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1 **Pigeon odor varies with experimental exposure to trace metal pollution**

2

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18

19

20 **Abstract**

21 Trace metals are chemical pollutants that have well-known noxious effects on wildlife and  
22 that are current major environmental issues in urban habitats. Previous studies have  
23 demonstrated their negative (e.g. lead) or positive (e.g. zinc) effects on body condition,  
24 immunity and reproductive success. Because of their effects on condition, trace metals are  
25 likely to influence the production of condition-dependent ornaments. The last decade has  
26 revealed that bird odors, like mammal odors, can convey information on individual quality  
27 and might be used as secondary sexual ornaments. Here, we used solid-phase microextraction  
28 headspace sampling with gas chromatography - mass spectrometry to investigate whether  
29 plumage scent varied with experimental supplementation in lead and/or zinc in feral pigeons.  
30 Zinc supplementation (alone or in combination with lead) changed the proportion of several  
31 volatiles, including an increase in the proportion of hydroxy-esters. The production of these  
32 esters, that most likely originate from preen gland secretions, may be costly and might thus be  
33 reduced by stress induced by zinc deficiency. Although lead is known to negatively impact  
34 pigeon condition, it did not statistically affect feather scent, despite most of the volatiles that  
35 increased with zinc exposure tended to be decreased in lead-supplemented pigeons. Further  
36 studies should evaluate the functions of plumage volatiles to predict how trace metals can  
37 impact bird fitness.

38

39 **Keywords:** Scent; Zinc; Lead; Birds; Dove

## 40 **Introduction**

41 Trace metals (e.g. lead, iron, copper, zinc) are normally present in very low levels in the  
42 environment and small amounts are essential part of physiology in animals and plants (Powell  
43 2000, Prasad 2009, Plum et al. 2010). For instance, zinc is essential in regulating homeostasis,  
44 immune responses, and oxidative stress (Stefanidou et al. 2006), and zinc deficiency has  
45 serious consequences that have been recognized for many years and that include oxidative  
46 damages, diabetes and congenital malformations (Fosmire 1990; Oteiza et al. 1995; Prasad  
47 2003). However, excessive quantities of trace metals can be toxic. The large amount of trace  
48 metals that have been released in the environment by anthropogenic activities (Nriagu 1979)  
49 have well-known noxious effects on humans (Järup 2003; Tchounwou et al. 2012) and  
50 wildlife (Demayo et al. 1982; Goutte et al. 2014; Scheuhammer et al. 2007). The binding  
51 potential (Godwin 2001; Tainer et al. 1992) and the propensity of trace metals to catalyze  
52 oxidation reactions (Koivula and Eeva 2010) are responsible for immunosuppression  
53 (Chatelain et al. 2016c; Snoeijs et al. 2005; Snoeijs et al. 2004), oxidative stress (Berglund et  
54 al. 2007) and endocrine disruptive effects (Baos et al. 2006; Meillère et al. 2016). For  
55 instance, lead, whose widespread use has caused extensive environmental contamination  
56 (Nriagu 1996), is associated with impaired learning in herring gulls (*Larus argentatus*)  
57 (Burger and Gochfeld 2005), reduced sperm quality in red deer (*Cervus elaphus*) (Castellanos  
58 et al. 2015), and higher oxidative stress in Mozambique tilapias (*Oreochromis mossambicus*)  
59 (Kaya and Akbulut 2015).

60 Sexual ornaments are conspicuous phenotypic traits that can take various forms such  
61 as large antlers, bright plumage, or elaborated song, and that are displayed to attract mates.  
62 Because their expression can be influenced by genotype and environment, including various  
63 stress factors, such as food availability, extreme temperatures, noise and parasites (McGraw et  
64 al. 2002; Troïanowski et al. 2017; von Schantz et al. 1999), they often honestly reveal the

65 quality of the bearer (Zahavi and Zahavi 1999). By inducing physiological stress, trace metals  
66 can also alter ornament expression, as shown for the plumage coloration and singing  
67 behaviour of birds (Chatelain et al. 2017; Giraudeau et al. 2015; Gorissen et al. 2005;  
68 Vallverdú-Coll et al. 2015). Odor cues are also subjected to physiological and environmental  
69 influences (Ferkin et al. 1997; Johnston and Bronson 1982; Penn and Potts 1998), and are  
70 major secondary sexual traits in a wide range of species (Blaustein 1981; Havlicek et al. 2005;  
71 Martín and López 2006; Wyatt 2014). However, the effects of environmental pollution on  
72 their expression are largely unknown.

