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Heavy Metal Contamination of Animal Feed in Texas

Susie Y. Dai[a,*], Ben Jones[b], Kyung-Min Lee[b], Wei Li[b], Lynn Post[c], Timothy J. Herrman[a,**]

[a] *Office of the Texas State Chemist, Department of Pathobiology, Texas A&M AgriLife Research, Texas A&M University System, College Station, TX, 77841, United States*

[b] *Office of the Texas State Chemist, Texas A&M AgriLife Research, Texas A&M University System, College Station, TX, 77841, United States*

[c] *Department of Veterinary Physiology & Pharmacology, Texas A&M University, College Station, TX, 77841, United States*

Abstract

The management of animal feed safety risks using a risk management framework begins with identifying and quantifying the presence of hazards. For animal feed, a paucity of information exists about the presence of heavy metal in feed ingredients, premixes, and finished feed. This study examines 564 feed samples over a period of five years (2010-2015) collected by Texas Feed and Fertilizer Control Service (FFCS) investigators using official sampling and chain-of-custody techniques. Samples were prepared and analyzed in the Office of the Texas State Chemists laboratory (Agricultural Analytical Service) on the campus of Texas A&M University in College Station, TX. The heavy metals of concern included arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), molybdenum (Mo), nickel (Ni), selenium (Se), and thallium (Tl). Data were analyzed using descriptive statistic techniques and summarized by element, feed type, and year. During 2010, 28% of the samples contained detectable levels of heavy metals, a few of which contained higher than maximum tolerable levels of the elements [1]. The percentage of detectable heavy metals increased in subsequent years as the analytical technique used became more sensitive and an increased number of heavy metal contaminants were analyzed. A positive skewness was observed for most heavy metals in most ingredients resulting for the detection of high levels of contamination among a few samples. This study provides a comprehensive analysis of heavy metals during the prescribed time period and ingredient/finished feed type and will facilitate risk assessment and implementation of risk management techniques prescribed by the Food Safety Modernization Act requirements that impacts the United States and global feed industry.

Keywords:
heavy metal, feed, risk management, risk assessment

1. Introduction

Inorganic heavy metal contaminants in animal feed pose a hazard to animal health and human food safety. Heavy metals including copper (Cu), cadmium (Cd), chromium (Cr), and lead (Pb) are potential bio-accumulative toxicants that may cause severe health problems even at low concentrations [2, 3]. Furthermore, non-essential elements such as Cd and Pb may also present in animal feed as the result of feed processing and environmental pollution [4]. The United States and Europe reported element concentrations in feed [5, 6, 7, 1]. Among the few studies that document the level of heavy metal contamination of feedstuffs in the US, Li et al. [6] reported that more than half of the Wisconsin dairy farms used feed rations containing Cu and Zn above the recommended levels and Cd was generally present in those feeds. Heavy metal contaminants in animal are identified in the Codex hazard prioritization document [8].

In the US, regulatory limits for the maximum levels of minerals allowed in feed ingredients and finished feed exist for selenium. In livestock production, commercial feeds are often supplemented with copper (Cu), zinc (Zn) and iron (Fe) to promote optimum growth rate and antimicrobial properties [9, 10]. The maximum tolerable levels (MTL) for 38 essential and non-essential minerals are discussed in the 2005 NRC Mineral Tolerances for Animals [1]. The MTLs are established according to the scientific literature and are based on animal health but not human health. These MTLs are used extensively by Food and Drug Administration (FDA) to make regulatory decisions on tolerable and toxic levels of feed ingredients and finished feeds for animals. The Association of American Feed Control Officials (AAFCO) Official Publication contains guidance involving acceptable deviation of a nutrient from the label guarantee

*Corresponding author: Susie Y. Dai, Phone:979-845-1121. Fax:979-845-1389. Email:susie@otsc.tamu.edu,Office of the Texas State Chemist, Texas A&M AgriLife Research, Texas A&M University System, College Station, Texas 77841, United States

**Corresponding author: Timothy Herrman, Phone:979-845-1121. Fax:979-845-1389. Email:susie@otsc.tamu.edu,Office of the Texas State Chemist, Department of Soil and Crop Science, Texas A&M AgriLife Research, Texas A&M University System, College Station, Texas 77841, United States

[11].

In Europe, the Directive 2002/32/EC sets regulatory limits for undesirable substances such as arsenic (As), Cd, Pb, and mercury (Hg) in animal feed. Even though the maximum limits for As, Cd, Pb and Hg have been established, animal feed can be contaminated with other heavy metals such as nickel (Ni) and Cr due to manufacturing process. For example, Ni has been shown to be immunotoxic and neurotoxic and may be carcinogenic [12]. Particularly, the most toxic heavy metals and minerals include Hg, Cd, Pb, As, Cr, Cu, Zn, and tin (Sn) [2, 13].

Several heavy metals are essential elements and added to animal feed to balance the micro minerals. Copper is an essential trace element that plays vital roles in human and animal health. The World Health Organization (WHO) recommended a minimal acceptable intake of 1.3 mg/day for adults [14]. However, it is common to observe Cu deficiency in grazing cattle diets and the common symptom shows poor growth rates as well as severe periods of copper deficiency can lead to anemia. As such, Cu is usually supplemented as copper sulfate, or tribasic copper chloride [15]. The maximum tolerance limit for copper in animal feed is 40 ppm as recommended by NRC [1].

