

## Tissue Levels of Lead in Experimentally Exposed Zebra Finches (*Taeniopygia guttata*) with Particular Attention on the Use of Feathers as Biomonitor

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**Abstract.** We tested experimentally whether zebra finch feathers can be used as a biomonitor for lead pollution, and we examined whether lead exposure influences the accumulation of zinc into feathers. Two groups of eight adult male zebra finches were dosed with, respectively, 0 and 25 ppm lead as lead acetate in their drinking water. After 30 days, lead-treated zebra finches accumulated significantly higher lead concentrations in brain, fat, kidney, liver, muscle, testes, and regrown outer tail feathers than control individuals. Lead levels in regrown outer tail feathers were significantly higher than in original outer tail feathers in the exposed group. The concentration of lead in original (not regrown) fifth tail feathers at the end of the experiment was significantly higher than lead levels in the original outer tail feathers. Our results indicate that lead in regrown feathers originates both from internal deposition and external contamination through the excretion of the uropygial gland during preening. Lead levels in regrown feathers were significantly correlated with levels in liver, kidney, and muscle, suggesting that feathers can be used as a biomonitor for lead. We found that lead had an influence on the metabolism of zinc. Zinc concentrations in the regrown feathers were significantly lower in the lead-treated group although zinc levels in the liver did not differ significantly. Moreover, lead and zinc concentrations in the feathers were significantly negatively correlated.

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Lead is the most ubiquitous toxic heavy metal in the environment (Goyer 1996). Although it can originate from natural processes, such as erosion and volcanism, most lead enters the food chain from anthropogenic sources, such as industrial emissions and the combustion of leaded gasoline (Burger 1993). Numerous biomonitoring studies have been set up to evaluate lead contamination in the environment. Birds have been used frequently and successfully as bioindicators or monitors to assess environmental lead contamination (Pain *et al.* 1995; Mateo *et al.* 1997). However, to examine the risk lead

poses on birds, laboratory toxicity tests are also required (Burger 1995). Most laboratory studies focussed on bird species frequently used in biomonitoring programs, such as raptors (Custer *et al.* 1984; Hoffman *et al.* 1985) and waterfowl (Heinz *et al.* 1999; Beyer *et al.* 2000). Recently field studies have demonstrated the feasibility of small passerines as sentinels for terrestrial lead contamination (Llacuna *et al.* 1995; Eens *et al.* 1999; Johnson *et al.* 1999; Dauwe *et al.* 2000). Small passerines have several characteristics rendering them suitable as biomonitors for terrestrial point source contamination (Eens *et al.* 1999). Therefore, experimental studies on the accumulation, metabolism, and effects of heavy metals in passerines are necessary to be able to interpret field data.

Feathers have been used extensively as a biomonitoring tool for lead the past 20 years, and their use has increased dramatically (Burger 1993). Lead is incorporated in the feather during the short period of feather growth when the feather is connected with the blood stream through small blood vessels. When the feather is fully grown these vessels atrophy, isolating the feather from the rest of the body. Once feather growth has ceased, the feather cannot receive additional lead from the blood; however, there is still a potential for lead concentrations to increase from external deposition. Feathers have limitations as well as strengths as a pollution research tool, and the clearer our understanding of the factors that affect deposition in feathers, the more accurate our interpretation will be (Burger 1993). Several studies have reported that lead levels in feathers can originate partially from external deposition (Goede and de Bruin 1984; Burger 1993; Kim *et al.* 1998). Still, few experiments have investigated the transfer of lead into the feather.

The primary aim of this study is to investigate the accumulation of lead into the feather and in other tissues of a small passerine, the zebra finch (*Taeniopygia guttata*). To determine the accumulation of lead in the feathers we plucked the outermost tail feathers at the beginning of the experiment and at the end of the experiment when the feathers were fully regrown. To investigate the effect of external contamination on the concentration of lead in the feather, we also determined the lead level in other, not regrown tail feathers at the end of the experiment. In a field study with nestling great (Parus major) and blue tits (*P. caeruleus*), it was found that high lead exposure resulted in lower levels of zinc in the feathers (Dauwe *et*

*al.* 2000). Therefore, the present study also deals with the possible influence of high lead exposure on the accumulation of zinc in the feathers.

