Environmental Pollution 255 (2019) 113322

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Relationships between Pb, As, Cr, and Cd in individual cows' milk and milk composition and heavy metal contents in water, silage, and soil[☆]

Xuewei Zhou [a, b, c], Nan Zheng [a, c], Chuanyou Su [a, c], Jiaqi Wang [a, c, *], Hélène Soyeurt [b]

[a] *Key Laboratory of Quality & Safety Control for Milk and Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing 100193, PR China*

[b] *TERRA Research and Teaching Centre, Gembloux Agro-Bio Tech, University of Liège, Gembloux, Belgium*

[c] *Laboratory of Quality and Safety Risk Assessment for Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing 100193, PR China*

ARTICLE INFO

Article history:
Received 2 July 2019
Received in revised form
27 September 2019
Accepted 27 September 2019
Available online 28 September 2019

Keywords:
Lead
Arsenic
Cadmium
Milk composition
Relationship

ABSTRACT

Various industrial activities lead to environmental pollution by heavy metals. Toxic heavy metals enter the food chain of dairy cows through feed and water, then transferred into milk. This study investigated the correlations of heavy metal contents between individual cows' milk, water, silage and soil. The relationships between heavy metal contents in individual cows' milk with milk protein, fat, lactose, solid nonfat (SNF), and total solids (TS) were analysed. Concentrations of Pb, As, Cr, and Cd in milk, silage and water were measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Lead, Cr, and Cd in soil were measured by Atomic Absorption Spectrometry (AAS), and As was detected by Atomic Fluorescence Spectrometry (AFS). One-way non-parametric tests and Spearman correlation analyses were performed using SAS 9.4 software. Levels of Pb and Cd in milk from the unpolluted area were significantly lower ($P < 0.01$) than those from industrial area. Significantly higher ($P < 0.01$) As residue was recorded in milk from unpolluted area. Positive correlation of Pb was observed between milk and silage, and As in milk was positively correlated with As in water. Content of As in milk was slightly ($r = 0.09$) correlated with As in silage, even though strong positive correlation ($r = 0.78$) was observed between silage and water. Positive correlations were observed for Cr and Cd between milk and silage, as well as milk and soil. Positive correlations were observed in Pb-protein, Cr-protein, and Cd-lactose; other positive correlation coefficients were nearly equal to zero. The results suggest that industrial activities lead to possible Pb and Cd contamination in milk. Drinking water could be the main source of As contamination in cows. No clear relationship was found between milk composition and heavy metals contents in milk. Water and soil on the farm had a partial contribution to heavy metal contamination in milk.

1. Introduction

In daily life, milk and dairy products are an important component of human diets. The national consumption of liquid milk in 2016 was 20.3 kg/person in China, 59.4 kg/person in European Union and 69.2 kg/person in USA (Dairy Association of China, 2018). However, it is possible that arsenic (As), chromium (Cr), and cadmium (Cd) could enter the feed of farm animals by a variety of exposure routes and contaminate food products derived from those animals (Caggiano et al., 2005; Crout et al., 2004; Khan et al., 2010). These heavy metals can lead to serious systemic health problems when they are consumed excessively (Simsek et al., 2000; Zhao et al., 2012). In humans, the main target organ for lead (Pb) toxicity is the central nervous system; the developing brain is more vulnerable to the neurotoxic effects of lead than the mature brain (Chiodo et al., 2004; EFSA, 2010). Furthermore, Pb is classified as probably carcinogenic to humans (Group 2A) by the International Agency for Research on Cancer (IARC), the suspected target organs are lung and stomach (IARC, 2014). Cadmium is classified by IARC as a human carcinogen (Group 1) based on the convincing evidence that extensive exposure to Cd is associated with an increased prevalence in the occurrence of various types of cancer appearing

---

[☆] This paper has been recommended for acceptance by Christian Sonne

[*] Corresponding author. Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, No. 2, Yuanmingyuan West Road, Haidian District, Beijing, PR China.

E-mail address: wangjiaqi@caas.cn (J. Wang).

https://doi.org/10.1016/j.envpol.2019.113322
0269-7491/

in lung, kidney, breast, prostate, urinary tract of humans. This is related to the fact that Cd can affect cell proliferation, differentiation, and other cellular activities (IARC, 2014; Waisberg et al., 2003). Lead and Cd often coexist in polluted regions (Phillips et al., 2003). Arsenic contamination in drinking water is the most important route of exposure for humans and cows, and As absorbed by food crops as they grow is another exposure route (Ohno et al., 2007; Uddh-Soderberg et al., 2015; WHO, 2010). Inorganic As present mainly in natural groundwater can cause cancer such as skin, bladder and lungs; while organic As is abundant in seafood, and is less toxic than the inorganic form and can be eliminated rapidly by the body (EFSA, 2014; WHO, 2010). Chronic exposure to As is especially dangerous to infants and young children. Arsenic has been linked to lower intelligence quotients and poor intellectual function, diminished cognitive function, and cancer (Porova et al., 2014; Tyler and Allan, 2014). The IARC has classified As and inorganic As compounds as carcinogenic to humans (Group 1) (IARC, 2014). Chromium is an essential nutrient, but it is also a human carcinogen (Group 1, lung) (IARC, 2014), has lower toxicity with trivalent chromium and much more toxic with hexavalent (Costa and Klein, 2008; WHO, 1996). Considering the hepatotoxic or neurotoxic properties of heavy metals such as As or Pb, which cause damage to human brain and liver, it is important to minimise their contents in foods (Porova et al., 2014).

Previous studies have reported the formation of different Pb complexes in stems and leaves of alfalfa, but there was no change of oxidation state of Pb(II) when it was transported from roots to leaves (López et al., 2007, 2009). Rosas et al. (1999) found a good correlation between extractable As in soil and the content present in alfalfa roots. The root of plants uptake and mobilize As(V) through the phosphate transport channels (Tripathi et al., 2007). Once As is adsorbed in the roots, it is transported to the upper portion of the crop. Same as other cations, Cd drives from root into the root cells based on the electrochemical potential gradient of the plasma-membrane in the root cells of plants (Perriguey et al., 2008; Wang, et al., 1994). Chromium enters the plants by reduction and complexation with root exudates.

