Title: Blood lead increases and haemoglobin decreases in urban birds along a soil contamination gradient in a mining city

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Abstract: Lead contaminated soil is a persistent global threat to the health of animal populations. Nevertheless, links between soil lead and its adverse effects on exposed wildlife remain poorly understood. Here, we explore local geographic patterns of exposure in urban birds along a gradient of lead contamination in Broken Hill, an Australian mining city. Soil lead concentrations are linked to co-located blood lead measurements in rock pigeons (*Columba livia*), house sparrows (*Passer domesticus*), crested pigeons (*Ocyphaps Lophotes*) and white-plumed honeyeaters (*Lichenostomus ornatus*). Mean blood lead levels were highest in crested pigeons (82.1 µg/dL), followed by rock pigeons (47.8 µg/dL), house sparrows (43.0 µg/dL) and white-plumed honeyeaters (30.3 µg/dL). Blood lead levels in all species declined away from mining areas, the primary source of lead contamination in Broken Hill. Blood lead increased significantly and at the greatest rate relative to soil lead in the three ground foraging species (crested pigeons, house sparrows, rock pigeons). For these species, soil lead concentrations below 200 mg/kg and 900 mg/kg were needed to maintain a median blood lead under the subtoxic (20 µg/dL) and toxic (50 µg/dL) effect thresholds previously identified for some avian species. We also investigated the effects of lead exposure on blood haemoglobin levels as a general measure of physiological condition in birds exposed to different levels of soil lead contamination. Overall, for every 1 µg/dL increase in blood lead, haemoglobin decreased by 0.11 g/L. The rate of this decrease was not significantly different between species, which supports the measurement of haemoglobin as a robust though insensitive measure of physiological condition in chronically lead exposed birds. Our findings reflect the importance of lead contaminated soil as a widespread source of elevated blood lead and supressed haemoglobin levels in birds inhabiting urbanised and mining impacted environments.

Introduction: Over the last century, regulatory and environmental interventions have made substantial progress in reducing human exposure to lead (Fuller et al., 2022). Comparatively, progress in reducing lead exposure amongst non-human animal populations has been inconsistent (Levin et al., 2021). Amongst bird species, ingestion of lead ammunition is the most widely studied source of lead exposure and, despite its prohibition in many countries, remains a common source of lead poisoning in wildfowl, gamebirds, raptors and other scavenging birds (Pain et al., 2019). Fewer studies have explored the exposure and susceptibility of birds to soil contaminated by lead emissions from mining and smelting activities (Berglund et al., 2010; Beyer et al., 2013; Chapa-Vargas et al., 2010; Durkalec et al., 2022; Williams et al., 2018). Inputs of lead by mining and smelting operations are ongoing, and unlike many other common anthropogenic lead sources, remain poorly regulated in many

countries (Entwistle et al., 2019). Even in regions with developed regulatory systems, interventions aimed at reducing human exposure in mining contaminated areas are rarely protective of terrestrial animals. Soil lead contamination therefore presents a potentially widespread and sometimes overlooked threat to the health of birds that deserves deeper consideration.

Lead contaminated soil has the potential to impact a diverse range of bird species across a broad range of habitats. This includes bird species which are not exposed to lead ammunition, such as those which are not hunted by humans, or are unlikely to consume game animals (Pain et al., 2019). Additionally, whereas exposure to lead ammunition is typically acute, ingestion or inhalation of lead contaminated soil and dust is more often chronic, and its physiological effects may not be directly comparable between the two scenarios (Franson and Pain, 2011). Despite this, there remains a paucity of research that links soil lead contamination to biomarkers of lead exposure and effect across multiple bird species. These links are important to understanding lead exposure risks in birds, but also amongst humans and other animals, where similar sources of exposure contribute to range of adverse health outcomes (Gillings et al., 2024; Imagawa et al., 2023).

Previous research has identified interspecific differences in tissue lead concentrations amongst bird populations inhabiting areas with varying levels of soil lead contamination (Beyer et al., 2013; Chapa-Vargas et al., 2010; Gil-Jiménez et al., 2021; Hansen et al., 2011). However, in these studies, a focus at the population level (e.g. contaminated versus uncontaminated areas) means that links between soil and blood lead levels are not always established at the scales over which exposure occurs in different bird species. This is often the case in mining and smelting impacted environments where contamination gradients create significant variability in exposure levels over small geographic areas (Entwistle et al., 2019; Gil-Jiménez et al., 2021). At these spatial scales, identifying biochemical and physiological responses to lead exposure amongst different bird species can benefit the monitoring and management of animal populations in lead contaminated ecosystems.

One of the most consistently reported physiological effects of lead exposure in birds and other animals is the inhibition of the enzyme delta-aminolevulinic acid dehydratase (δ-ALAD). The δ-ALAD enzyme is involved in the synthesis of haem, the protein present in red blood cells and responsible for the delivery and uptake of oxygen and carbon dioxide to and from tissues (Williams et al., 2018). Declines in haemoglobin have been attributed to detrimental impacts in a broad range of fitness related traits, including parasite load (Krams et al., 2013; Motta et

al., 2013) and egg size (Minias, 2014). However, observed effects of lead exposure on blood haemoglobin levels are variable, and where detected, typically occur at significantly higher blood lead levels than are associated with δ-ALAD inhibition (Buekers et al., 2009). Identifying the conditions under which lead exposure causes a blood haemoglobin response is important because, while δ-ALAD is a sensitive biomarker of exposure, haemoglobin levels are more directly relatable to the physiological condition of an individual (Minias, 2015).

In this study, we explore links between the spatial distribution of soil lead, blood lead and haemoglobin levels in four species of urban bird inhabiting multiple sites across the Australian mining city of Broken Hill, located in far western New South Wales. Emissions from mining operations in Broken Hill have created a gradient of soil lead contamination away from local production point sources (Gillings et al., 2022). The effect of lead contamination is seen in elevated blood lead levels in local children (Dong et al., 2019). Within this urban context, we explore lead exposure in two introduced species: rock pigeons (ROPI; *Columba livia*) and house sparrows (HOSP; *Passer domesticus*); and two native species: crested pigeons (CRPI; *Ocyphaps Lophotes*) and white-plumed honeyeaters (WPHE; *Lichenostomus ornatus*). Urban populations of these species are widely distributed throughout Australia, are relatively sedentary, and their density is sufficient to enable spatially representative sampling across lead contamination gradients. CRPI, HOSP and ROPI are ground foragers, and so are directly and frequently exposed to lead contaminated soil, whereas WPHE forage almost exclusively in bushes and tree canopies. We predict that levels of blood lead contamination will reflect those measured in soil and will decrease with distance from mining operations. We also expect haemoglobin values to be negatively correlated to blood lead concentrations. Finally, we anticipate WPHE to show lower blood lead levels compared to the other species due to their largely arboreal foraging strategy.

Methods:

Environmental context

Broken Hill is located in the far western arid zone of New South Wales, Australia. Local mining operations are centred on one of the world's largest silver-lead-zinc orebodies, which intersects the two main residential districts of the city. Low rainfall and sparse vegetative cover facilitate dust generation and transport and contribute to the dispersal of lead (Pb) contaminated dust from mining operations into the surrounding urban areas (Gillings et al., 2022). Surface soils in Broken Hill are highly enriched in Pb and are an important source of Pb exposure in the

local community (Dong et al., 2019). Birds were captured from a total of 77 sites in and around urban areas in Broken Hill. The sampling sites in this study were selected based on the distribution of the target species, but also according to their location along the gradient of soil contamination in Broken Hill (Figure 1).

Soil sampling

To determine the approximate level of soil Pb contamination within the home range of the target bird species we sampled surface soil from most catch sites. Three samples were collected from the upper 2 cm of the soil profile at locations approximately 1 m equidistant. An additional 6 samples were taken around a smaller number of sites nearer to mining areas where variability in soil contamination was anticipated to be highest. Where possible, samples were collected from exposed soil, and clean fill and imported soil material was avoided. A total of 237 soil samples were collected at 63 of the 77 sites.

Soil analysis

Soil Pb concentrations were measured using an Olympus Vanta portable X-ray fluorescence spectrometer (pXRF) fitted with a 50 kV tungsten (W) anode tube. Soil samples were dried, sieved to < 250 µm, and 10 g of material was transferred to pXRF analysis cups. Samples were analysed using the proprietary soil mode for a total measurement time of 60 s, with 20 s per measurement condition. Soil Pb concentrations are reported in mg/kg. No measurements returned concentrations below the instrument limit of detection (1 mg/kg). Mean recoveries for analyses of National Institute of Standards and Technology (NIST) Standard Reference Materials (SRMs) 2711a (Montana II Soil; Pb = 1400 mg/kg) and 2709a (San Joaquin Soil; Pb = 17 mg/kg) were 96% (n = 25) and 93% (n = 15) for Pb, respectively. Corresponding relative standard deviations (RSD) for these analyses were 1% and 7%. Analysis of a silicate (SiO_2) blank returned no readings above instrument limits of detection for Pb.

To supplement our soil data, we incorporated a wider dataset of 364 surface soil Pb measurements from urban areas in Broken Hill dating from between 2012–2022 (Gillings et al., 2022; Kristensen and Taylor, 2016). These samples were collected at similar soil depths (0 – 2 cm) and were analysed with a particle size ≥ 250 µm. This brought the total number of individual soil measurements included in this study to 601.

Biological sampling

Capture and sampling of CRPI (*Ocyphaps Lophotes*), ROPI (*Columba livia*), HOSP (*Passer domesticus*) and WPHE (*Lichenostomus ornatus*) was authorised by the Macquarie University

Animal Ethics Committee (ARA #2020/011). Due to the different densities and distributions of the targeted bird populations and the methods used to capture them, most sites are specific to one bird species. CRPI (n = 41) and ROPI (n = 40) were caught at 11 sites each, HOSP (n = 446) at 49 sites, and WPHE (n = 49) at 13 sites (Table S1). HOSP were captured and sampled intermittently over a three-year period between 2020–2023 (as part of a related study (Gillings et al., 2024)), while CRPI, ROPI, and WPHE were mostly captured and sampled between April–May 2023. HOSP and WPHE were caught using mist nets, while CRPI and ROPI were caught using a ground-based clap trap. All birds were released at the site of their capture following sampling and measurement.

