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Seasonal variation in heavy metal intake and excretion by dairy cattle in an Indian megacity

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HIGHLIGHTS

- Metropolitan dairying is vital for milk supply in the Global South.
- Feed scarcity drives farmers to use lake fodder and food leftovers for their cows.
- There is a risk of regular intake of heavy metals from uncontrolled feed sources.
- While considerable heavy metal concentrations are found in cattle feces, cow milk is safe.
- Regulating lake fodder use and informing farmers about contamination risks can reduce the latter.

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ABSTRACT

Milk consumption plays a pivotal role in human nutrition, especially for children, due to its high protein, calcium, and vitamin contents that aid cognitive development. Nonetheless, potential hazards from heavy metal presence in milk, due to environmental exposure and intake through feed, have gained global attention. Given paucity of data we investigated the seasonal variation in the quantitative intake of heavy metals (cadmium: Cd, chromium: Cr, lead: Pb) by dairy cows in the South Indian megacity of Bengaluru, and the resulting heavy metal concentrations in milk and feces. The research involved 39 dairy farms across urban and peri-urban areas, where 281 feed, 329 milk, and 183 feces samples were collected along with management data during monsoon (July 2020 - August 2020), winter (November 2020 – February 2021) and summer (March 2022 – June 2022) seasons. During the summer season, Cd concentration in lake fodder averaged 1.6 mg/kg DM, which was higher than the 0.6 mg/kg DM during winter and 0.9 mg/kg DM during the monsoon season. The higher concentration resulted in an increased daily Cd intake of 13.7 mg/cow/day. Similarly, Cr and Pb intake were also highest in summer (Cr: 46.3 mg/cow/day, Pb: 11.7 mg/cow/day), whereby for both elements the concentrations were higher in non-lake feed than in lake fodder. With the exception of a few milk samples, Cr and Pb concentrations were below threshold levels and did not compromise milk safety, and Cd levels never raised concerns. Nevertheless, elevated Cr and Pb concentrations (Cr: 9.8–16.9 mg/kg DM, Pb: 4.1–10.6 mg/kg DM) were determined in feces across seasons, potentially endangering the environment and reintroducing these elements into the food chain through manure application on agricultural land. Rather than uniformly discouraging the use of lake fodder in Bengaluru, authorities on the one hand should caution fodder usage in pollution-prone areas, and on the other hand take measures to reduce contamination levels at those sites. Furthermore, it appears necessary to implement more stringent control measures for other types of feedstuffs. Raising farmers' awareness of the problem of heavy metals in the food chain could promote their compliance with regulations without the need for complete feed bans, which are difficult to enforce in a highly contested urban environment.

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1. Introduction

Due to its favorable nutritional profile, milk plays an important role in human nutrition, especially for children (Rumbold et al., 2022). However, the presence of persistent environmental contaminants, especially heavy metals such as Cd, Cr, and Pb, may pose a significant risk to the safety of dairy products for human consumption (Ismail et al., 2017; Boudebbouz et al., 2021; Khalef et al., 2022; FAO, 2023). In mammals, repeated consumption of Cd can impair cell proliferation, differentiation, and apoptosis, thereby increasing the risk of kidney, lung, breast, prostate, and urinary tract cancers (Waisberg et al., 2003; Baccarelli and Bollati, 2009). Pb mainly targets the central nervous system, causing psychiatric symptoms and impairing manual dexterity in humans. Because of its neurotoxic effect, it is more dangerous to the developing than to the mature brain. It is also carcinogenic to lung and stomach, and damages kidney and reproductive organs (Baccarelli and Bollati, 2009; EFSA, 2010; Chandrakar et al., 2018). Despite being an essential nutrient, Cr can also pose a carcinogenic risk to humans (Baccarelli and Bollati, 2009; Wang and Yang, 2023), and exposure to high Cr concentrations can lead to DNA damage, failure of the liver, kidney, and reproductive system (Costa and Klein, 2006; Chandrakar et al., 2018).

Due to the natural filtering function of the mammary gland (Nag, 2010), the transfer of heavy metals from cattle feed to milk is typically only 1 % (Blüthgen, 2000). Nevertheless, a presence of heavy metals in milk has been reported repeatedly: Milk from cows fed wastewater-irrigated fodder had higher concentrations of Cd, Cr, and Pb compared to milk produced in unpolluted areas of Pakistan (Farid and Baloch, 2012). Yasotha et al. (2021) reported that half of the milk samples from cows reared near industrial areas of the Indian cities Erode, Bhavani, Perundurai, Chennimalai, and Andiyur, had concentrations of Cd and Pb above the permissible threshold levels of 0.01 mg Cd and 0.02 mg Pb per kilogram of milk (FAO/WHO, 2021). Similarly, milk from cows reared in industrial and non-industrial areas in Haryana, India, showed Pb concentrations above the permissible limits, while Cd concentrations were uncritical. Yet, this study did not distinguish between milk from industrial *versus* non-industrial zones (Roy et al., 2009).

Across India, milk production is still dominated by rural farmers, although the contribution from urban farmers increases (DAHD, 2022). While rural farmers rely on self-produced forages and crop residues to feed their cows, urban dairy farmers encounter a general difficulty of procuring these resources (Reichenbach et al., 2021a). Consequently, they frequently use readily accessible alternative feed resources, including forages from lakeshores (Seto and Ramankutty, 2016; Reichenbach et al., 2021a, 2021b; Mundoli et al., 2022) and food leftovers (Prasad et al., 2019; Takiya et al., 2019; Reichenbach et al., 2021a). As urbanisation is typically accompanied by expansion of industrialization, transportation infrastructure and traffic, the risk of soil and water contamination with organic and inorganic chemical residues is considerable (Yang and Zhang, 2015), from where heavy metal contamination of (ruderal) vegetation along streets, recreational parks, and surface water bodies may result. The consumption of such vegetation by farm animals poses a risk to animal health and product safety (Zodape, 2017; Giri and Singh, 2020; Yasotha et al., 2021).

