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*). Median blood lead levels were highest in crested pigeons (59.6 µg/dL), followed by house sparrows (35.2 µg/dL), rock pigeons (35.1 µg/dL), and white-plumed honeyeaters (27.4 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 concentration under the lower threshold of the subtoxic (20–50 µg/dL) and toxic (≥ 50 µg/dL) effect ranges previously identified for some bird 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.

Keywords: biomonitoring, lead exposure, urban ecosystems, pigeons, house sparrows

1. 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 in 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 a range of adverse health outcomes (Gillings et al., 2024; Imagawa et al., 2023).

Previous research has identified interspecific differences in tissue lead concentrations in 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. in 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 (*Columba livia*) and house sparrows (*Passer domesticus*); and two native species: crested pigeons (*Ocyphaps Lophotes*) and white-plumed honeyeaters (*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. Crested pigeons, house sparrows and rock pigeons are ground foragers, and so are directly and frequently exposed to lead contaminated soil, whereas white-plumed honeyeaters 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 white-plumed honeyeaters to show lower blood lead levels compared to the other species due to their largely arboreal foraging strategy.

2. Methods:

2.1. 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).

2.2. 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.

2.3. 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 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 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 < 2 mm. This brought the total number of individual soil measurements included in this study to 601.

2.4. Biological sampling

Capture and sampling of crested pigeons (*Ocyphaps lophotes*), rock pigeons (*Columba livia*), house sparrows (*Passer domesticus*) and white-plumed honeyeaters (*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. Crested pigeons (n = 41) and rock pigeons (n = 40) were caught at 11 sites each, house sparrows (n = 446) at 49 sites, and white-plumed honey eaters (n = 49) at 13 sites (Table S1). House sparrows were captured and sampled intermittently over a three-year period between 2020–2023 (as part of a related study (Gillings et al., 2024)), while crested pigeons, rock pigeons, and white-plumed honey eaters were mostly captured and sampled between April–May 2023. House sparrows and white-plumed honeyeaters were caught using mist nets, while crested pigeons and rock pigeons were caught using a manually triggered spring-loaded clap net set horizontally at ground level. 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 house sparrows 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).

2.5. 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 house sparrows to the haematocrit (packed cell volume) of blood samples taken from the same individual. Previous studies have identified a proportional relationship between blood Hb concentrations and haematocrit (Turkson and Ganyo, 2015). We measured haematocrit 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 haematocrit and Hb measurements (Pearson $r = 0.70$, $p < 0.001$, $n = 55$).

2.6. Blood lead analysis

Blood samples (50 µL) were analysed for Pb concentrations using a Meridian Bioscience 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 (n = 48) from crested pigeons (n = 6), rock pigeons (n = 6), house sparrows (n = 30) and white-plumed honeyeaters (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 house sparrows and white-plumed honeyeaters underestimated ICP-MS measurements by a greater degree than crested pigeons and rock pigeons. An ASV measurement of 10 µg/dL, for example, equated to a back-calculated ICP-MS measurement of 23.7 µg/dL for house sparrows, 21.4 µg/dL for white-plumed honey eaters, 13.6 µg/dL for crested pigeons and 14.7 µg/dL for rock pigeons. Based on this finding and the low number of paired measurements available for white-plumed honeyeaters, crested pigeons and rock pigeons, we pooled our data for house sparrows and white-plumed honeyeaters (n = 36), and crested pigeons and rock pigeons (n = 12) and recalculated the models for each group. We applied the recalculated models for house sparrows and white-plumed honeyeaters ($\ln(\text{ICP-MS blood Pb } (\mu g/dL)) = 0.8275 \times \ln(\text{ASV blood Pb } (\mu g/dL)) + 1.2552$) and crested pigeons and rock pigeons ($\ln(\text{ICP-MS blood Pb } (\mu g/dL)) = 1.0428 \times \ln(\text{ASV blood Pb } (\mu g/dL)) + 0.2592$) 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 house sparrows and white-plumed honeyeaters, and 2.5–100.7 µg/dL for crested pigeons and rock pigeons.

No measurements from white-plumed honeyeaters (n = 0/49) and only 4% (n = 20/446) of those from house sparrows 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 rock pigeons (n = 4/40) and 22% of crested pigeons (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.

