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Mercury, Lead and Copper in Feathers and Excreta of Small Passerine Species in Relation to Foraging Guilds and Age of Feathers

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Abstract In the present study excreta and feathers of five species of adult passerine birds from Montepulciano wetland (Siena, Italy) were assayed for trace elements between January and August 2006. Lead concentrations varied from 16.31 to 26.50 mg/kg and were found strictly related to the age of feathers. Copper levels were found to be high mainly in insectivorous birds (9.68 mg/kg) and were probably influenced by local use of copper-based agricultural fungicides. Mercury accumulation in feathers varied between species from 0.08 to 0.73 mg/kg. The role of feeding habits on trace elements levels in feathers and excreta is discussed.

Keywords Heavy metals · Passerine birds · Feathers · Excreta · Copper-based agricultural fungicides

In the last few decades feathers have been widely used as non destructive materials for monitoring exposure of birds to heavy metals (Furness and Greenwood 1993). According to Burger (1993) metal accumulation in feathers is the sum of endogenous and predominantly exogenous processes, the latter due to preening and direct contamination from the environment (dry and wet deposition, sand, dust, immersion in water, contact with soil and vegetation; Weyers et al. 1988; Furness and Greenwood 1993; Burger 1993; Jaspers et al. 2004). The endogenous fraction in feathers comes from metals in the bloodstream incorporated in keratin during its formation (Burger 1993; Weyers 1986).

Various authors have postulated that cadmium and lead in feathers are mostly exogenous while mercury is endogenous, indicating bioavailability of mercury in food webs (Hahn et al. 1993; Furness and Greenwood 1993).

The first studies of contaminants such as heavy metals in excreta and guano were conducted in colonial sea birds (Petit and Altenbach 1983) and subsequently extended to other species of birds and mammals. Other studies used excreta of nestlings and adults of various species of birds to assess relations between metal levels and diet, monitoring trace elements and heavy metals on local, regional and global scales (Dauwe et al. 2004).

Levels of trace elements in feathers and excreta are sufficient to enable good detection and the use of these materials minimizes disturbance to populations and individuals (non destructive sampling), permitting easy repetition of sampling in time and over wide geographical areas (Leonzio and Massi 1989).

In the present study, excreta and feathers of five species of passerine birds were collected in a central Italian wetland and assayed for mercury, copper and lead, in order to investigate relationships between these metals and species ecology (foraging guild) and the influence of age of feathers on accumulation processes.

Materials and Methods

The study area included the lake of Montepulciano and neighbouring farmland in Val di Chiana (southeastern part

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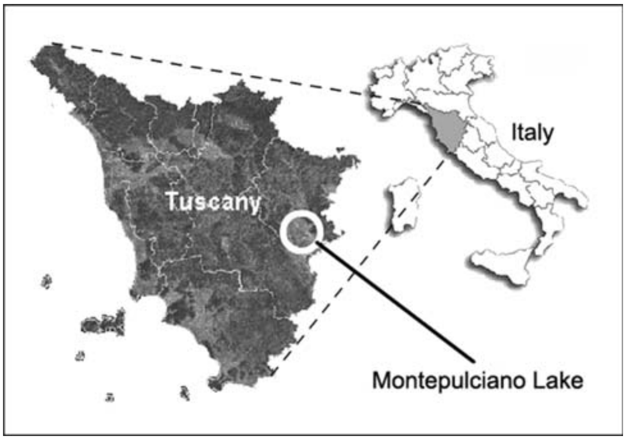

Fig. 1 Sampling site: Montepulciano lake in Tuscany (Italy)

of Siena Province, Tuscany, adjacent to Umbria; Fig. 1). The lake has an area of 400 ha, 90 ha of which is open water and the rest cane thicket, reed beds and other vegetation. Much of the basin is farmed, producing high yields of cereals, vegetables, fruit (apples and peaches), olives and quality wines. Cultivation of grapes and olives is widespread in northern Val di Chiana and the rest of the valley.