Previous studies have investigated the possible origins and exposure routes of heavy metals in raw milk, especially the effect of exposure to industrial pollution. Rahman et al. (2008) summarised multiple studies and combined their results, which suggested that plant-animal-human may be a potential food chain pathway of As accumulation in the human body. Natural exposure of lactating cows to the environmental contamination around steel manufacturing plants produced higher milk Cd levels (Patra et al., 2008). Swarup et al. (2005) found the similar results for Pb levels in milk. Concentrations of Pb were significantly higher in the milk of cows reared around a steel processing unit (501 ± 37 μg/L) than milk from unpolluted areas (252 ± 28 μg/L). Higher Pb levels in milk from farms near to areas of thermal power were also mentioned by Gonzalez-Montana et al. (2012). A positive correlation was also estimated by other authors between As contents in water and in milk (Pérez-Carrera et al., 2016b). Cano-Sancho et al. (2015) observed a weak positive correlation ($P > 0.05$, $r = 0.18$) between Cr concentrations in milk and fat or protein contents in milk.

In conclusion, a series of studies have focused on heavy metal contamination in raw cow milk and dairy products, and the relationships between heavy metal contents in raw milk with those in water, feed, and soil. However, milk samples collected in those studies were almost always from bulk tank; few studies have reported heavy metal concentrations in milk from individual cows, especially the correlations between water, soil, feed, and individual cow milk. Besides the impact on human health, the presence of heavy metals can impact the health of the cow and therefore directly or indirectly affect the milk composition. To our knowledge, there are no reports about the relationships between milk heavy metal concentrations and milk composition at the individual cow level. This study therefore aims to investigate 1) the relationships between heavy metals in raw milk and corn silage, as well as in water and soil, and 2) the links between the contents of Pb, As, Cr, and Cd in individual cow milk samples with the concentration of milk protein, fat, lactose, solid nonfat (SNF), and total solid (TS).

2. Materials and methods

2.1. Sampling of milk, water, silage, and soil

Samples were collected during autumn 2017 from 5 farms (B, C, D, E, F) in Tangshan and spring 2018 from 1 farm (A) in Qiqihar, China. The distance between farms and an industrial source is ranked as $A > B > E = F > D > C$. Tangshan is an industrialised city with factories that include steel plants, a cement plant, and waste incineration plant, which are potential sources of environmental pollution. Qiqihar is a city located in an agricultural area and has a wetland reserve. This preserved region was therefore supposed to be free from pollution and was used as a control farm. Sixty lactating Holstein cows in parity 3 were selected to collect milk samples (i.e., 10 milk samples per farm). Milk samples were collected from the udders of cows during the milking procedure. The mean days in milk was 130 ± 81 d; average milk yield for individual cows was 35.88 ± 11.76 kg/d. Milk samples (500 mL) were collected in polypropylene bottles from the udders of cows during milking and stored at 4 °C. The contents of milk protein, fat, lactose, SNF, and TS were quantified by milkoscan FT120 (Foss Electric, Hillerod, Denmark) within 4 h after sampling. The samples were stored at −20 °C before the quantification of heavy metals by reference chemical analysis. Cows' drinking water samples were collected from each farm and stored in glass bottle at 4 °C until measurement. Silage samples were dried in oven at 60 °C for 48 h and ground to a particle size of 1 mm. Soil samples were obtained from the depth of 0–30 cm from fields where corn plants were grown, and stored in bags. For each set, soil samples were collected at six random points and then mixed to form a 1 kg sample.

2.2. Quantification of heavy metals

All reagents used in the analysis procedure were analytically pure. Ultrapure water obtained with a Milli-Q Plus water purification system (18.2 MΩ cm, Millipore, USA), was used in the analytical procedure. Vessels used in the analytical procedure were soaked in 20% HNO_3 (v/v) for at least 12 h and washed with ultrapure water.

Individual cow milk samples (1 mL) were digested using 4 mL nitric acid (HNO_3, 65%, Merck, Germany) and 3 mL hydrogen peroxide (H_2O_2, 30%, Merck, Germany) in polytetrafluoroethylene (PTFE) tubes. Silage sample (0.5 g) added with 5 mL HNO_3 in PTFE tubes was digested for 4 h. Then, 1 mL H_2O_2 was added in the digest vessel. After 2 h digestion, 1 mL H_2O_2 was added again. The acid solutions of milk and silage samples were digested in a MARS 6 microwave digestion system (Xpress, CEM Corporation, USA). This microwave digestion was performed according to the procedure proposed by Zhou et al. (2017). The volume of digested samples was made up to 50 mL with ultrapure water after they had cooled to room temperature. Digested silage sample was filtered through a 0.45 μm membrane filter before ICP-MS analysis. Water samples were mixed with 1% v/v HNO_3 prior to ICP-MS analysis. The concentrations of Pb, As, Cr, and Cd were measured by inductively coupled plasma mass spectrometry (ICP-MS) (7700 x, Agilent, USA) with a helium gas reaction model.

Soil samples were naturally air-dried in the laboratory, and digested with hydrochloric acid (HCl), HNO_3, hydrofluoric acid (HF), and perchloric acid ($HClO_4$) for Pb, Cr, and Cd measurement (NEPA, 1997, 2009). Levels of Pb and Cd were detected using a graphite furnace atomic absorption spectrophotometer (GFAAS) (Z-2700, Hitachi, Japan), and Cr contents were analysed by flame atomic absorption spectrometry (FAAS) (ICE 3500, Thermo Scientific, USA). Soil samples were digested with HNO_3 and HCl for As detection, then analysed by atomic fluorescence spectrometry (AFS) (AFS 9800, Beijing Kechuang Haiguang Instrument Company, China) (CFDA and NHC, 2008). Blanks prepared with acid treatment, without samples, were subject to the same digestion procedures.