2.7. 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 median or 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 a 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 (crested pigeons, rock pigeons, house sparrows, white-plumed honeyeaters) 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 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 rock pigeons and house sparrows (Sol and Senar, 1995; Vangestel et al., 2010) and was assumed to be inclusive of the home range of crested pigeons and white-plumed honeyeaters 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 is 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 concentration ranges associated with subtoxic and toxic physiological effects in Columbiformes and Falconiformes (Franson and Pain, 2011). In Columbiformes (e.g., crested pigeons, rock pigeons) and Falconiformes, blood Pb levels between 20–50 µg/dL are associated with subtoxic physiological effects, which are unlikely to severely impair biological functioning. A blood Pb concentration above 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., house sparrows, white-plumed honeyeaters) and so the toxic effect range for Falconiformes (> 50 µg/dL) is used for these species since it is the closest phylogenetic relative for which data are available.

3. Results:

3.1. 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.

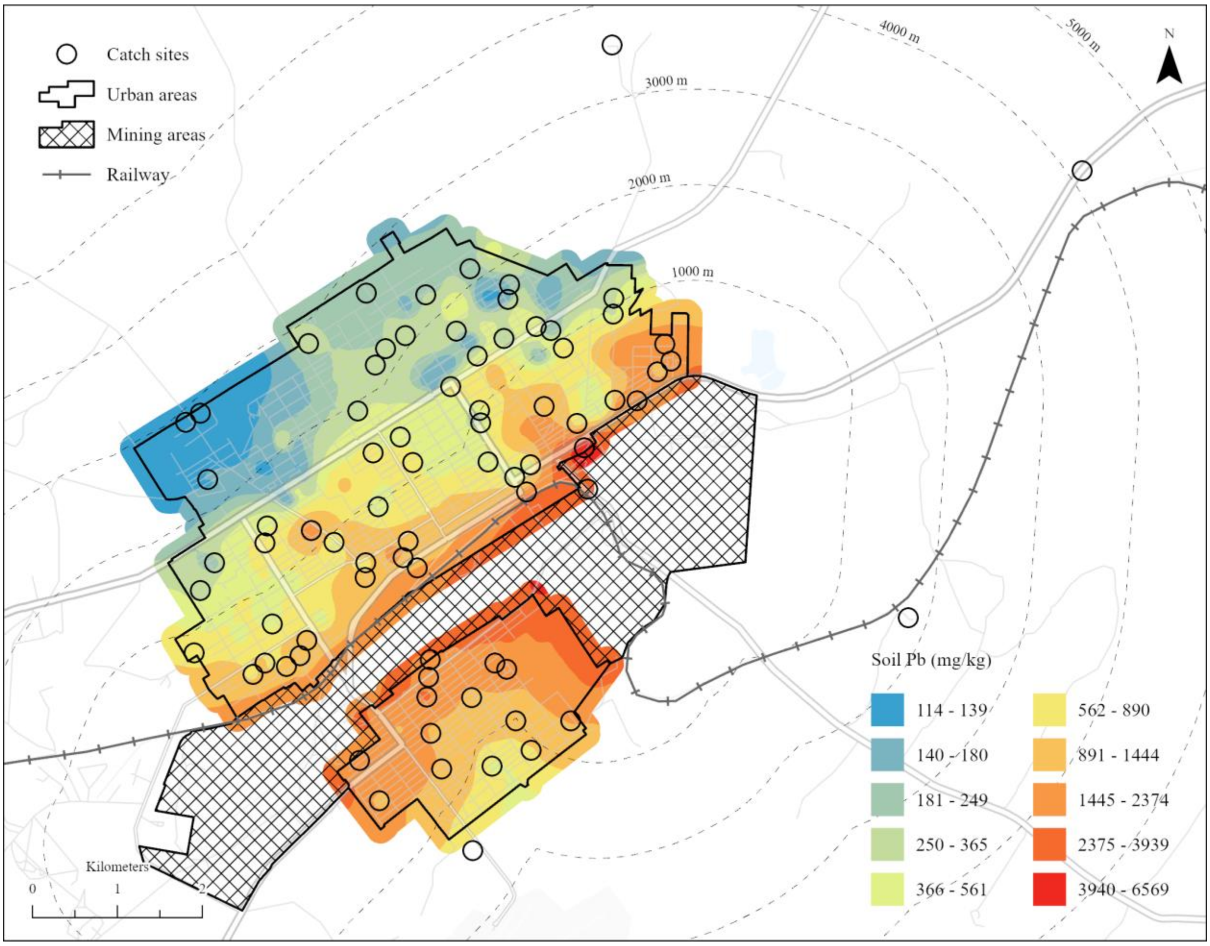

Figure 1. Map of Broken Hill catch sites. Contoured soil Pb concentrations are derived from the interpolation of 592 urban soil Pb measurements using empirical Bayesian kriging regression prediction. Concentrations are classified using a geometric interval. The interpolated surface is limited to within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

