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Research Article

Application of data ratio analysis of lead accumulation in cartilage and bones of goats and chickens in Yogyakarta, Indonesia

Djohan Djohan
Department of Biology, Satya Wacana Christian University, Diponegoro St. 52 - 60
Salatiga city, Central Java 50711 - INDONESIA

E-mail: djohan@uksw.edu

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Abstract
Cartilage and bones (CB) are primary organs for lead accumulation in domestic animals, but very few studies have addressed the occurrence of lead in both organs. CBs, especially from goat and chicken, although classified as non-edible food, are still being consumed by many local communities globally. Male goat and rooster were bought in traditional markets in Yogyakarta, Indonesia. Lead in CBs of humerus, radius, femur, and tibia (HRFT) were extracted by aqua regia digestible method and measured by Atomic Absorption Spectrophotometry. A set of data ratios, generated by data ratio analysis (DRA), namely concentration ratio (CR), amounts ratio (AR), variation ratio (VR), coefficient of variation ratio (CVR), joint probability (JP) and conditional probability (CP) based on the AR and CR, were determined to characterize the lead exposure in CB. The means of lead concentrations in CB in goat were 4.9 and 5.2 $\mu g.g^{-1}$, and those in chicken were 5.00 and 5.20 $\mu g.g^{-1}$, respectively. The CRs in goats and chickens were 0.95 and 0.96, and the ARs in goats and chickens were 1.34 and 1.16, respectively. A high linear relationship was found between Ln VR and Ln CVR, and the JP and CP supported decision-making on CB consumption. By targeting CB as primary organs for lead accumulation, considering its potential exposure for many communities, and comparing its further uses as animal feed, the DRA in this study is useful to provide broadened comparability in bioaccumulation studies.

Keywords: Cartilage and bone (CB), Data ratio analysis (DRA), Humerus radius femur tibia (HRFT), Goat and chicken, Lead (Pb)

INTRODUCTION

Concentrations and amounts of lead in farm animals' cartilage and bone (CB) have not been adequately studied. Lead concentrations are generally measured only in bones and do not include cartilage, for example, in goats (Orisakwe *et al.,* 2017) and birds (Ethier *et al.,* 2007). As bone is the main organ (~ 90%) for the accumulation of heavy metals (García-Fernández *et al.,* 2008) and CBs are still being consumed in many local communities globally, the exposure analysis, including the estimation of the body burden of lead in CB is an important factor in public health protection. Humerus radius femur tibia (HRFT) is the major bone component (39.8%) (w/w) based on the percentage of bone to carcass weight in goat (Alkass *et al.,* 2014). Had the differences between observatory and experimental results been determined, the validity of field and laboratory extrapolation of body burden of lead can be known and the appropriateness of using HRFT organs in body burden estimation can be evaluated.

Data ratio analysis (DRA) consists of the uses of data ratios such as concentration ratio (CR), amount ratio (AR), variability ratio (VR), and coefficient of variation ratio (CVR) which has not been adequately used in bioaccumulation studies of lead in CBs of farm animals. The first group of data ratio is concentration ratio. There is available data on lead concentrations in both CBs in dog and red fox (Lanocha *et al.,* 2013), and wild birds (Kalisinska *et al.,* 2007). However, those data are not transformed into CR, and the studied animals were not farm animals. There has been little data on cartilage lead concentrations, for example, in Kalisinska *et al.* (2007) with no reported CR.
The second group of data ratio is the VR and CVR. The VR is calculated from the ratios of the standard deviations (s), while the CVR is calculated from the ratios of the coefficients of variation (CV) of the means of concentrations. There are “s” and CV values reported in the literature (Lanocha *et al.,* 2013; Lanocha *et al.,* 2012a; Kalisinska *et al.,* 2007) but not transformed to VR and CVR. In addition, those s and CV values were

from the wild and not from farm animals. By providing VR and CVR and linear regression plots of VR – CVR, variabilities of lead accumulation in CBs among farm, wild and experimental animals can be pooled and compared.

A probability exposure analysis using the data ratios (AR and CR) and expressed as joint probability (JP) and conditional probability (CP) is the third group of of DRA in this study. JP and CP analyses have been used for aquatic chlorpyrifos risk assessment (Alvarez *et al.,* 2019) and Bayesian approach for exposure assessment (Orak *et al.,* 2019). The values of JP and CP displayed in a quadrant analysis (QA), as provided by Huang *et al.* (2020), are useful for evaluating and comparing the values in four patterns of exposure situations in which CR and AR are used in horizontal and vertical axis, respectively.

Comprehensive analysis using a set of data ratios for the broader context of animal habitats and products has not been adequately developed. Exposure conditions to lead in animals are diverse, such as lead exposure in CBs of farm (Shen *et al.,* 2019), wild (Gerofke *et al.,* 2018), experimental (Tehrani *et al.,* 2021), and surrogate animals (Lanocha *et al.,* 2013). Comprehensive analysis including CRs, VRs, and probabilities of data ratios in broader and diverse exposure situations of lead in CBs, is useful for providing general and specific patterns of exposure in decision-making for human and animal health protection.

The objectives of this study were (i) to determine the concentrations and amounts of lead in the CBs of goats and chickens in Yogyakarta, Indonesia; (ii) to apply a set of DRA (CR, AR, CVR, VR, probability of AR and CR) to assess the concentrations and amounts of lead in the CBs in this study and the literature; and (iii) to provide an example of the extended use of DRA in lead accumulation in CBs in farm and non-farm animals.

MATERIALS AND METHODS

Study area and collection of samples

The study area was Yogyakarta city, located at 110°24' - 110°28' E and 07°15' - 07°49' S in the south-central region of Java island, famous for its roles as a city of education, culture and tourism, with the current population of approximately 453,000 within an area of 32.5 km^{2}. The main samples collected were 48 goat cartilage, 48 goat bones, 36 chicken cartilage, and 36 chicken bones. The samples were collected from three local markets in Yogyakarta City in Indonesia (Fig.1). Condyle cartilages in the humerus, radius, femur, tibia, metatarsus, metacarpus, scapula, and costa were collected from each goat and separated from compact bones. Sternum was added as cartilage samples while no metatarsus, metacarpus, and scapula cartilage were sampled in roosters. Bone samples were obtained similarly to cartilage samples from the same animals. The other sample organs were muscle and trachea (goat and chicken), auricula (goat) and feather (chicken). The age of male goats was 16 months, at which the ossification was completed in goats (Alpdogan and Genccelep 2012), while the age of roosters was 6 months.