Selenium is a trace element naturally present in many foods and plays critical roles in reproduction, DNA synthesis, hormone metabolism, and protection from infection and oxidative damage [16]. Selenium deficiency alone or in combination with a second stress can lead to many diseases. Because of selenium deficiency, cattle can develop a nutritional myopathy which affects heart and skeletal muscles [15]. Furthermore, the reproductive complications may involve retained placenta in cows and reproductive failures in bulls. Feed concentrations of Se that slightly above nutritional requirements are toxic to animals and should be allowed only in a premix form. The current US FDA regulation allows the use of sodium selenite, sodium selenate, or selenium yeast as sources of selenium supplementation of complete feeds for chickens, swine, turkeys, sheep, cattle and ducks at the maximum level of 0.3 ppm [17]. The average annual feed tonnage distributed in Texas totals about 18 million tons during the 5 years of the current study (from 2010 to 2014) [18]. This accounts for approximately 11% of the US tonnage based on the International Feed Industry Federation report [19]. Texas leads the nation in cattle and calves, and sheep and goat operations and ranks No. 6 for poultry and milk operations [20]. The United States accounts for 21% of the worlds beef supply in 2011 and Texas shares more than 20% of the total US production [21]. Safe animal feed is not only a local and regional issue, but also has national and global impact on food safety.

Risk assessment as a discipline includes hazard identification, hazard characterization, exposure assessment and risk characterization outlined by the Food and Agriculture Organization [22]. Risk assessment is a preliminary step in a food safety risk management program similar to the one administered by FFCS of the Office of the Texas State Chemist (OTSC). This study was performed to establish a baseline for heavy metal contamination in animal feed ingredients and finished feed in Texas to facilitate future exposure assessment and regulatory risk management.

2. Materials and Methods

2.1. Study Design

A total of 564 feed samples were collected and analyzed for heavy metal content during the study period October 1, 2010 through May 31, 2015. In 2011 and 2012, samples included fish meal, minerals and vitamin/mineral premix. In 2013 the scope of product was expanded to include beef cattle feed and was further expanded in 2014 to include feed for horses, poultry, sheep and goats, and wildlife (Table 1).

2.2. Sample collection

All samples were collected in the state of TX at manufacturing and distributing establishments designated in the OTSC work plan. Dry feed samples were greater than 2 kg and collected using clean scoops or triers. The samples were placed in poly-lined leak resistant paper bags capable of holding 5 kg. Bags were sealed with a unique identifier containing year, product type, inspection area and sample number in two dimensional barcode and marked with a sticker designating heavy metal sample. The shipment adhered to a chain-of-custody protocol including placement in a sealed shipment bag that also contains accompanying paperwork including investigator notes and product label. Product categories were assigned to samples consistent with AAFCO terms and official definitions [23].

2.3. Sample preparation

Animal feed were ground through a Retsch Ultra Centrifugal Mill SR3, (Haan, Germany) using a 0.75 mm diameter screen, split using a commercial riffler (Carpco SS-16-25) twice, four corner mixed, and placed into a 100 ml plastic bottle.

2.4. Sample analysis and reagents

Samples were analyzed as follows: 0.5 gram of dry feed samples were predigested in 3:1 of HNO_3:HCl overnight before the microwave digestion (MARS Xpress, CEM Corp, Matthews, NC). The digestion was performed at 200 °C and then cooled samples were diluted with deionized water. The diluted samples were then analyzed by an inductively coupled plasma (ICP) optical emission spectrometer (OES) in 2011 (Vista Pro, Varian, Walnut Creek, CA) and by an ICP mass spectrometry (ICPMS Nexion 300 X, Perkin Elmer, Waltham, MA) in 2012-2015. The limit of quantification of the ICPMS method is 1 mg/kg for all elements except for Hg which is 0.01 mg/kg and for Cu which is 10 mg/kg. Reagent grade chemicals were used unless otherwise specified and deionized water (17 M or higher) was used to prepare the reagents and materials. Nitric acid and hydrochloride acid solutions are all of trace metal grade and obtained from VWR (Radnor, PA).

2.5. Statistical data analysis

For the purposes of statistical analysis, if the element concentration is below the limit of quantification it was treated as zero. Data were analyzed using the Excel descriptive data function. Pearsons correlation coefficients were calculated using SAS software (ver. 9.3, SAS Institute, Cary, NC) between all the heavy metals and other elements in this study.

Fiscal year	Feed type	Number of samples (N)	Number of detectable heavy metals (N)[b]
2011	Fish meal	9	0
	Minerals	58	23
	Premix	14	0
2012	Fish meal	18	18
	Minerals	47	38
	Premix	8	6
2013	Beef cattle	22	22
	Fish meal	16	16
	Horse	3	3
	Minerals	62	61
	Poultry feed	1	1
	Premix	7	7
	Swine feed	1	1
	Wildlife feed	1	1
2014	Beef cattle	100	99
	Horse feed	4	4
	Minerals	18	14
	Poultry feed	3	3
	Premix	8	8
	Sheep & goat feed	6	6
	Swine feed	2	2
	Wildlife life	4	4
2015	Beef cattle	40	40
	Fish meal	4	4
	Horse feed	8	8
	Liquid feed	13	13
	Minerals	37	36
	Poultry feed	7	7
	Premix	14	14
	Sheep & goat feed	14	14
	Swine feed	9	9
	Wildlife feed	7	7

Table 1.Feed samples analyzed for heavy metal contents during the fiscal years of 2011 and 2015.

[a] The definition of feed type follows the definition by AFFCO (2014). [b] The value represents the number of samples contaminated at least one of heavy metals analyzed during the period.

3. Results and discussion

3.1. Frequency of Heavy Metals

In 2011, twenty three of the 81 samples evaluated for heavy metal using ICP-OES possessed detectable levels of heavy metal including four samples with As, ten samples with Cd and twelve samples with Pb. The level of detectable As contamination ranged between 11 to 33 ppm (Table 2) with the highest concentration occurring in Zinc Oxide; for Cd, between 5 ppb to 82 ppb with the highest concentration occurring in ammonium polyphosphate solution; for Pb, 12 to 349 ppm with the highest concentration occurring in zinc oxide powder.

