

Table 6 presents the fugal population (TFC and fungal isolates) of the animals' samples. The findings revealed that the beef, goat meat, and bush animal meat had TFC populations of 2,755, 2,595, and 2,850 cfu/g, respectively. Likewise, the cows, goats, and bush animals liver's samples recorded TFC counts of 2,878, 2,461, and 2,940 cfu/g, respectively. Specifically, Aspergillus niger, Penicillium spp and Aflatoxins were the fungal strains recognized in the samples examined. Aflatoxins are toxic secondary metabolites mainly formed by certain fungal species, including some Aspergillus and Penicillium spp (Abrehame et al., 2023). The results revealed that the beef samples had Aspergillus niger, Penicillium spp., and Aflatoxins at concentrations of 65.30 cfu/g, 55.00 cfu/g, and 0.954 parts per billion (ppb), respectively; similarly, the cow liver specimens showed the same fungal isolates at levels of 77.30 cfu/g, 76.10 cfu/g, and 1.172 ppb, respectively. Also, the Chevon sample had the occurrences of Aspergillus niger, Penicillium spp., and Aflatoxins, with counts of 59.70 cfu/g, 52.20 cfu/g, and 0.887 ppb, respectively; and the goat liver sample also showed contamination with the same fungal isolates, with counts of 67.50 cfu/g, 57.90 cfu/g, and 0.965 ppb, respectively. Besides, the bush animals muscle and liver samplings had Aspergillus niger, Penicillium spp., and Aflatoxins contents of 70.30 cfu/g, 66.30 cfu/g and 0.963 ppb, and 80.50 cfu/g, 74.60 cfu/g and 1.609 ppb, respectively. These microbial loads can be aggravated, through the consumption of contaminated soil and vegetation by the animals.

The smaller Aflatoxins and other fungi amounts, identified in the goat's body can be associated with: the improved detoxification ability of the goat liver, and smaller exposure rate to the pathogen during feeding, which can be linked to their reduced grazing coverage and body size. Goats' smaller grazing range and body size can decrease their ingestion rate of fungi-infected materials, resulting in lower levels of aflatoxin contamination (Popescu et al., 2022). Regardless of the occurrence of Aflatoxins in the animals' bodies, the concentrations were below the maximum population approved by the International Agency for Research on Cancer (IARC) for food products and public health. Typically, WHO recommended maximum permissible aflatoxins level of

TABLE 6 The fungi load of the animal parts.

Parameter	Part	Cow	Goat	Bush animal	
TFC*	TFC* Muscle 27		$25.95 \pm 16.92 (5.5-64.20)$	$28.50 \pm 18.00 (1.70 65.00)$	
	Liver $28.78 \pm 17.20 (0.43 - 6.62)$		$24.61 \pm 13.50 \ (0.38-5.18)$	29.40 ± 16.84 (0.77-6.99)	
Aspergillus niger*	Muscle	$0.65 \pm 0.52 (0 1.84)$	$0.60 \pm 0.59 (0 - 1.84)$	$0.70 \pm 0.58 (0 1.84)$	
	Liver	$0.77 \pm 0.80 (0 - 2.21)$	$0.68 \pm 0.73 (0 - 1.94)$	$0.81 \pm 0.85 (0 2.33)$	
Penicillium sp.*	Muscle	$0.55 \pm 0.48 (0 - 1.47)$	$0.52 \pm 0.49 (0 - 1.47)$	$0.66 \pm 0.52 (0 1.48)$	
	Liver	$0.76 \pm 0.59 (0 - 1.86)$	$0.58 \pm 0.65 (0 - 1.95)$	$0.75 \pm 0.63 (0 - 1.96)$	
Aflatoxins**	Muscle	$0.95 \pm 1.64 (0-6.52)$	$0.89 \pm 1.70 \ (0-7.00)$	$0.96 \pm 1.59 (0 - 6.52)$	
	Liver	$1.17 \pm 1.97 (0 - 7.45)$	$0.97 \pm 1.69 (0 - 6.58)$	$1.61 \pm 2.37 (0 - 8.04)$	

TFC, Total viable fungal counts, $* = \times 10^2$ cfu/g, ** = ppb.

10 ppb for meat products (Dada et al., 2020). Aflatoxins have been verified of causing serious health complications, which can lead to liver toxicity, weakened immune response, and cancer (Dada et al., 2020).

