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Levels of Heavy Metals in Drinking Water, Blood and Milk of Buffaloes during Summer and Winter Seasons in Ludhiana, Punjab (India)

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ABSTRACT

The study investigated the influence of season on the concentrations of heavy metals chromium (Cr), nickel (Ni), arsenic (As)and lead (Pb) in blood and milk of buffaloes ingesting contaminated water in environmentally polluted areas of Ludhiana district of Punjab (India).Inductively coupled plasma- optical emission spectrometry (ICP-OES) instrument was used for estimating the heavy metals. The drinking water, blood and milk samples collected from the heavy metal exposed areas had significantly (p<0.01) higher Cr, Ni, As and Pb concentrations as compared to control. The drinking water, blood and milk samples collected during summer had significantly (p<0.01) higher concentrations of heavy metals as compared to that during winter season. The level of heavy metals Cr, Ni, As, and Pb in drinking water was positively correlated to their levels in blood and milk of buffaloes both winter and summer seasons. The concentration of all the heavy metals in all the samples of milk tested (control as well as exposed areas) were below permissible limits defined as per FSSAI regulations (2010) and hence do not possess any toxicological risks. It may be ascertained that assessment of the heavy metal status in the water is enough to give precise (P<0.0001) estimates of the same heavy metals in blood and milk of buffaloes in the area. The heavy metal pollution in exposed area might be due to intense distribution of industrial sites. However, further study is needed for the characterization of heavy metals in the context of human wellbeing and to ensure the protection of public health.

Key words: *Chromium, Nickel, Arsenic, Lead, Season, Bubalusbubalis*

INTRODUCTION

The worldwide attention has involved towards pollution of heavy metals in the aquatic environment due to their persistence and ecological toxicity[2,11]. Some of the heavy metals like arsenic (As), cadmium (Cd), lead (Pb), mercury (Hg) are persistent, accumulative and are not metabolized in environment; thus exerting toxic and harmful effects with challenging consequences for environmental, evolutionary, nourishing and ecological reasons[13].

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The existence of heavy metals has been identified in ground water of Ludhiana[23] which is supported by the findings presented by the heavy metal status of the cattle living in Buddha Nallah area of Ludhiana district in Punjab[7]. Thiscould be owing to the presence of heavy metals in surface water of Buddha Nallah and its seepage in the adjacent groundwater aquifers. The fabric industry is one of the most key and rapidly developing industrial areas in Ludhiana city of Punjab, India. The wastewater from several textile mills, woven textile and knit fabric finishing industries is drained into Buddha Nallah thus making it highly polluted[27,28].

In recent years, it has been widely believed that chemicals in the surroundings manifest their toxicity as mixtures[32]. In larger quantities, heavy metals affect human and animal health. They get accumulated in tissues of dairy animals and eventually secreted in milk for the reason of their non-biodegradable and persistent nature[12,18]. Heavy metals cause various biochemical, immunological and reproductive disturbances in animals. They are toxic, specifically with prolonged exposure; and certain forms such as hexavalent chromium or nickel carbonyl were labeled as carcinogens[20].

Heavy metals and metalloids, at the cellular level, lead to production of reactive oxygen metabolites, elicit oxidative stress, cause DNA damage, affect membrane function, nutrient assimilation and disturb protein function or activity[30]. Even at low levels, heavy metals can upset the normal body function, by playing as endocrine disruptors, production of reactive oxygen metabolites, hindering of essential functional groups or displacing the essential metal ions from biomolecules, leading to loss or inhibition of various enzyme activities and modification of metabolism[17], resulting in physiological or biochemical alterations in the animal. The toxic metals hinder antioxidant enzymes and exhaust intracellular glutathione due to the pro-oxidative properties of metals[22], and such changes may even override the adaptive potential of the animals. The provision of safe foods for human consumption is the first objective of the livestock production taking into account the welfare of animal. Bio magnification of food chain is due to the fact that heavy metals are abundant and generally persist in the environment[34]. Heavy metal contaminants enter animal systems through pollution of air, water, soil and feed/fodder. Bioaccumulation of toxic heavy metals, e.g. lead (Pb), cadmium (Cd), arsenic (As), mercury (Hg) etc., in milk has evoked great concern in recent years.

