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Original Article

# Concentrations of Cadmium and Lead in Milk and Feed in Dairy Farms in a Region Located in Kunming City, Yunnan Province, China.

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## Summary

Raw cow's milk (test milk) and feed samples were obtained from two dairy farms in the coastal area of southeast Dianchi Lake, Kunming City, Yunnan Province, China in July and September, 2013, to determine their concentrations of cadmium (Cd) and lead (Pb) by inductively coupled plasma mass spectrometry (ICP-MS). As references, the concentrations of Cd and Pb in raw milk (control milk) and feed samples of cows conventionally reared in Japan were also determined. Concentrations of Cd were not significantly different between test milk (0.39 ± 0.23 μg/l in July and 1.73 ± 2.47 μg/l in September) and control milk (0.55 ± 0.26 μg/l). However, concentrations of Pb were higher in test milk (23.37 ± 6.83 μg/l in July and 26.22 ± 10.83 μg/l in September) than in control milk (10.43 ± 5.23 μg/l) ($P < 0.01$). The mean concentrations of Pb in test milk in July and September exceeded the maximum recommended level established by the European Commission, and the concentrations of Pb in some feeds from the study farms (corn stem and leaf feed and broad-bean stem feed) were also above the values given in Japanese standards. These results indicate the possibility of Pb contamination in milk and feeds at dairy farms in the present study area. Hence, special attention should be paid to Pb residues in milk and feed at dairy farms in this region of China.

## Introduction

Cadmium (Cd) and lead (Pb) have received considerable attention for their adverse effects on human health, and children and pregnant women are particularly sensitive to such toxic elements[1)]. Regular exposure to small amounts of Pb may have serious effects on growing children, including difficulties in mental development (e.g. reading and learning disabilities), adverse effects on kidney function, blood chemistry and the cardiovascular system, and hearing degradation[1, 2)]. A previous study[3)] on pregnant women living in an area close to a copper smelter suggests that exposure to Cd and Pb could be related to the development of complications in pregnancy such as threatened spontaneous abortion, toxemia, and anemia, due to increased lipid peroxidation.

China is the world's most populous country and its economy is growing at the fastest rate of any major country; its environmental problems are getting worse. A recent investigation[4)] in global scale demonstrated that the levels of Cd and Pb in the blood of women from China were higher than those in the blood of women from Croatia, the Czech Republic, Poland, Slovakia, Slovenia, Sweden, Ecuador or Morocco. In addition, other studies[5-7)] have concluded that there is a high prevalence of Pb poisoning among children in China. Food is one of the main sources of Cd and Pb exposure, and Cd and Pb contamination in food has been a matter of great concern in China.

Kunming City, Yunnan Province, China has developed in its industrialization and urbanization. The coastal area of southeast Dianchi Lake, located in Kunming City, is the biggest vegetable and flower supplier for Kunming City. In order to increase the productivity of the farmland, large amounts of chemical fertilizer and animal manure are applied in the area. This kind of intensive agriculture practice can lead to the accumulation of Cd and Pb in the soil, and crops grown in such soil tend to be contaminated by Cd and Pb[8, 9)]. Dairy farmers in the area make use of large amounts of residues and market irregulars from the vegetables and flowers as roughage (e.g. 28.4-50.0 kg/head /day on a fresh weight basis[10)]). Therefore, dairy cows may absorb excessive quantities of Cd and Pb through

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vegetable and flower residues and other fodder grown in the area. Furthermore, Cd and Pb may be transferred into milk, which could cause a serious health problem, especially for infants and pregnant women. Therefore, it is essential to determine whether cow's milk is free from such toxic elements.

The present study aimed to determine the concentrations of Cd and Pb in cow's milk and feed in the coastal area of southeast Dianchi Lake, and to compare them with those in reference milk and feed produced in Japan.

## Materials and Methods

### Sample collection

A total of 21 raw milk (test milk) samples and 15 feed samples were collected from the coastal area of southeast Dianchi Lake (24.7° N, 102.7° E) where there were 30 dairy farms around a milking parlor located in the center of the village. Two farms were selected to obtain individual milk and feed samples during visits in July and September, 2013. On the farms, fermented corn residue, corn grain and purchased feed were fed as concentrates, and rice straw, broad-bean stem, corn stem and leaf and weeds from the coastal area of Dianchi Lake or around farms were fed as roughage. Vegetable and flower residues and seasonally available market irregulars, such as broccoli leaf, broccoli stem, carnation, Chinese cabbage and stem lettuce, were also fed to dairy cows. Furthermore, for reference, 5 raw milk (control milk) samples and 6 feed samples of cows conventionally reared in Japan were also collected from the Dairy Station at the Kyoto Prefectural Agriculture, Forestry and Fisheries Technology Center (35.3° N, 135.2° E) in August, 2013. All milk samples were taken in the morning.