Captured birds were banded and identified for age (juvenile/adult) and sex (female/male) (the latter only possible in the sexually dichromatic house sparrow). Measurements of mass, wing chord, and tarsus were also taken. Blood samples were taken by first puncturing the brachial vein with a 26 gauge hypodermic needle. A 50 µL blood sample was taken using a 75 µL plain glass Microhematocrit Capillary Tube (supplied by Livingstone). These samples were taken specifically for the quantification of blood Pb concentrations using a Meridian Bioscience LeadCare Plus blood Pb analyser. Blood samples were stored in proprietary sample tubes containing a dilute solution of HCl. These samples were stored on ice for a maximum of 9 hours and were returned to room temperature prior to analysis. A target of 5 or more birds were sampled for this purpose at most sites, totalling 576 blood samples (Table S1). Four of these samples are repeat measurements taken from the same HOSP captured from the same site at a time interval of 6–222 days.

Where permitted by blood flow, an additional 50–100 µL blood sample was also taken for the validation of blood Pb measurements obtained from the LeadCare Plus instrument using inductively coupled plasma mass spectrometry (ICP-MS). These blood samples were stored in 1.5 mL Eppendorf Tubes and frozen prior to analysis. An additional 10 µL of blood was sampled using Hemocue HB201 Microcuvette Strips for the in-field analysis of blood haemoglobin (Hb) levels using a HemoCue Hb 201+ point-of-care testing system. A total of 302 Hb measurements were taken across the four species (Table S1).

Blood haemoglobin analysis

Blood haemoglobin (Hb) levels (g/L) were measured immediately after sampling using a HemoCue Hb 201+ instrument. Within the microcuvette, Hb is converted to azide methemoglobin and the absorption of the sample is measured at two wavelengths, which are

used by the instrument to calculate Hb levels. The accuracy of Hb measurements obtained from this instrument has been validated by previous studies with comparison to measurements of Hb in capillary and venous blood using an automated cell counter (Jain and Chowdhury, 2020).

To further validate Hb measurements obtained for the HemoCue Hb 201+ instrument, we compared Hb measurements from 55 HOSP to the packed cell volume (PCV) of blood samples taken from the same individual. Previous studies have identified a proportional relationship between blood Hb concentrations and PCV (Turkson and Ganyo, 2015). We measured PCV based on the proportion of red blood cells to plasma in a 50 µL blood sample centrifuged at 10000 rpm in a 75 µL heparinised capillary tube. A significant positive correlation was found between paired PCV and Hb measurements (Pearson $r = 0.70$, $p < 0.001$, $n = 55$).

Blood lead analysis

Blood samples (50 µL) were analysed for Pb concentrations using a LeadCare Plus blood Pb analyser. This instrument uses anodic stripping voltammetry (ASV) to measure the blood Pb levels from a blood sample mixed with a dilute solution of HCl. The instrument has a detection range of 1.9–65.0 µg/dL. Measurements returning concentrations outside these limits are imputed with a value equivalent to either the lower (1.9 µg/dL) or upper limit of detection (65.0 µg/dL). Analysis of high and low controls for the LeadCare Plus instrument returned concentrations within the acceptable range. The accuracy of measurements obtained from this instrument was further validated with analysis of paired blood samples using ICP-MS, as detailed below.

Paired blood samples (total $n = 48$) from CRPI ($n = 6$), ROPI ($n = 6$), HOSP ($n = 30$) and WPHE ($n = 6$) were sent for analysis of Pb concentrations using an Agilent 7900 quadrupole ICP-MS at the National Measurement Institute, Sydney, Australia, with further details available in Gillings et al. (2024). Values reported in mg/kg were converted to µg/dL assuming an avian blood density of 1.05 g/mL (Scanes, 2015). Mean recoveries for analyses of laboratory control sample Seronorm Trace Elements Whole Blood ($n = 4$) and matrix spikes ($n = 4$) were 106% and 100%, respectively.

The Pb concentration of paired blood samples measured using ASV and ICP-MS were strongly correlated (Pearson $r = 0.89$, $p < 0.001$, $n = 48$). However, there was an overall negative bias for blood Pb concentrations measured using ASV compared to ICP-MS (mean of differences ± 95% confidence interval = -18.9 ± 4.4 µg/dL). Previous studies that have measured avian blood samples using ASV have also reported underestimations of blood Pb concentrations

compared to the results of other analytical methods, including ICP-MS (González et al., 2019; Herring et al., 2018). These studies recommend correction of ASV measurements according to more analytically robust spectrometric techniques (Herring et al., 2018).

To account for this underestimation, we fitted an ordinary least squares (OLS) model for natural log-transformed Pb concentrations obtained from ASV (independent variable) and ICP-MS (dependent variable), following established methods (Herring et al., 2018). We found that ASV measurements from HOSP and WPHE underestimated ICP-MS measurements by a greater degree than CRPI and ROPI. An ASV measurement of 10 µg/dL, for example, equated to a back-calculated ICP-MS measurement of 23.7 µg/dL for HOSP, 21.4 µg/dL for WPHE, 13.6 µg/dL for CRPI and 14.7 µg/dL for ROPI. Based on this finding and the low number of paired measurements available for WPHE, ROPI and CRPI, we pooled our data for HOSP and WPHE (n = 36), and CRPI and ROPI (n = 12) and recalculated the models for each group. We applied the recalculated models for HOSP and WPHE (ln(ICP-MS blood Pb µg/dL) = 0.8275 × ln(ASV blood Pb µg/dL) + 1.2552) and CRPI and ROPI (ln(ICP-MS blood Pb µg/dL) = 1.0428 × ln(ASV blood Pb µg/dL) + 0.2592) to the entire dataset of natural log transformed ASV blood Pb measurements of the corresponding species (Table S2). We then inverted the adjusted natural log-transformed data back to a linear scale. This shifted the ASV detectable blood Pb concentrations from the proprietary range of 1.9–65 µg/dL to 6.0–111.0 µg/dL for HOSP and WPHE, and 2.5–100.7 µg/dL for CRPI and ROPI.

No measurements from WPHE and only 3% (n = 20/576) of those from HOSP exceeded the upper limit of detection, suggesting that the upper range of Pb exposure in these species was captured by this analysis method. This was not the case for the other species, with blood Pb measurements exceeding the upper limit of detection for 10% of ROPI (n = 4/40) and 22% of CRPI (n = 9/41). To avoid underestimation of blood Pb levels in these species, blood samples paired to the right censored ASV measurements (n = 13) were analysed using ICP-MS, as previously detailed. The existing right censored ASV measurements were substituted with these ICP-MS measurements.

Spatial and statistical analysis

All statistical and spatial analyses were performed with Python 3.9.13 and ArcGIS Pro 3.02. For comparison with similar studies, geochemical and biochemical data reported here are summarised using the arithmetic mean. The distance of each site from mining areas in Broken Hill is calculated from the nearest outer boundary of mining zoned land.

Bivariate relationships are examined using Pearson and Spearman correlation coefficients. Due to the non-normality of our data, the non-parametric Kruskal-Wallis test was used for categorical comparisons of blood Pb, blood Hb, and soil Pb data. Dunn's test was used for post-hoc analysis of differences between individual groups.

The assumptions of generalised linear model were not met, and so quantile regression, which does not assume a normally distributed error term, was used to model the relationship between, firstly, soil Pb (independent variable) and the conditional median of blood Pb (dependent variable), and secondly, blood Pb (independent variable) and the conditional median of blood Hb (dependent variable). In both models, species (CRPI, ROPI, HOSP, WPHE) were included as a categorical covariate. In the second model, maturity (adult, juvenile) was included as an additional categorical covariate due to its known effect on blood Hb levels (Minias, 2015). For the initial iteration of the models, an interaction term was created between the continuous independent variables (soil Pb in the first model and blood Pb in the second model) and the categorical covariate of species to identify interspecific effects in the modelled relationships. To allow for robust comparisons of these interspecific differences, we conducted four iterations of each model, each time using a different reference species.

The geochemistry of urban soils is highly heterogenous, complicating characterisation of soil Pb contamination at site-specific scales. Soil samples were also only taken from 63 of the 77 sites. To account for these limitations, we used empirical Bayesian kriging (EBK) regression prediction to interpolate a continuous soil Pb surface from the wider dataset of 592 individual soil Pb measurements from urban areas in Broken Hill (excludes 9 samples from 3 sites outside urban areas). The distance of each soil sample from mining areas was included as an explanatory variable to improve the accuracy of the predicted soil Pb surface (further details provided in Table S3). Soil Pb concentrations at each catch site were summarised using the mean soil Pb of 100 m^2 grid cells located within a 150 m radius of that site. This distance was based on the 300 m radius reported as the maximum home range of urban ROPI and HOSP (Sol and Senar, 1995; Vangestel et al., 2010) and was assumed to be inclusive of the home range of CRPI and WPHE based on observational studies of these and similar species (Guppy et al., 2023; Mulhall and Lill, 2011). We did not summarise soil Pb concentrations over the full 300 m radius as this would bias estimates to the outer perimeter of this range. Three sites fell outside the interpolated area and so the mean of measured concentrations are used at these sites.

For comparison of blood Pb concentrations measured in our target species with similar studies, we classified soil Pb contamination levels for each site according to the geoaccumulation index

(*Igeo*) (Barbieri, 2016) (Table S4). We used a background Pb concentration of 100 mg/kg based on the median subsoil Pb concentration in Broken Hill (Kristensen and Taylor, 2016). Levels of soil Pb contamination are classified according to thresholds described in Barbieri (2016). Within these categories, blood Pb levels are compared based on the foraging strategy of different species, as detailed in Billerman (2020).

In contextualising levels of Pb exposure measured in the studied species, we draw on blood Pb toxicity thresholds derived for Columbiformes and Falconiformes (Franson and Pain, 2011). In Columbiformes (e.g., CRPI, ROPI) and Falconiformes, blood Pb levels above 20 µg/dL are attributed to subtoxic physiological effects, which are unlikely to severely impair biological functioning. A blood Pb of 50 µg/dL in Falconiformes or 200 µg/dL in Columbiformes is associated with toxic physiological effects, such as anaemia and weight loss (Franson and Pain, 2011). Similar thresholds are not available for Passeriformes (e.g., HOSP, WPHE) and so the toxic effect threshold for Falconiformes (50 µg/dL) is used for these species since it is the closest phylogenetic relative for which data are available.

Results:

Soil lead contamination

Levels of soil Pb contamination were highly variable across Broken Hill (Figure 1; Table S4). Across the 77 sites in this study, mean soil Pb ranged between 46–3664 mg/kg (Table S5). Mean soil Pb concentrations were significantly and negatively correlated with the distance of a site to mining areas; and this was the case for both interpolated site data (Spearman $r_s = 0.84$, $p < 0.001$, $n = 77$) and co-located site measurements (Spearman $r_s = 0.63$, $p < 0.001$, $n = 63$). We did not identify a significant difference in soil Pb concentrations between the groupings of catch sites where different species were caught (Kruskal-Wallis; $p = 0.077$), indicating that the location of sites for each species were similarly distributed with respect to soil Pb contamination in Broken Hill. This in turn suggests that pairwise comparison of blood Pb levels between species should be appropriate at the population scale.