3.2. 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; $p < 0.001$). Median blood Pb levels in crested pigeons (59.6 µg/dL) were significantly higher than house sparrows (35.2 µg/dL), rock pigeons (35.1 µg/dL), and white-plumed honeyeaters (27.4 µg/dL); there was no significant difference between blood Pb levels in house sparrows and rock pigeons; and median blood Pb levels in white-plumed honeyeaters were significantly lower than in crested pigeons, house sparrows and rock pigeons (Dunn's post hoc) (Figure 2; Table S6). Comparison of our data with blood Pb concentration ranges associated with subtoxic and toxic effects in similar species indicate that a majority of blood Pb measurements exceeded the lower concentration threshold for 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 (CRPI), white-plumed honeyeaters (WPHE), house sparrows (HOSP), and 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.*

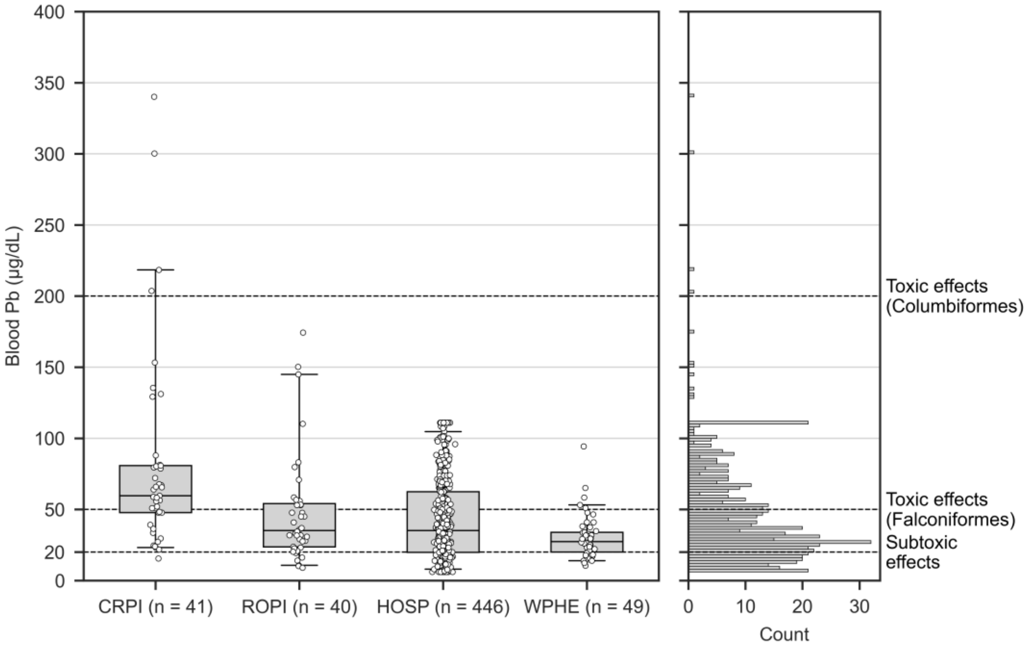

Figure 2. Distribution (5%, 25%, 50%, 75%, 95%) of blood Pb levels in crested pigeons (CRPI), rock pigeons (ROPI), house sparrows (HOSP), white-plumed honeyeaters (WPHE). Dashed lines indicate the lower threshold of the blood Pb concentration range associated with subtoxic physiological effects (20–50 µg/dL) and toxic physiological effects in Falconiformes (≥ 50 µg/dL) and Columbiformes (≥ 200 µg/dL).

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 relative standard deviation 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 relative standard deviation of blood Pb levels (mean ± standard deviation) in crested pigeons (35 ± 22%), rock pigeons (31 ± 27%), house sparrows (30 ± 13%), or white-plumed honeyeaters (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 house sparrows captured from the same site 6–222 days apart. The relative percent difference (mean ± standard deviation) of blood Pb concentrations between these repeated measurements was 13 ± 5% (Table S8).

