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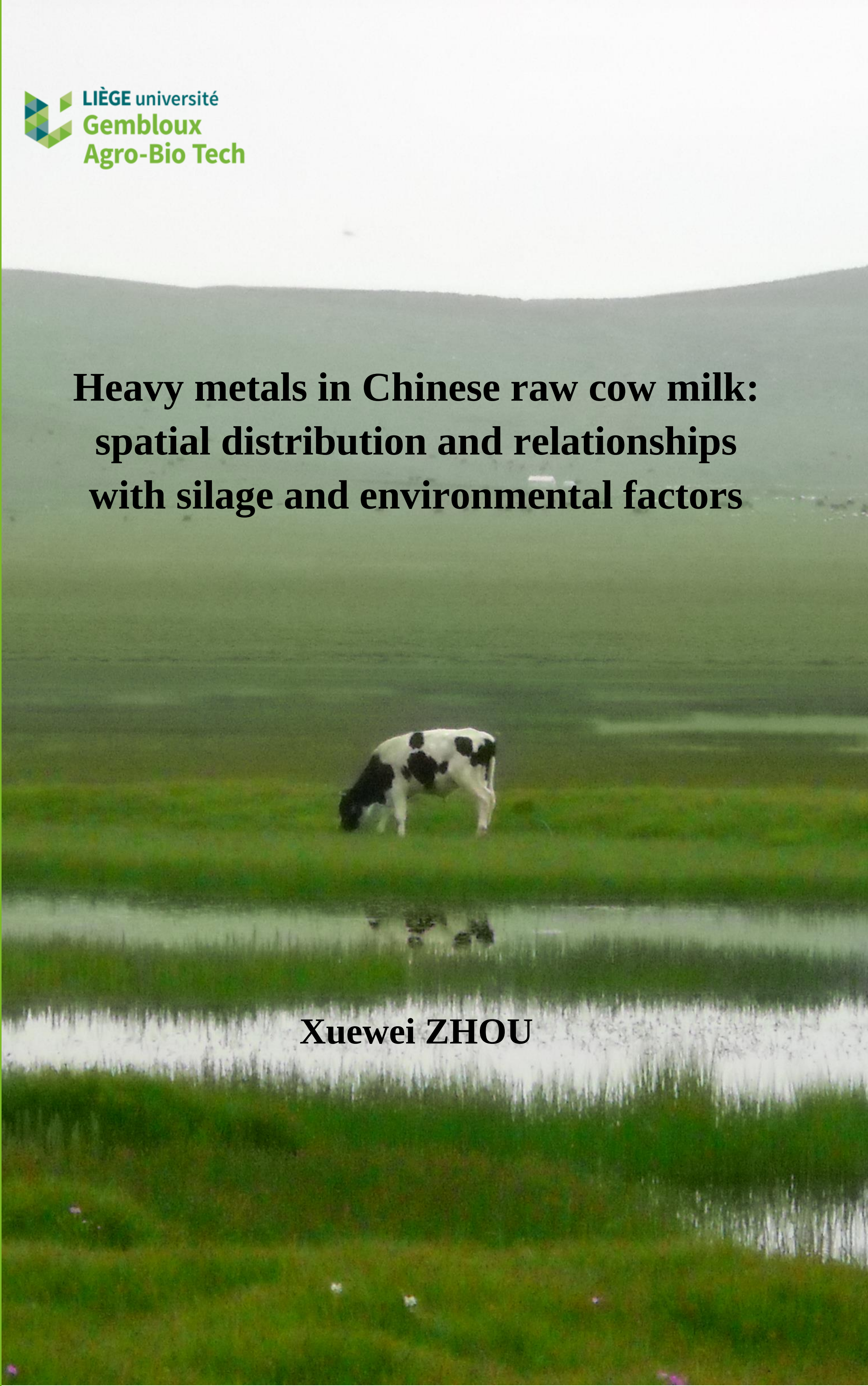

Heavy metals in Chinese raw cow milk: spatial distribution and relationships with silage and environmental factors

Xuewei ZHOU

COMMUNAUTÉ FRANÇAISE DE BELGIQUE UNIVERSITÉ DE LIÈGE –
GEMBLOUX AGRO-BIO TECH

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Résumé

ZHOU Xuewei (2019). ***Les métaux lourds dans le lait de vache produit en Chine : leurs variabilités spatiales et leurs relations avec le fourrage et les facteurs environnementaux*** (thèse de doctorat) Gembloux, Belgique, Liège Université, Gembloux Agro-Bio Tech, 125 p., 23 Tableaux, 10 Figures.

La consommation de lait et de produits laitiers joue un rôle important en alimentation humaine. Vu la consommation grandissante de produits laitiers en Chine, l’augmentation de la production laitière nationale et la présence d’activités industrielles qui pourraient potentiellement contaminer directement ou indirectement le lait en métaux lourds, il y a un besoin d’étudier la présence en métaux lourds dans le lait et son risque potentiel pour la santé humaine. Ainsi, une étude à large échelle a été conduite dans le cadre de cette thèse pour mesurer les concentrations et la variabilité spatiale en métaux lourds du lait produit dans les 11 principales régions de production laitière en Chine. Cette recherche visait également à estimer à partir d’un jeu limité de données les relations entre les contenus en métaux lourds du lait et ceux dans l’eau de consommation, la ration et le sol ainsi que leurs effets sur la composition globale du lait afin de détecter des routes potentiellement responsables de la contamination du lait. De plus, cette thèse a également eu pour objectif d’apprécier d’un point de vue théorique le risque pour la santé humaine de consommer un tel lait contaminé via l’estimation du quotient de risque. Comme les analyses visant à quantifier les teneurs en métaux lourds dans le lait sont onéreuses, il y a un intérêt à trouver un indicateur de cette présence disponible à faible coût. Cet indicateur pourrait être lié à la composition du lait. C’est pourquoi, les corrélations entre les teneurs en métaux lourds dans le lait et les composés majeurs du lait ont été estimées dans ce travail de thèse.

Les concentrations en métaux lourds ont été mesurées sur 1.043 échantillons de lait de tank, 60 échantillons de lait pris individuellement, 46 échantillons d’eau, 6 échantillons de fourrage et de sol et 40 échantillons de ration. Tous ces échantillons ont été mesurés par spectrométrie d’absorption atomique et par spectrométrie de fluorescence atomique. Les principaux résultats obtenus dans cette thèse sont repris dans les points suivants.

(1) Les concentrations moyennes en Pb, As and Cd dans le lait de tank étaient de 1.74 µg/L, 0.32 µg/L et 0.05 µg/L, suggérant une présence limitée de métaux lourds dans le lait produit en Chine. Seulement 12 échantillons (1.15%) excédaient la limite maximale de résidu pour Pb fixée par l’Union Européenne. Aucun risque sur la santé humaine n’est donc attendu sur base de ces concentrations ou des teneurs maximales observées dans cette étude. En effet, les quotients de risque calculés avec ces concentrations étaient toujours inférieurs à 1.

(2) Une variabilité régionale a été observée pour les teneurs en Pb, As and Cd du lait. Une hétérogénéité plus grande du contenu en métaux lourds du lait a été observée au sein des régions laitières plutôt qu'entre elles. Les contenus en Pb et Cd dans les échantillons de lait collectés individuellement dans des zones industrielles étaient significativement plus hauts que ceux observés dans les zones agricoles ; la teneur en As était significativement plus faible. Ces résultats suggèrent que les activités industrielles sont potentiellement responsables de la contamination en Pb et Cd du lait, confirmant ainsi le besoin de connaître et comprendre le micro environnement de la ferme afin d'interpréter les teneurs en métaux lourds observées.

(3) L'ingestion par les vaches d'aliments, d'eau et de sol pollués peut provoquer une contamination en métaux lourds du lait. Des corrélations faibles ont été obtenues entre les concentrations en As du sol et du lait de tank (r variant de 0.12 à 0.45). Les teneurs en As dans le lait était également positivement corrélées à celles mesurées dans l'eau (r = 0.37). La concentration en Cd dans le lait était négativement corrélée à celle de Cd dans l'eau. Aucune relation (r variant de -0.03 à 0.09) n'a été observée entre les teneurs en Pb dans l'eau et le lait. Les résultats obtenus indiquent que l'eau de consommation peut être la principale source de contamination en As du lait. Des corrélations faiblement positives ont été estimées entre les métaux lourds présents dans le lait et le fourrage. La concentration en Pb dans le lait de vache était positivement corrélée à la teneur en Pb dans le fourrage (r = 0.54). La présence de métaux lourds dans le fourrage pourrait donc être la source principale de métaux lourds dans le lait. Des corrélations modérément positives ont été trouvées pour les teneurs en Cr (r = 0.60) et Cd (r = 0.66) dans le lait et le sol ; des valeurs négatives ont été estimées pour celles dans l'eau (r = -0.60 pour Cr et -0.75 pour Cd). Ces corrélations suggèrent que l'eau et le sol contaminent différemment le lait.

(4) Aucune relation forte n'a été observée entre les contenus en métaux lourds dans le lait et sa composition. Cependant, la corrélation la plus fore a été estimée entre Pb et la teneur en protéines du lait (r = 0.11) et entre Cr et ce même composé du lait (r = 0.18). Par conséquent, la teneur en protéines semble être le composé laitier le plus affecté par la contamination en métaux lourds.

En conclusion, cette thèse a montré des teneurs en métaux lourds dans le lait produit en Chine en faibles quantités. C'est pourquoi, il a un besoin de mettre en place une procédure d'échantillonnage permettant de doser en routine les teneurs en métaux lourds du lait afin d'assurer au consommateur une qualité parfaite du lait qu'ils consomment. Malheureusement les résultats obtenus dans cette thèse ne sont pas suffisant pour proposer un protocole détaillé. En effet, les informations relatives au microenvironnement (par ex. présence d'activités industrielles, type d'industrie…) de la ferme n'étaient pas disponibles or cette connaissance est apparue cruciale à connaître au regard des résultats obtenus dans cette thèse. De

plus, il y a également un besoin d'augmenter le set de données pour avoir une plus grande confiance dans les relations décrites dans cette thèse.

Mots clés: lait, métal lourd, contamination, eau, fourrage, sol, corrélation.

Abstract

ZHOU Xuewei (2019). ***Heavy metals in Chinese raw cow milk: spatial distribution and relationships with silage and environmental factors*** (PhD thesis) Gembloux, Belgium, University of Liège, Gembloux Agro-Bio Tech, 125 p., 23 Tables, 10 Figures.

Milk and dairy products play important roles in human diets. Owing to the increasing consumption of milk and dairy products in China, the enhanced national production of milk and the presence of polluting industrial activities that could directly or indirectly contaminate milk with heavy metals, there is a need to screen for the presence of heavy metals and to assess the potential health risks of consuming contaminated milk. Therefore, this thesis conducts a large-scale research to study the spatial variability of heavy metals contents in raw cow's milk produced in the 11 main producing areas in China. This research also estimates from a limited set of records the relationships between the content of heavy metals in individual cow's milk, drinking water, feed and soil to isolate pathways potentially responsible for the milk contamination. Moreover, this thesis aims also to assess the human health risk of consuming contaminated milk by calculating using theoretical approach the hazard quotient (HQ). As the analysis cost to measure the contents of heavy metals in milk is high, there is an interest to find a cheap proxy. This one could be related to the milk composition. Therefore, the correlation values between the heavy metals' contents in milk and the main milk components were also estimated in this thesis.

The concentrations of heavy metals were measured in 1,043 bulk milk samples, 60 individual cow milk samples, 46 drinking water samples, 6 silage and soil samples and 40 TMR samples. All samples were analyzed by inductively coupled plasma mass spectrometry after microwave assisted acid digestion, except for soil samples, which were analyzed by atomic absorption spectrometry and atomic fluorescence spectrometry. The main results of this thesis are as follows:

(1) The average concentrations of Pb, As and Cd in bulk milk were 1.74 μg/L, 0.32 μg/L and 0.05 μg/L, thus suggesting limited cause for concern regarding heavy metal contamination in milk produced in China. Only 12 bulk milk samples (1.15%) exceeded the maximum residue limit (MRL) of Pb set by the European Union. There is no expected health risk when those average and highest levels were used to calculate HQ values. Indeed, those values were always lower than 1.

(2) Regional variability was observed in the Pb, As and Cd contamination in milk. A higher heterogeneity of heavy metal content in milk was observed within areas than between areas. The content of Pb and Cd in individual milk samples from industrial areas was significantly higher than that in agricultural areas, but the content of As was significantly lower. This result suggested that industrial activities (i.e., steel production, waste incineration plant and

cement plant) lead to potential Pb and Cd contamination in milk, thus confirming the need to know the micro-environment of the farm to interpret the observed contents of heavy metals.

(3) The ingestion of contaminated feed, water and soil by cows can induce the production of contaminated milk. Weak correlation values were obtained from As concentrations between water and bulk milk (r ranged from 0.12 to 0.45). As content in individual cow's milk samples was positively correlated with the content in water (r = 0.37). Conversely, Cd in milk was negatively correlated with Cd in water. Nearly no relation (r ranged from -0.03 to 0.09) was found between Pb levels in water and milk. This result indicated that drinking water may be the main source of As contamination in raw milk. Weak positive correlations were observed between heavy metals in milk and silage. The content of Pb in individual cow milk samples was positively correlated with Pb in silage (r = 0.54). Heavy metals in silage appeared to be the main source of heavy metals in raw milk. Moderate positive correlation values were found for Cr (r = 0.60) and Cd (r = 0.66) between milk and soil, and negative values were observed for Cr (r = -0.60) and Cd (r = -0.75) in water. Those correlations suggested that water and soil contaminate milk differentially.

(4) No strong relationships were observed between the content of heavy metal and milk composition. However, weak positive correlations were found between Pb and protein in milk (r = 0.11), and Cr and protein in milk (r = 0.18). Cd content in individual cow's milk was weak positively correlated with lactose. Beyond these, heavy metals and milk composition showed zero or negative correlations. Thus, milk protein appeared to be more affected by heavy metal pollution than other milk components.

In conclusion, this thesis highlights the presence of heavy metals at low quantities in milk produced in China. Therefore, there is a need to define a sampling protocol to screen dairy farms with a potential to produce contaminated milk, to ensure that high quality milk is provided to consumers. Unfortunately, the results obtained in this thesis were not sufficient to propose a detailed sampling procedure, because information on the micro-environment (such as the presence of industrial activities or the type of industry) around the farm was lacking. Such knowledge was highlighted in this thesis to be the most important factor to illustrate the sources of heavy metals in raw milk. Moreover, there is also a need to increase the sample size to provide higher confidence in the relationships described in this thesis.

Key words: milk, heavy metal, contamination, water, silage, soil, correlation.

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Zhou Xuewei

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List of Abbreviations

ICP-MS	Inductively coupled plasma-mass spectrometry
HMI	High matrix introduction
TMR	Total mixed ration
PTFE	Polytetrafluoroethylene
LOD	Limit of detection
LOQ	Limit of quantification
EU	European Union
MRL	Maximum residue limit
PC	Principal component
PCA	Principal component analysis
RMSE	Root mean squared error
RMSSE	Root mean squared standardized error
MSE	Mean of standardized error
ATSDR	Agency for toxic substances and disease registry
SNF	Solid nonfat
TS	Total solid
PTWI	Provisional tolerable weekly intake
TDI	Tolerable daily intake
HQ	Hazard quotient
THQ	Target hazard quotient

1

General introduction

1. Introduction

1.1 Milk and dairy products in China

Milk and dairy products are an important source of nutrients that are of interest to human health. Milk provides essential minerals for human body development and metabolism, such as copper (Cu), iron (Fe), zinc (Zn) and selenium (Se) (FAO, 2013). These trace elements are essential nutrients that participate in physiological processes, such as cell metabolism and enzyme synthesis, and play an important role in the body's metabolism, physiological functions, growth and development (WHO, 1996). For example, Fe is an essential trace element involved in the synthesis of hemoglobin and myoglobin, and is also a component of peroxidase in the human body (Mituniewicz et al., 2006). Se is a human immunomodulatory nutrient that activates both lymphocytes in cellular immunity and immunoglobulin and antibody production (Li and Zhang, 2011). Zn is involved in a large number of enzymes, and it participates in the synthesis of lipids and proteins (WHO, 1996). The physiological functions of Cu in the body is arise from its roles in several Cu-containing metalloenzymes (e.g., lysyl oxidase and superoxide dismutase) (WHO, 1996).

Table 1-1: Mineral contents in milk and their biological roles in the human body.

	g per 100 g of milk		Biological role [2]
	Average [1]	Range [1]	
Calcium	112	91-120	Major component of skeleton Mediators of intracellular signaling pathways
Iron	0.1	Tr-0.2	Carrier of oxygen by red blood cell haemoglobin An integrated part of enzyme systems
Magnesium	11	10-11	Co-factor of many enzymes in energy metabolism, protein synthesis, RNA and DNA synthesis
Potassium	145	132-155	Potassium/sodium balance, sodium-potassium pumps [3]
Sodium	42	38-45	Potassium/sodium balance, sodium-potassium pumps [3]
Zinc	0.4	0.3-0.4	Component of a large number of enzymes Plays a central role in the immune system
Copper	Tr	Tr-Tr	Cytochrome c oxidase, Ceruloplasmin [3]
Selenium	1.8	1.0-3.7	Protect body tissues against oxidative stress Component of selenium-containing enzymes
Manganese	8	4-10	Cofactor for a variety of enzymes [3]

[1]Food and Agriculture Organization of the United Nations (FAO, 2013); [2] FAO/WHO (2001); [3]Sigel et al. (2012-2016).

By studying the snacking habits of children having 4-8 and 9-13 years old, Wang et al. (2018) suggested that improving dairy consumption is important in China, since over 95% of children have inadequate calcium intake. So, the consumption of milk and dairy products is relevant for the Chinese population in the context of a balanced diet. In 2016, the Chinese National Health Commission recommended a dietary intake of 300 g of milk and dairy products/person/day in China (NHC, 2016). Unfortunately, the consumption is currently lower than this recommendation (NBSC, 2018) and stays low compared with the one in the European Union (59–65 kg/person/year) and USA (69–80 kg/person/year; Figure 1-1, D). However, an increase in the national consumption of liquid milk was observed in China between 2010 and 2016 (from 11 to 20 kg/person/year; Figure 1-1, D). This can be related to different factors like the age (under 18-years old persons had the highest milk consumption (He et al., 2016)), the rising incomes, the changing lifestyles, the presence of more sophisticated marketing channels and finally, a stronger promotion of the domestic dairy industry by the Chinese government (Cheng, L. et al., 2015).

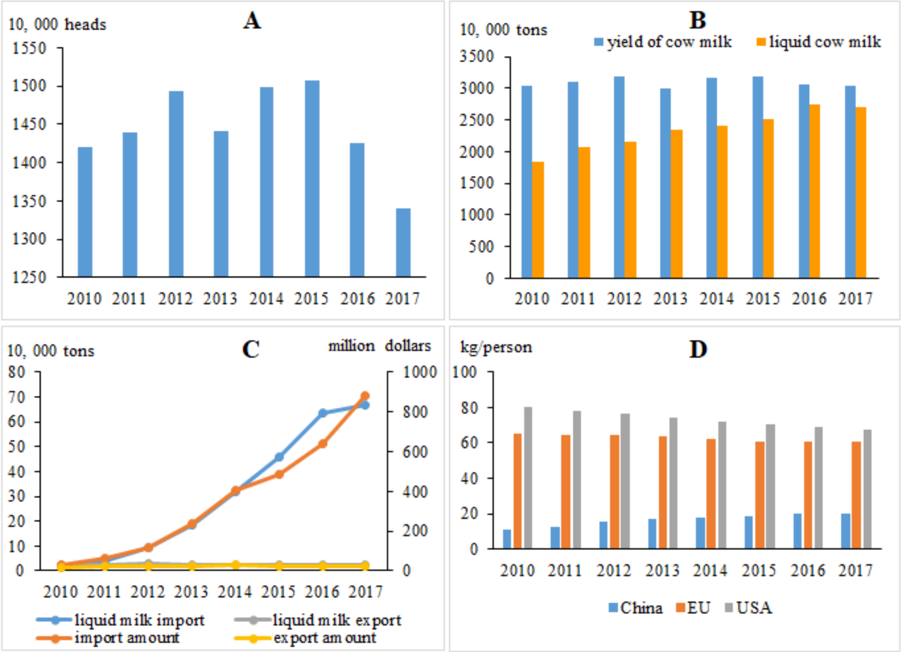

Figure 1-1: Milk production and consumption in China (NBSC, 2018): A - Number of dairy cows; B -Yields of cows' milk and liquid cow milk; C - Imported/exported volume of milk and their economic revenues; D - Consumption of liquid milk per person.

Although the consumption of milk in China is not as high as that in other developed countries, the national production of milk is not sufficient to meet demand. The self-sufficiency of China's milk production declined rapidly from

100% in 2008 to 60% in 2017 (Bai et al., 2018). Therefore, imported milk in China is mainly from Germany, New Zealand and France, and the trade of milk increased strongly during recent decades. Indeed, the quantity of imported liquid milk increased more than 40 times from 2010 to 2017 (from 15,891 to 667,555 tons, Figure 1-1, C). However, milk production in China is currently increasing and the import of liquid cow's milk decreased to 14 million tons for the first time in 2018 (Chang, 2019).

Simultaneously with the increasing milk production, the number of dairy cows in China increased from 2005 to 2015 and reached 15,072,000 heads in 2015. However, a decrease was observed between 2015 and 2017, although the milk yield per cow increased (Figure 1-1 B, D). In 2018, the annual national milk production reached 30.75 million tons, and the output of cow's milk was estimated to be 26.87 million tons (Chang, 2019). Beyond this increasing national production and the pressure placed by Chinese consumers after the melamine scandal in 2008, producers and processors, as well as the regulatory authorities, have been paying increasing attention to the quality and the safety of the cow's milk produced in China. Therefore, in China, there is currently interest in locally producing high-quality milk.

1.2 Contamination of milk by heavy metals

Heavy metals is often used as a group name for metals and semimetals (called metalloids) that have been associated with contamination and potential toxicity or ecotoxicity (Duffus, 2002). However, the terms of heavy metals have different definitions following the regulations and scientific articles. No definition existed before 1936. A summary of heavy metals definition was given by Duffus (2002). Those authors proposed to call heavy metals, elements presenting a density greater than 3.5-7 g/cm^3, a high atomic mass, an atomic number greater than 20, specific chemical properties, and a certain toxicity but without a clear basis. For instance, elements like Pb, Hg, Cd, Cr, As, Cu, Zn, Mn, Ni, Al were assumed by other authors to be heavy metals (Barenys et al., 2014; Ge et al., 2012; Khan et al., 2013; Salazar et al., 2012).

Heavy metals such as Pb, Hg, As, Cr and Cd are ubiquitous in the environment. Their presence is mainly a result of human activities in the mining, steel plant, chemical plant and leather industries (Cheng, S., 2003). These industrial activities lead to heavy metals transferred into air, soil and water; subsequently, plants and animals accumulate heavy metals in their tissues (Rehman et al., 2017; Srinivasa Gowd et al., 2010) and, in the case of dairy mammals, in milk. Finally, the consumption of products derived from contaminated animals and/or plants leads to a human health risk.

Heavy metal contamination in milk has been widely studied, especially that caused by industrial activities (Khan, K. et al., 2014; Licata et al., 2004;

Norouzirad et al., 2018; Patra et al., 2008). The presence of heavy metals in milk is often observed. Table 1–2 shows the number of samples, analyzed by different authors, that have been found to have a heavy metal content exceeding their limit of detection (LOD). According to those results, most of the analyzed milk samples contained heavy metals. These studies were conducted not only on milk but also on yogurt, cheese and milk powder (Ikema et al., 2002; Khan, N. et al., 2014; Kim et al., 2016; Miguel Navarro-Alarcón, 2011).

Table 1-2: Occurrence of heavy metals contamination in milk produced by different mammals.

	N[1]	Number of samples > LOD[2]					Country	References
		Pb	Hg	As	Cr	Cd		
Buffalo	68	49	2	18	40	30	Italy	(Esposito et al., 2017)
Goat	37	11		11	ND	ND	Italy	(Licata et al., 2012)
Ovine	10	1		2	1	ND	Italy	(Licata et al., 2012)
Bovine	40	40		36	6	3	Italy	(Licata et al., 2004)
Cow	249	249					Croatia	(Bilandzic et al., 2016)

[1]N = total number of samples analyzed; [2]LOD = limit of detection; ND = not detected.

The concentration of heavy metals was also studied in cow's milk from different regions and countries. Some of the results were shown in Table 1-3. In those studies, Pb was almost detectable in every studied area (country), followed by Cd. Farms used for sampling were located in rural region, urban region, industrial region or near some special pollution sources.

Table 1-3: Concentrations of heavy metals measured in raw cow’s milk by different authors.

N[1]	Means ± SD[2] (μg/L)			Country	Area	References
	Pb	As	Cd			
160	46±2.75	35±1.5	2±0.8	Mexico	Industrial Urban	(Castro Gonzalez et al., 2017)
6	3.89±4.04			Spain	Industrial	(Gonzalez-Montana et al., 2012)
118	47±8.45		4.7±0.89	Iran	Industrial	(Norouzirad et al., 2018)
30	68±63		20±4	Pakistan	Rural	(Khan, K. et al., 2014)
143	1.48±1.37			South Korea	Not mentioned	(Kim et al., 2016)
90	58.7±82.9	18.5±38.9	1.76±1.94	Croatia	Urban	(Bilandžić et al., 2011)
67	36.2±72.3	43.5±131.6	3.4±3.89	Croatia	Rural	(Bilandžić et al., 2011)
21	32.7±12.4		7±4	Egypt	Industrial	(El Sayed et al., 2011)
19	15.8±5.45	< 1.0	2.56±1.53	Romania	Rural	(Oana et al., 2016)
347	5.23±2.85		0.4±0.28	Spain	Area with As contamination	(Sola-Larrañaga and Navarro-Blasco, 2009)
736	2	< 2	1	Poland	Not mentioned	(Szkoda et al., 2013)
60	47.6±5.21		44.2±2.31	Pakistan	Heavy traffic	(Kazi et al., 2009)
40		69±3		India	Area with As contamination	(Sarkar et al., 2016)
249	11.4±8.08			Croatia	Rural	(Bilandzic et al., 2016)

[1]N is the number of samples; [2]SD means standard deviation.