Wang et al. (2023) in during the clinical exploration of livestock management reported that, consumption of pathogenic contaminated soils increases the prospect of diarrhea and other foodborne infections. Microbial infestation has a lot of numerous challenges in animal production, affecting both animal health and productivity. Carcasses of infected animals carry viable pathogens that pose a risk of food-borne illnesses to humans (Gabriël et al., 2023). Staphylococcus aureus is an opportunistic microorganism that affects a variety of animals, leading to conditions such as inflammation of the mammary gland, contamination of milk products, pyoderma, osteomyelitis, and declined reproductive efficiency (Haag et al., 2019). Aspergillus niger is a fungal species with zoonotic potential, that its toxicity can lead to gastrointestinal issues, respiratory problems, nasal discharge, and generally poor performance in animals (Seyedmousavi et al., 2015). Pathogenic strains of E. coli have diverse range of impacts on animals, as they produce toxins that lead to severe diarrhea, inflammation, septicemia, urinary tract infections, and reproductive disorder (Pokharel et al., 2023). Remarkably, this study's TBC and TFC populations were within the range of results documented for cow and goat muscles (Rani et al., 2023).

Health risk assessment

Estimated daily intake

The EDI and non-carcinogenic risk assessment (HQ and HI) results of the examined HMs are presented in Table 7. The computed findings depicted that the EDI results varied from 4.36 \times 10⁻⁵ to 2.89 \times 10⁻³ mg/kg/day, and 2.18 \times 10⁻⁵ to 1.44 \times 10⁻³ mg/kg/day for the children and adults, respectively. Equally, in the liver specimens, the EDI levels ranged from 5.73 \times 10⁻⁵ to 3.18 \times 10⁻³, and 2.86 \times 10⁻⁵ to 1.59 \times 10⁻³ mg/kg/day in the children and adults categories respectively. Specifically, this study's specified that the liver accumulates greater EDI values, and also the children category recorded greater EDI values compared to the adult category. These results were comparable to Kia et al. (2024) findings, when investigating basic health issues linked to

animal consumption. The elevated EDI results observed in the liver depicts greater health implications, associated with liver's ingestion. Correspondingly, the higher EDI content noted in the children can be linked to their relative smaller body weight, compared to the adult individuals (Uguru et al., 2023). Interestingly, the EDI values of the HMs, irrespective of the age group, were lower than the provisional tolerable daily intake (PTDI) levels for HMs establish by the FAO/WHO. It was recommended that the PTDI values for Cd, Pb, As, Ni, Zn, and Cu should not surpass 0.001, 0.00357, 0.0018, 0.005, 1, and 0.5 mg/kg/day, respectively (FAO/WHO, 2003).

Non-carcinogenic risk

From the results shown in Table 7 (HQ and HI for the animals muscle and liver), it was noted that in muscle tissue, the HQ values ranged from 5.60×10^{-3} to 1.84×10^{-1} for children and from 2.81 \times 10⁻³ to 9.20 \times 10⁻² for adults. In the liver, the HQ values were observed to vary from 7.55×10^{-3} to 2.57×10^{-1} for the children and from 3.77×10^{-3} to 1.29×10^{-1} for the adults. Furthermore, the muscle HI values were recorded as 0.58 (children) and 0.29 (adults); whereas the liver HI values were 0.75 for children and 0.38 for adults, respectively. These outcomes depicted that there are no non-carcinogenic risks, associated with both muscle and liver consumption by the children and adults age groups, since their HI results were >1 (HI >1) for both the animals' muscle and liver. According to FAO/WHO, consumption of items with heavy metals HI values higher than 1 could lead to detrimental noncarcinogenic consequence. According to FAO/WHO guidelines, the consumption of items with heavy metal HI result exceeding 1 portrays a latent risk of detrimental non-carcinogenic health effects (Atikpo et al., 2021; Uguru et al., 2024).

Remarkably, the HI values of the HMs in the meat and liver followed this descending order: Cr > As > Pb > Cd > Cu > Zn > Ni. This specifies that Ni posed the least health hazards, while Cr had the uppermost health challenges, which are licked to animal consumption. Furthermore, this experimental investigation outcome highlighted that the ingestion of either tamed or wild animal's meat products, has no substantial health danger to the human (children and adult) populations. Emurotu et al. (2024) stated that goat and cow muscle's HI values were considerably lower, compared to those of goat and cow liver; their findings aligned with this study's observations. Particularly, the computed

TABLE 7 EDI values of the HM (mg/kg/day), HQ values (unit less), and HI values (unit less).