Standard calibrations were developed to quantify the amounts of Pb, As, Cr, and Cd in raw milk, water, silage, and soil samples. The standard solutions were prepared with multi-element standard solution (10 mg/L, SPEX, USA), and the calibration curves for Pb, As, Cr, and Cd were prepared based on five points. Limits of detection (LOD) for Pb, As, Cr, and Cd in milk samples were 0.050, 0.004, 0.035, and 0.001 μg/L, respectively. The LOD for Pb, As, Cr, and Cd in water and silage samples were 0.015, 1.500, 0.130, 0.003 μg/L and 2.40, 0.03, 0.04, and 0.40 μg/kg, respectively. For calculations, values of Pb, As, Cr, and Cd which under the LOD were replaced by a constant value of half the LOD (Cano-Sancho et al., 2015; Potortì et al., 2013). Samples were spiked with standard solutions to verify the accuracy of the analytical procedure.

The contents of heavy metals obtained from milk samples were based on wet weight. For silage and soil samples, it was based on dry weight.

2.3. Statistical analysis

Calculations were performed using SAS 9.4 software (Cary, NC, USA). Univariate processing was carried out to describe the distribution of Pb, As, Cr, and Cd data. As the data were non-normal distribution, heavy metal contents in milk from unpolluted and industrial areas were compared using one-way Kruskal-Wallis tests. The same tests were used to compare the levels of heavy metals on the studied farms. Pairwise two-sided multiple comparisons were performed using the Dwass, Steek, Critchlow-Fligner method (Caroli et al., 2009). Mean differences were assumed to be statistically significant when the *P*-value of the Kruskal-Wallis test was lower than 0.05. To study the relationships between Pb, As, Cr, and Cd contents in milk with protein, fat, lactose, SNF, and TS contents in individual raw milk samples (N = 60), Spearman correlation coefficients were estimated. The significantly different correlation was observed when P-value lower than 5%. Mean concentrations of heavy metals in milk for each farm were used to calculate the Spearman correlation coefficients between heavy metals in milk, water, silage, and soil.

3. Results

3.1. Concentrations of heavy metals in milk, silage, water, and soil samples

The ranges of Pb, As, Cr, and Cd in milk samples were 0.03–10.46 μg/L, 0.004–1.53 μg/L, 0.02–5.01 μg/L, and 0.01–0.27 μg/L, respectively (Table 1). All Pb contents in milk were under the maximum residual limit (0.02 mg/kg wet weight for Pb) set by the EU (EC, 2006). The highest concentrations of Pb, As, Cr, and Cd in raw milk samples were observed in farms C, E, F, and D located in industrial areas. Lower Pb and Cd contents but higher As concentrations were observed in milk samples from farm A (Table 1); Pb, As, and Cd profiles in milk samples from the control farm (i.e., farm A) were significantly different to some of the selection farms in industrial area. No significant differences were observed for Cr between all farms. The average concentrations of Pb, As, Cr, and Cd were 0.07 ± 0.05 μg/L, 3.58 ± 5.23 μg/L, 1.80 ± 2.04 μg/L, and 0.01 ± 0.004 μg/L in water, and 17.57 ± 2.55 mg/kg, 7.91 ± 2.06 mg/kg, 39.93 ± 15.37 mg/kg, and 0.16 ± 0.08 mg/kg in soil, respectively. Mean contents of Pb, As, Cr, and Cd in silage samples were 1.38 ± 0.87 mg/kg, 0.36 ± 0.34 mg/kg, 1.57 ± 0.77 mg/kg and 0.05 ± 0.04 mg/kg.

Table 1
The concentrations of Pb, As, Cr, and Cd (μg/L) measured in raw milk from individual cows reared on 6 farms (N = 60).

Farms		Pb	As	Cr	Cd
A	Mean	0.16^{b}	0.10^{ab}	0.83^{nd}	0.04^{b}
	SD	0.27	0.08	1.13	0.01
	Min	0.03	0.002	0.02	0.02
	Max	0.86	0.26	2.89	0.06
	CV	164.71	80.9	137.15	32.69
B	Mean	1.47^{a}	0.002^{c}	0.57^{nd}	0.14^{a}
	SD	0.93	0.002	0.76	0.06
	Min	0.44	0.002	0.02	0.06
	Max	3.80	0.002	2.10	0.26
	CV	63.43	0.00	133.98	44.34
C	Mean	2.71^{a}	0.005^{cde}	0.55^{nd}	0.08^{abc}
	SD	3.08	0.01	0.52	0.05
	Min	0.41	0.002	0.02	0.03
	Max	10.46	0.03	1.74	0.20
	CV	113.95	190.24	94.47	59.19
D	Mean	0.30^{b}	0.02^{be}	0.96^{nd}	0.15^{ac}
	SD	0.38	0.06	1.30	0.07
	Min	0.03	0.002	0.02	0.02
	Max	1.20	0.18	3.49	0.27
	CV	125.89	283.66	134.96	49.56
E	Mean	1.13^{ab}	0.16^{a}	0.91^{nd}	0.06^{bc}
	SD	1.16	0.48	0.68	0.04
	Min	0.03	0.002	0.02	0.01
	Max	4.00	1.53	1.79	0.14
	CV	102.35	307.98	74.69	71.81
F	Mean	1.52^{a}	0.06^{bd}	1.39^{nd}	0.08^{ac}
	SD	0.62	0.06	1.41	0.02
	Min	0.17	0.002	0.02	0.05
	Max	2.42	0.16	5.01	0.12
	CV	40.98	101.18	101.49	30.02
Total	Mean	1.22	0.06	0.87	0.09
	SD	1.62	0.20	1.02	0.06
	Min	0.03	0.002	0.02	0.01
	Max	10.46	1.53	5.01	0.27
	CV	133.10	353.03	117.35	66.62

Superscripts a, b, and c denote significant differences between farms ($P < 0.05$); nd denotes no difference detected.

At the farm level, the contents of Pb and Cd in milk from unpolluted areas were significantly lower ($P < 0.01$) than those measured in milk from industrial areas (Table 2). Significantly ($P < 0.01$) higher As residue was recorded in milk from the unpolluted area (Table 2), but no difference between areas was noted for Cr ($P > 0.05$).