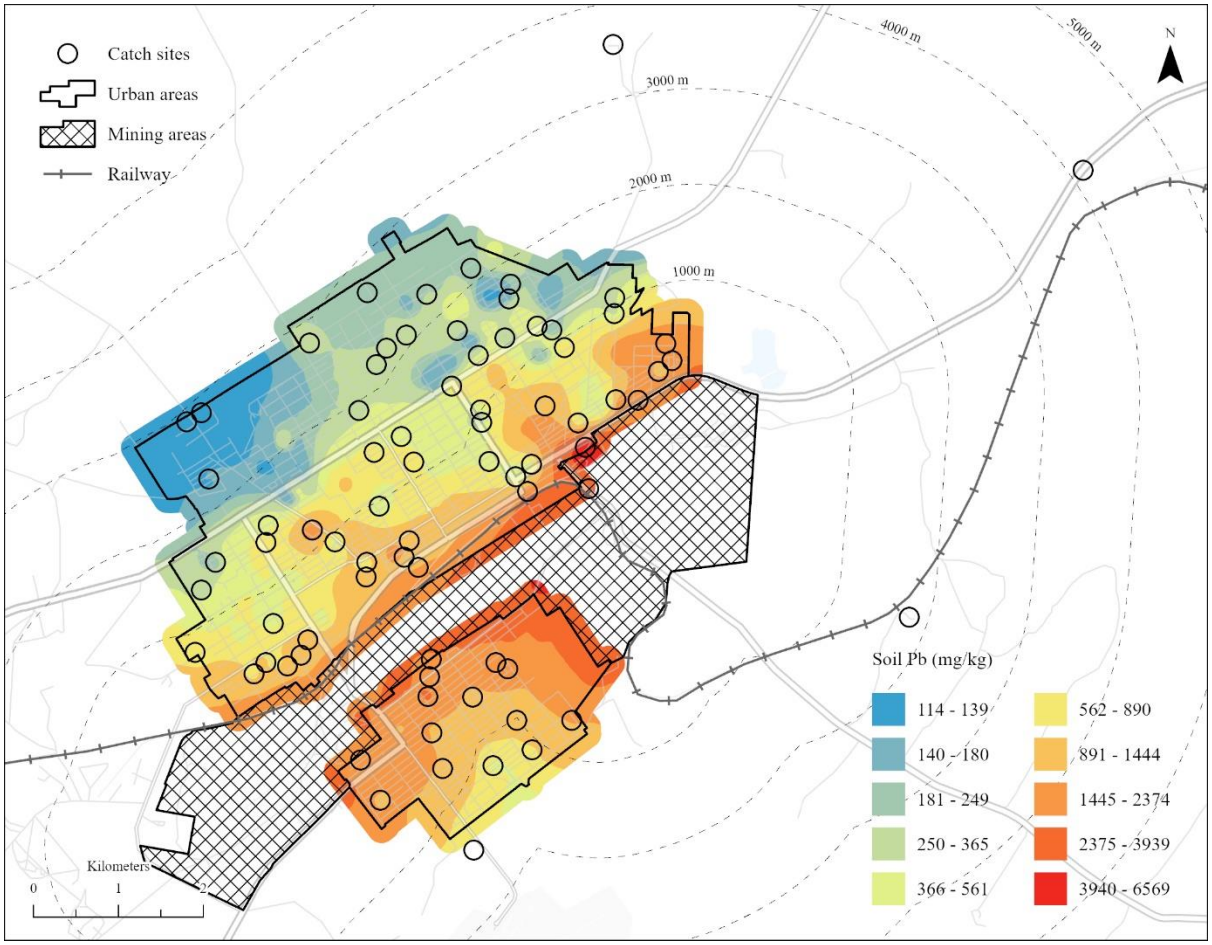


Figure 1. Map of Broken Hill catch sites. Contoured soil Pb concentrations are derived from the interpolation of 592 urban soil Pb measurements using EBK regression prediction. Concentrations are classified using a geometric interval. The interpolated surface is limited to within 100 m of urban areas due to the sparsity of soil data outside this extent.

Blood lead levels

Summary statistics for blood Pb levels in each species are reported in Table 1. We identified significant differences in blood Pb between species (Kruskal-Wallis; $p < 0.001$). Blood Pb levels (mean ± SD) in CRPI (82.1 ± 70.9 µg/dL) were significantly higher than ROPI (47.8 ± 38.0 µg/dL), HOSP (43.0 ± 29.3 µg/dL), and WPHE (30.3 ± 15.3 µg/dL); there was no significant difference between blood Pb levels in ROPI and HOSP; and WPHE blood Pb levels were significantly lower than in CRPI, ROPI and HOSP (Dunn's post hoc) (Figure 2; Table S6). Comparison of our data with blood Pb toxicity thresholds indicate that a majority of blood Pb measurements exceeded concentrations associated with subtoxic effects in Columbiformes and Falconiformes (20 µg/dL) (Figure 2; Table S7).

Table 1. Summary statistics for blood Pb concentrations (µg/dL) measured in crested pigeons (CPRI), white-plumed honeyeaters (WPHE), house sparrows (HOSP), rock pigeons (ROPI).

Species	Count	Mean	SD	Min	25%	50%	75%	Max
CRPI	41	82.1	70.9	15.5	47.8	59.6	80.8	340.2
WPHE	49	30.3	15.3	10.4	20.0	27.4	33.9	94.3
HOSP	446	43.0	29.3	6.0 [a]	19.8	35.2	62.4	111.0 [b]
ROPI	40	47.8	38.0	9.0	23.6	35.1	54.0	174.3

[a] *Lower limit of detection for ASV blood Pb measurements in HOSP.*
[b] *Upper limit of detection for ASV blood Pb measurements in HOSP.*

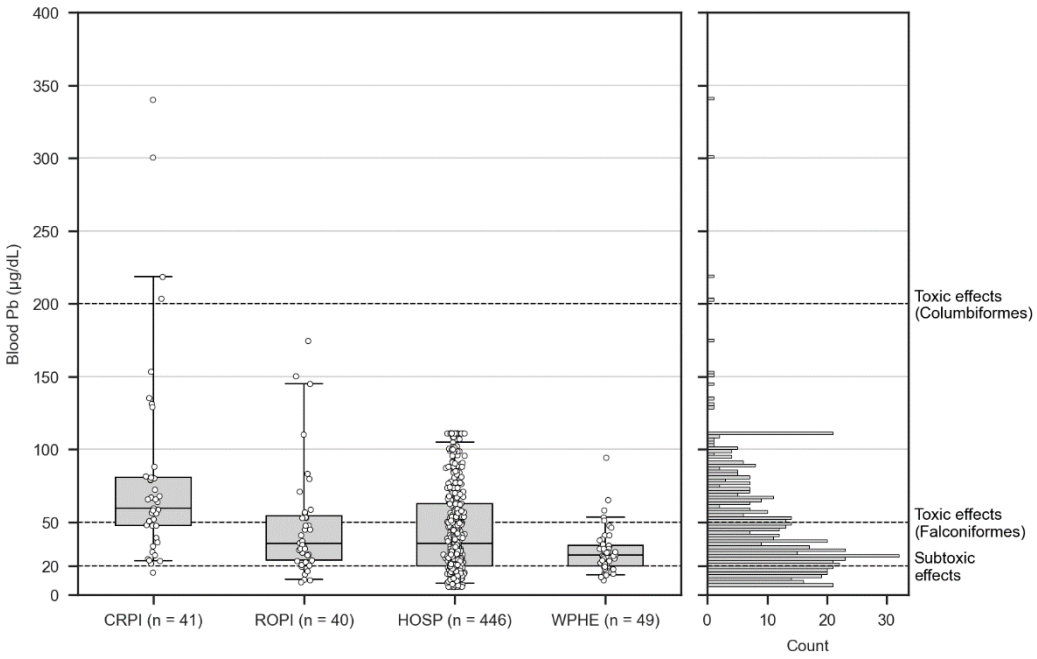


Figure 2. Distribution (5%, 25%, 50%, 75%, 95%) of blood Pb levels in crested pigeons (CPRI), rock pigeons (ROPI), house sparrows (HOSP), white-plumed honeyeaters (WPHE). Dashed lines indicate blood Pb thresholds associated with subtoxic effects (20 µg/dL), with the different toxic effect thresholds for Columbiformes (200 µg/dL) and Falconiformes (50 µg/dL) indicated.

Blood Pb levels in all species declined with distance from mining areas (Figure 3). As a localised measure of variability in exposure, we calculated the RSD of blood Pb levels for each species at sites where two or more individuals of that species were caught. We did not identify a significant difference between the RSD of blood Pb levels (mean ± SD) in CRPI (35 ± 22%), ROPI (31 ± 27%), HOSP (30 ± 13%), or WPHE (27 ± 10%) (Kruskal-Wallis; $p = 0.061$), suggesting that intra-site variability in blood Pb levels was somewhat consistent between these species. Furthermore, repeated blood Pb measurements were available for 4 HOSP captured from the same site 6–222 days apart. The relative percent difference (RPD) (mean ± SD) of blood Pb between these repeated measurements was 13 ± 5% (Table S8).