### Chemical analysis

A micro wave system (ETOS-D, Milestone General, Kawasaki, Japan) was used for acid digestion. One ml of milk was combined with 8 ml of concentrated nitric acid and 2 ml of hydrogen peroxide. The milk digestion program consisted of 4 steps: (1) 250 W for 2 minutes, (2) 0 W for 3 minutes, (3) 250 W for 5 minutes and (4) 400 W for 10 minutes. Feed samples were dried in a drying oven at 60℃. Dried samples of 0.5 g were combined with 7 ml of concentrated nitric acid and 1 ml of hydrogen peroxide. The feed digestion program consisted of 5 steps: (1) 250 W for 2 minutes, (2) 0 W for 3 minutes, (3) 250 W for 5 minutes, (4) 400 W for 5 minutes and (5) 500 W for 5 minutes. After digestion, the samples were diluted to 50 ml. Concentrations of Cd and Pb in milk and feed were determined by inductively coupled plasma mass spectrometry (ICP-MS) (Elan6000, Perkin Elmer, Norwalk, CT, USA). The limits of detection (LODs) were calculated as the triplicate of the standard deviation of the mean reagent blank values. The LODs used in the present study were 0.16 μg/l for Cd and 4.09 μg/l for Pb in the milk analysis and 0.33 μg/kg for Cd and 2.56 μg/kg for Pb in the feed analysis.

The accuracy of the analysis was checked using certified reference materials (CRMs; milk powder NCS ZC73015 and tomato leaves ARM 1573a). Because CRMs have no data for Cd in milk powder and Pb in tomato leaves, spiked milk or feed samples for 10 μg/l were used to calculate spiked recovery. The CRM recoveries were 92% for Pb with milk powder and 87% for Cd with tomato leaves. The spiked recoveries were 84% for Cd in the spiked milk samples and 105% for Pb in the spiked feed samples. The results of quality assurance testing were acceptable.

### Statistical analysis

All data were analyzed using GLM procedure of Statistical Analysis System (SAS 1998). The values below the LODs were treated to be half of the LODs. Datasets were not normally distributed and thus log-transformation was conducted before analysis. Differences in concentrations of Cd and Pb in milks among two test milks in the study area and control milk were tested by Tukey-Kramer method. A probability level of $P < 0.05$ was considered statistically significant.

## Results

The concentrations of Cd and Pb in test and control milk are shown in Table 1. The mean concentrations of

**Table 1** Concentrations (μg/l) of Cd and Pb in milk samples

| | | Cd | Pb |
|---|---|---|---|
| Test milk * | | | |
| July | Mean (±SD) | 0.39 ± 0.23 | 23.37 ± 6.83[b] |
| (n = 12) | range | ND – 0.80 | 12.45 – 37.10 |
| | CV (%) | 59 | 29 |
| September | Mean (±SD) | 1.73 ± 2.47 | 26.22 ± 10.83[b] |
| (n = 9) | range | ND – 7.85 | 11.20 – 46.30 |
| | CV (%) | 142 | 41 |
| Control milk ** | | | |
| August | Mean (±SD) | 0.55 ± 0.26 | 10.43 ± 5.23[a] |
| (n = 5) | range | 0.20 – 0.90 | ND – 15.63 |
| | CV (%) | 48 | 51 |

[a, b] Values with different superscripts significantly differ ($P < 0.05$).
* Collected in the dairy farms near Dianchi Lake.
** Collected in the Dairy Station in Kyoto, Japan.
CV: Coefficient of variation.
ND: Not detected.
SD: Standard deviation.

Cd in test milk in July and September and control milk were 0.39 μg/l, 1.73 μg/l and 0.55 μg/l, respectively. The concentrations of 3 samples in July and 2 samples in September were below the LOD. Although the mean concentration of Cd was three to four times higher in test milk in September than test milk in July and control milk, concentrations of Cd were not significantly different among 3 milk groups due to the large variation of Cd in test milk in September (CV: 142%). The mean concentrations of Pb in test milk in July and September and control milk were 23.37 μg/l, 26.22 μg/l and 10.43 μg/l, respectively. The concentration of 1 sample in control milk was below the LOD. In contrast to Cd, concentrations of Pb were significantly higher in test milk samples from both July and September than in control milk ($P < 0.01$).