## Materials and Methods

Twenty-four male zebra finches were housed individually in small breeding cages (60 × 50 × 40 cm) on a 14:10 light:dark cycle. All groups received *ad libitum* access of food, water, and extra calcium supply. After an acclimatization period of 18 days, we randomly divided the zebra finches into three groups of eight individuals: one group was kept as control, and the other two groups were given drinking water with, respectively, 25 and 100 ppm ( $\mu\text{g ml}^{-1}$ ) lead as lead acetate in reconstituted water. Water was provided in small drinking tubes and freshly dosed water was prepared every 2 days (Scheuhammer 1996). After 30 days' exposure all birds were sacrificed by decapitation; brain, fat, kidney, liver, pectoral muscle, and testes tissue were removed from each bird for lead analysis. To determine the accumulation of lead in the feathers we plucked the outermost tail feathers (sixth tail feather) at the beginning of the experiment and at the end of the experiment, when the feathers were fully regrown. Additionally we collected the original (not regrown) fifth tail feathers at the end of the experiment to assess the external contamination of lead on the feather surface caused by excretion of the uropygial gland during preening. To establish the effect of lead on the accumulation of zinc we determined zinc levels in the regrown feathers and in the liver.

Both internal tissues and feathers were washed with ultra-pure water (Milli-Q), dried and their weights recorded. Samples were digested in a 1:1 mixture of  $\text{HNO}_3$  and  $\text{H}_2\text{O}_2$  with the microwave digestion procedure as described by Blust *et al.* (1988). Blanks and standard reference materials were prepared in the same manner as the samples and analyzed for quality assurance. Lead levels in internal organs were analyzed by atomic absorption spectrophotometry (AAS) using a Varian SpectrAA-800. Lead and zinc levels in feathers and zinc levels in the liver were determined by inductively coupled plasma-atomic emission spectrophotometry (ICP-AES) using a Varian Liberty Series II (De Wit and Blust 1998). All lead and zinc concentrations are reported on a dry-weight basis.

We used Statistica statistical software (StatSoft 1994) to perform statistical analysis. Nonparametric Mann-Whitney U tests were used to compare results between groups. To compare lead concentrations between the original and the regrown sixth tail feathers and the fifth tail feathers we used nonparametric Wilcoxon signed rank tests. Nonparametric Spearman rank correlation coefficients were calculated between lead levels in the different tissues. Also, Spearman rank correlations were used to examine correlations between lead and zinc levels in both feathers and liver tissue. For comparison between groups the level of significance was set at  $\alpha = 0.05$ . However, when carrying out multiple comparisons (*i.e.*, for the correlation matrix),  $\alpha$  was adjusted using a Bonferroni correction (Sokal and Rohlf 1981) to correct for the increased probability of type I errors. To maintain an experiment-wise error rate at the 0.05 level across all six individual comparisons, the alpha level was set at 0.008.

## Results

Three males exposed to 100 ppm lead died within 10 days, presumably from lead poisoning. Although the sample size was small, concentrations in brain, liver, muscle, and testes were significantly higher than in the males given 25 ppm lead during 30 days (Mann-Whitney U test,  $p < 0.05$ ). Lead levels in kidney did not differ significantly (Mann-Whitney U test,  $p >$

0.05), however mean lead concentrations were two times higher in the males that died than in the males given 25 ppm lead. We stopped the treatment with 100 ppm, and all remaining males survived.

Before the start of the experiment the outermost tail feathers were plucked. Lead (Mann-Whitney U test,  $p > 0.9$ ) and zinc concentrations (Mann-Whitney U-test,  $p > 0.5$ ) in the initial feathers did not differ significantly between the two groups.

Zebra finches exposed to 25 ppm lead had significantly higher levels of lead in brain, fat, kidney, liver, muscle, testes, and the regrown feathers than the control group (Table 1). The difference between groups was highest for liver and kidney. Concentrations were almost 1,000 times higher in the lead-treated group.

Zebra finches not exposed to lead showed no significant differences among the original and the regrown outermost tail feathers and the fifth tail feathers (Wilcoxon signed rank tests,  $p > 0.1$ , in all cases; see Figure 1). The original outermost tail feathers of zebra finches exposed to 25 ppm, on the other hand, had significantly lower lead concentrations than the regrown outermost tail feathers (Wilcoxon signed rank test,  $p = 0.012$ ) and significantly lower concentrations than the fifth tail feathers (Wilcoxon signed rank test,  $p = 0.012$ ). The lead concentration in the regrown tail feathers and the fifth tail feathers of exposed zebra finches did not differ significantly (Wilcoxon signed rank test,  $p = 0.16$ ; see Figure 1).