In 2012, the samples with detectable heavy metal increased to 78% in response a change in the instrumentation using an ICP-MS and the addition of Mercury to the contaminants analyzed in feed samples. The level of detectable As contamination ranged between 1 to 77 ppm with the highest concentration occurring in manganous oxide; for Cd, between 1 ppm to 13.4 ppm with the highest concentration occurring in copper sulfate crystals; for Pb, 1 to 69 ppm with the highest concentration occurring in zinc oxide powder; for Hg, between 0.02 ppm to 0.456 ppm with the highest concentration in a mineral premix containing Calcium Carbonate, Zinc Oxide, Manganous Oxide, Ferrous Sulfate, Ethylenediamine Dihydriodide and Cobalt Carbonate.

In 2013-2015, the samples with detectable heavy metal increased to 98% resulting from the addition of Chromium, Copper, Molybdenum, Selenium and Nickel to elements analyzed in feed samples and low detection levels achieved by the ICP MS.

3.2. Heavy Metal Results

The concentrations of heavy metals by product type and year from 2012-2015 are presented in Table 3. The heavy metal level was significantly higher in mineral products compared to complete feed considered with the exception of Ni in horse feed. Mineral products displayed a positive skewness above one for all heavy metals analyzed in this study indicating a few

Heavy metal	Mean (mg/kg)	SD[b] (mg/kg)	Median (mg/kg)	Kurtosis	Skewness	Max (mg/kg)
As	1.2	5.1	0.0	28.5	5.1	33.0
Cd	1.5	3.7	0.0	7.4	2.7	18.0
Pb	20.5	62.7	0.0	17.1	4.0	349.0

Table 2. Concentration of arsenic (As), cadmium (Cd), and lead (Pb) in mineral feeds (N=58) produced in Texas during the fiscal year of 2011[a].

[a] Fish meals (N=9) and premixes (N=14) analyzed in 2011 did not contain As, Cd, and Pb elements. [b] Standard deviation.

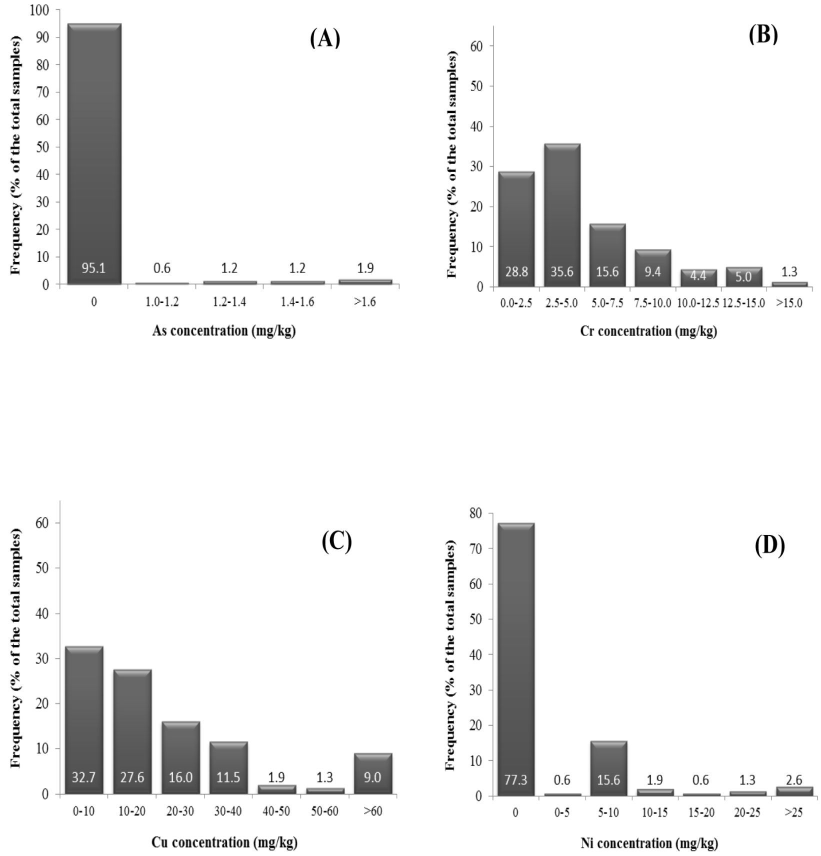

Figure 1. Histogram showing the relative frequency of As (A), Cr (B), Cu (C), and Ni (D) contamination in beef cattle during 2012-2015.

products containing levels well above the mean, as evident by the maximum value (Table 3). In some cases, these high levels result from the product delivering the element such as the nutrient Cu in premix (label guarantee 11060 ppb with Cu from Copper Chloride and Copper Sulfate) and mineral supplement (label guarantee 50,000 ppm with Cu from Copper Sulfate). The premix category contained heavy metal content and distribution that aligned more closely with mineral ingredients rather than complete feed. For finished feed, the level of heavy metal contaminant mean concentration, standard deviation and skewness were lower and the kurtosis value higher compared to the mineral and premix products. Complete feed contains protein and energy sources as well as micro-ingredients, thus exerting a dilution effect on heavy metal contaminants.

