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1 **Variation in personality traits across a metal pollution gradient in a free-living songbird**

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3 **Metal pollution and animal personality traits**

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27 **Abstract**

28 Anthropogenic contaminants could alter traits central to animal behavioral types, or personalities,  
29 including aggressiveness, boldness and activity level. Lead and other toxic metals are persistent inorganic  
30 pollutants that affect organisms worldwide. Metal exposure can alter behavior by affecting neurology,  
31 endocrinology, and health. However, the direction and magnitude of the behavioral effects of metal  
32 exposure remain equivocal. Moreover, the degree to which metal exposure simultaneously affects suites  
33 of correlated behavioral traits (behavioral syndromes) that are controlled by common mechanisms  
34 remains unclear, with most studies focusing on single behaviors. Using a model species for personality  
35 variation, the great tit (*Parus major*), we explored differences in multiple behavioral traits across a  
36 pollution gradient where levels of metals, especially lead and cadmium, are elevated close to a smelter.  
37 We employed the novel environment exploration test, a proxy for variation in personality type, and also  
38 measured territorial aggressiveness and nest defense behavior. At polluted sites birds of both sexes  
39 displayed slower exploration behavior, which could reflect impaired neurological or physiological  
40 function. Territorial aggression and nest defense behavior were individually consistent, but did not vary  
41 with proximity to the smelter, suggesting that metal exposure does not concurrently affect exploration and  
42 aggression. Rather, exploration behavior appears more sensitive to metal pollution. Effects of metal  
43 pollution on exploration behavior, a key animal personality trait, could have critical effects on fitness.

44

45 **Keywords:** metal pollution; anthropogenic disturbance; animal personality traits; exploration behavior;  
46 aggressiveness; hissing behavior

47

48 **1. Introduction**

49 Organisms are exposed to an array of anthropogenic contaminants, which can have cascading effects on  
50 physiological and neurological systems, individual fitness and population viability (Burger and Gochfeld,  
51 2000; Carere et al., 2010a; Peterson et al., 2017). Metal pollution is a worldwide biological problem,  
52 which continues to have lethal and sublethal effects on organisms, despite increasing government

53 regulations. Lead and other toxic metals are persistent inorganic pollutants, which can remain in the  
54 environment and affect organisms long after remedial policies have been adopted to reduce emissions  
55 (Ercal et al., 2001; Tchounwou et al., 2012; Vermeulen et al., 2015).

56 A shortage of studies exists regarding the sublethal behavioral ramifications of exposure to pollutants,  
57 including toxic metals, despite growing evidence that the neurotoxic and health effects of contaminants  
58 can have behavioral implications (Peakall, 1996; Burger and Gochfeld, 2000; Dell'Omo, 2002; Brodin et  
59 al., 2013; Kobiela et al., 2015; Ecke et al., 2017; Peterson et al., 2017). Studies predominantly focused on  
60 humans and laboratory animals indicate that exposure to metals, particularly lead, can impair learning,  
61 memory and performance in cognitive tasks (Koller et al., 2004; Liu et al., 2014; Engstrom et al., 2017).  
62 In addition, there is some evidence that exposure to contaminants (Brodin et al., 2013), including metals  
63 (Janssens et al., 2003a; White and Briffa, 2017), can affect behavioral traits central to animal behavioral  
64 types, or personalities. Animal personalities are defined by consistent inter-individual variation in  
65 behavioral traits such as aggressiveness, risk-taking and activity levels, and can have important effects on  
66 fitness (Dall et al., 2004; Dingemanse et al., 2004; Smith and Blumstein, 2008). Rather than being one-  
67 dimensional, personalities can consist of suites of correlated traits, referred to as behavioral syndromes  
68 (Sih et al., 2004). For instance, consistently more aggressive individuals are often also less risk-adverse  
69 and have higher activity levels. This co-occurring suite of traits is characterized by bold-shy and  
70 aggressive-passive behavioral continuums, with the bold-aggressive personality type potentially  
71 associated with a life-history strategy that favors early reproduction over survivorship (Wolf et al., 2007;  
72 Dingemanse and Wolf, 2010). Behavioral traits associated with personalities often have a heritable,  
73 genetic component (Drent et al., 2003; van Oers et al., 2004), but can also be affected by permanent  
74 environment effects (Quinn et al., 2009; Nicolaus et al., 2012) and state-dependent behavioral dynamics  
75 (Dall et al., 2004; Sih et al., 2015), which may be altered by exposure to contaminants (Montiglio and  
76 Royauté, 2014).

77 Past studies have yielded conflicting results regarding how metal exposure affects behavioral traits  
78 along the continuums described above. For instance, humans (Nevin, 2000) and golden hamsters

79 (*Mesocricetus auratus*) (Deville, 1999) exposed to lead during development show increases in  
80 aggressiveness, and some studies in humans also report that metal (cadmium, lead) exposure increases  
81 testosterone, which could elevate aggressiveness and boldness (Zeng et al., 2002; Meeker et al., 2010). In  
82 contrast, aggressive behavior is reduced in two species of crabs exposed to copper (Dissanayake et al.,  
83 2009; White et al., 2013), and zebra finches (*Taeniopygia guttata*) exposed to mercury become more risk-  
84 adverse (Kobiela et al., 2015), perhaps reflecting neurotoxic effects on fearfulness. In a variety of  
85 species, activity level also declines with metal exposure, perhaps reflecting poor health (Cheung et al.,  
86 2002; Ecke et al., 2017). Toxic metal exposure can reduce survivorship (Alissa and Ferns, 2011;  
87 Hallinger et al., 2011), and increases in aggressiveness and boldness with metal exposure could thus  
88 represent a shift in life-history strategy to promote early reproduction (Bélanger-Deschênes et al., 2013;  
89 Sih et al., 2015). However, either increases or decreases in the expression of behavioral traits could also  
90 reflect neurological and physiological changes that impair normal behavioral regulation.

91 If suites of behaviors are controlled by common mechanisms, these traits could be simultaneously  
92 affected by metal exposure, potentially magnifying behavioral syndromes in exposed populations (Brodin  
93 et al., 2013). On the other hand, if certain behavioral traits are more sensitive to metal exposure than  
94 others, exposure could disrupt behavioral syndromes relative to non-exposed populations (Swaddle et al.,  
95 2017). However, most studies focus on single behavioral traits, often within a single sex (e.g. aggression  
96 in males, Janssens et al., 2003a; White et al., 2013), leaving these hypotheses largely unexplored.

97 We used a model species for the study of personality types, the great tit (*Parus major*), to investigate  
98 behavioral ramifications of metal exposure. Great tits exhibit distinct personality types, with birds that  
99 rapidly explore a novel environment (fast explorers) also displaying boldness in the presence of novel  
100 objects (van Oers et al., 2004), high levels of aggressiveness (Verbeek et al., 1996; Carere et al., 2005;  
101 Thys et al., 2017), and low glucocorticoid stress responses (Carere et al., 2003; Baugh et al., 2017). Thus,  
102 fast explorers generally display a bold-aggressive personality type, whereas slow explorers are shy and  
103 non-aggressive. A few recent studies in great tits have linked urbanization to personality type, and  
104 specifically suggest that urbanization is associated with increased boldness and fast exploration behavior

105 (Charmantier et al., 2017; Riyahi et al., 2017; Sprau and Dingemanse, 2017). However, despite growing  
106 interest in the effects of urbanization on personality type, very few studies have examined how  
107 personality traits vary along known pollution gradients. Those studies that have pursued such  
108 examinations suffer from methodological limitations, including small sample sizes and a focus on single  
109 behavioral traits (e.g. Janssens et al., 2003a), whereas animal personalities are multidimensional and  
110 potentially characterized by a diverse array of interrelated traits (Koski et al. 2014).

