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Interspecific variation in the spatially-explicit risks of trace metals to songbirds

Béatrice V. Hernout^{a,1}, Louise J. Gibson^a, Adam J. Walmsley^{a,2} and

Kathryn E. Arnold^{a*}

^a Environment Department, University of York, Heslington, York, UK

¹ Present address: Texas A&M Galveston Campus, Department of Marine Biology, 1001 Texas Clipper Road Galveston, TX 77554, USA.

² Present address: Ribble Rivers Trust, Clitheroe, Lancashire, UK

*Author for correspondence: Dr Kathryn Arnold, Environment Department, University of York, Heslington, York YO10 5NG, UK

Tel: +44 (0) 1904 322997 Email: Kathryn.Arnold@york.ac.uk

Running Head: National assessment of metal risks to birds

1

2 **KEY WORDS**

3 Avian populations, Trace Metal toxicity risk, Invertebrate Prey, GIS modelling framework,

4 Environmental risk assessment

5 **HIGHLIGHTS**

6

7 • A spatially explicit modelling framework is presented to estimate risks of metal to
8 birds.

9 • The model has been applied to soil metal contamination and thirty songbird species.

10 • Our spatial model showed interspecies variation in metal toxicity risks to UK
11 songbirds

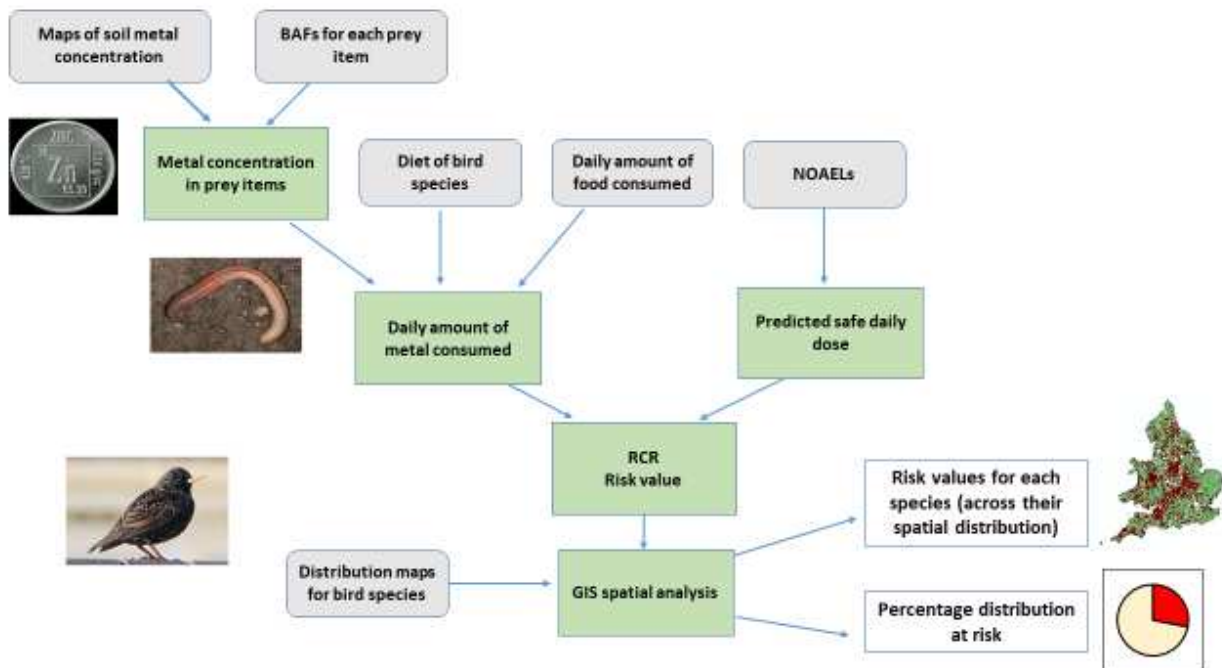
12 • Pb and Zn exposure posed high toxicity risks to adults and nestlings via diet as
13 indicated by the model

14 • Despite the model limitations, this study can be a useful for Environmental Risk
15 Assessment

16

17

18 **GRAPHICAL ABSTRACT**



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20

21

ABSTRACT

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Many wild animals can be adversely affected by trace metals around point sources but little

23

is known about the risks to birds across their ranges. Trace metals in the soil are

24

ubiquitously, if heterogeneously distributed, across the world due to natural and

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anthropogenic sources. Here, we built, parameterized and applied a spatially explicit

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modelling framework to determine the risks of soil-associated metals to 30 invertebrate-

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consuming passerine species across their spatial distribution in England and Wales. Our

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model highlights significant differences in toxicity risks from Cd, Cu, Pb and Zn across the UK

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distributions of different species; Pb and Zn posed risks to all species across most of species'

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distributions, with more localised risks to some species of conservation concern from Cd and

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Cu. No single taxa of invertebrate prey drove avian exposure to metal toxicity. Adults were

32

found to be at higher risk from Pb and Zn toxicity across their distributions than nestlings.

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This risk was partially driven by diet, with age differences in diets identified. Our spatially

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explicit model allowed us to identify areas of each species' national distribution in which the

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population was at risk. Overall, we determined that for all species studied an average of

36

32.7 ± 0.2 %, 8.0 ± 0.1 %, 86.1 ± 0.1 % and 93.2 ± 0.1 % of the songbird spatial distributions in

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the UK were characterized at risk of Cd, Cu, Pb and Zn, respectively. Despite some

38

limitations, our spatially explicit model helps in understanding the risks of metals to wildlife

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and provides an efficient method of prioritising areas, contaminants and species for

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Environmental Risk Assessments. The model could be further evaluated using a targeted

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monitoring dataset of metal concentration in bird tissues. Our model can assess and

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communicate to stakeholders the potential risks of environmental contaminants to wildlife

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species at a national and potentially international scale.

44

1. INTRODUCTION

45 Wildlife species, such as birds, are considered to be good indicators of ecosystem health
46 because they live in a wide range of habitats and generally occupy a high trophic level
47 (DEFRA, 2010). Direct monitoring of local bird populations around point sources of metal
48 pollution can be successful in identifying the adverse effects of metal exposure (Eeva et al.,
49 2009a; Llacuna et al., 1995; Swaileh and Sansur, 2006). For example, several studies have
50 demonstrated adverse effects of trace metal contamination on breeding success of
51 passerine birds in the vicinity of metal smelting sites (Belskii et al., 1995; Belskii et al., 2005;
52 Eeva and Lehtikoinen, 1996; Janssens et al., 2003a). Such localised studies, however, yield no
53 indication of the potential risks to birds of differing concentrations of trace metals found
54 across species' ranges. Notably, most studies lack a spatial dimension and thus variations of
55 exposure risks over an entire range remain poorly understood (but see Hernout et al., 2015;
56 Hernout et al., 2013; Hunsaker et al., 1990). Moreover, monitoring studies are time and cost
57 consuming and only provide an assessment of a limited number of species and regions. In
58 contrast, modelling exercises can utilise existing monitoring data, and can relatively quickly
59 and cheaply provide predictions about risk (Hernout et al., 2011; Hernout et al., 2013;
60 Schmolke et al., 2010; Smart et al., 2006). Indeed, they are strongly encouraged in
61 environmental risk assessment (ERA) (Schmolke et al., 2010; Smart et al., 2006; Suter, 2006).
62 Spatially explicit models can also extend the predictive scope and the geographic scale of
63 experiment investigations, as well as integrate relevant ecological parameters of food chain
64 models in ERA.

65 Trace metals are present naturally in the environment and their concentrations in
66 soils vary spatially according to local geology (Fairbrother et al., 2007). In addition, human

67 activity can increase the deposition rate of trace metals to the soil. The main anthropogenic
68 sources of trace metals in the environment are point sources, such as mines, chemical
69 factories, smelters and landfill sites (EA, 2009). Trace metal contamination in soil can last
70 long after emissions from initial point sources cease, and therefore, they remain
71 bioavailable to living organisms and can accumulate through the food chain (EA, 2009). The
72 main exposure route of trace metal elements in higher trophic level organisms, including
73 birds, is ingestion (Dauwe et al., 2005).

74 Birds have high energetic requirements (Klasing, 2000), so they need to consume
75 large quantities of food compared to their body mass. Insectivorous birds are thought to
76 have more exposure to metals than granivorous birds due to their higher trophic levels and
77 the potential accumulation in invertebrate species (Dauwe et al., 2004; Fritsch et al., 2012;
78 Swaileh and Sansur, 2006; Zhuang et al., 2009), especially invertebrates known as 'hyper-
79 accumulators', such as Lumbricidae (Qiu et al., 2014). Although several monitoring studies
80 have presented actual metal residues for a few terrestrial songbird species (Cooper et al.,
81 2017), the exposure to and risks posed by metals to a wide range of species are still poorly
82 understood (Godwin et al., 2016).

83 For passerine birds, data on the effects of metals are limited when compared with
84 small mammals. Non-essential metals' effects on birds include and are not-limited to: for
85 Cd, anaemia, intestinal damage, impaired digestion, kidney damage, changes to bone
86 mineralization, diseases and oxidative and histopathological damage, reduced reproductive
87 success and endocrine disruption (Wayland and Scheuhammer, 2011); for Pb, anaemia,
88 renal and haematological toxicity, possible brain damage, weight loss, immunosuppression,
89 lesions of tissues, lethargy, ataxia, neurological effects, reduced reproductive success, and
90 possibly death (Franson and Pain, 2011). Thus, multiple fitness-related traits can be

91 affected by Pb and Cd, but not all studies report such effects in birds living in contaminated
92 areas (Eeva et al., 2014; Rainio et al., 2015; Ruuskanen et al., 2015). In addition, since
93 oxidative stress can affect the fitness of bird population (growth, survival, and
94 reproduction), metal-related oxidative stress could affect populations of free-living birds
95 (Koivula and Eeva, 2010). Cu and Zn, in contrast, are essential elements to all vertebrates,
96 and are under homeostatic control. At normal physiological concentrations, Cu binds to the
97 blood protein ceruloplasmin but excess Cu is carried 'free' in the blood and causes oxidative
98 stress and intracellular oxidative damage by increasing ROS formation (Brewer, 2010);
99 (Berglund et al., 2007). Chronic Zn toxicity is not well studied in wild or captive birds (Beyer,
100 2006), with just a few studies on waterfowl (Taggart et al., 2006), which can be exposed
101 from acid mine drainage (Gasaway and Buss, 1972). Zn toxic effects can decrease the
102 pancreas and the body mass (Koivula and Eeva, 2010). As flying vertebrates that require
103 high levels of motor-control and muscular activity, the physiological effects of exposure to
104 metals may be critical to birds. Indeed, flight performance is known to be a sensitive
105 indicator of environmental perturbations, such as a poor quality diet (Larcombe et al.,
106 2008).

107 Sex and age can alter both the exposure to and consequences of metal residues. In
108 wild and captive birds, females have been shown to be more sensitive (Eeva et al., 2009b)
109 and/or simply accumulate metals at varying rates in different organs than males (Taggart et
110 al., 2006) but see (Cooper et al., 2017). Juveniles are known to be more sensitive to damage
111 due to Pb and other pollutants than adults, for example due to their immature digestion,
112 and especially underdeveloped blood–brain barrier (Scheuhammer, 1987). Pb poisoning
113 effects, including chick deformities and reductions in fertility, are well documented in
114 waterfowl as they are often exposed to lead shots (Fisher et al., 2006). Nestlings also seem

115 to be more exposed to Pb than adult birds through the diet and because they are unable to
116 escape polluted sites (e.g. (Grue and Franson, 1986).

