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# 1 Honey bees cannot sense harmful concentrations of metal 2 pollutants in food

3  
4 Coline Monchanin<sup>1,2</sup>, Maria Gabriela de Brito Sanchez<sup>1</sup>, Lorelei Lecouvreur<sup>1</sup>, Océane  
5 Boidard<sup>1</sup>, Grégoire Méry<sup>1</sup>, Jérôme Silvestre<sup>3</sup>, Gaël Le Roux<sup>3</sup>, David Baqué<sup>3</sup>, Arnaud Elger<sup>3</sup>,  
6 Andrew B. Barron<sup>2</sup>, Mathieu Lihoreau<sup>1</sup>, Jean-Marc Devaud<sup>1\*</sup>

7  
8 <sup>1</sup> Research Center on Animal Cognition (CRCA), Center for Integrative Biology (CBI); CNRS,  
9 University Paul Sabatier – Toulouse III, France

10 <sup>2</sup> Department of Biological Sciences, Macquarie University, NSW, Australia

11 <sup>3</sup> Laboratoire écologie fonctionnelle et environnement, Université de Toulouse, CNRS,  
12 Toulouse, France

13  
14 \*Corresponding author: [jean-marc.devaud@univ-tlse3.fr](mailto:jean-marc.devaud@univ-tlse3.fr)

## 17 Abstract

18 Whether animals can actively avoid food contaminated with harmful compounds through taste  
19 is key to understand their ecotoxicological risks. Here, we investigated the ability of honey bees  
20 to perceive and avoid food resources contaminated with common metal pollutants known to  
21 impair their cognition at low concentrations (lead, zinc and arsenic). In behavioural assays, bees  
22 did not discriminate food contaminated with field-realistic concentrations of these metals. Bees  
23 only reduced their food consumption and displayed aversive behaviours at very high, unrealistic  
24 concentrations of lead and zinc that they perceived through their antennae and proboscis.  
25 Electrophysiological analyses confirmed that high concentrations of the three metals in sucrose  
26 solution induced a reduced neural response to sucrose in their antennae. Our results thus show  
27 that honey bees can avoid metal pollutants in their food, but only at very high concentrations  
28 above regulatory levels. Their inability to detect lower, yet harmful, concentrations in a field-  
29 realistic range suggests that metal pollution is a major threat for pollinators.

30  
31 **Keywords:** *Apis mellifera*, metal pollution, feeding behaviour, learning, electrophysiology,  
32 taste

## 33 1. Introduction

34

35 Pollinators play major economic and ecological roles by facilitating the reproduction of  
36 flowering plants. Worryingly, pollinating insects are declining due to many stressors derived  
37 from human activities, among which are pesticides, reduced floral diversity, pests and viruses  
38 [1]. Exposure to potentially harmful metal pollutants may have additional impact, though  
39 largely overlooked despite raising ecological and public health concern worldwide [2]. The  
40 release of metal pollutants into the environment, as a result of industrial manufacturing and  
41 mineral extraction, has resulted in their accumulation in ecosystems at levels far beyond  
42 concentrations that would be considered natural [3,4]. Because metallic compounds cannot be  
43 degraded and can be poisonous at low levels, they represent a potential threat to animals  
44 exploiting contaminated resources [2].

45 In the case of pollinators, such as bees, the effects of metal pollutants could have  
46 ecosystemic consequences [2]. Bees are exposed to metal pollutants while flying [5] and  
47 collecting food resources (water, pollen and nectar) [6,7]. Metals then bio-accumulate in the  
48 bodies of the bees [8,9], as well as in hive products [10,11]. Many deleterious effects of metal  
49 pollutants, which vary depending on doses and durations (i.e. chronic vs. acute) of exposure,  
50 have been described in mammals [12], birds [13], and specifically on human health [14], and  
51 there is clear evidence that exposure to metals can have deleterious effects on the survival [15],  
52 physiology [16,17] and behaviour [18,19] of bees. However, whether bees can detect metal  
53 pollutants in food is not known.

54 Bees can detect natural deterrent substances produced by plants and recognize them as  
55 harmful, at least in specific experimental conditions [20–23]. Even when ingested, such  
56 substances trigger subsequent aversive responses due to a delayed malaise-like state [20,22–  
57 24]. If endowed with sensitivity to harmful metal concentrations, bees could actively avoid  
58 contaminated food. They could also consume food containing low concentrations, that are  
59 harmless or even profitable, as some metallic compounds are micronutrients needed for  
60 physiological functions [25].