The South Indian megacity of Bengaluru is praised as "garden city" (Sudhira et al., 2007), but newspaper reports (Thakur, 2001; HT, 2017; AN, 2018) and scientific studies (Jumbe and Nandini, 2009; Ramachandra et al., 2017) have repeatedly lamented the pollution of lake water by industrial effluents, which has led to a repeated "burning" of lakes (Hamsa and Prakash, 2020). A study conducted by Ramachandra et al. (2017) on lake macrophytes revealed elevated dry matter (DM) concentrations of Cd, Cr, and Pb that surpassed threshold levels (Cd: 0.5 mg/kg DM, Cr: 1.3 mg/kg DM, Pb: 2.0 mg/kg DM) set for animal feed by the World Health Organization (FAO/WHO, 1999, 2021). These findings were corroborated by other studies conducted in the same region (Ramachandra et al., 2017, 2020; Hamsa and Prakash, 2020; Alam et al., 2023). However, lake (shore) macrophytes are a highly solicited feed for dairy cattle in the Greater Bengaluru region due to their high crude protein and low neutral detergent fiber content compared to conventional forages (Reichenbach, 2020; Alam et al., 2022, 2023). To date, there is a paucity of information on the seasonal variation in lake fodder utilization by dairy farmers, on the quantitative intake of these forages by their cattle, and on the consequences of lake fodder-based feeding strategies on milk safety (Alam et al., 2023). Therefore, we aimed at quantifying the seasonal variation in the intake of Cd, Cr, and Pb by urban and peri-urban dairy cows in Bengaluru and the impact on milk safety as well as heavy metal concentration in cow feces.

2. Materials and methods

2.1. Study area and sample farms

The present study was conducted in the Greater Bengaluru (12.9716° N, 77.5946° E) region in India (Fig. 1). Bengaluru is the capital city of the southern Indian state of Karnataka, with a human population of approximately 13 million in 2022 (United Nations, 2022). Situated on the Deccan Plateau, the city rises between 600 and 900 meters above sea level in the southern half and 300 to 600 meters above sea level in the northern half. Bengaluru has an average ambient temperature of approximately 26 °C throughout the year, while annual rainfall averaged 1146 mm during the period spanning from 1989 to 2018 (Guhathakurta et al., 2020; WWO, 2020). According to the 2015 report by the National Dairy Development Board (NDDB), the Greater Bengaluru region hosts approximately 137,000 cattle, which collectively produce about 295,000 liters of milk per year (NDDB, 2015). For specific locations (circles 1-6 in Fig. 1) defined within this region (Hoffmann et al., 2017), details on dairy cattle management were collected from 145 farms in a previous study (Alam et al., 2022). From these, 39 farms were selected for the present study based on whether they obtained their cattle feed from lake shores or from outside the lake areas.

2.2. On-farm monitoring and sampling

The implementation of farm monitoring and all associated sampling procedures obtained ethical approval from the Institutional Animal Ethics Committee (IAEC) at the National Institute of Animal Nutrition and Physiology (NIANP) in Bengaluru, India, under the reference number NIANP/IAEC/1/2020/6. Each of the selected farmers was informed in detail about the intended farm monitoring across three seasons (monsoon: July 2020–August 2020, 39 farms; winter: November 2020–February 2021, 34 farms; summer: March 2022–June 2022, 33 farms) (Islam and van Amstel, 2021). Only farmers giving their full consent were included in the study; the decreasing number of participants was due to attrition in the aftermath of the COVID-19 pandemic (Alam et al., 2022). During each full-day monitoring session per season, individual records per cow were taken for breed, parity, stage of lactation, heart girth, daily milk yield, and type and amount of feeds offered. Depending on the type of feed offered, the cows were divided into a group receiving only non-lake feed (NF-cows) and a group receiving (some) lake fodder (LF-cows) (Table S1). Cows of the NF-cow group received a share of pelleted concentrate; groundnut cake; finger millet straw (*Eleusine coracana* Gaertn.); green maize (*Zea mays* L.); mixed feed consisting of wheat flour (*Triticum aestivum* L.), with or without bran, corn flour (*Zea mays* L.), and chickpea husks (*Cicer arietinum* L.); mixed grass (locally available cultivated grasses); and food leftovers. In addition, the LF-cow group received a certain share of lake fodder (any vegetation collected on lakeshores or in the lake) composed of various herbaceous species (Alam et al., 2023).

The quantitative and qualitative determination of on-farm feed intake and milk yield was performed following the approach of Reichenbach et al. (2021a). Briefly, during one full-day farm visit per season, the components of each meal were weighed (as fed) for each cow using a

PCE-HS 50N hanging scale (range: 0.2 kg to 50.0 kg, accuracy 0.08 kg; PCE Instruments, Hampshire, UK). If applicable and feasible, feed refusals were re-weighed to determine individual feed intake. For all feed types, a total of 124, 64, and 93 representative samples were collected across farms in the monsoon, winter, and summer seasons, respectively, retaining approximately 500 g of fed material for roughages and 160 g for concentrates in each case. The milk yield of each lactating cow was quantified per milking (once or twice) during each full-day farm visit and summed up to a daily value. The tared milking bucket was weighed (PCE-HS 50N hanging scale, see above) immediately after milking, and two milk samples were collected for analysis (see below). To determine heavy metal concentration in feces, 116 samples of freshly excreted material (approximately 500 g fresh matter) from individual cows was collected in the monsoon season, and 34 and 33 pooled samples (of similar mass) were collected at farm level in the winter and summer season, respectively.