117 To investigate the potential risks of soil-associated metals to a large range of
118 songbird species at a national scale, we applied a previously developed modelling
119 framework to 30 insectivorous songbird species (strictly speaking passerines that include
120 invertebrates in their diet but we use 'insectivorous' for brevity) breeding in England and
121 Wales. This modelling framework was initially developed to assess the risks of metal to bats
122 and uses a risk characterization approach (Hernout et al., 2013). The model has been
123 evaluated for insectivorous bats against monitoring data which showed that the model
124 provides satisfactory predictions (Hernout et al., 2015; Hernout et al., 2013). Of our focal
125 species, several are on the British Trust for Ornithology's (BTO) red or amber lists of high
126 conservation concern in the UK (Eaton et al., 2009). To improve our knowledge on the
127 potential risks of metal exposure to insectivorous passerine birds, our aims were to
128 parameterize and apply a modelling framework to insectivorous passerine birds in order to:
129 1) identify which species are the most exposed to metals and which diet items and metals
130 drive exposure risk; (2) investigate age-related effects on exposure risks and 3) Map spatial
131 variation in toxicity risk from individual metals at a national level to identify areas for further
132 investigation.

133

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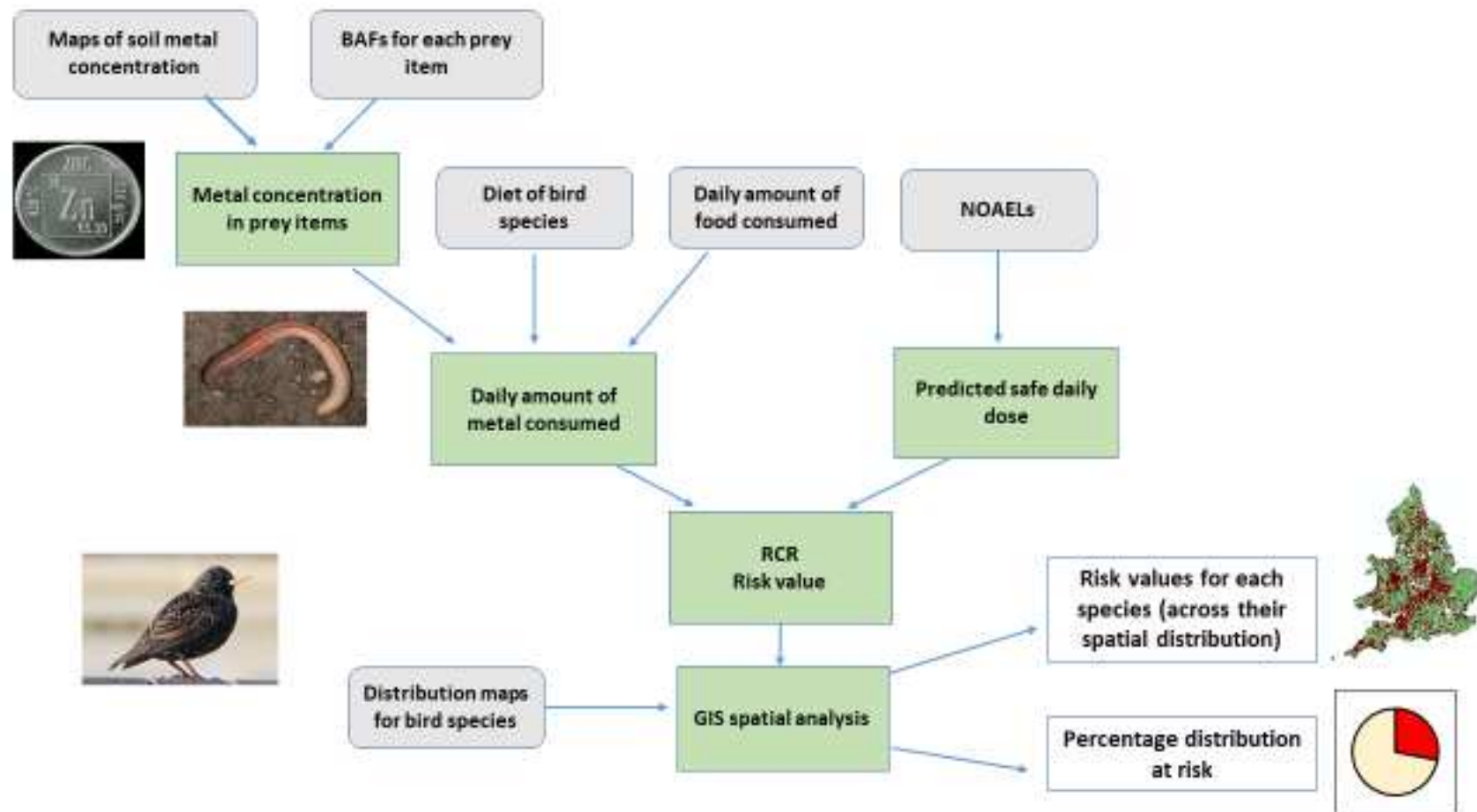
2. MATERIAL AND METHODS

135

136

137 To investigate the risks from soil-associated metals to passerine birds breeding in the UK, we
138 applied our modelling framework (Fig. 1) to four metals (Cd, Cu, Pb and Zn) and 30 passerine
139 species (listed in Table A1 Appendix). Our model is based on a basic risk characterization
140 ratio (RCR) approach in which the ratio is composed of a daily oral dose of a compound
141 ingested and a “safe” dose (as described in (Hernout et al., 2013)). The qualitative
142 information about the risk (acceptable or not) is given when the derived ratio is compared
143 to a trigger value (of 1)(Hernout et al., 2013).

144 The model (Fig. 1) integrates information on concentrations of metals in soils,
145 bioaccumulation from soil into invertebrates, bird diet, spatial range of each species and
146 toxicity data on each metal (see Appendix for full details of parameters and outputs). Data
147 for both adult and nestling diet was available for 26 of the 30 species (except for *Corvus*
148 *monedula*, *Delichon urbica*, *Passer domesticus* and *Turdus merula* for which only the data on
149 the diet of nestlings were accessible) (Table A2 Appendix). The final output was a risk
150 characterization ratio (RCR) for each 10 × 10 km cell defined by the ratio between the daily
151 dose of metal that a bird receives ($\mu\text{g/g}$ body weight/d) and predicted safe daily dose for the
152 metal ($\mu\text{g/g}$ body weight/d), within the spatial distribution of the bird (Hernout et al. 2013).
153 The final risk maps were used to calculate the percentage of each species’ range at risk from
154 trace metal toxicity, the species most at risk from metal toxicity and also to identify areas of
155 the England and Wales which pose the highest risks to invertebrate-consuming birds.



156

157 **Figure 1:** A flow chart showing the major stages, inputs and outputs of our model used to determine risk to an avian species to metals from soils via
 158 diet items. Oval-edged blue boxes represent parameters from the literature entered into the model. Green boxes with right angles represent the
 159 model steps. White, square- edged, boxes contain outputs from the models used in statistical analyses. See methods for further details.

160 **2.1 Model parameterisation**

161 The modelling framework (Fig. 1) was parameterised to thirty species breeding in the UK
162 (see Sections 2.1.1-5 and Appendix).

163 *2.1.1 Maps of soil metal concentrations:* The monitoring data on concentrations of
164 metals (Cd, Cu, Pb and Zn) in soils ($\mu\text{g/g dw}$) across England and Wales, measured after acid
165 extraction, were provided by the National Soil Resources Institute (NSRI). The data were
166 collected between 1979 and 2003 at a resolution of $5\times 5\text{ km}^2$. Data from the more recent
167 dataset (1994 and 2003) were used in preference to data from the older dataset (1979 and
168 1987) so the older data were only used to fill gaps in the more recent dataset. We used
169 approximately 70% of the data from the first dataset in the model. (see Hernout et al., 2013
170 for details). Inorganic metal concentration in soils persists for a long time after their
171 introduction (since most metals do not undergo microbial or chemical degradation).

172 *2.1.2 Metal concentrations in diet items:* Biota accumulation factors (BAFs) for
173 uptake of Cd, Cu, Pb and Zn from soils or sediments were collected for each diet item (i.e.
174 Araneida, Coleoptera, Collembola, Dermaptera, Diptera, Ephemeroptera, Gastropoda,
175 Hemiptera, Hymenoptera, Isopoda, Lepidoptera, Lumbricidae, Myriapoda, Odonata,
176 Opiliones, Orthoptera, Plecoptera, Trichoptera, carrion; and vegetation (plant material,
177 cereal grains, seeds and fruits)) from several field and experimental studies (See Appendix
178 Table A3 for BAF values for each diet item and Table A4 for search terms). The BAFs
179 represented the uptake of metal contained in the soil into the given prey or food items and
180 were expressed as the ratio between the soil metal concentrations and the metal
181 concentration in prey or food items. Studies reporting total metal concentrations in both
182 soil or sediments and prey or food items were selected (reference list given in Appendix).

183 Median BAFs were used for the model as there were several significant outliers in the
184 collected dataset. A mean value of all invertebrate BAFs was used to calculate the uptake
185 for each metal for any unspecified part of the diet (Table A3).

186 *2.1.3 Diet of bird species:* Data for adult birds were mostly collected from studies
187 that had used stomach contents to determine diet proportions, and mostly collected from
188 studies using a collar sampling method for nestlings (Table A2 for diet data and Table A4 for
189 search terms used to find diet data). The diet data were expressed in percentage number
190 for most species. Using these methods, it was possible to characterize 100% of the diet in
191 most cases. Prey items were generally reported to the taxonomical order level.

192 *2.1.3 Daily intake of food:* Experimental data were lacking on the feeding rates of
193 the different passerine species investigated in this study. Therefore, we used an
194 allometric equation to derive the daily amount of food eaten per day (g dry weight/g
195 body weight/d) given the body weight of the passerine species (Nagy, 1987; Snow and
196 Perrins, 1998) (Table A1). Built from empirical field metabolic rates, allometric
197 equations are of great use for biologists to predict the energetic and food requirements
198 (further details about the allometric equation, see Nagy, 1987). Since the model used
199 the same daily amount of food ingested per day for nestling and adult, our risks
200 predicted for nestlings were conservative because ingestion rates of nestlings reported
201 from field studies were higher than those of adults (Cramp, 1994a).

202 *2.1.4 Daily dose of metals:* The amount of metal that a bird uptakes per day was
203 calculated from the concentration of metal in each diet item, the proportion of each diet
204 item in the diet and their daily consumed amount of food per day. The calculation predicted
205 the daily dose of metal that a bird receives in $\mu\text{g/g}$ body weight/d (Appendix). The daily dose
206 of metals was calculated for each species and each life stage (nestling or adult).

207 *2.1.5 Predicted safe daily dose:* The predicted safe daily was estimated by using No
208 Observable Adverse Effect Level (NOAEL) values, defined as the highest dose ingested where
209 adverse effects are not observed during the experimental toxicological study. Since few data
210 are available on wild bird species, we used NOAELS data from test species measured
211 experimentally (Table A5, (Sample et al., 1998). The toxicological endpoints involved in the
212 test species experiments were reproduction (for Cd, Pb and Zn), and growth and mortality
213 (for Cu) (Table A5). These endpoints are crucially important in the risk assessment of
214 chemicals to wildlife populations. For avian species, the derivation of NOAELs data from test
215 species and simply applying this to other target species based on the body weight of the
216 organism, as is done for small mammals, is not relevant (Sample et al., 1998). Therefore, we
217 used the same predicted safe daily dose for each bird species for a given metal. As described
218 in Hernout et al. 2013, the predicted safe daily dose was calculated by dividing the NOAEL
219 value by an uncertainty factor of five to account for possible uncertainty in the toxicological
220 data (e.g. inter-laboratory difference, inter and intra species differences, differences in
221 sensitivity of different life stages). The value of five is commonly used in the regulatory
222 assessment of the long-term risks of pesticides to birds and mammals species (EFSA, 2009).

