

*Short Communication*

# Heavy Metal Concentrations in Five Tissues of Chickens from a Mining Area

Ping Zhuang\*, Bi Zou, Huanping Lu, Zhian Li

<sup>1</sup>Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510560, China

*Received: 5 June 2013*

*Accepted: 9 July 2014*

## Abstract

This study presents the effect of metal-enriched rice on concentrations of Pb, Cd, Zn, and Cu in the tissues of chicken. The experimental group of chicken (fed with contaminated rice) accumulated high concentrations of Pb (1.00, 0.73, 0.52, 0.99, and 13.8 mg/kg dw in kidney, liver, muscle, blood and feather, respectively), being 2, 1.2, 3.5, 3, and 3.5 times the corresponding tissue Pb in the control group of chicken. Liver seemed to be the primary tissue for Cd accumulation. High concentrations of Pb and Cu were found in feathers compared to other tissues. Lead concentrations in the muscle and liver of the experimental group and liver Cd concentrations in both experimental and control groups exceeded the maximum permissible limits of Pb and Cd in foods of PR China, respectively. The results indicated that there were significant effects of metal-enriched rice on Pb and Cd accumulations in chicken, and this dietary exposure pathway poses a potential health risk to local residents.

**Keywords:** heavy metals, chicken, accumulation, health risk, Dabaoshan Mine

## Introduction

Heavy metal contamination is of worldwide concern due to food safety issues and human health risk through the food chain. Once contaminated with metals, soils can be a potential source of contamination for plants and animals for a long time [1]. Several studies have focused on the transfer of heavy metals from soil to animals either by direct contamination or via vegetation [2, 3]. Heavy metals may accumulate in the vegetation, posing a risk for animals and humans, although soil is a biochemically active filter for most metals [4]. Previous toxicological investigations on bovines, poultry, etc. have shown a direct correlation between metal concentrations found in animal feed and in animal tissues [2, 5]. Thus information about heavy metal concentrations in animals through the food chain is important for assessing their risks to human health.

Avian species such as birds and ducks are susceptible to bioaccumulation of pollutants mainly through the consumption of contaminated food [6, 7]. For the higher trophic level of the food web, chickens can serve as a useful bioindicator species for environmental monitoring [8]. Concentrations of environmental contaminants in tissues of chickens can be used to evaluate chronic or acute exposure, as chickens are fed a wide variety of feed stocks [9, 10]. The metal accumulations in chickens via contaminated diets have been investigated in a few studies to assess the potential human risk from poultry consumption [4, 11].

Dabaoshan Mine is the largest metal mine located in southern China. The soil and water around the mine area have been contaminated by acid mine drainage discharged from the mine site [12]. It has been reported that rice and vegetables growing in the vicinity of the mine are contaminated by heavy metals and pose a great potential health risk for residents, who are suffering from serious cancers [12]. In particular, remarkably high Pb and Cd levels were mea-

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\*e-mail: zhuangp@scbg.ac.cn

sured in rice, which serves as the staple food for local residents and feed for poultry. Due to increasing concern about the toxic metal intake of chickens fed with contaminated rice, this study was undertaken. Thus the aims of this study are to determine whether metal-enriched rice affects the concentrations of Pb, Cd, Zn, and Cu in chicken tissues, and hence to estimate if the edible parts consumed by local residents exceed the Chinese and international permissible limits.

## Materials and Methods

Forty-eight chicks (100-150 g) were divided into two groups. The experimental group was fed with rice (grown in contaminated soil around the mine area and containing high metal levels), and the control group was fed with uncontaminated fodder (bought from market). The concentrations of Pb, Cd, Zn, and Cu in the metal-enriched rice and chickenfeed are shown in Table 1. After feeding for 150 days, blood samples of the chicken were obtained by caudal venipuncture using a chilled, heparinized disposable needle and syringe. Following feather collection, kidney, liver and the pectoral muscle were separately dissected from the body of the specimens, frozen in liquid nitrogen, and freeze-dried. Feather samples were washed vigorously three times in deionized water, alternating with acetone, to remove loosely adherent external contamination, then air-dried overnight.

Chicken tissues and feather samples were digested by  $\text{HNO}_3$  (16 mol/L) and  $\text{H}_2\text{O}_2$  (30%) as described by Jeffrey [7]. The concentrations of Zn and Cu were determined by flame atomic absorption spectrophotometry (AAS, Model 3030, Perkin-Elmer, USA), and tissues with low Pb and Cd concentration were measured by graphite-furnace atomic absorption spectrometry (GAAS, Model 3030, Perkin-Elmer, USA). Spikes and blanks were run at a frequency of at least 5% of the total number of samples. Certified reference materials GBW 08552 (pig muscle) and GBW 08551 (pig liver) for chicken samples were used for quality control. Spike recoveries ranged from 96% to 102%. Recovered concentrations of the certified samples were within 5% of the certified values. All elemental concentrations (mg/kg) in tissues were estimated on a dry weight basis.