2.3. Sample processing

Upon collection, feed samples were stored in micro-perforated polythene bags, and feces samples were kept in open trays. Both types of samples were weighed (range 0.01–820 g; precision: 0.01 g, Kern & Sohn GmbH, Balingen-Frommern, Germany) before and after oven drying to constant weight (at 60 °C for 48-72 h; Equitron-stream series No. 7051-250, Equitron Medica Private Ltd., Mumbai, India). Immediately thereafter, the dried roughage, concentrate and feces samples were grinded with a mixer grinder and sieved to pass a 0.5 mm mesh. Ground samples were stored air-dry in plastic zipper bags for heavy metal analysis. For milk, 116, 106, and 107 samples were collected from each lactating cow (2 × 25 ml) in the monsoon, winter, and summer season, and taken to the NIANP Animal Nutrition Division laboratory in Bengaluru using a cool box equipped with ice packs to prevent spoilage. On each collection day, one part (25 ml) of the individual milk sample was analyzed for fat, protein, and lactose content using a Lactoscan Milk Analyzer (Softrosys Technologies, Bengaluru, India), and the other 25 ml of the milk sample were stored at -20 °C for heavy metal analysis.

2.4. Heavy metal analysis

The determination of Cd, Cr, and Pb concentrations followed the procedures described by Alam et al. (2023). Samples of feed, feces, and milk underwent microwave-assisted digestion (Anton Paar GmbH, Graz, Austria). Approximately 0.5 g fully dried and ground (0.5 mm particle size) feed and fecal sample material, respectively, was placed in a marked polytetrafluoroethylene tube. A volume of 6 ml conc. HNO_3 (Supra 69 %, Roth, Germany) was added and the vessel was placed in the microwave digester. The digester was pre-heated (100 °C for 10 min, holding time 5 minutes), heated (180 °C for 10 minutes, holding time 5 min), digested (190 °C for 5 min, holding time 15 min), and cooled (55 °C for 23 min). After acid digestion, 1 ml of HCl (Supra 30 %, Carl Roth GmbH, Karlsruhe, Germany) was added to each vessel, along with demineralized water to complete a volume of 50 ml. For milk, approximately 3 g of liquid sample along with 5 ml conc. HNO_3 was placed in the digestion tube. The microwave digester was heated (180°C for 20 min, holding time 10 min), and cooled (55 °C for 21 minutes). After acid digestion, 1 ml of HCl along with demineralized water was added to complete a volume of 25 ml. Afterwards, the thus treated feed, feces, and milk sample solids were recovered by filtering (Whatman No. 40 paper) and preserved in a polyethylene bottle for heavy metal analysis (Paar, 2020). A reagent blank sample was also prepared to detect the concentration of Cd, Cr, and Pb for each batch of feed, feces, and milk.

Concentrations of Cd, Cr, and Pb in feed, feces, and milk were determined by inductively coupled plasma - optical emission spectroscopy (ICP-OES) using a Spectrogreen ICP-OES analyzer (Spectro Analytical Instruments GmbH, Kleve, Germany). Argon was used as plasma gas. Calibration standards were prepared by serial dilution using a dilute HNO_3 and HCl-matrix based aqueous solution of 100 mg/l (ppm) (Supelco, Centipur® ICP multi-element standard solution IV, Cat. No. 1.11355.0100, Merck KGaA, Darmstadt, Germany). Afterwards, from this solution, different concentrations of Cd, Cr, and Pb (0.005,