Heavy metal contamination problem is one of the alarming environmental hazards in Ludhiana, the industrial hub of Punjab, India as evidenced from their presence detected in soil, water, and fodder[26,27,28]. Milk contamination by heavy metals is considered unsafe for animal or human consumption[4]. The present study is therefore focused on the relationship between the levels of heavy metals in drinking water and blood and/or milk of buffaloes during summer and winter seasons in polluted areas of Ludhiana district of Punjab, India.

MATERIAL AND METHODS

Ethical approval

Experimental procedures using buffaloes in this study have been conducted after approval from the Institutional Animal Ethical Committee (IAEC) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India. All the research investigations with buffalo were carried out according to the IAEC guidelines.

Location of study area and animals

The water samples (100) were collected from the dairy farms located along side of Buddha Nallah on Haibowal Road (Haibowal, Choorpur and Balloake) and Mattewara (25 kilometers away from the Buddha Nallah where there were not any form of human activities that could generate wastes). The levels of heavy metals in drinking water were above the permissible limit (Chromium-0.05 µg /ml, Nickel-0.02 µg /ml, Arsenic –0.05 µg /ml and Lead-0.05µg/ml) in the dairy farms adjoining Buddah Nallah; which were taken as exposure area. The water levels of heavy metals in Mattewara area were below the permissible limits; therefore it was taken as uncontaminated or control area. Tube well water, blood and milk samples were collected during summer (June 2017 - August 2017) and winter (November 2017 - December 2017).

The buffaloes were maintained by their owners and provided with standard diet and *adlibitum* water.

Experimental procedure

Collection of samples

Drinking water

Drinking water samples were collected from tube wells in the exposed areas (n=60) and control area (n=40) in both seasons. These tube wells were used for irrigation and supply of drinking water to the buffaloes. Drinking water samples were collected in polypropylene bottles prewashed with 1ml/L nitric acid[19], preserved in 4 ml/L hydrochloric acid and refrigerated at 4^{o}C till analyzed.

Blood Samples

Blood samples (5 ml) were collected in heparinized vials by jugular venipuncture from the selected buffaloes both from exposed (n=60) and control areas (n=40) during summer and winter seasons using a sterile syringe/needle, and kept at -20°C for quantitation of heavy metals.

Milk samples

The fresh milk samples (50 ml) were collected in clean sterilized screw capped glass tubes. Immediately after collection, milk samples were transported to the laboratory in ice packs, and stored at -20°C until further analysis[9].

Digestion of water samples:

The drinking water samples were digested by following the method of Mc Graths and Cunliffe[21] with slight modifications. To 10 ml of water sample was added the10 ml of diacid (70 ml of Nitric acid + 30 ml of Perchloric acid) and left overnight. Then these mixtures were digested on hot plate at 250ºC until a colourless solution reducing of its volume to 1-3ml. After cooling, those digested samples were filtered using Whatman filter paper no.42 and made to10 ml with double glass distilled water.

Acid digestion of blood samples:

All glass wares were first cleaned with 10% HNO_3solution and then further washed with the distilled water and then sterilized in hot air oven at 160^{0} C for 60 minutes. To 1ml of whole blood samples, added 15 ml of a tri-acid mixture (10:4:1 HNO_3, H_2SO_4, and $HClO_4$) and left overnight. Then these mixtures were digested on hot plate at 250ºC until a transparent solution and volume reduced to 1-2 ml. After cooling, the digested sample was filtered using Whatman No. 42 filter paper and the volume of filtrate was made up to 10 ml with double glass distilled water[3].

