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Assessment of heavy metal contamination in faeces of cattle grazing in wastewater treatment plants in Limpopo Province, South Africa

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Abstract

In Limpopo Province, South Africa, livestock commonly graze freely around wastewater treatment plants (WWTPs), raising contamination concerns and prompting the assessment of heavy metal levels in palatable grasses, sludge liquors, and cow faeces. Twenty-one cow faecal samples were collected from two WWTPs and a control farm, while 12 palatable grass samples and 16 sludge liquor samples were collected from two WWTPs. Samples were collected from February to March 2024 and analysed for heavy metals using inductively coupled plasma-mass spectrometry. Heavy metal concentrations in the grass samples were within the maximum tolerable limits for cattle feed and in the order aluminium > zinc > copper > chromium > strontium > nickel > arsenic > selenium > cadmium. In the sludge liquor, metals such as aluminium, copper, mercury, and lead exceeded permissible limits for livestock drinking water, posing a health risk for both the animals and the consumers of the animal products. The mean concentrations in the faecal samples were in the order aluminium > zinc > strontium > copper > chromium > nickel > lead > arsenic > selenium > cadmium; in most cases, the control farm faecal samples contained significantly higher concentrations than the WWTP faecal samples. The strong correlations found between the heavy metal concentrations in the faeces, grass, and sludge liquor samples suggest that the grass and sludge liquor are contaminating the animal tissue. These findings indicate that livestock should be prevented from grazing near WWTPs and that heavy metal concentrations in animal products from the study area need regular monitoring.

Keywords: contamination, ICP-MS, livestock, palatable grass, sludge liquors

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Introduction

Meat and animal products are key components of the human diet and are important sources of protein, minerals, and vitamins (Zhou *et al.*, 2019; Numa Pompilio *et al.*, 2021; Hassan Emami *et al.*, 2023). However, animal products can also be sources of hazardous substances, such as heavy metals. Previous

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studies, such as Zhou *et al.* (2019), Hejna *et al.* (2019), and Numa Pompilio *et al.* (2021), have reported on the accumulation of metals in animal products through various exposure routes, and the resultant contamination of food products from such animals. The heavy metal contamination of animal products presents a public health concern because of the various disorders and health impacts such contaminants cause in consumers, particularly children (Numa Pompilio *et al.*, 2021). In addition to human health concerns, the bioaccumulation of heavy metals can impact animal health, as these metals are not biodegradable. This means that they are likely to accumulate in the animal's internal organs, potentially causing damage (Ding *et al.*, 2022).

The major sources of metals and trace elements in livestock are the diet and drinking water. This suggests the need to control and prevent their accumulation in these media. Heavy metals in animal diets can be present as undesirable contaminants or as essential nutrients (Hejna *et al.*, 2019). Undesirable contaminants can occur in the diet through forage and water contaminated with industrial waste, pesticide residues, or urban runoff, or through natural events such as volcanic processes (Numa Pompilio *et al.*, 2021). The addition of minerals such as arsenic, copper, and zinc to animal feed is also a common practice, as these minerals can stimulate growth and have antimicrobial properties (Zhang *et al.*, 2012).

Upon uptake by animals, minerals and nutrients that are not retained in the animal system or transferred to consumed products, such as milk, are excreted in the urine and faeces. Factors such as animal status (e.g. age and health), the source of the minerals (inorganic or organic), and the composition of the diet all determine the bioavailability of these minerals (Hejna *et al.*, 2019). Research has shown that animals retain only 5%–15% of the metals they consume, with the majority being excreted via urine and faeces, thereby contaminating the surrounding environment (Ntui *et al.*, 2014).

Limpopo Province is one of South Africa's nine provinces and is found in the northern part of the country. The province's population is predominantly rural and poor, with about 89% living in rural areas (Rootman *et al.*, 2015). As in many communal areas in South Africa, much of the land (80%–85%) is used as grazing land, and these areas support 50% of the country's livestock (Rootman *et al.*, 2015). Livestock farming, and particularly cattle farming, therefore plays an important role as the main source of livelihood for the rural poor in the province. A common practice in the province is letting cattle graze freely with little monitoring. The authors observed cattle grazing in many areas, including within the perimeters of sewage treatment plants. This occurs because the fences securing the treatment plants are often vandalised, and the grasses inside these plants remain evergreen because of the nutrient-rich water emanating from the treatment processes. In addition, nutrient-rich sludge is normally spread on the grass around the treatment plants.

Such easy access to sewage treatment plants by cattle has prompted concerns about their well-being, as the wastewater and sludge present in these areas are known to be contaminated with pollutants such as heavy metals. In addition to the animals' well-being, there are also risks to human health from indirect exposure to heavy metals through the consumption of contaminated meat and animal products. This study, therefore, aimed to establish the levels of selected heavy metals in cattle faecal samples from the selected wastewater treatment plants (WWTPs), as well as in the grass and sludge liquors around the WWTPs where the animals regularly graze.