3.3. *Copper (Cu)*

Copper was detected in most of the complete feed and feed supplements (Table 3). In beef cattle feed, the mean Cu content was 37.8 mg/kg with a skewness value of 7.7 resulting from supplements used for cattle on pasture with a Cu content between 53 to 1050 mg/kg. A total of 19 beef feeds contained greater than 40 mg/kg of which 3 products were the sole feed consumed by the cattle. Two of the 19 products used Copper Chloride as the source of Cu, the remaining 17 contained Copper Sulfate. Eight of the products contained Cu on the label, all products contained feeding instructions. The AAFCO Nutrient Guarantee Required by Species under the AAFCO Model Bill and Regulations does not require Cu guarantee for beef, swine, poultry, or dairy complete and supplement feeds [23]. One poultry feed contained 1170 mg/kg Cu from Copper Sulfate in a vitamin and trace mineral concentrate and did not contain a Cu label guarantee. One goat feed with a label guaranteed

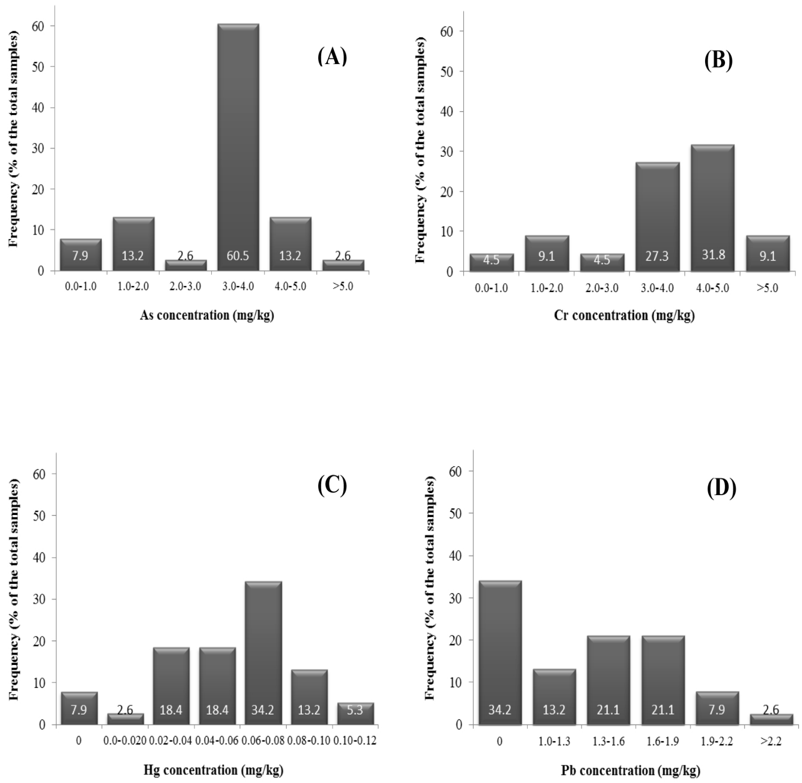

Figure 2. Histogram showing the relative frequency of As (A), Cr (B), Hg (C), and Pb (D) contamination in fish meal during 2012-2015.

analysis for Cu (15 mg/kg minimum and 25 mg/kg maximum) contained 115 mg/kg from added Copper Sulfate. Of the 12 swine feeds, five were concentrates that contained greater than 200 mg/kg with added Copper Sulfate and no label guarantee.

Copper is essential for enzyme function and stabilization of collagen and elastin, in energy metabolism, pigmentation, antioxidant defense system, and iron metabolism [1]. Sheep are sensitive to Cooper toxicity followed by cattle and goats. Ruminants typically develop hemolytic anemia, with liver and kidney lesions common to all species. The maximum tolerable level for cattle, sheep and goats is 40 and 15 ppm in complete feed [1]. Earlier report by Li et al. [6] presented the mean Cu concentration in alfalfa hay, haylage, corn silage and corn grain ranges between 3.7 to 6.8 mg/kg with the standard deviation between 1.2 to 5.3 mg/kg. Furthermore, the mean for corn grain mix, soybean protein mix ranges between 38.2 to 45.9 mg/kg with the standard deviation between 46.7 to 72.8 mg/kg based on Wisconsin dairy rations. Another study also suggested that a plant barrier would typically limit Cu concentrations in plant tissue, even though the plants grew in soils of varied Cu concentrations or not [24].

In the present study, the feed type resembled the grain mix or soybean protein mix presented in the earlier report. Since the earlier complete data set from Li et al. [6] was not accessible, a comparison between results of the two studies is not possible. While earlier studies suggested that excessive amounts of metals in animal feed are usually the outcome of human actions [25], our results indicate Cu is added to beef, poultry, goat and swine feed rations in TX as a nutrient.

3.4. Selenium (Se)

Selenium was detected in 8 of the 162 beef cattle feed samples, 6 of which had label guarantees ranging from 5 to 570 ppm. The Code of Federal Regulations [17] 21:573.920 does not require Selenium be included on the label for feed premixes. Of the 6 premixes with a Selenium guarantee, Sodium selenite was the principle ingredient used to add Selenium and one premix contained Selenium Yeast. Four of the six feeds with a Selenium guarantee exceeded the AAFCO analytical variation, containing level approximately three fold above the label guarantee.

The mean concentration for fish meal is 1.1 mg/kg with a standard deviation of 1.5 mg/kg. Most feed formulators take fish meal into consideration as part of a diet. The understanding of the Se concentration in fish meal is important to estimate the Se concentration in the final complete feed even though the total ration is not likely to be based on the Se concentration of the fish meal. The mean value for Se in our sample premixes is 74.6 mg/kg with a standard deviation being 69.5 mg/kg. Fish meal shows the lowest Se concentration compared to other feed types ($P < 0.05$).