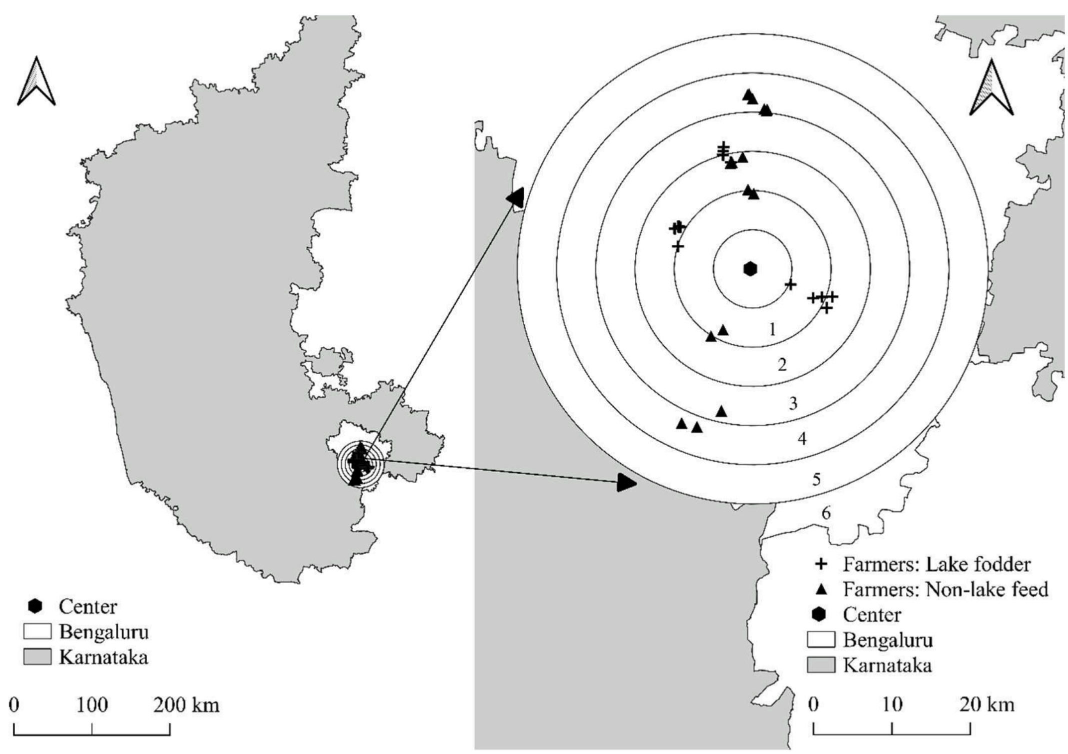

Fig. 1. Karnataka State (left) and Greater Bengaluru Metropolitan Area in India (right) with the location of the 39 surveyed dairy farms. Urban regions are represented by circles 1 and 2, peri-urban regions encompass circles 3 to 5, and rural areas are situated in circle number 6 and beyond.

0.01, 0.1, and 1.0 mg/l) were prepared for the calibration in the analysis of feeds and feces. Similarly, standard solutions with 0.005, 0.01, 0.05, 0.1, and 1.0 mg/l of Cd, Cr, and Pb were prepared for milk sample analysis. The analyzed elements were quantified using calibration curves plotted from analytical standards. The limits of detection were as follows: Cd= 0.000130 mg/l, Cr= 0.000752 mg/l, and Pb= 0.003022 mg/l. Three technical replicates were run for all samples. For Cd, Cr, and Pb, the accuracy of the analytical procedure was tested by analyzing a reference material (Rye grass, ERM®-CD281, Institute for Reference Materials and Measurements, Geel, Belgium) together with the feed samples. Obtained concentrations (Cd= 0.126 mg/kg DM, Cr= 23.6 mg/kg DM, Pb= 1.67 mg/kg DM) were within in the certified range of the reference material (Cd= 0.120 ± 0.007 mg/kg DM, Cr= 24.3 ± 1.3 mg/kg DM, Pb= 1.67 ± 0.11 mg/kg DM). The analytical method was tested by analyzing blank samples (n = 10) and no major interferences were found in the quantitative element analysis. The results were also validated by inter-laboratory comparison of 10% of the samples with analysis performed by AGROLAB LUFA GmbH (Kiel, Germany); differences in concentrations of Cd, Cr, and Pb were insignificant ($P > 0.05$).

2.5. Data analysis

2.5.1. Estimation of body weight and feed dry matter intake

Animal body weight (BW) was obtained from the heart girth (HG) measurements using the regression equation of Grund (2018), based on a cohort of 527 animals in Bengaluru:

$$BW = -8.119 + 2.738 \times \log(HG).$$

where BW is a cow's body weight in kg, and HG the heart girth in cm. The correlation coefficient (R^2) of the equation is 0.92.

According to the farmers' responses, 40 % (monsoon), 13 % (winter), and 35 % (summer) of the cows in the present study grazed on lakeshores for approximately 4, 7, and 6 hours per day. Furthermore, 16 %, 24 %, and 28 % of the cows grazed on non-lake pastures for approximately 4, 7, and 3 hours per day in the monsoon, winter, and summer season. To estimate the seasonal intake of feed dry matter from grazing, the following equation (Schlecht et al., 2019) was applied:

$$DMI_P = t_e * IR * BW^{0.75}$$

where DMI_P is the cow's daily feed dry matter intake on pasture in kg, t_e is the total daily grazing time in hours, IR is the hourly intake rate of 8 g DM per kg metabolic weight (MW), calculated from body weight (BW) scaled to the power of 0.75. Finally, on-farm (provided by the farmer) and on pasture (grazed) DM intakes for individual cows were calculated for non-lake feed, lake fodder, and per kilogram of MW.

2.5.2. Statistical analysis

All data analyses were performed in R (version 4.2.1) (RCoreTeam, 2022). Normality was tested separately for each variable by visually assessing individual Q-Q plots. Since all variables were normally distributed, one-way ANOVA was computed for the dependent variables, namely concentration of Cd, Cr, and Pb in non-lake feed, lake fodder, and feces, intake of DM from non-lake feed, lake fodder and total (g/kg MW), intake of Cd, Cr, and Pb from non-lake feed, lake fodder and total, milk yield (kg/cow/day, kg/kg total dry matter intake), and milk fat, protein, lactose, Cd, Cr, and Pb concentration. Independent variables considered were season (monsoon, winter, summer) and cow group (NF-cows, LF-cows). For all data, estimated marginal means separation was performed using the '*emmeans*' package (Midway et al., 2020). The Bonferroni post-hoc test was computed for pairwise comparisons (*multcomp* package; Hothorn et al., 2016). Differences were declared significant at $P < 0.05$.

3. Results

3.1. Feed quality

Lake fodder and non-lake feed showed a higher Cd concentration in summer (Table 1) than in monsoon and winter ($P < 0.05$); similarly, lake fodder and non-lake feed showed a lower Cr concentration in winter compared to monsoon and summer ($P < 0.05$). Pb concentrations in lake fodder were similar across seasons, whereas non-lake feed showed a lower concentration in winter compared to monsoon and summer ($P < 0.05$). Commercial as well as farm-produced concentrate feeds showed average Cd concentrations of 0.07, 0.12, and 0.05 mg/kg DM during monsoon, summer, and winter seasons, respectively. The average concentration of Cr in concentrates was 2.02, 1.98, and 1.81 mg/kg DM in monsoon, summer, and winter, and the respective concentrations of Pb averaged 0.44, 0.79, and 0.19 mg/kg DM.