1.3 The toxicology of heavy metals in humans

Heavy metals can cause health disorders after they accumulate in the body; the target organs are the liver, kidney and nervous system (Costa and Klein, 2008; Miranda et al., 2006; WHO, 1996). The toxicity of Pb, Hg, As, Cr and Cd in humans presents different clinical symptoms, and the toxic levels also differ. A series of parameters have been set by governments and organizations for evaluating heavy metal poisoning in humans, such as the minimum risk level

(MRL) in food, tolerable daily intake (TDI), provisional tolerable weekly intake (PTWI) and tolerable weekly intake (TWI). The reference safety limits for Pb, Hg, As, Cr and Cd in adults are shown in Table 1–4 and are regulated by the U. S. Department of Health and Human Services Public Health Service Agency for Toxic Substances and Disease Registry (ATSDR), Joint FAO/WHO Expert Committee on Food Additives (JECFA), European Food Safety Authority (EFSA) and World Health Organization.

Table 1-4: Parameters used to assess the toxic levels of heavy metals in adults.

	MRL [1] μg/kg body weight/day	PTWI [2] μg/kg body weight	TWI [3] μg/kg body weight
Pb		25 (FAO/WHO, 1999)	
Hg	2 (ATSDR, 2019a)	4 (EFSA, 2012)	4 (EFSA, 2012)
As	0.3 (ATSDR, 2019a)	15 (EFSA, 2014)	
Cr	0.9 (ATSDR, 2012)		
Cd	0.1 (EFSA, 2009)	7 (JECFA, 2003)	2.5 (EFSA, 2009)

[1]MRL = minimum risk level based on an oral exposure; [2]PTWI = provisional tolerable weekly intake; [3]TWI = tolerable weekly intake.

Pb is an environmental contaminant that occurs both naturally and to a greater extent from anthropogenic activities, such as mining and smelting (EFSA, 2010). Pb exists in organic and inorganic forms, but primarily the inorganic form is present in the environment (EFSA, 2010). Pb has neurotoxicity and neurodevelopmental toxicity for humans; the central nervous system is the main target organ for Pb toxicity (EFSA, 2010). Pb can cause neurobehavioral effects (Miranda et al., 2006), such as nervous system developmental delays and retardation of mental development (Ataro et al., 2008; Rahimi, 2013). Beyond neurological toxicity, Pb poisoning decreases the activity of several heme biosynthesis enzymes, as well as birth weight and size (ATSDR, 2019c). Human exposure to Pb is probably due to the ingestion of contaminated food and drinking water (ATSDR, 2019c; Barenys et al., 2014; EFSA, 2010). Exposure can also occur via inadvertent ingestion of contaminated soil/dust or lead-based paint (ATSDR, 2019c).

Cd and inorganic salts formed by Cd are considered carcinogenic (group 1) by the International Agency for Research on Cancer (IARC, 2014). Cd is introduced into the food chain through agricultural soils, which may naturally contain Cd, or from anthropogenic sources. Regardless of the exposure route, Cd is widely distributed in the body, and the highest levels are found in the liver and kidneys

(ATSDR, 2019b). The PTWI of Cd is 400–500 μg/person, and the intake of Cd per kg of body weight (kg b.w.) is 7 μg/kg b.w. (JECFA, 2003).

Hexavalent Cr and trivalent Cr are the two major stable oxidation states of elemental Cr (Costa and Klein, 2008). Trivalent Cr is an essential nutrient required for normal energy metabolism. Trivalent Cr has beneficial effects in the regulation of insulin and glucose levels (Costa and Klein, 2008). An adequate intake of trivalent Cr is 20–45 μg/day for adolescents and adults (ATSDR, 2012). The primary targets of trivalent Cr compounds are the respiratory and immune systems, but the IARC has indicated that the carcinogenicity of trivalent Cr and metallic Cr to humans is not classifiable (ATSDR, 2012). Hexavalent Cr compounds usually exist as oxides or oxohalides. Generally, hexavalent Cr compounds are more toxic than trivalent Cr compounds, because hexavalent Cr has high solubility and higher oxidizing potential than trivalent Cr (Singh et al., 2013). The IARC and Environmental Protection Agency (EPA) have classified hexavalent Cr as a human carcinogen (ATSDR, 2012). The most sensitive targets of hexavalent Cr are the respiratory, gastrointestinal, hematological and reproductive systems. The population may be exposed to Cr daily through contaminated food and drinking water.

Hg is a naturally occurring element found in air, water and soil. However, this element can also be released into the environment from anthropogenic sources. The toxicokinetic of Hg is highly dependent on the form of Hg to which a receptor has been exposed (EFSA, 2012). Organic compounds are more toxic than inorganic forms (USEPA, 1997). Methylmercury is the most common form of organic Hg in the food chain. People are exposed to Hg in daily life through foods, vaccines, antiseptics, ointments, amalgams or occupational exposure (Oz et al., 2012). Chronic exposure to Hg may induce central nervous system symptoms including tremors, delusions, memory loss and neurocognitive disorders (Syversen and Kaur, 2012). The PTWI is 1.6 μg/kg b.w. for methylmercury and 4 μg/kg b.w. for inorganic Hg. In line with the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the CONTAM Panel has established a TWI for inorganic Hg of 4 μg/kg b.w., expressed as Hg (EFSA, 2012). Oral absorption of inorganic Hg occurs through the gastrointestinal tract. Subsequently, elemental Hg is readily distributed throughout the body, and it can cross both the placental and blood-brain barriers (USEPA, 1997).

As is present in water, air and soil and is absorbed by food crops as they grow (Bassil et al., 2018). The organic forms of As are relatively nontoxic compared with the inorganic forms, which have been classified by IARC as type 1 carcinogens (IARC, 2014). As is a naturally occurring element widely distributed in the earth’s crust. In the environment, As combines with oxygen, chlorine and sulfur, forming inorganic As compounds. As in animals and plants combines with carbon and hydrogen, forming organic As compounds (ATSDR, 2007). Inorganic As can increase the risk of skin cancer and cancer in the liver, bladder and lungs. Human exposure to elevated levels of inorganic As occurs mainly through the consumption of groundwater containing naturally high levels of inorganic As, food prepared with contaminated water and food crops irrigated with high-As water sources (WHO, 2010). A study conducted by JECFA in 2010 determined that the lower limit on the benchmark dose lower confidence for a 0.5% ($BMDL_{0.5}$) increased incidence of lung cancer, on the basis of epidemiological data, is 3.0 µg/kg b.w./day (2–7 µg/kg b.w./day, on the basis of the range of estimated total dietary exposure). The JOINT FAO/WHO EXPERT Committee noted that the previously established PTWI of 15 µg/kg b.w. (equivalent to 2.1 µg/kg b.w./day) for inorganic As was within the $BMDL_{0.5}$ and therefore was no longer appropriate. Therefore, this PTWI was withdrawn by the Committee, and no new tolerable intake level has been established. In areas where the levels of As in water are below the WHO drinking-water guideline value, human health effects are presumed unlikely (WHO, 2010).

2. Objective and outline of the thesis

Owing to the increasing Chinese demand of milk and dairy products, the enhanced national production of milk and the presence of polluting industrial activities that could lead to direct or indirect contamination of milk, there is a need to screen the quality of milk produced in China, specifically the content of heavy metals, which potentially damages human health. Many researchers have already studied the heavy metals contamination in cow's milk in different countries but only few information is available for China. Even if some studies were conducted in China, they focused only on short geographical areas and used a limited number of samples. Therefore, the innovative aspect of this thesis is not the methodology but the realization of a large-scale study to assess the spatial variability of heavy metals contamination in raw cow's milk.

Determining the sources of heavy metals in cow's milk is complex, because heavy metals can be transferred into milk through various pathways, such as contaminated feed, drinking water and soil (i.e., direct and/or indirect ingestion due to grass or crops grown in this soil), as well as air. In previous studies, the contents of heavy metals in bulk milk related to feed and environmental factors were studied. However, in our knowledge, no study reported the relationships between milk and feed at individual cow level, especially cows reared near to an industrial pollution source.

Consequently, this thesis had the following aims:

1. Assessing the spatial variability of heavy metals contamination in Chinese cow's milk through a large-scale study;
2. Estimating the relationships between heavy metal contents in individual cow's milk, drinking water, feed and soil in order to highlight pathways potentially responsible for the presence of heavy metals in cow's milk;

3. Estimating at individual scale the links between the content of heavy metals and the main components in milk in order to observe potential proxies highlighting the presence of heavy metals in milk;
4. Assessing theoretically the human health risk caused by the ingestion of such contaminated raw cow's milk.

3. Thesis of framework

The current thesis is the result of a joint PhD project between Gembloux Agro-Bio Tech - University of Liege (GxABT-ULiège) and Graduate School of Chinese Academy of Agricultural Sciences (CAAS). The work related to the articles included in chapters 2 and 3 were totally realized in CAAS. Part of statistical analysis and the revising for the second article were corporate with Prof. Hélène Soyeurt. Sample collection and analysis used in the third article (i.e., chapter 4) were realized in CAAS but the statistical analysis and the article writing and revising were done in GxABT.

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4. Reference

Ataro, A., McCrindle, R. I., Botha, B. M., McCrindle, C. M. E., & Ndibewu, P. P., 2008. Quantification of trace elements in raw cow's milk by inductively coupled plasma mass spectrometry (ICP-MS). Food Chemistry, 111(1), 243-248.

ATSDR. (2007). Arsenic. Division of toxicology and environmental medicine ToxFAQs, from http://www.atsdr.cdc.gov/toxfaq.html

ATSDR. 2012. Toxicological profile for chromium. (Vol. Atlanta, GA, American: Agency for Toxic Substances and Disease Registry.

ATSDR. (2019a). Minimal risk levels (MRLs). Agency for Toxic Substances and Disease Registry.

ATSDR. (2019b). ToxGuide profile for cadmium.

ATSDR. (2019c). Toxicological profile for lead.

Bai, Z., Lee, M. R. F., Ma, L., Ledgard, S., Oenema, O., Velthof, G. L., Ma, W., Guo, M., Zhao, Z., Wei, S., Li, S., Liu, X., Havlik, P., Luo, J., Hu, C., & Zhang, F., 2018. Global environmental costs of China's thirst for milk. Global Change Biology, 24(5), 2198-2211.

Barenys, M., Boix, N., Farran-Codina, A., Palma-Linares, I., Montserrat, R., Curto, A., Gomez-Catalan, J., Ortiz, P., Deza, N., & Llobet, J. M., 2014. Heavy metal and metalloids intake risk assessment in the diet of a rural population living near a gold mine in the Peruvian Andes (Cajamarca). Food and Chemical Toxicology, 71, 254-263.

Bassil, M., Daou, F., Hassan, H., Yamani, O., Kharma, J. A., Attieh, Z., & Elaridi, J., 2018. Lead, cadmium and arsenic in human milk and their socio-demographic and lifestyle determinants in Lebanon. Chemosphere, 191, 911-921.

Bilandžić, N., Đokić, M., Sedak, M., Solomun, B., Varenina, I., Knežević, Z., & Benić, M., 2011. Trace element levels in raw milk from northern and southern regions of Croatia. Food Chemistry, 127(1), 63-66.

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. Bulletin Environmental Contamination and Toxicology, 96(5), 645-649.

Castro Gonzalez, N. P., Moreno-Rojas, R., Calderon Sanchez, F., Moreno Ortega, A., & Juarez Meneses, M., 2017. Assessment risk to children's health due to consumption of cow's milk in polluted areas in Puebla and Tlaxcala, Mexico. Food Additives & Contaminants: Part B, 10(3), 200-207.

Chang, L. (2019). Milk consumption continues to grow rigidly (In Chinese). from http://paper.ce.cn/jjrb/html/2019-05/23/content_391921.htm

Cheng, L., Yin, C., & Chien, H., 2015. Demand for milk quantity and safety in urban China: evidence from Beijing and Harbin. Australian Journal of Agricultural and Resource Economics, 59(2), 275-287.

Cheng, S., 2003. Heavy metal pollution in China: Origin, pattern and control. Environmental Science and Pollution Research, 10(3), 192-198.

Costa, M., & Klein, C. B., 2008. Toxicity and carcinogenicity of chromium Compounds in Humans. Critical Reviews in Toxicology, 36(2), 155-163.

Duffus, J., 2002. "Heavy metals" a meaningless term? (IUPAC Technical Report). Pure and Applied Chemistry, 74(5), 793-807.

EFSA. 2009. Scitific opinion, Cadmium in food, Scientific opinion of the panel on contaminants in the food chain. The EFSA Journal, 980, 1-139.

EFSA, 2010. Scientific opinion on lead in food. Parma, Italy: European Food Safety Authority.

EFSA. 2012. Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA Journal, 10(12), 2985.

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

El Sayed, E. M., Hamed, A. M., Badran, S. M., & Mostafa, A. A., 2011. A survey of selected essential and heavy metals in milk from different regions of Egypt using ICP-AES. Food Additives & Contaminants: Part B, 4(4), 294-298.

Esposito, M., Miedico, O., Cavallo, S., Pellicano, R., Rosato, G., Baldi, L., & Chiaravalle, A. E., 2017. Trace elements in raw milk of buffaloes (Bubalus bubalis) from Campania, Italy. Food Chemistry, 233, 378-384.

FAO. 2013. Milk and dairy products in human nutrition. Roma.

FAO/WHO. (1999). The Joint FAO/WHO expert committee on food additives. Paper presented at the Fifty-third meeting, Rome, Italy.

FAO/WHO. 2001. Human Vitamin and Mineral Requirements. Bangkok, Thailand.

Ge, F., Li, M. M., Ye, H., & Zhao, B. X., 2012. Effective removal of heavy metal ions Cd2+, Zn2+, Pb2+, Cu2+ from aqueous solution by polymer-modified magnetic nanoparticles. Journal of Hazardous Materials, 211-212, 366-372.

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). Environmental Monitoring and Assessment, 184(7), 4029-4034.

He, Y., Yang, X., Xia, J., Zhao, L., & Yang, Y., 2016. Consumption of meat and dairy products in China: a review. Proceedings of the Nutrition Society, 75(3), 385-391.

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

Ikema, A., Nwankwoala, A., Odueyungbo, S., Nyavor, K., & Egiebor, N., 2002. Levels of 26 elements in infant formula from USA, UK and Nigeria by microwave digestion and ICP–OES. Food Chemistry, 77, 439–447.

JECFA. (2003). Summary and conclusions. Paper presented at the Joint FAO/WHO expert committee on food additives, Sixty-first meeting, Rome, Italy.

Kazi, T. G., Jalbani, N., Baig, J. A., Kandhro, G. A., Afridi, H. I., Arain, M. B., Jamali, M. K., & Shah, A. Q., 2009. Assessment of toxic metals in raw and processed milk samples using electrothermal atomic absorption spectrophotometer. Food and Chemical Toxicology, 47(9), 2163-2169.

Khan, K., Khan, H., Lu, Y., Ihsanullah, I., Nawab, J., Khan, S., Shah, N. S., Shamshad, I., & Maryam, A., 2014. Evaluation of toxicological risk of foodstuffs contaminated with heavy metals in Swat, Pakistan. Ecotoxicology and Environmental Safety, 108, 224-232.

Khan, K., Lu, Y., Khan, H., Ishtiaq, M., Khan, S., Waqas, M., Wei, L., & Wang, T., 2013. Heavy metals in agricultural soils and crops and their health risks in Swat District, northern Pakistan. Food and Chemical Toxicology, 58, 449-458.

Khan, N., Jeong, I. S., Hwang, I. M., Kim, J. S., Choi, S. H., Nho, E. Y., Choi, J. Y., Park, K. S., & Kim, K. S., 2014. Analysis of minor and trace elements in milk and yogurts by inductively coupled plasma-mass spectrometry (ICP-MS). Food Chemistry, 147, 220-227.

Kim, D. G., Kim, M., Shin, J. Y., & Son, S. W., 2016. Cadmium and lead in animal tissue (muscle, liver and kidney), cow milk and dairy products in Korea. Food Additives & Contaminants: Part B, 9(1), 33-37.

Li, J., & Zhang, Z., 2011. Trace element selenium and human health. Studies of Trace Elements Health, 28(5), 59-63.

Licata, P., Di Bella, G., Potorti, A. G., Lo Turco, V., Salvo, A., & Dugo, G. M., 2012. Determination of trace elements in goat and ovine milk from Calabria (Italy) by ICP-AES. Food Additives & Contaminants: Part B, 5(4), 268-271.

Licata, P., Trombetta, D., Cristani, M., Giofre, F., Martino, D., Calo, M., & Naccari, F., 2004. Levels of toxic and essential metals in samples of bovine milk from various dairy farms in Calabria, Italy. Environment International, 30, 1-6.

Miguel Navarro-Alarcón, C. C.-V., Ma Dolores Ruiz-López ⥣, Manuel Olalla, Reyes Artacho, Rafael Giménez, Verónica Quintana, Triana Bergillos. 2011. Levels of Se, Zn, Mg and Ca in commercial goat and cow milk fermented products Relationship with their chemical composition and probiotic starter culture. Food Chemistry, 129, 1126-1131.

Miranda, M., Lo´pez-Alonso, M., Garcı´a-Partida, P., Velasco, J., & Benedito, J. L., 2006. Long-term Follow-up of Blood Lead Levels and Haematological and Biochemical Parameters in Heifers that Survived an Accidental Lead Poisoning Episode. Journal of Veterinary Medicine Series A, 53, 305-310.

Mituniewicz, T., Iwańczuk-Czernik, K., Wójcik, A., Sowińska, J., & Witkowska, D., 2006. Iron management in two meat breeds of cattle in selected elements of the trophic chain. Polish Journal of Environmental Studies, 15(2a), 431-435.

NBSC. (2018). National Bureau of Statistics of China. China statistical yearbook. from http://www.stats.gov.cn/tjsj/ndsj/2018/indexeh.htm

Norouzirad, R., Gonzalez-Montana, J. R., Martinez-Pastor, F., Hosseini, H., Shahrouzian, A., Khabazkhoob, M., Ali Malayeri, F., Moallem Bandani, H., Paknejad, M., Foroughi-Nia, B., & Fooladi Moghaddam, A., 2018. Lead and cadmium levels in raw bovine milk and dietary risk assessment in areas near petroleum extraction industries. Science of the Total Environment, 635, 308-314.

Oana, C., Tănaselia, C., Miclean, M., Level, E., Șenilă, M., & Șenilă, L., 2016. Analysis of Minor and Trace Elements in Cow, Goat and Sheep Milk in the NW Part of Romania. ProEnvironment, 9, 87-90.

Oz, S. G., Tozlu, M., Yalcin, S. S., Sozen, T., & Guven, G. S., 2012. Mercury vapor inhalation and poisoning of a family. Inhalation Toxicology, 24(10), 652-658.

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. Science of the Total Environment, 404(1), 36-43.

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

Rehman, Z. U., Khan, S., Brusseau, M. L., & Shah, M. T., 2017. Lead and cadmium contamination and exposure risk assessment via consumption of vegetables grown in agricultural soils of five-selected regions of Pakistan. Chemosphere, 168, 1589-1596.

Salazar, M. J., Rodriguez, J. H., Leonardo Nieto, G., & Pignata, M. L., 2012. Effects of heavy metal concentrations (Cd, Zn and Pb) in agricultural soils near different emission sources on quality, accumulation and food safety in soybean [Glycine max (L.) Merrill]. Journal of Hazardous Materials, 233-234, 244-253.

Sarkar, P., Ray, P., Ghatak, P., & Sen, M., 2016. Arsenic concentration in water, rice straw and cow milk - a micro level study at Chakdaha and Haringhata block of West Bengal. Indian Journal of Dairy Science, 6(69), 676-679.

Sigel, A., Sigel, H., & Sigel, R. K. O., 2012-2016. Metal Ions in Life Sciences (Vol. 13). New York London: Springer, Dordrecht.

Singh, H. P., Mahajan, P., Kaur, S., Batish, D. R., & Kohli, R. K., 2013. Chromium toxicity and tolerance in plants. Environmental Chemistry Letters, 11(3), 229-254.

Sola-Larrañaga, C., & Navarro-Blasco, I., 2009. Chemometric analysis of minerals and trace elements in raw cow milk from the community of Navarra, Spain. Food Chemistry, 112(1), 189-196.

Srinivasa Gowd, S., Ramakrishna Reddy, M., & Govil, P. K., 2010. Assessment of heavy metal contamination in soils at Jajmau (Kanpur) and Unnao industrial areas of the Ganga Plain, Uttar Pradesh, India. Journal of Hazardous Materials, 174(1-3), 113-121.

Syversen, T., & Kaur, P., 2012. The toxicology of mercury and its compounds. Journal of Trace Elements in Medicine and Biology, 26(4), 215-226.

Szkoda, J., Żmudzki, J., Nawrocka, A., & Kmiecik, M., 2013. Concentrations of toxic elements in tissues of slaughtered animals and cows milk - a five year study. Bulletin of the Veterinary Institute in Pulawy, 57, 529-533.

USEPA. (1997). Health effects of mercury and mercury compunds. In U. S. E. P. Agency (Ed.), Mercury study report to congress (Vol. 5): Office of air quality planning and standards and office of research and development.

Wang, D., van der Horst, K., Jacquier, E. F., Afeiche, M. C., & Eldridge, A. L., 2018. Snacking Patterns in Children: A Comparison between Australia, China, Mexico, and the US. Nutrients, 10(2).

WHO. 1996. Trace elements in human nutrition and health. Geneva, Switzerland: World Health Organization.

WHO, 2010. Exosure to arsenic: a major public health concern. Geneva, Switzerland: Public Health and Environment, World Health Organization.

2

Analysis of 22 elements in milk, feed and water of dairy cow, goat and buffalo from different regions of China

From Xuewei Zhou, Xueyin Qu, Shengguo Zhao, Jiaqi Wang, Songli Li and Nan Zheng, 2017. Analysis of 22 Elements in Milk, Feed, and Water of Dairy Cow, Goat, and Buffalo from Different Regions of China. Biological Trace Element Research, 176: 120-129.

This chapter is part of the work that have been done during my master degree. Several studies have already focused on the quantification of heavy metal contents in milk produced by cows and other mammals in different regions and countries. From those results, it appeared that regional variations exist due to their different environmental conditions. However, there was no data to characterize the contents of those elements in milk produced by Chinese cows, goats and buffalo. In this study, the contents of 22 elements, classified followed the regulation established by WHO (1996), were measured in cow, goat, and buffalo milk samples. Those results were used to assess the regional differences of heavy metals concentrations in China as well as the differences of contents between mammals. Relationships of heavy metals' contents between milk, drinking water and feed were also studied.

Following the suggestions formulated by CDC (2009) and WHO (1995), the measured content was replaced by the half of the limit of detection (LOD) when the sample content was below LOD for a specific heavy metal.

When no certified reference material (CRM) for a specific matrix is available, the European Commission (2002/657/EC) accepts that the measurement trueness is assessed through the recovery of known amounts of analytes added to a blank matrix. Due to the lack of CRM related to fluid milk with known amounts of heavy metals, a methodology based on spiked recovery (i.e., 150 µg/L, 400 µg/L and 2 mg/L) was used to do the quality control of milk analysis performed in this study. The quality of water analysis was also performed using spiked recovery with the following concentrations: 0.5 µg/L, 1.0 µg/L, 1.5 µg/L and 4 µg/L. The quality of feed analysis was assessed using CRM (i.e., cabbage).

Abstract

The objectives of this study were to measure the concentrations of elements in raw milk by inductively coupled plasma-mass spectrometry (ICP-MS) and evaluate differences in element concentrations among animal species and regions of China. Furthermore, drinking water and feed samples were analyzed to investigate whether the element concentrations in raw milk are correlated with those in water and feed. All samples were analyzed by ICP-MS following microwave-assisted acid digestion. The mean recovery of the elements was 98.7% from milk, 103.7% from water, and 93.3% from a certified reference material (cabbage). Principal component analysis results revealed that element concentrations differed among animal species and regions. Correlation analysis showed that trace element Mn, Fe, Ni, Ga, Se, Sr, Cs, U in water and Co, Ni, Cu, Se, U in feed were significantly correlated with those in milk ($P < 0.05$). Toxic and potential toxic elements Cr, As, Cd, Tl, Pb in water and Al, Cr, As, Hg, Tl in feed were significantly correlated with those in milk ($P < 0.05$). Results of correlation analysis revealed that elements in water and feed might contribute to the elements in milk.

Key words: Milk; Cow; Goat; Buffalo; Trace elements; ICP-MS

1. Introduction

Milk and milk products represent an important source of macro- and micronutrients, including minerals. Trace elements such as iron (Fe), zinc (Zn), copper (Cu), and selenium (Se), are essential in human metabolism, growth, and development (Stawarz et al., 2007), while toxic elements such as lead (Pb) and cadmium (Cd) induce mental retardation and cardiovascular diseases (Ataro et al., 2008; Rahimi, 2013). Therefore, element concentration in milk and milk products is indicative of their safety and nutritional value. The concentration of trace and toxic elements in raw cow milk vary significantly by region (Król et al., 2012; Pilarczyk et al., 2013; Rey-Crespo et al., 2013). For example, Pb, Cd, and Cu concentrations are 47.45 µg/L, 1.68 µg/L, and 890.15 µg/L, respectively, in raw milk from Croatia (Bilandžić et al., 2011), and 5.23 µg/L, 0.40 µg/L, and 51.8 µg/L, respectively, in raw milk from Spain (Sola-Larrañaga and Navarro-Blasco, 2009). Additionally, element concentration in milk varies by animal species (Farida M. Al-awadi, 2001; Najarnezhad and Akbarabadi, 2013; Rahimi, 2013). Najarnezhad et al. has studied the concentration of Pb and Cd in ewe and cow milk, the results showed Pb and Cd in ewe milk were significantly higher than in cow milk (Najarnezhad and Akbarabadi, 2013). Lin Bo (2014) reported that the concentrations of Fe and Zn in buffalo milk were higher than those in cow milk.

The concentrations of elements in raw milk are also affected by animal forage, feed, and water (Al-Wabel, 2008; Thomas Müller, 1996; Z. Dobrzański, 2005). Concentrations of health-beneficial elements, e.g., cobalt (Co), Fe, Zn, etc., in milk are dependent on the animal species, feed, milk sample collection time, environmental conditions, and manufacturing processes (Coni et al., 1996; Herwig et al., 2011). Arsenic (As) and Fe in cow milk are possibly related to a higher consumption of concentration feed (Rey-Crespo et al., 2013). Potortì et al. has reported that elements in donkey milk were related with those in feed and water (Potortì et al., 2013). Cu levels in milk are attributed to feed Cu concentrations (Havemose et al., 2006).

In China, cattle represent a vital part of the economy. Cattle breeding stock reached 14.9 million in 2012, contributing to approximately 38.75 million tonne of cow milk (China, 2014). In 2014, the population of dairy goats was approximately 1.2 million, and goat milk was the second most important type of milk in China (Zhao et al., 2014). Buffalo milk, which represents an important source of income in southern China, had a yield of approximately 33,000 tonne in Guangxi zhuang autonomous region in 2012 (Tang, 2014).