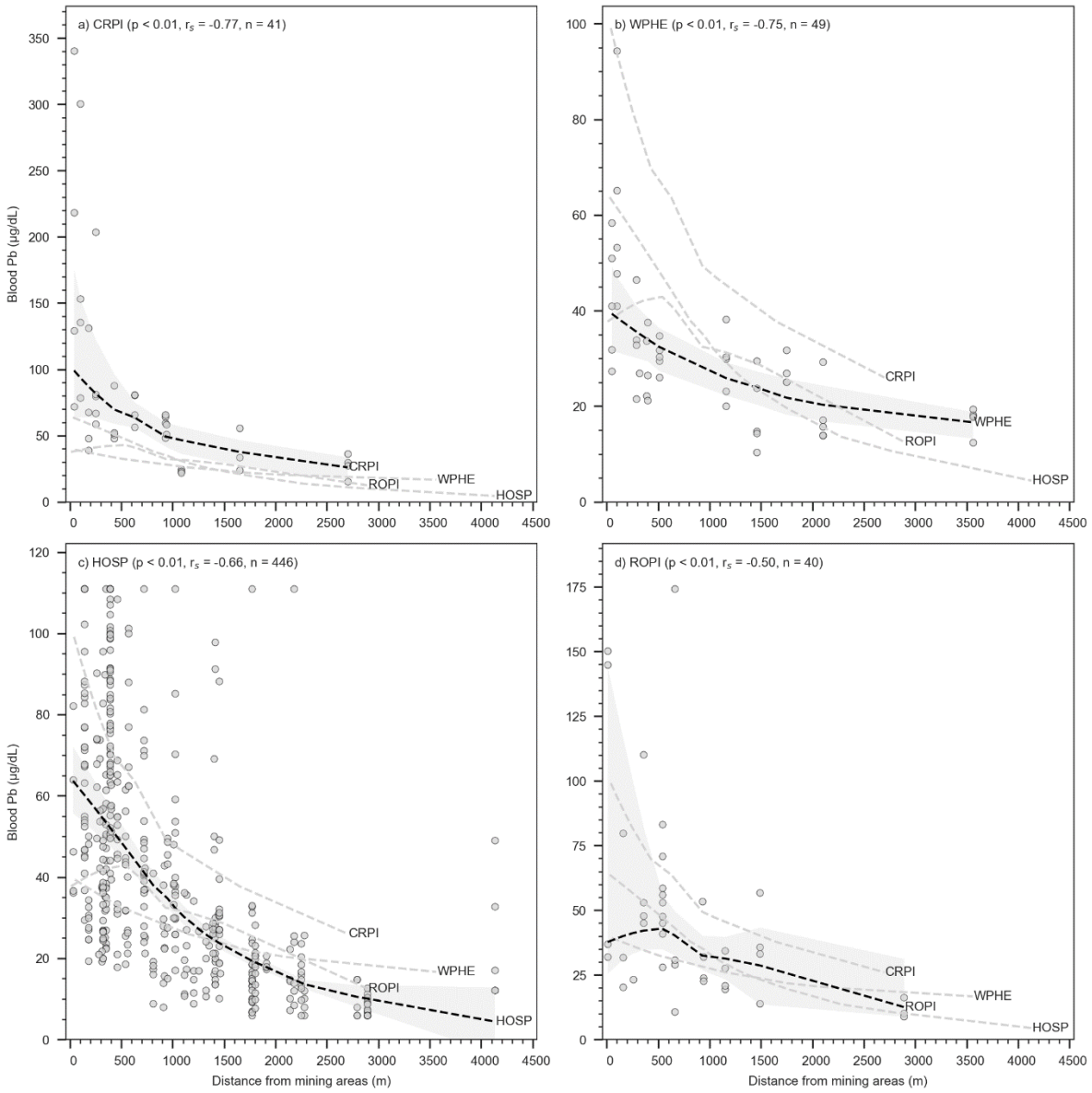


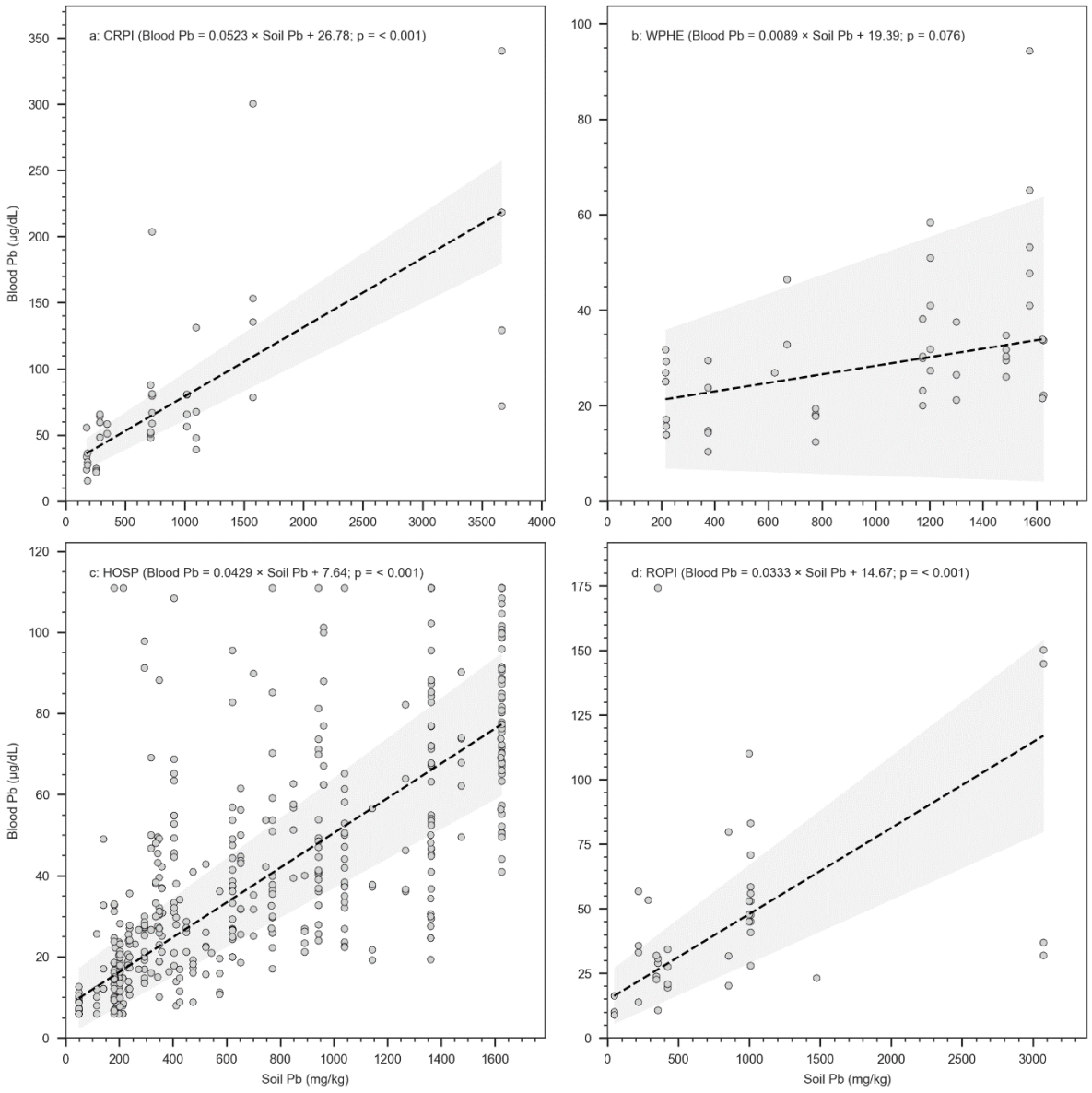
Figure 3. Relationship between blood Pb levels (µg/dL) of (a) crested pigeons (CPRI), (b) white-plumed honeyeaters (WPHE), (c) house sparrows (HOSP), (d) rock pigeons (ROPI), with distance of sites from the nearest mining area. The dashed line indicates a smoothed Loess regression (± 95% bootstrapped confidence interval) for this relationship. Spearman's correlation coefficient (r_s) describes the strength and significance of the correlation.

Relationship between soil and blood lead

We used quantile regression to examine the relationship between soil and blood Pb levels in the target species (Figure 4; Table S9 (Model A)). Soil Pb and species accounted for approximately 36% (R^2 = 0.36) of the variability in median blood Pb. Adjusting for species-specific effects derived from the soil Pb × species interaction, the change in blood Pb as a function of soil Pb was largest in CRPI ($\beta_1 + \beta_{CRPI}$ = 0.0523, p < 0.01), followed by HOSP (β_1

+ β_{HOSP} = 0.0429, p < 0.01), ROPI (β_1 + β_{ROPI} = 0. 0333, p < 0.01) and WPHE (β_1 + β_{HE} = 0.0089, p = 0.08), and was significant in all species except for WPHE (Figure 4; Table S9 (Model A)). Based on the soil Pb × species interaction, the slope of this relationship differed significantly between all species (p < 0.05), with the largest difference between WPHE and CRPI, HOSP and ROPI (Figure 4; Table S9 (Model A)).

Due to the significant association between soil and blood Pb levels amongst CRPI, HOSP, and ROPI, we recalculated our quantile regression model to derive a single exposure slope factor for the blood Pb response to changes in soil Pb for these species (Table S9 (Model B)). We did not use the species specific intercepts or slopes derived from the soil Pb × species interaction term for this purpose due to the relative sparsity of data available for CRPI and ROPI. Amongst these species, for every 1 mg/kg increase in soil Pb, the overall median blood Pb changed by β_1 = 0.0433 µg/dL (SE = 0.001, t = 30.969, p < 0.01) (Table S9 (Model B)). The associated linear model (blood Pb µg/dL = 0.0433 × soil Pb mg/kg + 11.33) indicates that a soil Pb of approximately 200 mg/kg, 900 mg/kg and 4350 mg/kg equates to a median blood Pb approximating to the subtoxic effect threshold of 20 µg/dL and the toxic effect thresholds of 50 µg/dL in Falconiformes and 200 µg/dL in Columbiformes, respectively.



360 **Figure 4.** Relationship between soil Pb (mg/kg) and blood Pb (µg/dL) in (a) crested pigeons (CPRI), (b) white-plumed honeyeaters (WPHE), (c) house sparrows (HOSP), (d) rock pigeons (ROPI). The dashed line indicates the quantile regression model (median ± 95% confidence interval) for blood Pb as a function of soil Pb across the different bird species.

Relationship between blood lead and haemoglobin

365 Blood Hb levels differed significantly between species and were highest in CRPI and lowest in HOSP (Kruskal Wallis with Dunn's post hoc; $p < 0.001$) (Table S6; Table S10). Blood Hb levels also differed significantly between adult and juvenile WPHE ($p < 0.001$), but not adult and juvenile HOSP (Kruskal-Wallis; $p = 0.304$). The number of juvenile CRPI ($n = 0$) and ROPI ($n = 2$) were too low to test for differences in blood Hb levels based on maturity.

We fitted another quantile regression model to examine the relationship between blood Pb and Hb levels amongst juveniles and adults of the target bird species (Figure 5; Table S11 (Model A)). Blood Pb, species and maturity accounted for 25% ($R^2 = 0.25$) of the variability in blood Hb. Adjusting for species-specific effects derived from the blood Pb × species interaction, the change in blood Hb levels was largest in ROPI ($\beta_1 + \beta_{ROPI} = -0.1691$, $p = 0.013$), followed by WPHE ($\beta_1 + \beta_{HE} = -0.1617$, $p = 0.302$), HOSP ($\beta_1 + \beta_{HOSP} = -0.1251$, $p < 0.001$) and CRPI ($\beta_1 + \beta_{CRPI} = -0.0790$, $p = 0.055$), although the relationship was only significant in ROPI and HOSP (Figure 5; Table S11 (Model A)). From the blood Pb × species interaction term, we did not identify any significant interspecific differences in the relationship between blood Pb and blood Hb (Table S11 (Model A)). We therefore removed this interaction term from the model while retaining species as a categorical covariate. In the simplified model, for every 1 mg/kg increase in blood Pb, the overall median blood Hb changed by $\beta_1 = -0.1118$ µg/dL (SE = 0.026, t = -4.379, p = < 0.001) (Table S11 (Model B)). Based on the overall relationship established between soil Pb and median blood Pb in CRPI, ROPI and HOSP, we used this relationship to estimate decreases in median blood Hb, relative to the baseline Hb level in adults from each of these species, expected at different soil Pb concentrations (Table 2).