Heavy metal	Feed type[a]	N[b]	Mean (mg/kg)	SD[c] (mg/kg)	Median (mg/kg)	Kurtosis	Skewness
As[d]	Beef cattle	162	0.15	0.92	0.0	104.0	9.6
	Fish meal	38	3.21	1.34	3.6	0.8	-0.8
	Mineral	164	6.38	10.80	2.45	17.6	3.7
	Poultry	11	1.11	3.68	0.0	11.0	3.3
	Premix	36	3.39	2.44	3.2	-0.3	0.5
	Swine	12	0.11	0.38	0.0	12.0	3.5
Cd[e]	Beef cattle	162	0.08	1.00	0.0	162.0	12.8
	Liquid feed	13	0.59	1.00	0.0	1.5	1.6
	Minerals	164	1.42	2.31	0.0	5.8	2.2
	Premix	36	0.54	1.04	0.0	2.6	1.8
Cr	Beef cattle	160	4.91	3.92	4.0	1.9	1.3
	Fish meal	22	3.32	1.75	3.2	1.3	0.7
	Horse feed	15	7.28	4.98	6.0	0.6	1.3
	Liquid feed	13	8.31	6.18	8.0	-1.1	0.3
	Mineral	124	40.43	87.48	9.3	16.5	3.9
	Poultry feed	11	4.02	4.70	2.2	10.2	3.2
	Premix	29	26.51	44.40	13.0	21.2	4.4
	Sheep & goat feed	20	4.15	2.89	3.0	0.9	1.3
	Swine feed	12	3.00	2.30	2.5	1.5	1.4
	Wildlife feed	12	5.04	2.56	4.0	-0.5	0.7
Cu	Beef cattle	156	37.8	99.0	18.0	72.6	7.7
	Fish meal	4	29.5	34.3	27.0	-5.6	0.1
	Horse feed	13	25.9	30.6	18.0	7.1	2.4
	Liquid feed	13	64.2	28.3	72.0	-0.8	-0.7
	Mineral	55	2845.8	7409.5	5.4	13.4	3.4
	Poultry feed	10	132.7	364.6	15.5	10.0	3.2
	Premix	22	2267.7	2748.5	1582.0	5.1	2.1
	Sheep & goat feed	20	22.0	23.5	17.0	12.9	3.4
	Swine feed	12	169.9	236.8	66.0	7.4	2.6
	Wildlife feed	11	41.9	23.7	45.0	-1.14	0.1
Pb[f]	Beef cattle	162	0.14	0.57	0.0	31.3	5.2
	Fish meal	38	1.11	0.86	1.45	-1.3	-0.2
	Horse feed	15	0.07	0.28	15.0	15.0	3.9
	Minerals	164	9.90	24.2	1.95	47.5	6.3
	Premix	36	2.34	3.13	1.45	2.3	1.7
	Swine feed	12	0.18	0.61	0.0	12.0	3.5
Hg[g]	Beef cattle	162	0.00	0.002	0.0	162.0	12.7
	Fish meal	38	0.056	0.029	0.062	-0.5	-0.3

	Mineral	164	0.032	0.102	0.0	16.2	4.0
	Premix	36	0.002	0.007	0.0	9.4	3.3
Mo[h]	Beef cattle	151	0.950	0.793	1.1	1.4	0.7
	Horse feed	13	1.569	0.926	1.4	-0.6	-0.4
	Liquid feed	13	0.408	0.539	0.0	-1.9	0.6
	Minerals	55	4.078	6.222	1.3	2.8	1.9
	Poultry feed	10	1.340	0.622	1.35	1.5	-0.9
	Premix	22	2.977	2.391	2.45	4.0	1.8
	Sheep & goat feed	20	1.125	0.973	1.1	4.5	1.5
	Swine feed	12	1.467	0.677	1.3	1.1	-0.4
	Wildlife	11	1.918	0.610	1.8	-0.8	0.5
Ni[i]	Beef cattle	154	2.81	7.43	0.0	17.6	4.0
	Horse feed	13	5.62	8.65	0.0	5.0	2.1
	Liquid feed	13	0.39	1.39	0.0	13.0	3.6
	Mineral	55	56.91	138.38	15.0	21.1	4.5
	Poultry feed	10	1.00	3.16	0.0	10.0	3.2
	Premix	22	26.46	31.92	19.0	14.9	3.6
	Sheep & goat feed	20	2.30	6.43	0.0	14.9	3.7
	Swine feed	12	1.50	5.20	0.0	12.0	3.5
	Wildlife feed	11	2.09	2.95	0.0	-1.5	0.8
Se[j]	Beef cattle	162	3.5	37.0	0.0	159.7	12.6
	Fish meal	22	1.1	1.5	0.0	-1.1	0.8
	Mineral	121	110.7	199.8	4.0	6.8	2.3
	Poultry feed	11	3.4	11.2	0.0	11.0	3.3
	Premix	28	74.6	69.5	59.5	0.3	0.9
Tl[k]	Mineral	124	0.27	1.79	0.0	56.8	7.5

Table 3. Concentration of arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), molybdenum (Mo), nickel (Ni), selenium (Se), and thallium (Tl) in livestock feeds produced in Texas during the fiscal years of 2012–2015.

[a] The definition of feed type follows the definition by AFFCO. [b] N = sample size. [c] Standard deviation. [d] As was not detected in horse feeds (N=15), liquid feeds (N=13), sheep & goat (N=20), and wildlife feeds (N=12). [e] Cd was not detected in fish meals (N=38), horse feeds (N=15), poultry feeds(N=11), sheep & goat feeds (N=20), swine feeds (N=12), and wildlife feeds (N=12). [f] Pb was not detected in liquid feeds (N=13), poultry feeds (N=11), sheep & goat feeds (N=20), and wildlife feeds (N=12). [g] Hg was not detected in horse feeds (N=15), liquid feeds (N=13), poultry feeds (N=11), sheep & goat feeds (N=20), swine feeds (N=12), and wildlife feeds (N=12). [h] Mo was not detected in beef cattle feeds (N=154), fish meals (N=4), horse feeds (N=13), liquid feeds (N=13), poultry feeds (N=11), sheep & goat feeds (N=20), swine feeds (N=12), and wildlife feeds (N=11). [i] Ni was not detected in fish meals (N=4). [j] Se was not detected in horse feeds (N=15), liquid feeds (N=13), sheep & goat feeds (N=20), swine feeds (N=12), and wildlife feeds (N=12). [k]Tl was not detected in beef cattle feeds (N=159), fish meals (N=22), horse feeds (N=15), liquid feeds (N=13), poultry feeds (N=11), premixes (N=29), sheep & goat feeds (N=20), swine feeds (N=12), and wildlife feeds (N=12).