There is little information on the concentrations of trace, potentially toxic, and toxic elements in goat and buffalo milk in China. The correlations of elements content between milk and feed, drinking water are also little. Therefore, the objectives of this study were to measure the concentrations of elements in raw milk by inductively coupled plasma-mass (ICP-MS) and evaluate differences in element concentrations among animal species and regions. Furthermore, drinking water and feed samples were analyzed to investigate whether the element concentrations in milk are correlated with those in drinking water and feed.

2. Materials and methods

2.1 Instrumentation

Vanadium (V), manganese (Mn), Fe, Co, nickel (Ni), Cu, Zn, gallium (Ga), Se, rubidium (Rb), strontium (Sr), silver (Ag), cesium (Cs), barium (Ba), uranium (U), aluminum (Al), chromium (Cr), As, Cd, mercury (Hg), thallium (Tl), and Pb were measured by ICP-MS (7700x, Agilent, USA), which was equipped with a quadrupole hyperboloid, Scott double pass spray chamber, concentric nebulizer, and sample introduction system. A microwave dissolver (CEM Corporation, USA) with PTFE tubes was used for milk and feed sample digestion; the operating conditions of ICP-MS are presented in Table 2-1. Vessels used in the digestion were previously immersed in 20% HNO_3 (v/v) for at least 12 h and rinsed with ultrapure water.

Table 2-1: Operating conditions and measurement parameters for the ICP-MS.

Parameter	
Nebulizer	Concentric Nebulizer
Spray chamber	Dual channel Scott type
Mass analizator	Quadruple
RF power	1550 W
Ar gas flow rates	
Plasma	15 L/min
Auxiliary	1.10 L/min
Lens voltage	12.2 V
Scanning mode	Peak hopping
Dwell time	45 s
Sample uptake rate	0.15 mL/min
Isotopes	^{45}Sc, ^{72}Ge, ^{103}Rh, ^{115}In, ^{159}Tb, ^{175}Lu, ^{209}Bi

2.2 Preparation of standard solutions

A mercury calibration solution was prepared from 10 mg/L mercury standard solution (SPEX, USA), while the calibration solutions of the other 21 elements were prepared from 10 mg/L multi-element stock standard solution (SPEX). An internal standard solution was an aqueous multi-element standard solution containing 100 mg/L of Li, Sc, Ge, Rh, In, Tb, Lu and Bi (SPEX). The internal stock standard solution was further diluted and used to correct any fluctuations of the instrument due to the matrix. Cabbage certified reference material (CRM) was obtained from the National

Institute of Metrology (GBW10014, China). Nitric acid (65%, Sigma, USA) and hydrogen peroxide (30%, Merck, Germany) were used in sample digestion. To maintain the same percentage of acid in the samples, the calibration solutions were diluted with 6% (v/v) HNO_3 for milk, 10% (v/v) HNO_3 for feed, and 1% (v/v) HNO_3 for water.

2.3 Sample collection and digestion

A total of 299 samples were analyzed, including 100 milk samples (20 cow milk samples from Shandong, 20 cow milk samples from Shaanxi, 20 goat milk samples from Shandong, 20 goat milk samples from Shaanxi, and 20 buffalo milk samples from Guangxi), 100 feed samples, and 99 water samples (feed and water samples were collected from the same sites as the milk samples). Feed samples were total mixed ration (TMR), and collected from where the animal feeding. All samples were collected in April and July of 2014.

Milk and water samples were stored in 200 mL polypropylene centrifuge tubes at -20 ℃. Water samples were preserved by acidification with 2 mL HNO_3. Feed samples were oven-dried at 65 ℃ for 48 h and ground to a particle size of 1 mm.

Milk (1 mL) was digested with 3 mL HNO_3 (65%) and 4 mL H_2O_2 (30%) in polytetrafluoroethylene (PTFE) tubes. Feed (0.5 g) was first added with 1 mL of ultrapure water, then digested with 5 mL HNO_3 (65%) and 2 mL H_2O_2 (30%). Water was mixed with 1% v/v HNO_3 prior to ICP-MS analysis. Sample digestion was performed at room temperature in open vessels.

A MARS 6 microwave sample digestion system (MARS 6, CEM Corporation, USA) with a power of 1,600 W was used for the digestion of milk and feed samples. The milk samples were digested according to the following program: (1) ramp time 5 min, temperature 90 ℃, hold time 5 min; (2) ramp time 5 min, temperature 150 ℃, hold time 10 min; (3) ramp time 5 min, temperature 180 ℃, hold time 20 min. The milk samples were digested according to the following program: (1) ramp time 5 min, temperature 90 ℃, hold time 5 min; (2) ramp time 5 min, temperature 150 ℃, hold time 10 min; (3) ramp time 5 min, temperature 200 ℃, hold time 20 min. Digested samples were allowed to cool to room temperature, transferred to polypropylene tubes (Corning, USA), and diluted to 50 mL with ultrapure water. Blanks, devoid of samples, were subjected to similar digestion procedures.

2.4 Quality assurance

Limit of detection (LOD) and limit of quantification (LOQ) were calculated from three and 10 times, respectively, the standard deviation of the sample blank relative to the slope of the analytical curve. The digestion procedures were different for milk, water, and feed samples; therefore, LOD and LOQ were calculated separately. The digested milk, feed, and water samples were used in the calculation of LOD and LOQ (D'Ilio et al., 2008), as shown in Table 2-2.

Table 2-2: Detection limits of 22 elements in milk, feed and water.

Element	Milk (μg/L)		Feed (μg/kg)		Water (μg/L)	
	LOD	LOQ	LOD	LOQ	LOD	LOQ
V	1.35×10^{-2}	0.04	0.05	0.16	0.04	0.12
Mn	1.83	6.11	0.19	0.64	1.47	4.91
Fe	9.83	32.76	9.40	31.33	6.25	20.85
Co	0.03	0.11	0.06	0.21	1.15×10^{-2}	0.04
Ni	0.12	0.41	0.15	0.51	0.13	0.43
Cu	1.16	3.86	1.71	5.71	0.16	0.52
Zn	3.16	10.53	1.51	5.02	1.65	5.50
Ga	9.73×10^{-3}	0.03	0.04	0.12	5.49×10^{-3}	0.02
Se	0.13	0.44	0.11	0.38	0.09	0.29
Rb	4.03	13.45	1.44	4.82	0.05	0.15
Sr	0.32	1.08	3.92	13.07	15.75	52.49
Ag	0.20	0.66	0.05	0.16	0.03	0.10
Cs	0.03	0.11	0.06	0.19	0.02	0.08
Ba	0.29	0.97	0.59	1.96	11.26	37.52
U	0.08	0.27	0.03	0.08	0.02	0.08
Al	1.69	5.64	1.53	5.11	2.91	9.71
Cr	0.82	2.74	0.39	1.28	0.07	0.24
As	0.09	0.30	0.18	0.61	0.15	0.51
Cd	2.89×10^{-3}	0.01	3.35×10^{-3}	0.01	2.88×10^{-3}	0.01
Hg	0.22	0.73	0.47	1.57	0.09	0.32
Tl	1.33×10^{-2}	0.04	4.13×10^{-3}	0.01	1.07×10^{-2}	0.04
Pb	0.28	0.94	0.16	0.53	0.05	0.16

To assess the accuracy of the method, cabbage CRM and spiked samples were analyzed. The recovery of the elements from cabbage CRM and spiked samples is shown in Table 2-3. The recovery of 22 elements in water and milk samples was 94.3%–123.3% and 91.4%–113.4%, respectively, except for Hg (86.5% in water and 71.0% in milk). For cabbage CRM, the recovery of 18 elements was 71.2%–114.8%. The results of recovery accord with precision of quantitative methods (EU, 2002).

Table 2-3: Spike recovery and quality control of certified reference material, cabbage.

Element	Recovery (%)		Certified reference material		
	Water	Milk	Certified values (µg/kg)	Observed Values (µg/kg)	Recovery (%)
Al	123.3	103.6	-- [b]	-- [b]	-- [b]
V	105.1	100.1	-- [b]	-- [b]	-- [b]
Cr	103.7	97.4	1800	1616.80	89.8
Mn	107.1	99.1	18700	19366.70	103.6
Fe	111.8	96.7	98000	89836.62	91.7
Co	96.2	97.7	89	63.34	71.2
Ni	95.3	97.1	930	922.70	99.2
Cu	94.3	94.7	2700	2190.18	81.1
Zn	107.3	94.0	26000	23543.38	90.5
Ga	100.3	99.6	-- [a]	-- [a]	-- [a]
As	100.6	104.2	62	56.82	91.7
Se	101.3	113.4	200	229.66	114.8
Rb	105.3	91.4	19600	18882.77	96.3
Sr	114.3	98.5	48000	48460.21	101.0
Ag	103.2	103.3	--[a]	-- [a]	-- [a]
Cd	100.1	99.7	35	36.60	104.6
Cs	105.1	101.5	82	74.87	91.3
Ba	112.3	104.1	12000	11321.96	94.3
Hg	86.5	71.0	10.9	10.56	96.9
Tl	102.4	101.1	6.3	4.63	73.4
Pb	103.2	100.6	190	173.92	91.5
U	102.1	103.2	20	19.42	97.1
Mean ± SD	103.7±7.45	98.7±7.4			93.3±10.25

a: Certified value not available; b: Measured value not available.

2.5 Statistical analysis

Element concentrations below LOD were replaced by half the value of the respective detection limits. The data were not normally distributed; therefore, non-parametric test was used in the analysis. Spearman rank correlation was used to determine the magnitude of the correlation among elements in milk, water, and feed samples. Data analyses were performed using SPSS 17.0 (IBM, USA). Statistical significance was set at $p < 0.05$. Principal component analysis (PCA) was performed with Canoco 5.0.

3. Results and discussion

3.1 Concentrations of trace elements in milk samples

Mn, Fe, Cu, Zn, Se, Rb, Sr, Cs, and Ba were present in all milk samples (positive rate: 100.0%). In buffalo milk, Ga had a positive rate of 100.0% (Table 2-4). V was present in 50%–100% of all milk samples, and Ga and U were present in 50%–100% of cow and goat milk samples (Table 2-4). Other elements had lower positive rates: Ni, Co, and U were present in 10.0 to 50.0% of all milk samples, goat milk, and buffalo milk, respectively. Ag had positive rate was lower than 10.0% in all milk samples. As a result of the large percentage range, the mean values were affected by the high concentrations of the elements, which contributed to mean values that were higher than the median values.

Mean values were compared with those previously reported. Values above LOD were used for mean value calculation. Fe and Zn concentrations in our cow milk samples were similar to those reported in cow milk from Northern Spain (Rey-Crespo et al., 2013), but lower than those reported from Turkey (Temiz and Soylu, 2012). In this study, Zn concentrations in cow milk were similar to those in cow milk from Silesia (Z. Dobrzański, 2005) and higher than those from Pakistan (Khan, N. et al., 2014). Mostly, Cu, Co, and Mn concentrations were lower than those previously reported. In our study, Cu and Co concentrations were lower than those measured in cow milk from Northern Spain (Rey-Crespo et al., 2013) and Turkey (Z. Dobrzański, 2005). Similarly, Cu concentrations in cow milk from Croatia and Pakistan (Bilandžić et al., 2011; Khan, N. et al., 2014), and Mn concentrations in cow milk from South Africa (Ataro et al., 2008) were higher than those obtained in our study (Table 2-4).

Table 2-4: Concentration of 22 elements in raw milk from different specie.

Element	Cow (n=40)				Goat (n=40)				Buffalo (n=20)			
	positive rate	median	p75	mean	positive rate	median	p75	mean	positive rate	median	p75	mean
V	75.0	0.12	0.32	0.29	80.0	0.21	0.58	0.39	75.0	0.15	0.30	0.26
Mn	100.0	20.58	27.10	23.35	100.0	33.78	40.26	36.32	100.0	56.30	61.29	51.49
Fe	100.0	230.15	329.80	352.08	100.0	361.15	525.19	462.96	100.0	497.60	567.47	421.33
Co	80.0	0.21	0.30	0.29	27.5	0.02	0.04	0.14	85.0	0.25	0.53	0.40
Ni	20.0	0.06	0.06	5.76	50.0	1.75	5.84	5.60	15.0	0.06	0.06	1.05
Cu	100.0	25.42	32.08	32.02	100.0	81.52	113.30	84.67	100.0	32.11	61.89	48.42
Zn	100.0	3307.37	3623.15	3233.96	100.0	2983.43	3281.77	2953.93	100.0	4689.09	5504.16	4629.55
Ga	80.0	0.05	0.07	0.08	60.0	0.05	0.08	0.09	100.0	0.09	0.17	0.25
Se	100.0	19.50	23.53	20.72	100.0	17.67	22.23	17.34	100.0	19.59	24.69	20.21
Rb	100.0	1659.44	1861.72	1828.84	100.0	2670.54	3418.40	2982.71	100.0	2757.65	3848.80	2965.06
Sr	100.0	518.79	658.39	589.20	100.0	1242.59	1827.51	1357.31	100.0	374.35	552.25	409.82
Ag	2.5	0.10	0.10	0.23	7.5	0.10	0.10	0.45	5.0	0.10	0.10	0.55
Cs	100.0	6.57	7.61	6.74	100.0	7.04	9.87	8.43	100.0	26.15	33.73	26.78
Ba	100.0	34.47	57.88	60.94	100.0	92.28	132.09	98.06	100.0	184.30	278.53	209.55
U	80.0	1.03	1.53	2.07	75.0	0.79	1.56	1.60	45.0	0.04	0.46	0.69
Al	92.5	48.22	74.54	56.91	92.5	69.40	138.62	102.54	50.0	0.99	101.64	89.18
Cr	15.0	0.41	0.41	12.52	35.0	0.41	2.36	5.26	ND	NC	NC	NC
As	82.5	0.48	0.81	0.86	45.0	0.05	0.44	1.26	100.0	2.30	2.73	2.49
Cd	32.5	0.00	0.02	0.07	27.5	0.00	0.02	0.05	ND	NC	NC	NC
Hg	47.5	0.11	1.74	5.20	15.0	0.11	0.11	5.07	15.0	0.11	0.11	0.33
Tl	77.5	0.03	0.06	0.07	80.0	0.14	0.27	0.20	5.0	0.01	0.01	0.02
Pb	95.0	1.16	2.10	1.46	100.0	1.52	2.40	1.75	100.0	6.77	8.84	7.16

On the other hand, Mn concentrations were comparable to those obtained in cow milk from Northern Spain (Rey-Crespo et al., 2013).

The trace elements in goat milk were lower than those previously reported (Table 2-4). Fe, Cu, and Zn concentrations in our goat milk samples were lower than those from Turkey (Güler, 2007) and Saudi Arabia (Al-Wabel, 2008). Additionally, the concentrations of most elements in goat milk were higher than those in cow milk (Table 2-4), as previously reported (Jan et al., 2011; Khan et al., 2006; Nestares et al., 2008). In present study, Zn concentration in buffalo milk was five times higher than that in buffalo milk from India, while Fe concentrations were similar between the two studies (Shailaja et al., 2014).

3.2 Concentrations of trace elements in drinking water and feed samples

One water sample of goat is missed, the corresponding milk and feed sample was used when calculated mean values, while dropped when correlation test. The concentrations of trace elements in drinking water are presented in Table 2-5. Buffalo drinking water had higher trace element concentrations than those of cows and goats. Buffalo drinking water samples were collected from different province; therefore, region might account for such differences (Khan, K. et al., 2014; Kifayatullah Khan, 2013). The concentrations of V, Co, Ni, Cu, Ga, Se, Rb, and Cs in drinking water were lower than 10 µg/L; Ag was not detected in any of the samples. The concentrations of Mn, Cu, and Zn in all drinking water samples were lower than those reported in northern Pakistan (Kifayatullah Khan, 2013).

V, Mn, Fe, Co, Ni, Cu, Zn, Ga, Se, Rb, Sr, Cs, and Ba were present in 100% of all feed samples. Both Ag and U had positive rates of 100% in buffalo feed samples (Table 2-6). The concentrations of trace elements in feed samples were higher (tenfold) than those in drinking water (Table 2-5 and 2-6).

3.3 Concentrations of toxic and potential toxic elements in raw milk, water and feed samples

The concentrations of toxic and potentially toxic elements (Al, Cr, As, Cd, Hg, Pb, and Tl) in milk samples are presented in Table 2-4. In cow milk, Pb had the highest positive rate (95.0%), followed by Al, As, and Tl. Chromium, Cd, and Hg had positive rates less than 50.0%. In goat milk, Pb had the highest rate, followed by Al and Tl, for Cr, Cd, As, and Hg had the positive rate less than 50%. In buffalo milk, Pb and As were detected in all samples (100.0%), Al, Hg, and Tl were detected in less than 50% of the samples, and Cr and Cd were not detected.

Table 2-5: Concentration (µg/L) of 22 elements in drinking water from different species.

Element	Cow (n=40)				Goat (n=39)				Buffalo (n=20)			
	positive rate	median	p75	mean	positive rate	median	p75	mean	positive rate	median	p75	mean
V	100.0	2.05	7.61	4.26	100.0	3.86	7.67	5.12	80.0	0.09	0.16	0.26
Mn	17.5	0.74	0.74	5.30	30.8	0.74	2.27	16.50	100.0	16.85	98.47	69.09
Fe	80.0	7.24	28.58	93.98	79.5	11.12	25.61	54.45	95.0	18.93	53.55	37.95
Co	85.0	0.02	0.06	0.06	100.0	0.07	0.12	0.11	100.0	0.51	3.17	1.89
Ni	35.0	0.06	0.19	0.44	61.5	0.18	0.30	0.69	100.0	0.72	3.11	1.78
Cu	40.0	0.08	0.30	1.19	71.8	0.24	0.60	1.81	95.0	1.04	2.93	1.82
Zn	65.0	2.44	4.64	9.54	71.8	2.59	6.88	17.07	95.0	7.44	10.52	8.52
Ga	27.5	0.00	0.00	0.01	23.1	0.00	0.00	0.02	90.0	0.02	0.28	0.62
Se	95.0	0.90	3.06	2.07	100.0	0.99	3.40	4.85	100.0	0.18	0.50	0.74
Rb	100.0	0.43	0.54	1.02	100.0	0.48	0.89	1.29	100.0	4.98	8.53	6.15
Sr	100.0	512.63	1517.11	1159.69	100.0	813.77	1428.44	1096.84	45.0	7.87	18.15	24.73
Ag	ND	NC	NC	NC	ND	NC	NC	NC	ND	NC	NC	NC
Cs	15.0	0.01	0.01	0.38	10.3	0.01	0.01	0.12	95.0	0.18	0.55	0.41
Ba	90.0	20.11	32.10	31.87	89.7	28.50	40.19	43.30	65.0	23.14	52.34	47.11
U	100.0	2.50	18.96	9.24	100.0	7.95	14.95	17.20	45.0	0.02	0.04	0.23
Al	57.5	3.18	5.36	9.61	61.5	3.69	7.24	20.17	100.0	17.75	45.93	139.88
Cr	100.0	9.60	83.03	45.34	100.0	1.55	28.54	22.63	100.0	0.25	0.40	0.45
As	77.5	0.52	2.47	1.76	87.2	1.03	2.64	1.65	70.0	0.27	0.50	0.69
Cd	55.0	0.00	0.01	0.01	76.9	0.01	0.01	0.03	100.0	0.03	0.06	0.05
Hg	ND	NC	NC	NC	ND	NC	NC	NC	ND	NC	NC	NC
Tl	7.5	0.01	0.01	0.02	2.6	0.01	0.01	0.02	95.0	0.03	0.11	0.08
Pb	47.5	0.02	0.09	0.10	71.8	0.10	0.17	0.24	100.0	0.48	1.23	2.93

ND: not detected. NC: not computable (Ag and Hg were not detected in water samples). PR: positive rate.

Table 2-6: Concentration (mg/kg) of 22 elements in feed from different species.

Element	Cow (n=40)				Goat (n=40)				Buffalo (n=20)			
	positive rate	median	p75	mean	positive rate	median	p75	mean	positive rate	median	p75	mean
V	100.0	1.00	1.45	1.26	100.0	1.01	1.97	1.37	100.0	0.29	0.99	0.97
Mn	100.0	102.74	128.30	117.96	100.0	97.39	139.82	115.77	100.0	123.15	176.77	124.37
Fe	100.0	573.35	802.76	664.16	100.0	428.06	791.66	687.38	100.0	387.67	574.80	624.28
Co	100.0	0.56	1.00	0.84	100.0	0.37	0.62	0.53	100.0	0.18	0.97	0.45
Ni	100.0	1.06	1.40	1.18	100.0	1.20	1.65	1.44	100.0	1.11	1.42	1.29
Cu	100.0	15.57	18.26	19.52	100.0	7.91	12.62	10.68	100.0	6.83	17.57	10.85
Zn	100.0	77.85	92.27	98.42	100.0	45.90	67.65	58.75	100.0	45.54	110.64	70.00
Ga	100.0	0.12	0.17	0.14	100.0	0.14	0.25	0.19	100.0	0.07	0.08	0.12
Se	100.0	0.33	0.42	1.50	100.0	0.18	0.33	0.25	100.0	0.12	0.36	0.21
Rb	100.0	8.98	10.75	9.16	100.0	7.98	10.45	9.01	100.0	18.48	30.98	21.04
Sr	100.0	31.57	41.73	34.28	100.0	16.43	55.57	38.60	100.0	9.29	12.49	10.46
Ag	50.0	0.00	0.00	0.01	77.5	0.00	0.01	0.01	100.0	0.00	0.01	0.01
Cs	100.0	0.12	0.14	0.12	100.0	0.11	0.17	0.12	100.0	0.09	0.12	0.13
Ba	100.0	11.22	14.14	10.99	100.0	8.59	26.95	15.84	100.0	8.63	13.22	13.53
U	97.5	0.05	0.11	0.08	82.5	0.03	0.12	0.10	100.0	0.02	0.05	0.03
Al	100.0	453.19	663.61	492.00	100.0	474.10	798.54	626.34	100.0	266.59	381.42	454.08
Cr	100.0	3.16	6.34	5.23	100.0	1.30	5.13	6.89	100.0	1.48	4.45	6.19
As	100.0	0.29	0.38	0.34	100.0	0.21	0.45	0.34	100.0	0.22	0.37	0.33
Cd	100.0	0.05	0.06	0.06	100.0	0.05	0.18	0.15	100.0	0.10	0.13	0.11
Hg	75.0	0.01	0.01	0.01	85.0	0.00	0.01	0.01	90.0	0.01	0.01	0.01
Tl	97.5	0.01	0.01	0.01	92.5	0.01	0.02	0.02	100.0	0.01	0.02	0.02
Pb	100.0	0.87	1.27	0.99	100.0	0.72	1.34	1.08	100.0	0.85	1.32	1.26

The concentrations of toxic and potentially toxic elements in water and feed samples are presented in Tables 2-5 and 2-6, respectively. Cr was detected in all drinking water samples, and Hg was not detected. The positive rate of the other elements in cow and goat drinking water was 47.5%–87.5%. Aluminum, Pb, and Cd were present in 100.0% of buffalo water samples. For feed samples, Al, Cr, As, Cd, and Pb showed the highest positive rates. Thallium had the highest positive rate in buffalo feed.

The results in Table 2-4 showed that Cd, Cr, and Pb concentrations in our cow milk samples were lower than those reported in Pakistan and Turkey, whereas As concentrations in our cow milk samples were higher than that reported in Turkey (Kazi et al., 2009; Khan, N. et al., 2014; Temiz and Soylu, 2012). There were higher Cd concentrations in our cow milk samples than those reported in cow milk from South Africa (Ataro et al., 2008). Additionally, the concentrations of Al, Cd, Cr, and Pb in goat milk were lower than those reported by Coni et al. (1996). The concentrations of As in all drinking water samples were lower than those reported in Argentina (Mirna et al., 2010). Most of the toxic and potentially toxic elements detected in our samples were lower than those from other regions. Concentrations of Pb, Cr, Hg, and As in all milk samples were below MRL established by China and the European Union (EU) (EC, 2002). Therefore, the raw milk samples used in this study had no health risks.

3.4 Difference analysis by principal component analysis

Figure 2-1 showed PC1 × PC2 plots, where loadings and scores were simultaneously represented. PCA allowed the reduction of 17 variables to four PCs, which explained 68.12% of the total variance. The samples were collected from different animal species and regions. Element concentrations in milk and drinking water were analyzed by PCA to evaluate the effect of animal species and regions on raw milk element concentration.

The PCA results revealed that the concentrations of elements differed among animal species and regions (Figure 2-1B), in agreement with the findings of Rahimi (2013). Similar results were obtained with drinking water. Element concentrations in water samples differed among regions. Element concentrations in buffalo water samples were different to those in cow and goat water samples, with no significant differences between cow and goat water samples (Figure 2-1A). These results might be attributed to the location of sample collection. Cow and goat drinking water samples were collected from the same provinces, while buffalo water samples were collected from another distant region. The concentrations of elements in underground water from different regions are considerably different (Kifayatullah Khan, 2013). Differences in element concentrations in underground water might contribute to differences in element concentration in milk, water, and feed. PCA for feed samples revealed no significant differences among animal species or regions (data not shown).

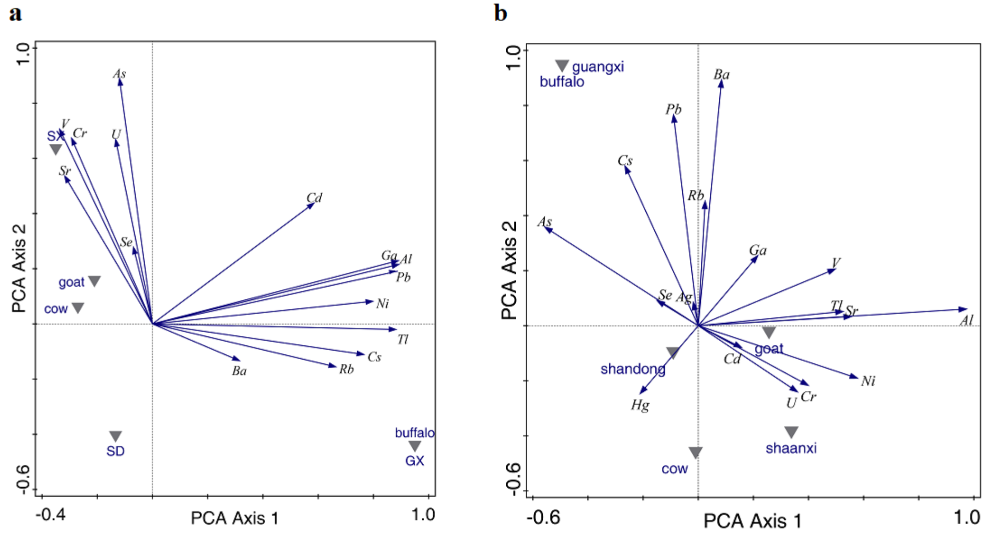

Figure 2-1: The result of difference analysis by PCA for milk and water. SD Shandong province, SX Shaanxi province, GX Guangxi province. X-axis is principal component 1 and Y-axis is principal component 2. a The result of PCA for water samples. b The result of PCA for milk samples. Variables used in the principal component analysis were V, Se, Ni, Ga, Rb, Sr, Ag, Cs, Ba, U, Al, Cr, As, Cd, Hg, Tl, and Pb in water and milk samples.