223

224 ***2.2 Application of spatially explicit model***

225 The GIS spatial analysis were done using ArcGIS (ArcGIS 9, ArcMap Version 9.3.1) to assess
226 the variation of the risks of soil-associated metals to birds via the diet, in their respective
227 ranges, across England and Wales. Based on the data of metal concentrations in the soil,
228 uptake of metals into diet items and the ingestion of different prey types by adults and
229 nestling of different species, we calculated an RCR value for each grid cell (resolution of 10 X

230 10 km²) (See Appendix and Hernout et al., 2013). Finally, the breeding distribution of the
231 bird species were provided by British Trust for Ornithology (BTO) 1991 atlas data (Gibbons
232 et al., 1993). These were overlaid on the RCRs maps to obtain risks predictions within the
233 range of each species. This resulted in two quantitative outputs that were analysed
234 statistically: 1); mean RCRs per species across their distribution which provide further
235 information about the risk amplitude for each species and are useful to compute further
236 quantitative analyses; and 2) percentage of a species' distribution at risk of metal toxicity
237 (number of grid cells with a RCR >1 divided by the total number of grid cells for the species'
238 distribution) which provides general information about how the risk extends on the spatial
239 range for a particular species. These two outputs data were obtained for each metal (Cd, Cu,
240 Pb and Zn), for each species and for each life stage (adult-nestling).

241

242 **2.3 Data analysis**

243 Statistical outputs were created using SPSS Ver 24 and software R version 3.2.5 (R
244 Development Core team, 2016). The model output data did not conform to the assumptions
245 of parametric tests so non-parametric tests were used to analyse the data. Kruskal-Wallis
246 test was used to compare the differences in RCRs values across species for the same metal.
247 To investigate the influence of the diet in driving the risk, Kendall's Tau was used to analyse
248 the correlations between RCRs and the percentage of a given diet item. Wilcoxon signed
249 rank tests were used to compare the differences in risk between adults and nestlings. Mann-
250 Whitney U-tests identified differences in the percentage of invertebrates in the diets of
251 adults and nestlings. Where we applied multiple tests to the same data, we adjusted the p-
252 value using a Holm-Bonferroni adjustment.

253 3. RESULTS AND DISCUSSION

254

255 **3.1 Interspecific variation in the risk from metal toxicity**

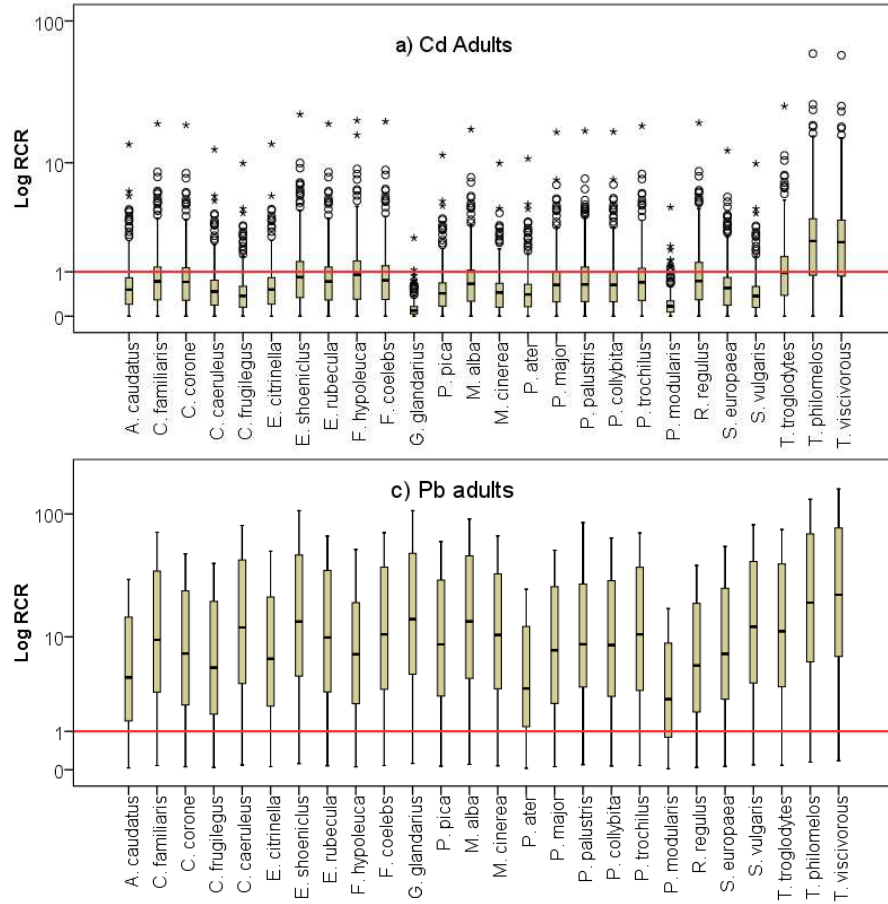
256 Among adults, species varied significantly in their median RCRs calculated across species'
257 distributions for Cd, Cu, Pb and Zn (Kruskal-Wallis test $P < 0.0001$ in all cases) (See Table 1
258 for full outputs). Starting with adults: For Cd (Fig 2a) and Cu (Fig 2b) only the outliers, i.e.
259 from locations with extremely high values of metal concentration and/or species consuming
260 large amount of prey types with high BAFs, appear to go above the risk line (red line). For Pb
261 (Fig 2c) and Zn (Fig 2d) exposure, all species have a median RCR above 1 indicating that a
262 high percentage of that species' distribution was assessed as being at risk of toxic effects.
263 This included species of conservation concern in the UK because of large population
264 declines, including the Starling *Sturnus vulgaris*, Song thrush *Turdus philomelos* and Reed
265 bunting *Emberiza schoeniclus*.

266 For nestlings, there were significant differences among species for all metals
267 (Kruskal-Wallis test $P < 0.0001$ in all cases) (See Table 1A6 for full outputs). In terms of Cd
268 (Fig 3a) the median RCRs for most species were below the red line with only some outliers
269 at risk of toxicity. Only four extremely widely distributed species had a significant
270 percentage of their population at risk of Cd toxicity (Rooks *Corvus frugilegus*, Jackdaws
271 *Corvus monedula*, Blackbird *Turdus merula* and Mistle Thrush *Turdus viscivorus*) (Table 1).
272 Cu toxicity did not pose a risk to nestlings across the vast majority of species' distributions
273 (Fig 3b). Nestlings of all species analysed were found to be at risk from Zn and Pb toxicity
274 (Fig 3c and 3d). This is concerning for two reasons; At least in other vertebrates, juveniles
275 are more sensitive to trace metal toxicity than adults, resulting in permanent changes to

276 brain function and limb development (Sfakianakis et al., 2015). Second, our analyses suggest
277 that nestlings are more exposed to trace metals than adults (see also bats (Korstian et al.,
278 2018). Thus, we suggest that while a lot of research effort is currently focused on the effects
279 of so called 'emerging contaminants' (Arnold et al., 2014), trace metals are also likely to be
280 causing a range of lethal and sublethal effects on individuals with the potential for
281 population level changes in some heavily contaminated, industrial or post-industrial areas.
282 The proliferation of the outliers (Fig 2a, 2b, 3a and 3b) is due to the distribution of the soil
283 concentrations data, which is our initially input value in the model. The soil concentration
284 data are ranging from <0.05 to 40.60 µg/g and <0.04 to 1507.70 µg/g for Cd and Cu,
285 respectively (median 0.50 and 17.30 µg/g, respectively).

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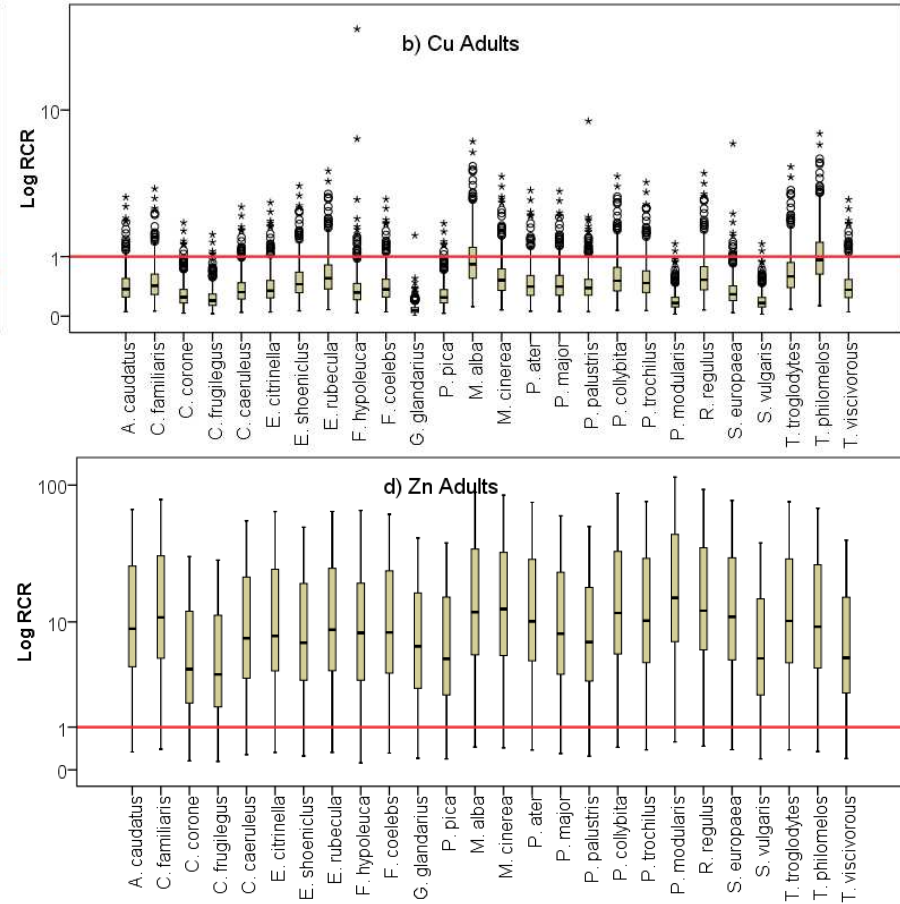
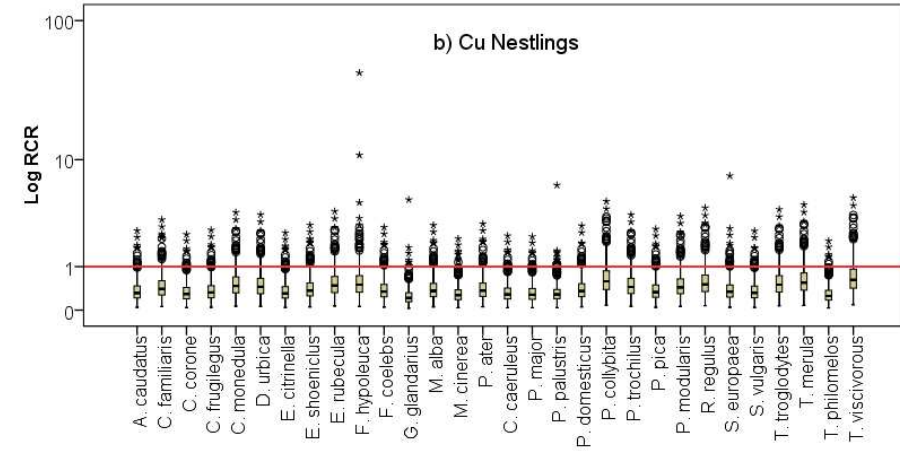
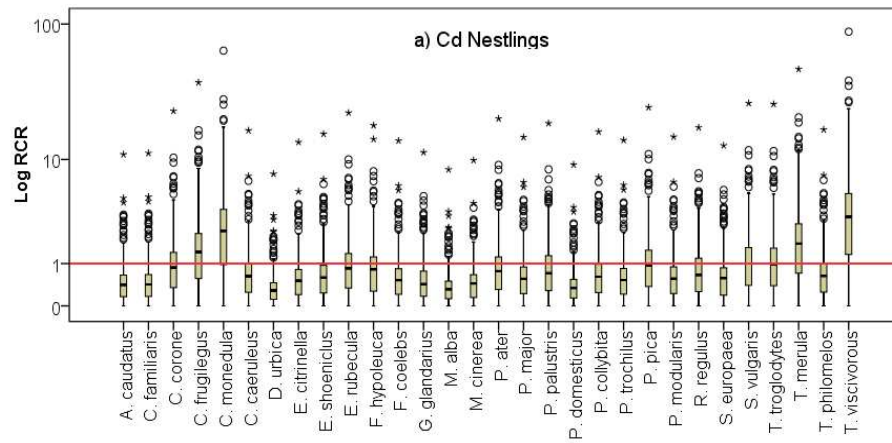
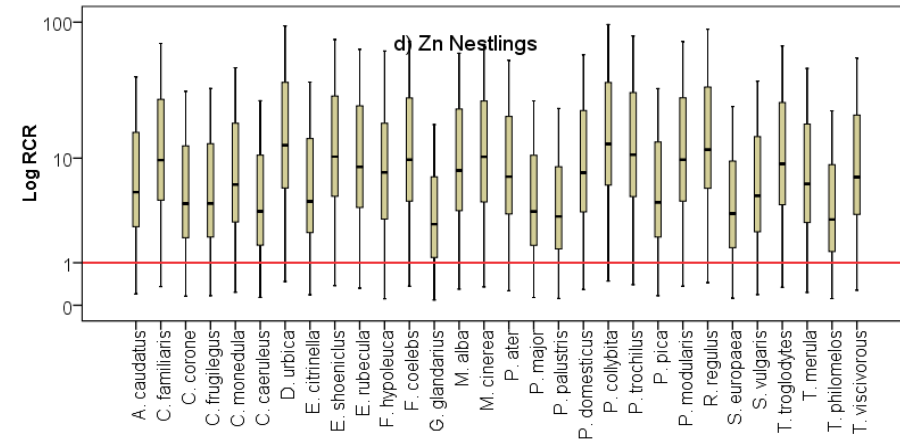
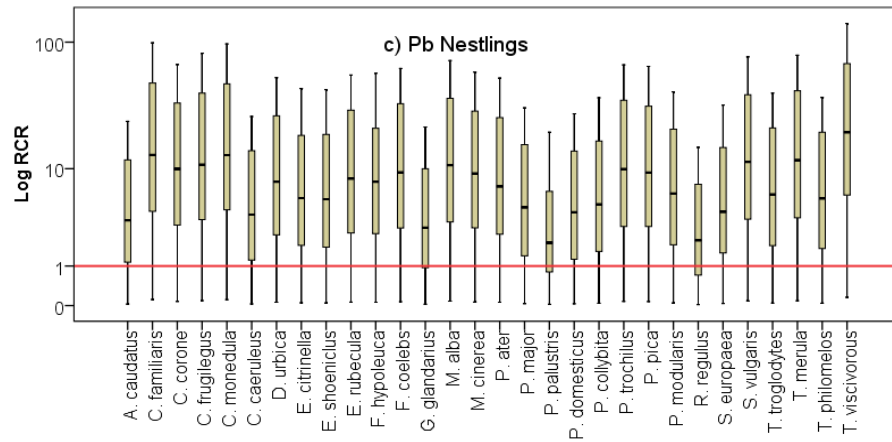