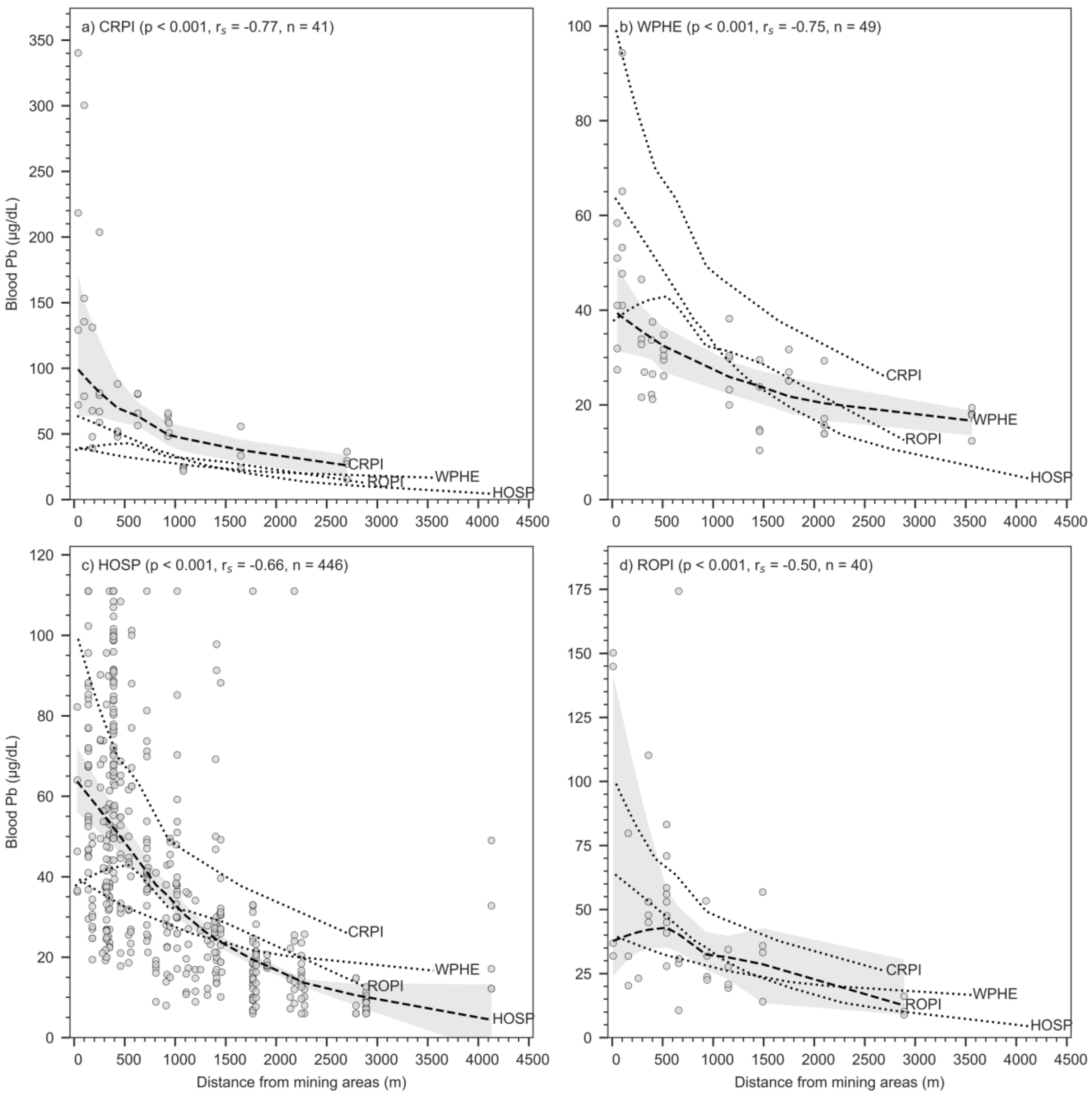

Figure 3. Relationship between blood Pb levels (µg/dL) of (a) crested pigeons (CRPI), (b) white-plumed honeyeaters (WPHE), (c) house sparrows (HOSP), (d) rock pigeons (ROPI), with distance of sites from the nearest mining area. The dashed line indicates a smoothed Loess regression (± 95% bootstrapped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