Preparation of samples

The CBs were set apart from adhering tissues (meat, connective tissues) using stainless steel knives, washed with deionized water, and stored at -18°C in a freezer until preparation. CB samples were washed with deionized water, ground to smaller pieces, and dried in an oven at 105^{0} C for 24 hours. The samples were reweighted to obtain the dry weight and placed in plastic sample bottles. Plastic and glass were soaked in dilute HNO_3 (2%) overnight and then rinsed with deionized water. The average water content in goat and chicken cartilage was 57 ± 13% and 54 ± 12%, respectively, while that in goat and chicken bone were 29 ± 8% and 37 ± 12%, respectively, showing higher water content in the cartilage than that in the bones.

Chemical analysis of samples

The tissue samples were digested using the *aqua regia* digestion procedure. The chemical analysis followed the method reported by Djohan and Rahardjo (2016) with a few adjustments. Two grams of dry-weight samples were extracted by a mixture of 18 mL HCl and 6 mL HNO_3 (3:1; v/v) for 10 minutes by an electric stove, and this extraction step was repeated once by using the same volume of the mixture of acid solutions. The extracted sample was filtered through Whatman filter paper and made up to 10 mL using deionized water. The concentrations of lead were determined by Atomic Absorption Spectrophotometer (Perkin Elmer PinAAcle 900T) at the Chemistry Laboratory of Indonesia Islamic University in Yogyakarta. The setup of instrument parameters was as follows: wavelength of lead at 283.3 nm; lamp current 7.5 mA; flame type air – acetylene with air and acetylene flow rate were 9.5 $L.min^{-1}$ and 2.3 $L.min^{-1}$, respectively; and slit width 1.3 mm. The standard curve was plotted based on six standard solutions with lead concentrations ranging from 0.5 to 10 $\mu g.mL^{-1}$. The detection limit in this analysis was 0.0005 $\mu g.g^{-1}$ and duplicate samples were analysed for quality assurance. The lead concentrations in the samples were expressed in units of $\mu g.g^{-1}$ d.w.

Data Ratio Analysis (DRA)

The DRA was used as a method for the interpretation of results in this study. The DRA consists of concentration and amount ratios, variability ratios, and probability analysis. Detailed steps of DRA consisting of a series of equations are described in Supplementary Information (SI: App.1-6). Complete scope and applicability

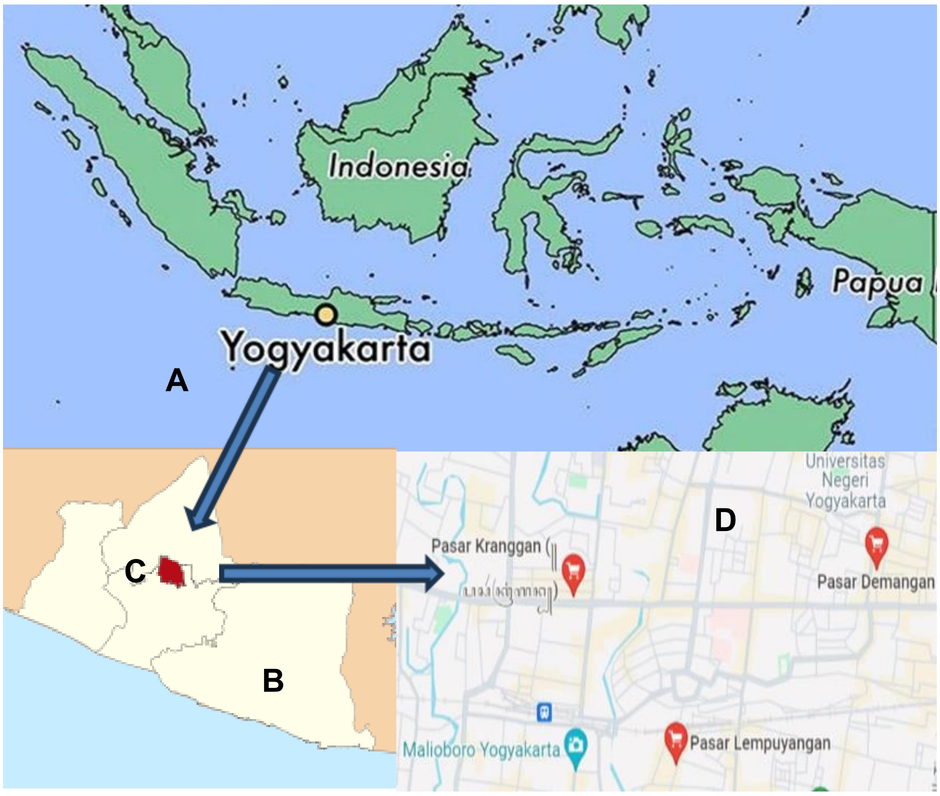

Fig. 1. *Map location of traditional markets of collection of cartilage and bones of goat and chicken in Yogyakarta special province (Sub- Fig.B) in Java island, Indonesia (Sub-Fig.A) with its capital Yogyakarta city (Sub-Fig.C). The three selected markets in the city (Sub-Fig.D) are Kranggan, Demangan, and Lempuyangan markets (map not to scale). Malioboro street (Sub-Fig.D) is one of the most famous tourist destinations in the city.*

of DRA is provided in Fig. 2.

RESULTS AND DISCUSSION

Concentrations of lead in cartilage, bones, and other tissues

The C_{LT} and C_{LN} in this study were 4.94 ± 0.17 $\mu g.g^{-1}$ and 5.67 ± 1.20 $\mu g.g^{-1}$, respectively and the C_{BT} and C_{BN} were 5.16 ± 0.52 and 5.16 ± 0.46 $\mu g.g^{-1}$, respectively (Table 1). There was a significant difference between the C_{LT} and C_{BT} ($p < 0.05$). However, no statistically significant difference was found between the C_{LN} and C_{BN} ($p > 0.05$). Similarly, no statistically significant differences were obtained between C_{LT} and C_{LN} ($p > 0.05$) and between C_{BT} and C_{BN} ($p > 0.05$). The C_{Ls} in non-HRFT cartilage tissues (metatarsus, metacarpus, scapula, costa) were generally lower than those in the HRFT except for sternum, while the C_{Bs} in non-HRFT were relatively similar to those in the HRFT (Table 1). The average C_L in the present study was comparable

Table 1. Concentrations of lead in cartilage (C_L) and bones (C_B) of goat and chicken