Acid digestion of milk samples:

Milk samples were digested by the method described by Ahmad *et al*., 2017 with slight modifications. To 10 ml of milk sample, added a mixture of 12 ml of1:3 H_2 O_2 (30%) and HNO_3 (65%) in acid prewashed Teflon vessels. After standing overnight, samples were heated on a hot plate until their volume reduced to 2 ml. After cooling, the digested samples were filtered using Whatman No. 42 filter paper and the filtrate diluted to 20 ml with double glass distilled water.

Detection of heavy metals:

Inductively coupled plasma-optical emission spectrometry (ICP-OES)(PERKINS ELMER OPTIMA 2100 DV MODEL)instrument was used for estimating the heavy metals. The working standards were analyzed at the beginning and end of a run and intermittently during longer runs. According to the absorbance, the concentration was measured directly, when the sample was well within the linear working range of the standard curve. All analyses were performed in duplicate. Analytical blanks were run along with each batch of digestion set[8].

Statistical analysis:

The experimental data were subjected to two way analysis of variance with interaction using SYSTAT 13 software (Inc., CA, USA, ver. 13.00.05)[30]

$$Y_{ijk} = \mu + S_i + T_j + I_{SxT} + e_{ijk}$$

Where, Y_{ijk}: The parameter being studied; μ: overall mean; S_i: Effect of ith season (i=1,2); T_j: Effect of jth treatment (j=1,2); I_{SxT}: Interaction between season and treatment and e_{ijk}: random error.

The differences between the mean values of the parameters belonging to the experimental groups reared during summer and winter seasons were analyzed for significant differences using Duncan's multiple range post hoc test. The normality of the data and autocorrelation between the groups were tested by Kolmogorov–Smirnov test (Lilliefors) and Durbin-Watson D-Statistic, respectively. Pearsonian correlation between the heavy metals (Cr, Ni, As, Pb) in water, blood and milk was determined. Heavy metal content in water was regressed to that of blood as well as milk was determined. The graphs have been generated using R programming language.

RESULTS AND DISCUSSION

The exposed areas showed significantly ($p<0.01$) higher concentrations of heavy metals Cr, Ni, As and Pb (Table 1) in water, blood and milk as compared to control area of Ludhiana district during both summer and winter seasons. The mean heavy metals levels in water, blood and milk were observed to be approximately 2-3 folds higher in the summer compared to winter season in both the areas (Fig.1). The heavy metal concentrations in the drinking water samples of the exposed area were found above the permissible limit. Considerable variations were observed in the concentrations of all heavy metals (Cr, Ni, As and Pb) in both the groups with lesser levels during winter season and higher during summer. The concentration of all the heavy metals in all the milk samples analyzed were below permissible limits defined by FSSAI regulations[10] and hence did not possess any toxicological risks. The Pearson correlation matrix of four heavy metals collected from drinking water, blood and milk are versed in the Table 2.Significant positive relationship was observed among Cr , Ni, As and Pb concentrations (Table 2) in drinking water, blood and milk in both the areas. It is evident that water, blood and milk showed significant positive correlation among themselves for all heavy metals studied (Table 3). It means there may be a source of pollution that is increasing the heavy metals concentration in water. The regression analysis is presented in (Table 3) between water and blood for Cr(r=0.782), Ni (r=0.844), As (r=0.888) and Pb (r=0.838) at ($P<0.01$) and between blood and milk Cr(r=0.811) , Ni (r=0.619), As (r=0.895) and Pb (r=0.845) at $P<0.01$). The regression output shows that concentrations of all heavy metals in water, blood and milk values are statistically significant ($p<0.01$). The P values of the coefficients indicate these relationships are significantly correlated. The coefficients describe the mathematical relationships between all heavy metal levels of blood and milk (dependent variable) and water (Independent variable).The higher R^2 values are indicative of better degree of accuracy and least chances of deviation from the plotted line (regression), thus these equations derived can be used for general predictions and interpolation for intermediate values. It can be ascertained that simple estimation of the heavy metals in the water are enough to give precise ($P<0.0001$) estimates of the same heavy metals in blood and milk of buffaloes in the area.