73         In this study, we tested the hypothesis of the impact of trace metals exposure (zinc and  
74 lead) on plumage odor in feral pigeon (*Columbia livia*) by an experimental supplementation  
75 approach. Although birds have long been considered to rely primarily on visual and acoustic  
76 cues, the past decade has revealed that birds use olfaction in various contexts including mate  
77 choice or parental care (reviewed in Caro et al. 2015). Bird odors, like mammal odors, can  
78 vary with genotype (Leclaire et al. 2014b), sex-hormone levels (Whittaker et al. 2017) and  
79 diet (Thomas et al. 2010), and are mainly composed of alkanes, alkanols, esters and fatty  
80 acids (reviewed in Campagna et al. 2012) that may be energetically costly to produce. Using a  
81 similar experimental design, we have already described beneficial effects of zinc on body  
82 mass, immunity and female investment into eggs (Chatelain et al. 2016b; Chatelain et al.  
83 2016c), and detrimental effects of lead on iridescent coloration, the maternal transfer of  
84 antibodies into eggs and nestling growth (Chatelain et al. 2016b; Chatelain et al. 2017). We  
85 predict therefore that if pigeon odor is condition-dependent, it should vary in opposite  
86 direction with experimental exposure to lead and zinc.

87

## 88 **Material and methods**

### 89 *Capture and housing*

90 From February 20 to March 27 2013, we captured 96 free-living adult feral pigeons in several  
91 locations in Paris. We immediately transferred them in 8 outdoor aviaries (2.20m x 2.20m) at  
92 the CEREEP field station (Centre d'Ecologie Expérimentale et Prédictive - Ecotron Ile-de-  
93 France, UMS 3194, Ecole Normal Supérieure, Saint-Pierre-lès-Nemours, France).

94

#### 95 *Metal supplementation*

96 This study is part of a bigger study investigating the effect of zinc and lead on several life  
97 history traits of pigeons. Details on the protocol have thus been published elsewhere  
98 (Chatelain et al. 2016b; Chatelain et al. 2016c; Chatelain et al. 2017). Briefly, on April 8  
99 2013, after an acclimation period, aviaries were divided into 4 metal treatments (2 aviaries per  
100 treatment). Birds were evenly distributed among aviaries according to their flock, sex and  
101 plumage eumelanin level (Chatelain et al. 2016b). Treatments were lead supplementation (tap  
102 water enriched with 1 ppm lead acetate), zinc supplementation (tap water enriched with 10  
103 ppm zinc sulphate), lead and zinc supplementation (tap water enriched with 1 ppm lead  
104 acetate and 10 ppm zinc sulphate) and no supplementation, i.e. control (tap water with no  
105 metal added). We chose these concentrations based on the lead concentration measured in  
106 blood of urban birds (Roux and Marra 2007) and on the gastrointestinal absorption rate of  
107 lead in zebra finches (Dauwe et al. 2002). Zinc concentrations were approximated using the  
108 zinc/lead concentration ratio measured in the environment and in bird feathers (on average,  
109 zinc is 10 times more concentrated than lead; Azimi et al. 2005; Chatelain et al. 2014; Frantz  
110 et al. 2012). Drinking bowls and baths were filled every other day with treated water. The  
111 efficiency of our supplementation protocol to increase bird exposure to lead and zinc has been  
112 previously validated (Chatelain et al. 2016b).

113

#### 114 *Sample collection*

115 After 20 weeks of treatment, a few feathers (~33 mg) were cut in the lower back region of 74  
116 birds (37 males and 37 females), and stored in 4 ml PTFE-faced septum sealed glass vials at -  
117 80°C until analysis. Birds from five aviaries (two lead-supplemented aviaries, one zinc-  
118 supplemented aviary, one zinc-and-lead-supplemented aviary and one control aviary) were  
119 sampled on 27 August 2013, while birds from the remaining three aviaries (one zinc-  
120 supplemented, one zinc-and-lead supplemented and one control aviaries) were sampled on the  
121 next day. The 74 birds sampled included 20 control birds, 19 lead-supplemented birds, 17  
122 zinc-supplemented birds and 18 zinc-and-lead-supplemented birds.