Materials and methods

Sample location and site description

Cow faeces, palatable grass, and sludge liquor samples were collected from three WWTPs and one farm, all located in the Limpopo Province of South Africa. Figure 1 shows the location of the sampling points within the Limpopo Province. Wastewater treatment plant 1 (WWTP1) consists of three waste stabilisation ponds (two primary and one secondary maturation pond), while WWTP2 and WWTP3 are used for activated sludge wastewater treatment processes. Cow faecal samples were collected at WWTP1 and WWTP2, as these have damaged perimeter fences and regularly receive visits from cows from neighbouring villages that graze within the facilities. Cow faecal samples were also collected from a privately-owned farm some distance from the WWTPs, for comparison. Plant (palatable grass) and sludge liquor samples were collected from WWTP2 and WWTP3, as these facilities generate sludge. The dried sludge residues are normally spread on the nearby grass or given to community members for use as manure. The three WWTPs were selected because of their accessibility and the visible presence of cow faeces and cattle grazing within their perimeter fences.

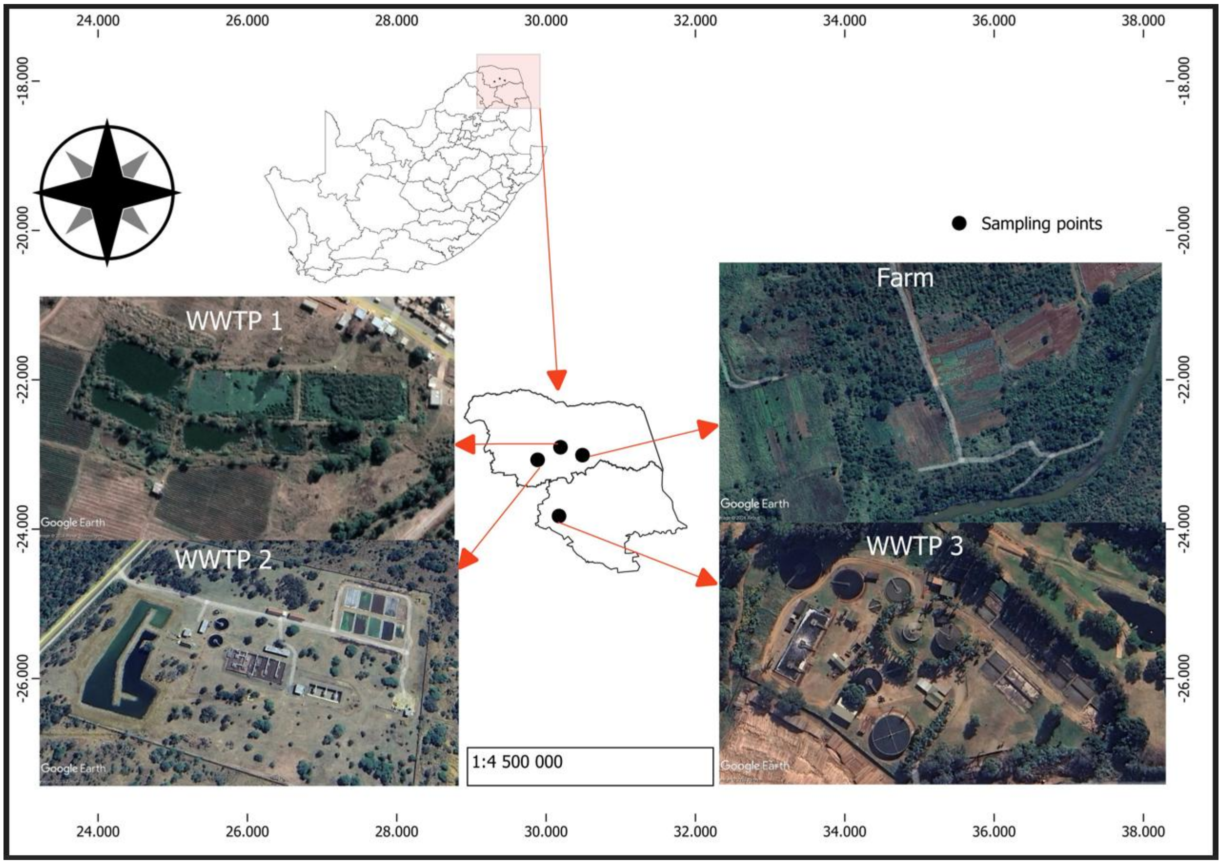

Figure 1 The location of the Limpopo Province within South Africa, and the location of the three wastewater treatment plants (WWTP 1–3) and one farm from which samples were collected, within the Limpopo Province.