3.2. Relationships between heavy metals in milk, water, silage, and soil

The estimated correlation coefficients between heavy metal

Table 2
Mean concentrations (μg/L) of Pb, As, Cr, and Cd in milk samples from unpolluted and industrial farms.

Areas	Pb	As	Cr	Cd
Unpolluted (N = 10)	0.16**	0.10	0.83	0.04**
Industrial (N = 50)	1.43	0.05**	0.88	0.10

** Significant difference according to Kruskal-Wallis test for different areas ($P < 0.01$).

contents in milk, water, silage, and soil are presented in Table 3.

Concentrations of As in milk and water samples were weakly positively correlated ($r = 0.37$). Otherwise, As levels in milk showed nearly zero correlation ($r = -0.03$) with As in soil. Lead levels in milk had a negative correlation ($r = -0.37$) with Pb in soil. A moderate positive correlation was found for Cr ($r = 0.60$) and Cd ($r = 0.65$) between milk and soil; a negative value was observed for Cr ($r = -0.60$) and Cd ($r = -0.75$) in water. Concentrations of Pb and Cr in water samples were positively correlated with those in soil samples, however As and Cd showed opposite results. Heavy metals in milk samples were all positively correlated with those in silage samples. The contents of Pb and As in silage samples were positively correlated with those of water samples. However, Cr and Cd showed negative correlations between silage and water samples. Based on Table 3, we can note that the relationships of heavy metals between silage and soil samples showed an opposite trend compared to those observed with water samples.

3.3. *Relationships between heavy metals and milk composition*

Spearman correlations between the contents of protein, fat, lactose, SNF, TS, Pb, As, Cr, and Cd in milk samples are given in Table 4. Weak positive correlations were estimated for Pb-protein, Cr-protein, and Cd-lactose. Lead, As, Cr, and Cd concentrations in milk were negatively related with SNF. The same result was also observed between Pb, As, and Cr with lactose. The contents of heavy metals had almost no effect on fat contents in milk.

Levels of protein, fat, SNF, and TS were positively correlated with each other. The content of lactose showed a negative correlation with protein, fat, and TS. Slightly positive correlations were observed for Pb—Cr, Pb—Cd, Cr—As, and Cr—Cd. Negative correlations were obtained for Pb—As and As—Cd.

4. Discussion

4.1. *Comparison of heavy metal profiles between industrial and unpolluted areas*

Significant differences in milk heavy metal profiles were observed in this study between the farm located in an unpolluted area and the farms in industrial areas. The unpolluted area (i.e., farm A) had lower contents of Pb and Cd, a higher content of As, and a similar concentration of Cr. Patra et al. (2008) obtained the same results for Pb and Cd in industrial areas (steel manufacturing plant, aluminum processing plant, thermal power plant, and lead zinc smelter). They found significantly higher ($P < 0.05$) average contents of Pb and Cd in milk produced by cows reared in those areas compared to an unpolluted area. The observed concentrations of Pb, As, Cr, and Cd in the present study are comparable with the results obtained by other researchers in industrial and unpolluted areas (Erdogan et al., 2004; Gonzalez-Montana et al., 2012; Patra et al., 2008).

Table 3
Spearman correlation coefficients among heavy metal contents in milk, water, silage, and soil ($N = 6$).

	Pb	As	Cr	Cd
Milk-water	0.03	0.37	−0.60	−0.75
P values	0.96	0.48	0.21	0.08
Milk-silage	0.54	0.09	0.14	0.49
P values	0.27	0.87	0.79	0.33
Milk-soil	−0.37	−0.03	0.60	0.66
P values	0.47	0.96	0.21	0.16
Silage-water	0.26	0.78	−0.09	−0.12
P values	0.62	0.07	0.87	0.83
Silage-soil	−0.14	−0.31	0.31	0.71
P values	0.79	0.54	0.54	0.11
Water-soil	0.03	−0.03	0.14	−0.32
P values	0.96	0.95	0.79	0.54

Table 4
Spearman correlation coefficients between heavy metals and milk composition ($N = 60$).

	Fat	Lactose	SNF	TS	Pb	As	Cr	Cd
Protein	0.35	−0.29	0.69	0.59	0.11	−0.16	0.18	0.00
Fat		−0.26	0.12	0.92	0.00	0.06	0.00	−0.04
Lactose			0.35	−0.15	−0.05	−0.11	−0.26*	0.16
SNF				0.42	−0.05	−0.18	−0.02	−0.11
TS					0.00	−0.05	0.00	−0.01
Pb						−0.17	0.17	0.33**
As							0.10	−0.47**
Cr								0.05

*Significant correlation at $P < 0.05$.
**Significant correlation at $P < 0.01$.

The content of Pb observed in milk produced on the 6 studied farms was 1.22 ± 1.62 µg/L ($N = 60$) with the lowest content observed from farm A. The minimum and maximum values were 0.03 and 10.46 µg/L. These concentrations were nearly ten times lower than those observed by Bilandzic et al. (2016) from 249 cows (11.4 ± 8.08 µg/kg of milk) reared in a rural area of Croatia, and Rahimi (2013) from 52 cows (9.88 ± 4.75 µg/L) in Iran. Gonzalez-Montana et al. (2012) measured 36 raw bovine milk samples collected in an area of industrial and mining activity. The observed Pb (3.89 ± 4.04 µg/kg, 0.71—16.06 µg/kg) concentrations in their study were a little higher than our study. The contents of Pb observed in this study on farms located close to polluting factories were higher (Table 1). This was expected based on previous studies. Higher Pb average concentrations (4.48 µg/L) in raw milk ($N = 85$) collected from bulk tanks on farms near to the industrial activities and factories of Boroujerd city in Iran were observed by Noori et al. (2016) compared to a region (0.76 µg/L) that was located away from industrial zones. Those results suggested that industrial activities could lead to higher Pb contents in raw cow milk. Bischoff et al. (2014) studied the clinical signs of Pb toxicosis over 2.5 years in 8 Holstein cows; a peak of Pb content in milk was observed from 128 to 306 d after silage Pb exposure. Unfortunately, the design of our study was not appropriate to confirm those results because we have not realized repeated measurements on the same cows.