123

#### 124 *Chemical analyses*

125 We extracted chemical compounds with solid phase microextraction (SPME) using a  
126 stableflex 50/30µm DVB (divinylbenzene) / CAR (carboxen) / PDMS (polydimethylsiloxan)  
127 fiber (Supelco, Sigma-Aldrich, Bellefonte, PA, USA). To clear headspace for SPME  
128 extraction, we packed feathers at the bottom of the vial, using a glass rod cleaned with  
129 dichloromethane and ethanol between samples. The sample was then placed for 20 min in a  
130 laboratory incubator maintained at 34°C. We chose 34°C to mimic the natural temperature of  
131 bird body surface during a Parisian summer. During the summer 2013, the average daily  
132 maximal temperature in Paris was 25.3°C (public data of Meteo France). At 25°C ambient  
133 temperature and low humidity, surface temperature of birds is 33.4°C (Chilgren and King  
134 1973). The SPME fiber was then exposed to the headspace (without touching the feathers) for  
135 25 minutes at 34°C, after which the fiber was retracted and the adsorbed chemicals were  
136 analyzed by gas chromatography-mass spectrometry (GC-MS). Only one SPME fiber was  
137 used in the study.

138 We analyzed the samples using a Shimadzu GCMS-QP2010plus (Shimadzu Scientific  
139 Instruments, Kyoto, Japan) equipped with an Optima®-5MS column (30 m x 0.25 mm x 0.25

140  $\mu\text{m}$ , Macherey-Nagel, Düren, Germany) at the Plateforme d'Analyses Chimiques en Ecologie  
141 (PACE), technical facilities of the LABORatoire d'Excellence named Centre Méditerranéen de  
142 l'Environnement et de la Biodiversité (CEMEB). Helium was used as the carrier gas, at a  
143 constant 1ml/min flow rate. Fibers were exposed in a 200 °C injection port to desorb volatiles.  
144 We ran the following temperature protocol: 40° for 5 min, 40°C to 180°C ramped at 5°C/min,  
145 180°C to 250°C ramped at 10°C/min, and finally held at 250°C for 1 min. The ion source and  
146 transfer line temperatures was 200°C and 250°C respectively. Mass were scanned from 38 -  
147 350 m/z in electron ionization mode. Fibers were removed from the injection port after 10  
148 min of desorption. We regularly interspersed blanks throughout the sample analyses to detect  
149 contamination. We ran an alkane standard solution (Alkanes standard solution, 04070, Sigma  
150 Aldrich®, Germany) under the same conditions for retention index calculation. The samples  
151 from 9 controls, 9 lead-supplemented pigeons, 10 zinc-supplemented birds and 11 zinc-and-  
152 lead-supplemented birds were ran in March 2015, while the other samples were ran in June  
153 2015. Within each analysis time period (March vs. June 2015), samples were ran randomly.  
154 Consequently, there was no difference in date of analysis between zinc-supplemented and  
155 non-zinc supplemented samples or between lead-supplemented and non-lead supplemented  
156 samples (all  $P > 0.15$ ). Ten samples had very low GCMS signal and were not included in the  
157 statistical analyses.

158 To confirm that the feather odor captured from captive pigeons was representative of  
159 that of wild pigeons, we compared the chromatograms with those of two samples of back  
160 feathers collected just after capture in February 2013. In addition, to shed light on the  
161 potential origin of plumage chemicals, we compared the feather profiles to those of preen  
162 secretion samples collected on two pigeons just after capture.

163 Chromatograms were analyzed blind to treatment using the Shimadzu GC-MS  
164 Solution software. Analytes were identified by comparison of mass spectral data using the



165 NIST library 2011 and cross-checking linear retention index of the spectral match found in  
166 the literature (PubChem database) with the calculated linear retention index of the analytes.  
167 Four monoterpenes were detected in pigeon feathers ( $\alpha$ -Pinene,  $\beta$ -Pinene, p-Cymene and  
168 Bornyl acetate; Table 1). Most probably of plant origin (Charpentier et al. 2012), they were  
169 excluded from the analyses. One peak corresponded to a co-elution of several compounds  
170 including one contaminant (Table 1), and it was also not included in the analyses.

171 To ascertain that our GCMS analyses were repeatable, 5 samples were analyzed twice  
172 with a few days interval (range: 5 - 8 days). Results show that total chromatogram area was  
173 lower in the second analysis than in the first analysis (paired t-test:  $t_4 = 5.32$ ,  $P = 0.006$ ) but  
174 that the composition of the two samples from the same bird was repeatable (PERMANOVA  
175 analyses with bird identity as fixed factor:  $F_{4,4} = 8.93$ ,  $P = 0.001$ ).