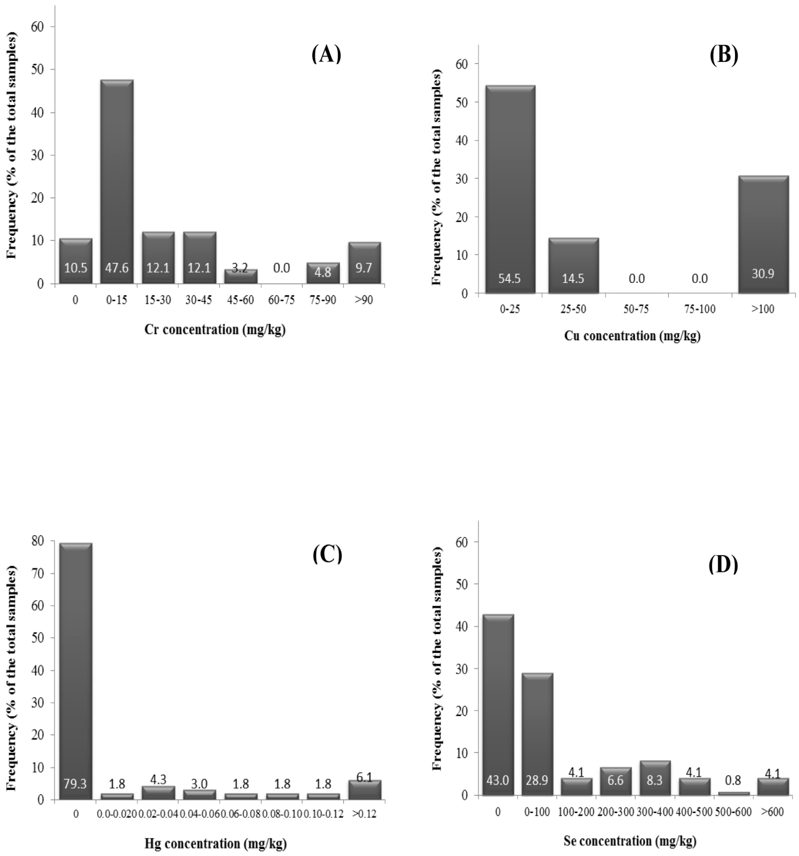

Figure 3. Histogram showing the relative frequency of Cr (A), Cu (B), Hg (C), and Se (D) contamination in mineral during 2012-2015.

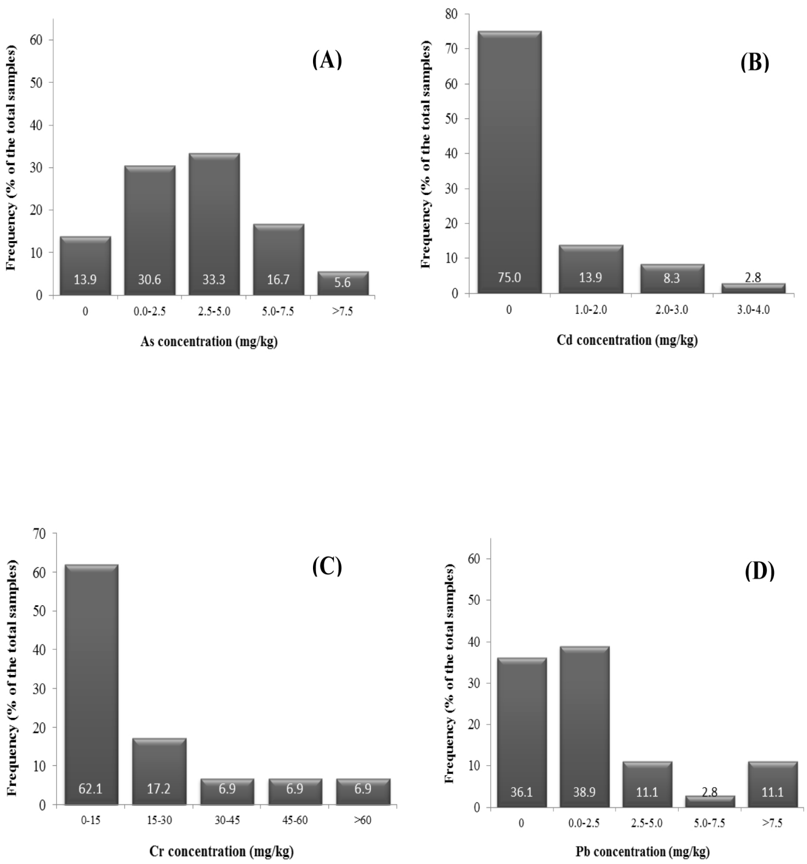

Figure 4. Histogram showing the relative frequency of As (A), Cr (B), Hg (C), and Pb (D) contamination in premix during 2012-2015.

Under the current FDA regulation, 0.1 ppm (equivalence of 1 mg/kg) of Se can be added in complete feed for broilers, chicken layers and breeders. A level of 0.3 ppm is allowed for addition of Se to sheep, beef, and dairy complete feed. For addition of Se to a salt-mineral mix, FDA limits are 90 and 120 ppm, for sheep and cattle respectively [26]. Selenium deficiency is a more widespread problem than selenium toxicity [1]. Most of the clinical signs associated with selenium deficiency are associated with vitamin E deficiency, such as white muscle disease in ruminants. For this reason, selenium injections are often administered to ruminants especially calves. Acute signs are manifested by vomiting, difficult breathing, muscle spasms and death. Calves die of respiratory and cardiac failure. Chronic poisoning of all species is produced by grazing selenium accumulator plants, or overfeeding inorganic selenium feeds. Animals exhibit bilateral alopecia, loss of appetite, lameness and dystrophic hoof growth. The maximum tolerable level of selenium in cattle feed is 5 mg/kg of complete feed [1].