3.3. 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 variance in median blood Pb. Adjusting for species-specific effects derived from the soil Pb × species interaction, the change in blood Pb (µg/dL) as a function of soil Pb was largest in crested pigeons (0.0523 × soil Pb (mg/kg), $p < 0.001$), followed by house sparrows (0.0429 × soil Pb (mg/kg), $p < 0.001$), rock pigeons (0.0333 × soil Pb (mg/kg), $p < 0.001$) and white-plumed honeyeaters (0.0089 × soil Pb (mg/kg), $p = 0.076$), 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 white-plumed honeyeaters and crested pigeons, house sparrows and rock pigeons (Figure 4; Table S9 (Model A)).

Due to the significant association between soil and blood Pb levels amongst crested pigeons, house sparrows, and rock pigeons, 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 the slopes derived from the soil Pb × species interaction term for this purpose due to the relative sparsity of data available for crested pigeons and rock pigeons. Amongst these species, for every 1 mg/kg increase in soil Pb, the overall median blood Pb changed by 0.0433 µg/dL (SE = 0.001, t = 30.969, $p < 0.001$) (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 lower concentration thresholds of the subtoxic effect range of 20–50 µg/dL and the toxic effect range of ≥ 50 µg/dL in Falconiformes and ≥ 200 µg/dL in Columbiformes, respectively.