Organ set	Organ	Goat		Chicken	
		C_{LT} ($\mu g.g^{-1}$)	C_{BT} ($\mu g.g^{-1}$)	C_{LN} ($\mu g.g^{-1}$)	C_{BN} ($\mu g.g^{-1}$)
HRFT	Humerus	5.19 ± 0.45	5.06 ± 1.33	5.26 ± 0.26	5.06 ± 0.47
	Radius	4.89 ± 0.29	5.28 ± 0.50	4.77 ± 0.46	5.00 ± 0.72
	Femur	4.89 ± 0.34	5.33 ± 0.33	4.78 ± 0.41	5.12 ± 0.49
	Tibia	4.79 ± 0.29	5.08 ± 0.28	5.19 ± 0.49	5.60 ± 0.43
	$\bar{x}$ ± s	4.94 ± 0.17	5.19 ± 0.14	5.00 ± 0.26	5.20 ± 0.27
	CV (%)	3.0	2.6	5.2	5.3
Non HRFT	Metatarsus	4.32 ± 0.44	5.13 ± 0.31	4.71 ± 1.56	4.90 ± 0.34
	Metacarpus	4.74 ± 0.30	5.19 ± 0.61	NM	NM
	Scapula	4.70 ± 1.02	5.19 ± 0.23	NM	NM
	Costa	4.37 ± 0.42	5.21 ± 0.46	NM	NM
	Sternum	NM	NM	5.28 ± 1.66	5.30 ± 0.32
	$\bar{x}$ ± s	4.53 ±0.22	5.18 ± 0.03	5.00 ±0.40	5.10 ± 0.28
	CV (%)	4.9	1.0	8.0	5.5

NM = not measured

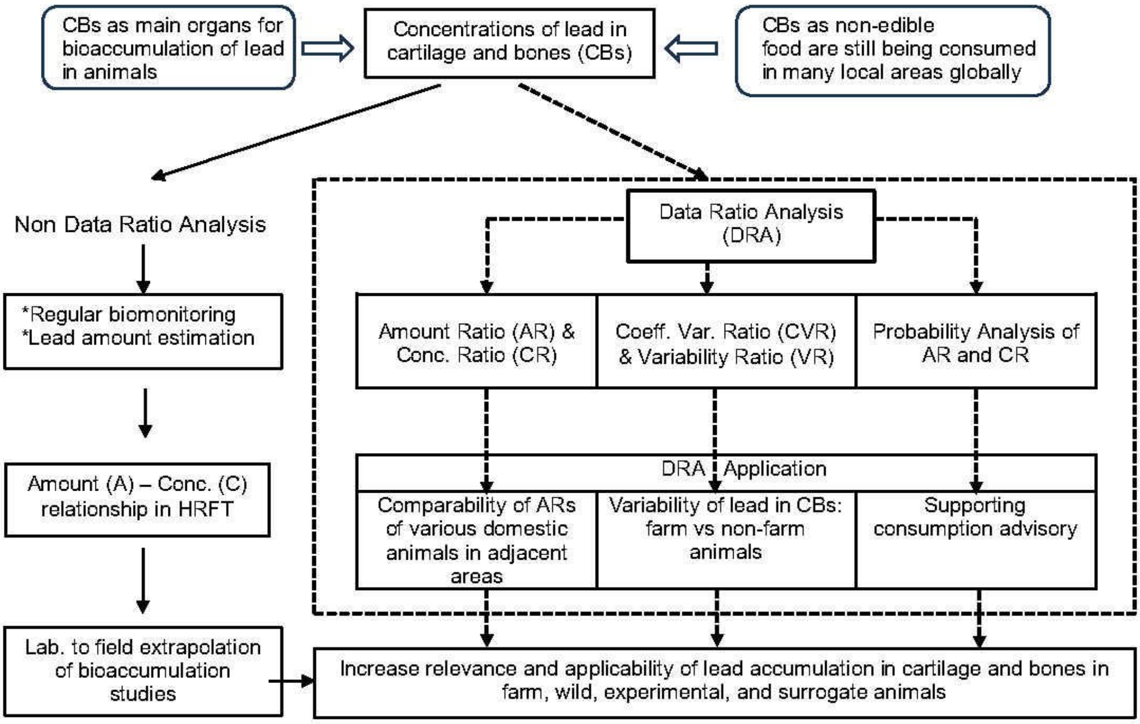

Fig. 2. *Applicability of data ratio analysis (DRA) in lead accumulation in cartilage and bones in farm and non farm-animals*

to the range of the C_L in the literature which was within three orders of magnitude (0.50 - 27.7 μg.g^{-1}). Similarly, the C_B in this study was also comparable to the range in the literature (0.54 - 19.3 μg.g^{-1}) (Table 2), including those reported by Ruszkowski *et al*. (2022) and Bratty et al., 2018).

Lead concentrations in organs other than CBs were measured in meat and trachea (goat and chicken), teeth (goat), and feathers (chicken). The average concentrations of lead in goat and chicken meat were 1.41 and 1.35 μg.g^{-1}, respectively, while those in the trachea were 1.19 and 4.71 μg.g^{-1}, respectively (Table 2). Similarly, the average lead concentrations in goat teeth and chicken feathers were 5.01 and 1.66 μg.g^{-1}, respectively. The average concentrations of lead in four organs in this study were slightly higher than those reported in the literature (Table 3), except for the average concentration in the meat of sheep (Shen *et al.*, 2019) and trachea of pochard (Kalisinska *et al.,* 2007) (Table 2). The C_{LT}, C_{LN}, C_{BT}, and C_{BN} of lead in this study were within the values reported in the literature, ranging within three orders of magnitude, including those reported by Mukhtar *et al.*(2020) and Shen *et al.*(2019).

Amount of lead in CBs

The average amounts of goat cartilage (A_{LT}) was approximately three times higher than those in chicken cartilage (A_{LN}). Similar comparison was also observed for average amounts in goat bone (A_{BT}) and that in chicken bone (A_{BN}), and these findings were likely related to differences between cartilage weights or bones. The A_{LT} (188.2 μg) was higher than the A_{BT} (139.8 μg), and the A_{LN} (55.9 μg) was also similarly higher than the A_{BT} (48.3 μg). A statistically significant difference ($p < 0.05$) was found between A_{LT} and A_{BT}, while a non-significant difference ($p > 0.05$) was noticed between A_{LN} and A_{BN}. The total amount of lead in HRFT of goat (A_{XT}) was 1,312 μg while that of chicken (A_{XN}) was 417 μg (Table 3).

Comparison between estimated amount and dosed lead in experimental goats

The calculated amount of lead in HRFT in this study, having known its proportion to whole body weight, was used to determine the estimated body burden in this study (A_{BT}^{BW}). Equally, the C_{BT} in this study (tibia) was used to determine the estimated body burden (A_{BT}^{cp}) based on the linear regression relationship in experimentally dosed goats provided by Cretacci and Parsons (2010). Details of calculation are provided in SI (App.2).