Feathers of yellow wagtail (*Motacilla flava*), Cetti's warbler (*Cettia cetti*), Italian sparrow (*Passer italiae*), Eurasian tree sparrow (*Passer montanus*) and reed bunting (*Emberiza schoeniclus*) were sampled between January and August 2006 during a banding campaign. A primary remex, a secondary remex and excreta were obtained from each bird. Italian sparrow, Eurasian tree sparrow, reed bunting, yellow wagtail and Cetti's warbler were caught in mist nets in a *Phragmites australis* thicket on the edge of the lake.

The species were classified in foraging guilds according to information in Cramp and Simmons (1989; Table 1).

It was assumed that levels of certain trace elements in feathers are influenced by atmospheric deposition and contact with the environment and therefore that the exposure time of feathers varies with feather age (Weyers et al. 1988). The species were grouped according to exposure time (in days) counted from the end of the previous moult to the date of sampling (Table 1).

The feathers were later brushed and cleaned with an air jet to remove gross particulate (rock particles) according to Bianchi et al. (2008), as classic cleaning methods with deionized water and/or acetone may partially remove the exogenous fraction (Weyers et al. 1988).

Aliquots (about 0.1 g) of lyophilized feathers and excreta were placed in teflon containers; 2 mL nitric acid and 0.5 mL hydrogen peroxide were added for mineralization. The containers were sealed and maintained at 160°C for 12 h. The clear solutions obtained were transferred to plastic tubes and made up to 10 mL with milli-Q water. Mercury, copper and lead were assayed in these solutions. A blank was included in each analytical series to verify reagent purity, as well as six tests of reference material with concentrations certified by the *Community Bureau of Reference* to determine analytical accuracy (Bovine Liver no. 1377b, NIST Standard Reference Material). Recovery on Certificate vary from 89% to 94% and instrumental detection limits are 0.001 mg/L for lead and mercury and 0.040 mg/L for copper.

Metal concentrations were calculated by the external addition method: graded quantities of a solution containing known concentrations of the metals to be analysed were added to equal aliquots of the same sample.

A Perkin Elmer Analyst 700 atomic absorption spectrophotometer with graphite furnace for lead analysis, a Perkin Elmer Analyst 400 atomic absorption spectrophotometer with cold vapour technique for mercury analysis, a Perkin Elmer OPTIMA 5300 DV ICP plasma emission spectrometer for copper analysis, were used.

Differences between and within groups were tested by the Mann–Whitney *U* test and Kruskal–Wallis test. Correlations between exposure time and heavy metal concentrations were analysed by the Spearman correlation test (StatSoft Inc. 1984–2000).

Results and Discussion

Figure 2 shows mercury concentrations in feathers of five species of passerine birds. The concentrations found in reed buntings, Italian sparrows and tree sparrows were similar to

Table 1 Classification of species on the basis of exposure time of feathers and foraging guild

Species	n	Date of sampling	Foraging guild	Exposure time (days)
Tree sparrow (*Passer montanus*)	5	20-07-2006	Omnivorous	321
Cetti's warbler (*Cettia cetti*)	10	01-12-2005	Insectivorous	105–137
Reed bunting (*Emberiza schoeniclus*)	10	17-01-2006	Granivorous	
Italian sparrow (*Passer italiae*)	4	15-01-2006	Omnivorous	
Tree sparrow (*Passer montanus*)	5	10-12-2005	Omnivorous	46–67
Italian sparrow (*Passer italiae*)	5	08-12-2005	Omnivorous	
Yellow wagtail (*Motacilla flava*)	5	20-07-2006	Insectivorous	Moulting