111 We explored whether metal exposure concurrently affects multiple animal personality traits and the  
112 correlations between these traits in great tits of both sexes, using five great tit populations located across a  
113 well-characterized metal pollution gradient, which emanates from the Umicore smelter and refinery  
114 facility, located south of Antwerp, Belgium (Fig. 1). Past research has established that pollution from the  
115 Umicore facility causes metal exposure in great tits (Janssens et al., 2001; Van Parys et al., 2008;  
116 Vermeulen et al., 2015), with lead and cadmium levels in kidneys being respectively >150 and 5 times  
117 higher close to the smelter than farther away (Van Parys et al., 2008). Exposure in liver tissue is similarly  
118 highly elevated close to the smelter (Van Parys et al., 2008). A previous study comparing aggressive  
119 behavior between the most and least polluted study sites tentatively suggested higher aggressiveness in  
120 birds breeding close to the Umicore smelter (Janssens et al., 2003a), but relied on much smaller sample  
121 sizes than the current study. To characterize personality traits across the pollution gradient, we measured  
122 novel environment exploration and territorial aggression. Moreover, we also measured female  
123 aggressiveness during nest defense as an important aspect of parental investment (Barash, 1975;  
124 Montgomerie and Weatherhead, 1988). To establish individual consistency in behavior and the existence  
125 of personality types, we tested the repeatability of territorial aggression and nest defense behaviors. We  
126 did not measure the repeatability of exploration behavior at sites across the pollution gradient because of  
127 logistical constraints, but exploration behavior is repeatable at our intensively monitored and centrally  
128 located study population (Thys et al., 2017), and in other European study populations of great tits  
129 (Dingemanse et al., 2012; Stuber et al., 2013). Based on past work along the pollution gradient (Janssens  
130 et al., 2003a), we predicted that aggression might increase with proximity to the pollution source and that

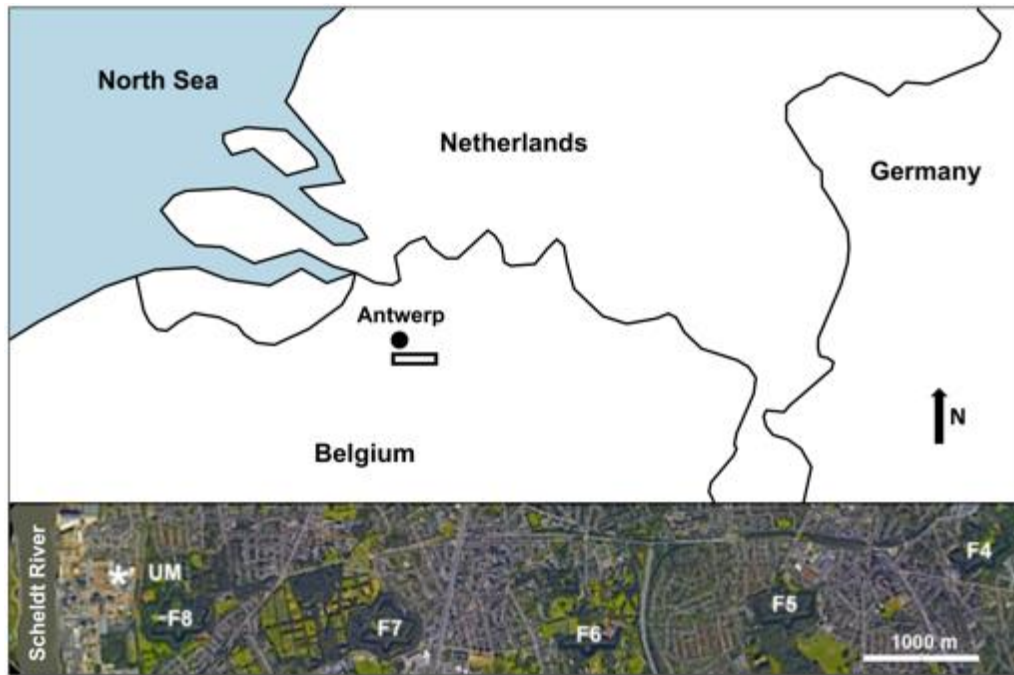
131 exploration behavior might increase in parallel. Our study informs how behavioral changes could affect  
132 population integrity in metal polluted areas, while also providing insights to behavioral researchers  
133 seeking to understand the mechanisms underlying individual variation in behavior.

134

## 135 **2. Methods**

136 **2.1. Study system:** We conducted our study in 2017, using five populations of great tits located along the  
137 metal pollution gradient produced by the Umicore smelter and metal refinery facility. The Umicore  
138 facility is a major point source for metal pollution, especially cadmium and lead. Near the smelter,  
139 emission limits (or target values) set by Flemish and European authorities are sometimes exceeded  
140 (VMM, 2016). The Behavioral Ecology and Ecophysiology Group at the University of Antwerp has  
141 intensively studied great tit populations across this pollution gradient since 1999 (Van Duyse et al., 2000;  
142 Rivera-Gutierrez et al., 2010; Vermeulen et al., 2015; Casasole et al., 2017; Raap et al., 2017; Thys et al.,  
143 2017). The study populations are located 0–600 m (site UM), 2500 m (F7), 4000 m (F6), 5000 m (F5)  
144 and 8500 m (F4) from Umicore (Fig. 1). For this study, we grouped two previously separated study  
145 populations (UM and F8; Eens et al., 1999; Janssen et al., 2001) into one site (referred to hereafter as  
146 UM), because these two sites are immediately adjacent, with some nest boxes at F8 being as close to  
147 sources of metal pollution (waste piles) as some nest boxes at UM (Fig. 1). Metal deposition levels  
148 exponentially decrease with distance. Thus, differences in metal exposure levels between adjacent study  
149 sites are highest between UM, F7 and F6, and lower (sometimes non-detectable) between F6, F5 and F4  
150 (see Janssens et al., 2001; Van Parys et al., 2008; Vermeulen et al., 2015; for specific metal exposure  
151 levels across the different study sites). All study sites contain ~30-60 nest boxes, except for the F6 study  
152 site (~140 nest boxes). However, nest box density is similar across all sites, and study sites are similar in  
153 habitat type and urbanization levels, minimizing the potential for these variables to confound the effects  
154 of metal pollution (Eens et al., 1999; Janssens et al., 2001; Geens et al., 2009). The location of nest boxes  
155 has been mapped using GPS. Thus, in addition to analyzing how behavioral traits varied between study  
156 sites, we also measured the distance of each nest box to the Umicore facility using Google Earth (version

157 7.1.8.3036) measurement tools. Measurements were made to the closest edge of the Umicore complex,  
158 where the smelter and waste piles are located.



159  
160 **Figure 1.** Location of great tit study sites in relationship to (top) Antwerp, Belgium, and (bottom) the  
161 Umicore smelter facility, which is a major source of lead and cadmium emission. The location of  
162 Umicore is indicated by the white asterisk. UM = Umicore study site, F8 = Fort 8, F7 = Fort 7, F6 = Fort  
163 6, F5 = Fort 5, and F4 = Fort 4. For this 2017 study, UM and F8 were grouped into one study area,  
164 referred to as UM. The rectangle on the upper panel shows the location of the pollution gradient.

165  
166 **2.2. Behavioral assays**

167 **2.2.1. Novel environment exploration test**

168 To perform the novel environment exploration test, we captured great tits sleeping in nest boxes over a  
169 relatively short timeframe, between January 25 and March 2, 2017. We transported birds to the  
170 University of Antwerp, where they were housed overnight in individual cages (0.83 × 0.4 × 0.5 m) with  
171 access to food and water. The following morning, between 8:15 and 10:00, we used standardized  
172 protocol to perform the novel environment exploration test (Dingemanse et al., 2002; van Overveld and



173 Matthysen, 2010; Thys et al., 2017). Sliding doors directly connected individual cages to a novel  
174 environment exploration room ( $4.0 \times 2.4 \times 2.3$  m) containing 5 artificial trees. Thus, we were able to  
175 release birds into the novel environment exploration room without handling them. We observed the  
176 movement of birds around the novel environment for a period of 2 minutes, and noted the number of hops  
177 and flights (cf. Dingemanse et al., 2002). Each individual's exploration score was calculated as the total  
178 number of hops and flights within 2 minutes.