3.5 Correlation analysis of milk, water and feed

Trace elements Mn, Fe, Ni, Ga, Se, Sr, Cs and U in milk were significantly correlated with those in drinking water, while Co, Ni, Cu, Se and U were significantly correlated ($p < 0.05$) with those in feed (Table 2-7). Similarly, toxic and potentially toxic elements in milk were significantly correlated ($p < 0.05$) with those in water and feed. Cr, As, Cd, Tl, and Pb in milk had significant correlations with those in drinking water, while Al, Cr, As, Hg, and Tl in milk samples were significantly correlated with those in feed samples ($p < 0.05$, Table 2-7).

Therefore, elements in drinking water and feed might contribute to the elements in milk. This result was consistent with previously reported correlations between elemental mass fractions in milk and ingested feed and water (Herwig et al., 2011). Fe contamination in drinking water may directly affect cow milk Fe concentrations (Mann et al., 2013). Deka et al. (2015) reported that Cr concentrations in milk is increased by adding Cr to the feed; however, As in drinking water showed a low biological transference to cow milk (Mirna et al., 2010). Compared to water, fewer elements in feed were significantly correlated with those in milk (Table 2-7). The feed of dairy animals is more likely to be collected from different regions rather than locally produced. On the other hand, drinking water is usually local.

Table 2-7: Correlation analysis of elements in milk with that in drinking water and feed.

Element	Water		Feed	
	R	*p*	R	*p*
Mn	0.421	0.000	--	--
Fe	0.237	0.018	--	--
Co	--	--	0.238	0.018
Ni	-0.369	0.000	-0.321	0.001
Cu	--	--	-0.206	0.041
Ga	0.261	0.009	--	--
Se	-0.210	0.037	0.365	0.000
Sr	0.431	0.000	--	--
Cs	0.512	0.000	--	--
U	0.207	0.040	0.218	0.030
Al	--	--	-0.075	0.005
Cr	0.481	0.000	-0.228	0.023
As	-0.398	0.000	0.199	0.048
Cd	-0.252	0.012	--	--
Hg	--	--	0.297	0.003
Tl	-0.483	0.000	-0.237	0.018
Pb	0.434	0.000	--	--

--: $p > 0.05$, no relationship between milk and water or feed. R: correlation coefficient.
p: statistically significant at $p < 0.05$.

In our study, 22 elements in raw milk, drinking water and feed from different species were analyzed. Those results revealed the conditions of elements content from cow, goat and buffalo. correlations between milk and feed, water are investigated, furthermore, the relations among milk, drinking water and feed need further study.

4 Conclusion

There were differences in element concentrations based on animal species and regions. Drinking water samples from different regions had different element concentrations. On the other hand, there were no significant differences in element concentrations in feed samples among animal species or regions. Correlation analysis revealed that the concentrations of elements in water and feed might contribute to those in milk. From the correlation results, toxic and potentially toxic elements in raw milk were associated with those in feed and drinking water, which emphasizes the importance of element control in the feed and drinking water of dairy animals. However, further longitudinal studies are required to clarify the way that element in drinking water and feed secreted into milk, especially toxic elements.

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6 Reference

Al-Wabel, N. A., 2008. Mineral contents of milk of cattle, camels, goats and sheep in the central region of Saudi Arabia. Asian Journal of Biochemistry, 3(6), 373-375.

Ataro, A., McCrindle, R. I., Botha, B. M., McCrindle, C. M. E., & Ndibewu, P. P., 2008. Quantification of trace elements in raw cow's milk by inductively coupled plasma mass spectrometry (ICP-MS). Food Chemistry, 111(1), 243-248.

Bilandžić, N., Đokić, M., Sedak, M., Solomun, B., Varenina, I., Knežević, Z., & Benić, M., 2011. Trace element levels in raw milk from northern and southern regions of Croatia. Food Chemistry, 127(1), 63-66.

China, D. A. o., 2014. 2014 China Dairy Statistical Summary. Beijing, China: Dairy Association of China.

Coni, E., Bocca, A., Coppolelli, P., Caroli, S., Cavallucci, C., & Marinucci, M. T., 1996. Minor and trace element content in sheep and goat milk and dairy products. Food Chemistry, 57(2), 253-260.

D'Ilio, S., Petrucci, F., D'Amato, M., Di Gregorio, M., Senofonte, O., & Violante, N., 2008. Method validation for determination of arsenic, cadmium, chromium and lead in milk by means of dynamic reaction cell inductively coupled plasma mass spectrometry. Analytica Chimica Acta, 624(1), 59-67.

Deka, R. S., Mani, V., Kumar, M., Shiwajirao, Z. S., & Kaur, H., 2015. Chromium supplements in the feed for lactating murrah buffaloes (Bubalus bubalis): influence on nutrient utilization, lactation performance, and metabolic responses. Biological Trace Element Research, 168, 362-371.

EC. 2002. European commission decision (EC). 2002 /32/EC of the European parliament and of the council of 7 May 2002 on undesirable substances in animal feed: European Journal of Communication.

EU. 2002. 2002/657/EC. implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results.

Farida M. Al-awadi, T. S. S., 2001. Trace elements and their distribution in protein fractions of camel milk in comparison to other commonly consumed milks. Journal of Dairy Research, 68, 463-469.

Güler, Z., 2007. Levels of 24 minerals in local goat milk, its strained yoghurt and salted yoghurt. Small Ruminant Research, 71, 130-137.

Havemose, M. S., Weisbjerg, M. R., Bredie, W. L. P., Poulsen, H. D., & Nielsen, J. H., 2006. Oxidative stability of milk influenced by fatty acids, antioxidants, and Cu derived from feed. Journal of Dairy Science, 89, 1970-1980.

Herwig, N., Stephan, K., Panne, U., Pritzkow, W., & Vogl, J., 2011. Multi-element screening in milk and feed by SF-ICP-MS. Food Chemistry, 124(3), 1223-1230.

Jan, F. A., Ishaq, M., Khan, S., Shakirullah, M., Asim, S. M., & Ahmad, I., 2011. Bioaccumulation of metals in human blood in industrially contaminated area. Journal of Environmental Sciences, 23(12), 2069-2077.

Kazi, T. G., Jalbani, N., Kandhro, J. A. B. G. A., & Shah, H. I. A. M. B. A. M. K. J. A. Q., 2009. Assessment of toxic metals in raw and processed milk samples using electrothermal atomic absorption spectrophotometer. Food and Chemical Toxicology, 47, 2163-2169.

Khan, K., Khan, H., Lu, Y., Ihsanullah, I., Nawab, J., Khan, S., Shah, N. S., Shamshad, I., & Maryam, A., 2014. Evaluation of toxicological risk of foodstuffs contaminated with heavy metals in Swat, Pakistan. Ecotoxicology and Environmental Safety, 108, 224-232.

Khan, N., Jeong, I. S., Hwang, I. M., Kim, J. S., Choi, S. H., Nho, E. Y., Choi, J. Y., Park, K. S., & Kim, K. S., 2014. Analysis of minor and trace elements in milk and yogurts by inductively coupled plasma-mass spectrometry (ICP-MS). Food Chemistry, 147, 220-227.

Khan, Z. I., Ashraf, M., Hussain, A., McDowell, L. R., & Ashraf, M. Y., 2006. Concentrations of minerals in milk of sheep and goats grazing similar pastures in a semiarid region of Pakistan. Small Ruminant Research, 65(3), 274-278.

Kifayatullah Khan, Y. L., Hizbullah Khan, Shahida Zakir, Ihsanullah, Sardar Khan, Akbar Ali Khan, Luo Wei, Tieyu Wang. 2013. Health risks associated with heavy metals in the drinking water of Swat, north Pakistan. Journal of Environmental Sciences, 25(10), 2003-2013.

Król, J., Litwiñczuk, Z., Brodziak, A., & Kêdzierska-Matysek, M., 2012. Content of selected essential and potentially toxic trace elements in milk of cows maintained in Eastern Poland. Journal of Elemntology, 17(4).

Lin Bo, L. Y., Li Ling, Du Han chun, Tang Yan, Nong Hao ru, Zeng Qing kun. 2014. Analysis and comparation of minerals content in bufallo milk from diferent breeds (in Chinese). China Dairy Industry, 42(3), 15-17.

Mann, G. R., Duncan, S. E., Knowlton, K. F., Dietrich, A. D., & O'Keefe, S. F., 2013. Effects of mineral content of bovine drinking water: does iron content affect milk quality? Journal of Dairy Science, 96(12), 7478-7489.

Mirna, S., Horacio, B., & M., R. R., 2010. Evaluation of the influence of arsenical livestock drinking waters on total arsenic levels in cow's raw milk from Argentinean dairy farms. Food Chemistry, 121(2), 487-491.

Najarnezhad, V., & Akbarabadi, M., 2013. Heavy metals in raw cow and ewe milk from north-east Iran. Food Additives & Contaminants Part B-Surveillance, 6(3), 158-162.

Nestares, T., Díaz-Castro, J., Alférez, M. J. M., López-Aliaga, I., Barrionuevo, M., & Campos, M. S., 2008. Calcium-enriched goat milk, in comparison with similarly enriched cow milk, favours magnesium bioavailability in rats with nutritional ferropenic anaemia. Journal of the Science of Food and Agriculture, 88(2), 319-327.

Pilarczyk, R., Wójcik, J., Czerniak, P., Sablik, P., Pilarczyk, B., & Tomza-Marciniak, A., 2013. Concentrations of toxic heavy metals and trace elements in raw milk of Simmental and Holstein-Friesian cows from organic farm. Environmental Monitoring and Assessment, 185(10), 8383-8392.

Potortì, A. G., Bella, G. D., Turco, V. L., Rando, R., & Dugo, G., 2013. Non-toxic and potentially toxic elements in Italian donkey milk by ICP-MS and multivariate analysis. Journal of Food Composition and Analysis, 31(1), 161-172.

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

Rey-Crespo, F., Miranda, M., & Lόpez-Alonso, M., 2013. Essential trace and toxic element concentrations in organic and conventional milk in NW Spain. Food and Chemical Toxicology, 55, 513-518.

Shailaja, M., Reddy, Y. S., Kalakumar, B. D., Brinda, S. A., Manohar, G., & Kumar, B. D., 2014. Lead and trace element levels in milk and blood of buffaloes (Bubalus bubalis) from Hyderabad, India. Bulletin of Environmental Contamination and Toxicology, 92, 698-702.

Sola-Larrañaga, C., & Navarro-Blasco, I., 2009. Chemometric analysis of minerals and trace elements in raw cow milk from the community of Navarra, Spain. Food Chemistry, 112(1), 189-196.

Stawarz, R., Formicki, G., & Massányi, P., 2007. Daily fluctuations and distribution of xenobiotics, nutritional and biogenic elements in human milk in Southern Poland. Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering, 42(8), 1169-1175.

Tang, S., 2014. Dairy buffalo industry developmental current situation and considerations in Guangxi (in Chinese). Journal of Guangxi Agriculture, 29(1), 50-55.

Temiz, H., & Soylu, A., 2012. Heavy metal concentrations in raw milk collected from different regions of Samsun, Turkey. International Journal of Dairy Technology, 65(4), 516-522.

Thomas Müller, H. F., Heribert Berger, Wilfried Müller. 1996. Endemic tyrolean infantile cirrhosis an ecogenetic disorder. The Lancet, 347, 877-880.

Z. Dobrzański, R. K., H. Górecka, K. Chojnacka, A. Bartkowiak. 2005. The content of microelements and trace elements in raw milk from cows in the Silesian region. Polish Journal of Environmental Studies, 14(5), 685-689.

Zhao, S., Li, Z., Dong, Y., Gu, X., Deng, L., Guo, C., Wang, Y., & Zhang, S., 2014. Strategy research on goats industry development and goat milk quality and safety condition in China (in Chinese). Quality and Safety of Agro-Products, 6, 12-15.

3

Large scale study of the within and between spatial variability of lead, arsenic, and cadmium contamination of cow milk in China

From Xuewei Zhou, Xueyin Qu, Nan Zheng*, Chuanyou Su, Jiaqi Wang and Hélène Soyeurt, 2019. Large scale study of the within and between spatial variability of lead, arsenic, and cadmium contamination of cow milk in China. Science of the Total Environment, 650: 3054–3061.

As shown by previous studies, the results of chapter 2 confirms the regional variability of heavy metals contamination in raw cow milk. However, this last study focused on 2 Chinese regions. So, a large-scale study was needed to assess the global problematic of heavy metal contamination in cow milk produced in China. So, in this chapter, Pb, As and Cd contents were measured in raw cow milk collected from the ten main milk producing areas in China to assess their spatial variabilities. Concentrations of Hg and Cr in raw milk were not shown in this chapter as nearly all samples had content of those elements under the limit of detection.

Kriging method based on samples collected from scattered areas can characterize the area of contamination and smooth the pollutant concentration data over the entire studied area (Desiato et al., 2014). Ordinary kriging algorithm provides the best linear unbiased estimate for spatial variables by using the nearby points to generate a continuous surface (Ran et al., 2016).

Abstract

This large-scale study investigated the spatial variability of Pb, As, and Cd contents in raw milk within and between the 10 main milk producing areas in China. A total of 997 raw milk samples were analyzed by inductively coupled plasma mass spectrometry (ICP-MS). Mean values of Pb, As, and Cd in milk were 1.75 μg/L, 0.31 μg/L, and 0.05 μg/L, respectively. The highest level of Pb and As was present in area C, and Cd was highest in area J. The standard deviation suggested a higher heterogeneity of milk heavy metal contamination within area than between areas. Levels of Pb, As, and Cd showed significant differences between studied areas. The estimated root mean squared standardized error obtained by the cross-validation suggested a differentiated quality of Pb, As, and Cd modelling between areas: the predictions obtained were sometimes overestimated or underestimated. These results can be used to define a more appropriate sampling procedure for heavy metal contaminate distribution in raw milk for improved future control of milk contamination by heavy metals in the studied areas. The significant positive correlations between concentrations of Pb-Cd, As-Cd, and Pb-As were observed in nine, six and five areas, respectively. No significant negative correlations were observed. The observed variability of correlation values suggested a different pollution source for Pb, As, and Cd in milk between areas. Further studies are required to clarify the relationships between the contamination of raw milk by heavy metals and the herd environment.

Keywords: heavy metals; milk; spatial distribution; variability

1. Introduction

Milk and dairy products contain protein, fat, and other elements essential for human health, especially for body metabolism, growth, and development. Minerals are one of these essential elements. For instance, calcium, copper, and selenium are essential minerals for normal human body functions, as they are involved in many physiological processes (Alasalvara et al., 2002; Licata et al., 2012). However, heavy metals such as lead (Pb), arsenic (As), and cadmium (Cd) can also be found in milk (Swarup et al., 2005; Shailaja et al., 2014). If they are consumed in excessive concentrations, these heavy metals can lead to serious systemic health problems (Oliver, 1997). This can occur when the foodstuff is contaminated by heavy metals present in the production environment. For instance, Swarup et al. (2005) showed that blood Pb levels in lactating cows reared around lead-zinc smelting factories were significantly higher than those in non-polluted areas. Shailaja et al. (2014) suggested that contaminated fodder given to buffalos might be one factor responsible for elevated Pb levels in blood and milk. The same observations can be made for other heavy metals such as As. For instance, contaminated drinking water and food are the most important routes of As exposure (Ohno et al., 2007; WHO, 2010). Therefore, there is a link between the environment/localization of the dairy farms and the number of contaminated ruminants.

Few investigations have been conducted to study this relationship in raw cow milk. Qu et al. (2013) measured Pb, As, and Cd in 192 raw milk samples from 7 districts of Tangshan city, to find an average of 35.13 µg/kg, 95.85 µg/kg, and 41.35 µg/kg, respectively. The levels of these metals were relatively higher in the industrial district. These findings confirmed the results obtained previously by Malhat et al. (2012) from 100 milk samples, and by Temiz et al. (2012) from 144 raw milk samples. Rahimi (2013) extended this study to 137 goat, cow, sheep, and buffalo milk samples. Those authors confirmed that an environmental relationship was responsible for milk contamination. More recently, from a limited number of samples ($n = 40$), Zhou et al. (2016) showed differences in heavy metal concentrations in raw milk collected in two different Chinese provinces (Shandong and Shaanxi province). In conclusion, all these studies suggest the existence of spatial variability in milk heavy metal contamination related to the production environment.

Unfortunately, there is a lack of large-scale studies to allow detailed investigation of the spatial distribution of these heavy metal contaminants in raw cow milk. Therefore, the present research aims to study the variability of Pb, As, and Cd within and between the main milk producing areas in China, thanks to a large-scale design. This study will contribute to improve knowledge of the existing variability of this type of milk contamination in China. This can be used to refine the definition of an appropriate sampling procedure for regular control of the evolution of heavy metal contamination in milk.

2. Materials and methods

2.1 Sample collection

The study was conducted in the ten main Chinese milk producing areas marked in Figure 3-1 with A (part of Beijing: 39°54′ - 40°58′ N, 115°39′ - 117°52′ E), B (part of Inner Mongolia: 40°53′ - 41°11′ N, 109°71′ - 112°30′ E), C (part of Shandong: 36°30′ - 36°95′ N, 118°38′ - 119°32′ E), D (part of Shanghai: 30°72′ - 31°02′ N, 121°00′ - 121°55′ E), E (part of Xinjiang: 43°54′ - 45°45′ N, 84°11′ - 87°67′ E), F (part of Sichuan: 30°31′ - 31°18′ N, 103°41′ - 104°81′ E), G (part of Hebei: 39°01′ - 39°92′ N, 118°81′ - 118°97′ E), H (part of Tianjin: 39°01′ - 39°86′ N, 116°96′ - 118°97′ E), I (part of Heilongjiang: 45°00′ - 47°23′ N, 124°13′ - 126°68′ E), and J (part of Anhui: 31°64′ - 34°29′ N, 115°09′ - 118°75′ E). The farms were selected randomly for sampling. A total of 997 milk samples were collected on 568 farms. This represented 100 milk samples per area studied, except for area H (n = 97). The sampling was realized between May and August 2016. All samples were collected in 200 mL polypropylene bottles and stored at -20 ℃ in the laboratory.

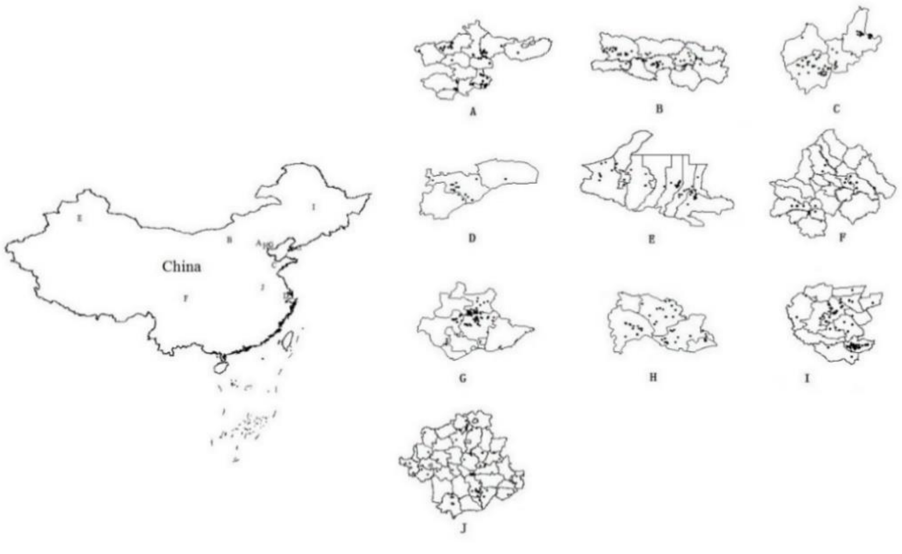

Figure 3-1: Map of the sampling area. Dots on the map are the locations of sampling.

2.2 Heavy metal measurement

All reagents were analytically pure. Water used in the analytical process was obtained from a Milli-Q Plus water purification system (18.2 MΩ cm) (Millipore, USA). Nitric acid (65%) and hydrogen peroxide (30%) used in sample digestion were obtained from Merck (Germany). All the vessels were immersed in 20% HNO_3 (v/v) for at least 12 h and rinsed with ultrapure water before analysis.

Raw milk samples were digested with microwaves after adding 4 mL HNO_3 and 3 mL H_2O_2 in polytetrafluoroethylene (PTFE) tubes. A MARS 6 microwave sample digestion system (MARS6, CEM Corporation, USA) with a power of 1600 W was used for the digestion of milk samples. This digestion was performed according to the method by Zhou et al. (2016): (1) ramp time 5 min, temperature 90 ℃, hold time 5 min; (2) ramp time 5 min, temperature 150 ℃, hold time 10 min; and (3) ramp time 5 min, temperature 180 ℃, hold time 20 min. Digested samples were allowed to cool to room temperature, transferred to polypropylene tubes (Corning, USA), and diluted to 50 mL with ultrapure water. Blanks, devoid of samples, were subject to the same digestion procedures during sample preparation. The contents of Pb, As, and Cd were measured by ICP-MS.

Standard calibrations were developed to quantify the contents of Pb, As, and Cd in raw milk samples. For this, standard solutions were prepared from multi-element stock standard solution (10 mg/L, SPEX, USA). The linear regression coefficients (R) of the calibration curves were considered acceptable when $R > 0.9995$. During the whole measurement, the calibration curves for Pb, As, and Cd were prepared with five points (1, 2, 3, 4, 5 μg/L, blank not included). Limit of detection (LOD) was calculated from 3 times the standard deviation of the sample blank, relative to the slope of the analytical curve. The digested milks were used in the calculation of LOD. The LOD for Pb, As, and Cd were 0.28 μg/L, 0.09 μg/L, and 2.89×10^{-3} μg/L, respectively.

Samples spiked with standard solution were used to verify the accuracy of the method. The recovery rates of Pb, As, and Cd in milk were 97.1%, 94.4%, and 95.5%. Concentrations below LOD were replaced by half the value of the respective detection limits, as proposed by Potortì et al. (2013) (i.e., 0.14, 0.045, and 1.445×10^{-3} μg/L for Pb, As, and Cd).

2.3 Statistical analysis

All statistical analyses were performed using SAS 9.4 software (Cary, NC, USA).

The variability of milk heavy metal contamination within milk producing areas was first assessed by calculating the standard deviation of measured Pb, As, and Cd levels, as well as by estimating the positive rate defined by the number of contaminated samples per area. Second, the spatial distribution patterns of Pb, As, and Cd within studied areas were modelled by ArcGIS 10.2 mapping software (ESRI, New York, USA) using the ordinary kriging algorithm. A leave-one-out cross-validation was performed to assess the prediction performances of the obtained distribution map. To evaluate the unbiased characteristic of the predictions and their uncertainty, some statistical parameters were calculated. The uncertainty was assessed by estimating the root mean squared error (RMSE). As the Pb, As, and Cd levels presented at different scales and in order to compare the different developed kriging models, the root mean squared error was standardized. This parameter was calculated using the prediction errors divided by its corresponding prediction standard error (RMSSE). A RMSSE value higher than 1 means an underestimation of the variability of the studied traits in the predictions. Conversely, RMSSE lower than 1 means an overestimation. Therefore, the difference in RMSSE-1 was calculated to compare the uncertainty between the developed kriging models. The predictions were estimated to be unbiased when their mean of standardised prediction error (MSE) was close to zero.

To assess the variability of milk heavy metal contamination between studied areas, non-parametric Kruskal-Wallis tests were performed, because the data were not normally distributed based on the results of the Shapiro-Wilk test. Area was considered as a fixed effect in the model. Pairwise two-sided multiple comparisons were performed using the Dwass, Steek, Critchlow-Fligner method. Finally, Spearman correlations were calculated to assess the relationships between observed Pb, As, and Cd contents within each studied area.

3. Results

3.1 Concentrations of Pb, As, and Cd in raw milk samples

The average level of Pb, As, and Cd in studied milk samples were equal to 1.75, 0.31, and 0.05 µg/L, respectively. Average levels of Pb in milk produced in the ten studied areas showed the descending average content as follows: E > D > A > G > J > C > B > F > H > I (Table 3-1). Average levels of As and Cd ranged from 0.13-0.80 µg/L and 0.02-0.09 µg/L, respectively, and the highest average contents were found in areas J and A. The maximum levels of Pb and As were detected in the same sample from area C, while Cd was highest in area J (Table 3-1).

Levels of Pb in 1.20% of collected samples (i.e., 12 samples out of 997) were over the maximum residue limit (MRL) imposed by the European Union (0.02 mg/kg) (EC, 2015), for 4 of the 10 studied areas. However, the observed concentrations of Pb in all samples were below the Chinese MRL (i.e., 0.05 mg/kg for Pb) (CFDA&NHC, 2017). The MRL for As imposed by the Chinese government is 100 µg/kg (China, 2017). The measurements for As were never higher than this threshold for all studied samples. To our knowledge, no MRL for Cd in raw milk exists. However, Cd globally had the highest positive rate (77.4%) showing a strong presence of Cd in the studied raw milk. Lead and As had a global lower positive rate: 68.4% and 46.5%, respectively (Table 3-1).

3.2 Between area variability

The positive rate differed between studied regions. The highest positive rate for Pb was observed for area G (96%), followed by areas J (91%) and A (90%). The lowest was measured in area I (40%). Area J had the highest positive rate for As

(66%) and area E the lowest (28%). For Cd, the highest positive rate was observed for area A (98%) and the lowest for area C (55%). The ranges observed for the positive rate were different between heavy metals: 68.4% for Pb, 46.5% for As, and 77.4% for Cd.