Sample collection and preparation

Nine cow faecal samples were collected from WWTP1, six from WWTP2, and six from the control farm. Eight sludge liquor samples were collected from WWTP2 and WWTP3 (16 in total), and six plant samples were collected from each of these two WWTPs (12 in total). Samples were collected on 19 February 2024 and again on 25 March 2024. Cow faeces and plant samples were collected in polyethylene sampling bags, which were tightly closed to prevent air from entering, and were dried in an oven at 80 °C for 24 hours. The dried samples were then ground to a fine powder using a mortar and pestle, and this powder was sieved to ensure a particle size of less than 100 µm. The ground and sieved samples (500 mg) were then microwave-digested using aqua regia digestion.

For microwave digestion, 500 mg of the dried sample was placed into the digestion vessel, and 12 mL of the aqua regia solution (3 mL HNO_3 + 9 mL HCl) was added. The vessels were tightly closed, and the samples were microwave-heated using a program adapted from Niemelä *et al.* (2012). In brief, the vessels were heated at 200 °C for 15 minutes and held at 200 °C for 10 minutes. Upon digestion, the mixture was allowed to cool, then filtered using Macherey-Nagel No.1 filter paper (0.45 µm) (Macherey-Nagel, Germany), and the filtrate was diluted to the 50 mL mark in a volumetric flask using deionised water (Milli-Q). The diluted samples were then stored at 4 °C in a refrigerator until they were sent to the

Stellenbosch University Central Analytical Facility for the determination of major, minor, and trace elements (combined). The concentrations of the elements were determined using an Agilent 7900 (USA) inductively coupled plasma-mass spectrometer (ICP-MS) and a Thermo iCap 6300 (USA) inductively coupled plasma-atomic emission spectrometer (ICP-AES). The ICP-AES was used for the elements present in relatively high concentrations (major elements), such as sodium, magnesium, phosphorus, potassium, and calcium, because of its lower sensitivity, while the ICP-MS was used for the trace elements, such as boron, aluminium, silicon, vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc, arsenic, selenium, strontium, molybdenum, cadmium, tin, antimony, barium, mercury, and lead, because of its lower detection limits.

Sludge liquor samples were collected in 250 mL polyethylene bottles and immediately acidified with a drop of HNO_3 to prevent precipitation, and then transported in a cooler to the laboratory. The samples were filtered upon arrival using Macherey-Nagel No.1 filter paper (0.45 µm). The filtrate was kept at 4 °C in a refrigerator and then sent to the Stellenbosch University Central Analytical Facility for determination of the major, minor, and trace elements (combined) using the ICP-MS and ICP-AES, as described above.

Data analysis

The Kolmogorov–Smirnov and Bartlett tests were used to test for normality and variance homogeneity at $P \leq 0.05$. Data that passed these tests were compared using analysis of variance (ANOVA), while data that did not pass these tests were compared using the Kruskal–Wallis test. The ANOVA/Kruskal–Wallis tests were used to compare the mean concentrations of the heavy metals in the cow faeces and plant samples from the different sampling sites using GraphPad InStat 3 (GraphPad Software, California, United States), with significance declared at $P < 0.05$. The concentrations of the metals in the different media are reported as the means ± the standard deviations.

Where there were significant differences between locations, the Tukey–Kramer multiple comparisons test and Dunn's multiple comparisons test were used as post-hoc assays for data that passed the normality test and data that did not pass the normality test, respectively. In addition, the mean levels of the analysed metals in the sludge liquors and plant samples were compared to the permissible limits for these metals in livestock diets and drinking water. To correlate the concentrations of the metals in the various media tested (i.e. sludge liquor versus cow faeces, and palatable grass versus cow faeces), linear regression using IBM SPSS (Statistical Package for the Social Sciences) version 29 was used.

Results and discussion

Metals in plant material

The levels of metals in the palatable grass samples collected from the WWTPs are presented in Table 1. The concentrations of the metals were in the order: aluminium > zinc > copper > chromium > strontium > nickel > arsenic > selenium > cadmium. Of the ten heavy metals commonly found in wastewater treatment sludges, none exceeded the maximum tolerable limits for heavy metals in cattle feed in either the grass samples from sites next to the sludge beds or those further away. Other than for strontium, nickel, and chromium, significantly higher levels of heavy metals were detected in plants collected near the sludge beds than in plants collected further away from the sludge beds.

In the samples collected near the sludge beds, aluminium had the highest mean concentration of 473.56 mg/kg, followed by zinc, which had a mean concentration of 82.05 mg/kg. The lowest mean concentrations were recorded for cadmium and selenium (0.04 and 0.07 mg/kg, respectively). The levels of heavy metals recorded in this study were comparable to those reported in similar studies. Adelusi *et al.* (2024) reported mean concentrations of 2.372 mg/kg for chromium, 0.594 mg/kg for copper, and 1.977 mg/kg for zinc in cattle feed collected in Limpopo Province. In the current study, slightly higher mean concentrations were reported; for example, chromium averaged 5.34 mg/kg, copper 2.64 mg/kg, and zinc 16.89 mg/kg in the samples collected from the sites further away from the sludge beds. Even though the samples were collected within the same province, the higher concentrations in our study can be attributed to the proximity of the sampling sites to the WWTP sludge beds and the sludge being washed away by rain to contaminate the surrounding areas.