In this study, the contents of As (i.e., 0.10 µg/L) in milk were significantly higher ($P < 0.05$) in the unpolluted area than concentrations of As (i.e., 0.05 µg/L) observed in milk from farms located in industrial areas. This was different to the results obtained by Arianejad et al. (2015). These authors observed no difference in the content of As between traditional and industrial farms. The observed range of As ($N = 32$) was 15.20—25.90 µg/L which was higher than that found in this study. Higher As content (35.47 ± 21.08 µg/L) was also observed by Dobrzański et al. (2005) in cow milk collected from udders in Upper Silesia, which is a Polish region with a presence of coal mining, industrial power, and iron and steel metallurgy factories. Simsek et al. (2000) reported that contents of As in raw milk from industrial regions were higher than samples collected in rural regions. Arsenic predominantly enters the body of a cow through drinking water (Kazi et al., 2016). In the present study, contents of As in water were higher in the unpolluted area (4.75 µg/L) than average As in industrial areas (1.36 µg/L). The area supposed to be unpolluted in this study was mainly used for agricultural productions. So, the applications of fertiliser could be the source of As contamination observed in soil and groundwater. This would be in agreement with Atafar et al. (2010) and Campos (2002). This may explain why the contamination of milk by As in the hypothesised unpolluted area came from the drinking water.

From Patra et al. (2008), higher levels of Cd in milk were observed from lactating cows near the steel manufacturing plant (265 μg/L) compared to an unpolluted area (33 μg/L). Muhammad et al. (2009) confirmed this observation; these authors found higher levels of Cd (80 μg/L) in milk samples collected along the main sewage drains of a city. The results of the present study are in agreement with this statement (Table 2), but the observed contents were largely lower than those studies. A similar concentration of Cd in cow milk (0.92 ± 0.47 μg/L, N = 52) was obtained in Iran (Rahimi, 2013). In the same city as our study, the concentration of Cd was previously found to be higher (N = 192, 8.30–74.40 μg/kg) (Qu et al., 2018).

A similar content of Cr was observed between all farms (0.87 ± 1.02 μg/L). However, higher Cr concentrations were observed in milk produced in industrial areas by Dobrzański et al. (2005) (75.06 ± 44.80 μg/L) and by Muhammad et al. (2009) (1070 μg/L). Mean concentrations of Cd and Cr were 40.8 ± 0.07 μg/L and 0.1 ± 0.00 μg/L Lutfullah et al. (2014). The mean concentrations of Cr and Cd were assessed as 1–232 μg/L and 1–53 μg/L (Perween et al., 2016).

Results obtained by the analysis of individual cow milk samples showed variability between farms in the heavy metal profiles of milk (Table 1). The coefficient of variation (CV) values for Pb, As, Cr, and Cd are 133, 353, 117, and 67%, respectively. The higher CV values for As might be due to the fact that fewer samples were detected above the LOD (19/60). This results from an individual cow variability in heavy metal distribution and elimination; cows could have different capacities to transfer heavy metals to milk at the same level of environmental pollution exposure.

4.2. Relationships between heavy metals in milk, water, silage, and soil

The findings reported previously suggest a link between the contents of heavy metals in milk and the production environment. To study these potential relationships, Spearman correlation values were estimated between the contents of heavy metals in milk, water, silage, and soil.

The average concentrations of heavy metals in individual cow's milk from each studied farm showed weak or moderate positive correlations with water, silage, and soil. Negative correlations were observed for Cr and Cd between milk and water, as well as for Pb between milk and soil. The concentration of Pb in milk was positively correlated (r = 0.54) with Pb in silage. A positive Pearson's correlation (r = 0.153) was found by Iftikhar et al., (2014) between the levels of Pb in maize in cow feed and in milk. The content of Pb in silage observed in this study was weakly positively correlated (r = 0.26) with Pb in water, but negatively correlated with Pb in soil. However, the literature has reported that a decreasing or increasing trend in Pb and Cd concentrations in soil have a direct effect on concentrations of Pb and Cd in the soil-fodder-milk chain (Vidovic et al., 2003). These results suggest that water-silage-milk could be one of the pathways of Pb transference to milk.

Levels of As in water showed a weak positive correlation value (r = 0.37) with milk in our study. Previously, a significantly positive Pearson correlation coefficient in the range 0.926–0.974 was reported by Kazi et al. (2016) between the As concentration in milk samples (N = 100) and in drinking water collected from the same farm. Sigrist et al. (2010) reported a low biological transference level of As to cow milk from drinking water ingestion, since higher levels of As (29.8 and 307.6 μg/L) were observed in drinking water (N = 20) but only 3 cow milk samples (total N = 36) were above the LOD. The contents of As showed a positive correlation (r = 0.78) between silage and water samples, but almost no relation between (r = 0.09) milk and silage samples. So, water-silage-milk may not be suggested as a transfer pathway of As contamination for cows. Water has a direct effect on As contamination in cow milk based on the relationships of As concentrations between milk, water, and silage.

A previous study found positive correlations existed for Cr and Cd in soil-forage (r = 0.68 for Cr, r = 0.63 for Cd) and forage-milk (r = 0.55 for Cr, r = 0.71 for Cd) in an industrial area (Sargodha, Pakistan) (Batool et al., 2016). Similar results were observed in this study with positive correlations of Cr and Cd in milk-silage, milk-soil, and silage-soil. This result indicates that Cr and Cd in milk may derive from the soil, which contributes to contamination in the soil-silage-milk chain. These findings corroborate an earlier study which found that crops and pasture grown on soils that contain high levels heavy metals may accumulate high levels of metals (Miranda et al., 2009). Therefore, the contents of Cr and Cd in milk could be related to cow feed produced from Cr and Cd polluted soil.