Differences in the contents of Pb, As, and Cd were also observed between areas (Table 3-1). Kruskal-Wallis tests were performed to evaluate the significance of these differences between regions. The obtained *P*-values confirmed the existence of significant differences between areas. Lead content in area G was significantly ($P < 0.05$) different between all areas except A and J. Furthermore, areas C and I showed no difference ($P > 0.05$) with area H, nor between areas C and I. There were no significant differences in Pb content in areas A, J, and E ($P > 0.05$). The mean value of As in area J was significantly higher than areas C, D, E, F, and I ($P < 0.05$). The contents of Cd in area A showed a significant difference with all other areas, followed by areas J and G ($P < 0.01$).

3.3 Within area variability

For all studied heavy metals, the standard deviations observed within area were higher than those observed between areas (Table 3-1). To study the spatial distribution of this contamination in more detail, the levels of Pb, As, and Cd measured in milk samples were modelled using the ordinary kriging method (Figures 3-2 to 3-4).

Arsenic and Cd in raw milk from area A shared a similar pattern of higher levels in western areas, and Pb showed higher levels in the northwest. Lead and As in raw milk from area C present the same spatial distribution trait of decreasing from northeast to southwest, while Cd showed a different distribution from Pb and As. Arsenic and Cd in area F presented same pattern with higher concentrations in the north, whereas Pb in raw milk had a different distribution pattern with higher concentrations in the east region. In addition, Pb, As, and Cd in area G showed a similar southwest to northeast distribution trend with higher concentrations in the southwest, whereas Pb also showed higher levels in the north region. The distribution properties of Pb, As, and Cd in areas B and D presented a similar trend with higher contents in the mid and west. However, the distribution pattern of Pb, As, and Cd in area E, H, and J showed a different trend. The higher levels of these metals were concentrated in limited ranges.

Table 3-1: Descriptive statistics of Pb, As, and Cd contents measured in raw cow milk from the 10 main milk producing areas in China (N = 997 samples).

	Pb (μg/L)			As (μg/L)			Cd (μg/L)		
	Mean[a] ± SD	Range	Positive rate[b]	Mean ± SD	Range	Positive rate	Mean ± SD	Range	Positive rate
A	2.11 ± 2.37	0.14-19.62	90.00%	0.27 ± 0.36	0.05-2.17	58.00%	0.09 ± 0.07	0.001-0.39	98.00%
B	1.43 ± 2.26	0.14-16.15	66.00%	0.51 ± 0.90	0.05-6.21	46.00%	0.02 ± 0.03	0.001-0.20	59.00%
C	1.60 ± 5.39	0.14-38.61	42.00%	0.33 ± 1.59	0.05-15.77	49.00%	0.07 ± 0.10	0.001-0.36	55.00%
D	2.73 ± 5.92	0.14-36.73	60.00%	0.14 ± 0.18	0.05-0.94	35.00%	0.05 ± 0.09	0.001-0.54	72.00%
E	2.96 ± 6.66	0.14-35.74	82.80%	0.34 ± 1.05	0.05-9.12	28.00%	0.04 ± 0.05	0.001-0.21	59.00%
F	1.35 ± 1.77	0.14-9.70	76.00%	0.13 ± 0.12	0.05-0.68	44.00%	0.02 ± 0.02	0.001-0.09	82.00%
G	2.01 ± 1.16	0.14-6.45	96.00%	0.17 ± 0.24	0.05-1.90	51.00%	0.05 ± 0.04	0.001-0.18	92.00%
H	1.16 ± 3.16	0.14-28.94	41.20%	0.19 ± 0.27	0.05-1.93	45.40%	0.03 ± 0.03	0.001-0.17	74.20%
I	0.46 ± 0.62	0.14-4.19	40.00%	0.18 ± 0.33	0.05-2.96	43.00%	0.03 ± 0.03	0.001-0.12	89.00%
J	1.70 ± 1.39	0.14-8.73	91.00%	0.80 ± 2.27	0.05-15.18	66.00%	0.06 ± 0.09	0.001-0.69	94.00%
Total	1.75 ± 3.73	014-38.61	68.40%	0.31 ± 1.02	0.05-15.77	46.50%	0.05 ± 0.07	0.001-0.69	77.40%

[a] Mean value is calculated by all samples, concentrations below the limit of detection were replaced by half the value of the LOD.

[b] PR: Positive rate is the sample number that values upper than LOD divided total samples’ number.

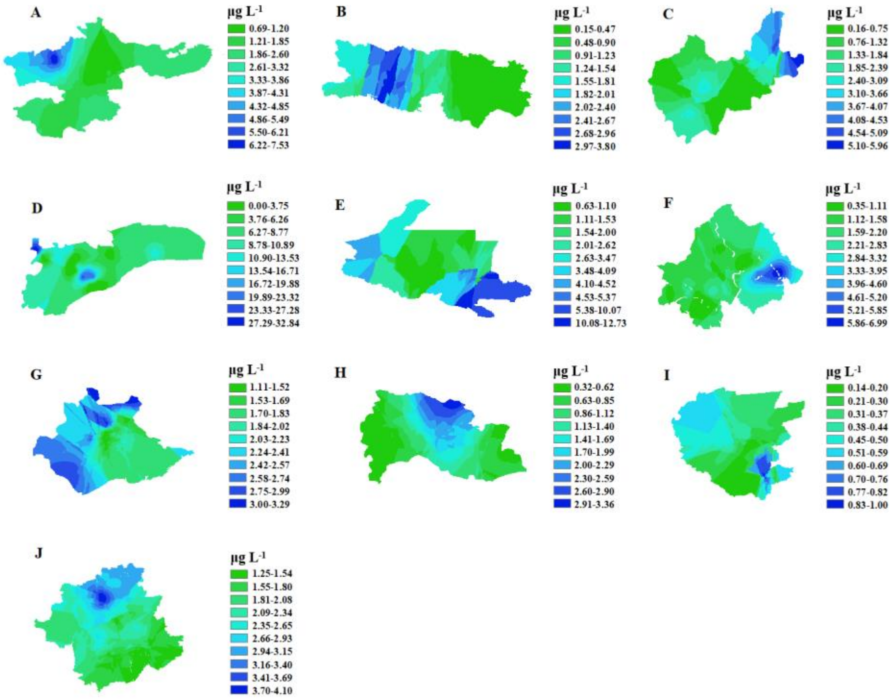

Figure 3-2: Spatial distribution of the lead concentrations in raw milk (µg/L) in the main milk producing areas in China.

To assess the prediction performance of the distribution maps, a leave-one-out cross-validation was performed. The statistical parameters calculated from this procedure are given in Table 3-2. For MSE, values were near zero, this means that the predictions were globally unbiased. The mean value for MSE was -0.004 for Pb, 0.001 for As, and 0.004 for Cd. Throughout the studied traits, the MSE in absolute values varied between 0.000 and 0.043. To assess the uncertainty of the predictions, RMSE and RMSSE values were estimated. If RMSSE is higher than 1, the variability of the studied trait is underestimated by the predictions. Conversely, if RMSSE is lower than 1, the variability is overestimated. The calculation of RMSSE-1 values gives direct information about the estimation of the variability by the predictions. For all studied heavy metals, the values of RMSSE-1 changed between regions. Globally, throughout the studied metals, half of the RMSSE-1 estimations were negative. A total of 15 absolute values of RMSSE-1 were lower than 0.1 suggesting a good representation of the uncertainty. This represented 50% for Pb, 40% for As, and 60% for Cd. An extremely high RMSSE-1 value was observed for the As model in area E. A high value was also observed for the As model in area G.

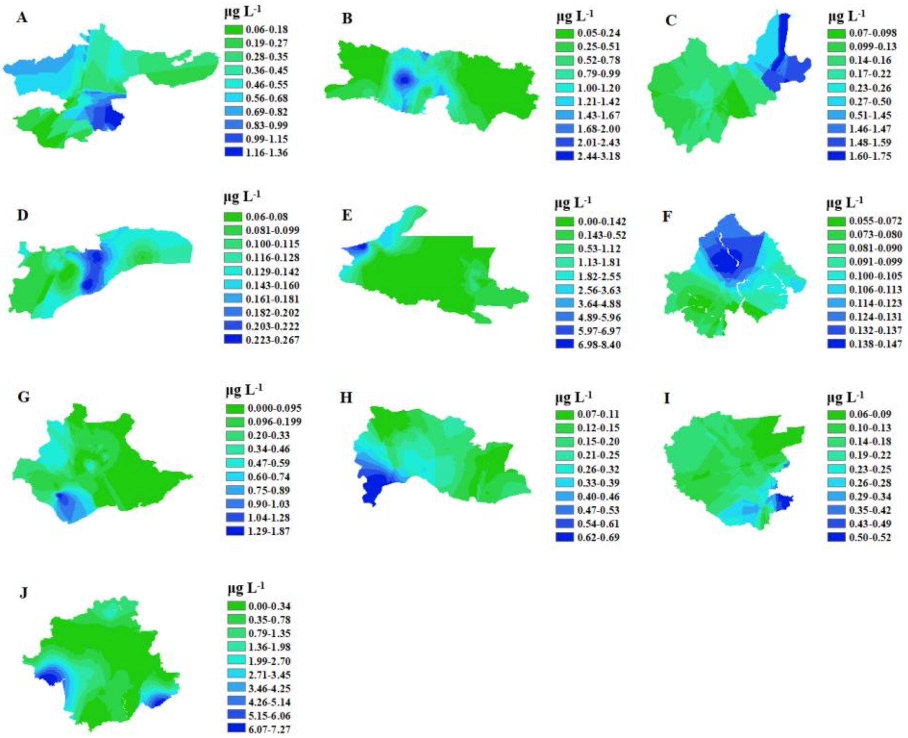

Figure 3-3: Spatial distribution of the arsenic concentrations in raw milk (µg/L) in the main milk producing areas in China.

3.4 Correlations between Pb, As, and Cd

Spearman correlations calculated from measured Pb, As, and Cd contents within specific areas are presented in Table 3-3. Lead and Cd showed significant positive correlations for all studied areas, except area I where the correlation value was positive but not significant. Lead-As showed positive correlations for areas A, B, C, H, and J. The remaining correlations were also positive for areas D, E, and G, and slightly negative for areas F and I, but all of those values were not significantly different to zero. Positive correlations were also found for As-Cd in areas E, F, G, H, I, and J. Positive correlations were observed between As and Cd in areas A and C but were not significant. No significant negative correlations were observed for areas B and D.

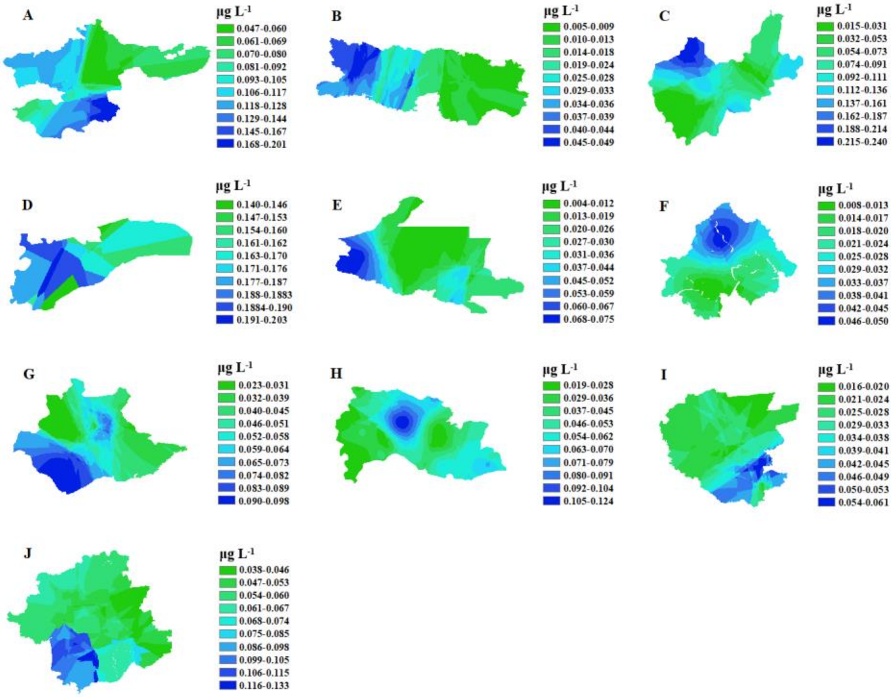

Figure 3-4: Spatial distribution of the cadmium concentrations in raw milk (µg/L) in the main milk producing areas in China.

Table 3-2: Root mean squared error (RMSE), mean of standardised error (MSE), and root mean squared standardised error (RMSSE) obtained after a k-fold cross-validation for each studied area.

	Pb (µg/L)			As (µg/L)			Cd (µg/L)		
area	RMSE	MSE	RMSSE-1	RMSE	MSE	RMSSE-1	RMSE	MSE	RMSSE-1
A	2.255	0.004	0.191	0.334	0.009	-0.053	0.064	0.024	0.190
B	3.463	-0.012	-0.225	0.893	-0.002	-0.006	0.050	-0.020	0.157
C	5.139	-0.022	-0.120	2.185	0.000	-0.142	0.098	0.004	-0.102
D	6.053	-0.010	-0.224	0.186	0.000	-0.081	0.090	-0.043	-0.093
E	7.082	0.013	0.047	1.257	-0.021	1.011	0.041	0.034	-0.331
F	1.797	-0.003	0.024	0.085	0.000	-0.405	0.017	0.024	-0.088
G	1.148	-0.009	0.037	0.325	0.033	0.620	0.037	0.024	-0.064
H	3.151	0.011	-0.081	0.273	-0.007	-0.005	0.036	0.020	0.062
I	0.777	0.003	-0.232	0.347	-0.005	0.167	0.027	0.012	0.043
J	1.491	-0.014	0.002	2.311	0.004	0.284	0.106	0.006	-0.043

Table 3-3: Spearman correlations between Pb-As, Pb-Cd, and As-Cd milk concentrations (µg/L) in the ten main milk producing areas in China (A-J).

Area	Pb-As	Pb-Cd	As-Cd
A	0.20^{*}	0.36^{**}	0.19
B	0.31^{**}	0.33^{**}	- 0.16
C	0.28^{**}	0.32^{**}	0.05
D	0.03	0.38^{**}	- 0.15
E	0.14	0.46^{**}	0.22^{*}
F	- 0.09	0.30^{**}	0.41^{**}
G	0.18	0.31^{**}	0.51^{**}
H	0.48^{**}	0.48^{**}	0.21^{*}
I	- 0.07	0.11	0.32^{**}
J	0.52^{**}	0.60^{**}	0.43^{**}

* $P < 0.05$, ** $P < 0.01$.

Based on the significance levels, we can observe that areas A, B, and C seemed to have similar relationships between Pb-As and Pb-Cd. Areas E, F, and G also showed similar correlations for As-Cd and Pb-Cd. Areas H and J seemed to also have similar relationship patterns between Pb-As, Pb-Cd, and As-Cd. Areas D and I were different to the other studied areas.

4. Discussion

Although milk and dairy products contain many active biomolecules for human health, these food products can also contain unfavourable components such as Pb, As, and Cd. An excessive consumption of these heavy metals can lead to serious systemic health problems (Oliver, 1997). The presence of Pb, As, and Cd are related to the production environment of the milk. Currently few studies with a limited dataset have been conducted to study the variability of milk heavy metal contamination (Swarup et al., 2005; Shailaja et al., 2014; Zhou et al., 2016). The objective of the current large-scale investigation was to estimate the variability of this contamination within and between the main milk producing regions in China. This study is needed to develop appropriate sampling procedures in order to control the evolution of this contamination of milk, which is potentially dangerous for human health as well as for dairy cows.

The presence of Pb and Cd was observed in the majority of analysed milk samples: 68.4% for Pb, and 77.4% for Cd. This positive rate was slightly lower for As with a percentage equal to 46.5%. This means that these heavy metals are largely present in milk. Some sources of contamination therefore exist in the studied milk production areas. However, the contents of Pb and As metals in the studied samples were not higher than the MRL set by the Chinese government (CFDA&NHC, 2017). However 1.20% of collected samples (i.e., 12 samples out of 997) were higher than the MRL imposed by the European Union (0.02 mg/kg) (EC, 2015), for 4 of the 10 studied areas. This second observation allows a nuanced conclusion of the observed positive rate to be made. Therefore, Pb and As were present in many measured samples but the contents are less likely to lead to serious health problems as they have globally low contents of these heavy metals. The mean value observed for As was lower than that reported by Pérez-Carrera et al. (2016) in Argentina (4.0 µg/kg, 3.5 µg/kg) and by Sarkar et al. (2016) in India where the cows were highly contaminated with As (69 µg/L). The average levels of Pb in the present study were also lower than those recorded by Bilandzic et al. (2016) in rural areas in Croatia (11.4 µg/kg, range 5.11-131 µg/kg) and by Kazi et al. (2009) in Pakistan (47.6 µg/L, range 41.8-58.7 µg/L). The observed mean Pb value was similar to that obtained by Kim et al. (2016) in Korea (1.48 µg/kg) and by Tang et al. (2014) in China. Unfortunately no MRL is available for Cd but the measured content (0.06 µg/L) was globally quite low when compared with the literature (Ismail et al. (2015): 1.0 µg/kg; Kazi et al. (2009): 44.2 µg/L, 42.9-60.2 µg/L; Tang et al. (2014): 2 µg/kg). One sample from area J had an extremely high value for Cd (8.74 µg/L). This is much higher than other values and a future investigation must be conducted to confirm this extreme value.

The positive rate as well as the measured contents for the studied heavy metals differed between regions. This could explain the differences observed between these results and the studies by Tang et al. (2014) and Zhou et al. (2016) who also conducted studies in China. As mentioned previously, the results obtained by Tang et al. (2014) from Chinese regions showed similar values for Pb and higher values for Cd. In this latter study, the samples were collected in Zhejiang province which is close to areas D and J of the present study. Area J showed an extremely high variability for Cd content which can relate to the higher value observed by Tang et al. (2014) from a lower number of samples. Zhou et al. (2016) observed a higher positive rate for As (82.5%) and a lower rate for Cd (32.5%). In Zhou et al. (2016), only 40 raw cow milk samples were collected in two regions (20 samples per region instead of 100 in the present study). Significant differences in the levels of Pb, As, and Cd between the main Chinese milk producing regions were confirmed in this study. However, based on the standard deviation observed in this study, we can suggest a higher heterogeneity of milk heavy metal contamination within areas than between areas. This was expected as the contamination of milk by heavy metals is mainly due to contamination of the environment and also the contamination of fodder given to the dairy cows (Mohajeri et al., 2014; Shailaja et al., 2014). For instance, the proximity of lead-zinc factories favoured contamination (Mohajeri et al., 2014; Patra et al., 2008; Swarup et al., 2005). These last authors found that the mean blood Pb level in cows around the smelter was higher (1.09 ± 0.26 mg/L) than the cows from the reference area (0.72 ± 0.25 mg/L). Higher As (65% detectable residue) concentrations in milk found in the winter sampling are possibly related to a higher consumption of concentrate in the feed, and soil ingestion when grazing (Rey-Crespo et al., 2013). The contents of As in industrial regions close to the Black Sea had higher levels, whereas regions also near the Black Sea but far from the industrial region showed higher Pb levels (Temiz and Soylu, 2012). The steel industry and traffic activities also lead to Cd and Pb pollution (Xiao et al., 2015). A previous study indicated that the lower reach of the Yellow River near Inner Mongolia contains higher metal concentrations in comparison with the middle and upper reaches of the river near Ningxia and Gansu, China (Ma et al., 2016). The spatial distribution of Cd in wheat grains showed similar geographical patterns with soils, whereas Pb showed an opposite pattern (Ran et al., 2016). All these results indicate that conditions in the local environment, such as those of the soil, water, river, industry, mining, and smelting, may contribute to Pb, As, and Cd contamination in animals and its transference to milk.

As it is not possible to make a sampling collection among all potential pollutant industries, there is an interest in realising a primary screening of the variability of milk heavy metal contamination. This was the objective of this study and this explains why we have decided to randomly select the studied farms. Based on the nearly 1000 measured samples and by using an ordinary kriging method, it was possible to model the distribution of Pb, As, and Cd contamination in milk in all studied areas (Figures 3-2 to 3-4). The cross-validation confirmed that the predictions were mainly unbiased. However, an uncertainty was observed for some models. A little over half of the estimated RMSSE-1 values were lower than 0.01 suggesting good modelling of data. The predictions obtained in some regions overestimated or underestimated the variability of the considered contamination. This is related to an inappropriate sampling procedure, but this cannot be known before making the different measurements realised in this study. However, the distribution map presented in this study, although imperfect, could be used to define a more appropriate sampling procedure if we want to control the evolution of milk heavy metal contamination in China in the future.

Based on Table 3-2, we can see that there are no values of RMSSE-1 lower than 0.001 for the 3 metals modelled in the same region. This could indicate different behaviours of Pb, As, and Cd milk contamination. This was confirmed by the estimation of Spearman correlations between all studied heavy metals per area. The values were globally moderate positive, some were negative but these values were not significant. Therefore, a moderate relationship exists between the studied metals. However, the values of this correlation changed between the studied regions. A hypothesis should be that the sources of contamination are different between regions. Pilarczyk et al. (2013) also found significant positive correlations between the concentrations of Pb and Cd in the milk of Simmental and Holstein-Friesian cows (r = 0.86, r = 0.87). These correlations were also visible on the obtained distribution maps (Figures 3-2 to 3-4).

5. Conclusion

Lead, As, and Cd were measured in many analysed samples. However, their average contents were lower than those reported in the literature. Moreover, no samples had a content of Pb and As higher than the MRL imposed by the Chinese Government. The heterogeneity of milk heavy metal contamination was higher within areas than between areas. Significant differences were observed between regions. Spatial distribution concentrations in raw milk samples were modelled using an ordinary kriging method to illustrate the distribution of milk Pb, As, and Cd contamination. Unfortunately, for a little less than 50% of the developed models, the cross-validation results showed a moderate uncertainty leading to an overestimation or an underestimation of the variability of those contaminates by the predictions. This was explained by the sampling procedure used. As no information was available about the potential pollution with Pb, As, and Cd, a random selection was done by farm. This was therefore not appropriate enough for some models. In the future, the distribution maps created in this study could be used to better define the sampling procedure needed. This could allow a programme to be implemented to follow the evolution of this contamination in milk in the main milk producing areas in China.

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7. References

Alasalvara, C., Taylora, K. D. A., Zubcovb, E., Shahidic, F., & Alexisd, M., 2002. Differentiation of cultured and wild sea bass (Dicentrarchus labrax): total lipid content, fatty acid and trace mineral composition. Food Chemistry, 79 145-150.

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. Bulletin Environmental Contamination and Toxicology, 96(5), 645-649.

CFDA&NHC. 2017. Maximum levels of contaminants in foods. (Vol. GB 2762-2012). China: China Food and Drug Adiministration; National health commission of the People's Republic of China.

Desiato, R., Bertolini, S., Baioni, E., Crescio, M. I., Scortichini, G., Ubaldi, A., Sparagna, B., Cuttica, G., & Ru, G., 2014. Data on milk dioxin contamination linked with the location of fodder croplands allow to hypothesize the origin of the pollution source in an Italian valley. Science of the Total Environment, 499, 248-256.

EC. 2015. Commossion Regulation (EU) 2015/1005 amending Regulation (EC) No 1881/2006 as regards maximum levels of lead in certain foodstuffs. Official Journal of the European Union, L 161, 11.

Ismail, A., Riaz, M., Akhtar, S., Ismail, T., Ahmad, Z., & Hashmi, M. S., 2015. Estimated daily intake and health risk of heavy metals by consumption of milk. Food Additives and Contaminants: Part B Surveillance, 8(4), 260-265.

Kazi, T. G., Jalbani, N., Baig, J. A., Kandhro, G. A., Afridi, H. I., Arain, M. B., Jamali, M. K., & Shah, A. Q., 2009. Assessment of toxic metals in raw and processed milk samples using electrothermal atomic absorption spectrophotometer. Food and Chemical Toxicology, 47(9), 2163-2169.

Kim, D.-G., Kim, M., Shin, J. Y., & Son, S.-W., 2016. Cadmium and lead in animal tissue (muscle, liver and kidney), cow milk and dairy products in Korea. Food Additives & Contaminants: Part B, 9(1), 33-37.

Licata, P., Di Bella, G., Potorti, A. G., Lo Turco, V., Salvo, A., & Dugo, G. M., 2012. Determination of trace elements in goat and ovine milk from Calabria (Italy) by ICP-AES. Food Additives & Contaminants: Part B Surveillance, 5(4), 268-271.

Ma, X., Zuo, H., Tian, M., Zhang, L., Meng, J., Zhou, X., Min, N., Chang, X., & Liu, Y., 2016. Assessment of heavy metals contamination in sediments from three adjacent regions of the Yellow River using metal chemical fractions and multivariate analysis techniques. Chemosphere, 144, 264-272.

Malhat, F., Hagag, M., Saber, A., & Fayz, A. E., 2012. Contamination of cows milk by heavy metal in Egypt. Bulletin of Environmental Contamination and Toxicology, 88(4), 611-613.

Mohajeri, G., Norouzian, M. A., Mohseni, M., & Afzalzadeh, A., 2014. Changes in blood metals, hematology and hepatic enzyme activities in lactating cows reared in the vicinity of a lead-zinc smelter. Bulletin of Environmental Contamination and Toxicology, 92(6), 693-697.

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. Science of the Total Environment, 381(1-3), 68-76.

Oliver, M. A., 1997. Soil and human health: a review. European Journal of Soil Science, 48(4), 573-592.

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

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. Science of the Total Environment, 404(1), 36-43.

Pilarczyk, R., Wojcik, J., Czerniak, P., Sablik, P., Pilarczyk, B., & Tomza-Marciniak, A., 2013. Concentrations of toxic heavy metals and trace elements in raw milk of Simmental and Holstein-Friesian cows from organic farm. Environmental Monitoring and Assessment, 185(10), 8383-8392.

Potortì, A. G., Bella, G. D., Turco, V. L., Rando, R., & Dugo, G., 2013. Non-toxic and potentially toxic elements in Italian donkey milk by ICP-MS and multivariate analysis. Journal of Food Composition and Analysis, 31(1), 161-172.

Qu, X. Y., Zheng, B. Q., Zheng, N., Wang, J. Q., Xu, X. M., Han, R. W., Zhen, Y. P., & Li, S. L., 2013. Survey of heavy metal residues in raw milk in Tangshan City of China. Journal of Food, Agriculture & Environment, 11(3&4), 259-262.

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

Ran, J., Wang, D., Wang, C., Zhang, G., & Zhang, H., 2016. Heavy metal contents, distribution, and prediction in a regional soil-wheat system. Science of the Total Environment, 544, 422-431.