179 After all birds were tested, we sexed and aged birds based on plumage characteristics (Svensson,  
180 1984), and took standard body measurements (tarsus length and mass). We used residuals of a regression  
181 of body mass on tarsus length as a metric of body condition (Schulte-Hostedde et al., 2005). Birds were  
182 aged as yearlings (hatched in 2016) or older. We gave all birds a metal band and a unique color band  
183 combination, and additionally fitted females with passive integrated transponder (PIT) tags (IB  
184 Technology, Aylesbury, UK), to allow identification. We then released birds near the nest boxes where  
185 they had been captured.

186 In total, we performed the novel environment exploration test on 249 birds, consisting of 32 birds from  
187 F4, 26 from F5, 81 from F6, 48 from F7, and 62 from UM. The larger sample size from F6, which is  
188 located at the University of Antwerp's Campus Drie Eiken, reflects the larger size of this intensively  
189 monitored population. Most of the birds that we tested from the F6 population had been previously tested  
190 for exploration behavior, either earlier (November to December) in the winter or during a different year  
191 (Thys et al., 2017). To ensure that all birds were tested during a relatively short timeframe and thus under  
192 similar environmental and physiological conditions, we used exploration scores derived during the period  
193 of this study (see above), rather than using scores from the first exploration test on each bird, as done by  
194 some past studies (e.g. Stuber et al., 2013).

195

### 196 2.2.2. *Simulated territory intrusions (STIs; territorial aggression)*

197 We performed STIs at nest boxes between March 19 and April 18, 2017, during the egg laying stage  
198 (between 1 and 9 eggs present; mean  $\pm$  SE =  $3.142 \pm 0.145$ ) of first clutch nests. All tests were performed

199 between 8:00 and 12:00 by one of three observers. We performed tests by placing a taxidermic mount  
200 (decoy) and song recording of an adult male great tit on top of the nest box. Decoys were enclosed in  
201 metal cages to prevent extensive damage. To avoid pseudoreplication and ensure generalizable results  
202 (McGregor et al., 1992), we used three different decoys and 6 different song recordings. Song recordings  
203 all consisted of a single, species typical song type, which derived from males recorded at F6, where we  
204 did not perform aggression tests. We manually compiled recordings in Raven Pro (Cornell Laboratory of  
205 Ornithology, New York, USA) so that 10 strophes (song bouts) containing 10 songs each were played  
206 across a period of 1 minute, followed by 10 seconds of silence. We used an Olympus VN-  
207 8100PC digital voice recorder placed on the nest box adjacent to the decoy to project song at natural  
208 amplitude across the entire trial period.

209 After placing the decoy and recording, we retreated to a distance of ~10-15 meters from the box and  
210 waited for the focal male to come within 15 meters. We then recorded the behavioral response of males  
211 for 3 minutes. Specifically, we recorded the male's minimum distance from the decoy (approach  
212 distance), time spent on the decoy, and the number of alarm calls, songs, dives at the decoy, and attacks  
213 on the decoy. An attack on the decoy was defined as a discrete episode of pecking at the decoy. Similar  
214 protocols and behavioral metrics have been utilized by past studies of territorial aggressiveness in the  
215 great tit (Araya-Ajoy and Dingemanse, 2014; Sprau and Dingemanse, 2017).

216 Our territorial aggression trials focused firstly on males, which are easily distinguished from females  
217 based on the larger size of the black, melanin-based breast stripe (Svensson, 1984; Nicolaus et al., 2016).  
218 Past studies indicate that female (and male) great tits respond most strongly to same-sex territorial  
219 intruders (Slagsvold, 1993), and we used a male decoy and recording during STIs. Thus, we expected a  
220 weaker territorial response from the female than if we had used a female decoy. However, when the  
221 female was present during the STI and came within 15 meters of the decoy, we also quantified her  
222 response to the male territorial intruder. Due to logistical constraints, we could not simultaneously  
223 tabulate all behaviors for both males and females. We thus used a simplified procedure to quantify the  
224 female's territorial response. We recorded the female's approach distance, and scored her aggressive

225 response on a scale of 0-3, with 0 corresponding to sitting near the box, 1 to alarm calling, 2 to sitting on  
226 the decoy, and 3 to attacking the decoy. The female's aggression score corresponded to the most  
227 aggressive behavior observed, regardless of time spent performing different behaviors.

228 We performed 223 STIs on 128 pairs. We tested a subset (63) of pairs multiple (2 to 3) times for  
229 territorial aggressiveness, to allow calculation of repeatability. A summary of sample sizes across study  
230 sites is given in Table 1a. Because of time and logistical constraints, we did not perform STIs at F6. Not  
231 all birds included in STIs were banded, as also summarized in this table. Great tits in our study  
232 populations are highly resident and tightly associated with a single nest box during each nesting attempt,  
233 and we only observed a single pair responding to STIs. Thus, the presence of unbanded birds did not lead  
234 to double counting of individuals. However, data on age, which can affect aggressiveness, was available  
235 only for banded birds. A subset of birds was captured during the nestling period, after the performance of  
236 behavioral tests, so that age could be determined (yearling/older).

237

### 238 *2.2.3. Female aggression during nest defense (hissing behavior)*

239 When confronted by a predator entering the nest cavity, female great tits perform a dramatic hissing  
240 display, which consists of producing loud vocalizations, shaking the wings, and sometimes lunging at the  
241 predator (Sibley, 1955; Krams et al., 2013; Koosa and Tilgar, 2016; Zub et al., 2017). Hissing behavior  
242 varies greatly between individual females, with some females producing no hisses, and others producing  
243 between one and >40 hisses per minute. To quantify this behavior, we introduced the head of the  
244 taxidermic mount (decoy) of a great spotted woodpecker (*Dendrocopos major*) into the hole of the nest  
245 box and counted the number of hissing vocalizations produced within 1 minute. To avoid  
246 pseudoreplication and ensure generalizable results, 3 different decoys were used. Following the 1 minute  
247 trial, we opened the box to confirm the presence of the female. The identity of PIT tagged females was  
248 verified by scanning the box with a handheld transponder reader (GR-250 RFID Reader, Trovan, Aalten,  
249 Netherlands), which allowed us to confirm individual identity without further disrupting the female.

250 Hissing tests were performed by one of seven observers. We performed tests between March 29 and

251 April 26, between 8:00 and 17:00. Tests were performed during the incubation stage (day 1 to 12; mean  
 252  $\pm$  SE = 5.524  $\pm$  0.177) of first clutches, with 123 females tested multiple (2 to 3) times to calculate  
 253 repeatability. We performed a total of 273 hissing tests on 152 females. The distribution of hissing tests  
 254 across sites is summarized in Table 1b. Information on age was available only for banded females.  
 255

256 **Table 1.** Summary of sample sizes for simulated territory intrusions (STIs; a) and hissing tests (b)  
 257 performed on great tits at study sites across the metal pollution gradient in Antwerp, Belgium, 2017. The  
 258 number of STIs for males and females differed because in some cases the female was not seen, in which  
 259 case the trial was not used for the female.