3.2. Daily feed intake

DMI of non-lake feed was higher than of lake fodder across seasons (Table 2 and Table S2). Daily DMI of lake fodder was highest in the monsoon season (5.6 kg/cow) and lowest in summer (4.9 kg/cow), while for non-lake feed it was highest ($P < 0.05$) in winter (9.1 kg/cow) and lowest ($P < 0.05$) in the monsoon season (7.5 kg/cow). Across cows, total daily DMI was highest in winter (127 g/kg MW) and lowest in summer (115 g/kg MW; $P < 0.05$), with overall higher values ($P < 0.05$) in NF-cows (128 g/kg MW) than in LF-cows (116 g/kg MW). Daily DMI of concentrate feed per cow averaged 3.97 kg during the monsoon season, 3.27 kg during summer, and 3.14 kg during winter ($P < 0.05$). With a daily average DMI of 4.09 kg/cow across seasons, daily concentrate consumption was higher for NF-cows than for LF-cows, which only consumed 2.73 kg/cow per day ($P < 0.01$).

3.3. Daily intake of heavy metals

Daily intake of Cd (Table 3) from lake fodder was higher in summer than in monsoon and winter ($P < 0.05$), whereas for non-lake feed, intake was higher in winter than in monsoon and summer ($P < 0.05$). Daily Cr intake from lake fodder was lower in winter than in summer ($P < 0.05$), while no seasonal differences were observed for Cr intake from non-lake feed. Pb intake from lake fodder was higher in winter than in the monsoon season and summer ($P < 0.05$), while Pb intake *via* non-lake feed was lower in monsoon than in winter and summer ($P < 0.05$). LF-cows ingested a higher amount of Cd, Cr, and Pb from lake fodder than from non-lake feed ($P < 0.05$).

Total Cd intake per animal and day (Table 4) was higher in summer than in monsoon and winter ($P < 0.05$), with LF-cows ingesting a higher amount than NF-cows ($P < 0.05$). The total daily Cr intake was lower in winter than in monsoon and summer ($P < 0.05$), whereas the total daily Pb intake was lower in the monsoon season than in summer and winter ($P < 0.05$). No significant differences in total daily Cr and Pb intake, respectively, were observed between LF-cows and NF-cows. Consumption of concentrate feed entailed a daily Cd intake of 0.38 mg, 1.43 mg, and 0.49 mg per cow during monsoon, summer, and winter seasons, respectively ($P < 0.05$). The corresponding values for Cr intake were 7.41 mg, 7.83 mg, and 5.62 mg per cow ($P > 0.05$), and the daily concentrate-based intake of Pb averaged 1.70 mg, 1.54 mg, and 1.85 mg per cow in monsoon, summer, and winter ($P > 0.05$). Since NF-cows consumed higher amounts of concentrate feeds than LF-cows, their concentrate-based intake of heavy metals per cow and day (Cd: 1.05 mg; Cr: 8.59 mg; Pb: 2.03 mg) exceeded that of LF cows (Cd: 0.20 mg; Cr: 4.33 mg; Pb: 1.33 mg; $P < 0.01$).

3.4. Milk yield and quality

Daily milk yield and fat content did not differ ($P > 0.05$) between

Table 1
Seasonal variation in the average concentration of Cd, Cr, and Pb in lake fodder and non-lake feed in Bengaluru, India.

Variables	n		Cd (mg/kg DM)		Cr (mg/kg DM)		Pb (mg/kg DM)	
Season	Feed origin							
	Lake fodder	Non-lake feed	Lake fodder	Non-lake feed	Lake fodder	Non-lake feed	Lake fodder	Non-lake feed
Monsoon	59	116	0.93^{aB}	0.08^{aA}	2.41^{bA}	2.73^{bA}	0.34^{aA}	0.55^{bB}
Winter	39	103	0.63^{aB}	0.08^{aA}	0.95^{aA}	1.99^{aB}	0.35^{aB}	0.22^{aA}
Summer	49	104	1.59^{bB}	0.14^{bA}	2.18^{bA}	3.08^{bB}	0.31^{aA}	0.60^{bB}
SEM			*0.22*	*0.02*	*0.38*	*0.20*	*0.05*	*0.05*
*EU threshold**			*1.00*		*1.30***		*5.00*	

Mean values with different lowercase ($^{a-b}$) and uppercase ($^{A-B}$) superscript within the same column and row, respectively, differ at $P < 0.05$; n= number of samples considered; DM: dry matter; SEM: Standard error of mean; *EU threshold (López-Alonso, 2012); **World Health Organization threshold (Ramachandra et al., 2017).

Table 2
Seasonal variation in average body weight (BW), dry matter intake (DMI) from lake fodder and non-lake feed, and total daily DMI relative to metabolic weight (MW) of lactating cows in Bengaluru, India. Values in brackets indicate the number of observations.

Variables	BW (kg)	DMI from lake fodder (kg/cow/day)	DMI from non-lake feed (kg/cow/day)	Total DMI (g/kg MW)
Season				
Monsoon	366 (116)	5.6 (59)	7.5^{a} (116)	125^{b} (116)
Winter	375 (106)	5.2 (39)	9.1^{b} (103)	127^{b} (106)
Summer	388 (107)	4.9 (49)	8.0^{ab} (104)	115^{a} (107)
SEM	*6.00*	*0.34*	*0.34*	*2.98*
Cow group				
NF-cows	378 (183)	0	10.9^{b} (183)	128^{b} (183)
LF-cows	373 (146)	5.3 (146)	4.6^{a} (140)	116^{a} (146)
SEM	*4.95*	*0.19*	*0.22*	*2.43*

$^{a-b}$ Mean values with different superscript within the same column differ at $P < 0.05$; SEM: Standard error of mean, NF: Non-lake feed; LF: Lake fodder. MW = Body weight scaled to the power of 0.75.