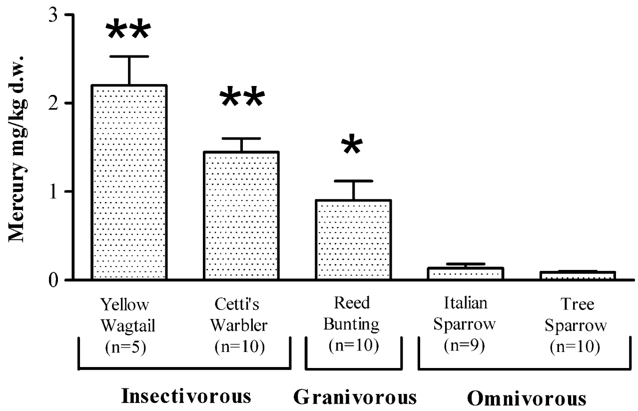

Fig. 2 Mercury concentrations (mean ± SD, mg/kg dry weight) in feathers of five species of birds (* and ** significantly high concentration, Mann–Whitney test: * = $p < 0.05$; ** = $p < 0.01$)

those encountered in other omnivorous/granivorous passerines sampled in areas not subject to mercury contamination (Jaspers et al. 2004; Swaileh and Sansur 2006). Comparison with species in different foraging guilds (Fig. 2) showed that reed buntings had significantly higher concentrations of mercury than Eurasian tree sparrows ($U = 2$, $p < 0.01$) and Italian sparrows ($U = 3$, $p < 0.01$). The insectivorous yellow wagtail and Cetti's warbler showed higher levels of mercury than granivorous and omnivorous species (Fig. 2). They frequent wetlands (Cramp and Simmons 1989) and mainly feed on aquatic invertebrates. The bioavailability of mercury is increased by methylation and biomagnification in the food chain.

Concentrations of lead in the five species were similar to those reported in other insectivorous passerines in anthropized areas (Nam et al. 2003). Considering age of feathers as an indicator of exposure time, lead concentrations showed a positive correlation with this parameter ($r = 0.4$; $p < 0.01$) and species that had moulted less recently showed significantly higher concentrations (Table 2). This indicates that lead levels increase with increasing exposure time of feathers.

Concentrations of copper (Fig. 3) did not seem related to exposure time. No significant differences were found between groups except the 321-day exposure group and the 46–67-day and 105–137-day exposure groups (Table 2). Copper concentrations were high and could not be due solely to incorporation during feather formation since ingested copper is mostly excreted and the rest accumulates in liver and kidneys (Skrivan et al. 2005). The high concentrations of copper in feathers of the five species could be due to exogenous contamination, particularly contact with copper-contaminated vegetation or soil. Indeed, copper is widely used as a fungicide for vines and olive trees in the study area (Calvino et al. 2008).

Comparison of lead and copper concentrations in feathers of two groups of Italian sparrows with different exposure times did not reveal significant differences, presumably because the difference in exposure time was small (about 40 days; Fig. 4). A highly significant difference ($U = 0$; $p < 0.01$; Fig. 4) between the two groups of Eurasian tree sparrows was found for lead but not copper. In this case, exposure times differed by 270 days (50 and 320 days; Fig. 4), demonstrating a relationship between exposure time and lead levels in feathers.

In excreta mercury concentrations of the five species were close to analytical detection limits and differences between species were not significant (Fig. 5). This is not surprising because methylmercury, the main bioavailable form of mercury in the environment, mostly accumulates in liver, muscle, nerve tissue and keratinized structures such as feathers, while renal and bile excretion is limited. Methylmercury binds strongly to feathers and is eliminated efficiently during moulting (Scheuhammer 1987). Such low levels of mercury in excreta probably indicate low exposure of the species to inorganic mercury (Scheuhammer 1987).

Concentrations of lead in excreta of the five species (Fig. 6) were similar to levels encountered in other small passerines from areas not contaminated by heavy metals (Dauwe et al. 2004). Significantly higher lead concentrations were found in excreta of omnivorous/granivorous Italian sparrows and insectivorous yellow wagtails (Fig. 6; Kruskal–Wallis test: $H = 17$; $p < 0.01$). These species also feed on soil invertebrates (anellids; Cramp and Simmons 1989) which may transmit lead from soil to predators.