The concentrations of Cd and Pb in feeds are shown in Table 2. The mean concentrations of Cd in concentrates on a dry matter basis were similar in the samples from the study farms (71.1 μg/kg) and those from Japan (67.1 μg/kg), while the mean concentration of Cd in roughage was higher in the samples from the study farms (372.1 μg/kg) than in those from Japan (30.6 μg/kg). The mean concentrations of Pb in concentrates and roughage on a dry matter basis were higher in the samples from the study farms (629.0 μg/kg and 2537.4 μg/kg, respectively) than in those from Japan (174.3 μg/kg and 264.1 μg/kg).

## Discussion

### Concentrations of Cd and Pb in cow's milk

Table 3 shows a comparison of the concentrations of Cd and Pb in cow's milk between the present study and those reported in the literature in studies on different countries[11-13]. The concentrations of Cd in the present milk samples were almost within the ranges of those reported in previous works, with the exception of the highest value (7.85 μg/l). However, the concentrations of Pb in the present test milk samples were higher than those reported in Spain[11] and Iran[12]. Furthermore, the mean concentrations of Pb in test milk were above the maximum recommended levels established by the European Commission (20 μg/kg) [14], even though they were below those established by China (50 μg/kg)[15]. Values exceeding 20 μg/l in the present study were measured in 66.67% of samples in July and 55.56% of samples in September in test milk.

The present results suggest the possibility of Pb con-

**Table 3** Comparisons of the concentrations (μg/l) of Cd and Pb in cow's milks between the present study and the literatures in studies on different countries

| | Cd | Pb |
|---|---|---|
| The present study | | |
| Test milk * | ND − 7.85 | 11.20 − 46.30 |
| Control milk ** | 0.20 − 0.90 | ND − 15.63 |
| The literatures *** | | |
| Spain | ND − 2.345 | ND − 5.613 |
| Iran | 0.28 − 3.43 | 1.84 − 20.7 |
| Croatia | 1.0 − 20.0 | 1.0 − 476.0 |
| MRL **** | | |
| European Commission | NE | 20 |
| China | NE | 50 |

* Collected in the dairy farms near Dianchi Lake.
** Collected in the Dairy Station in Kyoto, Japan.
*** Quoted from Rey-Crespo et al. (2013)[11]; Rahimi (2013)[12]; Bilandzic et al. (2011)[13].
**** Quoted from EC (2006)[14]; SAC (2010)[15].
ND: Not detected.
MRL: Maximum recommended level.
NE: Not established.

**Table 2** Concentrations (μg/kg) of Cd and Pb in feed samples on a dry matter basis

| The study farms | n | Cd | Pb | The Dairy Station in Kyoto, Japan | n | Cd | Pb |
|---|---|---|---|---|---|---|---|
| Concentrates | | | | | | | |
| Fermented corn residue | 1 | 134.1 | 1109.6 | Conventional dairy concentrates | 1 | 66.1 | 157.5 |
| Corn grain | 1 | 21.6 | 315.5 | Vitamin and mineral mix | 1 | 68.1 | 191.1 |
| Purchased feed | 1 | 57.6 | 461.8 | | | | |
| Mean | | 71.1 | 629.0 | | | 67.1 | 174.3 |
| Roughage | | | | | | | |
| Rice straw | 1 | 57.9 | 3060.5 | Alfalfa hay | 1 | 28.9 | 59.5 |
| Broad-bean stem | 1 | 159.3 | 3923.3 | Corn silage | 1 | 40.6 | 286.3 |
| Corn stem and leaf | 1 | 412.2 | 10171.7 | Crane hay | 1 | 26.1 | 365.1 |
| Amaranth weed | 1 | 149.9 | 2125.0 | Italian grass silage | 1 | 26.8 | 345.6 |
| Knotgrass | 1 | 35.8 | 2752.5 | | | | |
| Poaceae weed | 1 | 711.1 | 1669.4 | | | | |
| Manchurian wild rice | 1 | 13.9 | 1179.6 | | | | |
| Vegetable and flower residues | | | | | | | |
| Broccoli leaf | 1 | 289.4 | 420.1 | | | | |
| Broccoli stem | 1 | 309.0 | 695.9 | | | | |
| Carnation | 1 | 386.9 | 2516.0 | | | | |
| Chinese cabbage | 1 | 290.8 | 415.1 | | | | |
| Stem lettuce | 1 | 1649.2 | 1519.8 | | | | |
| Mean | | 372.1 | 2537.4 | | | 30.6 | 264.1 |