Figure 2: Adult RCRs. Differences in the median RCRs for the adults of passerine species across their distributions in England and Wales for a) Cd; b) Cu; c) Pb and d) Zn. Above the red line the risk from the metal is unacceptable; below it the risk is acceptable. Y axis is a log₁₀ scale due to the skew of the values and the number of outliers. Note the different scales on the y-axes. Medians, quartiles, outliers and extreme values are displayed.

293



294



295 **Figure 3:** Nestling RCRs. Differences in the median RCRs for the nestlings of passerine species across their distributions in England and Wales for a) Cd; b)
 296 Cu; c) Pb and d) Zn. Above the red line the risk from the metal is unacceptable; below it the risk is acceptable. Y axis is a log10 scale due to the skew of the
 297 values and the number of outliers. Medians, quartiles, outliers and extreme values are displayed.

298

299 **Table 1.** Descriptive statistics (median and range) of RCRs data and percentage of the bird range (in italics) predicted at risk for each metal and bird species
 300 (adults and nestlings).

301

Bird species	Cd		Cu		Pb		Zn	
	Adults	Nestlings	Adults	Nestlings	Adults	Nestlings	Adults	Nestlings
<i>Aegithalos caudatus</i>	0.5 (0.0 – 13.7) 24%	0.4 (0.0 – 10.9) 12%	0.4 (0.0 – 33.3) 6%	0.3 (0.0 – 28.4) 4%	2.8 (0.0 – 32.3) 77%	2.3 (0.0 – 26.0) 72%	8.0 (0.1 – 75.0) 95%	4.8 (0.0 – 45.1) 92%
<i>Certhia familiaris</i>	0.7 (0.0 – 19.3) 42%	0.4 (0.0 – 11.2) 14%	0.4 (0.0 – 38.0) 8%	0.4 (0.0 – 35.9) 7%	6.7 (0.1 – 77.2) 91%	9.2 (0.1 – 106.4) 93%	9.5 (0.1 – 88.6) 97%	8.5 (0.1 – 78.9) 95%
<i>Corvus corone</i>	0.7 (0.0 – 18.8) 35%	0.9 (0.0 – 23.3) 50%	0.3 (0.0 – 21.9) 2%	0.3 (0.0 – 26.1) 4%	4.5 (0.1 – 51.9) 89%	6.3 (0.1 – 72.3) 90%	3.7 (0.0 – 34.4) 88%	3.8 (0.0 – 35.4) 88%
<i>Corvus frugilegus</i>	0.4 (0.0 – 9.9) 9%	1.4 (0.0 – 37.8) 62%	0.2 (0.0 – 17.7) 1%	0.3 (0.0 – 28.8) 4%	3.7 (0.0 – 43.6) 84%	7.6 (0.1 – 88.4) 92%	3.5 (0.0 – 32.4) 87%	4.0 (0.0 – 37.2) 89%
<i>Corvus monedula</i>	NA	2.4 (0.0 – 64.3) 72%	NA	0.5 (0.1 – 41.7) 9%	NA	9.0 (0.1 – 104.4) 93%	NA	5.6 (0.0 – 52.5) 94%
<i>Delichon urbica</i>	NA	0.3 (0.0 – 7.7) 4%	NA	0.5 (0.1 – 39.8) 9%	NA	5.0 (0.1 – 57.3) 89%	NA	11.3 (0.1 – 105.0) 97%
<i>Emberiza citrinella</i>	0.5 (0.0 – 13.7) 24%	0.5 (0.0 – 13.6) 24%	0.4 (0.0 – 30.6) 5%	0.3 (0.0 – 27.0) 4%	4.7 (0.1 – 54.4) 89%	4.0 (0.0 – 47.2) 85%	7.6 (0.1 – 72.5) 95%	4.4 (0.0 – 41.4) 91%
<i>Emberiza schoeniclus</i>	0.8 (0.0 – 22.5) 50%	0.6 (0.0 – 15.7) 28%	0.5 (0.0 – 39.6) 9%	0.4 (0.0 – 32.1) 6%	9.8 (0.1 – 114.4) 92%	4.0 (0.0 – 46.2) 85%	5.9 (0.1 – 55.9) 95%	8.9 (0.1 – 83.9) 97%
<i>Erithacus rubecula</i>	0.7 (0.0 – 19.2) 41%	0.8 (0.0 – 22.6) 50%	0.6 (0.1 – 49.2) 15%	0.5 (0.1 – 42.6) 10%	6.2 (0.1 – 71.8) 90%	5.2 (0.1 – 60.0) 89%	7.7 (0.1 – 72.6) 96%	7.6 (0.1 – 71.4) 96%
<i>Ficedula hypoleuca</i>	0.9 (0.0 – 20.3) 42%	0.8 (0.0 – 18.2) 35%	0.3 (0.0 – 27.2) 4%	0.5 (0.1 – 43.0) 10%	7.0 (0.1 – 52.3) 90%	7.8 (0.1 – 57.6) 91%	8.2 (0.1 – 66.0) 95%	7.7 (0.1 – 62.1) 95%
<i>Fringilla coelebs</i>	0.7 (0.0 – 20.0) 42%	0.5 (0.0 – 14.0) 24%	0.4 (0.0 – 32.3) 6%	0.4 (0.0 – 30.6) 5%	6.6 (0.1 – 76.3) 91%	5.9 (0.1 – 67.5) 89%	7.4 (0.1 – 69.2) 96%	8.7 (0.1 – 81.2) 97%
<i>Garrulus glandarius</i>	0.1 (0.0 – 2.4) 0%	0.5 (0.0 – 11.4) 15%	0.1 (0.0 – 6.2) 0%	0.2 (0.0 – 19.1) 2%	10.0 (0.1 – 115.9) 93%	2.1 (0.0 – 23.8) 68%	5.0 (0.0 – 46.3) 91%	2.1 (0.0 – 19.9) 74%
<i>Motacilla alba</i>	0.4 (0.0 – 11.4)	0.3 (0.0 – 8.3)	0.2 (0.0 – 21.6)	0.4 (0.0 – 31.9)	5.6 (0.1 – 64.9)	6.7 (0.1 – 77.7)	4.6 (0.0 – 43.1)	7.1 (0.1 – 66.9)