Table 2 Comparison of copper and lead concentrations in feathers in relation to exposure time of feathers (U Test)

Different exposure time (day) compared		U test	
		Copper	Lead
321 days (n = 5)	Moulting (n = 5)	Not significant	$U = 0$; $p < 0.01$
321 days (n = 5)	46–67 day (n = 10)	$U = 6$; $p < 0.05$	$U = 1$; $p < 0.01$
321 days (n = 5)	105–137 day (n = 25)	$U = 0$; $p < 0.01$	$U = 13$; $p < 0.01$
105–137 days (n = 25)	Moulting (n = 5)	Not significant	$U = 23$; $p < 0.05$
105–137 days (n = 25)	46–67 day (n = 10)	Not significant	Not significant
46–67 days (n = 10)	Moulting (n = 5)	Not significant	$U = 2$; $p < 0.01$

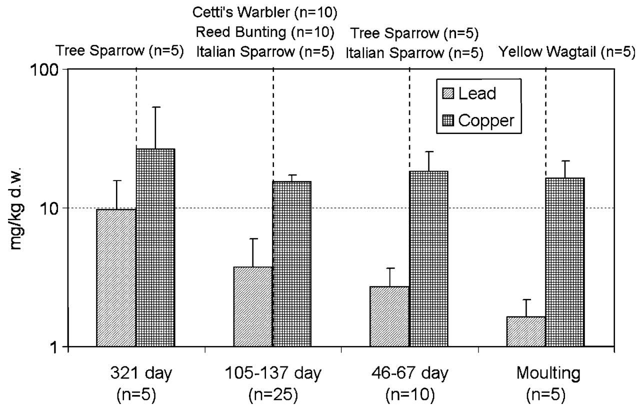

Fig. 3 Concentrations of lead and copper (mean ± SD, mg/kg dry weight) in feathers of five passerine bird species in relation to interval between sampling and moult, namely exposure time of feathers

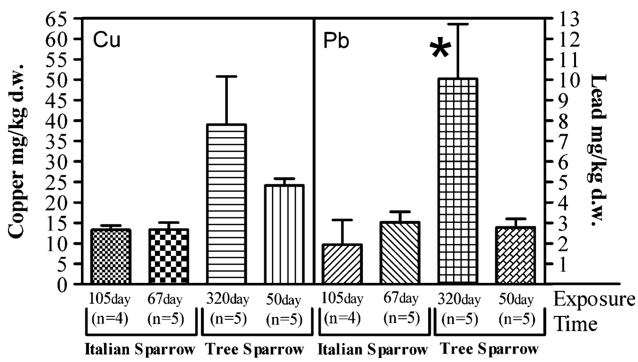

Fig. 4 Comparison of lead and copper concentrations (mean ± SD, mg/kg dry weight) in feathers of groups of Italian sparrows and tree sparrows with different exposure times (* = significantly high concentration, Mann–Whitney test $p < 0.01$)

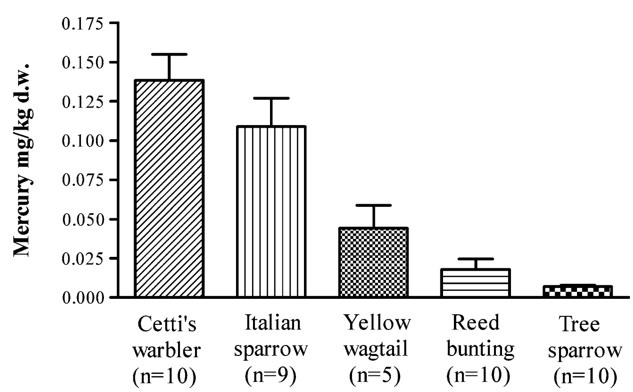

Fig. 5 Mercury concentrations (mean ± SD, mg/kg dry weight) in excreta of five passerine bird species

Concentrations of copper in excreta of Cetti's warblers (68.10 ± 24.5 mg/kg d.w.) and yellow wagtails (94.12 ± 58.47 mg/kg d.w.) were extremely high and similar to levels encountered in passerines from areas severely polluted with heavy metals (Dauwe et al. 2004). This indicates that the wetland and lake surroundings are contaminated