The levels of As and Pb in drinking water recorded in this study were higher whereas levels of Cr and Ni were similar to the levels reported by Singh *et al.*,[27,28]. They reported that the considerable variations were observed in the concentration of Cr during both seasons but the seasonal pattern for all the heavy metals was same i.e. high values during summer and low values during winter season. These findings were similar to that of the result obtained in this study. The levels of As, Pb, Ni and Cr in drinking water in this study were higher than the values reported by Balachennaiah *et al.*,[5]. The levels of Pb, As and Cr in drinking water of Malwa region of Punjab reported by Sharma and Datta,[25] were higher than the levels recorded in this study. The levels of As recorded in this study in both drinking water were higher to those reported by Dash *etal*.,[6]. The water levels of As, Cr, Ni and Pb during different seasons in some selected ponds of district Ludhiana were similar in accordance with those reported by Kaur and Hundal,[15]. The blood levels of Ni and Pb obtained in this study is much higher whereas blood levels of As much lower than those reported in cattle (Ni 0.04mg/L, Pb 0.58mg/L and As 0.13mg/L) by Dhaliwal and Chhabra,[7].In this study, the levels of heavy metals in buffalo's milk was much lowerthan the permissible limits which is in accordance with the values reported in cow's milk by Kabir A. *et al*.,[14]. The levels of As, Ni and Pb recorded in this study were higher than the levels reported by Dhanalakshmi and Gawdaman,[8] in goat milk of industrial area of Hosur, Tamilnadu. The levels of Pb were similar and that of As were higher in this study in comparison to that reported by Roy *et al*.,[24] in buffalo milk samples from Haryana. The content of Ni and Cr in buffalo milk in the present study was lower than the levels reported by Ahmad *et al*.,[1].

Increase in the concentration of heavy metals during summer season as compared to that of winter could be due to drought and decrease in water level[16]. The results indicate that drinking water might be the primary source of all heavy metals in the present study i.e., Cr, Ni, As and Pb which contaminate the blood and subsequently milk. The present results indicate that, Cr, Ni, as and Pb may have the same source of production and they may have entered into the body of these animals through bio augmentation process.

The authors are of the view that drinking water of exposed area is having higher concentrations of heavy metals because of some tannery industries nearby the area thus creating pollution of the water source. The results showed that blood and milk have similar trend with respect to these heavy metals levels in drinking water. The higher concentrations of heavy metals in drinking water may be a factor for increase in concentration of heavy metals in blood and milk of animals of heavy metal exposed area. Animals that drink from heavy metal polluted waters may accumulate such metals in their tissues, and milk, if lactating[31]. Therefore, drinking water might be the source of heavy metal contamination as evidenced from the increased blood and milk heavy metals level in the present study.

Table 1. Heavy metal (mean ± S.E.) concentration (µg /ml) in drinking water, blood and milk of buffaloes of exposed and control areas during different seasons