In our previous study, Pb and Cr concentrations in milk were significantly positively related with those in water (r = 0.434 for Pb, r = 0.481 for Cr), while As and Cd contents were significantly negatively related with those in water (r = −0.398 for As, r = −0.252 for Cd) (Zhou et al., 2017). Different results were obtained in this study. The correlation coefficient was nearly zero (r = 0.03) for Pb between milk and water, the concentrations of Cr showed a negative correlation between milk and water samples. Totally different results were obtained for As and Cd levels between milk and silage, a positive correlation was observed in this study. The number of milk samples used for correlation analysis in our earlier study was 100 (N = 40 for cow, N = 40 for goat, N = 20 for buffalo). Cows received total mixed rations, which including *Leymus chinensis*, oat grass hay, 35%–55% silage and concentrate. Forage and corn kernels were given to goats separately. Cow and goat milk samples were collected in Shandong and Shaanxi province in our previous study. The environmental factors for buffalos were different from the ones observed for cows and goats. Consequently, animal breed, feed components and breeding environment might lead to the different results obtained in these two studies.

The bio-transference factor from heavy metal contents in feed, soil, or water into milk could be interesting to know in order to interpret the results obtained in this study. Pérez-Carrera et al. (2016a) estimate the bio-transference factor from As content in drinking water into cow milk. Wang et al. (2018) estimated the bio-transferrence factors from Pb and Cr contents in feed into raw milk. Both studies need the estimation of heavy metal quantities ingested by the cows. Unfortunately, this record was not available in this study.

Heavy metal contamination in the environment may lead to the transformation of heavy metals through water-silage and soil-silage. The correlation results of heavy metals between water-silage and soil-silage may lead to a positive correlation of the studied metals between milk and silage. Overall, different kinds of heavy metal contamination in raw milk may travel through complex pathways from the environment, directly or indirectly, via drinking water and soil.

4.3. Relationships of heavy metals with milk protein, fat, lactose, SNF, and TS

Contents of Pb and Cr in milk exhibited weak correlations with milk protein. Based on the low positive correlation values, we can conclude that no relation between heavy metals in milk with fat and TS seem to exist. This result agrees with the relationship of heavy metal residues to milk fat observed by Muhammad et al. (2009). The regression coefficients estimated by those authors for

Pb, Cr, and Cd residues in cattle milk with respect to %fat in milk were 4.09, 0.096, and 0.023. The content of As in milk was also positively related with fat, while levels of Cd in milk showed a negative correlation with fat. Coroian et al. (2017) studied the contents of milk protein, fat, and lactose and levels of heavy metals in cow milk from a very polluted area; and the average contents in five locations were in the range 3.26–3.38 g/100 g, 3.62–4.12 g/100 g, and 4.36–4.74 g/100 g, respectively. In the present study, the mean contents of milk protein, fat, and lactose in industrial areas were 3.49 g/100 g, 3.83 g/100 g, and 4.80 g/100 g. For average Pb and Cd concentrations, Coroian et al. (2017) observed ranges of 11.53–43.22 μg/L and 4.32–10.93 μg/L. We observed slightly higher contents of milk protein and lactose, but much lower Pb and Cd contents. These results highlight potential relationships between heavy metals and milk protein, as well as lactose.

5. Conclusion

The results obtained suggest that the contamination of milk by Pb, As, Cr, and Cd came from multiple sources. Water and soil on the farms had a partial contribution to heavy metal contamination in milk based on the obtained correlation amplitude. Drinking water and soil contribute different types of heavy metals to raw milk. We found that Pb and As were positively correlated between milk and water, while Cr and Cd were correlated between milk and soil. Heavy metals in silage may be the main contributor to milk contamination, as Pb, As, Cr, and Cd in silage all showed positive correlations with those in milk. Heavy metals transferred into milk can not only come directly from water and soil, but also through the pathways water-silage-milk and soil-silage-milk. The links were not clear between heavy metals in milk and milk protein, fat, lactose, and TS based on the results of correlations. The smaller number of samples may be an explanation for those results as the coefficient variability is large within farms. The lactation, days in milk, and milk yield of cows was not totally similar between cows in our study. Furthermore, the number of cows should be larger to dilute the amplitude caused by individual cows.

Declaration of competing interest

All authors have approved this submission and none of the authors declare any conflicts of interest in the work performed or in the submission of the manuscript.

Acknowledgements

This study was financially supported by the Project of Risk Assessment on Raw Milk (GJFP2019008), the Modern Agro-Industry Technology Research System of the PR China (CARS-36), and the China Scholarship Council.

References

Arianejad, M., Alizadeh, M., Bahrami, A., Arefhoseini, S.R., 2015. Levels of some heavy metals in raw cow's milk from selected milk production sites in Iran: is there any health concern? Health Promot. Perspect. 5 (3), 176–182.

Atafar, Z., Mesdaghinia, A., Nouri, J., Homaee, M., Yunesian, M., Ahmadimoghaddam, M., Mahvi, A.H., 2010. Effect of fertilizer application on soil heavy metal concentration. Environ. Monit. Assess. 160 (1–4), 83–89.

EC, 2006. Commission Regulation (EC) No 1881/2006 of 19 December 2006 Setting Maximum Levels for Certain Contaminants in Foodstuffs.

Batool, F., Iqbal, S., Tariq, M., Akbar, J., Noreen, S., Danish, M., Chan, K.W., 2016. Milk: carrier of heavy metals from crops through ruminant body to human beings. J. Chem. Soc. Pak. 38 (1), 39–42.

Bilandzic, N., Sedak, M., Calopek, B., Luburic, D.B., Solomun Kolanovic, B., Varenina, I., Dokic, M., Kmetic, I., Murati, T., 2016. Lead concentrations in raw cow and goat milk collected in rural areas of Croatia from 2010 to 2014. Bull. Environ. Contam. Toxicol. 96 (5), 645–649.

Bischoff, K., Higgins, W., Thompson, B., Ebel, J.G., 2014. Lead excretion in milk of accidentally exposed dairy cattle. Food Addit. Contam. A 31 (5), 839–844.

Caggiano, R., Sabia, S., D'Emilio, M., Macchiato, M., Anastasio, A., Ragosta, M., Paino, S., 2005. Metal levels in fodder, milk, dairy products, and tissues sampled in ovine farms of Southern Italy. Environ. Res. 99 (1), 48–57.

Campos, V., 2002. Arsenic in groundwater affected by phosphate fertilizers at São Paulo, Brazil. Environ. Geol. 42 (1), 83–87.