Table 3
Seasonal variation in the average intake of Cd, Cr, and Pb through lake fodder and non-lake feed by lactating cows in Bengaluru, India.

Variables	n		Cd intake (mg/cow/day)		Cr intake (mg/cow/day)		Pb intake (mg/cow/day)	
	Lake fodder	Non-lake feed	Lake fodder	Non-lake feed	Lake fodder	Non-lake feed	Lake fodder	Non-lake feed
Season								
Monsoon	59	116	14.7^{a}	0.3^{a}	26.9^{ab}	26.6	4.5^{a}	5.1^{a}
Winter	39	103	12.4^{a}	1.5^{c}	16.9^{a}	25.5	7.1^{b}	7.8^{b}
Summer	49	104	27.3^{b}	1.0^{b}	37.7^{b}	29.7	4.9^{a}	9.7^{b}
SEM	-	-	*3.48*	*0.15*	*4.20*	*2.19*	*0.64*	*0.83*
Cow group								
NF-cows	0	183	0	1.2^{b}	0	38.7^{b}	0	8.9^{b}
LF-cows	146	140	18.3	0.5^{a}	27.9	12.3^{a}	5.3	5.6^{a}
SEM			*1.53*	*0.13*	*1.23*	*1.48*	*0.18*	*0.69*

$^{a-c}$ Mean values with different superscript within the same column differ at $P < 0.05$; SEM: Standard error of mean; NF: Non-lake feed; LF: Lake fodder; n = number of observations.

seasons and between LF-cows and NF-cows (Table 5). Milk protein and lactose content were higher in summer than in monsoon and winter, and lactose content was higher in milk from LF-cows than from NF-cows ($P < 0.05$). No traces of Cd were found in the milk unlike Cr and Pb. The highest Cr concentration in milk was determined in the monsoon season, followed by summer and winter ($P < 0.05$). Milk Pb concentrations were higher in winter season than in summer ($P < 0.05$).

3.5. *Presence of heavy metals in cattle feces*

Pb concentration in cattle feces (Table 6) varied between seasons ($P < 0.05$), whereas this was not observed for Cd and Cr. While the Cd

Table 4
Seasonal variation in the average total daily intake of Cd, Cr, and Pb by differently managed lactating cows in Bengaluru, India.

Variables	n	Total Cd intake (mg/cow/day)	Total Cr intake (mg/cow/day)	Total Pb intake (mg/cow/day)
Season				
Monsoon	116	8.1^{a}	40.4^{b}	7.5^{a}
Winter	106	6.2^{a}	30.9^{a}	10.2^{b}
Summer	107	13.7^{b}	46.3^{b}	11.7^{b}
SEM		*1.75*	*2.51*	*0.84*
Cow group				
NF-cows	183	1.5^{a}	38.7	8.9
LF-cows	146	19.1^{b}	39.8	10.8
SEM		*1.28*	*2.11*	*0.70*

$^{a-c}$ Mean values with different superscript within the same column differ at $P < 0.05$; SEM: Standard error of mean, NF: Non-lake feed, LF: Lake fodder; n = number of observations.

Table 5
Seasonal variation in average milk yield and concentration of fat, protein, lactose, Cd, Cr, and Pb in milk of differently managed lactating cows in Bengaluru, India.

Variable	n	Milk yield		Concentration (g/100 g milk)			Concentration (mg/kg milk)		
		(kg/cow/day)	(kg/kg DMI)	Fat	Protein	Lactose	Cd	Cr	Pb
Season									
Monsoon	116	9.2	0.8	4.2	3.2[b]	4.7[b]	0	0.020[b]	0.006[ab]
Winter	106	9.3	0.9	3.9	3.1[a]	4.5[a]	0	0.009[a]	0.036[b]
Summer	107	8.1	0.9	4.2	3.3[c]	4.9[c]	0	0.012[a]	0.002[a]
SEM		*0.42*	*0.04*	*0.09*	*0.02*	*0.03*		*0.002*	*0.006*
Cow group									
NF-cows	183	9.1	0.8	4.2	3.2	4.7[b]	0	0.015	0.017
LF-cows	146	8.6	0.9	4.1	3.2	4.8[a]	0	0.013	0.012
SEM		*0.35*	*0.03*	*0.07*	*0.02*	*0.02*		*0.002*	*0.005*
Permissible limit*							0.010	-	0.020

[a-c] Mean values with different superscript within the same column differ at $P < 0.05$; DMI: dry matter intake; SEM: Standard error of mean; NF: Non-lake feed; LF: Lake fodder; n = number of observations;
* FAO/WHO (2021); - no value given.

Table 6
Seasonal variation in average concentration (mg/kg DM) of Cd, Cr, and Pb in feces of differently managed lactating cows in Bengaluru, India.

Variable	n	Cd	Cr	Pb
Season				
Monsoon	116	1.2	14.3	4.1[a]
Winter	31	1.7	16.9	10.6[b]
Summer	28	1.2	9.8	9.4[b]
SEM		*0.45*	*1.82*	*1.57*
Cow group				
NF-cows	95	0.4[a]	11.0[a]	5.1
LF-cows	80	2.4[b]	17.6[b]	7.3
SEM		*0.29*	*1.24*	*1.12*

[a-c] Mean values with different superscript within the same column differ at $P < 0.05$; SEM: Standard error of mean, NF: Non-lake feed; LF: Lake fodder; n = number of observations.

concentration in the feces of LF-cows was three times higher than in those of NF-cows, fecal Cr concentration of LF-cows was 27 % higher than that of NF-cows ($P < 0.05$).