In another study, Abah *et al.* (2017) assessed the levels of heavy metals in pasture grass growing next to a dump site and reported mean concentrations of 0.37 mg/kg for arsenic, 0.54 mg/kg for lead, 1.07 mg/kg for nickel, 7.10 mg/kg for copper, and 8.85 mg/kg for chromium. Similar to our findings, Abah *et al.* (2017) reported higher mean levels in the contaminated site compared to the control site for all the heavy metals monitored, except for copper. A recent study by Raji & Palamuleni (2023) investigated metal contamination in soil and plants near a gold mining area in the North West Province of South Africa. The study measured the arsenic, cadmium, lead, and zinc concentrations in soil and plants within 500 m and 1000 m radii of the mine. The results indicated that while the concentrations of these metals were below national and international thresholds, they were higher than concentrations found several kilometres away from the mining area, suggesting localised contamination due to mining activities. The results reported by both Abah *et al.* (2017) and Raji & Palamuleni (2023) highlight the impact of anthropogenic activities, such as mining, on metal contamination in the environment, and emphasise the need for monitoring heavy metal levels in areas affected by such activities to mitigate potential ecological and health risks.

Although the heavy metal concentrations reported here were within the permissible limits for consumption by cattle, Mbangi *et al.* (2018) found that the long-term application of wastewater sludge to plants increased the concentrations of zinc, lead, cadmium, nickel, and chromium to levels beyond acceptable, thus posing health risks to consumers. According to Mbangi *et al.* (2018), 35% of surveyed sludge in South Africa exceeded the permissible limits for at least two metals. Agoro *et al.* (2020) similarly studied the accumulation of heavy metals in sewage sludge in the Eastern Cape Province of South Africa, assessing the distribution of five heavy metals (cadmium, lead, copper, zinc, and iron) across various stages of treatment in three sewage treatment facilities and their receiving waterbodies. The results indicated that the concentrations of these metals in the sludge samples were generally below the recommended limits for agricultural use. However, the study also noted that copper, cadmium, iron, and lead levels were higher than those reported in other regions, suggesting that local factors may influence metal concentrations in sewage sludge (Agoro *et al.*, 2020).

Table 1 Metal concentrations in grass samples collected from wastewater treatment plants in the Limpopo Province of South Africa (mean ± SD)

Metal (mg/kg)	Adjacent to sludge beds		>20 m from sludge beds		MTL (mg/kg)
	Mean ± SD	Range	Mean ± SD	Range	
Lead[1]	$1.07^a \pm 0.02$	1.05–1.09	$0.76^b \pm 0.31$	0.36–1.01	100
Cadmium[1]	$0.04^a \pm 0.02$	0.03–0.06	$0.02^b \pm 0.01$	0.01–0.04	10
Strontium[1]	$4.39^a \pm 1.01$	3.34–5.50	$3.52^a \pm 2.71$	1.06–6.90	2000
Selenium[1]	$0.07^a \pm 0.02$	0.04–0.08	$0.04^b \pm 0.02$	0.02–0.07	5
Arsenic[1]	$0.12^a \pm 0.01$	0.11–0.14	$0.08^b \pm 0.05$	0.04–0.14	-
Zinc[1]	$82.05^a \pm 4.13$	76.01–86.73	$16.89^b \pm 6.89$	8.28–23.12	500
Copper[1]	$6.96^a \pm 0.13$	6.82–7.10	$2.64^b \pm 1.02$	1.40–3.66	40
Nickel[1]	$2.73^a \pm 0.69$	2.00–3.52	$2.30^a \pm 1.53$	0.77–4.15	100
Chromium[1]	$5.05^a \pm 1.05$	3.95–6.06	$5.34^a \pm 3.50$	1.73–9.51	100
Alumium[2]	$473.56^a \pm 150.84$	304.61–617.88	$307.31^b \pm 45.42$	248.83–340.26	1000

[ab] Different superscript letters in the same row indicate significant differences between the mean concentrations in samples from different sites (P <0.05), as determined by the [1]unpaired *t*-test and [2]Mann-Whitney U test. SD: standard deviation; MTL: maximum tolerable level established for cattle by the National Research Council (NRC, 2005; Weiss, 2008).