Most lead concentrations were significantly positively correlated among internal tissues (Table 2). Lead levels in fat tissue were poorly correlated with lead levels in other tissues. Tissue levels in fat were only significantly positively correlated with levels in muscle tissue. Lead levels in the regrown feathers were significantly positively correlated with lead levels in liver, kidney, and muscle (Table 2). After Bonferroni correction, lead levels in regrown feathers and levels in brain and testes were not significantly correlated.

The zinc concentration in the regrown feathers of one individual from the lead-treated group was, compared to all other individuals, extremely high (495 ppm). We therefore excluded this individual from analysis. Zinc concentrations in the regrown feathers were significantly lower in the lead-treated than in the control group (Mann-Whitney U test,  $p = 0.021$ ; see Figure 2). Moreover, lead levels were significantly negatively correlated (Spearman rank correlation,  $R = -0.55$ ,  $p = 0.026$ ) with zinc levels in the feathers (Figure 3). The zinc concentration in the liver did not differ significantly between the two groups (Mann-Whitney U test,  $p = 0.8$ ) and was not significantly correlated with lead levels (Spearman rank correlation,  $R = 0.10$ ,  $p = 0.7$ ).

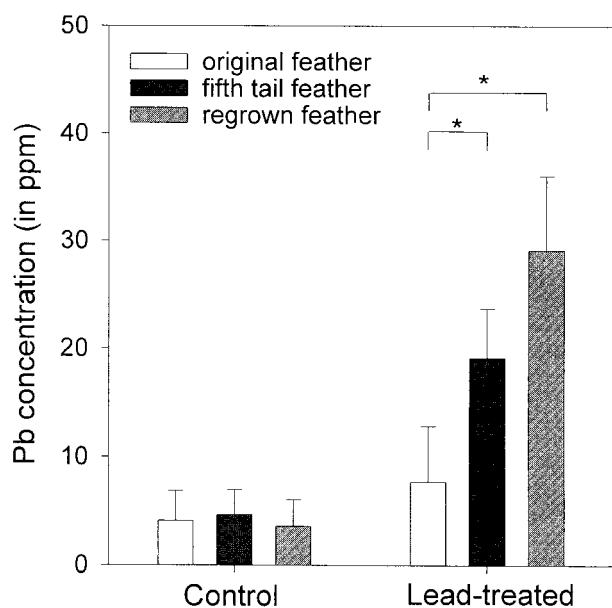
## Discussion

Several studies have suggested that lead in feathers can partially originate from direct external contamination onto the feather surface (Burger 1993; Furness 1993). Weyers *et al.* (1988) and Kim *et al.* (1998) even suggested that lead in unwashed feathers would be from direct atmospheric contamination rather than the food chain through excretion into growing feathers. However, few experimental studies have investigated the transfer of lead into the feather and related it to levels in important internal tissues.

**Table 1.** Lead concentrations (ppm [ $\mu\text{g g}^{-1}$  DW], mean  $\pm$  SE) in the tissues of the control (n = 8) and zebra finches exposed to 25 ppm lead (n = 8)

Tissue	Control	Exposed	U	p <sup>a</sup>
Brain	0.023 $\pm$ 0.010	1.5 $\pm$ 0.4	7.5	0.01
Fat	0.036 $\pm$ 0.022	0.119 $\pm$ 0.029	15.5	0.02
Regrown feather	3.6 $\pm$ 2.5	28 $\pm$ 6	4.0	0.003
Kidney	0.035 $\pm$ 0.010	33 $\pm$ 13	0	0.0008
Liver	0.0090 $\pm$ 0.0025	8.9 $\pm$ 2.5	0	0.0008
Muscle	0.0073 $\pm$ 0.0014	0.22 $\pm$ 0.03	0	0.0008
Testes	0.21 $\pm$ 0.14	2.8 $\pm$ 0.9	4.0	0.003

<sup>a</sup> p values from Mann-Whitney U tests applied to test for differences between the control and the lead-treated group.