---

**a) STIs (male and female territorial aggressiveness)**

Study site	Tests (Males/Females)	Males/Females	Banded males	Banded females
F4	39/38	24/23	14	14
F5	39/36	22/21	17	10
F7	66/65	44/41	17	19
UM	42/38	32/27	22	19
Total	186/177	122/112	70	62

---

**b) Hissing tests (female aggressiveness towards a nest predator)**

Study site	Tests	Females	Banded females
F4	34	20	10
F5	37	22	11
F6	70	35	35
F7	72	41	13
UM	60	34	19
Total	273	152	88

---

260

261 **2.3. Statistics**

262 We performed statistical analyses using R 3.4.0 (R Core Team, 2017). In the results section, we report  
263 mean  $\pm$  SE unless indicated otherwise. To test model assumptions, we used residual plots and Shapiro-  
264 Wilk tests for linear models, and also tested for overdispersion of Poisson mixed effect models using R  
265 function `dispersion_glmer` in the `blmeco` package (Korner-Nievergelt et al., 2015).

266

267 *2.3.1. Principal component analysis (PCA) on territorial aggression data*

268 We first used PCA (R function `prcomp`; package `stats`) to compress data from STIs into a few orthogonal  
269 variables. For males, we performed PCA on all of the behavioral variables measured (approach distance,  
270 calls, songs, dives, attacks, and time on decoy). For females, we performed PCA on approach distance  
271 and aggression score. We retained principal components with eigenvalues  $>1$ , and interpreted variable  
272 loadings  $>0.30$  as contributing substantially to the principal components (Tabachnick and Fidell, 1996).

273

274 *2.3.2. Repeatability of behaviors*

275 To examine the repeatability of territorial aggression and hissing behavior, we used R package `rptR`  
276 (Stoffel et al., 2017). Package `rptR` estimates repeatability based on variance components extracted from  
277 mixed effects models. Bootstrapping is used to provide 95% confidence intervals, and  $P$ -values are  
278 calculated based on log-likelihood ratio tests. We calculated conditional (adjusted) repeatability values,  
279 meaning that repeatability estimates were adjusted for predictor variables that were statistically significant  
280 in mixed effects models predicting behavioral traits (see below). Thus, for male territorial aggression, we  
281 calculated repeatability adjusted for the effect of male age (yearling versus older). However, since using  
282 only aged (banded) males restrictively reduced our sample size of repeated measures, we included males  
283 of unknown age in the analysis, with age coded as unknown. The repeatability estimate for female  
284 territorial aggression was adjusted for an effect of song recording number (random effect), and the  
285 repeatability estimate for hissing behavior was adjusted for effects of study site, trial number, and time of  
286 day. We report all repeatability estimates in the format:  $r \pm$  SE [95% CIs].

287

288 *2.3.3. Behavioral variation across the pollution gradient*

289 We next analyzed whether behavioral traits varied among study sites. To analyze exploration score, we  
290 used a generalized linear model (GLM) with a quasiPoisson distribution. We entered study site, date, age  
291 (yearling versus older) and sex as predictor variables. We used an F-test comparing nested quasiPoisson  
292 GLMs with and without study site included to test for an overall significant effect of study site. We used  
293 R package lsmeans (Lenth, 2016) to calculate least square means with 95% confidence intervals for each  
294 study site, and to perform post-hoc comparisons (Tukey method) between sites. We also applied this  
295 approach to perform post-hoc comparisons for territorial aggressiveness and hissing behavior. Repeated  
296 testing can influence exploration behavior (Dingemanse et al., 2002; Thys et al., 2017), and repeated  
297 testing of exploration behavior was performed only at F6. Thus, we conducted analyses of exploration  
298 behavior with and without birds from F6 included and compared results. Results were similar in both  
299 cases, so we only present the analysis that includes birds from all populations.

300 To analyze territorial aggressiveness, we used linear mixed effects models (LMM; R packages lme4  
301 and lmerTest; Bates et al., 2015; Kuznetsova et al., 2016) with Satterthwaite approximations for degrees  
302 of freedom. We entered study site, age, date, time and trial number (first to third observation on a given  
303 individual) as fixed effect predictors, and individual (some birds were tested multiple times), decoy, song  
304 recording, and observer as random effects.

305 Lastly, to analyze hissing behavior we used a generalized linear mixed effect model (GLMM; R  
306 package lme4) with a Poisson distribution. Study site, age, date, time and trial number were entered as  
307 fixed effects, and individual, decoy, and observer as random effects. We additionally added an  
308 observation level random effect to control for overdispersion (Harrison, 2014).

309 Following the comparisons of study sites, we assessed whether behaviors changed directionally across  
310 the pollution gradient by constructing models that used the distance of each individual's nest box from the  
311 pollution source, instead of study site, as the primary explanatory variable. For all behaviors (exploration,  
312 territorial aggression, hissing), we used mixed effects models with the distance of each nest box from the

313 pollution source as the primary fixed effect predictor and study site as a random effect. We modeled  
314 territorial aggressiveness using LMMs, and exploration and hissing behavior using Poisson GLMMs, with  
315 an observation level random effect to control for overdispersion. Other fixed and random effect predictor  
316 variables were as described above.

317 In addition, to assess whether associations between behaviors might shift across the pollution gradient,  
318 we tested whether territorial aggressiveness and hissing behavior (for females) could be predicted by two-  
319 way interactions between study site (or distance of each box from the smelter) and exploration score.  
320 These analyses used a smaller sample size than those described above, because not all individuals tested  
321 for aggressive behaviors were also tested for exploration behavior. When significant interactions were  
322 detected, we used the function `testInteraction` (R package `phia`; post-hoc interaction analysis; De Rosario-  
323 Martinez, 2015) to test whether the relationship between behaviors significantly differed between pairs of  
324 sites. These post-hoc tests employed a Holm correction for multiple comparisons.

325

#### 326 *2.3.4. Differences in body condition*

327 We investigated differences in body condition among study sites using linear models (LMs). We entered  
328 sex, age, date, and study site (or distance from Umicore) as predictor variables. We utilized data only  
329 from birds captured in the winter, because the majority of birds were captured and banded in the winter,  
330 in coordination with exploration testing. We also used a quasiPoisson GLM to test whether body  
331 condition was associated with exploration behavior, with date included in this model as a covariate.

332 All of the models described above were reduced using a stepwise procedure. We first removed  
333 random effects that explained none of the variance in the dependent variable, next removed non-  
334 significant interactions between fixed effect predictors, and then sequentially removed fixed effect  
335 predictor variables with the largest *P*-values. We retained fixed effect predictor variables at the  $\alpha = 0.05$   
336 threshold (Zuur et al., 2009). We report reduced models in the text, but full models can be found in the  
337 supplementary material (Appendix A).

338

339 **2.4. Ethical statement**

340 This study was approved by the University of Antwerp ethical committee (ID 33480) and performed in  
341 accordance with Belgium and Flemish laws. During handling and behavioral testing, we made all  
342 possible efforts to minimize the stress experienced by birds. The Belgian Royal Institute for Natural  
343 Sciences (Koninklijk Belgisch Instituut voor Natuurwetenschappen) provided banding licenses for  
344 authors and technical personnel.

345

346 **3. Results**

347 **3.1. PCA on territorial aggression data**

348 The PCA on male aggressive behaviors yielded 2 principal components with eigenvalues  $>1$ , which  
349 together explained 60.2% of the total variance in behavior. PC1 explained 32.8% of the variance and PC2  
350 an additional 27.4% (Table 2a). Loadings for time on the decoy and attacks were large and positive for  
351 PC1, whereas loadings for approach distance and songs were large and negative (Table 2b). Thus, birds  
352 with large scores on PC1 were more aggressive, with PC1 particularly separating non-aggressive males  
353 that sang at a distance from the decoy from aggressive males that approached and attacked the decoy.  
354 PC2 also exhibited large positive loadings for time on the decoy and attacks, but in contrast to PC1  
355 exhibited large negative loadings for calls and dives (Table 2b). Thus, birds with high scores on PC2  
356 were also characterized by more aggressive behavior, with PC2 particularly separating non-aggressive  
357 individuals that called and dove at the decoy from aggressive individuals that directly attacked the decoy.