The (A_{BT}^{BW}) in this study was also compared to the estimated body burden of lead based on the concentration of lead in goat teeth (C_{ET}) as provided by Bellis *et al.,* (2008) (A_{BT}^{bl}) (SI: App.3). Bone lead in tibia is considered as a lifelong cumulative exposure to lead in human (Wang *et al.,* 2017). Tibia bone char exhibited the highest maximum lead adsorption capacity compared to the humerus (Park *et al.,* 2019). Bone quality of tibia and

Table 2. Cartilage to bone concentration ratio (CR), cartilage to other tissue concentration ratio (COR), and bone to other tissue concentration ratio lead (BOR) in present study and literature

Parameter	Tissues (C_1: C_2)	C_1 (μg.g^{-1})	C_2 (μg.g^{-1})	Ratio Value	Animal	Reference
CR	Cartilage: Bone	4.94	5.19	0.95	Goat	Present study
		1.36	0.98	1.39	Red Fox	Lanocha *et al.,* 2012a
		1.65	0.98	1.68	Red Fox	Lanocha *et al.,* 2013
		2.83	1.55	1.82	Dog	Lanocha *et al.,* 2013
		1.90	1.54	1.23	Dog	Lanocha *et al.,* 2012b
		5.67	5.16	0.96	Chicken	Present study
		0.50	0.54	0.96	Chicken	Bratty *et al.* 2018
		10.2	9.5	1.07[a]	Scaup	Kalisinska *et al.,* 2007
		20.3	8.1	2.51[b]	Scaup	Kalisinska *et al.,* 2007
		27.7	19.3	1.44[a]	Pochard	Kalisinska *et al.,* 2007
		26.1	6.6	3.95[b]	Pochard	Kalisinska *et al.,* 2007
COR	Cartilage: Meat	4.94	1.41	3.50	Goat	Present study
		5.00	1.35	3.70	Chicken	Present study
		0.50	0.22	2.27	Chicken	Bratty *et al.,* 2018
	Cartilage: Trachea	4.94	1.19	4.15	Goat	Present study
		5.00	4.71	1.06	Chicken	Present study
BOR	Bone: Meat	5.19	1.41	3.68	Goat	Present study
		2.57	0.87	2.95[c]	Sheep	Shen *et al.* 2019
		22.53	2.85	7.90[d]	Sheep	Shen *et al.* 2019
		2.26	0.32	7.06	Pig	Prisnyi 2021
		5.20	1.35	3.85	Chicken	Present study
		0.54	0.22	2.45	Chicken	Bratty *et al.,* 2018
	Bone : Trachea	5.20	4.71	1.10	Chicken	Present Study
		9.70	21.10	0.46	Pochard	Kalisinska *et al.,* 2007
	Bone: Teeth	5.19	5.01	1.04	Goat	Present Study
		2.57	1.21	2.12[c]	Sheep	Shen *et al.,* 2019
		0.62	0.51	1.22	Deer	Demesko *et al.,* 2018
	Bone: Feather	5.20	1.66	3.13	Chicken	Present Study
		1.38	0.60	2.30	A. phoenicurus	Mukhtar *et al.,* 2020
		1.71[d]	1.14	1.50	Broiler chicken	Valera *et al.,* 2017
		2.46	0.99	2.48	Cow (hair)	Darwish *et al.,* 2018
		2.26	0.43	5.26	Pig (hair)	Prisnyi 2021

[a] Year 2000 [b] Year 2003 [c] Control group [d] Affected group

femur of chicken did not decline up to 56 days (Damaziak *et al.,* 2019). The difference by a factor of 1.48 (tibia) and 1.50 (teeth) between this study compared to experimentally lead-dosed goats (Cretacci and Parsons, 2010; Bellis *et al.,* 2008) was considered comparable.

Suitability of HRFT in body burden estimation of lead exposure

HRFT is suitable for exposure analysis of lead exposure due to four rationales: the practicality of long bones, the proportion of their weight component, protein compositions, and smaller values of coefficient of variation (CV). First, long bones have been proposed as indicators for lead accumulation, such as tibia in goat (Cretacci and Parsons, 2010), the femur in brown bear (Lazarus *et al.,* 2018), and wild birds (Lanocha *et al.,* 2013). Long bones are easier to be recognized and collected (Bocca *et al.,* 2018). Second, HRFT is the major component (39.8%) (w/w) based on the percentage of bone to carcass weight in goats, consisting of humerus 9.7%, radius 8.5%, femur 10.8% and tibia 10.8% (Alkass *et al.,* 2014). Third, HRFT bones in chicken contained major (52%) of the chondroitin sulphate – uronic acid, extracellular matrix protein binding to heavy metals (Nakano and Ozimek, 2014). Four, smaller values of CVs of HRFT bones (≤ 5%) compared to other non HRFT bones (Table 1), indicating less variability, indicating high appropriateness to be used in exposure analysis. In sequential orders, femur, tibia, and humerus have been proposed as organs to be sampled in vultures (van den Heever *et al.,* 2019) and meat-type chickens (Damaziak *et al.,* 2019).

Cartilage to bone concentration ratio (CR)

Five types of concentration ratios, namely concentration ratios between (a) cartilage to bone (CR), (b) cartilage of HRF to cartilage of tibia (CCR), (c) bone of HRF to bone of tibia (BBR); (d) cartilage to other tissues especially for meat (COR), and (e) bone to other tissues especially for meat (BOR) (Eq.4 - 8)(SI: App.1). CR values were measured as slopes between C_L and C_B (Fig.3), with CR_T as 0.95 and CR_N as 0.96, and very high regression coefficients (R^2 □ 1.0) were observed, although no significant difference was found between $C_{LT} – C_{BT}$ and C_{LN} - C_{BN} linear relationships (p > 0.05) (Fig.3). The decreasing individual CR_T values for HRFT were 1.03 (H) > 0.94 (T) > 0.93 (R) > 0.92 (F); while those for CR_N were 1.03 (H) > 0.95 (R) > 0.93 (F) and 0.93 (T). Only humerus (H) had both CR_T and CR_N higher than unity. The CR_N values in this study were comparable to the CR_N of 0.96 as reported by Bratty *et al.* (2018) (Table 2), although the C_{LN} and C_{BN} of lead in this study were approximately ten times of those in local chicken in Bratty *et al.* (2018). Monitoring of lead in CB of wild animals in long period would enhance the application of data ratio over time (Gizejewska *et al.,* 2020).