The elevated levels of these metals, as reported in this study, might not pose an acute health risk to the animals; however, the risk and likelihood of long-term impacts due to intense grazing in such contaminated areas remains a concern. Prolonged consumption of such contaminated pasture grass by animals leads to the bioaccumulation of metals in animal tissue, which can result in health risks to the consumers of the animal products (Abah *et al.*, 2017). Heavy metals are persistent and non-biodegradable and are eventually excreted in animal products such as milk (Abah *et al.*, 2017).

Metals in sludge liquors

The mean concentrations of metals in the sludge liquor samples are presented in Table 2. Aluminium had the highest mean concentration in the liquor samples from the two WWTPs, with the liquor samples from WWTP3 containing the highest concentration of aluminium (79 628.66 mg/L). Other metals with high mean concentrations in the liquors were zinc (7804.43 mg/L at WWTP3), manganese (1556.14 mg/L at WWTP3), and strontium (443.56 mg/L at WWTP3). The metals with the lowest mean concentrations were cadmium (1.35 mg/L at WWTP1) and mercury (0.51 mg/L at WWTP1). In general, liquor samples from WWTP3 contained higher concentrations of heavy metals than samples from WWTP2. This can be attributed to the intensity of industrial activities in and around WWTP3, which contribute high loads of heavy metal-laden wastewater to the plant.

As cows have been observed drinking these sludge liquors on several occasions, particularly at WWTP2, the mean concentrations of heavy metals recorded in these liquors were compared to the Department of Water Affairs and Forestry (DWAF) (1996) guidelines for livestock consumption. The mean concentrations of aluminium in liquor samples from both WWTPs exceeded the permissible limits for cattle consumption. The mean levels of copper and mercury in the liquor samples from WWTP3 also exceeded these limits. The mean concentrations of other metals, such as lead, did not exceed the limits, but were very high and exceeded the limits in some samples.

Generally, the levels of heavy metals reported here were much higher than those reported in previous studies. Giri *et al.* (2020) summarised the mean levels of heavy metals found in sludge liquors in a number of studies, and reported much lower concentrations than those found in our samples. For example, they reported ranges of concentrations in sludge liquors of 0.019–0.045 mg/L for cadmium, 0.0037–0.0054 mg/L for lead, 0.0268–0.0621 mg/L for arsenic, 1.03–1.04 mg/L for copper, 0.46–0.48 mg/L for zinc, and 0.85–0.86 mg/L for aluminium (Giri *et al.*, 2020). Generally, sludge and sludge liquors are known to be more contaminated with heavy metals than other water and waste streams. For example, Mesfin *et al.* (2020) reported much lower mean concentrations of lead (37.5 µg/L), chromium (62.25 µg/L), and cadmium (3.75 µg/L) in livestock drinking water. Such findings reiterate the high risks of allowing livestock to graze and drink from WWTPs.

Compared to other livestock, dairy cows, such as those observed at WWTP2, have much higher water requirements, as approximately 80% of their dietary water intake is used for milk production (Giri *et al.*, 2020). In addition, water is essential for digestion, maintaining electrolyte balance, transporting nutrients, proper excretion, and energy metabolism (Giri *et al.*, 2020). Poor water quality is thus associated with poor herd health, as it affects feeding habits, milk production, reproduction, and the balance of nutrients in the body (Schütz *et al.*, 2021). A recent study by Murtaza *et al.* (2022) reported on the bioaccumulation of potentially toxic elements such as cadmium, copper, lead, and zinc in animals consuming fodder irrigated with sewage water. This research underscores the importance of water quality in preventing the transfer of contaminants through the food chain, which can adversely affect both animal and human health. It is thus important to ensure good water quality to reduce the negative effects on the herd, particularly if it is a dairy herd (Wegener, 2012).

Table 2 Metal concentrations in sludge liquors from two wastewater treatment plants (WWTPs) in the Limpopo Province of South Africa

Metal (µg/L)	WWTP2		WWTP3		Upper-limit guideline (µg/L)[1]
	Mean ± SD	Range	Mean ± SD	Range	
Aluminium	19 093.24 ± 35 564.18	2.71–99 789.28	79 628.66 ± 65 679.66	15.12–15 5479.79	5000
Chromium	141.61 ± 224.40	0.23–529.22	308.46 ± 228.47	0.49–545.16	1000
Manganese	849.59 ± 1211.15	10.32–2695.94	1556.14 ± 324.38	1078.93–1799.79	10000
Nickel	91.85 ± 135.28	4.55–309.12	161.08 ± 89.14	43.85–259.42	2000
Copper	646.28 ± 979.99	9.06–1961.11	1791.25 ± 1282.84	12.06–2836.96	1000
Zinc	3705.55 ± 5739.32	2.01–11 309.43	7804.43 ± 5811.07	213.73–13 272.47	20000
Arsenic	7.74 ± 7.49	2.44–19.08	28.35 ± 17.93	6.19–43.22	1000
Selenium	7.73 ± 9.68	0.98–22.10	11.45 ± 7.83	1.05–19.63	50
Strontium	466.94 ± 468.31	69.31–1365.64	443.56 ± 153.01	258.21–621.95	-
Cadmium	1.35 ± 1.98	0.00–	6.24 ± 5.07	0.02–11.20	10
Mercury	0.51 ± 0.73	0.00–	6.85 ± 5.00	0.00–11.45	1
Lead	18.02 ± 27.95	0.00–	81.97 ± 56.43	0.09–128.34	100

SD: standard deviation. [1]DWAF (1996).