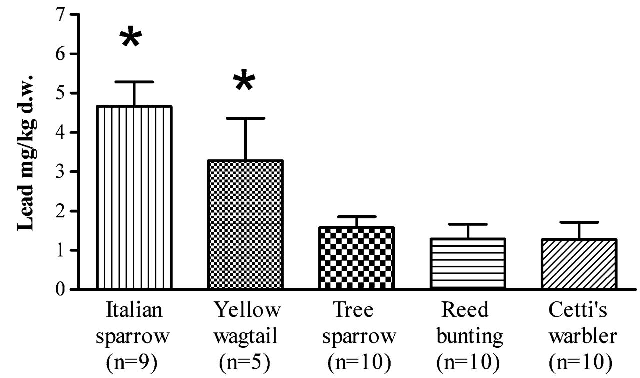

Fig. 6 Lead concentrations (mean ± SD, mg/kg dry weight) in excreta of five passerine bird species (* = significantly high concentration, Kruskal–Wallis test $p < 0.01$)

with copper, presumably due to its use as fungicide for olives, fruit trees and vines (Commission Regulation (EC) No 473/2002), widely cultivated throughout the municipality of Montepulciano.

Copper used in fungicides not only falls as aerosol on vines but also on other plants and animals in the vicinity. Many species of invertebrates that live in vineyards and farmland around the lake are therefore exposed to copper contamination by wet deposition during spraying and through accumulation of the bioavailable fraction of copper (Snyman et al. 2005; Eijsackers et al. 2005). Copper accumulating in invertebrates can be transmitted to certain insectivorous birds. Indeed, copper levels were significantly higher in insectivorous (Cetti's warblers and yellow wagtail) than granivorous and omnivorous birds [Kruskal–Wallis, (H(4, N = 44) = 25; $p < 0.01$; Fig. 7)].

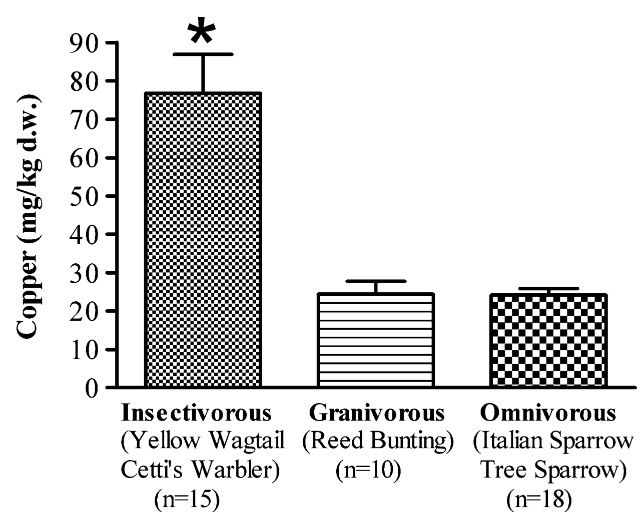

Fig. 7 Copper concentrations (mean ± SD, mg/kg dry weight) in excreta in relation to foraging guild (* = significantly high concentration, Kruskal–Wallis test $p < 0.01$)

The present data suggests that copper and lead concentrations in feathers of the five species of passerine birds are largely of exogenous origin. Indeed, lead concentrations were found to be correlated with age of feathers and presumably therefore with exposure time, whereas copper, concentrations of which were very high, seemed to be influenced by contact with vegetation or soil contaminated with copper-based agricultural fungicides. Mercury showed a different pattern and was probably influenced more by foraging guild and thus by ingested mercury.

In excreta, lead levels were higher in species (yellow wagtail and Italian sparrow) whose diet included soil invertebrates (anellids) that are known to transmit lead from the soil to higher consumers. Copper levels in excreta were very high and similar to those reported in passerines from environments contaminated by heavy metals. The highest copper concentrations were found in insectivorous birds yellow wagtail and Cetti's warbler that presumably ingest copper sprayed as agricultural fungicide.

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