	14%	5%	2%	6%	89%	91%	92%	95%
<i>Motacilla cinerea</i>	0.7 (0.0 – 17.6) 35%	0.4 (0.0 – 9.9) 9%	0.8 (0.1 – 73.9) 38%	0.3 (0.0 – 23.6) 3%	8.5 (0.1 – 98.2) 93%	5.7 (0.1 – 63.2) 90%	10.6 (0.1 – 99.4) 97%	8.6 (0.1 – 77.4) 95%
<i>Parus ater</i>	0.4 (0.0 – 9.9) 9%	0.8 (0.0 – 20.5) 42%	0.5 (0.1 – 45.4) 12%	0.4 (0.0 – 33.0) 5%	6.6 (0.1 – 72.1) 91%	4.9 (0.1 – 56.9) 90%	10.6 (0.1 – 94.8) 97%	6.6 (0.1 – 59.5) 94%
<i>Cyanistes caeruleus</i>	0.4 (0.0 – 10.7) 12%	0.6 (0.0 – 16.7) 35%	0.4 (0.0 – 37.0) 7%	0.3 (0.0 – 25.3) 3%	2.3 (0.0 – 26.9) 75%	2.5 (0.0 – 28.5) 75%	9.4 (0.1 – 84.3) 97%	3.2 (0.0 – 30.2) 85%
<i>Parus major</i>	0.5 (0.0 – 12.6) 18%	0.6 (0.0 – 14.8) 29%	0.3 (0.0 – 28.6) 4%	0.3 (0.0 – 24.8) 3%	7.6 (0.1 – 87.3) 92%	2.9 (0.0 – 33.3) 79%	6.7 (0.1 – 62.3) 95%	3.2 (0.0 – 30.2) 85%
<i>Parus palustris</i>	0.6 (0.0 – 16.8) 35%	0.7 (0.0 – 18.9) 36%	0.4 (0.0 – 36.5) 7%	0.3 (0.0 – 25.1) 3%	4.8 (0.1 – 55.4) 89%	1.6 (0.0 – 21.3) 64%	7.2 (0.1 – 67.5) 96%	2.8 (0.0 – 26.2) 81%
<i>Passer domesticus</i>	NA	0.3 (0.0 – 9.1) 6%	NA	0.4 (0.0 – 31.6) 5%	NA	2.6 (0.0 – 29.9) 76%	NA	6.9 (0.1 – 65.2) 95%
<i>Phylloscopus collybita</i>	0.6 (0.0 – 17.1) 36%	0.6 (0.0 – 16.3) 29%	0.4 (0.0 – 34.4) 6%	0.6 (0.1 – 51.9) 17%	7.0 (0.1 – 91.8) 93%	3.4 (0.0 – 40.0) 82%	6.0 (0.1 – 55.8) 95%	11.8 (0.1 – 107.4) 97%
<i>Phylloscopus trochilus</i>	0.6 (0.0 – 16.9) 35%	0.5 (0.0 – 14.1) 24%	0.5 (0.1 – 45.5) 13%	0.5 (0.1 – 39.8) 8%	6.0 (0.1 – 69.5) 89%	6.2 (0.1 – 71.9) 90%	10.7 (0.1 – 97.8) 97%	9.5 (0.1 – 88.9) 97%
<i>Pica pica</i>	0.7 (0.0 – 18.6) 35%	0.9 (0.0 – 24.9) 50%	0.5 (0.1 – 41.8) 9%	0.3 (0.0 – 29.4) 5%	6.6 (0.1 – 76.3) 91%	6.1 (0.1 – 70.0) 90%	9.1 (0.1 – 85.4) 97%	3.9 (0.0 – 37.1) 89%
<i>Prunella modularis</i>	0.1 (0.0 – 4.5) 1%	0.6 (0.0 – 14.9) 29%	0.2 (0.0 – 14.7) 1%	0.4 (0.0 – 38.6) 8%	1.6 (0.0 – 18.7) 62%	3.8 (0.0 – 44.4) 84%	13.6 (0.1 – 127.3) 98%	8.7 (0.1 – 81.3) 97%
<i>Regulus regulus</i>	0.7 (0.0 – 19.5) 42%	0.7 (0.0 – 17.5) 35%	0.5 (0.1 – 47.6) 13%	0.5 (0.1 – 45.6) 12%	3.6 (0.0 – 41.9) 83%	1.4 (0.0 – 16.1) 57%	11.0 (0.1 – 104.2) 97%	10.5 (0.1 – 99.5) 97%
<i>Sitta europaea</i>	0.6 (0.0 – 12.3) 19%	0.6 (0.0 – 12.8) 19%	0.3 (0.0 – 25.5) 3%	0.3 (0.0 – 29.8) 5%	5.2 (0.1 – 60.2) 90%	3.1 (0.0 – 35.4) 80%	9.1 (0.1 – 85.7) 96%	2.9 (0.0 – 27.0) 82%
<i>Sturnus vulgaris</i>	0.2 (0.0 – 9.9) 9%	1.0 (0.0 – 26.6) 50%	0.2 (0.0 – 14.7) 1%	0.3 (0.0 – 28.2) 4%	7.7 (0.1 – 88.5) 92%	7.2 (0.1 – 82.8) 91%	4.6 (0.0 – 43.2) 92%	4.5 (0.0 – 42.1) 91%
<i>Troglodytes troglodytes</i>	1.0 (0.0 – 25.6) 50%	1.0 (0.0 – 26.2) 50%	0.6 (0.1 – 52.2) 17%	0.5 (0.1 – 44.2) 11%	7.0 (0.1 – 81.1) 91%	3.8 (0.0 – 43.5) 83%	9.1 (0.1 – 85.0) 97%	8.1 (0.1 – 75.5) 96%
<i>Turdus merula</i>	NA	1.8 (0.0 – 47.2) 71%	NA	0.6 (0.1 – 48.6) 14%	NA	7.4 (0.1 – 85.4) 91%	NA	5.6 (0.0 – 52.0) 94%

<i>Turdus philomelos</i>	2.2 (0.0 – 59.7) 72%	0.6 (0.0 – 16.9) 35%	0.9 (0.1 – 82.0) 46%	0.3 (0.0 – 22.4) 2%	12.2 (0.1 – 140.6) 94%	3.5 (0.0 – 40.2) 83%	8.2 (0.1 – 76.7) 96%	2.7 (0.0 – 25.5) 81%
<i>Turdus viscivorus</i>	2.2 (0.0 – 58.1) 72%	3.3 (0.0 – 88.1) 83%	0.3 (0.0 – 32.1) 6%	0.6 (0.1 – 55.7) 21%	14.7 (0.2 – 169.6) 95%	13.0 (0.2 – 149.7) 94%	4.8 (0.0 – 45.1) 92%	6.5 (0.1 – 61.9) 95%

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304 **3.2 Influence of Diet on Exposure to Toxicity Risk**

305 Next, we investigated the influence of diet on determining the risk to species. Table 2 shows
306 the correlations between the incidence of different prey types in the diet and the mean risk
307 of toxicity (RCRs) to the four different metals for species. The most obvious point is that no
308 single diet item drives exposure to metal toxicity (see also Table A7); Consumption of
309 Araneidae was significantly correlated with toxicity risk to Cd for adult songbirds only.
310 Coleoptera intake was significantly linked with risk of Cd toxicity to adults and Pb toxicity
311 risk for nestlings. Diptera consumption posed more of a risk than other prey being
312 significantly linked with Pb and Zn risks to nestlings. Lumbricidae in the nestling diet
313 exposed them to Cd and Pb toxicity. There was a significant negative relationship between
314 consumption of Lepidopterans by nestling and relative risk of toxicity to both Pb and Zn.
315 This suggests that moths and butterflies do not bioaccumulate high levels of trace metals
316 and that their inclusion in the diet perhaps reduces the consumption of more high risk
317 invertebrates by specific bird species.

318 Our modeling exercise compiles several factors in determining the risks, with a
319 variety of risk scenario depending on the species, the diet, spatial range, ect. Therefore, our
320 results were not simply driven by the frequency of a prey type in the diet or by high BAF
321 values for a prey item. Diptera for example, contribute a large proportion to the diet of birds
322 overall but has a relatively low BAF value for Pb and a much higher one for Zn. Despite this
323 difference in BAF value between Pb and Zn, these two elements are are strongly linked with
324 toxicity risk of birds from Diptera at the national distribution level, which could suggest high
325 exposure of Pb due to the high Pb concentrations in the soil and a higher toxicity of Pb
326 compare to Zn. Thus, the distribution of both the songbird species and the metals in the

327 soils clearly impact upon toxicity risk. These two factors are important in predicting the
328 exposure of metals to wildlife species. In a field study of small mammals, for example, Cd
329 exposure was related to diet in the woodmouse (*Apodemus sylvaticus*), whereas local soil Cd
330 concentrations or soil properties were more important to determine Cd accumulation for
331 the common vole (*Microtus arvalis*), (van den Brink et al., 2011). We have shown that there
332 are high toxicity risks posed by Pb to avian distributions in both adults (Fig 2) and nestlings
333 (Fig. 3). Pb does not accumulate in prey at higher rates than other metals. Rather it seems
334 that the ubiquity and relatively high concentrations of Pb in the soils, as well as its low
335 NOAEL, result in it posing a risk to a high percentage of the distributions of passerines. Given
336 these relationships indicating that the RCRs across species' breeding distribution in the UK
337 at risk from metal toxicity can be linked to intake of specific invertebrates, the next step is to
338 investigate the differences in metal concentrations in different invertebrate taxa from high
339 and low toxicity risk areas of the country (such as in (Davison et al., 1999) and see list of
340 references in Appendix used to determine BAF values).

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344 **Table 2:** Correlations between the percentage of specific commonly consumed prey items in a species' diet for adults and nestlings and the mean risk (RCR)
345 from metal toxicity. Kendall's tau (tau), sample size (N) and p-value are presented. A Holm-Bonferroni correction was applied as four tests per prey type per
346 avian life stage were applied. Bold indicates that the relationship was significant according to the corrected p-values.

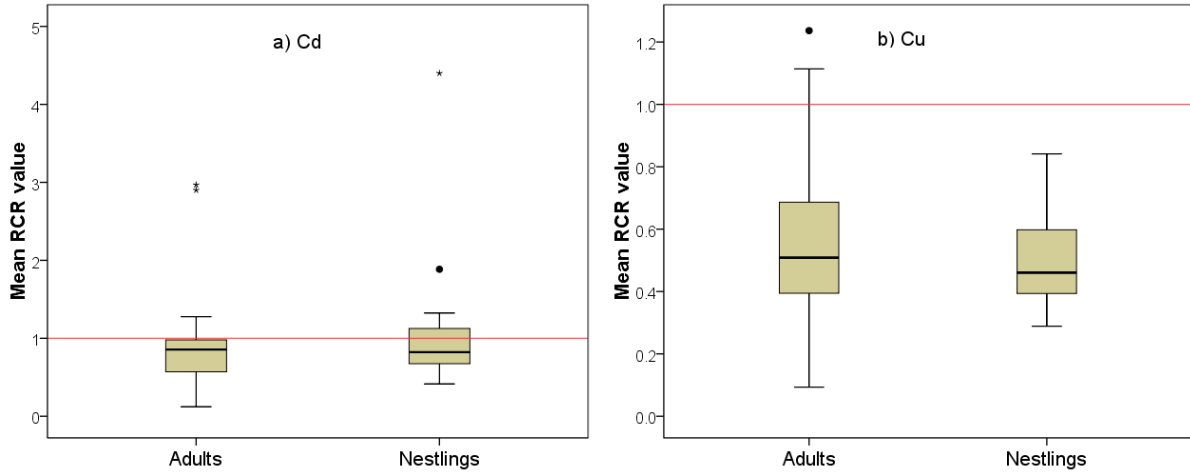
Prey	Bird age	Cd			Cu			Pb			Zn		
		tau	N	p	tau	N	p	tau	N	p	tau	N	p
Araneidae	Adult	0.442	20	0.006	0.263	20	0.105	-0.053	20	0.746	0.368	20	0.023
	Nestling	0.167	25	0.243	0.153	25	0.283	-0.313	25	0.028	0.173	25	0.225
Coleoptera	Adult	0.422	26	0.003	0.255	26	0.067	0.305	26	0.029	-0.114	26	0.415
	Nestling	0.331	30	0.011	-0.155	30	0.231	0.382	30	0.003	-0.280	30	0.031
Diptera	Adult	0.109	26	0.439	0.291	26	0.040	0.128	26	0.364	0.259	26	0.066
	Nestling	-0.118	30	0.363	0.200	30	0.121	0.463	30	0.0001	0.380	30	0.003
Hymenoptera	Adult	-0.009	26	0.947	-0.034	26	0.808	-0.122	26	0.388	-0.022	26	0.877
	Nestling	0.144	30	0.277	0.163	30	0.218	0.106	30	0.426	0.173	30	0.192
Lepidoptera	Adult	-0.101	26	0.478	0	26	1.00	-0.353	26	0.013	-0.025	26	0.859
	Nestling	-0.055	30	0.668	-0.226	30	0.080	-0.442	30	0.001	-0.460	30	0.0001
Lumbricidae	Adult	0.400	5	0.327	0.200	5	0.624	0.600	5	0.142	0.200	5	0.624
	Nestling	0.833	9	0.002	0.500	9	0.061	0.722	9	0.007	0.389	9	0.144

348 **3.3 Life-stage related variation in the toxicity risks of metals to birds**