Rey-Crespo, F., Miranda, M., & López-Alonso, M., 2013. Essential trace and toxic element concentrations in organic and conventional milk in NW Spain. Food and Chemical Toxicology, 55, 513-518.

Sarkar, P., Ray, P., Ghatak, P., & Sen, M., 2016. Arsenic concentration in water, rice straw and cow milk - a micro level study at Chakdaha and Haringhata block of West Bengal. Indian Journal of Dairy Science, 6(69), 676-679.

Shailaja, M., Reddy, Y. S., Kalakumar, B. D., Brinda, S. A., Manohar, G., & Kumar, B. D., 2014. Lead and trace element levels in milk and blood of buffaloes (Bubalus bubalis) from Hyderabad, India. Bulletin of Environmental Contamination and Toxicology, 92, 698-702.

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. Science of the Total Environment, 347(1-3), 106-110.

Tang, J., Huang, Z., & Pan, X. D., 2014. Exposure assessment of heavy metals (Cd, Hg, and Pb) by the intake of local foods from Zhejiang, China. Environmental Geochemistry and Health, 36(4), 765-771.

Temiz, H., & Soylu, A., 2012. Heavy metal concentrations in raw milk collected from different regions of Samsun, Turkey. International Journal of Dairy Technology, 65(4), 516-522.

WHO, 2010. Exosure to arsenic: a major public health concern. Geneva, Switzerland: Public Health and Environment, World Health Organization.

Xiao, Q., Zong, Y., & Lu, S., 2015. Assessment of heavy metal pollution and human health risk in urban soils of steel industrial city (Anshan), Liaoning, Northeast China. Ecotoxicology and Environmental Safety, 120, 377-385.

Zhou, X., Qu, X., Zhao, S., Wang, J., Li, S., & Zheng, N., 2016. Analysis of 22 elements in milk, feed, and water of dairy cow, goat, and buffalo from different regions of China. Biological Trace Element Research, 176(1), 120-129.

4

Relationships between lead, arsenic, chromium and cadmium in individual cows' milk with milk composition and heavy metals contents in water, silage and soil

From Zhou Xuewei, Zheng Nan, Su Chuanyou, Wang Jiaqi* and Hélène Soyeurt, 2019. Relationships between Pb, As, Cr and Cd in individual cows' milk with milk composition and heavy metals contents in water, silage and soil. Environmental pollution, 255 (Pt 2): 113322.

Concentrations of heavy metals in raw cow milk and their relationships with water and feed were studied in the previous chapters. Only bulk milk samples were used to conduct those studies. In this chapter, milk samples collected from individual cows were used to estimate the relationships between Pb, As, Cr and Cd contents in milk, drinking water, silage and soil. This was done to highlight potential pathways responsible for the heavy metal contamination in milk. Moreover, additional information about the milk composition was also available such as the contents of protein, fat, lactose, solid non-fat and total solids. This allows to conduct a short study to estimate the relationships between those main milk components and the measured heavy metals contents. The hypothesis is that the presence of heavy metals can influence the global milk composition. If this is revealed, this would mean that some milk components measured on routine by milk laboratories could be used as proxies to detect the presence of heavy metals.

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.

Keywords: Lead, Arsenic, Cadmium, milk composition, relationship

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 (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 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, M. 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, A. L. et al., 2016). 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 four hours 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&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, water, silage 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 4-1). 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.

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 4-2). 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 ($P > 0.05$).

Table 4-1: 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, c, d and e denote significant differences between farms ($P < 0.05$); nd denotes no difference detected.

Table 4-2: 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)

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

The estimated correlation coefficients between heavy metal contents in milk, water, silage, and soil are presented in Table 4-3.

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.

Table 4-3: Spearman correlation coefficients among heavy metals contents in milk, water, silage and soil (N = 6).

	Pb	As	Cr	Cd
Milk-water	0.03	0.37	-0.60	-0.75
Milk-silage	0.54	0.09	0.14	0.49
Milk-soil	-0.37	-0.03	0.60	0.66
Silage-water	0.26	0.78	-0.09	-0.12
Silage-soil	-0.14	-0.31	0.31	0.71
Water-soil	0.03	-0.03	0.14	-0.32

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 4-4. 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.

Table 4-4: Spearman correlation coefficient 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.

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).

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 4-1). 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 Dobrzañski 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 Dobrzañski et al. (2005) (75.06 ± 44.80 µg/L) and by Muhammad et al. (2009) (1,070 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 4-1). 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, A. et al. (2016) estimate the bio-transference factor from As content in drinking water into cow milk. Wang, H. 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/100g, 3.62-4.12 g/100g, and 4.36-4.74 g/100g, respectively. In the present study, the mean contents of milk protein, fat, and lactose in industrial areas were 3.49 g/100g, 3.83 g/100g, and 4.80 g/100g. 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. Conclusions

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.

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7. 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. Environmental Monitoring and Assessment, 160(1-4), 83-89.

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. Journal of the Chemical Society of Pakistan, 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. Bulletin Environmental Contamination and Toxicology, 96(5), 645-649.

Bischoff, K., Higgins, W., Thompson, B., & Ebel, J. G., 2014. Lead excretion in milk of accidentally exposed dairy cattle. Food Additives & Contaminants: Part 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. Environmental Research, 99(1), 48-57.

Campos, V., 2002. Arsenic in groundwater affected by phosphate fertilizers at São Paulo, Brazil. Environmental Geology, 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. Journal of Food Composition and Analysis, 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. Journal of Dairy Science, 92(11), 5335-5352.

CFDA&NHC, (2008). Soil quality-Analysis of total mercury, arsenic and lead contents-Atomic fluorescence spectrometry (in Chinese). Beijing, China: China Food and Drug Administration; National Health Commission of the Peoples's Republic of China.

China, D. A. o., 2018. Chinese Dairy Statistical Summary 2018 (in Chinese). Beijing: China Agriculture Press.

Chiodo, L. M., Jacobson, S. W., & Jacobson, J. L., 2004. Neurodevelopmental effects of postnatal lead exposure at very low levels. Neurotoxicology and Teratology, 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 Journal of Biotechnology, VIII(16), 69-74.

Costa, M., & Klein, C. B., 2008. Toxicity and carcinogenicity of chromium Compounds in Humans. Critical Reviews in Toxicology, 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. The Journal of Agricultural Science, 142(2), 203-212.

Dobrzañski, 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. Polish Journal of Environmental Studies, 14(5), 685-689.

EC, 2006. Commission regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.

EFSA, 2010. Scientific opinion on lead in food. Parma, Italy: European Food Safety Authority.

EFSA. 2014. Dietary exposure to inorganic arsenic in the European population. EFSA Journal, 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. Biological Trace Element Research, 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). Environmental Monitoring and Assessment, 184(7), 4029-4034.

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

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. Pakistan Journal of Botany, 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. Environmental Science & Technology, 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. International Journal of 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. The Scientific World Journal, 2014, 715845.

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. Journal of Trace Element Medicine Biology, 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. Toxicology and Industrial Health, 25, 177-181.

NEPA, (1997). Soil quality-Determination of lead, cadmium-Graphite furance atomic absorption spectrophotometry. Beijing, China: National Environmental Protection Agency.

NEPA, (2009). Soil quality-Determination of total chromium-Flame atomic absorption spectrometry. Beijing, China: National Environmental Protection Agency.

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 Reviews, 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. Science of the Total Environment, 381(1-3), 68-76.

Pérez-Carrera, A., Alvarez-Gonçalvez, C. V., & Fernández-Cirelli, A., 2016. Transference factors as a tool for the estimation of arsenic milk concentration. Environmental Science and Pollution Research, 23, 16329-16335.

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

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. Science of the Total Environment, 404(1), 36-43.

Perriguey, J., Sterckeman, T., & Morel, J.-L., 2008. Effect of rhizosphere and plant-related factors on the cadmium uptake by maize (Zea mays L.). Environmental and Experimental Botany, 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. Polish Journal of Environmental Studies, 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. Journal of the Science of Food and Agriculture, 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. Journal of Food Composition and Analysis, 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. Biological Trace Element Research, 183(1), 92-101.

Rahimi, E., 2013. Lead and cadmium concentrations in goat, cow, sheep, and buffalo milks from different regions of Iran. Food Chemistry, 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. Ecotoxicology and Environmental Safety, 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, and Soil Pollution, 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 Chemistry, 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. Molecular Nutrition and Food Research, 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. Science of the Total Environment, 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 in Biotechnology, 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. Current Environmental Health and Reports, 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. Science of the Total Environment, 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, 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 Additives & Contaminants: Part A, 35(10), 1990-1999.

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

WHO. 1996. Trace elements in human nutrition and health. Geneva, Switzerland: World Health Organization.

WHO, 2010. Exosure to arsenic: a major public health concern. Geneva, Switzerland: Public Health and Environment, World Health Organization.

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. Science of the Total Environment, 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. Biological Trace Element Research, 176, 120-129.

5

General discussion, conclusions and perspectives

In Chapters 2 to 4, the spatial distribution of the concentrations of Pb, As and Cd in bulk milk was described, and the concentrations of Pb, As, Cr and Cd in individual milk were measured. The relationships between milk, drinking water, silage/feed and soil were also estimated, as well as the links with milk composition. The present chapter synthetizes the results obtained in the previous chapters and discusses the pollution sources that may contaminate milk and plants. Then, using a theoretical way, the potential human health risk related to the ingestion of heavy metals contaminated milk will be assessed. Finally, the conclusions and perspectives will be mentioned.

1. Heavy metal contamination in raw cow's milk

1.1 Heavy metals studied

The first study presented in Chapter 2 can be considered a global study focusing on 22 elements measured in milk produced by different species such as cow, goat, and buffalo. The other studies conducted in this thesis focused specifically on heavy metal contamination in raw cow's milk. The reasons for using the cow's milk matrix are the higher yield and consumption of cow's milk and its derived dairy products than of goat and buffalo milk in China. Moreover, this discussion section will focus more on Pb, As and Cd, because the content of Cr and Hg measured in cow's milk was markedly lower.

1.2 Content of heavy metals in raw cow's milk in China

The content of heavy metals was measured on 1,043 bulk milk samples (40 in Chapter 2, 997 in Chapter 3 and 6 in Chapter 4) between 2014 and 2018 in 11 regions in China (Figure 5-1).

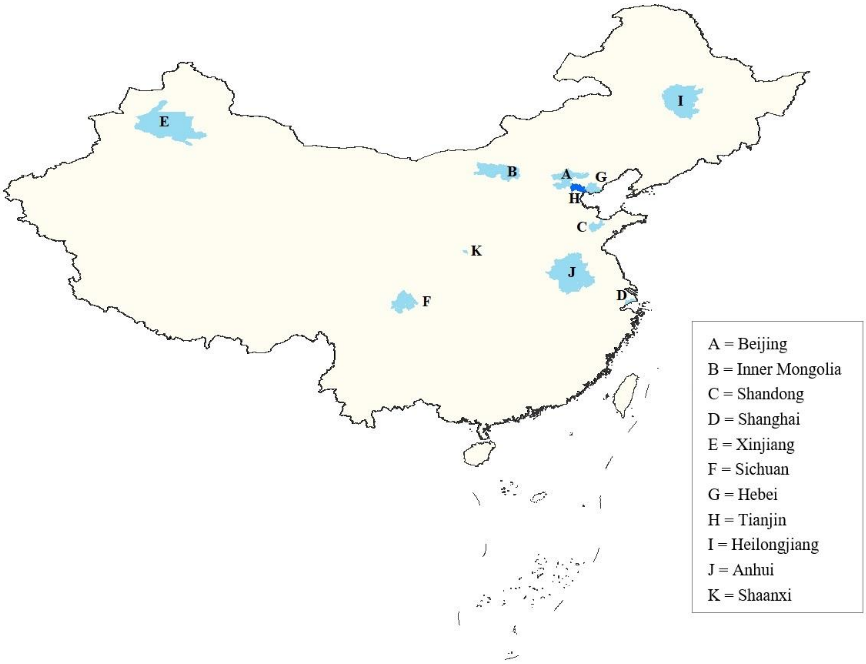

Figure 5-1: Location of regions where milk samples were collected.

Those regions were studied because they are the main milk producing areas in the country. Indeed, the yield of milk production in these areas accounted for 70%

to 74.5% of the total Chinese milk yield between 2010 and 2016. More specifically, areas B, I and G are the top three milk production areas in China (Figure 5-2).

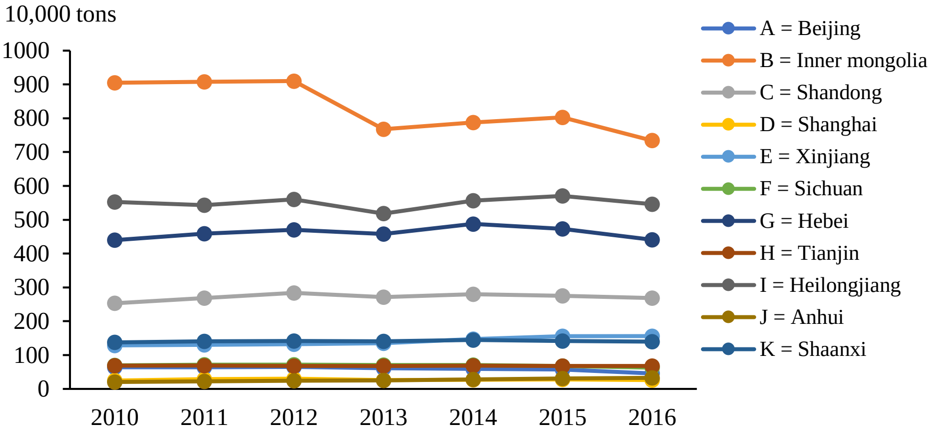

Figure 5-2: Evolution of milk yield in the 11 studied regions.

The descriptive statistics of Pb, As and Cd calculated from the entire dataset were shown in Table 5-1. The average concentrations and SD were calculated by the data above the LOD.

On average, 30% of the samples had a content lower than the LOD for Pb, 52% for As and 24% for Cd. However, different detection rates were observed across the 11 studied areas for each heavy metal. This finding might have been due to local sources of pollution. For instance, the samples with high Pb and Cd detected in area G might have been due to industrial pollution, as described in Chapter 4. Indeed, the steel industry and traffic activity lead to Cd and Pb pollution (Qing et al., 2015). Because we obtained varying detection rates across areas, we expected to observe low correlations between heavy metals measured in cow's milk. Positive significant Spearman correlation values were obtained from the entire dataset for Pb-Cd (r = 0.40), Pb-As (r = 0.22) and As-Cd (r = 0.15). The positive relationship between Pb and Cd was not shown in the thesis, but high detection rates were observed for Pb and Cd in areas A, J and G. These findings suggest the coexistence of Pb and Cd pollution, as already found by Phillips et al. (2003).

Table 5-1: Mean ± SD of Pb, As, and Cd content measured in 1,043 raw cow's milk samples collected from bulks in 11 regions of China.

Area[1]	N	Pb			As			Cd		
		N > LOD [2]	%N < LOD	Mean ± SD (µg/L)	N > LOD	%N < LOD	Mean ± SD (µg/L)	N > LOD	%N < LOD	Mean ± SD (µg/L)
A	100	90	**10**	2.33 ± 2.40	54	46	0.47 ± 0.41	98	**2**	0.09 ± 0.07
B	100	66	34	2.10 ± 2.54	46	54	1.05 ± 1.10	59	41	0.04 ± 0.03
C	120	61	49	2.90 ± 6.65	69	43	0.58 ± 1.90	63	48	0.11 ± 0.12
D	100	60	40	4.46 ± 7.12	35	65	0.33 ± 0.21	72	28	0.07 ± 0.10
E	100	81	19	3.59 ± 7.22	28	72	1.10 ± 1.80	59	41	0.06 ± 0.05
F	100	76	24	1.73 ± 1.88	44	56	0.24 ± 0.10	82	18	0.03 ± 0.02
G	105	101	**4**	2.04 ± 1.12	54	49	0.28 ± 0.28	97	**8**	0.06 ± 0.04
H	97	40	59	2.61 ± 4.57	44	55	0.37 ± 0.32	72	26	0.04 ± 0.03
I	101	41	59	0.93 ± 0.76	44	56	0.36 ± 0.44	90	11	0.04 ± 0.03
J	100	91	**8**	1.85 ± 1.36	66	34	1.19 ± 2.71	94	**6**	0.16 ± 0.90
K	20	19	**5**	1.84 ± 1.34	14	**30**	1.44 ± 0.76	5	75	0.06 ± 0.03
Total	*1,043*	*726*	*30*	*2.43 ± 4.19*	*498*	*52*	*0.63 ± 1.40*	*791*	*24*	*0.07 ± 0.32*

[1] A: part of Beijing; B: part of Inner Mongolia; C: part of Shandong; D: part of Shanghai; E: part of Xinjiang; F: part of Sichuan; G: part of Hebei; H: part of Tianjin; I: part of Heilongjiang; J: part of Anhui; K: part of Shaanxi; [2]N denotes number of samples above LOD, and LOD means limit of detection. N% < LOD is the number of not detected samples divided by total number of samples

The highest levels of Pb and As were observed in area C, with 38.61 µg/L and 15.76 µg/L, respectively. The highest Cd concentration was obtained in area J, with 0.69 µg/L. However, only a few samples exceeded the MRL. Indeed, the levels of Pb in 12 samples from 1,043 collected samples (1.15%) were above the MRL of 0.02 mg/kg set by the European Union (EC, 2015), but all samples were below the Chinese MRL set at 0.05 mg/kg (CFDA&NHC, 2017). There was no sample above the MRL of 100 µg/kg for As set by the Chinese government (CFDA&NHC, 2017). No MRL for As has been set by the European Union or the United States of America. To our knowledge, no MRL for Cd in raw milk exists.

In Chapters 2 to 4, an extensive comparison of the content of heavy metals measured in the samples analyzed in this thesis with those obtained in previous studies was performed. The main studies conducted in this field are shown in Table 1-3 along with the average content of Pb, As and Cd quantified in cow's milk. Comparison of the obtained (Table 5-1) and published values (Table 1-3) indicated that the average concentrations of As and Cd measured in this thesis were lower than those obtained in the previous studies, even when lower values of Pb were also found, such as 1.48 µg/L and 2 µg/L (Table 1-3). Those differences may be partly due to the regions where the samples were collected. Indeed, the aim of the studies presented in Chapter 3 (997/1043 samples) was to screen the existing variability in heavy metal contamination of milk in the main Chinese dairy producing regions. Consequently, there was no special focus placed on regions where possible pollution sources might be present. Although the possible pollution sources affecting cow's milk were studied in Chapter 4, this small number of samples did not substantially contribute to the overall results. In the other studies published in the literature, the locations of farms where the samples were collected were not chosen randomly. For instance, Bilandzic et al. (2016), Gonzalez-Montana et al. (2012) and Norouzirad et al. (2018) collected samples in industrial areas where the cows were exposed to higher pollution risk from steel production, cement production, waste incinerators and phosphate fertilizers, which are recognized to be heavy metal pollution sources (Salazar et al., 2012). For instance, Norouzirad et al. (2018) have reported that 82.2% (97/118) of raw cow's milk samples exceeded the MRL for Pb set by the EU (20 µg/kg) in areas near petroleum extraction industries.

The term "potential source of pollution" is important, because high variability in Pb, As and Cd contents was observed between samples. Indeed, the observed variability in Pb within a region estimated from the standard deviation (SD) calculated on samples with a content higher than the LOD ranged from 1.12 to 7.22 µg/L for Pb, 0.10 to 2.71 µg/L for As and 0.02 to 0.12 µg/L for Cd (Table 5-1). These variabilities were therefore higher than those observed between regions, thus suggesting high heterogeneity within dairy regions (Chapter 3) and a strong influence of the micro-environment around the farm. Therefore, knowledge about the potential sources of pollution present near the farm is necessary to understand and interpret the measured content of heavy metals.

2. Effects of the micro-environment around the farm

Industrial effluents and emissions are major sources of heavy metal contamination in air, groundwater and soil (Islam et al., 2015; Rahman et al., 2008). Those types of industrial sources are also the main pollution source of heavy metals in China. Indeed, Cheng (2003) has reviewed heavy metal pollution in China and concluded that the country has a low geological background level of heavy metals but faces high anthropogenic emissions of those elements in the biosphere, owing to industrial emissions into the air, wastewater and waste solids. Cd in the atmosphere is mainly from refuse incineration, iron and steel production. The atmospheric emissions of Cd and Pb from coal burning in China have rapidly increased from 31.14 t to 261.52 t and 2,671.73 t to 12,561.77 t, respectively, from 1980 to 2008 (Tian et al., 2012). The industrial sector contributes 88.3% and 81.8% of total Cd and Pb emissions, respectively (Tian et al., 2012). The steel industry is responsible for high heavy metal content in the soil (Xiao et al., 2015). Smelter dust fallout is the major source of soil Pb and Cd contamination (Li et al., 2015), but soil contamination can have a greater impact on the production and the quality of crops (Chen, 2003). Indeed, Bermudez et al. (2010) have reported that the content of As and Pb in topsoil samples collected around cement plant and waste incinerators was higher than that in agricultural soils. Moreover, Ogundiran et al. (2012) have found that improper disposal of metallurgical wastes can lead to contamination of forage plants and subsequent transfer to ruminants. For instance, several previous studies have concluded that plants can accumulate heavy metals from water and soil, thus leading to heavy metal exposure in dairy cows after feed ingestion (Diacono et al., 2008; Mingorance et al., 2007; Peralta-Videa et al., 2009; Sun et al., 2016). The heavy metals enter milk not only through water-plant-milk and soil-plant-milk pathways, but also directly through consumption of water and ingestion of pasture soil (Table 4-4). Consequently, the contamination of air, soil and water by heavy metals can clearly lead to direct and indirect contamination of milk (Figure 5-3). However, the intensity of contamination will vary in function of several factors such as the distance between the farm and the polluting source, the soil composition, the wind direction, the quantity of contaminated feed given to the cows, the exposure duration… All of those elements must be known to characterize the micro-environment around the farm in order to highlight the polluting source responsible to the contamination of feed, soil, water and milk. However, such information was not recorded in this thesis. Indeed, the 46 samples of underground water, soil, feed and milk samples were collected at the same time in the studied farm but the environmental conditions around the farms were only recorded in 6 farms. This is too limited to be used in this work.

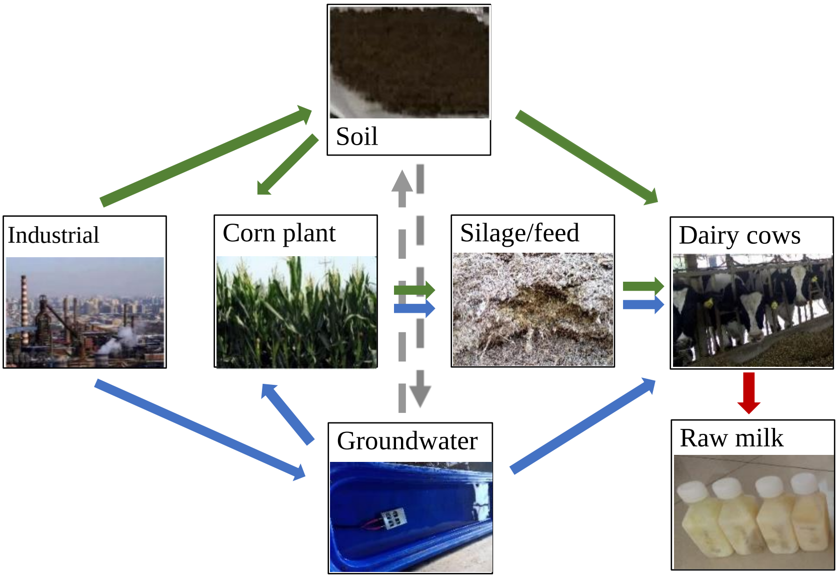

Figure 5-3: Representation of the interactions between potential sources of milk contamination.

2.1 Industrial vs. rural areas

As mentioned previously, milk samples measured in this thesis were collected from 11 regions in China (Figure 5-1). It was only recorded the location of farms, Unfortunately, no precise information about the micro-environments around the farms selected in those regions was available. However, global knowledge about the activities in the studied regions exists. Region I is a traditional agricultural area that can be presumed to be unpolluted. The industrial activity in region E is petroleum extraction. In addition, part of the land in this region are used for pasture. Regions G, B and C are industrial areas whose main industrial activities are steel production (G and B), rare earth mining (B), metal mining (B), and machinery and battery manufacturing (C). Farms C, D and E are close to the cement plant, and farm F is close to a waste incinerator plant. The distances between farms and these two industrial plants were no more than 15 km. By studying soil samples collected on sites located between 0.05 to 6 km from an abandoned copper mine, Wilson and Pyatt (2007) have reported that Pb concentrations in soil decreased with the distance. Similar results were also obtained in cattle organs. Indeed, Pb and Cd in liver, kidney and lungs were higher when the cattle were reared close to the industrial center (Farmera and Farmer, 2000). The Yangtze River estuary is in region D, and the river also flows through regions J and K. Regions A, G and H are adjacent; therefore, the environmental conditions in regions A and H may be affected by the industrial emissions in area G. Unfortunately, for the other samples used in Chapters 2 and 3, the environmental and pollution information around the farms was not recorded. Consequently, the dairy cows studied in this thesis were exposed to different environmental conditions. Knowing the distance between the farm and the source of pollution is important.

A significantly higher Pb content (1.43 μg/L) was observed in individual milk samples collected from industrial areas (Chapter 4). As shown in Table 5-1, the mean concentrations of Pb differed among regions and decreased as follows: D > E > C > H > A > B > G > J ≈ K > F > I. The average levels of Pb ranged from 0.93 μg/L to 4.46 μg/L. The rural region I had the lowest value among regions. The highest level of Pb in milk samples was obtained in region D, this could be owing to the Yangtze River estuary. The results suggested that cows reared in an industrial area are exposed to heavy metal contamination, and those elements might be excreted into milk. This finding is in agreement with the results obtained by Ogundiran et al. (2012), in which cows reared around the contaminated sites (auto battery slag dumpsites) were found to have higher concentrations of Pb in both environmental (leachate: 8.81 ± 0.06 mg/L; forage grasses: 425 ± 79.0 mg/kg) and biological samples (blood: 349 ± 82.0 μg/L; milk: 347 ± 144 μg/L; fecal: 2.08 ± 1.46 mg/kg).