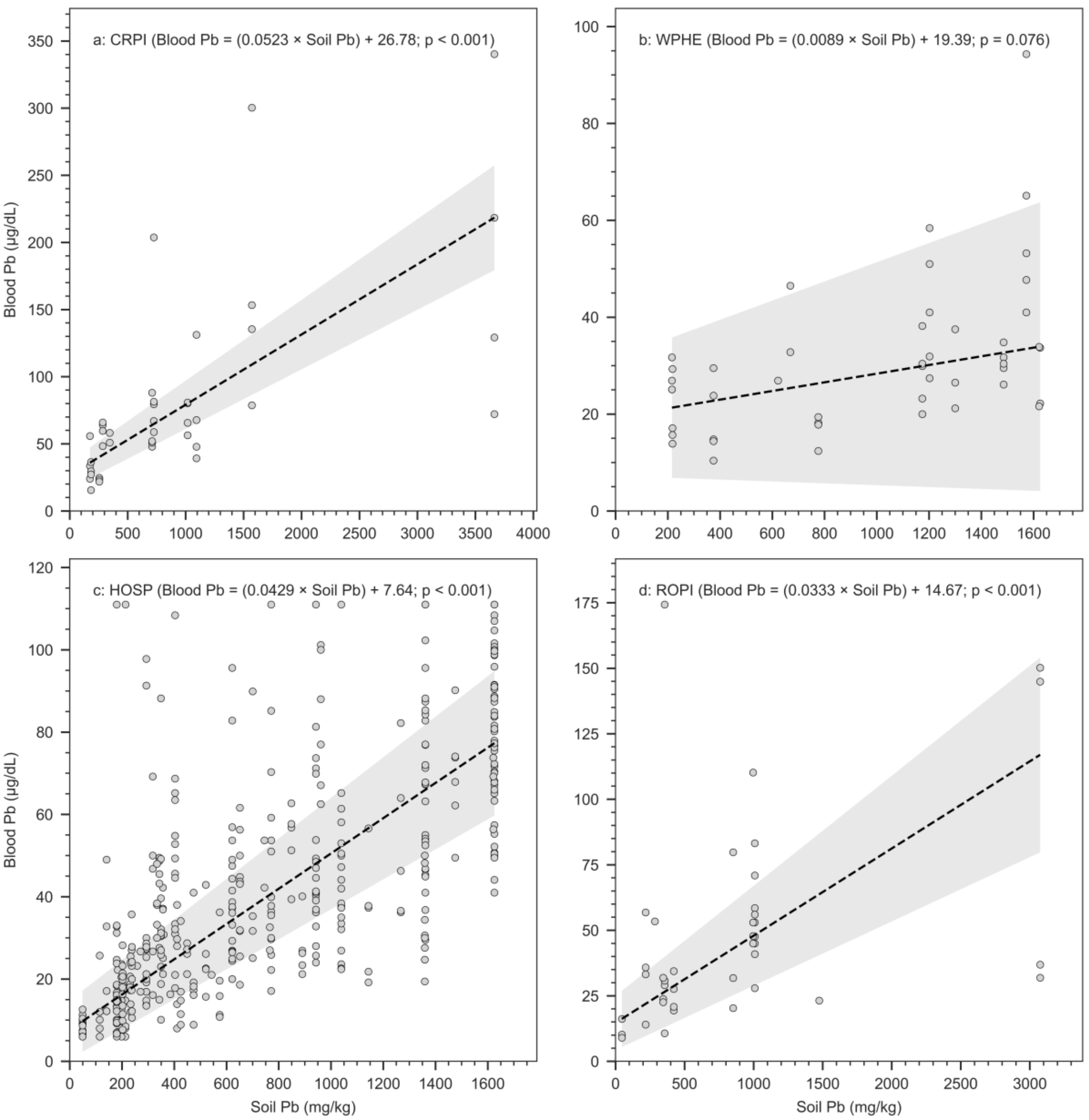

Figure 4. Relationship between soil Pb (mg/kg) and blood Pb (μg/dL) in (a) crested pigeons (CRPI), (b) white-plumed honeyeaters (WPHE), (c) house sparrows (HOSP), (d) rock pigeons (ROPI). The dashed line indicates the quantile regression model (median ± 95% confidence interval) for blood Pb as a function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

3.4. Relationship between blood lead and haemoglobin

Median blood Hb levels differed significantly between species and were highest in rock pigeons (196 g/L), followed by crested pigeons (186 g/L), white-plumed honeyeaters (175 g/L) and house sparrows (164 g/L) (Kruskal Wallis with Dunn's post hoc; $p < 0.001$) (Table S6; Table S10). Median blood Hb levels also differed significantly between adult (183 g/L) and juvenile (166 g/L) white-plumed honeyeaters ($p < 0.001$), but not adult (165 g/L) and juvenile (161 g/L) house sparrows (Kruskal-Wallis; $p = 0.304$). The number of juvenile crested pigeons (n = 0) and rock pigeons (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 variance in blood Hb. Adjusting for species-specific effects derived from the blood Pb × species interaction, the change in blood Hb levels was largest in rock pigeons (-0.1691 × blood Pb (µg/dL), $p = 0.013$), followed by white-plumed honeyeaters (-0.1617 × blood Pb (µg/dL), $p = 0.302$), house sparrows (-0.1251 × blood Pb (µg/dL), $p < 0.001$) and crested pigeons (-0.0790 × blood Pb (µg/dL), $p = 0.055$), although the relationship was only significant in rock pigeons and house sparrows (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 -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 crested pigeons, rock pigeons and house sparrows (Table S11 (Model B)), we estimated decreases in median blood Hb, relative to the baseline Hb level in adults from each of these species, expected at different soil Pb concentration ranges (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 (CRPI), house sparrows (HOSP), and rock pigeons (ROPI) (Table S11 (Model B)). White-plumed honeyeaters are not included here 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
Background	< 150	< 17.8	< 0.1	< 0.8	< 0.5
Background 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	2–3.8	1.6–3
Highly contaminated	1200–2400	63.3–115.2	2.8–5.7	3.8–7.1	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