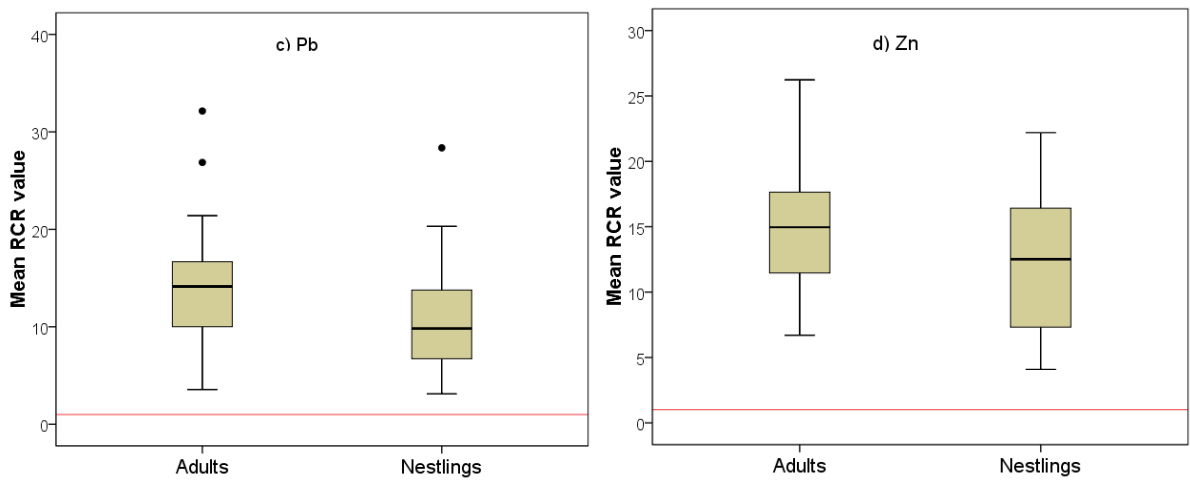
349 Few studies have compared specific risk from metals across different species and life stages,
350 so we were interested to see how diet and breeding range would combine to affect risk. For
351 the 26 species for which we had both adult and nestling diet data, we ran paired tests on
352 the mean RCR per age class per species: There were no age specific differences in the RCR
353 values for Cd (Wilcoxon signed-rank test $Z = 0.724$, $N = 26$, $p = 0.47$; Fig. 4a) or Cu (Wilcoxon
354 signed-rank test $Z = -0.241$, $N = 26$, $p = 0.81$; Fig. 4b). Moreover, the median RCR values
355 were below the toxicity cut-off of 1. Adults were found to have a significantly higher risk
356 from Pb (Wilcoxon signed ranks test $Z = -2.15$, $N = 26$, $p = 0.032$; Fig. 4c) and Zn (Wilcoxon
357 signed ranks test $Z = -2.83$, $N = 26$, $p = 0.005$; Fig. 4d) than nestlings of the same species.

358 The age specific differences in spatial risk must have been partially driven by
359 differences in diet between adults and nestlings, because other factors, such as age specific
360 sensitivity to contaminants or rates of excretion, were not taken into account within our
361 model. Moreover, due to data limitation in the literature, the daily amount of food ingested
362 and the NOAELs were similar for the adults and nestlings of the same species. Thus next, we
363 directly compared the diets of nestlings and adults. For the species that we studied, the
364 adult diets contained a higher percentage of Coleoptera (Mann-Whitney U-test $z = -3.63$,
365 Adult $N = 26$, nestling $N = 30$, $p < 0.0001$) and Hymenoptera (Mann-Whitney U-test $z = -$
366 2.06 , Adult $N = 26$, nestling $N = 30$, $p = 0.039$) than the diets of nestlings. In contrast, adult
367 diets consisted of a lower percentage of Araneidae (Mann-Whitney U-test $z = 3.08$, adult $N =$
368 20 , nestling $N = 25$, $p = 0.002$), Diptera (Mann-Whitney U-test $z = 3.21$, Adult $N = 26$, nestling
369 $N = 30$, $p = 0.001$), and Lepidoptera (Mann-Whitney U-test $z = 3.60$, Adult $N = 26$, nestling N
370 $= 30$ adults, $p < 0.0001$) compared with the nestling diets. Moreover, we can see from Table

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377 **Figure 4:** Difference between nestlings and adults in mean RCRs across species' distributions from a)
378 Cd (N.S.); b) Cu (N.S.); c) Pb ($p = 0.032$) and d) Zn ($p = 0.005$). See text for more information. NB the
379 very different scales on the y-axes. The red line indicates RCR = 1 above which there is a toxicity risk.
380 Medians, quartiles, outliers (black dots) and extreme values (stars) are displayed.

381

2 that there are some differences between adults and chicks in the diet items driving mean RCRs across species. A larger dataset on feeding requirement, metabolic rate, sublethal effects of metal toxicity, toxicokinetics and exogenous metal bioaccumulation and detoxification in adult versus nestlings for wildlife species could help to refine the model and better understand the differences in metal toxicity across life-stage.

3.4 Percentage of bird distribution predicted at risk

Our spatially explicit model allowed us to identify areas of each species' distribution at a national level in which the population is at risk from metal toxicity (Table 1). Overall, based on this modelling exercise we were able to determine that an average of $32.7 \pm 0.2\%$, $8.0 \pm 0.1\%$, $86.1 \pm 0.1\%$, and $93.2 \pm 0.1\%$ of the bird spatial distribution (for all the species studied) were characterized at risk of Cd, Cu, Pb and Zn, respectively. For adults, the highest average percentage of area at risk was for Zn (with $94.3 \pm 0.0\%$), followed by Pb ($87.5 \pm 0.1\%$), Cd ($31.4 \pm 0.2\%$) and Cu ($9.1 \pm 0.1\%$). For nestlings we obtained: Zn ($91.0 \pm 0.1\%$), Pb ($83.5 \pm 0.1\%$), Cd ($34.3 \pm 0.2\%$) and Cu ($7.1 \pm 0.0\%$) (Table 1). These results were consistent with our previous analysis (section 3.1). We believe that the percentage of area characterized at risk and the maps are model outputs of great use for risk communication to the public (including outreach activities) and environmental risk assessment's stakeholders (e.g. policy makers)(Lahr and Kooistra, 2010)

Figure 5 provides three examples of risk maps for Cd; Figure 5a shows the spatial variation in the risk of Cd toxicity to Pied Flycatcher *Ficedula hypoleuca* nestlings - a summer migrant, which has the smallest breeding range of all the bird species studied and consumes mainly aerial and arboreal invertebrates. In comparison, Figure 5b shows the wide distribution of the Blackbird *Turdus merula* which is resident in the UK, has the largest range of the species studied (with *Fringilla coelebs* and *Cyanistes caeruleus*) and is common in urban areas. Thus, Blackbirds due to their wide distribution and diet of ground-dwelling invertebrates are at relatively high risk of Cd toxicity over a large part of their range, not just from heavily polluted areas. Finally, Fig. 5c shows the risk map for Blue Tits *Cyanistes caeruleus* which are also

widespread but forage more on arboreal than soil dwelling invertebrates, so has a lower percentage of its UK distribution at Cd toxicity risk than that of Blackbirds.

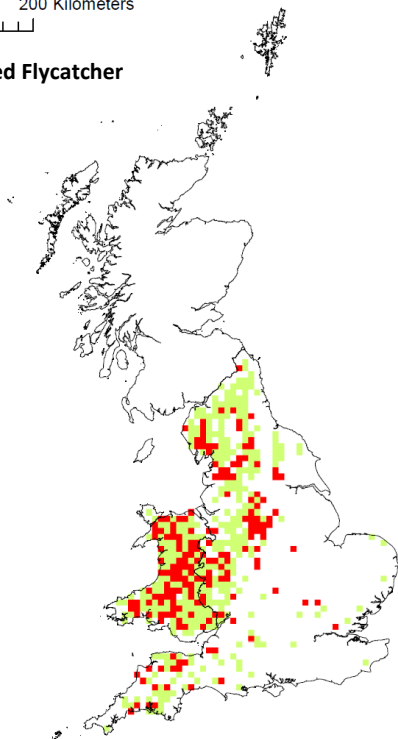
Next we investigated the relationships between metal exposure from the different metals studied by assessing the strength of the associations of the percentage of area at risk between metals for each life-stage. For nestlings, the percentage of avian distributions at risk from Cd was not correlated with risk from any other metal. There were significant positive relationships between the percentages of the songbird distributions at risk from Cu and Zn (Kendall's tau = 0.628, N = 22, P = 0.0001). For adult passerine species in our dataset, the percentage of species' distribution at risk from Cd was positively correlated with risk from Cu (Kendall's tau = 0.473, N = 16, P = 0.015), as was Cu and Zn (Kendall's tau = 0.679, N = 16, P = 0.001). So, although for most of their distributions, songbirds were not exposed to toxic levels of Cd or Cu (RCR < 1), in some areas affected by heavy industry, bird populations could be at risk from exposure to several trace metals simultaneously. Where different metals affect the same organ or body system, they might have an additive effect (EPA, 2010; EPA, 2004). For example, both Cd and Pb cause pathology of the kidneys so simultaneous exposure to both metals is likely to cause increased kidney damage. However, some metals have antagonistic effects such as that displayed by Cu and Zn (Oestreicher and Cousins, 1985). Zn can even cause toxicity through inducing deficiencies in other essential metals, particularly Cu (Beyer, 2006). The combined effects of several trace metals has been related to reduced survival in wild passerine populations (Belskii et al., 1995; Belskii et al., 2005; Eeva and Lehikoinen, 1996; Janssens et al., 2003b). The sublethal effects of different mixture of trace metals, as well as other pollutants associated with industrial activities, on birds at different life history stages remains a key knowledge gap (Heys et al., 2016; Cedergreen, 2014).

The risk areas identified by our maps reflect the trace metal concentrations in the soils and all four metals varied in concentration considerably throughout England and Wales (Fig. 5 and Fig A1 in Appendix). The main areas of significantly elevated metal concentrations are former mining areas or heavily industrialised conurbations such as the south Peak District and North Pennines. The peatland in these

areas is known to accumulate trace metals via atmospheric deposition (Rothwell et al., 2007). The industrialised coast of the Severn Estuary shows high concentrations of Cd and Zn, as do the south Wales valleys around the coal belt. There are also several areas where high concentrations of a single metal are not co-localised with other metals; e.g. Dorset and Hampshire have an elevated concentration of Cd only. In contrast, Pb appears to pose the spatially broadest risk of the four metals (Fisher et al., 2006).

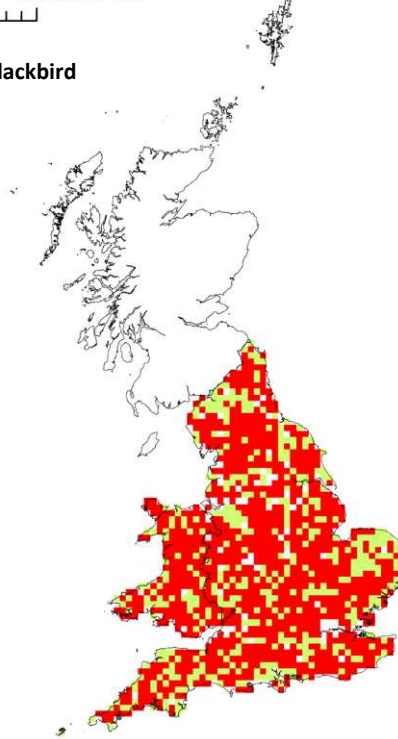
0 50 100 200 Kilometers

a) Pied Flycatcher



0 50 100 200 Kilometers

b) Blackbird



0 50 100 200 Kilometers

c) Blue Tit

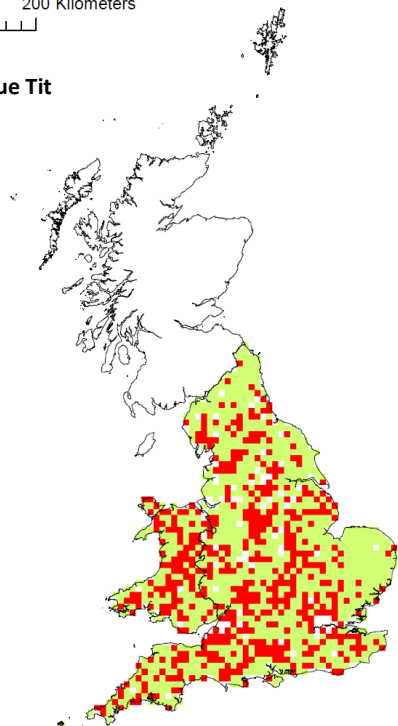


Figure 5: Cd toxicity risk maps for: a) Pied Flycatcher nestlings *Ficedula hypoleuca*; b) Blackbird nestlings *Turdus merula*; c) Blue Tit *Cyanistes caeruleus* nestlings in England and Wales. Red grid cells indicate that the population present in those cells are at risk from Cd toxicity, green indicates no risk in that area and white indicates that the species is not present in that area (or in a limited number of cells that there are no metal data).