3.5. Arsenic (As)

Arsenic was detected in 8 of the 162 beef cattle feed samples. Two of the products were poultry waste and three were hydrolyzed feather meal, suggesting the source of contamination could originate from As in poultry diets. Of the 8 products with As at detectable levels, 6 had violative levels of other heavy metals including Zn, Cu, Se, and Cd. Arsenic is widely present in fish meal (36 of the 38 samples), with the mean value of 3.21 mg/kg with the standard deviation of 1.34 mg/kg. In fish meal, As was significantly correlated with the two other elements, Pb ($r = 0.644$) and Hg ($r = 0.594$) ($P < 0.001$). This might be attributed to the fact that Pb, and Hg are preferentially bio-accumulated in fish tissues or the fish has recently been exposed to high dietary or water levels of these three elements. Previous studies reported that such elements are found to be at high levels in fish bone and internal organs and tissues, such as kidney, liver, gills, and muscles [27, 28, 29]. The 36 premix samples that we surveyed in this study have the mean value of 3.39 mg/kg with a standard deviation of 2.44 mg/kg.

Previous studies have suggested that arsenic has beneficial action in low amounts. Some organic arsenic compounds have been used in swine and poultry to improve weight gain [1]. Organic arsenicals as growth promoters in animals have been removed from the market due to human health concerns. The toxicity of As is dependent on the chemical valence and form, as inorganic As is usually more toxic than organic As [30]. The EU maximum allowable level of As in general feed materials is 2 mg/kg, and for feed material of fish, other aquatic animals and products derived thereof is 25 mg/kg [31]. The maximum level of As in premix is 12 mg/kg. The NRC maximum tolerable level is 30 ppm for cattle and 5 ppm for fish.

Thus, the As concentrations in beef cattle feed, and fish meal generally do not exceed the regulation limit set by EU and the NRC MTL. The highest concentration of As found in the premix is 9.2 mg/kg. The histogram of As concentration in fishmeal and premix is presented in Figs. 2(A) and 4(A). The figure shows that majority of the premix is below the allowable maximum level of As in mineral feed of 12 mg/kg[31]. Even though some of the premix samples contain more than the maximum allowable level of As in mineral feed, they are not intended as a complete feed but will be used after dilution and mixing with other ingredients.

3.6. Cadmium (Cd)

Cadmium was not detected in fish meals, horse feeds, poultry feeds, sheep & goat feeds, swine feeds, and wildlife feeds, but was detected in beef cattle, liquid feed, minerals and premix samples. The mean value of Cd concentration in premix is 0.54 mg/kg with a standard deviation of 1.04 mg/kg. Our findings are consistent with the previous report of Li et al. [6], where they reported the mean value of 1.58 mg/kg with a standard deviation of 0.7 mg/kg in mineral mix fed to Wisconsin dairy cattle. Nicholson, Chambers, Williams, & Unwin [7] reported detection of 0.19 mg/kg (mean value) of Cd in dairy cattle feed and 1.79 mg/kg (mean value) in minerals, which is also consistent with our results. A survey of Cd in cattle feed and cattle manure reported the presence of Cd in cattle feed in farms of different herd sizes, with the mean value between 0.38 to 2.31 mg/kg in Northeast China [32]. However, other reports suggested that mean value of Cd concentration in pig, cattle and chicken feed at 2.29, 2.79 and 8.13 mg/kg in Beijing and Fuxin, China [33]. It has been suggested that Chinese animal production might have a unique presence and distribution of the heavy metal Cd. The histogram of Cd concentration in premix is presented in Fig. 4(B).

Cadmium is not an essential nutrient for animals or humans. Accumulation of Cd was seen in human kidney and liver, which can lead to kidney disease including proteinuria and kidney stone formation [12]. Recently, cadmium was classified as a carcinogen, which is carcinogenic to experimental animals after oral exposure and has been shown to pose hazards to fish and aquatic invertebrates [34, 1]. In general, the liver and kidney are the primary target organs of cadmium toxicity in most species. Cadmium tends to bio-accumulate in the kidney and, to a lesser extent, in the liver. The NRC maximum tolerable level is 10 ppm for all species [1].

3.7. Chromium (Cr)

Chromium was detected in all feed types. The mineral shows the highest concentration with the mean value to be 40.43 mg/kg with a standard deviation of 87.48 mg/kg ($P < 0.05$). Cr was detected in all but 13 of the beef cattle feed samples with the mean value for Cr is 4.91 mg/kg and a standard deviation of 3.92 mg/kg. In fish meal, the mean value is 3.32 mg/kg with a standard deviation of 1.75 mg/kg. In the Wisconsin dairy farm study, Cr was reported to be 69 mg/kg with a standard deviation of 61 mg/kg. Nicholson reported the mean of 42 mg/kg of five mineral samples. Sullivan, Douglas, & Gonzalez [35] have found that the Cr content of minerals used for feed ingredients may contain Cr ranging from 60 mg/kg to 500 mg/kg. As a result, contamination of Cr in animal feed can occur with addition of phosphorous-containing minerals, and/ or the blending process during which stainless steel containers and processors, which typically contain 18% Cr. The histogram of Cr concentration in fishmeal and premix is presented in Figs. 2(C) and

4(B). The Cr concentration levels trended below the NRC [1] MTL level.