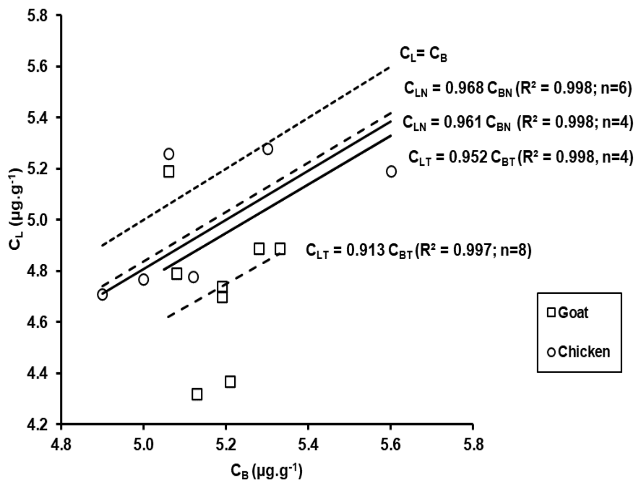

Fig. 3. *Relationships between concentrations of lead in cartilage (C_L) to concentrations of lead in bone (C_B) of goat and chicken in this study. Subscripts $_T$ and $_N$ refer to goat and chicken, respectively. Concentration ratios (CRs) are measured as slopes of regression lines. Slopes are determined graphically as the concentration ratios (CRs). Number of samples of 4 refer to HRFT bones, 8 refer to 4 HRFT and 4 non-HRFT bones, and 6 refer to 4 HRFT and 2 non-HRFT bones*

The CRs in this study and literature ranged from 0.95 (goat) to 3.95 (pochard) (Kalisinska *et al.,* 2007) or the difference was by a factor of 4.2, with high CR values were observed for wild animals. In addition to solid organ such as CB, CR can also be determined from the ratios of lead concentrations in liquid medium such as in chicken broth. For example, the concentration of lead in broth made from cartilage and skin was 9.5 µg.L^{-1} while that made from bone only was 7.01 µg.L^{-1} (Monro *et al.,* 2013) and the CR in this case could be determined as 1.36 which was comparable to the CR values listed in Table 2. The CR values in this study were determined both for groups and individuals of HRFT and were comparable to the literature values.

Cartilage of HRF to cartilage of tibia concentration ratio (CCR) and bone of HRF to bone of tibia concentration ratio (BBR)

The CCRs and BBRs values were close to unity and the deviations from unity could be tested for statistical differences between this study and a few experimental studies. Ranges of CCR_T and CCR_N in this study were 1.00-1.08 and 0.92-1.01 (Table 4), respectively. All of the individual CCR_T of HRF (humerus, radius, femur) (3 from 3) were higher than unity for goats, while most of CCR_N of HRF (2 from 3) were lower than unity for chicken . The range of BBR_T was 0.96 - 1.10, which was smaller than BBR_N in chicken (0.66-1.01) . All of

Table 3. Amount (A) and amount ratio of lead in cartilage and bones in goat (AR_T) and chicken (AR_N)

Bone organ	Goat				Chicken			
	A_{LT} (µg)	A_{BT} (µg)	A_{XT} (µg)	AR_T	A_{LN} (µg)	A_{BN} (µg)	A_{XN} (µg)	AR_N
Humerus (H)	179.6	130.0	309.6	1.38	31.0	58.7	89.7	0.53
Radius-ulna (R)	205.9	194.8	400.7	1.06	33.4	46.8	80.2	0.71
Femur (F)	219.1	114.6	333.7	1.91	73.1	45.7	118.8	1.60
Tibia (T)	148.0	119.9	267.9	1.23	86.2	41.8	128.0	2.06
Average	188.2	139.8	328.0	1.34	55.9	48.3	104.2	1.16
Subtotal (left or right part)	752.6	559.3	1,312	-	224	193	417	-

All value units are expressed in µg except for CV (%) and AR (unitless); A_{XT} = total amount = A_{LT} + A_{BT}

the BBR_T of HRF were higher than unity for goat, while all of BBR_N were lower than unity for chicken (Table 4). The range of BBR of lead in this study was 0.89 - 1.08 and comparable to those in literature (0.70 – 1.10), although the range in this study was smaller than that in the literature. The results of BBR_T in this study were comparable to those obtained from experimental goats (0.96 – 1.08) (Cretacci and Parsons, 2010) (Table 4), with no statistical difference ($p > 0.05$; paired t-test) was found between the two studies. Similarly, there was no significant difference ($p > 0.05$) for BBR_N in this study and that in Ethier *et al.* (2007) who investigated field sampled birds. A BBR_N for non HRFT (tarsus) was reported by Valera *et al.* (2017) as 1.25, which was higher than that in this study (1.04).

Cartilage to other tissue concentration ratio (COR) and bone to other tissue concentration ratio (BOR)

COR and BOR are especially related to the CB content in meat, which could be ingested incidentally or accidentally. COR is important due to the occurrence of cartilage in meat or meat products (Latorre *et al.,* 2015) as a source of dietary exposure of lead. The COR_T (meat) in this study was 3.5 while the COR_N (meat) was 3.7 in goat and chicken. Similarly, the BOR_T (meat) and BOR_N (meat) in this study were 3.68 and 3.85, respectively (Table 2). Meat deboning would decrease the BOR (meat) and risk of lead exposure by consuming bone-in meat (Molaee-aghaee *et al*., 2020). CB contents (w/w) in the breast of veal were reported as 2.7 ± 2.3 % and 0.5 ± 0.1%, respectively (Branscheid *et al.,* 2009). The BOR_T (teeth) in this study was 1.04 and this value was comparable to that in sheep (2.12) (Shen *et al.,* 2019) and deer (1.22) (Demesko *et al.,* 2018). The variation of BOR (teeth) in wild deer was influenced by age (Demesko *et al.,* 2018). A composite sample of bone and teeth was proposed as an indicator of air pollution of lead in wild ruminants (Ballova *et al.,* 2019).