Our model has identified species at potential risk of metal toxicity in different areas of England and Wales. This approach is useful in utilising existing data to make predictions concerning spatial variation in exposure to environmental contaminants. More specifically, we were able to refine the estimations of the amount of metal ingested per bird species and per life-stage, and per spatial areas. This refinement is often overlooked in environmental risk assessment. However, some limitations need to be borne in mind when interpreting the outputs. These include the assumptions used in deriving the diet of bird species which can vary within and between species depending on life history stage, but also vary within spatial locations and seasons, for example (Arnold et al., 2010; Nagy, 1987). In addition, bird energetic requirements and therefore the daily amount of food consumed can also vary within more refined parameters: such as the temperature, season etc. Further studies could therefore refine the metabolic rate requirement across species and across life-stage. Moreover, the model does not take into account the influences of soil parameters on uptake into food items, the spatial obtainability of prey items and the bioavailability of metals from food items into the birds (Fritsch et al., 2012; Hernout et al., 2011; Hernout et al., 2013; Schipper et al., 2012). Exposure to metals will of course differ between resident species and those migratory species, such as Pied flycatchers that overwinter in regions that have not been modelled here. Here, we considered only exposure via prey but Pb exposure via ingestion of spent Pb from ammunition, presumably as grit or as particles in vertebrate prey is not considered in the model. So, risk for Pb could be underestimated perhaps more so for predatory and scavenging, than insectivorous species (Pain et al., 2007). Finally, we have not taken soil or prey Ca availability into account and Ca affects the absorption of other metals (Eeva and Lehtikoinen, 2004; Scheuhammer, 1991).

The tolerance of the species to each metal (i.e. ratio NOAEL by uncertainty factor) were obtained from studies on model species which have different life histories and energetic requirements to passerines (Table A5). Surrogate species are often used to derive toxicological data for wildlife species from the same taxa. Further work is required to assess species-level effects and life-stage related effects of trace metals for almost all bird species (Korsman et al., 2016). Nestling may also have a different tolerance to metal

than adults, and different excretion rates of metal (excretion through growing feathers) (Dauwe et al., 2000). Moreover, there is increasing discussion in the literature of the limitations of simplified ecotoxicological descriptors, such as NOAEL, which do not describe the exposure-response curve fundamental in ecotoxicology (Landis and Chapman, 2011; but see (Green et al., 2013)).

Our model provides an indication of risk across different areas of a species' range but this requires ground-truthing via field sampling (Hernout et al., 2015). We have previously shown, for example, that insectivorous bats found in areas predicted to be the most "at risk", by a similar spatially explicit model, contained higher metal concentrations in their tissues than those found in areas projected to be "not at risk" by the model (Hernout et al., 2015). Although some monitoring studies presenting body burdens of metals in bird tissues are available (e.g.(Berglund and Nyholm, 2011; Berglund et al., 2015; Fritsch et al., 2012), these data are not directly comparable with our model predictions, since they refer to different study areas (Sweden, Finland and France, respectively). Further monitoring studies could investigate body burdens of metals in songbirds in the UK and compare these concentrations across a gradient of soil concentrations. However, unlike other monitoring studies, we have investigated risk and proposed a refined assessment of the amount of metal ingested for thirty species of bird across their breeding distribution in England and Wales, not just focussed on point sources of contamination.

Despite the limitations described above, the model framework shown in this paper provides a starting point for conservation and environmental risk assessment efforts and to orientate further research. In the context of moving forward spatially explicit and ecologically relevant risk assessment of chemicals for wildlife species, we believe that the model presented in this study is valuable. In addition, this paper shows that with adequate data, spatial models can be used to highlight regional and global areas and species of concern which can then be targeted for more in depth study and management.

4. Conclusions

Our study has mapped interspecies variation in risk of metal exposure to insectivorous passerines at a national scale. In particular, our model estimates that Pb contamination of soils poses the highest risk to insectivorous passerines in England and Wales given its ubiquity in soil, low NOAEL values and despite its relatively low BAF for most invertebrate prey. This risk was independent of age specific differences in sensitivity to environmental contaminants. There were no common dietary features that exposed multiple bird species to all metals across their ranges. The heterogeneity in soil concentrations of metals, diet of different passerine species and bioaccumulation of metals in different invertebrate prey meant that spatial variation in risk of toxicity varied widely across bird species. The next step is to validate this model through a targeted field sampling of metal concentrations in the tissues of different bird species collected across their ranges (Hernout et al., 2015). As funding for conservation management becomes ever more elusive, efficient targeting of efforts will become crucial. Specifically, this model highlights areas where birds are likely to be at risk, which species are in need of attention and thus, where mitigation and conservation efforts might be focussed at a national and potentially international scale.

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APPENDIX

Interspecific variation in the spatially-explicit risks of trace metals to songbirds

Béatrice V. Hernout, Louise J. Gibson, Adam J. Walmsley and Kathryn E. Arnold

Methods

Bird Distribution Data

Gibbons Atlas 10-km records. Data from the Gibbons Atlas -- fieldwork 1988-1991. Information on how the data collection was carried out can be found on <https://www.bto.org/volunteer-surveys/complete-survey-details>. Briefly, Records for 10-km squares were collated over the breeding seasons of 1988-1991 inclusive. The timed visits for any one tetrad (a 2km square within the 10-km square) were carried out between 1 April and 31 July (with a stated preference for one visit of one hour in April-May and one in June-July although in the more remote areas this was not always possible), and for any one tetrad such visits were only done in one of these years. Tetrads were eligible for coverage if their centre was on land. Coverage of a minimum of eight such tetrads in each 10-km square was requested although the choice of which ones was left to the observer(s) with the proviso that they should aim to represent the major habitats within the 10-km square. All species heard and seen were recorded.

Table A1: List of the avian species studied and their body weight (Snow and Perrins, 1998) used to derive the amount of food eaten per day based on an allometric equation (Nagy, 1987).

	Body weight (g) (adult)	Daily amount of food eaten (g dw/ g body weight/ d)
<i>Aegithalos caudatus</i>	9	0.286
<i>Certhia familiaris</i>	10	0.282
<i>Corvus corone</i>	510	0.156
<i>Corvus frugilegus</i>	310	0.168
<i>Corvus monedula</i>	220	0.177
<i>Parus caeruleus</i>	11	0.278
<i>Delichon urbica</i>	19	0.256
<i>Emberiza citrinella</i>	31	0.238
<i>Emberiza schoeniclus</i>	21	0.252
<i>Erithacus rubecula</i>	18	0.258
<i>Fringilla coelebs</i>	24	0.247
<i>Ficedula hypoleuca</i>	13	0.271
<i>Garrulus glandarius</i>	170	0.184
<i>Motacilla alba</i>	21	0.252
<i>Motacilla cinerea</i>	18	0.258
<i>Passer domesticus</i>	34	0.235
<i>Parus ater</i>	9	0.286
<i>Parus major</i>	18	0.258
<i>Parus palustris</i>	12	0.274
<i>Phylloscopus collybita</i>	9	0.286
<i>Phylloscopus trochilus</i>	10	0.282
<i>Pica pica</i>	220	0.177
<i>Prunella modularis</i>	21	0.252
<i>Regulus regulus</i>	6	0.304
<i>Sitta europaea</i>	24	0.247
<i>Sturnus vulgaris</i>	78	0.207
<i>Troglodytes troglodytes</i>	10	0.282
<i>Turdus merula</i>	100	0.199
<i>Turdus philomelos</i>	83	0.205
<i>Turdus viscivorus</i>	130	0.192

Table A2: Table summarizing the diet of bird expressed in percentage number in percentage number (%) from several literature data. (see reference list below).
(Abbreviations of food items: Araneida (Ar), Coleoptera (Coleo), Collembola (Col), Dermaptera (Der), Diptera (Dip), Ephemeroptera (Eph), Gastropoda (Gas), Hemiptera (Hem), Hymenoptera (Hym), Isopoda (Iso), Lepidoptera (Lep), Lumbricidae (Lum), Myriapoda (Myr), Odonata (Od), Opiliones (Op), Orthoptera (Orth), Plecoptera (Ple), Trichoptera (Tri), Unspecified (Uns), Carrion (Car), Plant material (P.m), Cereal grains (C. g), Seeds (See), Fruits (Fru).

	Invertebrates and animal material																				Plant material				
	Ar	Coleo	Col	Der	Dip	Eph	Gas	Hem	Hym	Iso	Lep	Lum	Myr	Od	Op	Orth	Ple	Tri	Uns	Car	P. m.	C. g.	See	Fru	
<i>Aegithalos caudatus</i>	Adults	2.7	13.1			0.6		27.2	17.6		35.8													1.4	
	Nestlings	5.0	1.9			5.7	0.5	3.1	16.2	0.0	37.8						29.8								
<i>Certhia familiaris</i>	Adults	4.1	44.4			8.1		13.4	2.6		1.6													18.9	
	Nestlings	4.9	2.2			89.8					0.0						3.1								
<i>Corvus corone</i>	Adults	0.1	27.5			0.0		5.1		1.3		0.0	3.1			0.1	1.5								
	Nestlings	4.4	65.8			16.9		1.1		1.1		3.1	3.1			4.4									
<i>Corvus frugilegus</i>	Adults	0.0	27.0			1.3		1.9	0.2	0.5		5.7	0.5			0.0	0.6								
	Nestlings	0.1	72.6			2.2		0.2	0.9	0.0		1.3	8.1			0.1	0.1								
<i>Corvus monedula</i>	Adults		0.0			0.0					0.0														
	Nestlings	9.7	22.3			13.1		2.4	2.5	3.3	1.8	7.8	14.3												
<i>Parus caeruleus</i>	Adults	1.0	18.5			11.7			12.6	11.7		23.3													
	Nestlings	14.9	5.6			1.3			1.9	3.7		71.0													
<i>Delichon urbica</i>	Adults		0.0			0.0					0.0														
	Nestlings		1.8			47.8	0.9		43.9	1.9		0.0						0.9	0.9		1.9				
<i>Emberiza citrinella</i>	Adults		44.7			0.3			0.8	4.3		3.2					18.3								
	Nestlings	5.3	22.2		6.1	10.9		1.0		5.3	2.0	29.6					3.6								
<i>Emberiza schoeniclus</i>	Adults		73.0			27.0					0.0														
	Nestlings	26.2	5.0			20.0	13.4		5.0	11.0		9.5			3.2		4.3								
<i>Erithacus rubecula</i>	Adults	0.8	36.5		1.9	2.2		0.3	4.8	31.2		3.5	0.2	5.7											
	Nestlings	8.8	14.0			16.6		0.4	12.5	8.8	6.6	16.6	0.4			8.8	0.2								
<i>Fringilla coelebs</i>	Adults	8.4	49.4			2.6			3.9	15.7		13.4													
	Nestlings	7.1	21.1			14.3	6.4	1.4	7.4	9.3		11.9					4.1	7.4							
<i>Ficedula hypoleuca</i>	Adults	16.0	25.0			6.0				45.0		5.0													
	Nestlings	14.3	13.8			17.0		0.9	4.8	9.1	1.7	30.8		1.5											
<i>Garrulus glandarius</i>	Adults	0.4	4.6			0.0					2.7						0.2								
	Nestlings	13.7	8.2		0.0	1.7		1.0	0.2	0.5		71.5			1.4		0.4								
<i>Motacilla alba</i>	Adults		45.0			0.0					0.0				30.0										
	Nestlings		0.0			71.3					0.0						7.6								
<i>Motacilla cinerea</i>	Adults	1.8	10.9			50.9			18.2	0.0		0.0			5.5		12.7								
	Nestlings	1.0	21.9			21.7	23.8	0.2	3.6	0.3		0.9					0.1	22.6	3.2						
<i>Passer domesticus</i>	Adults		0.0			0.0					0.0														
	Nestlings		7.7			9.7			36.4	0.0		39.9					0.5								
<i>Parus ater</i>	Adults	0.3	6.2			5.4			40.6	7.2		39.0													
	Nestlings	28.7	6.3			27.2			1.0	1.4		32.0													
<i>Parus major</i>	Adults	7.1	27.6			3.7		0.0	22.1	6.4		20.4													
	Nestlings	12.0	4.1			9.4		0.8	0.4	3.4		68.8													