Parameter	EDI*				HQ*			
	Meat		Liver		Meat		Liver	
	Children	Adult	Children	Adult	Children	Adult	Children	Adult
Cd	9.60 ×10 ⁻⁵	4.80×10^{-5}	1.22×10^{-4}	6.11×10^{-5}	9.60×10^{-2}	4.80×10^{-2}	1.22×10^{-1}	6.11×10^{-2}
Pb	1.48×10^{-4}	7.42×10^{-5}	1.80×10^{-4}	9.00×10^{-5}	1.06×10^{-1}	5.30×10^{-2}	1.29×10^{-1}	6.43×10^{-2}
Cr	5.52×10^{-4}	2.76×10^{-4}	7.72×10^{-4}	3.86×10^{-4}	1.84×10^{-1}	9.20×10^{-2}	2.57×10^{-1}	1.29×10^{-1}
As	4.36×10^{-5}	2.18×10^{-5}	5.73×10^{-5}	2.86×10^{-5}	1.45×10^{-1}	7.27×10^{-2}	1.91×10^{-1}	9.53×10^{-2}
Cu	1.26×10^{-3}	6.29×10^{-4}	1.60×10^{-3}	8.01×10^{-4}	3.15×10^{-2}	1.57×10^{-2}	4.00×10^{-2}	2.00×10^{-2}
Zn	2.89×10^{-3}	1.44×10^{-3}	3.18×10^{-3}	1.59×10^{-3}	9.63×10^{-3}	4.80×10^{-3}	1.06×10^{-2}	5.30×10^{-3}
Ni	1.12×10^{-4}	5.62×10^{-5}	1.51×10^{-4}	7.53×10^{-5}	5.60×10^{-3}	2.81×10^{-3}	7.55×10^{-3}	3.77×10^{-3}
НІ					0.58	0.29	0.75	0.38

^{*} average of all the samples animals.

TABLE 8 The CR and TCR values of the HMs.

Parameter	CR						
	Meat		Liv	ver			
	Children Adult		Children	Adult			
Cd	3.65E-05	1.82E-05	4.64E-05	2.32E-05			
Pb	1.26E-06	6.31E-07	1.53E-06	7.65E-07			
Cr	2.76E-04	1.38E-04	3.86E-04	1.93E-04			
As	6.54E-05	3.27E-05	8.60E-05	4.29E-05			
Ni	9.41E-05	4.72E-05	1.27E-04	6.33E-05			
ΣCR	4.73E-04	2.37E-04	6.47E-04	3.23E-04			

HI and HQ results of this study, were observed to be greater than the results documented for poultry products (Kia et al., 2024); though, this research's results were lower than the findings documented for animals' offal (Mohamed et al., 2023; Emurotu et al., 2024).

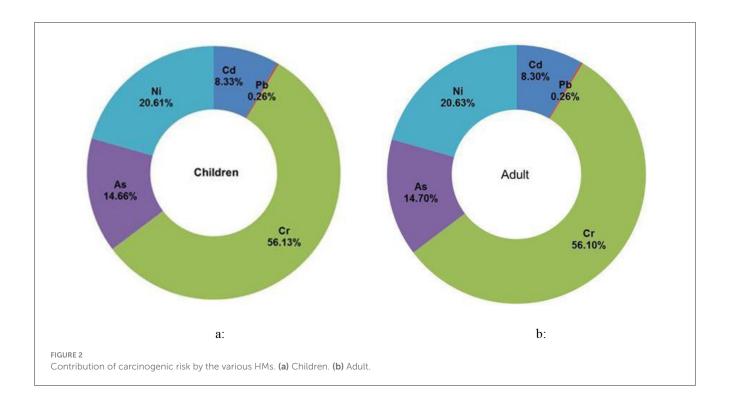
Carcinogenic risk (CR)

The CR and TCR results of the sampled animal's muscles and liver are given in Table 8. The children's CR results were: 3.65×10^{-5} for Cd, 1.26×10^{-6} for Pb, 2.76×10^{-4} for Cr, 6.54×10^{-5} for As, and 9.41×10^{-4} for Ni. Then for the adults, CR values for the meat samples for Cd, Pb, Cr, As and Ni, were 1.82×10^{-5} , 6.31×10^{-7} , 1.38×10^{-4} , 3.27×10^{-4} and 4.72×10^{-6} , respectively. Regarding the animals liver specimens, children CR values were $4.64\ 10^{-5}$ (Cd), 1.53×10^{-6} (Pb) 3.86×10^{-4} (Cr), 8.60×10^{-5} (As) and 1.27×10^{-4} (Ni). Similarly, the adult CR values for the liver samples were 2.32×10^{-5} , 7.65×10^{-7} , 1.93×10^{-4} , 4.29×10^{-5} and 6.33×10^{-5} for Cd, Pb, Cr, As and Ni, respectively. Additionally, the results highlighted that the meat TCR values were 4.73×10^{-4} and 2.37×10^{-4} for children and adult age groups; likewise, the liver TCR values were 6.47×10^{-4} and 3.23×10^{-4} for children and adults, respectively. It was observed that the CR

and TCR values were $<\!1.0\times10^{-4},$ regarding of the individual age bracket and animal part, signifying that the potential toxic metals did not posed significant cancer risk to human beings. This is an indication that the potential toxic metals evaluated in this research did pose major concern in the ingestion of the muscle and liver of the animals.