358 The PCA on female territorial behaviors yielded one principal component with an eigenvalue  $>1$ . PC1  
359 explained 79.7% of the total variation in aggressive behavior and had an eigenvalue of 1.595. Loadings  
360 on PC1 were -0.707 and 0.707, for approach distance and aggression score, respectively. Thus, females  
361 with higher PC1 scores were more aggressive.

362

363 **3.2. Repeatability of behaviors**



364 In our whole dataset, male territorial aggression, as quantified by PC1 was significantly repeatable after  
 365 correcting for the effect of male age ( $r = 0.321 \pm 0.110$  [0.114, 0.538],  $P = 0.003$ ,  $N = 186$  observations,  
 366 122 males), indicating individual consistency in behavior. When reducing the dataset to only known-aged  
 367 males, repeatability fell short of significance ( $r = 0.113 \pm 0.118$  [0, 0.405],  $P = 0.295$ ,  $N = 110$   
 368 observations, 70 males). This was due to a lack of statistical power, because of a reduced sample size.  
 369

370 **Table 2.** PCA characterizing territorial aggressiveness in male great tits in Antwerp, Belgium, 2017. (a)  
 371 Proportion of variance explained by PC1 and PC2, and (b) component loadings. Loadings on PC1 and  
 372 PC2 >0.30 are indicated by bold text. PC1 and PC2 had eigenvalues >1, and were thus retained for  
 373 statistical testing.

	<b>PC1</b>	<b>PC2</b>
<b>a) Variance explained</b>		
Prop. Variance	0.328	0.274
Cum. Variance	0.328	0.602
Eigenvalue	1.968	1.644
<b>b) Loadings</b>		
Approach distance	<b>-0.553</b>	0.198
Calls	0.143	<b>-0.443</b>
Songs	<b>-0.487</b>	0.269
Dives	0.272	<b>-0.428</b>
Attacks	<b>0.376</b>	<b>0.534</b>
Time on	<b>0.467</b>	<b>0.471</b>

374  
 375 Female territorial aggression (PC1) ( $r = 0.555 \pm 0.083$  [0.384, 0.695],  $P < 0.001$ ,  $N = 177$  observations  
 376 on 112 females) and hissing behavior ( $r = 0.638 \pm 0.059$  [0.518, 0.758],  $P < 0.001$ ,  $N = 273$  observations

377 on 152 females) were also repeatable. However, the dimension of male aggressive behavior captured by  
378 PC2 was not significantly repeatable ( $r = 0.078 \pm 0.096$  [0, 0.313],  $P = 0.262$ ).

379

### 380 **3.3. Behavioral variation across the pollution gradient**

#### 381 *3.3.1. Exploration behavior*

382 Exploration score varied from 0 to 61 movements during 2 minutes (mean =  $11.671 \pm 0.668$ ). Great tits  
383 inhabiting study sites farther from the pollution source (especially F4 and F6 compared to UM) displayed  
384 faster exploration behavior ( $F_{4,242} = 3.004$ ,  $P = 0.019$ ; Table 3a; Fig. 2a), as reflected by more movements  
385 produced within the 2-minute exploration test. Post-hoc comparisons revealed that scores were  
386 significantly lower for birds from UM than for birds from F4 ( $\beta = -0.533 \pm 0.191$ ,  $z = 2.786$ ,  $P = 0.042$ )  
387 and F6 ( $\beta = -0.578 \pm 0.198$ ,  $z = 2.906$ ,  $P = 0.030$ ), and UM scores tended to be lower than F5 scores, but  
388 were not significantly so ( $\beta = -0.520 \pm 0.210$ ,  $z = 2.468$ ,  $P = 0.098$ ; Fig. 2a). In addition, when distance  
389 between the metal pollution source and the nest box was used as the primary predictor variable, there was  
390 a significant relationship between distance to the pollution source and exploration score (Table 3b). This  
391 result indicates a directional pattern across the pollution gradient, with birds residing closer to the  
392 pollution source displaying slower exploration behavior. There was no evidence for a relationship  
393 between sex or age and exploration score ( $P > 0.90$ ), but some evidence for a relationship between test  
394 date and exploration score, with birds tested later in the year displaying lower scores (Table 3a). See  
395 Table A.1 for full models.

396

#### 397 *3.3.2. Male territorial aggression*

398 Male territorial aggression, as reflected by PC1, did not differ among study sites ( $F_{3,49} = 2.311$ ,  $P =$   
399  $0.088$ ; Fig. 2b). When using the distance from Umicore in the model rather than study site, there was also  
400 no effect of proximity to the pollution source on male aggression PC1 ( $\beta = -0.049 \pm 0.067$ ,  $t_{2,95} = -0.731$ ,  
401  $P = 0.519$ ). The component of aggressive behavior captured by PC2 was not related to study site ( $F_{3,105} =$

402 0.333,  $P = 0.801$ ) or distance from Umicore ( $\beta = -0.027 \pm 0.151$ ,  $t_{4,29} = -0.179$ ,  $P = 0.866$ ). Male  
 403 exploration behavior was not significantly related to aggression PC1 ( $\beta = -0.186 \pm 0.176$ ,  $t_{67} = -1.054$ ,  $P =$   
 404

405 **Table 3.** Results from (a) a quasiPoisson GLM assessing the relationship between exploration score and  
 406 study site, and (b) a Poisson GLMM assessing the relationship between exploration score and distance to  
 407 the metal pollution source for great tits in Antwerp, Belgium, 2017.

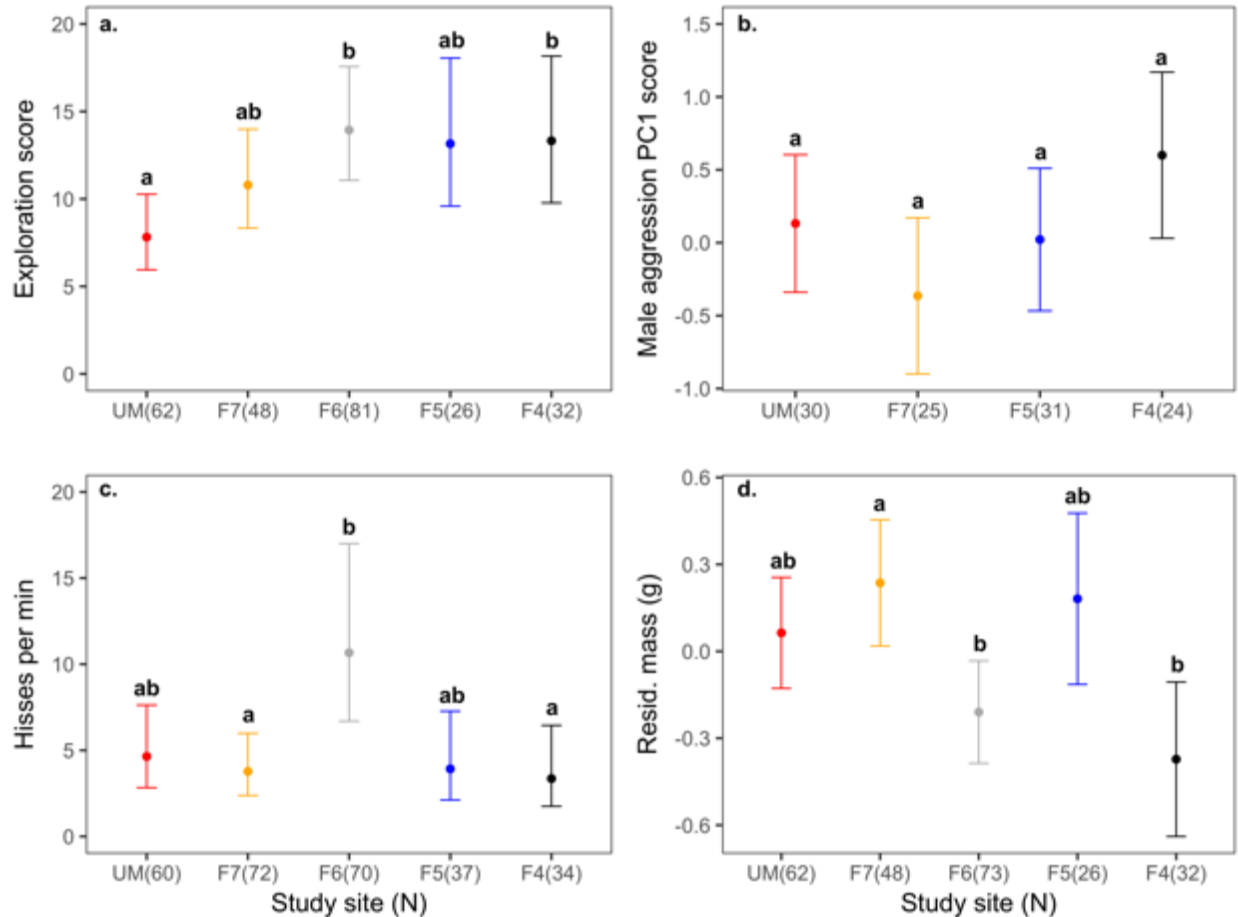
<b>a)</b>			
	$\beta \pm SE$	$t_{243}$	$P(> t )$
Intercept	$2.761 \pm 0.310$	8.899	<0.001
Site F4 <sup>a</sup>	$0.533 \pm 0.191$	2.786	0.006
Site F5	$0.520 \pm 0.210$	2.468	0.014
Site F6	$0.578 \pm 0.199$	2.907	0.004
Site F7	$0.323 \pm 0.184$	1.759	0.079
Date	$-0.016 \pm 0.007$	-2.152	0.032
<b>b)</b>			
<b>Fixed effects</b>	$\beta \pm SE$	<b>Z</b>	$P(> Z )$
Intercept	$1.537 \pm 0.162$	9.466	<0.001
Distance (km)	$0.071 \pm 0.034$	2.071	0.038
<b>Random effects</b>	<b>Variance</b>	<b>SD</b>	<b>N</b>
Study site	<0.001	<0.001	5
Observation <sup>b</sup>	1.874	1.369	249