Area/ Sample	Control (n=40)		Exposed (n=60)	
	Winter	Summer	Winter	Summer
Chromium				
Water	$0.013^a \pm 0.001$	$0.038^a \pm 0.001$	$0.164^b \pm 0.002$	$0.483^c \pm 0.003$
Blood	$0.021^a \pm 0.002$	$0.043^a \pm 0.001$	$0.314^b \pm 0.014$	$0.480^c \pm 0.008$
Milk	$0.009^a \pm 0.001$	$0.013^a \pm 0.001$	$0.037^b \pm 0.001$	$0.046^c \pm 0.001$
Nickel				
Water	$0.009^a \pm 0.001$	$0.017^a \pm 0.001$	$0.186^b \pm 0.002$	$0.557^c \pm 0.003$
Blood	$0.011^a \pm 0.001$	$0.018^a \pm 0.001$	$0.290^b \pm 0.020$	$0.342^c \pm 0.014$
Milk	$0.006^a \pm 0.001$	$0.010^a \pm 0.001$	$0.015^b \pm 0.001$	$0.018^c \pm 0.001$
Arsenic				
Water	$0.010^a \pm 0.001$	$0.016^a \pm 0.001$	$0.126^b \pm 0.001$	$0.237^c \pm 0.002$
Blood	$0.022^a \pm 0.001$	$0.034^a \pm 0.001$	$0.225^b \pm 0.008$	$0.320^c \pm 0.008$
Milk	$0.004^a \pm 0.001$	$0.011^a \pm 0.001$	$0.033^b \pm 0.001$	$0.047^c \pm 0.001$
Lead				
Water	$0.020^a \pm 0.001$	$0.031^a \pm 0.001$	$0.265^b \pm 0.002$	$0.377^c \pm 0.004$
Blood	$0.019^a \pm 0.001$	$0.029^a \pm 0.001$	$0.364^b \pm 0.017$	$0.445^c \pm 0.017$
Milk	$0.001^a \pm 0.001$	$0.002^a \pm 0.001$	$0.005^b \pm 0.001$	$0.008^c \pm 0.001$

Superscripts a, b and c : Means with different superscripts are significantly different ($p<0.01$) within each row between different seasons.

Note: Maximum Permissible Limits in drinking water in view of animal health: Chromium - 0.05 µg /ml, Nickel - 0.02 µg /ml, Arsenic - 0.05 µg /ml and Lead - 0.05µg /ml (WHO 2005).

Table 2: Pearson's Correlation Co-efficient (r) for the heavy metal concentrations in drinking water, blood and milk of buffaloes

	Water Vs blood	Water Vs Milk	Blood Vs Milk
Chromium	0.782**	0.722**	0.811**
Nickel	0.844**	0.680**	0.619**
Arsenic	0.888**	0.822**	0.895**
Lead	0.838**	0.857**	0.845**

**Indicates significant relationships ($p < 0.01$).

Table 3: Regression coefficient for heavy metal concentration in drinking water, blood and milk of buffaloes

Sr.No.	Metal	Y(Dependent)	X(Independent)	b_0@	b_1#	P##	$R^2$$$
1	Chromium	Blood	Water	0.060	1.626	0.000	0.611
		Milk	Water	0.014	0.012	0.000	0.611
2	Nickel	Blood	Water	0.032	1.912	0.000	0.713
		Milk	Water	0.009	0.001	0.000	0.463
3	Arsenic	Blood	Water	0.026	0.957	0.000	0.789
		Milk	Water	0.008	0.014	0.000	0.676
4	Lead	Blood	Water	0.008	2.843	0.000	0.702
		Milk	Water	0.001	0.019	0.000	0.734

@ indicates Intercept constant
# indicates Simple linear regression coefficient
indicates Probability value
$$ indicates Coefficient of determination