176

### 177 *Statistical analyses*

178 Because, for most samples, we did not control for the amount of feathers collected,  
179 and because an internal standard was not included in the GCMS analyses, we did not rely on  
180 the absolute abundance of chromatogram peaks for the analyses of chemical composition;  
181 rather, we quantified each peak as the proportion of the peak size relative to the total area of  
182 the chromatogram. Proportions of chemical compounds were then transformed using  
183 isometric log ratio "ilr" (Brückner and Heethoff 2017). In addition, to limit the effect of the  
184 differences in the amount of feathers on odor composition, we restricted the odor composition  
185 analyses on the peaks that each represented on average more than 0.75% of the chromatogram  
186 ( $n = 19$  peaks representing on average  $91.0 \% \pm 0.3 \%$  of the total chromatogram areas, Table  
187 1). All of these peaks, except one, were present in all birds.

188 To determine if the chemical composition of plumage odor was related to lead and  
189 zinc exposition, we used a PERMANOVA with 5000 permutations i.e. nonparametric

190 multivariate analysis of variance, (Adonis2 function, "vegan" package in R; Oksanen et al.  
191 2013), based on Euclidean distance. The two-way interaction between lead and zinc, the bird  
192 sex and the analysis time period (March vs. June 2015) were also included in the model. The  
193 date of feather sampling was used as strata, thus restricting permutations within date of  
194 sampling. In Adonis, significant differences may be caused by different within-group  
195 dispersion instead of different mean values of the groups (Warton et al. 2012). For significant  
196 effects, we therefore tested difference in dispersion between groups (Betadisper function,  
197 "vegan" package; Oksanen et al. 2013).

198 To determine the chemical compounds that were preferentially associated with a  
199 significant fixed effect, we used correlation indices (function "multipatt" in the "indicspecies"  
200 package in R; De Cáceres et al. 2016) with the point biserial coefficient of association on  
201 proportions of chemical compounds (De Cáceres and Legendre 2009). We restricted  
202 permutations within date of sampling. This method uses permutation tests to test significance  
203 of value, but does not correct for multiple tests. Multiple testing corrections are flawed  
204 because they assume multiple tests are independent, whereas multiple volatiles can show  
205 correlated effects (Fig. S1) because of the nature of the data (relative abundance vs. absolute  
206 abundance) and because the biosynthesis pathway of these volatiles may be related.  
207 Therefore, we also performed robust principal component analyses (RPCA) on the ilr-  
208 transformed dataset (Filzmoser et al. 2009) to identify the subset of compounds that varied  
209 with treatment. RPCA axis scores were compared between zinc-supplemented and non-zinc  
210 supplemented birds using linear mixed models with zinc treatment and analysis time period as  
211 fixed effects, and date of feather sampling as a random factor.

212 We performed all statistical analyses using the R statistical software (R Core Team  
213 2017) and used a significance level set at  $\alpha = 0.05$ .

214

## 215 **Results**

216 We detected a total of 45 peaks in the 63 odor profiles of pigeons, that included alkanes,  
217 aldehydes, alcohols, ketones, and esters (Table 1 and Fig. 1). Only 6 (13%) of these  
218 compounds were also detected in preen secretions (Table 1). In contrast, 39 of them (87%)  
219 were also detected in the plumage of wild Parisian pigeons, suggesting that captivity had only  
220 slight influence on the identity of feather volatiles.

221 Chemical composition varied with zinc supplementation ( $F_{1,61} = 4.27$ ,  $P =$   
222  $0.016$ ; Fig. 2 and Fig. S2), but not with lead supplementation ( $F_{1,60} = 0.62$ ,  $P = 0.89$ ) or the  
223 interaction between zinc and lead ( $F_{1,59} = 1.22$ ,  $P = 0.56$ ). It did not vary with sex ( $F_{1,60} = 0.14$ ,  
224  $P = 0.99$ ), but varied with the period when the samples were analyzed ( $F_{1,61} = 13.20$ ,  $P <$   
225  $0.001$ ). Multivariate dispersion in chemical composition between zinc-supplemented and non-  
226 zinc supplemented birds was similar ( $F_{1,62} = 0.59$ ,  $P = 0.45$ ). Correlation indices showed that  
227 the proportions of the two hydroxy esters and 3-Hexanone were increased in zinc  
228 supplemented birds, while the proportion of Nonanal was reduced (Fig. 3). Similarly, a robust  
229 PCA showed that the second principal component, which accounted for 26% of the variance  
230 and which was positively related to the two hydroxy esters and the two ketones, was higher in  
231 zinc-supplemented birds than in non-zinc supplemented birds ( $\chi^2_1 = 7.14$ ,  $P = 0.008$ ; Fig. 4).