**Fig. 1.** Mean (+ SE) lead concentration (ppm DW) in the original, the regrown outermost tail feathers, and the fifth tail feathers of control and lead-treated zebra finches

External contamination of lead on the feather can depend on two mechanisms: (1) direct uptake of lead caused by exogenous deposition, and (2) excretion of the uropygial gland on the feather during preening (Pilastro *et al.* 1993). Since no contact could occur between the feathers and the contaminated water in this experiment, feathers can only have been contaminated through excretion of the uropygial gland. We found a significant difference between the original and the regrown outer tail feathers. Although the lead concentration in the regrown outer tail feathers was higher than the concentration in the fifth outer tail feather, the difference was not significant. Our results therefore suggest that lead in the regrown feathers originates both from internal deposition via the blood and external contamination via the uropygial gland. Moreover, external contamination could not be removed by washing, suggesting that also in natural environments, lead in feathers partially originates from the excretion of the uropygial gland. Also for other metals external contamination can have an influence on the concentrations found in feathers. Pilastro *et al.* (1993) found significantly higher cadmium concentrations in old feathers of

**Table 2.** Correlation among lead levels in different tissues; given are Spearman rank correlation coefficients (n = 16)

Tissue	Brain	Fat	Regrown Feather	Kidney	Liver	Muscle	Testes
Brain	—	0.32	0.56*	0.85***	0.86***	0.62*	0.87***
Fat		—	0.45	0.51	0.55*	0.73**	0.50
Regrown feather			—	0.64**	0.75**	0.70**	0.61*
Kidney				—	0.93***	0.79**	0.87***
Liver					—	0.81***	0.86***
Muscle						—	0.75**
Testes							—

\* p < 0.05.

\*\* p < 0.008 (after Bonferonni correction).

\*\*\* p < 0.0001.

starlings (*Stumus vulgaris*) than in new ones. As with lead, bioaccumulated cadmium is partially excreted through the uropygial gland, thus contaminating the feathers. Rose and Parker (1982) found that levels of copper, iron, and nickel in feathers in the postmolt condition of ruffed grouse (*Bonasa umbellus*) did not differ significantly between a highly contaminated and an uncontaminated site. Levels of copper, iron, and nickel in newly formed feathers probably reflect endogenous incorporation during the period of feather growth. The metal levels in feathers in the premolt condition, which were subjected to exogenous influences during the feather year, showed an increase in metal content of 7- to 20-fold over metal levels in feathers in postmolt condition at the contaminated site (Rose and Parker 1982).

We found a significant correlation between lead levels in the regrown feathers and levels in important target tissues, such as the liver and the kidney. This correlation suggests that feathers can be a measure of the exposure to lead and can be used as a biomonitor for environmental lead pollution. However, because lead levels increase due to external deposition the age of the feathers must be taken into account to interpret the obtained results.

Earlier research with nestling great and blue tits (Dauwe *et al.* 2000) suggested that lead might influence the accumulation of zinc in the feathers. The results in this experiment seem to support our field results. Levels of zinc were significantly lower in the lead-treated group. Moreover, lead and zinc levels in the feather were significantly negatively correlated. Petering (1973) and O'Flaherty (1998) have described the negative

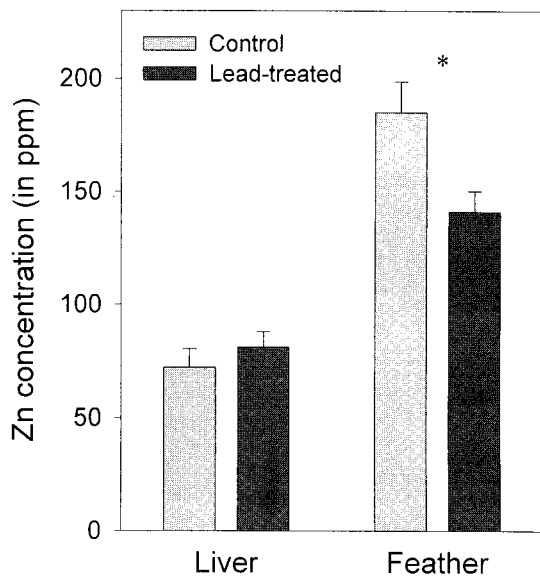


Fig. 2. Mean (+ SE) zinc concentration (ppm DW) in the liver and the feathers of control and lead-treated zebra finches