tamination of raw milk from the dairy farms under consideration. In addition, concentrations of Pb in bulk milk samples collected from the tank in the milking parlor in the study area were investigated as a reference of the mean values of milk in the study area, and the obtained reference values (18.15 μg/l in July and 19.05 μg/l in September) were slightly below 20 μg/l. However, since Pb is a potentially bioaccumulative toxin[16], bulk milk can be expected to exceed the recommended EU level in the near future unless special measures are taken.

With respect to human Cd and Pb tolerance, tolerable weekly intakes (TWIs) have been set for Cd at 2.5 μg/kg body weight by the European Food Safety Agency[17] and for Pb at 25 μg/kg body weight by the Codex Alimentarius Commission[18]. On the assumptions that daily milk intake is 200 ml[12] and that people drink milk with the same Cd and Pb levels as test milk, weekly intakes of Cd and Pb were calculated at 0.54 μg and 32.72 μg in July and 2.42 μg and 36.71 μg in September, respectively. The numerical example indicated that since the contribution of milk intake to a TWI of 70 kg adults of Cd (175 μg) and Pb (1750 μg) were small, Cd and Pb exposure from regular intake of milk would be small. Nevertheless, Cd and Pb exposure for children and pregnant women should be minimized as much as possible in order not to increase their exposure to toxic levels in the future.

### Concentrations of Cd and Pb in feed

The concentrations of Cd in roughage from the study farms were generally greater than the ranges reported in previous works: roughage, 62-74 μg/kg in America[16]; and grass silage, < 100 μg/kg in England and Wales[19], on a dry matter basis. In contrast, the concentrations of Cd in roughage in Japan were below or within these ranges. The concentrations of Cd in concentrates from the study farms and from Japan were below the ranges reported in previous works: corn grain, 181 μg/kg and soybean mixture, 259 μg/kg in America[16]. Accordingly, it was inferred that the concentrations of Cd in feed were higher on the present study farms than in Japan. In spite of the large differences in the concentrations of Cd in feed, no significant differences were observed in Cd concentrations in milk samples. These results are consistent with previous findings[20-23] that the concentration of Cd in milk is not increased by a high dietary concentration of Cd due to limited transportation of Cd into milk by the mammary glands.

The concentrations of Pb in concentrates and roughage from the present study farms were generally above the ranges reported in previous works: corn grain, 134 μg/kg; soybean mixture, 237 μg/kg; and roughage, 198-271 μg/kg in America[16] and grass silage, < 1000 μg/kg in England and Wales[19], on a dry matter basis. In contrast, those in Japan were below or within these ranges. Moreover, concentrations of Pb in corn stem and leaf feed (9.20 mg/kg on a fresh weight basis) and broad-bean stem feed (3.33 mg/kg on a fresh weight basis) from the present study farms were higher than the Japanese standard of Pb (3 mg/kg on a fresh weight basis) [24]. The higher concentrations of Pb in feed can be related to the significantly higher values in test milk than in control milk.

The reason for the high Pb levels in feed, especially in roughage, appears to be the high levels of Pb released into the environment. In the present study area, Pb releases may be high since there is a phosphorus mining site and heavy traffic with old vehicles and large trucks that produce unclean exhaust and dust. Emitted Pb due to such pollution can penetrate and contaminate soil. Furthermore, intensive application of manure and chemical fertilizers to soil may also elevate soil contents of Pb[8] .

In addition, the high Pb levels in feed in the present study area could be partly explained by soil features. According to the 'plant nutrient uptake' theory, ions in soluble forms (exchangeable and carbonate) are readily taken up by plants[25, 26]. This suggests that Pb may be mobile and bioavailable in acidic soil[26]. The soil in the present study area was characterized as acidic[27], suggesting increased plant availability of Pb.

In conclusion, the present study found Pb contamination of milk and feed at the study farms. Hence, special attention should be paid to Pb residues in milk and dairy feed at farms in the study area.

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