Metals in cattle faecal samples

The metal concentrations in the cattle faecal samples are shown in Table 3. As found in the other media tested (plant material and sludge liquors), aluminium had the highest mean concentration, of 1044.27 mg/kg, in samples from the control farm. Other metals with high mean concentrations in the cattle faecal samples were zinc (19.79 mg/kg) and strontium (53.34 mg/kg). Generally, concentrations of metals were higher in the control farm samples than in the samples from the two WWTPs. The order of mean metal concentrations in the faecal samples from the three sites sampled was aluminium > zinc > strontium > copper > chromium > nickel > lead > arsenic > selenium > cadmium.

Table 3 Metal concentrations in faecal samples from cattle grazing at two wastewater treatment plants (WWTPs) and one private farm in the Limpopo Province of South Africa

Metal (mg/kg)	WWTP1		WWTP2		Control farm	
	Mean	Range	Mean	Range	Mean	Range
Lead	0.32^{a}	0.21–0.64	0.44^{a}	0.10–0.94	0.56^{a}	0.39–0.75
Cadmium	0.01^{a}	0.00–0.01	0.01^{a}	0.00–0.03	0.01^{a}	0.01–0.01
Strontium	11.37^{a}	7.55–14.65	9.71^{a}	6.42–11.46	53.34^{b}	42.40–67.16
Selenium	0.08^{a}	0.06–0.12	0.07^{a}	0.039–0.08	0.08^{a}	0.08–0.10
Arsenic	0.10^{a}	0.06–0.20	0.17^{a}	0.06–035	0.53^{b}	0.47–0.60
Zinc	26.34^{a}	12.63–44.33	16.46^{a}	9.50–23.14	19.79^{a}	15.67–24.31
Copper	7.54^{a}	3.67–12.34	3.60^{b}	2.608–5.21	4.60^{b}	4.022–5.29
Nickel	1.68^{a}	0.94–2.87	1.35^{a}	0.943–1.90	3.15^{b}	2.121–3.52
Chromium	1.79^{a}	0.99–2.48	1.59^{a}	1.169–2.52	3.88^{b}	3.077–4.97
Aluminium	290.99^{a}	72.20–719.35	364.13^{ab}	121.19–809.93	1044.27^{b}	304.17–1654.40

^{abc} Different superscript letters in the same row indicate significant differences between the mean concentrations in samples from the different locations (P <0.05), as determined by one-way analysis of variance.

Significantly higher mean concentrations of strontium, arsenic, nickel, chromium, and aluminium were found in the samples from the control farm than in the samples from the two WWTPs. Significantly higher mean concentrations of copper were found in the samples from WWTP1 than in the samples from WWTP2 and the control farm. Aluminium concentrations were significantly higher in the samples from the control farm than in the samples from WWTP1, with the samples from WWTP2 containing intermediate concentrations.

The higher concentrations of metals found in the control farm samples can be explained by the fact that communal farmers usually leave their livestock to graze freely, and do not control what they consume. In contrast, the cattle that usually infiltrate WWTP2 are owned by a commercial dairy farm. Consequently, while these cattle may infiltrate the premises of the WWTP and consume contaminated water and palatable grass, their diet is otherwise largely controlled and monitored by the farm owners. Zhang *et al.* (2012) similarly reported significant differences in the concentrations of heavy metal residues in cattle faecal samples from farms of different scales. Zhang *et al.* (2012) reported higher cadmium levels in samples from highly-intensive farms than in samples from medium-scale farms, and attributed this to the abuse of metal additives in livestock diets by small-scale farmers, the lack of supervision by government officials, and the farmers' lack of scientific knowledge. Recent studies, such as Mu *et al.* (2020), have corroborated these findings, highlighting the influence of farm scale and management practices on heavy metal contamination in animal faeces. According to Mu *et al.* (2020), a nationwide survey in China reported that pig faeces contained significantly higher concentrations of copper and other heavy metals than cattle faeces, with copper levels in pig faeces being 4.9–17.5 times higher than in cattle faeces. This disparity is partly because of the extensive use of copper additives in pig feed, which is a less prevalent practice in cattle farming.