Cano-Sancho, G., Perelló, G., Nadal, M., Domingo, J.L., 2015. Comparison of the nutritional composition and the concentrations of various contaminants in branded and private label yogurts. J. Food Compos. Anal. 42, 71–77.

Caroli, A.M., Chessa, S., Erhardt, G.J., 2009. Invited review: milk protein polymorphisms in cattle: effect on animal breeding and human nutrition. J. Dairy Sci. 92 (11), 5335–5352.

CFDA, NHC, 2008. Soil Quality-Analysis of Total Mercury, Arsenic and Lead Contents-Atomic Fluorescence Spectrometry (In Chinese). China Food and Drug Administration; National Health Commission of the Peoples's Republic of China, Beijing, China.

Chiodo, L.M., Jacobson, S.W., Jacobson, J.L., 2004. Neurodevelopmental effects of postnatal lead exposure at very low levels. Neurotoxicol. Teratol. 26 (3), 359–371.

Coroian, A., Miresan, V., Cocan, D., Raducu, C., Longodor, A.L., Pop, A., Feher, G., Andronie, L., Marchis, Z., 2017. Physical-chemical parameters and the level of heavy metals in cow milk in the Baia Mare area. Banat's J. Biotechnol. VIII (16), 69–74.

Costa, M., Klein, C.B., 2008. Toxicity and carcinogenicity of chromium compounds in humans. Crit. Rev. Toxicol. 36 (2), 155–163.

Crout, N.M.J., Beresford, N.A., Dawson, J.M., Soar, J., Mayes, R.W., 2004. The transfer of 73As, 109Cd and 203Hg to the milk and tissues of dairy cattle. J. Agric. Sci. 142 (2), 203–212.

Dairy Association of China, 2018. Chinese Dairy Statistical Summary 2018 (in Chinese). China Agriculture Press, Beijing. In press.

Dobrzański, Z., Koacz, R., Górecka, H., Chojnacka, K., Bartkowiak1, A., 2005. The content of microelements and trace elements in raw milk from cows in the Silesian region. Pol. J. Environ. Stud. 14 (5), 685–689.

EFSA, 2010. Scientific Opinion on Lead in Food. European Food Safety Authority, Parma, Italy.

EFSA, 2014. Dietary exposure to inorganic arsenic in the European population. EFSA J. 12 (3).

Erdogan, S., Celik, S., Erdogan, Z., 2004. Seasonal and locational effects on serum, milk, liver and kidney Cr, Mn, Cu, Zn, and Fe concentration of dairy cows. Biol. Trace Elem. Res. 98, 51–61.

Gonzalez-Montana, J.R., Senis, E., Gutierrez, A., Prieto, F., 2012. Cadmium and lead in bovine milk in the mining area of the Caudal River (Spain). Environ. Monit. Assess. 184 (7), 4029–4034.

IARC, 2014. World Cancer Report. International Agency for Research on Cancer, Lyon, France.

Iftikhar, B., Arif, S., Siddiqui, S., Khattak, R.A., 2014. Assessment of toxic metals in dairy milk and animal feed in Peshawar, Pakistan. British biotechnology journal 4 (8), 883–893.

Kazi, T.G., Brahman, K.D., Afridi, H.I., Arain, M.B., Talpur, F.N., Akhtar, A., 2016. The effects of arsenic contaminated drinking water of livestock on its total levels in milk samples of different cattle: risk assessment in children. Chemosphere 165, 427–433.

Khan, Z.I., Ahmad, K., Raza, N., Al-Qurainy, F., Ashraf, M., Hussain, A., 2010. Assessment of chromium concentrations in soil-plant-animal continuum: possible risk for grazing cattle. Pak. J. Bot. 42 (5), 3409–3414.

López, M.L., Peralta-Videa, J.R., Parsons, J.G., Benitez, T., Gardea-Torresdey, J.L., 2007. Gibberellic acid, kinetin, and the mixture indole-3-acetic acid-kinetin assisted with EDTA-induced lead hyperaccumulation in alfalfa plants. Environ. Sci. Technol. 41, 8165–8170.

López, M.L., Peralta-Videa, J.R., Parsons, J.G., Gardea-Torresdey, J.L., Duarte-Gardea, M., 2009. Effect of indole-3-acetic acid, kinetin, and ethylenediaminetetraacetic acid on plant growth and uptake and translocation of lead, micronutrients, and macronutrients in alfalfa plants. Int. J. Phytoremediation 11 (2), 131–149.

Lutfullah, G., Khan, A.A., Amjad, A.Y., Perveen, S., 2014. Comparative study of heavy metals in dried and fluid milk in Peshawar by atomic absorption spectrophotometry. Sci. World J. 715845, 2014.

Miranda, M., Benedito, J.L., Blanco-Penedo, I., Lopez-Lamas, C., Merino, A., Lopez-Alonso, M., 2009. Metal accumulation in cattle raised in a serpentine-soil area: relationship between metal concentrations in soil, forage and animal tissues. J. Trace Elem. Med. Biol. 23 (3), 231–238.

Muhammad, F., Akhtar, M., Javed, I., ZU-Rahman, Jan, I., Anwar, M., Hayat, S., 2009. Quantitative structure activity relationship and risk analysis of some heavy metal residues in the milk of cattle and goat. Toxicol. Ind. Health 25, 177–181.

NEPA, 1997. Soil Quality-Determination of Lead, Cadmium-Graphite Furance Atomic Absorption Spectrophotometry. National Environmental Protection Agency, Beijing, China.

NEPA, 2009. Soil Quality-Determination of Total Chromium-Flame Atomic Absorption Spectrometry. National Environmental Protection Agency, Beijing, China.

Noori, N., Noudoost, B., Hatami Nia, M., 2016. The assessment of lead pollution in milk collected from all dairy farms in Lorestan province, Iran. Toxin Rev. 35 (3–4), 196–200.

Ohno, K., Yanase, T., Matsuo, Y., Kimura, T., Rahman, M.H., Magara, Y., Matsui, Y.,

2007. Arsenic intake via water and food by a population living in an arsenic-affected area of Bangladesh. Sci. Total Environ. 381 (1–3), 68–76.