408 <sup>a</sup>Contrasts between sites are to UM, the most polluted site.

409 <sup>b</sup>Adding the observation level random effect reduced the dispersion parameter from 3.215 to 1.100.

410  $N = 249$  birds, F4: 32, F5: 26, F6: 81, F7: 48, and UM: 62.

411 See Table A.1 for the full model.



412

413 **Figure 2.** Variation in (a) exploration behavior, (b) male aggression, (c) hissing behavior and (d) residual  
 414 body mass for great tits at study sites across the metal pollution gradient in Antwerp, Belgium, 2017.

415 Distance from the metal pollution source increases along the x-axis. Plotted values are least square means  
 416 with 95% confidence intervals. Different letters over error bars indicate statistically significant

417 differences between sites. Sample sizes (number of tests) are given in parentheses following site names.

418 For male aggression (b), sample sizes are the number of tests on aged (banded) males.

419

420 0.296) or aggression PC2 ( $\beta = 0.200 \pm 0.137$ ,  $t_{67} = 1.467$ ,  $P = 0.147$ ), and did not interact with study site  
 421 or distance from Umicore to predict either metric of aggressiveness ( $P > 0.80$ ). Time, date and trial

422 number had no effect on the aggressive behavior of males (either PC1 or PC2;  $P > 0.15$  in all cases).

423 Yearling males were more aggressive than older males as reflected by higher PC1 scores ( $\beta = -0.651 \pm$

424 0.276,  $t_{45} = -2.619$ ,  $P = 0.012$ ), but male age was not related to PC2 scores ( $\beta = -0.264 \pm 0.226$ ,  $t_{108} = -$   
425 1.171,  $P = 0.244$ ). See Table A.2 for full models.

426

### 427 3.3.3. Female territorial aggression

428 Female territorial aggression (PC1) was not related to study site ( $F_{3, 54} = 1.319$ ,  $P = 0.278$ ) or the distance  
429 to Umicore ( $\beta = -0.115 \pm 0.153$ ,  $t_{3.84} = -0.751$ ,  $P = 0.496$ ), and was also unrelated to age ( $\beta = -0.449 \pm$   
430 0.273,  $t_{52.01} = -1.648$ ,  $P = 0.105$ ) and exploration behavior ( $\beta = -0.128 \pm 0.135$ ,  $t_{42.20} = -0.949$ ,  $P = 0.348$ ).

431 Female exploration behavior did not interact with either site or distance from Umicore to predict  
432 aggressiveness ( $P > 0.60$ ). There was no effect of time, date or trial number on the aggressive behavior of  
433 females ( $P > 0.10$  in all cases). See Table A.3 for full models.

434

### 435 3.3.4 Female aggression during nest defense (hissing behavior)

436 Female great tits exposed to a woodpecker invading the nest box produced 0 to 44 hisses per minute  
437 (mean =  $11.144 \pm 0.643$ ). Females hissed more at F6 than at other study sites ( $\chi_1^2 = 12.846$ ;  $P = 0.012$ ;  
438 Fig. 2c). The difference in hissing behavior was statistically significantly between F6 and F4 ( $\beta = 1.155 \pm$   
439 0.400;  $Z = 2.887$ ;  $P = 0.032$ ) and F6 and F7 ( $\beta = 1.038 \pm 0.321$ ,  $Z = 3.227$ ,  $P = 0.011$ ; Fig. 2c; Table 4).  
440 Hissing behavior did not differ significantly between UM and the other study sites (Fig. 2c), and there  
441 was not a directional relationship between distance to Umicore and hissing behavior ( $\beta = -0.026 \pm 0.045$ ,  
442  $Z = -0.573$ ,  $P = 0.566$ ). Female hissing behavior decreased later in the day and increased during later trial  
443 numbers, when females were tested multiple times (Table 4). Date ( $\beta = 0.030 \pm 0.023$ ,  $Z = 1.270$ ,  $P =$   
444 0.204) and female age ( $\beta = -0.100 \pm 0.310$ ,  $Z = -0.324$ ,  $P = 0.745$ ) were unrelated to hissing behavior.

445 When we tested the relationship between exploration score and hissing behavior, there was a  
446 significant interaction between study site and exploration score (Table 5), which also emerged as a  
447 significant interaction between the distance from Umicore and exploration score ( $\beta = 0.490 \pm 0.160$ ,  $Z =$   
448 3.061,  $P = 0.002$ ). However, the relationship between hissing and exploration score was statistically

449 significant only at F4, where a positive relationship existed ( $\beta = 0.142 \pm 0.037$ ;  $Z = 3.773$ ;  $P < 0.001$ ), and  
 450 was non-significant at UM ( $\beta = -0.041 \pm 0.058$ ;  $Z = -0.712$ ;  $P = 0.476$ ), F7 ( $\beta = 0.006 \pm 0.034$ ;  $Z = 0.180$ ;  
 451  $P = 0.857$ ), F6 ( $\beta = -0.0002 \pm 0.016$ ,  $Z = -0.015$ ,  $P = 0.988$ ) and F5 ( $\beta = 0.026 \pm 0.030$ ;  $Z = 0.894$ ;  $P =$   
 452  $0.371$ ). Post-hoc comparisons indicated that the relationship between hissing and exploration was  
 453 significantly different between F4 and UM ( $\chi_1^2 = 11.907$ ;  $P = 0.005$ ), and F4 and F6 ( $\chi_1^2 = 10.562$ ;  $P =$   
 454  $0.010$ ), whereas other post-hoc comparisons were non-significant ( $P > 0.30$ ). Thus, the statistical  
 455  
 456 **Table 4.** Results from a Poisson GLMM assessing how the hissing behavior of female great tits varied  
 457 among study sites located across a metal pollution gradient in Antwerp, Belgium, 2017.