232

## 233 **Discussion**

234 By analyzing feather volatiles of feral pigeons chronically exposed to zinc and/or lead, we  
235 provide evidence for an effect of zinc on pigeon odor composition. In particular, we found  
236 that zinc mainly increased the relative abundance of the two hydroxy esters and 3-Hexanone.  
237 All the compounds affected by zinc supplementation may potentially be produced  
238 endogenously by the bird. However, in contrast to several species, where most plumage

239 chemicals originate from the preen gland (Leclaire et al. 2011; Mardon et al. 2011; Zhang et  
240 al. 2009), only the two hydroxy-esters were detected in pigeons' preen secretions. Although  
241 the sample size used to study the difference between preen secretion and feather volatiles was  
242 low (n = 2 birds sampled for preen secretions), this result is consistent with a study on  
243 common wood pigeons (*Columba palumbus*), where 93% of the whole plumage lipids do not  
244 originate from preen secretions (Jacob and Grimmer 1975).

245         The other compounds affected by zinc exposure were not detected in preen secretions,  
246 and may rather originate from other lipid-producing areas in the integument (Jacob and  
247 Ziswiler 1982) or from feather bacteria metabolism (Whittaker and Theis 2016). The role of  
248 bacteria in animal olfactory signaling has recently received renewed interests (Archie and  
249 Theis 2011; Douglas and Dobson 2013; Ezenwa and Williams 2014) and a few experimental  
250 studies in mice, elephants (*Loxodonta africana*), Indian mongooses (*Herpestes*  
251 *auropunctatus*) and European hoopoes (*Upupa epops*) have shown that some major odorants  
252 require commensal bacteria for their production (Goodwin et al. 2016; Gorman et al. 1974; Li  
253 et al. 2013; Martin-Vivaldi et al. 2010). Methyl-branched alkanes, methyl ketones, and  
254 aldehydes can all be released by bacterial degradation of fatty acids (Schulz and Dickschat  
255 2007). Feathers of zinc-supplemented pigeons have lower bacterial load and different  
256 bacterial assemblage than feathers of birds non-exposed to zinc (Chatelain et al. 2016a),  
257 which may have led to differences in the composition of feather volatiles between birds.  
258 Further studies on the mechanisms of avian odor production are clearly needed to understand  
259 how environment can affect feather volatiles.

260         We found that zinc increased the proportion of preen-gland-related compounds, which  
261 might suggest higher production of preen oil in zinc-exposed birds. Several studies have  
262 suggested that birds in higher condition are able to produce higher quantity of preen oil. For  
263 instance, experimental infections and immune challenge reduce the size of the preen gland in

264 house sparrows (*Passer domesticus*) and tawny owls (*Strix aluco*) (Moreno - Rueda 2015;  
265 Pap et al. 2013; Piau et al. 2008). Zinc provided at low dose is essential for many biological  
266 processes (Stefanidou et al. 2006), and accordingly, zinc supplementation has various  
267 beneficial effects on pigeon condition (Chatelain et al. 2016b; Chatelain et al. 2016c).  
268 Therefore, zinc, through its positive effects on bird condition, may increased the ability of  
269 bird to produce wax esters and other costly volatiles. Although avian odors have repeatedly  
270 been shown to vary with genetics (i.e., Krause et al. 2012; Leclaire et al. 2017) and  
271 physiological status (Douglas et al. 2008; Whittaker et al. 2018), a very few studies have  
272 shown the effect of environmental stressors on their expression (Bombail et al. 2018). For  
273 instance, infection influences the wax ester profiles of preen oil in song sparrows (*Melospiza*  
274 *melodia*) and the volatile profiles of feces in mallards (*Anas platyrhynchos*) (Grieves et al.  
275 2018; Kimball et al. 2013). Our study, which shows that the volatile profiles of feather is  
276 related to zinc levels in the environment, is therefore consistent with the stress-induced effects  
277 on odors demonstrated in other birds.