A two-tailed student t-test was used to test for significant differences of heavy metal concentrations between the experimental group of chickens fed with metal-enriched rice and the control group of chickens.

## Results and Discussion

The feeding experiment lasted for 150 days, with no apparent toxic response observed in the experimental group. However, the average weight of chickens fed with metal-enriched rice was lower than that of control chickens, and the experimental chickens contained darker red livers and smaller testicles than the control group. The final

Table 1. Heavy metal concentrations (mg/kg, n=4) in the metal-enriched rice and fodder for both groups of chicken.

|                      | Pb        | Cd          | Zn        | Cu        |
|----------------------|-----------|-------------|-----------|-----------|
| Rice                 | 1.27±0.41 | 0.24±0.03   | 37.8±6.01 | 5.97±1.15 |
| Fodder               | 0.29±0.04 | 0.013±0.001 | 121±10.1  | 17.8±3.64 |
| p-value <sup>a</sup> | < 0.0001  | < 0.0001    | < 0.0001  | < 0.0001  |

<sup>a</sup> p-value for t-test comparing between rice and fodder

weight of chickens was 1,087 g and 1,526 g for the experimental group and control group, respectively. The heavy metal accumulations in kidney, liver, muscle, blood, and feathers of chickens fed with metal-enriched rice and fodder are shown in Tables 2 and 3. Levels varied among heavy metals and tissues. There was a significant difference ( $p < 0.05$ ) between the two groups of chickens in tissue Cd concentrations.

Lead concentrations of chickens fed with metal-enriched rice ranged from a minimum of 0.52 mg/kg in the muscle to 13.8 mg/kg in the feathers (Table 2). For control chickens, the lowest and highest concentrations were recorded in the muscle (0.15 mg/kg) and feathers (3.95 mg/kg), respectively. There were significant differences ( $p < 0.01$ ) between the two groups of chickens in tissue Pb concentrations, except for livers. Lead concentrations in livers of chickens (0.62-0.73 mg/kg) in this study were lower than those in black-crowned night heron chicks (mean 0.92 mg/kg) reported by Kim et al. [8], and chickens fed with insects (3.62 mg/kg) reported by Zhuang et al. [4]. Lead has been responsible for acute incidents of bird poisoning, and Burger and Gochfeld [6] suggested that Pb levels as low as 0.4 mg/kg in blood can result in adverse physiological effects, while 4 mg/kg in feathers is associated with negative effects on behavior, locomotion, and depth perception resulting in lowered nestling survival. Compared with the control chickens, feather and blood Pb concentrations of chickens fed with metal-enriched rice exceeded these toxicity levels, which were directly related to relatively high Pb levels in rice grown on the soil contaminated by the discharge of effluents from Dabaoshan mine [12].

There were some significant differences ( $p < 0.05$ ) for all tissue concentrations of Cd between the experimental and control group of chickens (Table 2). Concentrations of Cd in tissues of both groups of chickens were in the descending order of liver > kidney > feather > muscle > blood. Cadmium concentrations in the liver (9.36 mg/kg) and kidney (4.64 mg/kg) of chickens fed with metal-enriched rice were higher than those of Siberian gull from Hara biosphere reserve in southern Iran (1.1 and 2.2 mg/kg, [13]), and black-crowned night heron chicks (1.00 and 2.20 mg/kg, [8]). As a result, it is suggested that high Cd concentrations in livers of chickens fed with metal-enriched rice are a consequence of the diet containing a high Cd level (0.24 mg/kg) in rice grown near Dabaoshan Mine. Scheuhammer [9] reported that liver-to-kidney ratios for Cd more than 1 indicate acute exposure to relatively high

Table 2. Concentrations of Pb and Cd (mg/kg, dry weight) in different tissues of experimental and control groups of chickens.