Table 2. Predicted median blood Pb as a function of soil Pb and predicted decrease in median blood Hb relative to baseline Hb across different levels of soil Pb contamination for crested pigeons (CPRI), house sparrows (HOSP), rock pigeons (ROPI). White-plumed honeyeaters (WPHE) are not included as blood Pb levels in this species were not significantly correlated with soil Pb. Levels of soil Pb contamination are derived from the soil Pb geoaccumulation index (*Igeo*) (Table S4).

			Predicted Hb decrease (%)		
Soil Pb contamination level	Soil Pb (mg/kg)	Predicted blood Pb (µg/dL)	CRPI	HOSP	ROPI
Uncontaminated	< 150	< 17.8	< 0.1	< 0.8	< 0.5
Uncontaminated to moderately contaminated	150–300	17.8–24.3	0.1–0.5	0.8–1.2	0.5–0.8
Moderately contaminated	300–600	24.3–37.3	0.5–1.3	1.2–2	0.8–1.6
Moderately to highly contaminated	600–1200	37.3–63.3	1.3–2.8	2–3.7	1.6–3
Highly contaminated	1200–2400	63.3–115.2	2.8–5.7	3.7–7.1	3.0–5.9
Highly to extremely contaminated	2400–4800	115.2–219	5.7–11.7	7.1–13.9	5.9–11.6

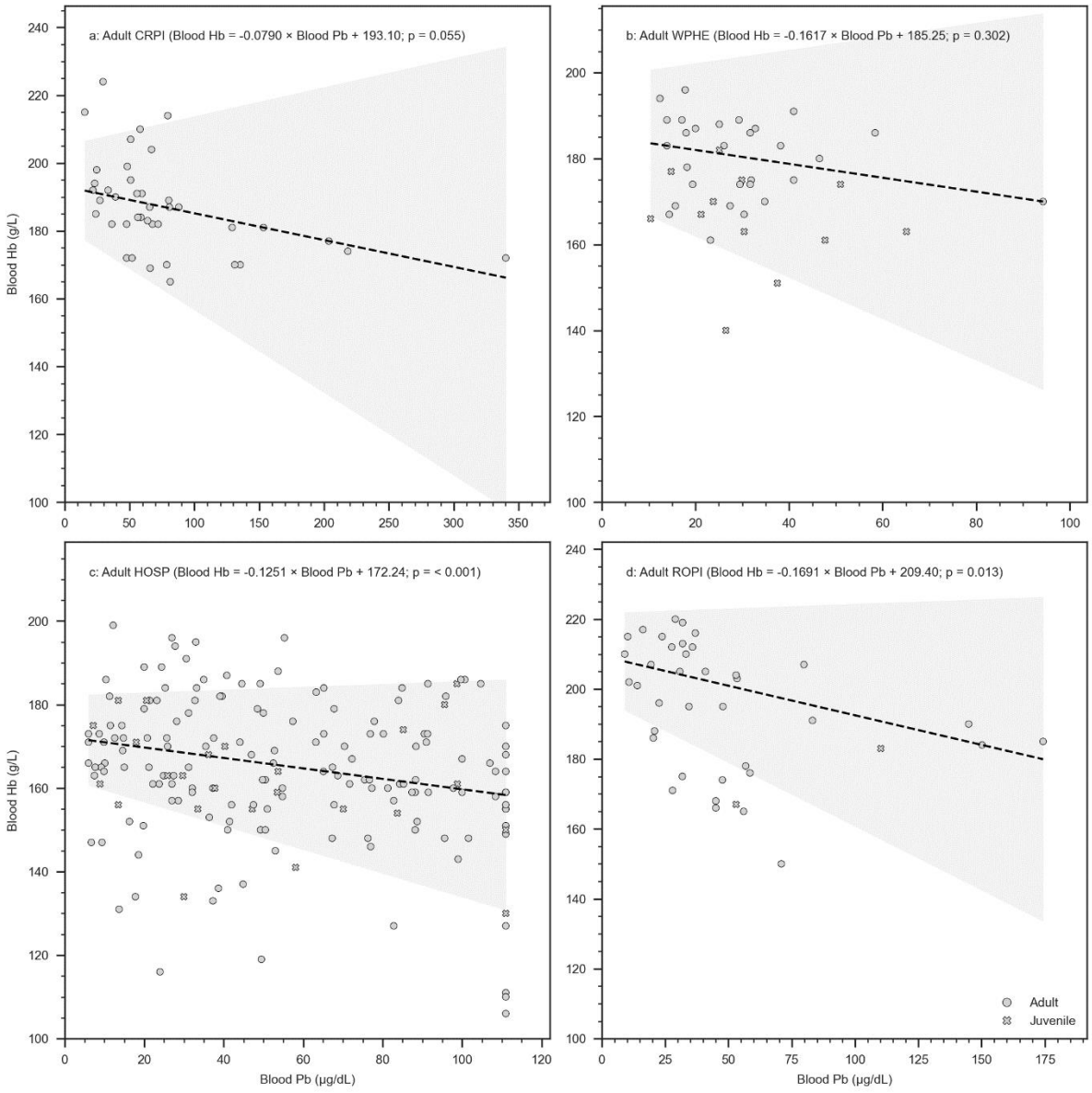


Figure 5. Relationship between blood Pb (µg/dL) and blood Hb (g/L) in adult (a) crested pigeons (CPRI), (b) white-plumed honeyeaters (WPHE), (c) house sparrows (HOSP), (d) rock pigeons (ROPI). The dashed line indicates the quantile regression model (median ± 95% confidence interval) for blood Hb as a function of blood Pb across adults of the different bird species. Datapoints for juveniles are plotted but are not included in the regression calculation.

Discussion: This study examined links between soil Pb contamination and biomarkers of Pb exposure and effect in four species of urban bird living in an Australian mining city. We observed significant differences in the blood Pb concentration of crested pigeons, rock pigeons, house sparrows and white-plumed honeyeaters which corresponded closely to the relationship observed between soil and blood Pb in these species (Table 1; Figure 2; Figure 4; Table S6). These differences can be partly attributed to interspecific variation in foraging strategy. The

arboreal foraging white-plumed honeyeater, for example, has limited direct contact with Pb contaminated soil. This is reflected in the lower blood Pb of this species, and the lower rate at which blood Pb is observed to increase relative to soil Pb compared to the ground foraging species in this study (crested pigeons, rock pigeons, house sparrows). In other Australian honeyeaters, atmospherically deposited Pb has been attributed to elevated levels of Pb exposure, even in contexts where levels of soil Pb contamination are low (Gulson et al., 2012). This would account for the significant declines in the blood Pb of white-plumed honeyeaters away from mining emission sources and also suggests that atmospherically deposited Pb could be a more relevant source of Pb exposure than soil Pb in arboreal foraging bird species.

In crested pigeons, rock pigeons and house sparrows, factors influencing significantly different blood Pb levels and their rate of increase relative to soil Pb are less clear. These species have similar diets and foraging behaviours, and often forage together in groups (Mulhall and Lill, 2011). It is possible the opportunistic feeding tendencies of the rock pigeon and house sparrow contribute to a more diversified diet and a lower overall soil ingestion rate than crested pigeons with a strong dietary preference for seeds (Anderson, 2007; Frith et al., 1974). House sparrows and rock pigeons could also be more physiologically efficient at regulating blood Pb levels than crested pigeons. A recent genomic analysis of house sparrow populations in Australia found a higher incidence of genes relevant to Pb exposure in populations inhabiting mining cities, including two involved in the transport of Pb and other metals across cell membranes (Andrew et al., 2019). Human commensalism in rock pigeons and house sparrows likely began with the advent of agriculture (Marom et al., 2018; Sætre et al., 2012), and some resilience to Pb contamination may well have evolved in both species. Crested pigeons instead have only recently expanded their range into urban habitats (Mulhall and Lill, 2011). However, while the influence of foraging strategy and diet on contaminant uptake is well established (Durkalec et al., 2022), further research is needed to understand how evolutionary processes may influence susceptibility to Pb exposure in different species.

A limited number of studies have spatially linked soil Pb contamination from mining to blood Pb levels measured in birds (Beyer et al., 2013; Brasso et al., 2023; Chapa-Vargas et al., 2010; Hansen et al., 2011). As reported here, most of these studies show a consistent increase in blood Pb with increasing soil Pb, regardless of species. The magnitude of this increase appears closely related to foraging strategy, with the largest differences in blood Pb evident at moderate to extreme levels of soil Pb contamination (600–4800 mg/kg; Table 3). Additionally, higher blood Pb levels were observed in both ground and arboreal foraging species in this study compared

to previously published research on species with equivalent foraging strategies and at sites with similar levels of soil Pb contamination (Table 3; Table S12). This variability is possibly related to other factors influencing soil Pb exposure, such as habitat, nesting behaviour, and diet (Durkalec et al., 2022), as well as environmental factors such as climate (Noyes et al., 2009). For example, in arid, sparsely vegetated environments such as Broken Hill, a lack of ground cover, including leaf litter, may increase exposure to Pb contaminated surface soil by ground foraging species. A lack of ground cover also enhances the generation and deposition of Pb contaminated dust onto other foraging substrates, including vegetation. These factors may contribute to overall lower blood Pb levels reported by studies from mining impacted contexts in North America where there is higher levels of rainfall and vegetative cover (Beyer et al., 2013; Brasso et al., 2023; Hansen et al., 2011) (Table 3). However, our findings also indicate that even amongst species inhabiting the same environmental context and with similar food preferences and foraging behaviours, the blood Pb response to exposure to soil Pb contamination can still differ significantly.

Table 3. Summary of literature data on blood Pb levels in birds with different foraging strategies inhabiting different soil Pb contamination ranges. Only studies with site-specific soil Pb measurements from Pb mining and smelting contaminated environments are included (Beyer et al., 2013; Brasso et al., 2023; Hansen et al., 2011). Levels of soil Pb contamination are derived from the geoaccumulation index (*Igeo*), assuming a background soil Pb concentration of 100 mg/kg. Foraging strategies are assigned according descriptions in Billerman (2020). A significant difference between groups is indicated by * ($p < 0.05$) or ** ($p < 0.01$) (Kruskal-Wallis). Comparative statistics for individual species, along with information on foraging guild and diet, are reported in Table S12.