4. Discussion

4.1. Feed quality, feed intake, and milk production

The present analysis of dairy farming systems in Greater Bengaluru provided insights into the practice of feeding macrophytes from lakes and lake shores, the associated quantitative intake of some relevant heavy metals, and their transfer to milk and feces. Due to increasing land scarcity for food and feed cultivation, 54 % of the sampled 39 farmers included a share of lake fodder in their cows' rations. In terms of feed quality (crude protein, neutral detergent fiber, gross energy), this fodder was of satisfactory nutritional quality (Alam et al., 2023; Feedpedia, 2023) and comparable to conventional forages (Reichenbach, 2020; Alam et al., 2022, 2023).

However, as also reported elsewhere (Jumbe and Nandini, 2009; Ramachandra et al., 2020), lake macrophytes from Bengaluru contained Cd, Cr, and Pb at concentrations exceeding, in 55 %, 100 %, and 100 % of the cases, permissible limits for animal feed set by WHO threshold values (Ramachandra et al., 2020). Such findings are not limited to Bengaluru but were also reported from the Indian megacity of Hyderabad along the Musi river (Raj et al., 2006) and the Erode district of Tamil Nadu (Yasotha et al., 2021). Yet, heavy metal concentrations in feed are just one aspect to be considered in the evaluation of related human and animal health risks. Equally important are the consumed amounts of contaminated feed (Li et al., 2005), the regularity of such intake (Hejna et al., 2018), seasonal variations (Ahmad et al., 2013), and the bioavailability of the heavy metals in the animals' metabolism (Miroshnikov et al., 2021).

In the present study, daily DMI (g/kg MW) was lower for LF-cows than NF-cows. These differences can be explained by the fact that farmers using lake fodder often lack access to cultivated forage, leading to the extensive utilization of lake fodder albeit at reduced quantities. In addition, seasonal variation in lake fodder use is linked to the growth cycle and availability of major crops such as finger millet, maize, wheat, and pulses (Mundoli et al., 2015; Sharmili et al., 2022), as well as natural vegetation. Crop residues are abundant in winter, but deplete as summer begins and progresses; therefore, farmers increasingly use common pool fodder resources (Mundoli et al., 2015). This is reflected in the higher frequency of lake fodder use in summer compared to winter, when cows rely more on non-lake feed. However, the quantitative intake of lake fodder per cow is lower in summer than in winter because the growth and abundance of these macrophytes is highest during the monsoon season, mainly due to increased rainfall (Mundoli et al., 2015; Bareuther et al., 2020; Sharmili et al., 2022).

Regarding milk production, there were contrasting results between LF-cows and NF-cows, with the former having a lower overall milk production. This might be attributed to the stage of milk production for an individual cow (lactation stage and number), which was not accounted for in the current study. However, LF-cows produced a higher amount of milk per kg DMI, which might be attributed to the good quality of the lake fodder (Alam et al., 2023).

4.2. Intake of heavy metals and transfer to milk and feces

The seasonal variations in the overall intake of Cd, Cr, and Pb by cows through their feed were due to the different proportions of feed from different sources in their rations and the concentration of specific heavy metals in those feeds. In our study, higher daily average intake of heavy metals (Cd: 13.7 mg, Cr: 46.3 mg, Pb: 11.7 mg) per animal were observed in summer and in LF-cows (Cd: 19.1 mg, Cr: 39.8 mg, Pb:10.8 mg) due to a higher share (13 %) of lake fodder in their diet. Unfortunately, only few studies report on the quantitative intake of heavy metals by cattle. Johnson et al. (1981) reported that beef cattle consuming forage manured with high-Cd sewage sludge ingested 101 mg Cd and 480 mg Pb per animal and day. The authors reported that the overall retention of Cd and Pb in muscle, bone, and organs (liver, kidney, lung, brain, spleen) was 0.09 % and 0.3 %, respectively, with retention in liver and kidney being five to 20 times higher than in the other tissues.

Despite the substantial intake of Cd, Cr, and Pb by the studied cows in summer season, Cd was not detected in milk. A recent study by Yasotha et al. (2021) on cows reared near industrial areas in Erode district, Tamil Nadu, India, found that 73 % of the 40 milk samples they analysed for Cd were below detection limits, and the concentration in the detected samples was very low (0.045 mg Cd/kg milk). Similarly, the concentration of 0.006 mg Cd/kg milk reported for Haryana cows kept in 19 districts of Haryana State, India, was below the permissible limits (Roy et al., 2009). This suggests that the Cd concentration in milk is too low to pose a health risk to human consumers.