Chromium was found to be involved with sugar and lipid metabolism [36] and was shown to be a necessary trace element for mammalian diet. Chromium dietary supplements have been a common practice in the United States and that is only second to calcium supplements. Although Cr (+3) has been proven to be essential for biological pathways, such as glucose metabolism, studies also showed that high concentrations of Cr can reduce weigh gain for chicks and rats [1]. However, there is no daily allowance established for Cr (+3) in human consumption. Hexavalent Cr (+6) is the most toxic, and a carcinogen, allergen and acute irritant in humans. Organic Cr (+3), such as chromium picolinate, is more bioavailable than inorganic Cr (+3), but both are much less bioavailable than Cr (+6). The greater toxicity of the hexavalent form may be due to its reduction to +3, +4 and +5 intermediates that induce free radical which binding to intracellular macromolecules. The NRC maximum tolerable level is 100 ppm for soluble Cr (+3) in swine, horse, cattle sheep and 500 ppm for poultry.

3.8. *Mercury (Hg)*

Mercury was not detected in beef cattle premixes or complete feed, but was detected in both fish meal and premixes. The fish meal shows a higher mean value of Hg (0.056 mg/kg) with a standard deviation of 0.029 mg/kg than the premix with a mean value of 0.002 mg/kg and a standard deviation of 0.007 mg/kg ($P < 0.05$). In fish meal, Hg concentration is significantly correlated with Pb concentration ($r = 0.564$, $P < 0.01$). Johnston & Savage [37] reported mean concentration of Hg in fish meal of 0.02 to 7.7 mg/kg. It has been suggested that the Hg concentration in fish meal may depend on the fish species and geographic locations [29]. For example, fish meal produced from whole fish caught in the North Sea, Norwegian Sea and Barents Sea, the Hg concentration was 0.01 kg/mg [38]. Contrary to that, the mean Hg concentration in whale meal has been recorded to be 10.5 mg/kg in previous study [39]. As bioaccumulation of heavy metals by aquatic organisms has been reasonably documented by the research field, our analysis showed that there is Hg presence in the majority of the fish meal samples but was not detected in the complete beef cattle feed. About 16% of the premix shows contamination of Hg. The histogram of the Hg in fish meal samples was shown in Fig. 2 (C). The range of Hg concentration in fish meal was between 0 to 11mg/kg.

Mercury is quite toxic to both animals and humans. Fish have a the natural tendency to accumulate organic mercury [40]. Inorganic mercury primarily targets the species but also causes peripheral neurotoxicity while chronic organic mercury exposure affects the peripheral and central nervous system [1]. The maximum tolerable level is 0.2 and 1 to 2 mg/kg of feed for inorganic and organic Hg, respectively.

3.9. *Lead (Pb)*

The mean value for beef cattle feed is 0.14 mg/kg with the standard deviation of 0.57 mg/kg. In fish meal the mean value for Pb is 1.11 mg/kg with the standard deviation being 0.86 mg/kg. Premixes had a mean Pb concentration of 2.34 mg/kg with the standard deviation of 3.13 mg/kg and the highest concentration detected is 11.6 ppm. The EU regulation sets the maximum level for Pb in feed material is 10 ppm and the NRC MTL is 100 ppm for cattle and sheep, 10 ppm for swine, horse and poultry. Lead is the most common contaminate observed by other research groups. Li et al. [6] reported that the mean concentration of Pb in the feed samples were all below 1 mg/kg with the exception of mineral premixes, whose mean value was 2.857 mg/kg with the standard deviation being 2.483 mg/kg. Earlier reports by Nicholson, Chambers, Williams, & Unwin [7] suggested that Pb concentrations in corn silage were lower than 1 mg/kg in the United Kingdom. The same study suggested that the mean Pb concentration for beef cattle feeds was less than 1 mg/kg. A study by Garcia & Rosentrater [5] showed that the median Pb concentration in meat and bone meal in North America to be 1.16 mg/kg. As some samples in the meat and bone meal study contained Pb below the method limit of detection (LOD) of 0.3 mg/kg, the study only reported the median value for the survey. The trends in our study are similar for Pb contamination in other studies, which suggest that the mineral premixes tend to contain higher Pb content than other feed types. Previous studies show that lead biotransfer factor can be approximately 50 times greater than cadmium from feed to milk [41]. The histogram of Pb concentration in fishmeal and premix is presented in Figs. 2(D) and 4(D). While 10 ppm in premix is generally not a concern to the animals considering the final inclusion rate for feed manufacturing, high concentrations at hundreds of ppm lead in premix can be a potential problem.

Lead is a commonly used element in industry, such as lead batteries. Humans, especially children, are susceptible to lead poisoning by causing neurological and cognitive deficits [42]. Exposure to high concentrations of Pb can lead to blood disorders in mammals [42]. In animals and humans, cardiovascular, hematological and neurological signs occur at the lowest levels of Pb exposure whereas renal, gastrointestinal, hepatic and immunologic signs occur with higher doses or longer exposure times [1]. Lead impairs normal bone growth and remodeling as indicated by decreased bone density and bone calcium content. The maximum tolerable level in animal feed is 10 mg/kg for poultry, swine, fish and horses; and 100 mg/kg for cattle and sheep.

3.10. *Thallium (Tl)*

Thallium and its compounds are highly toxic. Historically Tl has been used for rodenticide but abandoned in many countries due to human safety concerns. Thallium has rarely been reported in the literatures for animal feed contamination. Our study shows that there is no incidence of Tl contamination in beef cattle feed and fish meal. Currently there are no regulatory limits established for Tl in animal feed. The US Environment Protection Agency (EPA) has set the maximum contamination level goals is 0.0005 mg/L and the maximum contamination at 0.002 mg/L in drinking water. All 5 samples tested containing more than 1 mg/kg Tl are minerals, as Tl has been largely associated with potassium based minerals in granites, soil and clays

[43]. Our analysis suggested that the probability of Tl contamination in animal feed is low.

4. Declaration of Conflicting Interest

The authors declare no conflicts of interest.

5. Acknowledgment

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6. Article information

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