		Other studies		This study	
Soil Pb contamination level	Soil Pb (mg/kg)	Ground forager	Other forager	Ground forager	Other forager
Uncontaminated	< 150	5.5 ± 8.6 (n=129)	5.8 ± 9.3 (n=24)	11.7 ± 9.1 (n=31)	
Uncontaminated to moderately contaminated	150–300	8.9 ± 5.4 (n=5)	6.1 ± 3.3 (n=4)	23.8 ± 19.2 (n=116)	22.1 ± 6.9 (n=9)
Moderately contaminated	300–600			33.5 ± 22.5 (n=96)	24.0 ± 14.9 (n=6)
Moderately to highly contaminated	600–1200	24.7 ± 31.7 (n=171) *	10.7 ± 7.2 (n=14)	49.6 ± 25.9 (n=154) **	26.3 ± 9.8 (n=12)

Highly contaminated	1200–2400	45.3 ± 35.9 (n=113)	**	21.3 ± 14.9 (n=59)	76.4 ± 33.3 (n=122)	**	39.1 ± 17.2 (n=22)
Highly to extremely contaminated	2400–4800	55.2 ± 44.0 (n=38)	*	26.5 ± 23.8 (n=5)	140.5 ± 102.4 (n=8)		
Extremely contaminated	≥ 4800	40.5 ± 18.3 (n=44)					
All data		27.9 ± 33.1 (n=500)		16.0 ± 14.9 (n=106)	46.3 ± 36.4 (n=527)		31.0 ± 15.5 (n=49)

Some of the interspecific variability observed in the relationship between soil Pb and blood Pb could also be related to differences in behaviours such as site fidelity and home range (Durkalec et al., 2022). However, following the same trend found for soil, blood Pb levels in all target bird species declined with distance from Pb emission sources in mining areas (Figure 1; Figure 3). This suggests a relatively high degree of sedentarism in these species, with blood Pb levels in each bird reflecting levels of Pb contamination within their immediate environment. Recorded home ranges for these species vary but are often shortest in urban areas where food sources and favourable nesting sites are abundant (Sol and Senar, 1995; Vangestel et al., 2010). Amongst these species, similar intra-site variability in blood Pb levels provides additional evidence for their sedentarism and suggests that any of these species could be used as spatially sensitive biomonitors of Pb exposure risks to bird populations in urban ecosystems. As well as being spatially representative, low variability observed in repeated blood Pb measurements from house sparrows also indicates a high degree of temporal consistency in Pb exposure, at least in this species (Table S8).

More broadly, our findings also align with previous studies indicating physiological differences in the uptake of Pb by different animal classes. We detected an apparently linear dose-response of blood Pb as a function of soil Pb, which is contrary to sublinear relationships previously identified by Pb dosing experiments in mammals (Casteel et al., 2006; Freeman et al., 1992; Freeman et al., 1991). In studies of birds dosed with Pb contaminated soil and sediment a linear response is most frequently reported (Beyer et al., 2014; Day et al., 2003; Heinz et al., 1999; Hoffman et al., 2000a; Hoffman et al., 2000b). This has implications for the characterisation of risk associated with exposure to soil Pb and its bioaccumulation in birds.

Estimated soil ingestion rates in ground foraging bird species range between 9–20% of their diet (Beyer et al., 1994; Beyer et al., 2013). Assuming this range is applicable to the ground foraging bird species examined here (crested pigeon, rock pigeon, house sparrow), we can

apply it to compare the relationships established between soil and blood Pb levels to those previously identified in experimental dosing studies. At a soil ingestion rate of 9–20%, the slope for the change in blood Pb as a function of soil Pb in these ground foraging species ranges from 0.22 at 20% to 0.48 at 9% (Figure 6). This range fits within that identified by Beyer et al. (2014), where a review of soil Pb dosing studies reported unit equivalent slopes of 0.16 in Canada geese (*Branta canadensis*) to 0.75 in mallards (*Anas platyrhynchos*) (values converted from mg/kg wet weight to µg/dL assuming an avian blood density of 1.05 g/mL (Scanes, 2015)) (Beyer et al., 2014; Day et al., 2003; Heinz et al., 1999; Hoffman et al., 2000a; Hoffman et al., 2000b) (Figure 6). This supports the relevance of these soil ingestion rates for ground foraging birds. It also suggests that the relationships established between soil and blood Pb levels in this study are consistent with those identified in more controlled soil Pb dosing studies.

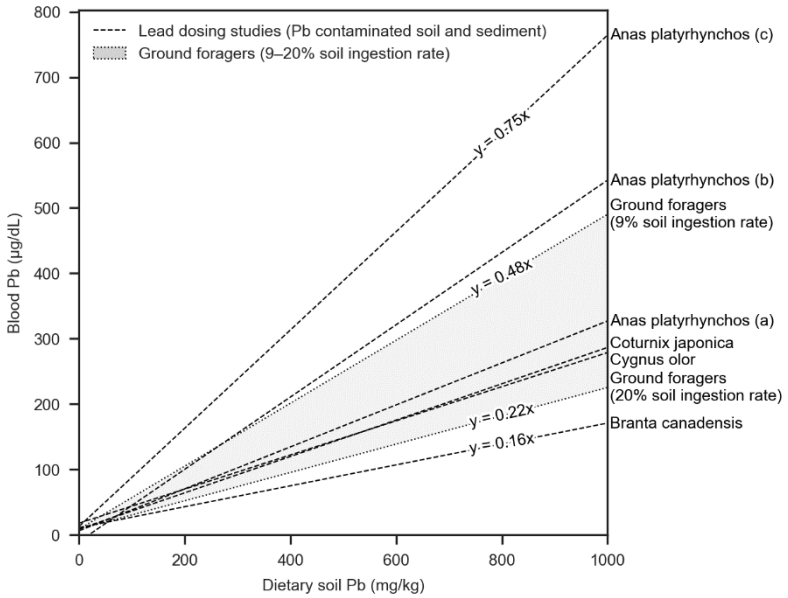


Figure 6. Comparison of modelled slopes for relationship between dietary soil Pb and blood Pb. Only dosing studies using Pb contaminated soil from mining areas are included (values converted from mg/kg wet weight to µg/dL assuming an avian blood density of 1.05 g/mL (Scanes, 2015)) (Beyer et al., 2014; Day et al., 2003; Heinz et al., 1999; Hoffman et al., 2000a; Hoffman et al., 2000b). Equivalent slopes (shaded area) for ground foraging birds in this study (crested pigeons, rock pigeons, house sparrows) are calculated based on the combined exposure slope factor for these species (β_1 = 0.0433), expressed as a proportion of the estimated soil Pb ingestion rate of similar species (9–20%) (Beyer et al., 1994; Hansen et al., 2011).

The consistency of the relationship between dietary soil Pb and blood Pb identified here, and by previous studies, suggests that soil Pb thresholds could be derived for subtoxic and toxic

blood Pb thresholds, at least for ground foraging species with similar soil ingestion rates. For the ground foraging species in this study (crested pigeons, rock pigeons, house sparrows), the overall modelled relationship between soil and blood Pb indicates that to maintain a median blood Pb concentration below the subtoxic threshold of 20 µg/dL, soil Pb concentrations should be below 200 mg/kg. This is only slightly higher than the 166 mg/kg soil Pb concentration identified as maintaining domestic chicken (*Gallus gallus domesticus*) blood Pb levels below this same subtoxic threshold (Yazdanparast et al., 2022). The accuracy of these soil Pb thresholds will vary according to behavioural, physiological, and environmental factors influencing exposure to, and uptake of, Pb in different bird species.

Soil Pb concentrations (200 mg/kg) exceeding those associated with the subtoxic blood Pb effect threshold (20 µg/dL) in this study are widespread in mining impacted contexts, and account for approximately 88% of our catch sites in Broken Hill (n = 68/77). However, they are also common in unindustrialised urban areas. In an analysis of 17,256 soil samples from residential areas throughout Australia, 20% had soil Pb concentrations exceeding 300 mg/kg, and 35% of residences had at least one sample which exceeded this concentration (Taylor et al., 2021). Consequently, if the relationship between soil Pb and blood Pb established for the ground foraging birds in this study is representative of similar species, blood Pb concentrations associated with subtoxic effects may be widespread in urban bird populations.

In addition to the relationship established between soil and blood Pb, we observed a consistent yet gradual decline in blood Hb levels across the range of blood Pb concentrations measured within our target species (Table 2; Figure 5). This aligns with our understanding of the effects of elevated blood Pb on δ-ALAD activity and Hb synthesis in birds (Minias, 2015). However, while δ-ALAD inhibition is commonly observed at elevated blood Pb levels, the resultant effects on haematological parameters are more variable (Blus et al., 1993; Buekers et al., 2009; Custer et al., 1984; Espín et al., 2015; Grue et al., 1986). This might be related to the severity and duration of Pb exposure (Redig et al., 1991), or possibly interspecific factors influencing sensitivity to Pb. We did not, however, detect any significant difference in the relationship between blood Pb and blood Hb levels in different species (Table S11 (Model A)). This is despite previous research indicating that Columbiformes are more resilient to the toxic effects of Pb exposure, at least compared to Falconiforms (Franson and Pain, 2011). The blood Hb response to Pb exposure observed here is also comparable to previous studies of other bird species in experimental Pb dosing studies. For example, compared to a background blood Pb of 3–19 µg/dL, Beyer et al. (2000) observed significant decreases in blood Hb of between 9–

22% over a blood Pb concentration range of 242–315 µg/dL in dosed mute swans (*Cygnus olor*), Canada geese (*Branta canadensis*) and mallards (*Anas platyrhynchos*). Over this same concentration range, we would expect a decline in median blood Hb levels of 12.9–20.2% in adults of our target species (Table 2; Table S11 (Model B)). The blood Hb response to Pb exposure may therefore be more consistent between species than has been previously indicated by pairwise comparisons of exposed and unexposed individuals.

In this study, we investigated blood Pb and Hb levels in four species of urban bird along a gradient of soil Pb contamination in an Australian mining city. Globally, there are many mining impacted environments where levels of soil Pb contamination are comparable to those reported here (Frank et al., 2019; Landrigan et al., 2018). Our findings show that exposure to these levels of soil Pb contamination lead to elevated blood Pb and supressed Hb levels. However, even in urban areas with minimal industry and comparatively low levels of soil Pb contamination, subtoxic levels of Pb exposure have been linked to adverse behavioural and physiological outcomes in birds (Espín et al., 2015; Hitt et al., 2023; McClelland et al., 2019; Work and Smith, 1996). Our data indicates that, at least in ground foraging species, blood Pb levels associated with these subtoxic effects may occur at soil Pb concentrations that are widespread within urban areas (Laidlaw et al., 2017; Taylor et al., 2021). This has implications for the health of vulnerable bird populations inhabiting the growing extent of land impacted by urbanisation and mining (Maus et al., 2022; Seto et al., 2011). The findings of this study should provide context for monitoring the health of bird populations in environments with varying levels of soil Pb contamination.