The milk concentration of Cr determined in Bengaluru was very low compared to milk from cows reared in industrial areas of Erode district in India (Yasotha et al., 2021), the reason being the variation in the Cr content of the diet supplied in the above-mentioned industrial areas. For cows reared in mining areas of Singhbhum in India's Jharkhand State (Giri and Singh, 2020), higher milk concentrations (0.10 mg Cr/kg) were reported than what was detected in Bengaluru (0.01 mg Cr/kg). Milk concentrations of Pb were below the permissible limits, except during the winter season, when milk of 29 % of the sampled cows contained more Pb than considered safe for human consumption (FAO/WHO, 2021). These results align with findings of Yasotha et al. (2021) who reported that Pb concentrations in cow milk were below the permissible limits in three out of four locations within Erode district in India. Similarly, Roy et al. (2009) found that Pb concentrations in milk (0.01 mg/kg) were within permissible limits in six districts of Haryana State, while they exceeded the limits (0.10 mg/kg) in another 13 districts of the same state. Furthermore, contrasting results were obtained by the same scientists in different years: focusing on cows kept in mining areas, Giri et al. (2011) reported absence of Pb in milk in a first study, while ten years later they detected milk Pb concentrations above the permissible limits (Giri et al., 2021). However, these studies did not examine whether the soil and the plants contained heavy metals. Overall, the differences in milk heavy metal concentrations between the present and other studies may be attributed to differences in the (environmental) sources of heavy metals, industrialization and urbanization rates, mining activities, traffic density, transfer rates from water and soil to (fodder) crops, and subsequent transfer to cow's milk, the latter also depending on feeding practices (Nag, 2010; Wuana and Okieimen, 2011; Boudebbouz et al., 2021).

Our study did not show significant seasonal variations in fecal concentrations of Cd, Cr, and Pb. The present findings match with data from Wang et al. (2013) who reported concentrations (per kg DM) of 0.92 mg Cd, 8.1 mg Cr, and 40.1 mg Pb in manures of cattle reared in industrial areas of Jiangsu, China. Similarly, Nicholson et al. (1999) reported concentrations (per kg DM) of 0.36 mg Cd, 5.48 mg Cr, and 4.74 mg Pb in cattle manures from commercial farms in England and Wales.

In the present study, the higher intake of heavy metals - particularly Cd - through feed and the lower excretion through feces in the summer season suggests that Cd deposition in liver and kidney might have been high at that moment. For cultural reasons, the assessment of Cd, Cr, and Pb in blood, muscle, or organs was not intended nor possible in this study. However, Hashemi (2018) examined the concentrations of Cd and Pb in muscle, liver, and kidney of cows in Fars Province, Iran, where animals from the three most polluted and three least polluted counties were grouped into a polluted and an unpolluted group. With the exception of muscle Pb concentrations, this author reported no significant differences in Cd and Pb concentrations in the organs of the two cow groups. This indicates that despite variations in the heavy metal concentration of cattle feed, there is not necessarily a substantial impact on the animal, due to a low retention rate (Cd: 0–0.9 %, Pb: 0.3 %) in the organs (Johnson et al., 1981). Nevertheless, presence of heavy metals in the organs (liver, kidney, brain) of cows is associated with various negative health effects (EFSA, 2010; Kazakova et al., 2021). Furthermore, accumulated heavy metals can be gradually released and excreted over time (Li et al., 2005; EFSA, 2010; Kazakova et al., 2021). Thus, even if the current intake of heavy metals from feed is low, the cumulative effect of past exposure and storage may lead to higher concentrations in feces (Li et al., 2005; Makridis et al., 2012; Hejna et al., 2018). This underlines the need to quantify heavy metal intake and excretion, to understand transfer processes, and to track the fate of heavy metals in excreta that may be applied to fields or washed into sewers (Reichenbach et al., 2021a), as both practices pose threats to environmental, animal, and human health (Hashemi, 2018; Boudebbouz et al., 2021).

5. Conclusions

In the rapidly urbanising Greater Bengaluru region, regular harvesting and feeding or grazing of lake fodder provides up to half of the daily dry matter intake of cows on farms that actively utilise this freely available feed, despite its heavy metal concentrations that often exceed recommended thresholds. Nevertheless, seasonal fluctuations and variations in farmers' feeding practices influence the heavy metal intake of dairy cows. With the exception of a few instances, milk safety was not compromised by Cr and Pb concentrations, and Cd was not problematic at all. Conversely, elevated heavy metal concentrations in cattle feces may threaten environment health and re-enter the food chain via manure application to cropland. While farmers should not be discouraged from using lake fodder at all, exempting the use of particularly polluted sites would reduce the contamination risk along the feed-food chain. Rather than banning cost-effective feeding practices on which land-constrained urban farmers rely, informing them of the fate of heavy metals along the feed-food chain would enhance compliance with specific regulations.

Ethics statement

Farm animal monitoring did not involve any direct intervention with the cows, except for heart girth measurements. Animal handling, such as feeding or milking, was always performed by the farmers themselves, with the scientists only weighing and sampling feed, milk and feces (from the barn floor). All animal-related procedures were described in detail before the start of the study and were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of the National Institute of Animal Nutrition and Physiology (NIANP) in Bengaluru, India, under the reference number NIANP/IAEC/1/2020/6.

Data availability statement

The datasets generated and/or analyzed in the current study are available from the corresponding author for scientific purposes upon written request.

Data protection and participation consent

All farmers participating in this study were fully informed about the study purpose and were assured that their information would be treated anonymously. Therefore, only persons giving explicit informed oral consent were interviewed and included in this study.

Declaration of generative AI and AI-assisted technologies in the writing process

AI and AI-supported tools played no role in the preparation and production of this manuscript.

CRediT authorship contribution statement

Shahin Alam: Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Silpa Mullakkalparambil Velayudhan:** Writing – review & editing, Methodology. **Christian Adjogo Bateki:** Writing – review & editing, Formal analysis. **Pradeep Kumar Malik:** Writing – review & editing, Resources. **Raghavendra Bhatta:** Writing – review & editing, Resources. **Andreas Buerkert:** Writing – review & editing, Conceptualization. **Sven König:** Writing – review & editing, Supervision, Methodology. **Eva Schlecht:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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Supplementary materials

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