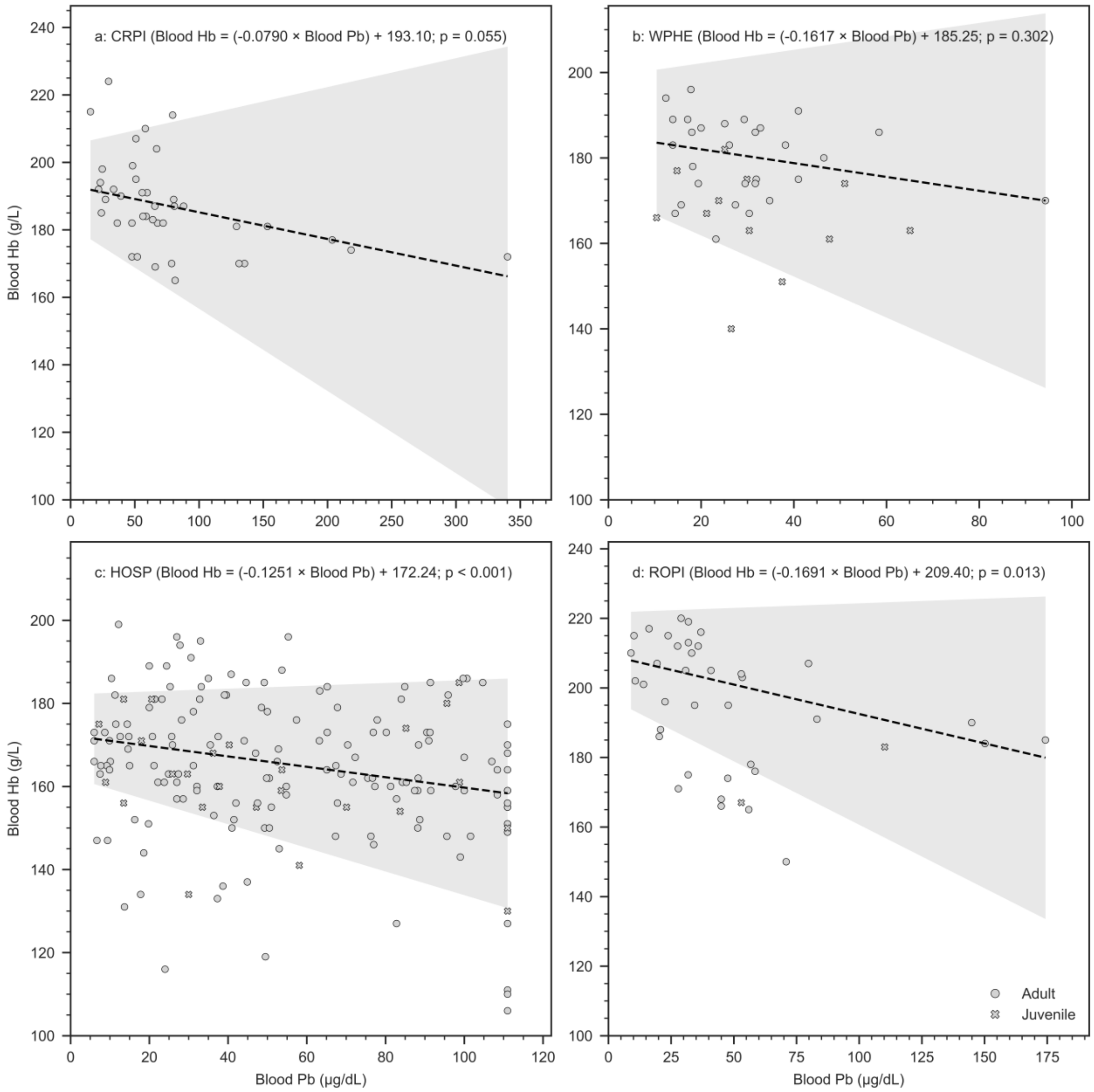

Figure 5. Relationship between blood Pb (µg/dL) and blood Hb (g/L) in (a) crested pigeons (CRPI), (b) white-plumed honeyeaters (WPHE), (c) house sparrows (HOSP), (d) rock pigeons (ROPI). The dashed line indicates the quantile regression model (median ± 95% confidence interval) for blood Hb as a function of blood Pb in the different bird species. Datapoints for adult and juvenile individuals are displayed, but the intercept of the plotted regression equation is based on the baseline Hb of adults of each species.

4. 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. These differences correspond to those observed in the relationship between soil and blood Pb in these species (Table 1; Figure 2; Figure 4; Table S6) and 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 dust could be a more relevant source of Pb exposure than soil 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). All also ingest grit, which would contribute to their ingestion of soil Pb. 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 statistics (median | mean blood Pb ± SD (µg/dL)) for 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 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 in the blood Pb of groups is indicated by * ($p < 0.05$) or ** ($p < 0.01$) (Kruskal-Wallis). Comparative statistics for species, along with information on foraging guild and diet, are reported in Table S12.