According to USEPA, TCR value lowers than 1.0×10^{-4} poses significant carcinogenic risk to human beings (Uguru et al., 2024). As shown by the results, the CR and TCR of the children were greater compared to the results recorded for the adults, which is similar to the observations made by Hossain et al. (2023). Also the higher liver CR values in children suggest that prolonged exposure of children to liver consumption must not be ignored. The TCR values observed in this study was lower than those reported by these researchers (Kia et al., 2024; Zhou et al., 2022). Emurotu et al. (2024) stated in their study into animal nutrition and toxicity that the TCR values for cow and sheep in both children and adult exceeded 1.0, making their ingestion having serious toxicological concerns. In contrast, the CR and TCR level recorded in this study were lower, when equated to those reported for poultry meat (Chowdhury and Alam, 2024). The differences in the HI, CR and TCR values observed among the different authors could be attributed to the sampling methods, number of animals species captured during the sampling process, number of individual samples collected, climatic conditions and other anthropogenic errors. The total numbers of samples and animals species examined during sampling operation have a significant impact on the experimental results, as different species possess distinct nutritional and physiological characteristics, while total number of samples significantly affects the descriptive statistics (Kia et al., 2024). Likewise, Uguru et al. (2024) stated that Environmental factors and Anthropogenic Factors play crucial roles in influencing health hazards associated with concentration and absorption of heavy metals and other contaminants.

Figure 2 shows the contribution of health hazards associated with the different HMS metals according to their CR levels for the two age categories. It was noted that Cr had the maximum contribution to the TCR level, which is 56.13 and 56.10% for children and adults respectively. Also, it was noted that Pb



contributed the least quota to the TCR level, contributing only 0.26% for both children and adults. This is similar to the reports of from other researchers (Emurotu et al., 2024; Kia et al., 2024), which reported that Pb had a negligible contribution to the carcinogenic risk related with meat product consumption. Interestingly, this research's outcomes will enhance livestock production, through several innovations in animal husbandry practices, such as proper waste management, controlled grazing and supplementary feeding.

Conclusion

Free range animal rearing system is viable tool for monitoring terrestrial pollution in livestock management. The amount of heavy metals (Cd, Pb, As, Cr, Ni, Zn, and Cu) and microbial levels in the muscles (meat) and livers of cows, goats, and wild animals were determined, to evaluate the effects of environmental contamination on livestock management. It was noted that the heavy metals (HMs) and pathogens levels, in the soil were greater than the amount verified for the vegetation. Remarkably, the results highlighted that there is a link between the environmental contamination and HMs and microbial loads in the animals' bodies. Furthermore, the results specified that cow tissues have significantly higher levels of HMs and pathogens compared to those from cows, goats, and bush animals. Additionally, the study outcomes depicted that animal's liver was more susceptible to HMs accumulation and microorganisms' infestation than the muscle. The HPLC analysis detected traces of aflatoxins in the animal bodies; however, the levels were below the maximum limit of 10 ppb established by World Health Organization for edible meat products. Interestingly, the pathogenic microorganisms (Aspergillus niger, Penicillium spp, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa) detected in the animals' bodies can significantly impact their productivity. The computed hazard index and cancer risk values portrayed that the ingesting the animal samples tends to posed no non-carcinogenic or carcinogenic risks to human beings. Conclusively, this research outcome emphasizes the need for environmental monitoring to prevent the accumulation of HMs and pathogens in animal and human bodies, along with the associated risks in animal production and the food supply chain.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

Ethical approval was not required for the study involving animals in accordance with the local legislation and institutional requirements because only commercially available samples were used.

Author contributions

SAlhar: Writing – original draft, Writing – review & editing. HU: Writing – original draft, Writing – review & editing. OA:

Writing – original draft, Writing – review & editing. RS: Writing – original draft, Writing – review & editing. MA: Writing – original draft, Writing – review & editing. SAlo: Writing – original draft, Writing – review & editing. SA-O: Writing – original draft, Writing – review & editing. JA: Writing – original draft, Writing – review & editing. RZ: Writing – original draft, Writing – review & editing. HH: Writing – original draft, Writing – review & editing. RHK: Writing – original draft, Writing – review & editing. SAlm: Writing – original draft, Writing – review & editing. SAlhaj: Writing – original draft, Writing – review & editing. RA: Writing – original draft, Writing – review & editing. AA: Writing – original draft, Writing – review & editing. SQ: Writing – original draft, Writing – review & editing. SQ: Writing – original draft, Writing – review & editing.

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The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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