|    |                           | Kidney    | Liver     | Muscle      | Blood       | Feather    |
|----|---------------------------|-----------|-----------|-------------|-------------|------------|
| Pb | Experimental group (n=25) |           |           |             |             |            |
|    | Mean±SD                   | 1.00±0.51 | 0.73±0.29 | 0.52±0.22   | 0.99±0.29   | 13.8±3.83  |
|    | Min-Max                   | 0.22-1.22 | 0.30-1.41 | 0.30-1.07   | 0.67-1.56   | 6.04-20.8  |
|    | Control group (n=23)      |           |           |             |             |            |
|    | Mean±SD                   | 0.58±0.31 | 0.62±0.22 | 0.15±0.09   | 0.38±0.15   | 3.95±1.16  |
|    | Min-Max                   | 0.52-2.61 | 0.34-0.85 | 0.04-1.21   | 0.16-0.47   | 2.58-6.76  |
|    | p-value <sup>a</sup>      | < 0.0001  | 0.11      | 0.0002      | < 0.0001    | < 0.0001   |
| Cd | Experimental group (n=25) |           |           |             |             |            |
|    | Mean±SD                   | 4.64±1.56 | 9.36±3.60 | 0.059±0.021 | 0.042±0.016 | 0.51±0.16  |
|    | Min-Max                   | 1.87-7.73 | 4.69-20.4 | 0.02-0.08   | 0.02-0.07   | 0.22-0.89  |
|    | Control group (n=23)      |           |           |             |             |            |
|    | Mean±SD                   | 1.73±0.52 | 7.34±1.18 | 0.039±0.018 | 0.027±0.021 | 0.18±0.12  |
|    | Min-Max                   | 1.03-2.64 | 5.24-9.84 | 0.02-0.08   | 0.004-0.07  | 0.063-0.58 |
|    | p-value                   | < 0.0001  | 0.016     | 0.002       | 0.015       | < 0.0001   |

<sup>a</sup>p-value for t-test comparing the experimental and control groups of chicken

Table 3. Concentrations of Zn and Cu (mg/kg, dry weight) in different tissues of experimental and control groups of chickens.

|    |                           | Kidney    | Liver     | Muscle    | Blood     | Feather   |
|----|---------------------------|-----------|-----------|-----------|-----------|-----------|
| Zn | Experimental group (n=25) |           |           |           |           |           |
|    | Mean±SD                   | 259±53.8  | 190±29.0  | 42.0±12.2 | 27.3±3.76 | 157±14.9  |
|    | Min-Max                   | 146-356   | 142-238   | 20.6-58.5 | 19.7-33.1 | 136-202   |
|    | Control group (n=23)      |           |           |           |           |           |
|    | Mean±SD                   | 229±52.6  | 221±42.5  | 169±31.2  | 107±14.3  | 166±15.3  |
|    | Min-Max                   | 148-305   | 174-382   | 112-232   | 90.5-123  | 129-195   |
|    | p-value <sup>a</sup>      | 0.064     | 0.008     | < 0.0001  | < 0.0001  | 0.051     |
| Cu | Experimental group (n=25) |           |           |           |           |           |
|    | Mean±SD                   | 1.31±0.12 | 2.34±0.64 | 1.43±0.41 | 0.91±0.24 | 26.8±7.12 |
|    | Min-Max                   | 1.11-1.58 | 1.55-3.21 | 0.94-2.42 | 0.56-1.36 | 17.7-34.6 |
|    | Control group (n=23)      |           |           |           |           |           |
|    | Mean±SD                   | 1.26±0.11 | 5.18±0.99 | 1.43±0.33 | 0.71±0.16 | 11.8±4.62 |
|    | Min-Max                   | 1.03-1.58 | 2.04-6.66 | 1.00-2.09 | 0.48-1.01 | 7.96-26.0 |
|    | p-value                   | 0.18      | < 0.0001  | 0.99      | 0.003     | < 0.0001  |

<sup>a</sup>p-value for t-test comparing the experimental and control groups of chicken

dietary cadmium concentrations, whereas less than 1 indicates chronic exposure to low levels. In this study, the ratio of Cd liver-to-kidney in chickens was consistent with acute background exposure. This trend was reported in some wild birds, suggesting that the local and species differences of acute and/or chronic contamination by cadmium are attributable to cadmium concentrations in avian diets and environmental pollution [9].

Zinc concentrations in the kidney, liver, muscle, blood, and feather of both groups of chickens are presented in Table 3. There was a 4-fold difference between the two groups for Zn levels in the muscle and blood. The concentrations of Zn in liver (190-221 mg/kg) in this study were below the Zn toxicity level (more than 280 mg/kg in liver) reported by Sileo et al. [10], who described the clinical signs and diagnosis of Zn poisoning in birds in the laboratory and natural

Table 4. Maximum permissible level of metals (mg/kg) in poultry set by Chinese and international standards.

|                      | Pb  | Cd                              | Zn  | Cu |
|----------------------|-----|---------------------------------|-----|----|
| FAO/WHO <sup>a</sup> | 0.1 | 0.05 for meat,<br>0.5 for liver | 20  | 1  |
| EC <sup>b</sup>      | 0.1 | 0.05 for meat,<br>0.5 for liver | 20  | 1  |
| CN <sup>c</sup>      | 0.2 | 0.1 for meat,<br>0.5 for liver  | 100 | 10 |

<sup>a</sup> FAO/WHO 2002 Codex Alimentarius, Schedule 1 of the proposed draft Codex general standards for contaminants and toxins in food. Joint FAO/WHO Food Standards Programme, Codex Committee, Rotterdam. Reference CX/FAC 02/16.