	Invertebrates and animal material																				Plant material			
	Ar	Coleo	Col	Der	Dip	Eph	Gas	Hem	Hym	Iso	Lep	Lum	Myr	Od	Op	Orth	Ple	Tri	Uns	Car	P. m.	C. g.	See	Fru
<i>Parus palustris</i>	Adults	0.3	39.4			2.8			3.1	3.7	14.7										36.0			
	Nestlings	27.2	0.0			0.0				0.0	72.8													
<i>Phylloscopus collybita</i>	Adults	1.5	33.9			15.3			36.6	7.6	2.4								2.7					
	Nestlings	13.7	0.3			13.9	0.5	5.3	44.9	3.2	15.4			0.4			1.1	0.7	0.6					
<i>Phylloscopus trochilus</i>	Adults	4.3	35.1			16.6	1.3	0.1	21.2	8.3	6.7						0.0	0.2	6.2					
	Nestlings	10.1	0.0			49.7	0.5	2.0	14.2	7.8	9.6						0.4	0.6	5.1					
<i>Pica pica</i>	Adults	3.5	39.6			1.1		0.3	3.8	3.6	1.8	2.7			3.5	4.7				4.3		10.1	11.8	9.5
	Nestlings	1.6	41.5			13.1		0.6	1.2	1.7	1.1	22.4	4.1	1.2	1.6	1.4				1.3	7.4			
<i>Prunella modularis</i>	Adults		1.2			0.0		3.1		0.5	0.5												94.8	
	Nestlings	21.2	0.5			30.3		1.0	30.3	1.0	12.6								3.0					
<i>Regulus regulus</i>	Adults	19.9	8.5	0.1		6.2		0.9	38.4	3.9	12.7				0.4		1.1	0.7	7.1				0.2	
	Nestlings	22.0	0.0			0.0			46.0	0.0	24.0								8.0					
<i>Sitta europaea</i>	Adults	3.7	25.0			24.4			3.7	5.5	1.8				1.2								34.8	
	Nestlings	1.5	8.9			5.7		1.2	1.1	0.5	76.8		1.4						2.9					
<i>Sturnus vulgaris</i>	Adults	1.1	21.5			5.3				46.8	1.1											2.7	0.6	21.0
	Nestlings		38.4			24.0				5.7	21.8	2.4							6.7					1.2
<i>Troglodytes troglodytes</i>	Adults	11.7	42.5			4.7		6.0	20.7	11.5	2.9												1.0	
	Nestlings	41.2	2.0			9.4		1.3	13.1	2.0	12.4								17.6					
<i>Turdus merula</i>	Adults		0.0			0.0				0.0	0.0													
	Nestlings	2.5	12.4			8.8		9.4	1.2	4.4	10.7	34.4	9.4		2.5				1.3				3.1	
<i>Turdus philomelos</i>	Adults		32.4			7.3		39.3		0.0	0.0	8.2							1.6		11.3			
	Nestlings	6.5	8.1			14.5		1.6		0.0	67.7	1.6												
<i>Turdus viscivorus</i>	Adults		36.0			0.0		3.5		0.0	0.0	14.0							1.5		4.5		12.0	28.5
	Nestlings		23.6			27.2		15.0		0.0	12.0	22.2												

References used for diet data calculations (Table A2). References cited by Cramp, 1988 (Vol 5); Cramp 1992 (Vol 6); Cramp and Perrins, 1993 (Vol 7); Cramp and Perrins, 1994 (Vol 8); Cramp and Perrins, 1994 (Vol 9), Handbook of the birds of Europe, the Middle East and North Africa.

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Table A3: BAF values used in the model. Values represent the values of BAF values from soil into various invertebrates and vegetation food items for birds. The values were gathered from the literature (reference listed below) and the median value was used in our model.

	Invertebrates and carrion											
	Araneida	Coleoptera	Collembola	Dermaptora	Diptera	Ephemeroptera	Gastropoda	Hemiptera	Hymenoptera	Isopoda	Lepidoptera	Lumbricidae
Cd	3.125	2.387	0.197	0.134	0.735	0.245	5.525	0.507	0.882	1.489	0.890	37.407
Cu	0.894	1.041	0.663	0.003	0.810	0.273	4.478	1.222	0.291	3.656	0.449	2.778
Pb	0.047	0.245	0.475	0.039	0.210	0.066	0.379	0.012	0.030	0.180	0.038	1.034
Zn	0.553	0.508	0.468	0.055	0.772	1.378	1.446	1.400	0.783	1.048	0.136	1.261

								Missing order/ unspecified	Vegetation			
	Myriapoda	Odonata	Opiliones	Orthoptera	Plecoptera	Trichoptera	Carrion		Plant material	Cereal grains	Seeds	Fruits
Cd	0.956	0.432	3.591	0.238	0.001	0.318	0.327	4.460	0.582	0.404	0.193	0.080
Cu	8.611	3.057	0.557	1.303	0.001	0.783	0.644	1.380	0.642	0.189	0.222	0.090
Pb	0.137	0.147	0.083	0.005	0.000	0.295	0.267	0.230	0.179	0.002	0.025	0.681
Zn	1.500	1.800	0.780	1.166	0.001	3.286	0.326	0.817	0.598	0.465	1.333	0.001

Table A4: Search terms used in the literature review for diet, bioaccumulation and toxicity data. Terms with an asterisk (*) denote wildcard searches. The search operator 'OR' is denoted in capitals.

Data type	Search terms
Bird diet	diet* OR food OR feeding (common bird name) OR (scientific bird name) bird* passeri* quantitative nestling* gizzard OR stomach OR collar insectivorous OR granivorous
Bioaccumulation (BSAFs)	BSAF OR BAF OR BCF concentration* accumulation bioaccumulation bioavailability trace metal* OR trace metal* (metal name) OR (metal chemical symbol) (food item common name) OR (food item scientific name)
Toxicity (NOECs)	NOEC OR NOAEL toxicit* poison* trace metal* (metal name) OR (metal chemical symbol) bird* mammal*

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1 **Table A5:** NOAELs in mg kg_{bw}⁻¹ d⁻¹ data used in the model (Sample et al. 1996). The NOAELs are
 2 considered chronic, and the metals were orally administered in the diet.

Metal	NOAEL	Test Species	Endpoint	Study duration	Reference
Cd	1.45	Mallard duck	Reproduction	90 days	White and Finley, 1978
Cu	46.97	<i>Young Chicks</i>	Growth/mortality	10 weeks	Mehring et al. 1960
Pb	3.85	<i>American kestrel</i>	Reproduction	7 months	Pattee 1984
	1.13	Japanese Quail	Reproduction	12 weeks	Edens et al. 1976
Mean	2.49				
Zn	14.5	White Leghorn Hens	Reproduction	44 weeks	Stahl et al. 1990

3 **References used for the NOAELs data:**

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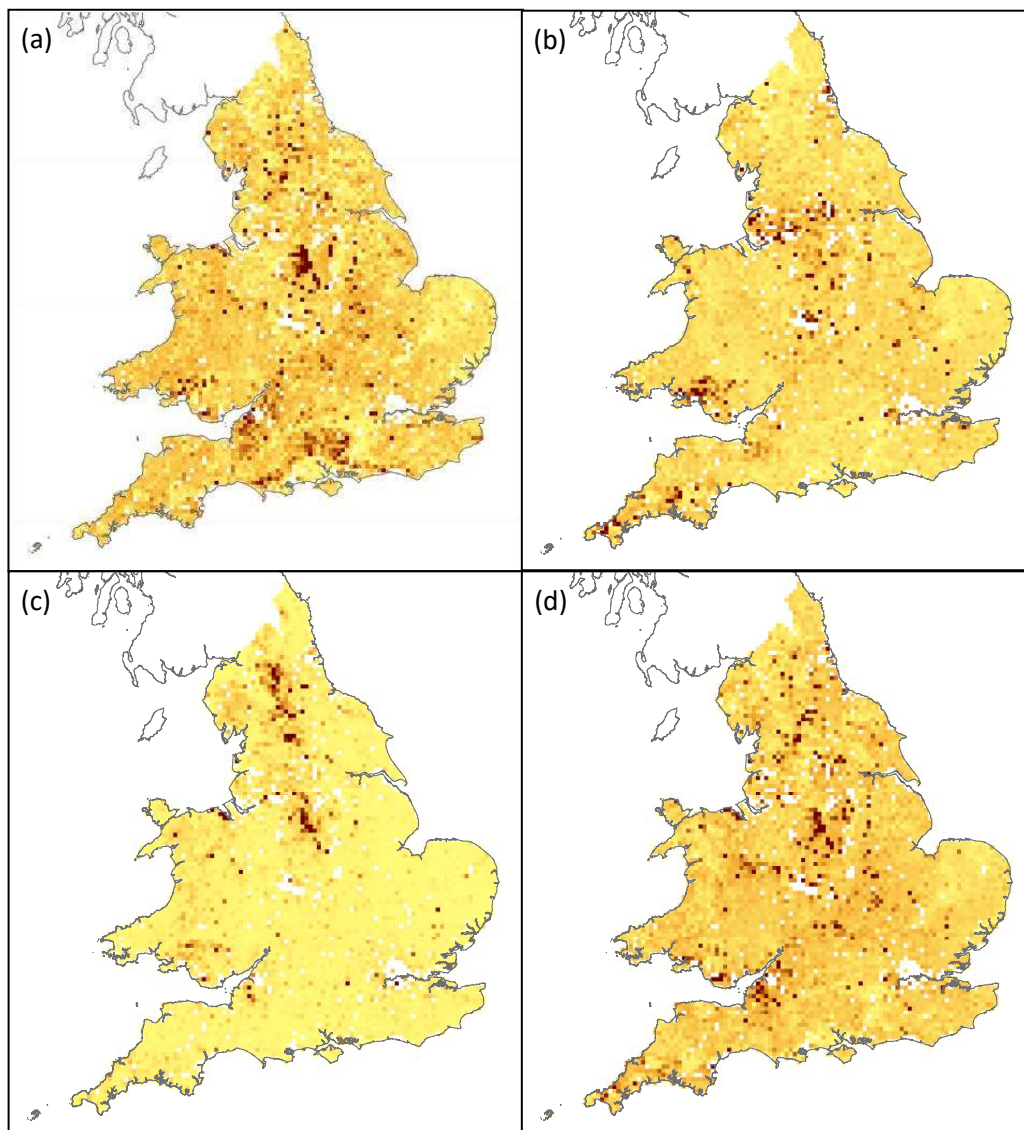


Fig. S1. The relative concentration of four heavy metals in the soil. Higher concentrations are seen as darker shading. The metals shown in each distribution map are a) Cd; b) Cu; c) Pb and d) Zn.

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