Fixed effects	$\beta \pm SE$	Z	$P(> Z )$
Intercept	$2.287 \pm 0.468$	4.886	<0.001
Site F4 <sup>a</sup>	$-0.324 \pm 0.406$	-0.800	0.424
Site F5	$-0.169 \pm 0.392$	-0.431	0.666
Site F6	$0.830 \pm 0.334$	2.483	0.013
Site F7	$-0.208 \pm 0.332$	-0.625	0.531
Trial	$0.314 \pm 0.093$	3.365	<0.001
Time	$-0.115 \pm 0.035$	-3.337	<0.001
Random effects	Variance	SD	N
Female band	1.577	1.256	152
Decoy	0.012	0.108	3
Observation <sup>b</sup>	0.347	0.589	273

458 <sup>a</sup>Contrasts between sites are to UM, the most polluted site.

459 <sup>b</sup>Adding the observation level random effect reduced the dispersion parameter from 1.473 to 1.069.

460  $N$  (observations, females) = total: 273, 152; F4: 34, 20; F5: 37, 22; F6: 70, 35; F7: 72, 41; UM: 60, 34.

461 **Table 5.** Poisson GLMM predicting the number of hisses produced by female great tits from the  
 462 interaction between exploration score (ES) and study site, along a metal pollution gradient in Antwerp,  
 463 Belgium, 2017.

<b>Fixed effects</b>	$\beta \pm SE$	<b>Z</b>	<b>P(&gt; Z )</b>
Intercept	0.107 ± 0.451	0.236	0.813
Site F4	-1.514 ± 0.653	-2.318	0.020
Site F5	-1.551 ± 0.630	-2.461	0.014
Site F6	1.328 ± 0.344	3.855	<0.001
Site F7	0.985 ± 0.416	2.370	0.018
ES	-0.372 ± 0.360	-1.034	0.301
Site F4 × ES	1.909 ± 0.553	3.451	<0.001
Site F5 × ES	0.832 ± 0.642	1.295	0.195
Site F6 × ES	0.428 ± 0.402	1.066	0.286
Site F7 × ES	0.440 ± 0.522	0.843	0.399
Date	0.074 ± 0.020	3.694	<0.001
<b>Random effects</b>	<b>Variance</b>	<b>SD</b>	<b>N</b>
Female ID	1.026	1.102	84
Decoy	0.004	0.066	3
Observation <sup>b</sup>	0.125	0.354	158

464 <sup>a</sup>Contrasts between sites are to UM, the most polluted site.

465 <sup>b</sup>Adding the observation level random effect reduced the dispersion parameter from 1.237 to 1.098.

466 *N* (observations, females) = total: 158, 84; F4: 17, 10; F5: 11, 7; F6: 70, 35; F7: 23, 13; UM: 36, 19.

467 See Table A.5 for full model.

468

469 interaction was driven by the pattern at F4. Among females tested for exploration behavior, females  
470 hissed less when tested later in the year (Table 5). See Table A.5 for full models.

471

### 472 **3.4. Body condition**

473 When controlling for a positive effect of male sex ( $\beta = 0.760 \pm 0.099$ ,  $F_{1, 235} = 62.355$ ,  $P < 0.001$ ), birds  
474 from F4 and F6 displayed poorer body condition than birds from other sites ( $F_{4, 235} = 4.788$ ,  $P < 0.001$ ;  
475 Fig. 2d). Birds from F4 were in poorer condition than those from F7 ( $\beta = -0.608 \pm 0.174$ ,  $t_{235} = -3.480$ ,  $P$   
476  $= 0.005$ ), and tended to be in poorer condition than those from F5 ( $\beta = -0.553 \pm 0.202$ ,  $t_{235} = -2.740$ ,  $P =$   
477  $0.051$ ) and UM ( $\beta = -0.436 \pm 0.166$ ,  $t_{235} = -2.615$ ,  $P = 0.070$ ). Birds from F6 were also in poorer  
478 condition than those from F7 ( $\beta = -0.445 \pm 0.142$ ,  $t_{235} = -3.139$ ,  $P = 0.016$ ), whereas other pairwise  
479 comparisons were non-significant (Fig. 2d). The overall relationship between distance from Umicore and  
480 body condition was negative ( $\beta = -0.046 \pm 0.018$ ,  $t_{238} = -2.516$ ,  $P = 0.012$ ). However, this association was  
481 not clear cut, and was predominately driven by low body condition at F4 (farthest from Umicore) and F6,  
482 rather than high body condition at UM. The variation in body condition between study sites did not  
483 contribute to differences in exploration behavior between sites, because we found no relationship between  
484 body condition and exploration score ( $\beta = -0.03 \pm 0.06$ ;  $t_{237} = -0.565$ ,  $P = 0.572$ ). A summary table  
485 comparing least square mean values for behavioral traits and body condition across study sites is available  
486 in online Appendix A (Table A.6).

487

## 488 **4. Discussion**

489 The results of our study are consistent with the existence of distinct personality types and suggest an  
490 important behavioral effect of metal pollution on exploration behavior, but not on the two other  
491 behavioral traits that we measured. These results contradict the prediction that, because of reduced  
492 survival prospects, great tits at the highly polluted site would show increases in territorial aggression, nest  
493 defense and exploration behaviors to promote early reproductive success (Wolf et al., 2007; Dingemanse



494 and Wolf, 2010). Great tits resident at the site closest to Umicore displayed slower rather than faster  
495 exploration behavior compared to those from study sites farther away. Moreover, we detected no  
496 differences in territorial aggression or hissing behavior (nest defense) in association with proximity to the  
497 pollution source, suggesting that exploration, territorial aggression, and nest defense behaviors are not  
498 simultaneously affected by metal exposure.

499

#### 500 ***4.1. Repeatability of behaviors***

501 We found significant levels of repeatability for all of the behaviors for which we had repeated measures  
502 on individuals (male aggression in response to STI, female aggression in the contexts of both STI and  
503 nest defense), supporting the existence of distinct personality types. These results are consistent with past  
504 studies that have demonstrated repeatability of territorial aggression (Araya-Ajoy and Dingemanse, 2014)  
505 and hissing behaviors (Krams et al., 2013) in the great tit. Repeatable differences in individual behavior  
506 form the basis of animal personalities, can have a genetic component, and can be acted on by selection  
507 (Dingemanse et al., 2002; Drent et al., 2003). Unfortunately, although exploration behavior is repeatable  
508 at our central study population, F6 (Thys et al., 2017), we did not repeatedly measure exploration  
509 behavior, the main behavioral metric that varied across the pollution gradient. Determining whether  
510 metal pollution is associated with individually consistent differences in exploration behavior is an  
511 important subject for future research.

512

#### 513 ***4.2. Effects of metal exposure on exploration behavior***

514 The observed negative correlation between exposure to metal pollution and exploration behavior could  
515 arise through effects of metal exposure on the health of neurological and physiological systems. Past  
516 work on the same pollution gradient has demonstrated negative correlations between metal exposure and  
517 a wide range of physiological and fitness parameters including hematological status measured in the  
518 winter (Geens et al., 2010), immunological traits (Snoeijs et al., 2004), and breeding performance  
519 (Janssens et al., 2003b). Consistent with one previous study (Dauwe et al., 2006), we found no evidence

520 that adult great tits at the most polluted sites were in poorer body condition (as assessed by residual mass)  
521 than birds at other sites. Rather, residual mass was lowest at F4, the study site farthest from the pollution  
522 source. However, even if not under nutritional stress as reflected by body condition, birds at polluted  
523 sites could suffer changes in stress responsiveness and neurological damage (Gonçalves et al., 2012;  
524 Orisakwe, 2014). Indeed, song rate and repertoire size, indicator traits for neurological integrity (Spencer  
525 and MacDougall-Shackleton, 2011; Suthers et al., 2012), are low in great tit populations near Umicore  
526 (Gorissen et al., 2005), suggesting that great tits exposed to metals could suffer neurotoxic effects.