The BOR_N (feather) in this study was 3.13, which was within the ranges of the BORs of some birds in the literature (1.50 – 5.26) (Table 2). A high correlation coefficient (0.60) was reported between lead concentration in the femur and that in resident birds' feathers (Mukhtar *et al.,* 2020). In an experimental study, no significant difference was found for lead concentrations in femur, tarsus, and feathers following exposure of chicken with three different levels of lead in the diet (Valera *et al.,* 2017). The data ratios were determined as meaningful variables (Dhanoa *et al.,* 2018), for example the BOR (feather) value was calculated by considering the occurrence of lead in calcified tissue (bone) and keratinized tissue (feather) (Tehrani *et al.,* 2021). COR and BOR have practical uses, such as CORs (meat) and BORs (meat) related to lead exposure through consuming cartilage-in-meat and bone-in-meat. BOR (teeth) and BOR (feather) have been proposed as bioindica-

Table 4. Concentration ratio (CR), cartilage to cartilage concentration ratio (CCR), and bone to bone concentration ratio (BBR) of lead in cartilage and bones of goat and chicken in present study and literature

Bone Organ	Goat									Chicken						
	C_{LT} [a] (µg.g^{-1})	C_{BT} (µg.g^{-1})			CR_T	CCR_T	BBR_T			C_{LN}	C_{BN} (µg.g^{-1})		CR_N	CCR_N	BBR_N	
	TS[a]	TS	CP[b]	CP[c]	TS	TS	TS	CP[b]	CP[c]	TS	TS	ET[d]	TS	TS	TS	ET[d]
Humerus (H)	5.19	5.06	40.1	34.7	1.03	1.08	1.00	1.08	0.96	5.26	5.06	0.72	1.04	1.01	0.90	0.66
Radius (R)	4.89	5.28	39.3	37.9	0.93	1.01	1.04	1.05	1.05	4.77	5.00	0.76	0.95	0.92	0.89	0.70
Femur (F)	4.89	5.33	38.4	39.7	0.92	1.01	1.05	1.03	1.10	4.78	5.12	1.02	0.93	0.92	0.91	0.93
Tibia (T)	4.79	5.08	39.7	36.2	0.94	1.00	1.00	1.00	1.00	5.19	5.60	1.09	0.93	1.00	1.00	1.00

[a] Present Study; [b] Cretacci & Parsons 2010 (left bones); [c] Cretacci & Parsons 2010; (right bones) [d] in C.eider (Ethier et al. 2007)
Statistical t-test: BCR_T : Present study vs Cretacci & Parsons (2010) ns ($p > 0.05$); BCR_N : Present study vs Ethier et al. (2007) ns ($p > 0.05$)

tors of exposure for wild animals (Ballova *et al.,* 2019).

AR - CR relationship

After the A-C relationship was evaluated to estimate body burden as described previously, the AR – CR relationships were plotted to compare the linear regression line of AR_T – CR_T with that of AR_N – CR_N as a measurement of interspecies difference. The interval between the maximum (1.91) and the minimum AR_T (1.06) was 0.85 while that between the maximum (2.06) and minimum AR_N (0.53) was 1.53. The interval of the maximum – minimum of AR_T was smaller than that of AR_N, while the AR_T (1.34) was higher than that of the AR_N (1.16) . The values of AR > 1 indicated potential exposure to lead in higher amount through consumption of cartilage than of bone (Table 3). The CR_T (0.95) and CR_N (0.96) in this study were less than 1.0. Linear AR – CR relationship (Fig.4), with a wider CR range, can be compared to experimental and estimated linear A - C relationships to know its application in body burden analysis. The average AR_T and AR_N in this study were 1.4 and 1.2, respectively, which indicated that the amount of lead in cartilage was slightly higher than that in bone.

In this study, both ART – CRT and ARN – CRN linear regression lines were above the ideal AR = CR line (slope of 1.0) but below the AR = 2 CR line. Fewer variation was confirmed for AR_T – CR_T (R^2 0.95) than for AR_N – CR_N (R^2 0.76), all were based on data of this study. Chicken bone consumed as chicken broth contains lead in very small amounts (1.5 µg.serving^{-1}) (Hsu *et al.,* 2017). There was no significant difference ($p > 0.05$) between AR_T – CR_T and AR_N – CR_N relationships in this study (Fig.4). No significant interspecies difference was observed for the amount of lead released from bones of cow and pig in bone broths (Hsu *et al.,* 2017). No differences in X-ray diffractometry of decomposed goat's bones and chicken's bones at 800^{0}C and 900-1,100^{0}C (Lesbani *et al.,* 2015). The AR – CR regression lines are useful to evaluate interspecies differences in the amount of lead in CBs of HRFT organs.

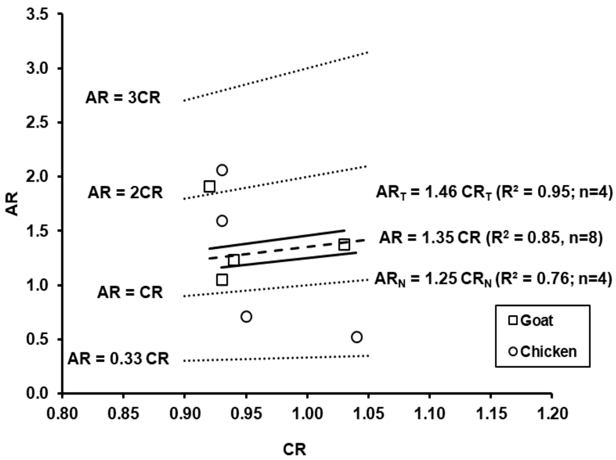

Fig.4. *Relationship between amount ratio (AR) and concentration ratio (CR) of lead in cartilage and bones of HRFT of goat and chicken. Four hypothetical lines of AR – CR relationship is shown. Subscripts $_T$ and $_N$ refer to goat and chicken, respectively*

CV, VR and CVR

The variability analysis in this study consisted of CVs, VRs, and CVRs. The CVs of lead in CBs showed relatively low values, ranging from 5% ($CV_{LN,}$ humerus) to 26% (CV_{BT}, humerus) . A wider range of CVs was found for literature data, ranged from 16% (CV_{LN}) (Bratty *et al.,* 2018) to 169% (CV_L, scaup) (Kalisinska *et al.,* 2007) (SI: App.4). Wider ranges of CVs were observed mainly for wild animals (Lanocha *et al.,* 2013; Kalisinska *et al.,* 2007) and a shorter range of 9 – 17% was observed for experimental goats (Cretacci and Parsons, 2010). As CV is an estimator of relative variability (Ospina and Marmolejo-Ramos, 2019), for which a high variation is determined for 100% or higher (Pelabon *et al.,* 2020). This study found highCVs for wild animals compared to farm animals (SI: App.4).