It is important to note the pattern of heavy metal concentrations in the sludge liquor, plant, and cattle faecal samples from WWTP2 (Figure 2 and Table 4). As depicted in Figure 2 and confirmed in Table 4, a similar pattern/trend can be noted in the concentrations of the heavy metals in the three different media. The results of the linear regression analysis suggest that the concentrations of the heavy metals in the sludge liquors have a significant relationship with the concentrations of these metals in the palatable grass and cow faecal samples.

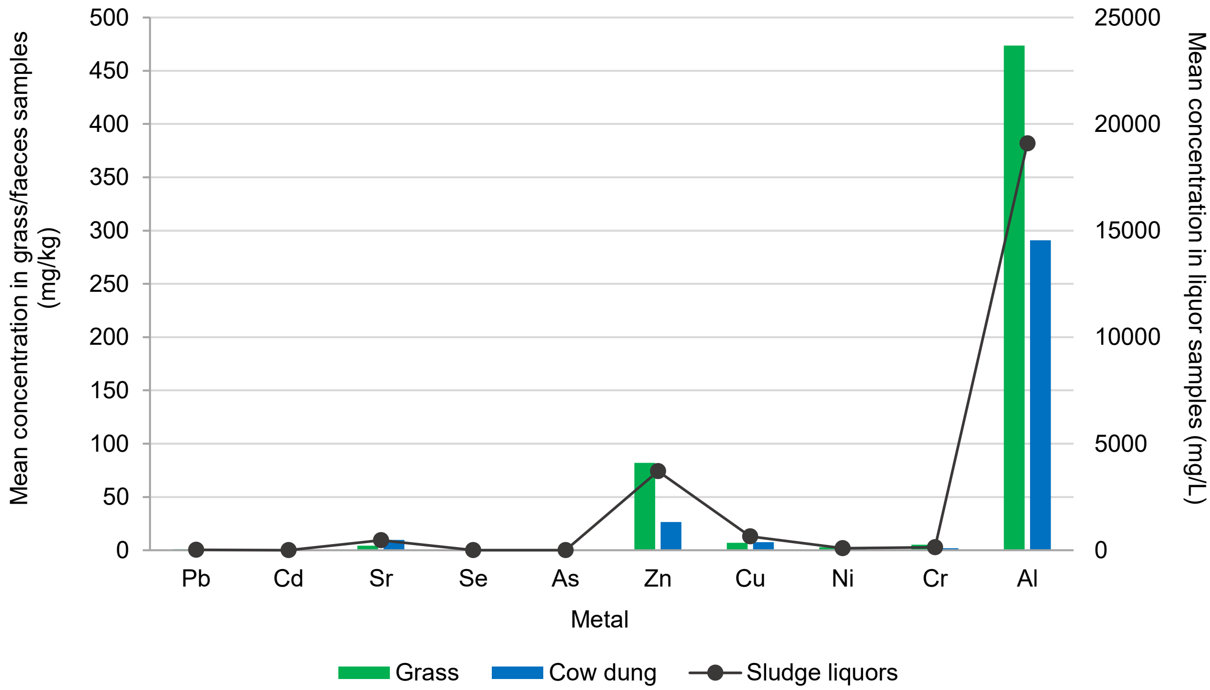

Figure 2 Metal concentrations in palatable grass, cattle faeces, and sludge liquor samples collected from a wastewater treatment plant in the Limpopo Province of South Africa (Pb: lead, Cd: cadmium, Sr: strontium, Se: selenium, As: arsenic, Zn: zinc, Cu: copper, Ni, nickel, Cr: chromium, Al: aluminium).

Table 4 Relationships between metal concentrations in sludge liquors and in palatable grass and cow faecal samples collected from wastewater treatment plants in the Limpopo Province of South Africa

Dependent variables	F	df	*P*-value	R^2 (adjusted)
Palatable grass[1]	8910.103	1, 8	<0.001[2]	0.999
Cattle faeces[1]	708.362	1, 8	<0.001[2]	0.987

[1] Dependent variables: palatable grass, cattle faeces. [2] Predictors (constant): sludge liquors.

Previous studies have assessed the levels of heavy metals in cow faeces, mainly as potential sources of contamination for soils and water when applied as manure or fertilisers. Zhang *et al.* (2012) monitored the levels of heavy metals in faecal samples from large cattle farms in Northeast China and found mean concentrations of 31.37 mg/kg for copper, 136.13 mg/kg for zinc, 2.71 mg/kg for arsenic, 0.24 mg/kg for chromium, 0.73 mg/kg for cadmium, and 2.68 mg/kg for lead. These reported concentrations of copper, zinc, arsenic, cadmium, and lead in cattle faeces are much higher than those found in the current study. Compared to our findings, Ntui *et al.* (2014) found higher concentrations of nickel and lead, and lower concentrations of zinc, chromium, and copper, in cattle faecal samples collected in the Bauchi urban area of Nigeria. Ntui *et al.* (2014) found mean concentrations of 4.79 mg/kg for zinc, 4.64 mg/kg for manganese, 2.80 mg/kg for nickel, 2.12 mg/kg for lead, 0.79 mg/kg for chromium, and 0.42 mg/kg for copper. However, Yasotha *et al.* (2014) reported copper concentrations in the range of 2.58 to 20.03 mg/kg in cattle manure, with a mean concentration of 7.06 mg/kg, which is comparable to the highest mean of 7.54 mg/kg we recorded for copper in samples from WWTP1.