527 At our highly polluted sites, lead and cadmium concentrations are elevated in eggs and in the feathers  
528 of juvenile great tits (Dauwe et al., 1999, 2000; Janssens et al., 2002), suggesting that early-life  
529 organizational effects could contribute to slower exploration behavior in great tits residing near the  
530 pollution source. A recent experimental study on a different population of great tits found no evidence  
531 that early-life exposure to lead affects exploration behavior or a number of other behavioral traits in adults  
532 (Ruuskanen et al., 2015). However, the sample size of this study was limited, and the exposure period  
533 relatively short. Other studies have shown that exposure to metals during development can translate into  
534 differences in adult behavior and cognition in birds (Burger and Gochfeld, 1995a, b; Douglas-Stroebe et  
535 al., 2005; Carvalho et al., 2008; Muller et al., 2008) and other taxa (Rodrigues et al., 1996; Morgan et al.,  
536 2000; Chen et al., 2012).

537 Differences in exploration behavior across the pollution gradient could additionally reflect evolved  
538 differences in behavior between study sites (Jacquin et al., 2017). Our study sites are in relatively close  
539 proximity and separated by distances that dispersing great tits can conceivably cross (Dhondt, 1979; van  
540 Overveld et al., 2011). Thus, some gene flow could occur between sites. However, we have documented  
541 rare cases of dispersal only between F6 and F7, suggesting that gene flow is limited and that genetic  
542 changes due to metal exposure could exist.

543 Past studies regarding effects of lead and cadmium on exploration behavior and general activity levels  
544 have yielded mixed results (Lasky and Laughlin, 2001; Ecke et al., 2017). For instance, in contrast to our  
545 results, rhesus monkeys (*Macaca mullatta*) exposed to lead showed faster exploration behavior,

546 potentially reflecting psychotic increases in anxiety levels (Lasky and Laughlin, 2001). Discrepancies  
547 between studies could reflect differences in metal exposure levels, or species-specific responses to  
548 behavioral assays (Minderman et al., 2010; Carter et al., 2013). In great tits, fast exploration behavior  
549 appears to be associated with boldness, rather than anxiety, as assessed by other behavioral assays such as  
550 novel object tests (van Oers et al., 2004; Stuber et al., 2013). Thus, slow exploration of the novel  
551 environment could be associated with anxiety and risk-aversion. Interestingly, an aversion to novelty and  
552 risk-taking could have particularly negative effects on fitness in urban great tit populations exposed to  
553 contaminants, because boldness may be beneficial in urban habitats (Riyahi et al., 2017; Sprau and  
554 Dingemans, 2017). Determining whether changes in exploration behavior affect reproductive success  
555 and survivorship in metal exposed birds is an important objective for future research.

556

#### 557 *4.3. No effect of metal exposure on territorial aggression or nest defense*

558 In contrast to some past studies (Nevin, 2000; Janssens et al., 2003a; White et al., 2013), and despite  
559 relatively large sample sizes, we found no evidence that male or female aggressive behavior during  
560 territorial challenge, or female aggression during nest defense (hissing behavior) varied along the  
561 pollution gradient. These results suggest that, contrary to our predictions, metal pollution does not have  
562 similar effects on exploration, territorial aggression, and nest defense behaviors. For instance, neurotoxic  
563 effects on fearfulness could affect novel environment exploration behavior more than aggressiveness in  
564 familiar environments. Moreover, exploration behavior was measured during the winter, whereas  
565 aggressive behaviors were measured during the breeding season. Thus, effects of metal exposure on risk-  
566 taking propensity and associated behavioral traits could depend on season, which influences hormonal  
567 status (Romero, 2002; Greggor et al., 2016).

568 Fast exploration, territorial aggression, and aggression during nest defense are all generally associated  
569 with greater risk-taking, and are sometimes correlated (Sih et al., 2004; Carere et al., 2010b; Thys et al.,  
570 2017), which is why we expected that these behaviors might be similarly affected by metal exposure.  
571 Moreover, some past studies report that metal exposure elevates testosterone levels (e.g. Meeker et al.

572 2010), which could simultaneously increase boldness and aggressiveness. However, Janssens et al.  
573 (2003a) found no evidence for elevated testosterone levels in male great tits residing near Umicore.

574

#### 575 *4.4. Effects on behavioral correlations*

576 Because multiple behavioral traits did not concurrently shift across the metal pollution gradient, our  
577 results suggest that metal exposure does not strengthen or induce behavioral correlations between these  
578 traits. One might expect contaminants that disrupt shared mechanisms, such as hormonal or neurological  
579 controls (Sih and Bell, 2008), to concurrently affect all behavioral traits within behavioral syndromes  
580 (Brodin et al., 2013). However, each behavioral trait is likely to be affected by many different genetic  
581 and state variables. Thus, exposure to some contaminants could affect only certain behaviors within  
582 suites of related behavioral traits, potentially decoupling previously correlated behaviors (Sih and Bell,  
583 2008; Swaddle et al., 2017). Our sample sizes to test for correlations between behavioral traits were  
584 reduced because not all birds were tested for both aggressive and exploration behavior. Therefore, more  
585 data is needed to assess whether metal exposure could disrupt behavioral correlations.

586

#### 587 *4.5. Direct versus indirect behavioral effects of metal exposure?*

588 As a caveat, although great tits near the Umicore facility are exposed to high levels of lead and cadmium  
589 (Janssens et al., 2001; Van Parys et al., 2008; Vermeulen et al., 2015), we cannot prove a causal  
590 relationship between metal exposure and reductions in exploration behavior because of the correlational  
591 nature of our data. Indeed, the relationship between metal pollution and exploration behavior could arise  
592 through indirect effects. For instance, lower food availability in metal exposed areas (Eeva et al., 2003;  
593 Janssens et al., 2003b), rather than direct biological effects of toxic metals, could explain reduced  
594 exploration behavior. However, the absence of poor body condition near Umicore, and the lack of a  
595 relationship between body condition and exploration behavior, both appear to argue against this  
596 mechanism. In addition, differences between study sites besides those arising from metal pollution could  
597 explain our results. However, we find this unlikely because study sites were especially selected to

598 minimize differences in urbanization levels and habitat characteristics, and contain similar nest box  
599 densities. Experimental work and more physiological and neurological data (e.g. data on stress  
600 physiology and neurological integrity) will help to clarify the relationship between exploration behavior  
601 and metal pollution. In addition, determining individual levels of exposure to metals is a future objective,  
602 which will solidify our findings, and allow us to link concentrations of specific metals (e.g. lead,  
603 cadmium) to behavioral patterns.

604

## 605 **5. Conclusions**

606 We found that metal pollution was associated with slower exploration behavior in adult great tits of both  
607 sexes, but was not associated with the other behavioral traits that we measured. The difference in  
608 exploration behavior could arise through effects of toxic metals, especially lead and cadmium, on the  
609 integrity of physiological and neurological systems, potentially reflecting neurotoxic effects on  
610 fearfulness. Exploration behavior affects how individuals interact with the environment, and can hence  
611 affect fitness, for instance, by altering foraging patterns and dispersal. Thus, changes in exploration  
612 behavior could represent an unappreciated mechanism whereby exposure to metal pollution affects  
613 individual fitness and population dynamics. On the other hand, territorial aggression and nest defense  
614 behavior were repeatable, but did not vary systematically across the metal pollution gradient. These  
615 results suggest that relationships between environmental contamination and behavioral traits may emerge  
616 only for specific behavioral traits, or in specific contexts, and thus underscore the importance of  
617 measuring multiple behavioral traits in studies of behavioral ecotoxicology.

618

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627

## 628 **Appendix A.** Supplementary material

629 Full statistical models, before model reduction, can be found in the online appendix.

630

631 **Data availability:** Data will be available in the Dryad Digital Repository.

632

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