The values of VR in this study were comparable to those in literature, and the values of CVR in this study were less variable than those in literature. The ranges of VRs of HRFT in goats (0.34 – 1.04) and chickens (0.55 - 1.14) in this study were comparable. VRs from field studies of wild mammals such as red fox and dog ranged from 1.34 – 2.04 (Lanocha *et al.,* 2013; Lanocha *et al.,* 2012b) and VRs of wild birds ranged wider as 1.93 - 6.98 (Kalisinska *et al.,* 2007) (SI: App.4).

Log CVR can be used in meta-analysis of variability (Senior *et al.,* 2020), which gave values of CVR in this study as 0.34 – 1.17, while the range from the literature was much wider as 1.00 to 1.82 for dog (Lanocha *et al.,* 2013), and 1.13 to 1.80 for scaup (Kalisinska *et al.,* 2007). The slope of Ln VR – Ln CVR relationship in this study was closer to unity (1.03) compared to that from literature (2.19), and higher regression coefficient (0.98) was obtained for farm animals in this study than those of wild animals in literature (0.62) (Fig.5).

Probability analysis of AR and CR

Probability analysis in this study consisted of SP, JP, and CP. All probability analyses were based on AR and CR values and provided with a QA to display JP and CP graphically (SI:App.6). The SP values in goat were higher than those in chicken, as for P ($AR_T \geq 1$) was higher than P ($AR_N \geq 1$), and similarly, P ($CR_T \geq 1$) was higher than P ($AR_N \geq 1$) (SI:App.5).

The JP values in goat followed the sequential order as displayed in QA as Q_1 (0.32) > Q_2 (0.26) > Q_4 (0.24) > Q_3 (0.18). However, the JP values in chicken did follow the sequence differently in QA as Q_3 (0.30) > Q_2 (0.26) > Q_4 (0.24) > Q_1 (0.20). The CP values, written as P (AR_T ≥1 **|** CR_T ≥1) in goat was 0.57 which was higher than the P (AR_N ≥ 1 | CR_N ≥1) in chicken of 0.45 (S1: App.5). If the CP with high importance is proposed as ≥ 0.5, the CP in goat met that standard while that in chicken did not. In QA by quick graphical evaluation (SI:App.6), it can be observed that the JPs of goat followed sequential order and CPs of goat outside of line of P of 0.5. All probability values of goat were higher than those of chicken, except for that in Q_4 (SI:App.6). All patterns of SP, JP, CP in goat differed from those in chicken, despite of closeness of the CR values between the two species as described previously. This implies complementary roles of probability analysis to the AR and CR in the DRA.

Extended DRA application in farm animals raised in polluted sites

More specific exposure of lead from farm animals to humans, can be supported by COR and BOR uses (Table 5) such as ingestion of CB particles in meat (Latorre *et al.,* 2015); consumption of CBs in processed food such as sausages (Nagdalian *et al.,* 2021); consumption of chicken broths from cartilage and or bones (Hsu *et al.,* 2017; Monro *et al.,* 2013). Another possible exposure route is the incidentally consumption of small bone, such as chicken bone (Radicic *et al.,* 2019). In this case the A – C relationship is useful to estimate such amount of lead.

BBR is the important parameter in comparing and contrasting lead concentrations in bones of farm animals in polluted sites with those in the reference site, as one of the suggestions in Table 5. For example, the lead concentration in the sheep's radius in the zinc smelting site was almost nine times higher than in the control site. There were similar orders of magnitude of the C_B in radius (22.53 $\mu g.g^{-1}$) (Shen *et al.,* 2019), and the highest C_B 19.3 $\mu g.g^{-1}$ in pochard (Kalisinska *et al.,* 2007) (Table 2) and lead poisoning level in bearded vulture as 20.92 $\mu g.g^{-1}$ (Kruger and Amar, 2018). The BBR can also be used in comparing the C_B in deer containing intact rifle bullets, which was almost 5 times higher than that in unshot deer (Zimmer and Osier, 2018). A comparison of the C_{Bs} of lead in wild to that in farm red deer was reported as 0.98 to 0.55 $\mu g.g^{-1}$, respectively (Tajchman *et al.,* 2020), or the ratio between the two C_{Bs} was close to two.

Extended DRA application in game animals

Some game animals reported in the literature were wild goat (Kanda, 2022), wild cows (Scott *et al.,* 2020), wild chicken and red jungle fowl (Min, 2020) with game animal consumption rate was provided by Gerofke *et al.* (2018). Important topics related to exposure to wild

Table 5. Comprehensive analysis and broader comparison of concentration ratio, amount ratio, variability ratio, and probability of data ratio of exposure analysis of lead in cartilage and bones of farm and non-farm animals

Type of Data Ratio	Comprehensive analysis (Present study)		Extended application of DRA in Pb accumulation				
	Parameter	**Results**	**Farm Animals**	**Game Animals**	**Wild Animals**	**Experimental Animals**	**Surrogate Animals**
Concentration Ratio	C_L	4.94 – 5.00 $\mu g.g^{-1}$	Food Safety		Internal Toxic Conc.	Small Ruminant as Animal Model	Pet Animal as Human Exposure Model
	C_B	5.19 – 5.20 $\mu g.g^{-1}$					
	CR	0.95 – 0.96					
	COR	3.50 – 3.70	Incidentally Consumption				
	BOR	3.68 – 3.85					
	CCR	1.00 – 1.08					
	BBR	1.00 – 1.05	Polluted vs Reference Site		Bioindicator		
Amount Ratio	A – C	Estimation: Measured 1.5:1	Animal Feed Safety			Lab to Field Extrapolation	LADD Estimation
	AR – CR	AR = 1.35 CR					
Variability Ratio	VR	0.34 – 1.04	Spatio-temporal variations of lead accumulation in different species across different habitats				
	CVR	0.34 – 1.17					
	VR – CVR	Ln VR = 1.03 Ln CVR					
Probability of Data Ratio	P (AR ≥ 1 □ CR ≥ 1)	0.20 – 0.32	Consumption Advisory for Local Community				
	P (AR ≥1 **|** CR ≥ 1)	0.45 – 0.57					
	Quadrant Analysis	$Q_1 > Q_2 > Q_4 > Q_3$					

LADD = lifetime average daily dose

animals are determining low to high levels of C_{Bs} including internal toxic C_B, BOR for bone meat ratio, and ratios between the C_{Bs} in wild to that in farmed animals. The JP and CP can likely be applied in local communities of frequent game meat consumers. The P ($AR_T \geq 3$ $CR_T \geq 1$) (0.06) and the P ($AR_N \geq 3$ $CR_N \geq 1$) (0.04) for farm animals in this study can be used to estimate risks for frequent cartilage and or bone consumers.