		Other studies		This study	
		Median | mean blood Pb ± SD (µg/dL)		Median | mean blood Pb ± SD (µg/dL)	
Soil Pb contamination level	Soil Pb range (mg/kg)	Ground forager	Other forager	Ground forager	Other forager

Background	< 150	3.7 | 5.5 ± 8.6 (n=129)		3.7 | 5.8 ± 9.3 (n=24)	9.0 | 11.7 ± 9.1 (n=31)		
Background to moderately contaminated	150–300	9.4 | 8.9 ± 5.4 (n=5)		5.0 | 6.1 ± 3.3 (n=4)	18.6 | 23.8 ± 19.2 (n=116)		25.1 | 22.1 ± 6.9 (n=9)
Moderately contaminated	300–600				28.9 | 33.5 ± 22.5 (n=96)		19.3 | 24.0 ± 14.9 (n=6)
Moderately to highly contaminated	600–1200	16.3 | 24.7 ± 31.7 (n=171)	*	8.4 | 10.7 ± 7.2 (n=14)	44.2 | 49.6 ± 25.9 (n=154)	**	25.0 | 26.3 ± 9.8 (n=12)
Highly contaminated	1200–2400	38.2 | 45.3 ± 35.9 (n=113)	**	15.9 | 21.3 ± 14.9 (n=59)	73.9 | 76.4 ± 33.3 (n=122)	**	33.8 | 39.1 ± 17.2 (n=22)
Highly to extremely contaminated	2400–4800	32.4 | 55.2 ± 44.0 (n=38)	*	20.6 | 26.5 ± 23.8 (n=5)	137.1 | 140.5 ± 102.4 (n=8)		
Extremely contaminated	≥ 4800	34.9 | 40.5 ± 18.3 (n=44)					
All data		18.4 | 27.9 ± 33.1 (n=500)		11.6 | 16.0 ± 14.9 (n=106)	36.8 | 46.3 ± 36.4 (n=527)		29.3 | 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 (µg/dL) as a function of soil Pb (mg/kg) in these ground foraging species ranges from 0.22 × dietary soil Pb (mg/kg) at 20% to 0.48 × dietary soil Pb (mg/kg) 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 × dietary soil Pb (mg/kg) in Canada geese (*Branta canadensis*) to 0.75 × dietary soil Pb (mg/kg) 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 soil Pb dosing studies.

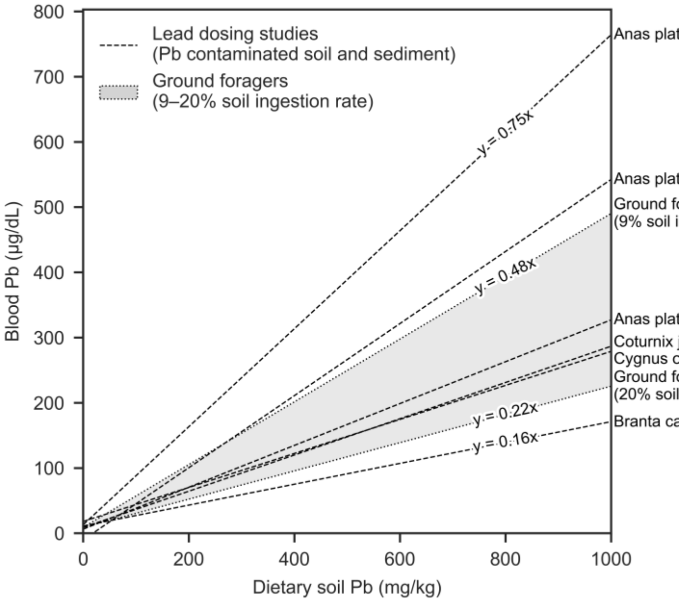

Figure 6. Comparison of modelled slopes for relationship between dietary soil Pb and blood Pb. Only dosing studies using Pb contaminated soil from mining areas are included (values converted from mg/kg wet weight to µg/dL assuming an avian blood density of 1.05 g/mL (Scanes, 2015)) (Beyer et al., 2014; 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 (blood Pb (µg/dL) = 0.0433 × soil Pb (mg/kg)), 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 ranges, 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 lower threshold of the subtoxic blood Pb effect range (20 µg/dL), soil Pb concentrations should be below approximately 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 concentration 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 equivalent to the lower threshold of the subtoxic blood Pb effect range (20–50 µg/dL) in this study are widespread in mining impacted contexts. For example, mean soil Pb concentrations above 200 mg/kg were present at approximately 88% of our sites in Broken Hill (n = 68/77). However, similar soil Pb concentrations 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 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 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 blood Pb concentration range (242–315 µg/dL), 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, exposure to Pb has 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 similar 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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Investigation: MMG, RT, TH, SCG

Visualization: MMG

Funding acquisition: SCG, MPT

Project administration: SCG

Supervision: MPT, SCG

Writing (original draft): MMG

Writing (review and editing): MMG, RT, TH, MPT, SCG

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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