The descending average content of As was K > J > E > B > C > A > H ≈ I ≈ D > G ≈ F (Table 5-1). The average concentrations of As in those regions ranged from 0.24 to 1.44 μg/L. The average concentrations of Cd obtained in milk decreased as follows J > C > A > D ≈ E = G = K > B = H = I ≈ F. The lowest levels of As and Cd were both present in region F. Regions I and F showed low levels of Pb, As and Cd, because of the rural environments around the farms selected in those areas. However, the relationship between heavy metals and industrial activities is not necessarily simple. Van Dijk et al. (2015) have measured the content of Cd in cow's milk collected in two farms in the vicinity of waste incinerators in the Netherlands during 2004–2013 and found that the Cd content was generally similar to that in samples in the reference location. This finding is consistent with the results observed in farm F, which was also close to a waste incinerator. Indeed, the levels of Cd measured in milk collected in this farm were not significantly different from those quantified in the other studied farms located in industrial areas (Chapter 4, Table 4–2). Interestingly, samples collected in this study in industrial areas did not have higher levels, and the mean values for Pb and As were the lowest (Chapter 4, Table 4-2).

2.2 Relationships among water, feed, soil and milk

As explained in Figure 5-3, the content of heavy metals in milk must be interpreted on the basis of detailed examination of the farm soil, feed and drinking water given to the cows and also potentially the individual variations in cows' ability to excrete heavy metals in milk. Consequently, relationships should exist among the concentrations of heavy metals in milk, silage and feed. If the feed is produced on the farm, the main driver of milk contamination is the drinking water and the crops/silage given to the cows.

In Chapters 2 and 4, the relationships of heavy metal contents between milk, water and feed were estimated. Table 5-2 summarizes the obtained Spearman correlation coefficients calculated using the samples collected in Chapters 2 and 4. More specifically, the relationships of Pb, As and Cd between bulk milk, drinking water and feed (TMR) were estimated in Chapter 2 using 40, 40 and 20 milk samples produced by cows, goats and buffalos. In Chapter 4, the correlation coefficients were calculated using records obtained from the analysis of individual cow's milk (N = 60) and bulk milk (N = 6) samples, separately. In order to compare the results obtained by these two studies, the correlations between milk, drinking water and TMR for cows were re-estimated in the present chapter using the data presented in Chapter 2 (Table 5-2).

Significant Spearman correlations between milk and water for Pb, As and Cd were observed for all milk samples collected from cows, goats and buffalos. However, those values markedly changed when only records collected from cows were included. Indeed, the levels of Pb showed no relationship between cow's milk and drinking water; however, calculation for milk from the three mammals resulted in a significant ($P < 0.05$) positive correlation for Pb content (Table 5-2). These strong changes in correlation values may be associated with the different ingredients of feed ingested by goats and buffalos compared with cows. For cows, the feed was TMR, including *Leymus chinensis*, oat grass hay and 35%–55% silage. Forage and corn kernels were given to the goats separately. The drinking water was the same for cows and goats, because the sampling area was the same. However, the micro-environment for buffalos was different from that observed for cows and goats. Buffalo milk samples were collected from Guangxi, which is in the south of China. The geological structure, soil type and human activities were different from those in Shandong and Shaanxi provinces. However, the correlation coefficients obtained by using data from the cows studied in Chapters 2 and 4 were more similar. Although the correlation values were not significant (mainly because of the small dataset), a zero correlation between cow's milk and water for Pb; a negative correlation for Cd and a positive correlation for As can be assumed to exist (Table 5-2). The correlation of As concentrations between milk and drinking water was in agreement with findings from Kazi et al. (2016). As contamination in cow's milk occurs mainly by drinking water (Rana et al., 2014). The intensity of contamination will be related to the amount of water drink by cows. This can fluctuate following per day in function of milk production, the temperature and the moisture of their feed. However, the relationships were different for Pb (milk-water and milk-soil) and As (milk-silage and milk-soil) when the correlation coefficients were calculated with data from industrial areas. The results suggested that water is the main source of As contamination in cow's milk, but Pb appears to have a more complex origin.

Table 5-2: Spearman correlation coefficients between Pb, As and Cd content in milk, water, feed and soil. Values were estimated from the data used in Chapters 2 and 4.

Source	Milk	N^1	Pb	As	Cd	Chapter
Water	Cow, goat, buffalo	100	0.43^*	-0.40^*	-0.25^*	2
Water	Cow (bulk)	40	-0.05	0.12	-0.19	2
Water	Individual cow	60	0.03	0.37	-0.75	4
Water	Cow (bulk)	6	0.09	0.45	-0.46	4
Water	Cow (bulk)	46	-0.03	0.01	-0.11	2&4
TMR	Cow, goat, buffalo	100	-0.05	0.20^*	-0.12	2
TMR	Cow (bulk)	40	-0.02	-0.07	0.24	2
Silage	Individual cows	60	0.54	0.09	0.49	4
Silage	Cow (bulk)	6	0.43	0.64	-0.03	4
Soil	Individual cow	60	-0.37	-0.03	0.66	4
Soil	Cow (bulk)	6	-0.71	-0.41	0.09	4
Factor						
Water	TMR	40	0.09	0.06	-0.28	2
Water	Silage	6	0.26	0.78	-0.12	4
Soil	Silage	6	-0.14	-0.31	0.71	4
Water	Soil	6	0.03	-0.03	0.14	4

^{1}N is the number of samples. $^*P < 0.05$, correlations were significant.

Before presenting the correlated values obtained for Pb, As and Cd, it is important to understand that a plant can uptake and accumulate those heavy metals. As exists in two different forms: inorganic and organic. The inorganic forms, such as As(III) and As(V), exist in rocks, soil and natural groundwater. The organic form is the most common form in seafood (EFSA, 2014). The roots of plants uptake and mobilize As(V) through phosphate transport channels (Tripathi et al., 2007). As(V) enters at the root in maize plants, is reduced to As(III) within the maize root system through complexation with phytochelatines and is then stored in cell vacuoles as an As(III)-tris thiolate complex (Mallick et al., 2011). After As is adsorbed in the roots, it is transported to the upper portion of the crop. As mobilization from roots to aerial parts of plants is controlled by the external As concentrations (Peralta-Videa et al., 2009). Concentrations of As in different maize plant parts decreases in the order root > stem > leaf > grain (Bruwaene et al., 1984; Rosas-Castor et al., 2014). The mechanism of maize uptake and transfer As from water can explain the high correlation between As content in water and in silage (r = 0.78). Whereas, a positive correlation was expected between As in soil and As in feed. The mean concentrations of As in milk samples (N = 60) was 0.06 μg/L, and 7.91 ± 2.06 mg/kg in soil samples (N=6). However, a negative correlation coefficient of -0.31 was found between silage and soil (Table 4-3). Interestingly, the measured correlations were not accordance with the mechanism of As uptake and transfer in maize. Previous studies have indicated that roots of maize plants accumulate higher levels of As than other parts of the maize plant, but this finding is not sufficient to explain the negative correlation. A weakly positive correlation was observed between silage and milk (r = 0.09). A nearly zero correlation was observed for the level of As in cow's milk samples with those in TMR (r = −0.07). Those results were not sufficient to explain the relationship of As contamination between raw cow's milk and feed ingestion. However, as summarized above, feed is a relatively low contributor to As contamination in cow's milk. However, the findings of this thesis provide some information supporting the results obtained in previous studies.

In previous studies, alfalfa plants have been grown hydroponically for 14 d with 40 mg/L Pb and 15 d in soil containing 80 mg/kg Pb (López et al., 2007; 2009). The formation of different Pb complexes has been reported in stems and leaves of alfalfa, but the oxidation state of Pb(II) was not changed after transport from roots to leaves. A nearly zero correlation was observed for the levels of Pb in cow's milk samples with those in TMR (r = −0.02). However, the concentration of Pb between milk samples from individual Holstein cows and TMR was positively correlated with low-, middle- and high dosages in TMR (Wang, H. et al., 2018). The correlation coefficients were nearly equal to zero for Pb content between bulk milk and TMR samples but were positive between silage samples and 60 individual milk samples as well as 6 bulk milk samples (Table 5-2). The components of TMR and the variability among individual cows may partly explain these differences between the findings from this thesis and the previous study. Indeed, the different correlations for TMR and silage might have been caused by the composition of TMR. The percentage of silage in the TMR ranged from 35% to 55%; thus, nearly half or more than half of feed ingested by cows is from other sources, such as *Leymus chinensis*, oat grass and concentrate. This composition might influence the content of heavy metal in feed. However, a correlation value does not lead automatically to a causality. To confirm the hypothesis about the feed composition, additional information about the micro-environment around the farm must be needed.

The electrochemical potential gradient of the plasma membrane in the root cells of plants drives Cd into the root cells (Perriguey et al., 2008; Wang, M. et al., 1994). In maize plants, the entrance of Cd into the root symplast is unregulated, but its translocation toward the shoots is controlled and restricted to some extent by unknown factors (Perriguey et al., 2008). Jamali et al. (2006) have calculated transfer factors (concentration in maize grains/concentration in 0.05 M EDTA soil) of the extractable fraction of Pb, As and Cd in soil to the respective maize grains of 4.01 ± 0.18, 4.24 ± 0.12 and 13.32 ± 0.79, respectively. Consequently, Cd is transferred in the plant more efficiently than are As and Pb. Owing to the transfer of heavy metals by water, soil and plants, the transfer of heavy metals is possible in dairy cows. Although the content of Cd (r = 0.24) in cow's milk samples was slightly correlated with Cd in TMR samples, the relationship for Cd content was stronger between silage and individual cow's milk samples (Table 5-2). However, the correlation coefficient was nearly equal to zero between Cd in silage and bulk milk, possibly because of the limited number of bulk milk samples (N = 6).

Soil and soil contaminant forage are the main sources of toxic metals exposure to livestock (López-Alonso, 2012). The relationships between bulk milk and farm soil were investigated in Chapter 4. As shown in Table 5-2, the correlation coefficients showed the same trend between soil with both individual and bulk milk. Additionally, the content of As ($r = -0.03$) in individual milk samples showed a nearly zero correlation with those in soil. Rey-Crespo et al. (2013) suggested that soil ingested during grazing maybe the reason for significantly higher ($P < 0.05$) As concentration in winter milk than in summer milk.

To understand the relationships among water, soil and plants, it is important to discuss the sources of heavy metal contamination in raw cows' milk. Figure 5-3 summarizes the potential pathways of heavy metals among water, soil, feed and milk. However, no significant relationships were observed among those factors. Although the concentrations of heavy metal in silage were all positively associated with those in milk, the pathways through which heavy metals are transferred from water and soil to silage are unclear. The content of Cd was negatively correlated with Cd in both TMR and silage. Pb and As showed nearly no relationship between water and TMR, as well as with soil. The concentrations of Pb, As and Cd in soil samples showed opposite correlations from water-silage/TMR. The lower correlation coefficient of Pb between soil and silage may have been caused by the concentrations in soil. A previous study has reported that accumulation of Pb in plants occurs only with high concentrations of lead in soils (Cambier, 1997).

These results together may indicate that the content of Pb, As and Cd measured in the milk samples collected in this thesis had different origins. The concentrations of Pb and Cd appeared to be more affected by feed, whereas As appeared to be more affected by the drinking water. Although no significant correlation was observed, stronger correlations were observed in heavy metal content between individual cows' milk samples and silage, compared with those estimated with bulk milk. Transfer of Pb, As and Cd in water, soil and silage was expected to follow the water-plant-silage or soil-plant-silage pathway. There was nearly no relationship or a slight relationship between water and soil, and those relationships did not support the evidence and illustrate the potential relationship between water and soil samples.

3. Effects of heavy metals on milk composition

3.1 Metabolic pathways of heavy metals in dairy cows

Heavy metals harm human health, they can be transferred and metabolized in the organism. Trace elements, such as Zn, Mg and Cr, can enter in animals' organism and are tightly regulated by a complex molecular machinery that controls the rate of absorption of those elements from the gut lumen as well as the amount of excretion via feces, urine and milk to maintain an internal equilibrium (Brugger and Windisch, 2015).

Lead is adsorbed in the duodenum but the absorption rate is better for soluble Pb compared to its insoluble form. Pregnancy and lactation could increase the efficiency of Pb absorption (NRC, 2005). Approximately 90% of absorbed Pb is taken up by red blood cells, whereas the remaining portion is largely bound to albumin (NRC, 2005). Finally, Pb is released from erythrocytes but remains firmly bound to cytosolic binding proteins (Suttle, 2010). Lactating cows with blood Pb levels above 0.20 µg/mL have significantly ($P < 0.05$) higher milk Pb excretion than those with blood Pb levels ranging from non-detectable to 0.20 µg/mL (Swarup et al., 2005). Pb exposure in livestock can lead to an accumulation in tissues and enhanced excretion in milk (Patra et al., 2008). The long bone is the main target for Pb and Cd deposition (Serdaru et al., 2001). Lead can be also transferred into milk, where 90 percentage is associated with casein (Beach and Henning, 1988). Bischoff et al. (2014) have studied the clinical signs of Pb toxicosis over 2.5 years in 8 Holstein cows. They found that the concentrations of Pb in milk showed a peak between 128 and 306 days after silage Pb exposure. In ruminants, 250 mg/kg Pb in the diet can be tolerated for several months without significantly affecting performance; however, levels of Pb in the kidneys are of concern if they are consumed by humans (NRC, 2005).

Arsenic can be accumulated in animal tissues, or can be excreted in feces or urine, or transferred into milk (Rana et al., 2014). Once absorbed, inorganic As could be transferred into various tissues. The excretion of ingested As is rapid, principally in the urine (NRC, 2005). In livestock, there are three mechanisms through which As perturbs normal metabolism: (1) impaired methylation through inhibition of sulfhydryl enzyme systems; (2) oxidative stress though inhibition of keto acid oxidation; and (3) antagonism of other elements (NRC, 2005). Animals are exposed to As through drinking water and forage. Bera et al. (2010) have found a significant ($P < 0.05$) correlation between As concentrations in drinking water and those in the urine and hair of cows. Moreover, Kazi et al. (2016) have reported a positive correlation ($P < 0.05$) between As concentrations in milk samples of cattle and corresponding drinking water. The bio-transference factor (BTF) of As concentrations in cow's milk from As in drinking water calculated by Pérez-Carrera et al. (2016), has been found to range from 1.5×10^{-5} to 7.3×10^{-4}. Drinking water may be considered the only source of As according to those authors. Indeed, no significant difference was observed between the BTF calculated by using only drinking water and the BTF estimated by considering drinking water and food. Therefore, drinking water appears to be the main source of As for cattle.

Cadmium is not transported efficiently into milk, after absorption, kidney is a major site of metallothionein synthesis and consequently accumulation (NRC, 2005). According to Makridis et al. (2007), Cd concentrations in the feed and the duration of exposure to feed are very important factors for predicting the Cd content in the liver and kidney. Cadmium transported in the blood bound primarily to albumin (Zalups and Ahmad, 2003). Su et al. (2017) have studied Cd concentrations in the blood, milk, hair, feces and urine of Holstein cows after oral exposure of 0.182 mg/kg b.w./day for 21 days. Cd residues were detected in blood and milk more than 63 days after Cd withdrawal. These findings may indicate that Cd accumulated in tissues of cows and was excreted into milk after Cd withdrawal. Sharma et al. (1979), in a Cd exposure experiment in dairy cows, have found that daily feed containing an equivalent of 11.3 mg/kg Cd in the total rations did not cause Cd accumulation in the milk, muscle and bones. However, significant accumulation was observed in the liver and kidney. In this thesis, the contents of Cd in feed and soil were positively associated with Cd in milk (Table 5-2) except for the small number of bulk milk (N=6). Those results could indicate that the ingestion of feed (TMR/silage) and farm soil can introduce Cd into dairy cows.

Consequently, on the basis of our results and those reported by other researchers, the possible metabolic pathways of heavy metals in dairy cows are shown in Figure 5-4. Heavy metals could enter into dairy cows through the drinking water and the ingestion of feed and even farm soil; then they are absorbed in the gut. Blood transports ions to other organs such as the liver, kidney and bone where ions are accumulated. The portion of ions which is not accumulated in the organs will be excreted in feces and urine as well as in milk for lactating cows. The mean concentrations of Pb in animals' (cattle, horse and sheep) tissues were highest in surface hair then followed by kidney and liver; for Cd, the order was liver, surface hair, lungs and muscles (Farmera and Farmer, 2000). The portion accumulated in liver, kidney and bone can be released again. Physiological states (pregnancy, parturition, osteoporosis, infection, or prolonged immobilization) that are associated with increased bone resorption promote the release of Pb and entry into blood and milk (ATSDR, 2019).

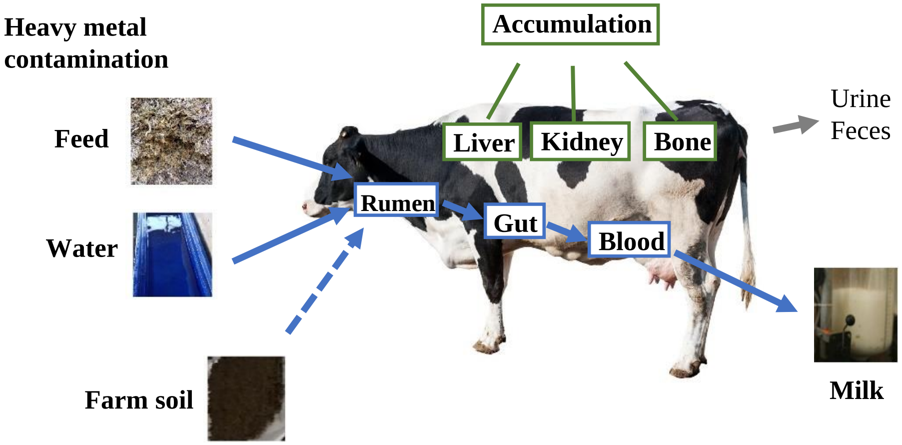

Figure 5-4: Summary of heavy metals transfer, accumulate and excrete after diet ingestion.

3.2 Effects of heavy metals on milk composition

Heavy metals can be accumulated in the blood, serum, muscle and bone (Crout et al., 2004; Su et al., 2017). A significant correlation (r = 0.469, $P < 0.01$) has been observed in Pb content between blood and milk when the blood Pb level exceeds 0.20 µg/mL (Swarup et al., 2005). Kim et al. (2016) have estimated the correlation coefficients between the concentration of Cd or Pb and animal age, as well as between the content of those heavy metals in milk versus the muscle, liver and kidney in cows. Good correlations were found between age and Cd in the kidney (r = 0.748) and between Cd in the liver and in kidney (r = 0.878). Cd can accumulate in cow tissue, and a significant increase in Cd concentrations in the kidney and liver tissue is expected with increasing age of the cow (Kim et al., 2016). Martino et al. (2001) have studied the content of heavy metals (Pb, Cd, Hg, Cr) in different fractions of milk (whole milk, skimmed milk and milk whey), no matter the source of skimmed milk (raw milk, UHT milk, formula milk), the content of Pb ranged from 73% to 78% of that in whole milk. No heavy metals were detected in milk whey. It is logical for Pb as it seems that 90% of Pb in milk is combined with casein (Beach and Henning, 1988), which was removed as fat from whole milk to obtain milk whey. Unfortunately, few studies have reported the effects of heavy metal contamination on global milk composition.

The main states of Pb are +2 and +4, that is, Pb(II) and Pb(IV). The prevalent forms in the environment are Pb(II) and Pb(IV), which are produced by industrial synthesis (EFSA, 2010). This metal can combine with protein fractions of bovine milk; the interaction between Pb(II) with α-casein occurs through cysteine and sulfhydryl residues. This reaction is not reversible because of the nature of the interaction between Pb(II) and amino acid residues (Srinivas et al., 2007). In this thesis, the concentration of Pb in 60 individual cow's milk samples was weakly positively correlated with milk protein (r = 0.11; Table 4-4). The positive correlation supports the ability of Pb in raw milk to combine with protein fraction. No relationship appeared to exist in Pb among milk and fat, lactose, SNF and TS.

Slight negative correlations were obtained between As with SNF (r = −0.18) and protein (r = −0.16). The fat and TS content in milk had nearly no correlation with heavy metals in milk (Table 4-4). Those results did not indicate a clear tendency of As to affect milk composition.

Cd induced effects on metallothionein synthesis. Additionally, disruption of trace element metabolism by relatively low levels of toxic metals can contribute to the pathogenicity of other metabolic disorders (López-Alonso et al., 2002). In mammals, Cd is transported as a Cd-protein complex, particularly Cd-metallothionein, and is stored in the liver, kidney and intestinal mucosa (Cooke and Johnson, 1996). However, in this thesis, Cd showed no relationship with milk protein, although lactose was positively correlated (Table 4-4). No possible explanation was found to interpret those results.

3.3 Heavy metal contamination in milk and its links with milk processing

Milk can be sold as liquid milk after sterilization procedure such as Ultra High Temperature (UHT) or pasteurization or this milk can be used to process dairy products like cream, yoghurt, butter and cheese. The use of milk contaminated by heavy metals could enhance the human risk if a such product is ingested. The production of dairy products increased in China: butter yield increased from 3.0×10^4 in 2005 to 7.0×10^4 tons in 2016, the yield of cheese increased from 1.5×10^4 tons in 2005 to 3.0×10^4 tons in 2016 (China, 2018).

Amer et al. (2005) used 36 raw cow milk samples to produce cream and soft cheese in laboratory. The obtained contents of Pb were 1.97, 0.49 and 1.18 μg/kg in raw cow milk, cream and soft cheese. After processing, the contents of Pb were therefore reduced in cream and soft cheese. Different results were obtained by Kodrik et al. (2011). From 11.7 µg/kg of Pb in raw milk, they found 125.7 µg/kg of Pb in semi-hard cheese. From 9–126 μg of Pb /kg dry weight in raw milk, Maas et al. (2011) found 20–925 μg of Pb/kg dry weight in cheese. This enhancement can be partly explained by the fact that the content of Pb in cheese is not only due to the content of Pb in milk, but also due to the processing methodology. Indeed, the lead pipes are used during cheese processing and can therefore contaminate the final product (Maas et al., 2011). Tona et al. (2013) also found that levels of Pb and Cd in soft cheese were significantly higher than those in raw cow milk, butterfat and yoghurt. This finding for Cd was also confirmed by Maas et al. (2011) where the contents of Cd increased from 0.34–1.01 μg/kg dry weight in raw milk to 0.68–11.37 μg/kg dry weight in cheese.

Enb et al. (2009) have compared the concentration of Pb and Cd in cow's milk and dairy products like cream, butter, samna and yoghurt produced from the same samples. Cream, butter and samna contained 3.7, 5.6 and 6.7 folds of Pb, whereas 4.3, 6.5 and 7.7 folds of Cd. However, the manufactured yoghurt obtained lower levels of Pb and Cd than the raw cow's milk. It maybe caused by the manufacture process and the fermentation by *S. thermophilus* and *L. bulgaricus*.

4. Health risk assessment for heavy metals intake from raw milk

As mentioned in the introduction section of this thesis, the consumption of contaminated milk could induce damages to humans. This potential risk can be assessed based on the hazard quotient (HQ). HQ is the ratio of a single substance exposure level over a specified time period (e.g., subchronic) to a reference dose (or concentration) for that substance derived from a similar exposure period (USEPA, 2001). In this thesis, HQ estimating using the estimated daily intake (EDI) and the reference dose of a hazardous substance (RfD).

EDI value depends on the concentration of heavy metal in milk, the amount of daily milk consumption and the body weight of the consumer. In China, the main dairy products consumed by consumers is liquid milk. And the consumption of cheese and butter is really less than liquid milk, that was, the average consumption of cheese and butter are both 0.1 kg/person in 2016. Therefore, the ingest of milk is calculate by the liquid milk consumption in the thesis. The EDI of metals can be determined with the following equation (Meshref et al., 2014; Muhib et al., 2016):

$$EDI(\mathrm{mg/kg/day}) = \frac{C \times \mathrm{W}}{BW}$$

where C (mg/kg) is the concentration of heavy metals in milk, W is the daily average consumption of milk (kg/day), and BW is the body weight (kg).

The mean concentrations for Pb, As and Cd in raw milk, calculated with the entire dataset available in this thesis, were 1.74 μg/L, 0.32 μg/L and 0.05 μg/L, respectively (measurements below the LOD were replaced by half the LOD). The highest concentrations for Pb, As and Cd in raw milk found in this study were 38.61 μg/L, 15.76 μg/L and 8.74 μg/L. From 2013 to 2017 (Figure 1-1), the average consumption of liquid cow's milk was 45.82 g/ person/day (NBSC, 2018). For an adult of 60 kg [reference proposed by WHO (2008)], the EDI values calculated by using the average and highest concentrations of Pb, As and Cd found in this thesis are shown in Table 5-4.

The non-carcinogenic health risk and hazard quotient (HQ) are used to estimate the health risk of heavy metals ingested from milk and dairy products (USEPA, 1989). HQ is computed by dividing the average daily dose by a specific reference dose (RfD) for Pb, As and Cd. The RfD is an estimation of the maximum acceptable lifetime risk of any metals/metalloids for humans of every age group.

$$HQ = \frac{EDI}{\mathrm{RfD}}$$

where RfD is the reference dose of a hazardous substance. The oral reference dose of inorganic As and Cd in diet for adults is 0.0003 mg/kg/day and 0.001 mg/kg/day, respectively (USEPA, 2014). Inorganic As is more toxic than its organic forms, the RfD for inorganic As was using for health risk assessment, The oral RfD of Pb (0.0036 mg/kg/day) was calculated from the limit of the PTWI of Pb fixed to 25 μg/kg b.w./week (Sipter et al., 2008).

Table 5-3: Parameters used for estimating the hazard quotient.

	Values	References
Average body weight	60 kg (adult)	(WHO, 2008)
Pb reference dose	0.0036 mg/kg/day	(Sipter et al., 2008)
As reference dose	0.0003 mg/kg/day	(USEPA, 2014)
Cd reference dose	0.0010 mg/kg/day	(USEPA, 2014)

Table 5-3 provides some reference values allowing estimation of the health risk of drinking heavy metal contaminated milk for adults. If the HQ value is lower than 1, the reference dose is greater than the average daily dose, and there is no negative impact on human health. In contrast, if the HQ value is greater than 1, the reference dose is lower than the average daily dose, and there is a chance of significant adverse health effects (Izhar et al., 2016). The average and highest values of Pb, As and Cd were used in the calculation of HQ (Table 5-4).

Table 5-4: Estimated daily intake (EDI) and hazard quotient (HQ) calculated with the average and highest concentrations of Pb, As and Cd observed in this thesis.