Extended DRA application in wild animals

The C_{Bs} in deer antlers have been monitored to evaluate the decreasing trends of lead concentrations in deer (from 1 to < 0.3 μg.g^{-1}) in Poland for 60 years (Gizejewska *et al.,* 2020). In other cases, bone and teeth have been proposed as bioindicators of air pollution of lead in wild ruminants (Ballova *et al.,* 2019). The higher C_B of lead in the femur of 2.7 years-old wild bear was reported as 1.9 - 12.5 μg.g^{-1} (Lazarus *et al.,* 2018). Similar level of C_{Bs} was reported as subclinical toxic concentration of lead in wild birds (10-20 μg.g^{-1}) (Pain *et al.,* 2019). These internal toxic concentrations were also comparable to the lethal concentration in bone of bearded vultures (20.9 μg.g^{-1}) (Kruger and Amar, 2018) and wild birds (19.3 μg.g^{-1}) (Kalisinska *et al.,* 2007).

Extended DRA application in experimental animals

Small ruminant fits as animal experimental model for human due to its proportional body weight and long bones to humans (Camassa *et al.,* 2017). Four points related to experimental animals can be further provided to develop the DRA: ossification, body burden, internal toxic concentration in bone, and animal models. This study used 18 months old goats and 6 months old

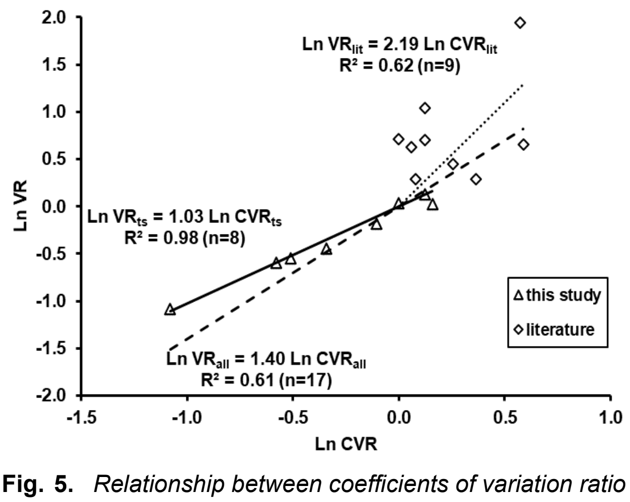

Fig. 5. *Relationship between coefficients of variation ratio (Ln CVR) and variability ratio (Ln VR) of lead in cartilage and bones of goat and chicken, in this study and literature (data from Table 4). Three lines of Ln VR – Ln CVR are shown, this study (ts); literature (lit), and combination of this study and literature (all).*

roosters, both times had already passed the ossification period. Ossification in male Mohair goat occurs at 15-17 months (Alpdogan and Genccelep, 2012), while that in pigeon occurs at day 35 after (Ojaghloo *et al.,* 2018). More detailed ossification ages are reported by Demesko *et al.* (2017). With experimental studies, bone can be used for internal exposure and toxic concentrations, with the latter referring to bone as the target organ of toxicity.

The use of experimental goats (Tehrani *et al.,* 2021; Cretacci and Parsons 2010), experimental cow (Scott *et al*., 2020), and experimental chickens (Valera *et al.,* 2017) are recommended in the accumulation of heavy metals, including their uses in ossification studies which is generally done by using rats (Rodriguez and Mandalunis, 2018). As an example of comparison, the C_{BT} in goat femur in this study was 5.33 μg.g^{-1} (Table 1), while the C_{BT} in the femur of the experimentally lead-dosed cow was 0.5 – 2.4 μg.g^{-1} (Scott *et al.,* 2020).

Extended DRA application in surrogate animals

Dogs can be considered the main surrogate animals for humans as dogs share the same environment as humans (Sumner *et al.,* 2020). Dog is more feasible to represent domestic exposure to human (Sumner *et al,.* 2020), than other animals, such as goat, which is more suitably used for the orthopaedic purpose (Cretacci and Parsons, 2010). Dogs that consume game meat can also be used as surrogate animals for humans (Fernández *et al*., 2021). Comparing the linear plots of Ln VR - Ln CVR of dog to that in human bones will highlight the closeness of dog as surrogate animal for humans.

The C_L and C_B of lead in dog was reported as 2.83 and 1.55 μg.g^{-1}, respectively (Lanocha *et al.,* 2012b) (Table 2), while the C_B of lead in human bones ranged from 0.53 to 54 μg.g^{-1} (Brodziak-Dopierata, 2020). The LADD (μg.kg^{-1} body weight.day^{-1}) of the surrogate animals can be used to estimate lead exposure in humans. Although less relevant, backyard chickens can also be used as surrogate animals due to their increasing presence in urban gardening for food production (Yazdanparast *et al.,* 2022), especially for egg consumption (Leibler *et al.,* 2018).

As described previously, variables among farm, game, wild, experimental, and surrogate animals can be compared using Ln VR - CVR relationships (Table 5). New field and laboratory data of accumulation studies in animals with diverse habitats will increase the understanding of lead bioaccumulation in CBs of animals related to species, habitat, and bone type differences.

Conclusion

The mean values of C_{LT} and C_{BT} in goats were 4.94 and 5.19 μg.g^{-1}, respectively, and the mean values of

C_{LN} and C_{BN} in chickens were 5.00 and 5.20 µg.g^{-1}, respectively. The average CRs in goats and chickens were 0.95 and 0.96, respectively, and the ranges of VR and CVR were 0.34 - 1.14 and 0.34 - 1.17, respectively. HRFT organs are suitable to be used as primary organs of lead accumulation in CBs for field biomonitoring and estimation of body burden. Comprehensive data ratio and broader connection analysis accentuated the applicability of DRA in exposure assessment of lead in CBs, but also highlighted CB as the main organ in bioaccumulation of lead in animals. The DRA is prospective to be developed for future research in bioaccumulation studies.

List of symbols

A list of symbols is provided in SI: App.7.

Ethics approval

The collection of cartilage, bones, and other organs of goats and chickens used in this research from the local markets and preparation in the laboratory were approved by the Animal Ethics Committee of Satya Wacana Christian University (Reference No.006/ KOMISIETIK/EC/1/2023), Salatiga, Central Java Indonesia.

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Supplementary information

All data generated and analyzed in this study are given as supplementary information (SI: App. 1 to 6). A list of symbols is provided in SI: App.7. The author is responsible for the content or functionality of any supplementary information. Any queries regarding the same should be directed to the corresponding author. The supplementary information is downloadable from the article's webpage and will not be printed in the print copy.

Conflict of interest

The author declares that he has no conflict of interest.

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