<sup>b</sup> EC (The Commission of the European Communities) 2006 Commission Regulation No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Communities, L 364/18-19.

<sup>c</sup> MHPRC (Ministry of Health of the People's Republic of China) the limits of pollutants in foods (GB 2762-2005 for Pb and Cd, GB 15199-1994 for Cu, GB 13106-1991 for Zn). Beijing, China: MHPRC [In Chinese]

environments. Zinc concentrations (229-265 mg/kg) in chicken kidneys from the present study were higher than those in Siberian gull (91.1 mg/kg, [13]) and in little egret chicks from Korea (18.5 mg/kg, [8]). Zn concentrations in liver, muscle and blood of control chickens were significantly higher ( $p < 0.01$ ) than those in experimental chickens – a result of the relatively high Zn level in the fodder fed the control group. It is also suggested that essential elements in chickens are in the normal range in this study, and are maintained there by homeostatic mechanisms.

There was no significant difference between the two groups of chickens with respect to Cu concentrations in kidneys and muscles (Table 3). Concentrations of Cu in tissues of the two groups were in the descending order of feather > liver > muscle > kidney > blood. The Cu concentrations in the liver of both groups were lower than those reported in black-crowned night heron chicks (47.9 mg/kg, [5]) and seabirds from the Pacific coast of Canada (20.5-25.4 mg/kg, [14]). Concentrations of Cu in feathers of chickens fed with metal-enriched rice were more than 2 times higher than those in control chickens, suggesting that concentrations of essential elements (e.g. Zn, Cu) in feathers were not related to dietary concentrations [5].

The high concentrations of Pb and Cu in feathers compared to other tissues imply that chickens are successful at sequestering Pb and Cu in feathers, a result in agreement with the finding that feathers can play the role both of storing and of eliminating metals [3, 15]. This pattern also suggests that feathers provide a reliable indication of the different metal concentrations in the environment.

Elevated heavy metals in edible parts of the chicken can pose a potential health risk to humans who consume them. It was a negative result that Pb concentration in the muscle of chickens fed with Pb-contaminated rice exceeded the maximum permissible level (MPL) for Pb in meat (Table 4)

prescribed by China (MHPRC 2005) and international standards. The Pb concentrations in the liver (0.31 mg/kg ww, estimated from dw concentration and moisture) and muscle (0.15 mg/kg ww, estimated from dw concentration and moisture) of the experimental group of chickens exceeded the Chinese (0.2 mg/kg) and EC (0.1 mg/kg) limits. The concentrations of Cd in the liver of both groups of chickens were 6-8 times higher than Chinese and EC limits (Table 4). The mean levels of Zn and Cu in both groups of chickens were below the Chinese and international MPL (Table 4). Therefore, the livers of chicken should not be consumed because of the high Pb and Cd levels in both the experimental and control groups.

Cultivation of foodcrops for human or livestock consumption on contaminated soil can potentially lead to the uptake and accumulation of trace metals in the edible plant parts, with a resulting risk to human and animal health [16]. In the present study, metal-enriched rice significantly affected the concentration of metals in chicken tissues compared with the control group, because the rice was grown in contaminated soil around the mine area. Therefore, we suggest that tissue Pb and Cd concentrations of chickens fed with metal-enriched diets can reflect environmental contamination of poultry production sites. In conclusion, some local residents, whose consumption of meat and internal organs of chickens fed with the local forages, is higher than average, and who also consume contaminated locally grown foodcrops, or breathe contaminated air, or drink contaminated water, might be exposed to a high potential human health risk from heavy metals.

## Conclusions

The concentrations of Pb, Cd, Zn, and Cu found in muscles, kidneys, feathers, and blood of chickens feeding on metal-enriched rice grown in the vicinity of Dabaoshan Mine are relatively higher than the control group of chickens. The highest levels of Cd and Zn were found in liver and kidney, which are known specific target organs of metal bioaccumulation. This study showed that the high Pb and Cu levels measured in the feathers of chickens imply that chickens are successful at sequestering metals through feathers. As Cd and Pb are known to be a highly toxic compound to which chronic exposure results in severe diseases or even death, there is an urgent need to initiate an extensive epidemiological study of people consuming products (vegetables and meat) originating from the Dabaoshan Mine area.

## Acknowledgements

This research was financially supported by the National Natural Science Foundation of China (No. 40871221 and No. 41301571). Authors are grateful to Prof. Murray B. McBride (Cornell University, USA) for checking language of the manuscript. We thank the anonymous reviewer for suggestions and comments.

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