	Average concentrations			Highest concentrations		
	Pb	As	Cd	Pb	As	Cd
EDI (mg/kg/day)	1.33×10^{-6}	2.44×10^{-7}	3.82×10^{-8}	2.95×10^{-5}	1.20×10^{-5}	6.67×10^{-6}
HQ	3.69×10^{-4}	8.15×10^{-5}	3.82×10^{-5}	0.0082	0.0401	0.0067

The values of HQ calculated with average and highest levels of Pb, As and Cd were both lower than 1. This suggests that no health risk would be introduced by consumption of cow’s milk. But there are still some samples with high contents of Pb, As and Cd in cow’s raw milk. This highlights the importance to prevent the contamination of milk by heavy metals as some high contents can be observed on field. This means that a protocol must be defined to ensure a good routine sampling in order to ensure to the customer a high quality of Chinese milk.

5. Variations from sampling

The coefficient of variation (CV) is used to evaluate the distribution of samples. The CV values for 60 Holstein cows were 133.10, 353.03, 117.35 and 66.62 for Pb, As, Cr and Cd, respectively (Table 4-1), thus suggesting high inter-individual variability. CV values at the farm level per study (i.e., chapter) are shown in Table 5-5.

Table 5-5: Mean ± SD of Pb, As and Cd concentrations (μg/L) in bulk milk samples.

Chapter	N[1] > LOD	Pb	N > LOD	As	N > LOD	Cd
2	38	1.46 ± 0.93	34	0.86 ± 0.72	13	0.07 ± 0.05
(CV[2])		63.7		83.7		71.4
3	682	1.75 ± 3.73	460	0.31 ± 1.02	772	0.05 ± 0.07
(CV)		213.1		329.0		140.0
4	6	1.13 ± 0.84	4	0.14 ± 0.15	6	0.11 ± 0.04
(CV)		74.3		107.1		36.4
Total	726	2.43 ± 4.19	498	0.63 ± 1.40	791	0.07 ± 0.32
(CV)		210.3		311.3		388.9

[1] N is the number of samples; [2] CV denotes coefficient of variation; The results were calculated from the values above the limit of detection (LOD).

CV values indicate the degree of variability for each studied heavy metal. According to the obtained CV values (Table 5-5), the concentrations of Pb, As and Cd in Chapter 3 showed an extremely high degree of variability compared with those in Chapters 2 and 4. Consequently, there is a highly heterogeneous distribution of concentrations of the studied heavy metals in Chinese raw cow's milk in the field. In Chapters 3 and 4, Cd had the lowest CV, as compared with Pb and As. However, the CV calculated from all data in this thesis were much higher (> 200%) than observed in Chapters 2 to 4, especially for Cd. This finding may have been due to the higher levels of Cd observed in Chapters 2 and 4. In a previous study by Lukáčová Anetta (2012), the levels of Cd was measured in 30 raw and UHT milk samples, and the obtained CV for Cd was 70.69% and 73.36% for raw and UHT milk, respectively. Their samples were collected randomly from dairy farms in the Nitra region in western Slovakia. The CVs observed in this thesis were higher, possibly because of the environmental status of the surrounding land.

Interestingly, a high degree of inter-farm variation was found for all elements analyzed in this thesis, thus indicating that the type of production system (organic or conventional) does not in itself determine higher or lower toxic metal exposure or trace mineral status. The variations were mainly due to the farm nutritional and management practices.

Xiao et al. (2015) have reported that CVs above 100% for Pb and Cd in urban soils can be considered to indicate exceptionally high variability. In Chapter 3, the CV values were all higher than 100% and were at least 140%, thus indicating that concentrations of Pb, As and Cd differed greatly with respect to the different sampling farms. The CV values estimated for Pb, As and Cd observed in Chapter 2 were moderate, thus suggesting that the content of those heavy metals had lower variability in the study described in this chapter. Only two different areas were chosen to collect samples in Chapter 2, and sampling occurred twice in each area for ten farms, which shared similar environmental factors. This potentially explaining the lower observed variability of heavy metals. The opposite may explain the higher CV observed for the data collected in the study described in Chapter 3 as well as that observed when all data were combined. Indeed, a total of 614 farms were selected for sampling in this thesis, which were located in 11 Chinese provinces. The soil type, feed composition and pollution sources were therefore different. As mentioned previously, those factors affect the heavy metal content in raw milk. The same findings have been reported by Lopez-Alonso et al. (2017).

6. Conclusions and perspectives

6.1 Conclusions

The presence of heavy metals in milk is mainly associated with the diet and micro-environment around the farm, especially the presence of industrial activities (e.g., steel or mining activity). Drinking contaminated milk can be damaging to humans if the content of heavy metals in milk is elevated. Therefore, monitoring the presence of heavy metals in milk in terms of composition and content is crucial for public health, especially in dairy producing areas where industrial activities are present. This thesis conducted the first large scale research in China to evaluate the spatial distribution of heavy metals in milk and to highlight the relationships between the heavy metals and micro-environmental aspects of the farm. More than 1,000 milk samples collected in the 11 main milk producing areas in China were measured to quantify the content of heavy metals.

According to those quantifications, the spatial variations in Pb, As and Cd in raw cow's milk were illustrated, and significant differences were found across milk producing areas. The average concentrations of Pb, As and Cd in bulk milk samples were 1.74 μg/L, 0.32 μg/L and 0.05 μg/L, respectively. Only 12 bulk milk samples (1.15%) exceeded the MRL for Pb set by the EU, thus suggesting limited importance of heavy metal contamination in milk produced in China. However, on the basis of the estimated EDI and HQ, no health risk for drinking contaminated milk by adults was found when the average and highest levels of heavy metals were used for the calculation. Consequently, even if the presence of high content of heavy metal content in milk was not observed frequently in this thesis, a sampling procedure should be designed by the ministry to routinely control those elements in the field.

The sampling protocol used in this thesis does not appear to be the best approach to develop routine screening, because the sampling was based on a random selection of farms in main milk producing areas. Indeed, the CV values revealed that higher variability was obtained within areas than between areas. This variability explained why our predicted spatial distributions of the heavy metal content in milk were not robust enough. Consequently, the sampling protocol for a routine screen must be defined on the basis of other criteria beyond the producing areas. Therefore, there is a need for better understanding of the factors in the farm micro-environment that affect the content of heavy metals in milk. Moreover, the various CVs observed for Pb, As and Cd suggested different sources of contamination.

The relationships of heavy metals among drinking water, feed, soil and bulk milk were estimated, as well as between these factors and individual cow's milk samples. Feed, water and soil can contribute to the contamination of milk by different heavy metals through direct or indirect pathways. Pb and As in water, and Cr and Cd in soil, were positively correlated with the content in milk. Pb, As and Cd in silage were all positively correlated with the content in raw milk, thus suggesting that silage may be the main contributor to heavy metal contamination in milk. However, As appeared to contribute less than Pb and Cd according to the obtained Spearman correlation values. Individual cow's milk samples showed clearer relationships with feed and environmental factors than bulk milk samples. The links among drinking water, silage and soil suggested that Pb, As and Cd were transferred through water-silage-milk and soil-silage-milk pathways. However, the values of the correlations remained low, possibly because of the low number of samples used. To confirm those trends, it will be important to increase the sample size to provide more evidence of the transfer of heavy metals from soil, feed and drinking water into cows and consequently into milk. Moreover, the low observed correlations may have been due to the existence of different sources of pollution, which differently affected the heavy metal content in drinking water and feed for dairy cows. To avoid this problem, information about the micro-environment around the farm must be determined. This information was largely unavailable in this study but is crucial knowledge for designing efficient sampling procedures.

To screen potential abnormal samples, investigating the effects of the presence of heavy metals on global milk composition may be interesting. Even if milk protein content is more affected by heavy metal pollution, especially Pb, no clear relationship was found between global milk composition (i.e., protein, fat, lactose and TS) and the content of heavy metals in raw milk. These results may have been due to the small number of samples used to estimate those correlations, because the CV was high within farms. Moreover, the levels of heavy metals measured in the milk samples analyzed in this thesis were insufficient to affect cow health, thus resulting in nearly zero correlations.

In conclusion, this thesis highlighted the presence of heavy metals in low quantity in milk produced in China. Therefore, there is a need for the ministry to define a sampling protocol to screen dairy farms with potential risk of producing contaminated milk to ensure that high quality milk is provided to consumers. Unfortunately, the results obtained in this thesis were not sufficient to propose a detailed sampling procedure, because information about the micro-environment (e.g., the presence of industrial activities and the type of industry) around the farm was lacking, and this knowledge was found to be the most important factor.

6.2 Perspectives

The results in this thesis indicated that the concentrations of heavy metals in raw cow's milk are affected by the cow breeding environment. Cows reared in farms near industrial areas have a higher risk of producing milk with higher heavy metal content than cows reared in unpolluted areas. In this thesis, agricultural areas were considered to be unpolluted. Only one farm was selected in this area and was considered a control farm. However, this farm had a certain amount of As in milk. Therefore, in further studies, more than one farm must be selected to compose the control group. Moreover, instead of knowing only the macro-environment (i.e., agricultural area), the micro-environment of those farms must be known to ensure that milk samples free of heavy metals are collected. Knowing the micro-environmental conditions of the farm (industrial activities near the farm, the type of industry, and the distance between farms and the pollution sources) is extremely important. Gaining this knowledge requires merging different databases and/or organizing a national meeting with all stakeholders in dairy industry to provide this information at the national level. This procedure will not be easy to implement but will help to focus on the most appropriate farms and to facilitate the interpretation of the heavy metal measurements obtained.

If the micro-environment of the farms sampled in this thesis were known, the data could be sorted according to industrial activities. This procedure would improve the distribution map created and aid in designing an appropriate sampling procedure. Sorting the data according to the industrial activity present near the farm would improve the relevance of estimated correlations. However, more samples must first be collected in the same area to increase the size of the current dataset. Indeed, the number of samples used for the correlations was too low, on the basis of the estimated SD within and between farms. Moreover, because CV values above 100% were observed for Pb, As and Cr, it will be important to increase the number of cows to dilute the effects of individuals and better isolate the herd effect. The number of samples of water and silage should be increased and taken at different times to be accordance with the samples of milk collected. Silage was collected and measured to assess the correlation with the content of heavy metals in milk. However, silage is part of the TMR feed. Therefore, TMR samples should also be collected and measured. All those improvements would clarify the relationships between the contamination of raw milk by heavy metals and the herd environment.

Heavy metals in 60 individual cows' milk samples (i.e., ten samples per farm) were measured to assess the relationships with drinking water, silage and farm soil. A high variability was observed, a finding associated with farming management as well as individual differences among cows. Indeed, animal characteristics such as the days in milk, stage of lactation, genetics and health status influence cows' ability to transfer the heavy metals ingested or stored in the tissues into milk. Future studies will require the acquisition and analysis of many samples collected in farms in industrial areas. Beyond the improvement in the knowledge about the transfer of heavy metals into milk, this kind of studies could confirm the levels of heavy metals when the cows poisoning. This information would be important to ensure cows' well-being. If those samples were also analyzed with the infrared technology used in milk recording, quantification of the content of fat, protein, lactose, urea, TS and many other minor components, such as fatty acids, lactoferrin or minerals, would be possible in milk. This information would enable the effects of the presence of heavy metals in milk to be studied.

According to other researchers, levels of Pb in milk are significantly related to those in the blood, especially when Pb levels exceed 0.2 mg/mL. Blood heavy metal content, such as Pb and Cd content, can serve as a biomarker for excretion of those metals. Measuring the content of heavy metals in blood samples could also improve understanding of the excretion of heavy metals in milk and enable cow and feed effects to be better isolated or differentiated.

All these further studies, if they are funded, could also be used to define better sampling procedures for routine quality control by the ministry to ensure the high quality of Chinese milk.

7. References

Amer, I. H., Sayed, M. S. E., & Aal, S. F. A. A.-E., 2005. The preliminary content of heavy metal residues in raw cow's milk and its distribution in some dairy products. Food Control, 33(1), 263-271.

ATSDR. 2019. Toxicological profile for lead. (Vol. draft).

Beach, J. R., & Henning, S. J., 1988. The distribution of lead in milk and the fate of milk lead in the gastrointestinal tract of suckling rats. Pediatric Research, 23, 58-62.

Bermudez, G. M., Moreno, M., Invernizzi, R., Pla, R., & Pignata, M. L., 2010. Heavy metal pollution in topsoils near a cement plant: the role of organic matter and distance to the source to predict total and hcl-extracted heavy metal concentrations. Chemosphere, 78(4), 375-381.

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. Bulletin Environmental Contamination and Toxicology, 96(5), 645-649.

Bischoff, K., Higgins, W., Thompson, B., & Ebel, J. G., 2014. Lead excretion in milk of accidentally exposed dairy cattle. Food Additives & Contaminants: Part A, 31(5), 839-844.

Brugger, D., & Windisch, W. M., 2015. Environmental responsibilities of livestock feeding using trace mineral supplements. Animal Nutrition, 1(3), 113-118.

Bruwaene, R. V., Kirchmann, R., & Impens, R., 1984. Cadmium contamination in agriculture and zootechnology. Experientia, 40.

Cambier, P., 1997. Evaluation of the mobility of toxic elements in contaminated soils. Analusis, 25(9-10), 35-38.

CFDA&NHC. 2017. Maximum levels of contaminants in foods. (Vol. GB 2762-2012). China: China Food and Drug Adiministration; National health commission of the People's Republic of China.

China, D. A. o., 2018. Chinese Dairy Statistical Summary 2018 (in Chinese). Beijing: China Agriculture Press.

Cooke, J., & Johnson, M. (1996). Cadmium in mammals. In B. WN, H. G & R.-N. AW (Eds.), Environmental contaminants in wildlife, SETAC special publication series (pp. 377-388). Boca Raton: CRC Lewis Publishers.

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. The Journal of Agricultural Science, 142(2), 203-212.

Diacono, E., Faye, B., Meldebekova, A., & Konuspayeva, G. (2008). Plant, water and milk pollution in Kazakhstan. In B. Faye & Y. Sinyavskiy (Eds.), Impact of Pollution on Animal Products (pp. 107-116). Dordrecht, The Netherlands: Springer.

EC. 2015. Commossion Regulation (EU) 2015/1005 amending Regulation (EC) No 1881/2006 as regards maximum levels of lead in certain foodstuffs. Official Journal of the European Union, L 161, 11.

EFSA, 2010. Scientific opinion on lead in food. Parma, Italy: European Food Safety Authority.

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

Enb, A., Donia, M. A. A., Abd-Rabou, N. S., Abou-Arab, A. A. K., & El-Senaity, M. H., 2009. Chemical composition of raw milk and heavy metals behavior during processing of milk products. Global Veterinaria, 3(3), 268-275.

Farmera, A. A., & Farmer, A. M., 2000. Concentrations of cadmium, lead and zinc in livestock feed and organs around a metal production centre in eastern Kazakhstan. The Science of the Total Environment, 257, 53-60.

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). Environmental Monitoring and Assessment, 184(7), 4029-4034.

Islam, M. S., Ahmed, M. K., Habibullah-Al-Mamun, M., & Masunaga, S., 2015. Assessment of trace metals in foodstuffs grown around the vicinity of industries in Bangladesh. Journal of Food Composition and Analysis, 42, 8-15.

Jamali, M. K., Kazi, T. G., Arain, M. B., Afridi, H. I., Jalbani, N., & Adil, R. S., 2006. The correlation of total and extractable heavy metals from soil and domestic sewage sludge and their transfer to maize (Zea maysL.) plants. Toxicological & Environmental Chemistry, 88(4), 619-632.

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.

Kim, D. G., Kim, M., Shin, J. Y., & Son, S. W., 2016. Cadmium and lead in animal tissue (muscle, liver and kidney), cow milk and dairy products in Korea. Food Additives & Contaminants: Part B, 9(1), 33-37.

Kodrik, L., Wanger, L., Imre, K., Polyak, K. F., Besenye, F., & Husveth, F., 2011. The effect of highway traffic on heavy metal content of cow milk and cheese. Hungarian Journal of Industrial Chemistry Veszprem, 39(1), 15-19.

López-Alonso, M. (2012). Animal feed contamination by toxic metals. In J. Fink-Gremmels (Ed.), Animal feed contamination. effects on livestock and food safety. (pp. 183-204). Cambridge: Woodhead publishing.

López-Alonso, M., Benedito, J. L., Miranda, M., Castillo, C., Hernández, J., & Shore, R. F., 2002. Interactions between toxic and essential trace metals in cattle from a region with low levels of pollution. Archives of Environmental Contamination and Toxicology, 42(2), 165-172.

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. Environmental Science & Technology, 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. International Journal of Phytoremediation, 11(2), 131-149.

Li, P., Lin, C., Cheng, H., Duan, X., & Lei, K., 2015. Contamination and health risks of soil heavy metals around a lead/zinc smelter in southwestern China. Ecotoxicology and Environmental Safety, 113, 391-399.

Lopez-Alonso, M., Rey-Crespo, F., Herrero-Latorre, C., & Miranda, M., 2017. Identifying sources of metal exposure in organic and conventional dairy farming. Chemosphere, 185, 1048-1055.

Lukáčová Anetta, M. P., Greń Agnieszka , Golian Jozef 2012. concentration of selected elements in raw and ultra heat treated cow milk. Journal of Microbiology, Biotechnology and Food Sciences, 2 (2), 795-802.

Maas, S., Lucot, E., Gimbert, F., Crini, N., & Badot, P.-M., 2011. Trace metals in raw cows' milk and assessment of transfer to Comté cheese. Food Chemistry, 129(1), 7-12.

Makridis, C., Svarnas, C., Rigas, N., Gougoulias, N., Roka, L., & Leontopoulos, S., 2007. The risk of contamination of food with toxic substances present in animal feed. Animal Feed Science and Technology, 133, 84-108.

Mallick, S., Sinam, G., & Sinha, S., 2011. Study on arsenate tolerant and sensitive cultivars of Zea mays L.: differential detoxification mechanism and effect on nutrients status. Ecotoxicology and Environmental Safety, 74(5), 1316-1324.

Martino, F. A. R., Sánchez, M. ı. L. F., & Sanz-Medel, A., 2001. The potential of double focusing-ICP-MS for studying elemental distribution patterns in whole milk, skimmed milk and milk whey of different milks. Analytica Chimica Acta, 442, 191-200.

Meshref, A. M. S., Moselhy, W. A., & Hassan, N. E.-H. Y., 2014. Heavy metals and trace elements levels in milk and milk products. Journal of Food Measurement and Characterization, 8(4), 381-388.

Mingorance, M. D., Valdes, B., & Oliva, S. R., 2007. Strategies of heavy metal uptake by plants growing under industrial emissions. Environment International, 33(4), 514-520.

Muhib, M. I., Chowdhury, M. A. Z., Easha, N. J., Rahman, M. M., Shammi, M., Fardous, Z., Bari, M. L., Uddin, M. K., Kurasaki, M., & Alam, M. K., 2016. Investigation of heavy metal contents in cow milk samples from area of Dhaka, Bangladesh. International Journal of Food Contamination, 3(1).

NBSC. (2018). National Bureau of Statistics of China. China statistical yearbook. from http://www.stats.gov.cn/tjsj/ndsj/2018/indexeh.htm

Norouzirad, R., Gonzalez-Montana, J. R., Martinez-Pastor, F., Hosseini, H., Shahrouzian, A., Khabazkhoob, M., Ali Malayeri, F., Moallem Bandani, H., Paknejad, M., Foroughi-Nia, B., & Fooladi Moghaddam, A., 2018. Lead and cadmium levels in raw bovine milk and dietary risk assessment in areas near petroleum extraction industries. Science of the Total Environment, 635, 308-314.

NRC. 2005. Mineral tolerance of animals (Second revised edition ed.). Washington, D.C.: The national academies press.

Ogundiran, M. B., Ogundele, D. T., Afolayan, P. G., & Osibanjo, O., 2012. Heavy metals levels in forage grasses, leachate and lactating cows reared around lead slag dumpsites in Nigeria. International Journal of Environmental Research, 6(3), 695-702.

Pérez-Carrera, A., Alvarez-Gonçalvez, C. V., & Fernández-Cirelli, A., 2016. Transference factors as a tool for the estimation of arsenic milk concentration. Environmental Science and Pollution Research, 23, 16329-16335.

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. Science of the Total Environment, 404(1), 36-43.

Peralta-Videa, J. R., Lopez, M. L., Narayan, M., Saupe, G., & Gardea-Torresdey, J., 2009. The biochemistry of environmental heavy metal uptake by plants: implications for the food chain. The International Journal of Biochemistry & Cell Biology, 41(8-9), 1665-1677.

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

Qing, X., Yutong, Z., & Shenggao, L., 2015. Assessment of heavy metal pollution and human health risk in urban soils of steel industrial city (Anshan), Liaoning, Northeast China. Ecotoxicology and Environmental Safety, 120, 377-385.

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. Ecotoxicology and Environmental Safety, 69(2), 317-324.

Rana, T., Bera, A. K., Das, S., Bhattacharya, D., Pan, D., & Das, S. K., 2014. Subclinical arsenicosis in cattle in arsenic endemic area of West Bengal, India. Toxicology and Industrial Health, 30(4), 328-335.

Rey-Crespo, F., Miranda, M., & López-Alonso, M., 2013. Essential trace and toxic element concentrations in organic and conventional milk in NW Spain. Food and Chemical Toxicology, 55, 513-518.

Rosas-Castor, J. M., Guzman-Mar, J. L., Hernandez-Ramirez, A., Garza-Gonzalez, M. T., & Hinojosa-Reyes, L., 2014. Arsenic accumulation in maize crop (Zea mays): a review. Science of the Total Environment, 488-489, 176-187.

Salazar, M. J., Rodriguez, J. H., Leonardo Nieto, G., & Pignata, M. L., 2012. Effects of heavy metal concentrations (Cd, Zn and Pb) in agricultural soils near different emission sources on quality, accumulation and food safety in soybean [Glycine max (L.) Merrill]. Journal of Hazardous Materials, 233-234, 244-253.

Serdaru, M., Avram, N., Medrea, N., & Vlădescu, L., 2001. Determination of trace elements content in biological materials-measure of industrial pollution. Analytical Letters, 34(9), 1437-1447.

Sharma, R. P., Street, J. C., Verma, M. P., & Shupe, J. L., 1979. Cadmium uptake from feed and its distribution to food products of livestock. Environmental Health Perspectives, 28, 59-66.

Sipter, E., Rozsa, E., Gruiz, K., Tatrai, E., & Morvai, V., 2008. Site-specific risk assessment in contaminated vegetable gardens. Chemosphere, 71(7), 1301-1307.

Srinivas, S., Kaul, P., & Prakash, V., 2007. Mechanism of Interaction of Pb(II) with Milk Proteins: A case study of α casein. Jouranl of Agricultural and Food Chemistry, 55, 9283-9288.

Su, C., Zhang, J., Li, Z., Zhao, Q., Liu, K., Sun, Y., & Wang, J., 2017. Accumulation and depletion of cadmium in the blood, milk, hair, feces, and urine of cows during and after treatment. Biological Trace Element Research, 175(1), 122-128.

Sun, Z., Chen, J., Wang, X., & Lv, C., 2016. Heavy metal accumulation in native plants at a metallurgy waste site in rural areas of Northern China. Ecological Engineering, 86, 60-68.

Suttle, N. F., 2010. Minerals in animal nutrition (4 ed.). UK.

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. Science of the Total Environment, 347(1-3), 106-110.

Tian, H., Cheng, K., Wang, Y., Zhao, D., Lu, L., Jia, W., & Hao, J., 2012. Temporal and spatial variation characteristics of atmospheric emissions of Cd, Cr, and Pb from coal in China. Atmospheric Environment, 50, 157-163.

Tona, G. O., Adetunji, V. O., Ameen, S. A., & ., A. O. I., 2013. Evaluation of lead and cadmium heavy metal residues in milk and milk products in Ogbomoso, Southewestrn Nigeria. Pakistan Journal of Nutrition, 12(2), 168-171.

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 in Biotechnology, 25(4), 158-165.

USEPA. 1989. Risk assessment guidance for superfund volume I human health evaluation manual (part A). (Vol. Washington, DC. U.S.: Office of Emergency and Remedial Response, U.S. Environmental Protection Agency.

USEPA. 2001. Risk assessment guidance for superfund: volume III -part A, process for conducting probabilistic risk assessment. (Vol. Washington, DC. U.S.: Office of Emergency and Remedial Response, U.S. Environmental Protection Agency.

USEPA. (2014). United States Environmental Protection Agency, 2014. Region 9, regional screening levels tables. from http://www.epa.gov/region9/superfund//prg/index.html

Van Dijk, C., Van Doorn, W., & Van Alfen, B., 2015. Long term plant biomonitoring in the vicinity of waste incinerators in The Netherlands. Chemosphere, 122, 45-51.

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 Additives & Contaminants: Part A, 35(10), 1990-1999.

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

Wilson, B., & Pyatt, F. B., 2007. Heavy metal dispersion, persistance, and bioccumulation around an ancient copper mine situated in Anglesey, UK. Ecotoxicology and Environmental Safety, 66(2), 224-231.

Xiao, Q., Zong, Y., & Lu, S., 2015. Assessment of heavy metal pollution and human health risk in urban soils of steel industrial city (Anshan), Liaoning, Northeast China. Ecotoxicology and Environmental Safety, 120, 377-385.

Zalups, R. K., & Ahmad, S., 2003. Molecular handling of cadmium in transporting epithelia. Toxicology and Applied Pharmacology, 186(3), 163-188.

6

Doctoral Trainings and Scientific Communication

Thematic training	Credits
Doctoral seminar for animal nutrition	1
RDCT1046-1 - Applied multivariate analysis: data mining and chemometrics	2
RDCT1047-1 - Guide to writing scientific papers	2
RDCT1048-1 - Training course for ICP-MS in Agilent corporation	2
Seminar for animal nutrition and feed science	4
5th International Symposium on Dairy Cow Nutrition and Milk Quality	3
70th Annual Meeting of the European Federation of Animal Science	5
Total	19
Transversal training	Credits
Chinese Academy for Agricultural Science	10
2016 World Life Science Conference	3
From international mix-ups to effective intercultural communication	1
Eurodoc annual conference 2019	1
Total	15
Scientific production	Credits
Analysis of 22 Elements in Milk, Feed, and Water of Dairy Cow, Goat, and Buffalo from Different Region	8
Spatial Distribution of Pb, As, Cd, Contents in Chinese Raw Milk and Risk Assessment for Human Health	5
Analysis and Risk Assessment of Seven Toxic Element Residues in Raw Bovine Milk in China	5
Contamination and spatial distribution of Pb, As and Cd contents in Chinese cow raw milk	3
Large scale study of the within and between spatial variability of lead, arsenic, and cadmium contamination of cow milk in China	8
Relationships between Pb, As, Cr, and Cd in soil and water in agricultural and industrial areas with heavy metals contents from individual cow milks	3
Relationships between heavy metals in milk, soil and water from agricultural and industrial areas	5
Total	37
In total	71