278         In several avian species, odor plays a crucial role in social interactions (Balthazart and  
279 Schoffeniels 1979; Balthazart and Taziaux 2009; Hirao et al. 2009). Zinc-induced changes in  
280 pigeon scent might therefore influence the behavior of conspecifics, with, for instance,  
281 females avoiding the scent of zinc-deficient males. However, although pigeons are well-  
282 known to use their olfactory abilities for homing (reviewed in Wallraff 2005), it is unknown  
283 whether they use also olfaction for social communication. Feather chemicals may play also a  
284 role in social communication by affecting feather coloration or condition (Moreno - Rueda  
285 2017). For instance, in house finches (*Carpodacus mexicanus*), preen oil enhances the  
286 coloration of red feathers (López-Rull et al. 2010). In pigeons, changes in the coloration of  
287 melanic feathers have been induced by zinc supplementation (Chatelain et al. 2017), and  
288 might therefore be mediated by changes in the chemical composition of feather volatiles.

289 Feather chemicals do not only play a role in social communication; they can protect  
290 feathers from wear and act as chemical defenses against parasites (Douglas 2013; Hagelin and  
291 Jones 2007). For instance, in rock pigeons, preen oil has positive effects on plumage condition  
292 (Moyer et al. 2003). 3-Hexanone, which was increased in zinc-exposed birds, is a common  
293 urine component of mammals, including humans (Burger et al. 2006; delBarco - Trillo et al.  
294 2011; Raymer et al. 1986; Smith et al. 2008), and has been detected in the feathers of white  
295 Leghorn chickens (*Gallus gallus domesticus L.*) (Bernier et al. 2008) and king penguins  
296 (Gabirot et al. 2018). It belongs to the volatile methyl-ketone family, whose some compounds  
297 can provide protection against parasites (Borges et al. 2015; Kirillov et al. 2017). According  
298 to the parasite-repellent function of volatile ketones, volatile methyl-ketones of dark-eyed  
299 juncos (*Junco hyemalis*) are increased during the breeding season (Soini et al. 2007), when  
300 the need for protection against nest parasites is high. Zinc, by inducing higher production of  
301 hydroxy-esters and 3-hexanone, might thus enhance plumage condition and the anti-parasite  
302 properties of feathers, thus adding to the numerous positive effects of zinc on pigeon  
303 (Chatelain et al. 2016b; Chatelain et al. 2016c).

304 Lead is a toxic trace metal that can impair various biological processes, leading to  
305 illness and mortality (Papanikolaou et al. 2005). In feral pigeons, lead decreases iridescent  
306 coloration, the maternal transfer of antibodies into eggs, and nestling growth (Chatelain et al.  
307 2016b; Chatelain et al. 2017). Despite these detrimental effects on pigeon condition, we did  
308 not detect effects of lead on pigeon scent. As our experimental exposure to lead  
309 underestimated the natural range (lead was 1.5 times less concentrated in the feathers of lead-  
310 supplemented pigeons than in those of wild feral pigeons; Chatelain et al. 2016b), we cannot  
311 exclude that, in urban areas, the high concentration of lead negatively impacts odor  
312 production in birds.

313 We did not find evidence for a sex signature in the feather odor of wild-caught pigeons  
314 kept in captivity. Similarly, no sex-differences in preen secretion composition have been  
315 detected in non-breeding rock pigeons (Montalti et al. 2005). Although sexual dimorphism in  
316 chemical cues from feathers or preen secretions has been reported in several bird species  
317 (Amo et al. 2012; Leclaire et al. 2011; Whittaker et al. 2010; Zhang et al. 2010), it is not  
318 ubiquitous. For instance, king penguins (*Aptenodites patagonicus*) and Cory's shearwaters  
319 (*Calonectris borealis*) seem to exhibit no sex-differences in scent (Gabirot et al. 2018;  
320 Gabirot et al. 2016). Pigeons display sexual polymorphism in iridescent coloration of neck  
321 feathers (Chatelain et al. 2017; Leclaire et al. 2014a), and might, therefore, use this other trait  
322 to discriminate between sexes. Alternatively, because the molecules detected using GC-MS  
323 depend on the analysis strategy (e.g., extraction protocol and GC column), the profile of  
324 chemical composition presented here is not exhaustive. For instance, carboxylic acids, which  
325 are better resolved using a polar column (Whelan et al. 2010), might encode sex information.