Overall, the presence of high concentrations of metals in the cow's faecal material, as reported in Table 3, could be an indication of potential contamination of the consumed products, such as meat and milk. Metals such as aluminium, zinc, copper, and strontium were found in high concentrations in the faecal material. However, of particular concern are heavy metals such as cadmium, lead, and arsenic, which are toxic even at trace levels and were present in all the samples. According to Guo *et al.* (2024), most heavy metals (70%–80%) are excreted in the faeces and utilisation in the animal's body is about 10%–20%; this implies that at least some of these heavy metals are accumulated in the animal's body.

The presence of heavy metals in animals' faecal excretions reflects the presence of the contaminants in their diet and the conversion efficiency of these metals by the animals (Zhang *et al.*, 2012). The presence of metals such as copper in cow faeces has been attributed to it being a significant ingredient in pigments, pesticides, and dyes that are taken up by plants through the soil and consumed in the pasture grass by cattle (Ntui *et al.*, 2014). This is supported by recent local research by Webster *et al.* (2022), who studied the presence of potentially toxic elements in South African mammalian wildlife. Webster *et al.* (2022) found that faecal material was an effective matrix for trace element assessment in free-ranging wildlife species, as it reflected the metal content of their diets and the efficiency of conversion and excretion processes. Such studies underscore the utility of faecal analysis as a non-invasive method to monitor heavy metal contamination in animals, providing insights into dietary exposure and metabolic processing efficiency.

Similar to our findings, Deng *et al.* (2020) reported a positive correlation between heavy metal concentrations in the feed and the faecal matter. This suggests that even though animals have different accumulation abilities and metabolise these heavy metals differently, diet remains the primary source of these contaminants, and the contaminants largely pass through into the faeces (Guo *et al.*, 2024). The addition of heavy metal additives to livestock feed and the unmonitored grazing of livestock, resulting in their consumption of sludge-contaminated palatable grasses and sludge liquors, will thus have far-reaching consequences for the animals themselves and for human consumers of the meat products. Recent studies, such as Muhib *et al.* (2016), Chirinos-Peinado *et al.* (2021), and Elafify *et al.* (2023), have all reported the presence of heavy metals in animal products such as milk.

This study was limited by its short sampling duration and our limited access to other WWTPs. Since data was collected from February to March only, it reflects the occurrence of these heavy metals during a particular season, and might not reflect their occurrence at other times of the year. When interpreting the study findings, consideration should also be given to potential differences in metal bioavailability arising from unassessed soil or plant factors. Variability in parameters such as soil pH, organic matter content, cation exchange capacity, plant species-specific uptake mechanisms, and root exudate composition could significantly influence the mobility and availability of metals. As these factors were not directly measured in this study, their potential impact on the observed metal concentrations and uptake patterns should not be overlooked.

Conclusions

This study highlights the significant health risks posed by the intrusion of livestock into WWTPs in the Limpopo Province of South Africa. Although metal concentrations in palatable grasses were within tolerable limits for cattle feed, sludge liquors contained elevated levels of potentially hazardous metals such as aluminium, copper, mercury, and lead, surpassing permissible limits for livestock drinking water. The presence of these metals and the strong correlations between the metal concentrations in the cow faecal samples, grass samples, and sludge liquor samples indicate the likelihood of metal accumulation in animal tissues and suggest a potential risk to human consumers of the resultant animal products. These findings underscore the urgent need to restrict livestock access to WWTPs and implement routine monitoring of heavy metals in both the environment and animal products to safeguard animal and public health. Future studies examining metal accumulation in animal tissues, especially in organs like the liver and kidneys, are recommended. Such studies are crucial because of their implications for animal health, food safety, ecotoxicology, and human health through the food chain.

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Authors' contributions

G.K. Pindihama: conceptualisation, methodology, investigation, data curation, writing (original draft, and review and editing). R. Mudzielwana: supervision, funding and resource acquisition, conceptualisation, investigation, data curation, writing (review and editing).

Conflict of interest declaration

The authors declare that they have no conflicts of interest related to this study.

Data availability

All data used in this study are included in this published article, and supporting data are available from: Pindihama, G., 2023. Assessment of toxic metal contamination in cow dung of cattle grazing in wastewater treatment plants in Limpopo Province, South Africa (2025-02-13) (dataset). DOI: https://doi.org/10.6084/m9.figshare.24799185

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