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Supervision: MPT, SCG

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Competing interests: Mark Patrick Taylor has undertaken work for, and received funding from, the Broken Hill Environmental Lead Program of the NSW Environment Protection Authority (EPA). He has received funding for lead and other trace metal related work from the Australian Federal Government. He has also prepared commissioned reports and provided expert advice on environmental contamination and human health for a range of bodies, including the Australian Building Codes Board (lead in plumbing fittings and materials), lawyers, governments, union agencies, and private companies. He has also served as an expert in plaintiff cases of childhood lead poisoning relating to Mount Isa, Queensland and Kabwe, Zambia. No other authors declare a competing interest.

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Supplementary information for:

Title: Blood lead increases and haemoglobin decreases in urban birds along a soil contamination gradient in a mining city

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Table S1. Number of catch sites, blood Pb measurements and blood Hb measurements for each species in this study. Some species were caught from the same site.

	Catch sites (n)	Blood Pb measurements (n)	Blood Hb measurements (n)
CRPI	11	41	40
HOSP	49	446	182
ROPI	11	40	39
WPHE	13	49	41
Total	77	576	302

Table S2. Summary statistics for the OLS models used to adjust natural log-transformed ASV blood Pb measurements according to paired natural log-transformed ICP-MS measurements. Separate models were used to adjust data for the species pairs CRPI and ROPI, and HOSP and WPHE.

Species pair	Slope	Intercept	Slope SE	Intercept SE	R^2	F statistic	P value
CRPI and ROPI	1.0428	0.2592	0.1216	0.4040	0.8804	73.5859	< 0.001
HOSP and WPHE	0.8275	1.2552	0.0643	0.1701	0.8297	165.6921	< 0.001

Table S3. EBK regression prediction method parameters and summary diagnostics. EBK regression prediction was used to derive a continuous soil Pb concentration surface across urban Broken Hill based on a dataset of 592 individual soil Pb samples. The distance of each sample from mining areas was included as an additional explanatory variable.

Method	
Output type	Prediction
Cumulative variance	95
Transformation type	Empirical
Semivariogram model type	Exponential
Subset size	100
Overlap factor	1
Number of simulations	100
Searching neighbourhood	Smooth circular
Smoothing factor	0.25
Radius	1000 m

Summary	
Count	247.75
Average Continuous Ranked Probability Score	90.54
Inside 90 Percent Interval	96.11
Inside 95 Percent Interval	-16.54
Mean	840.31
Root-Mean-Square	0.01
Mean Standardized	0.94
Root-Mean-Square Standardized	763.71
Average Standard Error	247.75

Table S4. Classification of soil Pb contamination levels (Barbieri, 2016) according to calculation of the geoaccumulation index (*Igeo*) based on a Broken Hill background soil Pb concentration of 100 mg/kg (Kristensen and Taylor, 2016). The number and percentage of catch sites falling within each soil Pb contamination level is also provided.

Soil Pb contamination level	Igeo	Soil Pb (mg/kg)	Catch sites (n)	Catch sites (%)
Uncontaminated	< 0	< 150	4	5.2%
Uncontaminated to moderately contaminated	0–1	150–300	20	26.0%
Moderately contaminated	1–2	300–600	19	24.7%
Moderately to highly contaminated	2–3	600–1200	22	28.6%
Highly contaminated	3–4	1200–2400	10	13.0%
Highly to extremely contaminated	> 5	2400–4800	2	2.6%

Table S5. Summary statistics for site mean soil Pb concentrations derived from EBK regression prediction and site measurements. Data for the EBK regression prediction includes measurements from three sites which fell outside the urban extent of Broken Hill, and therefore the EBK interpolated soil Pb surface.

Method	Site count	Mean	SD	Min	25%	50%	75%	Max
EBK regression prediction	77	685	616	46	255	449	961	3664
Site measurements	63	699	1021	59	208	369	683	6388

Table S6. Results of Kruskal-Wallis test with Dunn's post hoc test on differences in blood Pb and blood Hb between species.

Blood Pb

Kruskal-Wallis	H statistic	P value			
	29.8875	< 0.001			
Dunn's post hoc		CRPI	HOSP	ROPI	WPHE
	CRPI	1.000	0.000	0.003	0.000
	HOSP	0.000	1.000	0.519	0.018
	ROPI	0.003	0.519	1.000	0.030
	WPHE	0.000	0.018	0.030	1.000

Blood Hb

Kruskal-Wallis	H statistic	P value			
	104.4681	< 0.001			
Dunn's post hoc		CRPI	HOSP	ROPI	WPHE
	CRPI	1.000	0.000	0.669	0.180
	HOSP	0.000	1.000	0.000	0.035
	ROPI	0.669	0.000	1.000	0.078
	WPHE	0.180	0.035	0.078	1.000

Table S7. Distribution of species blood Pb concentrations relative to those associated with "background" levels of exposure and thresholds for subtoxic and toxic effects (Franson and Pain, 2011). CRPI and ROPI are compared to toxic effect thresholds for Columbiformes, while HOSP and WPHE are compared to the more conservative toxic effect thresholds for Falconiformes.

Species	Effect	Blood Pb threshold (µg/dL)	Individuals (n)	Individuals (%)
CRPI	"Background"	< 20	1	2.4%
	Subtoxic effects	20–200	36	87.8%
	Toxic effects	≥ 200	4	9.8%
ROPI	"Background"	< 20	6	15.0%
	Subtoxic effects	20–200	34	85.0%
	Toxic effects	≥ 200	0	0.0%
HOSP	"Background"	< 20	116	26.0%
	Subtoxic effects	20–50	186	41.7%
	Toxic effects	≥ 50	144	32.3%
WPHE	"Background"	< 20	13	26.5%
	Subtoxic effects	20–50	31	63.3%
	Toxic effects	≥ 50	5	10.2%

Table S8. Blood Pb concentrations and relative percent difference (RPD) for first and second capture of resampled HOSP.

		First Capture			**Second Capture**			
Species	Band	Site ID	Date	Blood Pb (µg/dL)	Site ID	Date	Blood Pb (µg/dL)	RPD (%)
HOSP	46657	15_LEX	1/10/2020	65.2	15_LEX	11/05/2021	75.5	14.6%
HOSP	78641	23_JOA	13/10/2020	20	23_JOA	10/05/2021	24.4	19.8%
HOSP	78667	23_JOA	4/05/2021	27.8	23_JOA	10/05/2021	25.3	9.4%
HOSP	79313	24_FRA	12/10/2020	97.8	24_FRA	28/04/2021	91.3	6.9%

Table S9. Results for quantile regression model examining relationship between the dependent variable blood Pb and the independent variables soil Pb and species. Model A incorporates all species and a soil Pb × species interaction, with comparisons based on the conditional median of blood Pb in each reference species. Model B includes only ground foraging species (CRPI, HOSP, ROPI), with no soil Pb × species interaction, and with comparisons based on the overall conditional median of blood Pb across these species. Italicised coefficients from Model B are used for blood Pb predictions.

Model A (Blood Pb ~ Soil Pb + Species + (Soil Pb × Species))

Reference species: CRPI	Coefficient	SE	T value	P value	Lower 95% CI	Upper 95% CI
Constant	26.777	3.978	6.732	< 0.001	18.964	34.59
Soil Lead	0.0523	0.003	18.153	< 0.001	0.047	0.058
Soil Pb × HOSP	-0.0094	0.003	-2.849	0.005	-0.016	-0.003
Soil Pb × ROPI	-0.019	0.005	-4.196	< 0.001	-0.028	-0.01
Soil Pb × WPHE	-0.0434	0.006	-7.477	< 0.001	-0.055	-0.032
Reference species: WPHE	Coefficient	SE	T value	P value	Lower 95% CI	Upper 95% CI
Constant	19.3944	5.499	3.527	< 0.001	8.594	30.195
Soil Pb	0.0089	0.005	1.779	0.076	-0.001	0.019
Soil Pb × CRPI	0.0434	0.006	7.477	< 0.001	0.032	0.055
Soil Pb × HOSP	0.0339	0.005	6.413	< 0.001	0.024	0.044
Soil Pb × ROPI	0.0243	0.006	3.966	< 0.001	0.012	0.036
Reference species: HOSP	Coefficient	SE	T value	P value	Lower 95% CI	Upper 95% CI
Constant	7.6408	1.494	5.115	< 0.001	4.707	10.575
Soil Pb	0.0429	0.002	26.302	< 0.001	0.04	0.046
Soil Pb × CRPI	0.0094	0.003	2.849	0.005	0.003	0.016
Soil Pb × ROPI	-0.0096	0.004	-2.485	0.013	-0.017`	-0.002
Soil Pb × WPHE	-0.0339	0.005	-6.413	< 0.001	-0.044	-0.024
Reference species: ROPI	Coefficient	SE	T value	P value	Lower 95% CI	Upper 95% CI
Constant	14.6699	4.189	3.502	< 0.001	6.442	22.898
Soil Pb	0.0333	0.004	9.492	< 0.001	0.026	0.04
Soil Pb × CRPI	0.019	0.005	4.196	< 0.001	0.01	0.028
Soil Pb × HOSP	0.0096	0.004	2.485	0.013	0.002	0.017
Soil Pb × WPHE	-0.0243	0.006	-3.966	< 0.001	-0.036	-0.012

Model B (Blood Pb ~ Soil Pb + Species (CRPI, ROPI, HOSP))

Overall median	Coefficient	SE	T value	P value	Lower 95% CI	Upper 95% CI
Constant	*11.3343*	*1.386*	*8.175*	*< 0.001*	*8.611*	*14.058*
Soil Pb	*0.0433*	*0.001*	*31.733*	*< 0.001*	*0.041*	*0.046*
CRPI	17.1039	2.378	7.193	< 0.001	12.433	21.775
HOSP	-3.775	1.249	-3.023	0.003	-6.228	-1.322
ROPI	-1.9946	2.38	-0.838	0.402	-6.669	2.68

Table S10. Summary statistics for blood Hb concentrations (g/L) measured in the target species.

Species	Count	Mean	SD	Min	25%	50%	75%	Max
CRPI	40	187	14	165	180	186	192	224
HOSP	182	164	17	106	156	164	175	199
ROPI	39	194	18	150	180	196	210	220
WPHE	41	176	12	140	169	175	186	196

Table S11. Results for quantile regression model examining relationship between the dependent variable blood Hb and the independent variables blood Pb, species and maturity. Model A incorporates all species and a blood Pb × species interaction, with comparisons based on the conditional median of blood Hb in each reference species. In Model B, the blood Hb × species is removed, and comparisons are based on the overall conditional median of blood Hb across the species and maturity levels. Italicised coefficients from Model B are used for blood Hb predictions.