326 In conclusion, our study showed that exposure to a moderate amount of zinc induced  
327 changes in the feather volatile composition of feral pigeons. Future studies should now  
328 evaluate the role of these volatiles in social communication, protection against parasites or  
329 feather wear to further predict how trace metals, which are chemical pollutants of prime  
330 concern in cities, can impact bird fitness.

331

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342

### 343 **Conflict of interest**

344 The authors declare that they have no conflict of interest.

345

### 346 **Compliance with Ethical Standards**

347 All experiments were carried out in strict accordance with the recommendations of the  
348 "European Convention for the Protection of vertebrate Animals used for Experimental and  
349 Other Scientific Purposes" and were conducted under the authorizations of the "Ministère de  
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353

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607

608

609 **Figure captions**

610 **Figure 1:** Representative chromatogram of feather volatiles in captive pigeons. Letters refer  
611 to compounds listed in table 1.

612

613 **Figure 2:** Partial unconstrained distance-based redundancy analysis plot showing separation  
614 between pigeons by zinc supplementation. The effect of the period when the samples were ran  
615 on the GCMS was partialled out to better show separation.

616

617 **Figure 3:** Mean  $\pm$  SE proportion of the four chemical compounds that varied with zinc  
618 exposure in zinc-supplemented birds and non zinc-supplemented birds. P values were  
619 calculated using a mixed model with date of sampling as a random effect and analyses time  
620 period as covariate.

621

622 **Figure 4:** Robust principal component analysis plot showing separation between pigeons by  
623 zinc supplementation. Vectors representing chemical compounds with high loading on axis 2  
624 are highlighted.

625

626

627 **Table 1:** Chemical compounds putatively identified in the feathers of feral pigeons.  
 628 Compounds in bold are those that were > 0.75 % of the total chromatogram and that were  
 629 used in statistical analyses.

630

Name	Letter (see figure 1)	Retention index calculated	Detected in preen secretions	Detected on the plumage of wild Parisian pigeons
<b>Ketones</b>				
<b>Hexan-3-one</b>	a	n.d.		x
<b>6-methyl-5-Hepten-2-one</b>	b	982		x
<b>Aldehydes</b>				
<b>Hexanal</b>	c	n.d.		x
<b>Heptanal</b>	d	896		x
<b>Nonanal</b>	e	1101	x	x
Decanal		1202	x	x
<b>Alkanes</b>				
<b>Octane</b>	f	n.d.		x
2, 4-dimethyl-Heptane		817		x
<b>Nonane</b>	g	899		x
<b>Branched alkane #1</b>	h	1060		x
Branched alkane #2	o	1065		x
<b>Branched alkane #3</b>	i	1104		x
Branched alkane #4		1109		x
Dodecane		1200		x

Branched alkane #5		1277		x
<b>Branched alkane #6</b>	j	1283		x
Branched alkane #7		1305		x
Branched alkane #8		1322		x
<b>Branched alkane #9</b>	k	1329		x
Branched alkane #10		1337		x
Branched alkane #11		1345		x
Branched alkane #12		1348		x
Tridecane		1300		x
Pentadecane		1500		x
<b>Alcohols</b>				
Pentanol		n.d.		x
2-butoxy-Ethanol		903		x
1-butoxy-2-Propanol		940		x
1-Octen-3-ol		978		x
<b>2-ethyl-Hexan-1-ol</b>	l	1028		x
2, 6-dimethyl-7-Octen-2-ol		1071		x
<b>Esters</b>				
Butanoic acid, methyl ester		n.d.		x
Propanoic acid, propyl ester		804		
<b>Acetic acid, butyl ester</b>	m	809		x
Hexanoic acid, methyl ester		922		x
<b>Hydroxy ester #1</b>	n	1357	x	x
<b>Hydroxy ester #2</b>	o	1376	x	x



<b>Fatty acids</b>				
Nonanoic acid		1273		x
<b>Unknown</b>				
<b>Unknown #1</b>	p	884		x
<b>Unknown #2</b>	q	910		
Unknown #4		1143		
Unknown #5		1232		
<b>Unknown #6</b>	r	1295		
Unknown #7		1307		x
Unknown #8		1316		
<b>2, 2, 4, 6, 6-pentamethylheptane + hexanoic acid <sup>a</sup></b>	s	993	x	x

n.d.: not determined

<sup>a</sup>: These two compounds were eluted together.

631

632