Patra, R.C., Swarup, D., Kumar, P., Nandi, D., Naresh, R., Ali, S.L., 2008. Milk trace elements in lactating cows environmentally exposed to higher level of lead and cadmium around different industrial units. Sci. Total Environ. 404 (1), 36–43.

Pérez-Carrera, A., Alvarez-Gonçalvez, C.V., Fernández-Cirelli, A., 2016a. Transference factors as a tool for the estimation of arsenic milk concentration. Environ. Sci. Pollut. Res. 23, 16329–16335.

Pérez-Carrera, A.L., Arellano, F.E., Fernández-Cirelli, A., 2016b. Concentration of trace elements in raw milk from cows in the southeast of Córdoba province, Argentina. Dairy Sci. Technol. 96 (5), 591–602.

Perriguey, J., Sterckeman, T., Morel, J.-L., 2008. Effect of rhizosphere and plant-related factors on the cadmium uptake by maize (Zea mays L.). Environ. Exp. Bot. 63 (1–3), 333–341.

Perween, R., Shaukat, S., Yasmeen, K., Ara, D., 2016. Evaluation of environmental impact on heavy metal load in Cattle Milk. Pol. J. Environ. Stud. 25 (3), 1161–1166.

Phillips, C., Győri, Z., Kovács, B., 2003. The effect of adding cadmium and lead alone or in combination to the diet of pigs on their growth, carcase composition and reproduction. J. Sci. Food Agric. 83 (13), 1357–1365.

Porova, N., Botvinnikova, V., Krasulya, O., Cherepanov, P., Potoroko, I., 2014. Effect of ultrasonic treatment on heavy metal decontamination in milk. Ultrason. Sonochem. 21 (6), 2107–2111.

Potortì, A.G., Di Bella, G., Lo Turco, V., Rando, R., Dugo, G., 2013. Non-toxic and potentially toxic elements in Italian donkey milk by ICP-MS and multivariate analysis. J. Food Compos. Anal. 31 (1), 161–172.

Qu, X.Y., Zheng, N., Zhou, X.W., Li, S.L., Wang, J.Q., Zhang, W.J., 2018. Analysis and risk assessment of seven toxic element residues in raw bovine milk in China. Biol. Trace Elem. Res. 183 (1), 92–101.

Rahimi, E., 2013. Lead and cadmium concentrations in goat, cow, sheep, and buffalo milks from different regions of Iran. Food Chem. 136 (2), 389–391.

Rahman, M.A., Hasegawa, H., Rahman, M.M., Miah, M.A.M., Tasmin, A., 2008. Arsenic accumulation in rice (Oryza sativa L.): human exposure through food chain. Ecotoxicol. Environ. Saf. 69 (2), 317–324.

Rosas, I., Belmont, R., Armienta, A., Baez, A., 1999. Arsenic concentrations in water, soil, milk and forage in Comarca Lagunera, Mexico. Water Air Soil Pollut. 112, 133–149.

Sigrist, M., Beldoménico, H., Rosa Repetti, M., 2010. Evaluation of the influence of arsenical livestock drinking waters on total arsenic levels in cow's raw milk from Argentinean dairy farms. Food Chem. 121 (2), 487–491.

Simsek, O., Gultekin, R., Oksuz, O., Kurultay, S., 2000. The effect of environmental pollution on the heavy metal content of raw milk. Mol. Nutr. Food Res. 44 (5), 360–363.

Swarup, D., Patra, R.C., Naresh, R., Kumar, P., Shekhar, P., 2005. Blood lead levels in lactating cows reared around polluted localities; transfer of lead into milk. Sci. Total Environ. 347 (1–3), 106–110.

Tripathi, R.D., Srivastava, S., Mishra, S., Singh, N., Tuli, R., Gupta, D.K., Maathuis, F.J., 2007. Arsenic hazards: strategies for tolerance and remediation by plants. Trends Biotechnol. 25 (4), 158–165.

Tyler, C.R., Allan, A.M., 2014. The effects of arsenic exposure on neurological and cognitive dysfunction in human and rodent studies: a review. Curr. Environ. Health Rep. 1, 132–147.

Uddh-Soderberg, T.E., Gunnarsson, S.J., Hogmalm, K.J., Lindegard, M.I., Augustsson, A.L., 2015. An assessment of health risks associated with arsenic exposure via consumption of homegrown vegetables near contaminated glassworks sites. Sci. Total Environ. 536, 189–197.

Vidovic, M.M., Vidovic, M.U., Rodic, M.N., 2003. Heavy metals in relation to soil-fodder-milk, Sixth International Symposium and Exhibition on Environmental Contamination in Central and Eastern Europe and the Commonwealth of Independent States 1e4 September (Prague, Czech).

Waisberg, M., Joseph, P., Hale, B., Beyersmann, D., 2003. Molecular and cellular mechanisms of cadmium carcinogenesis. Toxicology 192 (2–3), 95–117.

Wang, M., Class, A.D.M., Shaff, J.E., Kochian, L.V., 1994. Ammonium uptake by rice roots. Plant Physiol. 104, 899–906.

Wang, H., Jiang, Y., Tian, C., Pan, R., Dang, F., Feng, J., Li, M., Zhang, Y., Li, H., Man, C., 2018. Determination of the transfer of lead and chromium from feed to raw milk in Holstein cows. Food Addit. Contam. A 35 (10), 1990–1999.

WHO, 1996. Trace Elements in Human Nutrition and Health. World Health Organization, Geneva, Switzerland.

WHO, 2010. Exosure to Arsenic: a Major Public Health Concern. Public Health and Environment, World Health Organization, Geneva, Switzerland.

Zhao, H., Xia, B., Fan, C., Zhao, P., Shen, S., 2012. Human health risk from soil heavy metal contamination under different land uses near Dabaoshan Mine, Southern China. Sci. Total Environ. 417–418, 45–54.

Zhou, X., Qu, X., Zhao, S., Wang, J., Li, S., Zheng, N., 2017. Analysis of 22 elements in milk, feed, and water of dairy cow, goat, and buffalo from different regions of China. Biol. Trace Elem. Res. 176, 120–129.