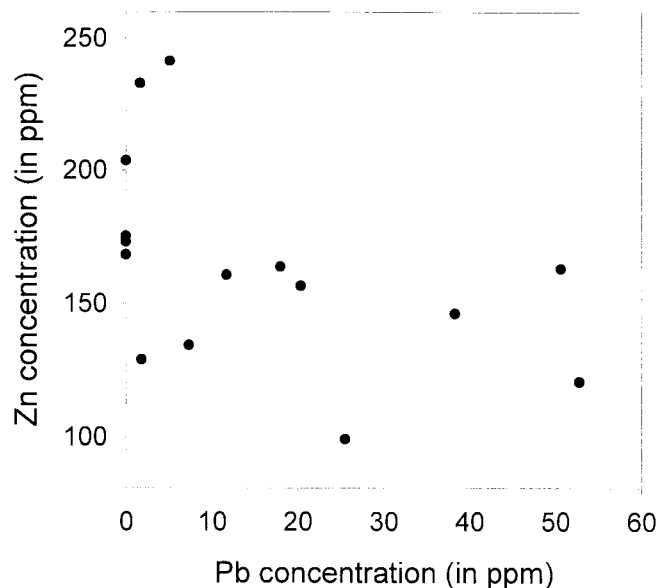


Fig. 3. Spearman rank correlation between the lead and the zinc concentration (ppm DW) in the feathers of control and lead-treated zebra finches ( $n = 15$ )

effect of toxic metals on the uptake of essential elements, such as copper and zinc. In our study, zinc levels in the liver were not significantly different between the two groups and are comparable to levels found in other passerines (Llacuna *et al.* 1996; Hogstad 1996). Therefore, it is unlikely that the lowered zinc concentrations in the feathers of lead-treated zebra finches are caused by a decreased absorption of zinc. It remains unclear what is responsible for the lowered accumulation of zinc in the feathers. A possible hypothesis is that zinc-binding proteins, such as zinc-protoporphyrin and delta-aminolevulinic acid,

which are induced by lead (Pain 1989; Redig *et al.* 1991; Heinz *et al.* 1999), render zinc unavailable for accumulation in the feather. More research into the interactions between lead and other heavy metals, especially the effect it has on the accumulation in the feathers, is necessary to determine the feasibility of feathers as a biomonitoring tissue for heavy metals.

Several experimental studies have investigated the accumulation of lead in internal tissues of birds. Kendall and Scanlon (1981) investigated the accumulation and effects of lead on ringed turtle doves (*Streptopelia risoria*). The mean lead concentration in the kidney and the liver of male doves was 145 ppm and 10.6 ppm, which is much higher than described in this study. Scheuhammer (1996) described lead levels in zebra finches exposed to 10 ppm lead as lead acetate via water. Levels in the liver and the kidney were considerably lower than found in the lead-treated group in this study. Zebra finches accumulated 1.5 ppm and 0.64 ppm of lead in, respectively, the kidney and the liver. Few experimental studies have examined lead accumulation in feathers although they have been used intensively as a biomonitor for lead pollution. Burger and Gochfeld (1990) injected herring gull (*Larus argentatus*) chicks with 0.1 and 0.2 mg/g lead. The chicks dosed with 0.2 mg/g lead had higher concentrations in liver (21.5 ppm) and kidney (41.1 ppm) but considerably lower concentrations in the feathers (9.2 ppm) than described in this study. However, we have to point out that between-laboratory comparison is difficult due to methodological differences (Burger and Gochfeld 1990).

Recently field studies have examined lead levels in tissues of passerines as a possible biomonitor for environmental pollution. Llacuna *et al.* (1995) reported lead levels in three species of free-living passerine birds. Lead concentrations in great tit, blackbird (*Turdus merula*) and rock bunting (*Emberiza cia*) tissues were much lower than described in this study. As with the zebra finches in this study, however, both great tit and rock bunting had similar lead concentrations in the feather (respectively, 4.6 and 8.0 ppm) and the kidney (respectively, 4.0 and 7.1 ppm) and lower levels in the liver (respectively, 1.4 and 0.8 ppm) (Llacuna *et al.* 1995). Sawicka-Kapusta *et al.* (1986) investigated the levels of lead in the liver and the feathers of several tit species. At a polluted site the lead concentrations in the liver (21.6 ppm) and the feather (145 ppm) were considerably higher than in our study. Eens *et al.* (1999) reported lead levels in the feathers of blue tits living near a waste incinerator. The mean lead concentration (64 ppm) was more than 2 times higher than in our study. The comparison with field studies shows that lead concentrations in the tissues of zebra finches exposed to 25 ppm are comparable or even lower than concentrations found in free-living passerines from polluted areas.

The feasibility of songbirds as a sentinel for terrestrial point source pollution with heavy metals such as lead has recently been demonstrated (Llacuna *et al.* 1995; Eens *et al.* 1999; Dauwe *et al.* 2000). However, experimental studies are necessary to determine the accumulation and the effects of lead and other heavy metals on passerines. The zebra finch has several features making it suitable as a study species. Zebra finch behavior and physiology are already well established, and they are easy to keep and breed in laboratory conditions. Therefore, the zebra finch may become an important model species in experimental avian toxicology.



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