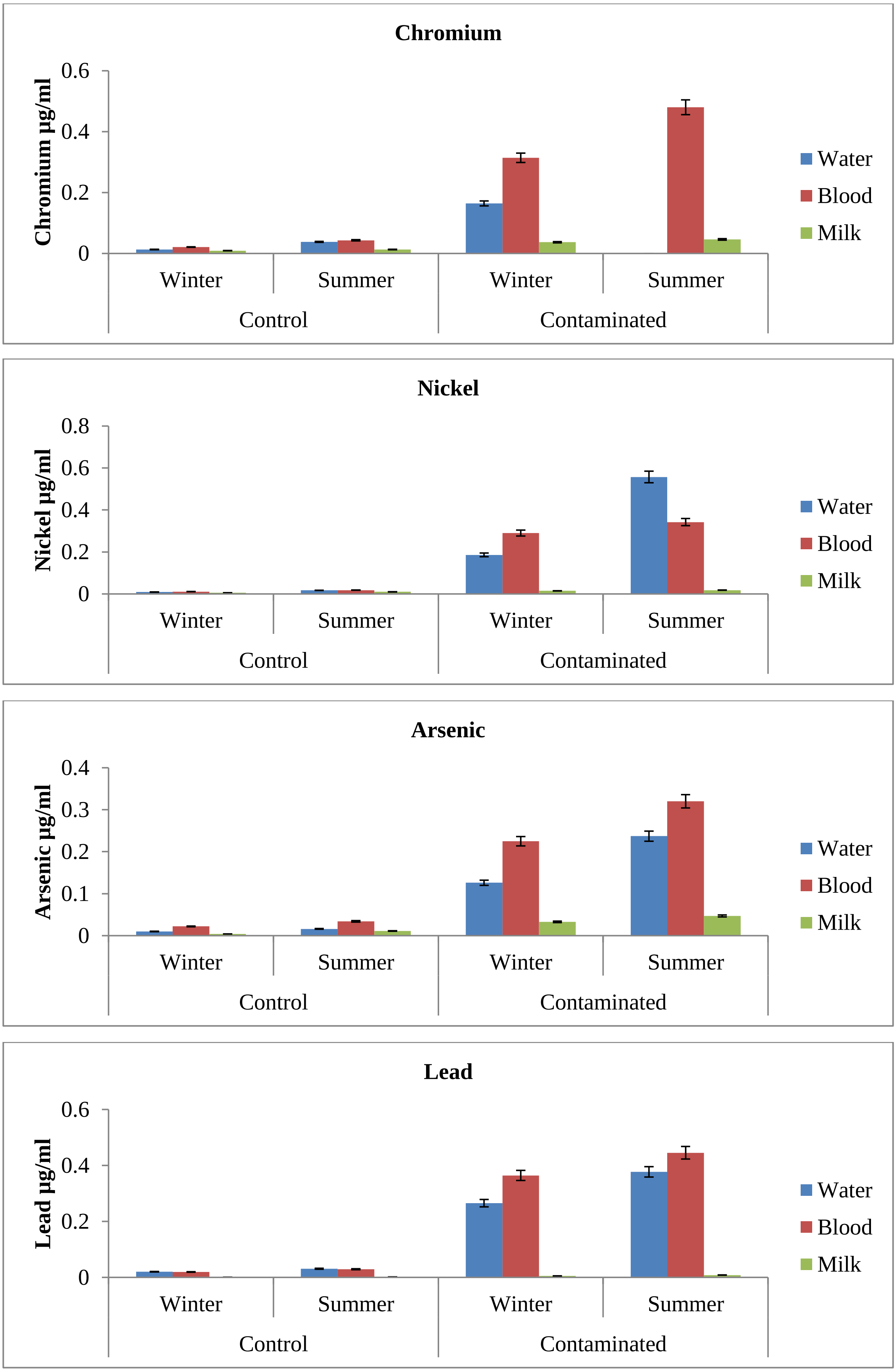

Fig. 1 : The seasonal variation of heavy metals in control and exposed groups in winter and summer seasons

CONCLUSION

Considerable variations were observed in the concentration of all heavy metals (Cr, Ni, As and Pb) in both the groups with lesser accumulation during winter season and higher during summer. The probable reason for elevated levels of all the heavy metals in drinking water, blood and milk of buffaloes in exposed area during summer might be due to environmental contamination, drought and decrease of water level. The heavy metal concentration in drinking water might have elevated their blood levels thus reflecting in milk samples. The concentration of all the heavy metals in all the samples analyzed in milk was below permissible limits and hence do not possess any toxicological risks. It can be ascertained that simple estimation of the heavy metals in the water are enough to give precise ($P<0.0001$) estimates of the same heavy metals in blood and milk of buffaloes in the area.

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