Model A (Blood Hb ~ Blood Pb + Species + Maturity + (Soil Pb × Species)

Reference species: CRPI	Coefficient	SE	T value	P value	Lower 95% CI	Upper 95% CI
Constant	126.1437	2.858	44.144	< 0.001	120.52	131.768
Blood Pb	-0.079	0.041	-1.923	0.055	-0.16	0.002
Adult	66.9541	1.648	40.625	< 0.001	63.711	70.198
Juvenile	59.1896	2.327	25.435	< 0.001	54.61	63.77
Blood Pb × HOSP	-0.0461	0.054	-0.85	0.396	-0.153	0.061
Blood Pb × ROPI	-0.0901	0.079	-1.134	0.258	-0.246	0.066
Blood Pb × WPHE	-0.0827	0.162	-0.512	0.609	-0.401	0.235
Reference species: WPHE	Coefficient	SE	T value	P value	Lower 95% CI	Upper 95% CI
Constant	120.9102	3.595	33.63	< 0.001	113.834	127.986
Blood Pb	-0.1617	0.156	-1.035	0.302	-0.469	0.146
Adult	64.3373	2.129	30.219	< 0.001	60.147	68.527
Juvenile	56.5728	2.445	23.136	< 0.001	51.76	61.385
Blood Pb × CRPI	0.0827	0.162	0.512	0.609	-0.235	0.401
Blood Pb × HOSP	0.0365	0.16	0.228	0.82	-0.279	0.352
Blood Pb × ROPI	-0.0074	0.17	-0.044	0.965	-0.343	0.328
Reference species: HOSP	Coefficient	SE	T value	P value	Lower 95% CI	Upper 95% CI
Constant	112.2378	1.65	68.008	< 0.001	108.99	115.486
Blood Pb	-0.1251	0.035	-3.534	< 0.001	-0.195	-0.055
Adult	60.0012	1.34	44.793	< 0.001	57.365	62.637
Juvenile	52.2367	1.902	27.468	< 0.001	48.494	55.979
Blood Pb × CRPI	0.0461	0.054	0.85	0.396	-0.061	0.153
Blood Pb × ROPI	-0.044	0.077	-0.573	0.567	-0.195	0.107
Blood Pb × WPHE	-0.0365	0.16	-0.228	0.82	-0.352	0.279
Reference species: ROPI	Coefficient	SE	T value	P value	Lower 95% CI	Upper 95% CI
Constant	137.0123	2.943	46.552	< 0.001	131.22	142.805
Blood Pb	-0.1691	0.068	-2.487	0.013	-0.303	-0.035
Adult	72.3884	1.68	43.08	< 0.001	69.081	75.695
Juvenile	64.6239	2.357	27.415	< 0.001	59.985	69.263
Blood Pb × CRPI	0.0901	0.079	1.134	0.258	-0.066	0.246
Blood Pb × HOSP	0.044	0.077	0.573	0.567	-0.107	0.195
Blood Pb × WPHE	0.0074	0.17	0.044	0.965	-0.328	0.343

Model B (Blood Hb ~ Blood Pb + Species (CRPI, HOSP, ROPI, WPHE) + Maturity (adult, juvenile))

Overall median	Coefficient	SE	T value	P value	Lower 95% CI	Upper 95% CI
Constant	*105.017*	*1.203*	*87.295*	*< 0.001*	*102.649*	*107.385*
Blood Pb	*-0.1118*	*0.026*	*-4.379*	*< 0.001*	*-0.162*	*-0.062*
CRPI	*34.2712*	*2.425*	*14.132*	*< 0.001*	*29.499*	*39.044*

HOSP	*9.9407*	*1.402*	*7.089*	*< 0.001*	*7.181*	*12.7*
ROPI	*41.7307*	*2.283*	*18.282*	*< 0.001*	*37.239*	*46.223*
WPHE	*19.0743*	*2.267*	*8.415*	*< 0.001*	*14.613*	*23.535*
Adult	*56.4482*	*1.26*	*44.796*	*< 0.001*	*53.968*	*58.928*
Juvenile	48.5688	1.86	26.108	< 0.001	44.908	52.23

Table S12. Summary of blood Pb concentrations in species from studies in mining impacted sites with co-located soil Pb measurements. Values initially reported in mg/kg dry weight are converted to µg/dL based on reported sample moisture content and assuming an avian blood density of 1.05 g/mL (Scanes, 2015). Locations include the Southeast Missouri Lead Mining District, USA (Beyer et al., 2013; Brasso et al., 2023), the Coeur d'Alene River Basin, Idaho, USA (Hansen et al., 2011), and Broken Hill, New South Wales, Australia (this study). Soil Pb contamination levels are based on the geoaccumulation index (Igeo). Information on diet and foraging strategy are derived from Billerman (2020).

				Soil Pb range (mg/kg)							
				< 150	150–300	300–600	600–1200	1200–2400	2400–4800	> 4800	Overall
Reference	Species	Dietary preference	Foraging strategy	Mean blood Pb ± SD (µg/dL)							
Beyer et al. (2013)	Cardinalis cardinalis	Granivore	Ground	2.3 ± 2.2 (n=18)			27.3 ± 16.7 (n=4)	54.6 ± 48.0 (n=6)	62.2 ± 12.1 (n=3)		21.5 ± 32.2 (n=31)
	Cyanocitta cristata	Omnivore	Ground				22.9 ± 15.3 (n=4)	12.3 ± 8.9 (n=3)	32.9 (n=1)		20.2 ± 13.3 (n=8)
	Dumetella carolinensis	Insectivore	Ground	3.4 ± 2.1 (n=3)							3.4 ± 2.1 (n=3)
	Hylocichla mustelina	Insectivore	Ground	7.9 (n=1)				37.7 (n=1)			22.8 ± 21.1 (n=2)
	Molothrus ater	Granivore	Ground	3.0 ± 0.8 (n=5)							3.0 ± 0.8 (n=5)
	Pipilo erythrophthalmus	Omnivore	Ground	3.2 ± 0.7 (n=2)			6.9 (n=1)	30.7 (n=1)	25.2 (n=1)		13.8 ± 13.1 (n=5)
	Progne subis	Insectivore	Aerial	4.2 (n=1)							4.2 (n=1)
	Sayornis phoebe	Insectivore	Flycatcher	0.3 (n=1)							0.3 (n=1)
	Turdus migratorius	Insectivore	Ground	27.2 ± 26.8 (n=7)			199.9 ± 71.8 (n=3)		83.1 ± 35.8 (n=3)		80.0 ± 81.6 (n=13)
Brasso et al. (2023)	Cardinalis cardinalis	Granivore	Ground	3.3 ± 0.9 (n=5)			12.4 ± 7.7 (n=27)	43.7 ± 31.2 (n=30)			26.8 ± 27.7 (n=62)

	Helmitheros vermivorum	Insectivore	Foliage				6.1 ± 3.9 (n=2)	15.1 (n=1)			9.1 ± 5.9 (n=3)
	Molothrus ater	Granivore	Ground				9.2 (n=1)	42.0 ± 14.3 (n=7)			37.9 ± 17.6 (n=8)
	Passerina cyanea	Insectivore	Foliage	8.9 ± 14.1 (n=10)			12.8 ± 8.0 (n=9)	29.1 ± 15.1 (n=29)			21.8 ± 16.4 (n=48)
	Pipilo erythrophthalmus	Omnivore	Ground				6.8 ± 4.4 (n=11)	37.3 ± 38.2 (n=5)			16.4 ± 24.8 (n=16)
	Sialia sialis	Insectivore	Ground	4.9 ± 5.1 (n=38)			19.2 ± 11.7 (n=32)	45.5 ± 22.4 (n=30)			21.7 ± 21.9 (n=100)
	Spizella pusilla	Insectivore	Ground	3.8 ± 2.6 (n=12)			15.9 ± 15.7 (n=41)	24.3 ± 6.1 (n=7)			14.5 ± 14.5 (n=60)
	Turdus migratorius	Insectivore	Ground	7.6 ± 6.0 (n=9)			41.6 ± 24.4 (n=28)				33.3 ± 26.0 (n=37)
	Zenaida macroura	Granivore	Ground				44.6 ± 33.7 (n=9)	272.4 (n=1)			67.3 ± 78.7 (n=10)
Hansen et al. (2011)	Catharus ustulatus	Insectivore	Foliage	3.7 ± 0.0 (n=12)	6.1 ± 3.3 (n=4)		7.5 ± 4.3 (n=3)	13.7 ± 10.5 (n=29)	26.5 ± 23.8 (n=5)		11.7 ± 12.1 (n=53)
	Melospiza melodia	Insectivore	Ground	3.8 ± 0.4 (n=23)	7.0 ± 3.8 (n=4)		20.8 ± 10.9 (n=5)	25.9 ± 17.8 (n=8)	26.6 ± 14.4 (n=16)	32.4 ± 11.2 (n=33)	21.6 ± 15.9 (n=89)
	Turdus migratorius	Insectivore	Ground	5.6 ± 3.2 (n=6)	16.5 (n=1)		12.1 ± 6.8 (n=5)	62.4 ± 32.7 (n=14)	84.1 ± 53.4 (n=14)	64.8 ± 13.3 (n=11)	56.4 ± 42.9 (n=51)
This study (2024)	Columba livia	Granivore	Ground	11.8 ± 3.9 (n=3)	38.6 ± 17.3 (n=5)	38.7 ± 45.5 (n=11)	54.4 ± 22.7 (n=16)	23.2 (n=1)	91.0 ± 65.4 (n=4)		47.8 ± 38.0 (n=40)
	Lichenostomus penicillatus	Omnivore	Foliage		22.1 ± 6.9 (n=9)	18.6 ± 7.8 (n=5)	25.7 ± 9.6 (n=13)	39.1 ± 17.2 (n=22)			30.3 ± 15.3 (n=49)
	Ocyphaps lophotes	Granivore	Ground		37.8 ± 17.4 (n=14)	54.5 ± 5.2 (n=2)	76.4 ± 39.3 (n=17)	166.9 ± 94.4 (n=4)	190.0 ± 116.9 (n=4)		82.1 ± 70.9 (n=41)
	Passer domesticus	Omnivore	Ground	11.7 ± 9.5 (n=28)	21.1 ± 18.5 (n=97)	32.5 ± 17.9 (n=84)	45.4 ± 21.4 (n=120)	73.8 ± 24.7 (n=117)			43.0 ± 29.3 (n=446)

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