61 Metal ions can distort the function of peripheral chemoreceptors involved in taste-  
62 mediated feeding behaviour [26], particularly by reducing the sensitivity of gustatory neurons  
63 to sugars in some insect species [27,28]. Honey bees can recognize a variety of potentially  
64 noxious substances through gustatory receptor cells located on their antennae, mouthparts and  
65 forelegs [29], but their capacity to detect and/or avoid metals in food seems limited. In a study  
66 of the proboscis extension reflex, restrained bees willingly consumed solutions containing field-

67 realistic levels of selenium [30] or cadmium [18] with no behavioural indications of avoidance.  
68 Copper and lead solutions appeared to be palatable at certain concentrations, and only lead  
69 solutions induced any aversive responses [18]. Field studies have reported either no  
70 discrimination between flowers grown in lead-contaminated or uncontaminated soils [31], or  
71 increased visitation of zinc- and lead-treated flowers [32]. Thus, it appears that the ability to  
72 detect and reject potentially toxic substances varies greatly with their chemical identities and  
73 concentrations, the body parts in contact with them (mouthparts, antennae or tarsi), and the  
74 experimental or ecological context (harnessed, free-flying individuals). Whether bee taste  
75 receptors actually respond to metals has never been tested to our knowledge, so that the  
76 mechanisms of metal perception remain unknown [18].

77 Here, we tested whether bees could detect common metals in food. We focused on salts  
78 of zinc (an essential nutrient at low concentrations [33]), as well as of lead and arsenic (two  
79 major environmental pollutants [34]). We first assessed whether bees modified their  
80 consumption of sucrose solutions containing metal pollutants in choice and no-choice  
81 conditions. We then investigated whether bees could detect metal pollutant salts through their  
82 antennae and proboscis. Finally, we tested the capacity of gustatory antennal neurons to respond  
83 to metal pollutant salts delivered alone or in combination with sucrose.

84

## 85 **2. Methods**

86

### 87 **(a) Bees and metals**

88 We collected honey bees (*Apis mellifera*) from fourteen hives at our experimental apiary  
89 (University Paul Sabatier – Toulouse III, France) between January 2019 and August 2020. For  
90 the experiments, we used lead ( $\text{PbCl}_2$ ; CAS #7758-95-4 and  $\text{PbC}_4\text{H}_6\text{O}_4 \cdot 3\text{H}_2\text{O}$ ; CAS #6080-56-  
91 4), zinc ( $\text{ZnCl}_2$ ; CAS # 7646-85-7 and  $\text{ZnC}_4\text{H}_6\text{O}_4$ ; CAS #557-34-6) and arsenic ( $\text{NaAsO}_2$ ; CAS  
92 #7784-46-5) (all from Sigma-Aldrich, St Louis, MO). The metallic compounds were either  
93 dissolved in 30% (w/v) sucrose solution (for feeding, proboscis response and  
94 electrophysiological assays) or in mineral water (for antennal response and electrophysiological  
95 assays). We tested both chloride or acetate salts of lead (Pb) and zinc (Zn). For arsenic (As)  
96 (for the sake of simplicity, we will refer to it as a metal pollutant), we chose arsenite AsIII, a  
97 highly toxic form [35] derived from smelting and found in insecticides [36,37]. We used  
98 nominal concentrations of 0.001, 0.013, 0.129 and 12.83  $\mu\text{M}$  of As; 0.36, 3.60, 35.96  $\mu\text{M}$  and  
99 3.6 mM of Pb; and 0.012, 0.12, 1.22 and 122.3 mM of Zn (see Table S1 for correspondences in  
100 ppm and  $\text{mg}\cdot\text{L}^{-1}$ ). The three lower concentrations of Zn and Pb and all concentrations of As

101 have been reported in field studies of metal pollution (Table S1). The highest concentrations  
102 were above the regulatory levels in food as defined by the WHO [38,39], and were assessed  
103 through chemical analysis, which gave a good recovery rate (Table S1).

104

#### 105 **(b) Feeding assays**

106 We tested the ability of bees to discriminate metal salts in food in assays in which groups of  
107 bees could self-select foods over several hours [40]. We collected workers of unknown age at  
108 the colony entrance of five different hives, as they returned from foraging. The bees were cold-  
109 anaesthetized and placed in groups of 20 in plastic cages (80 x 50 x 40 mm), for 3 days in an  
110 incubator (dark, 28 °C ± 1°C, 60% relative humidity). Each cage contained two 2 mL feeding  
111 vials (Eppendorf) pierced with two 2 mm holes at the bottom to allow drinking of the sucrose  
112 solutions they contained. In the no-choice condition, bees were offered only one type of food:  
113 either 30% sucrose solution or 30% sucrose solution containing either As, Pb or Zn salts at one  
114 of the concentrations in Table S1. In the choice condition, bees were offered one vial containing  
115 pure sucrose and one vial containing a sucrose and metal salt solution. Feeding vials were  
116 weighed prior to be placed in the experimental cages, then removed, weighed and replaced by  
117 fresh ones every 24 h during 3 days. Cages without bees were used to measure the evaporation  
118 rate from the feeding vials. The amount of solution consumed daily was estimated by measuring  
119 weight loss in each vial every 24 h. The average value for evaporation of each treatment was  
120 subtracted from this final value for each vial. The number of dead bees in each cage was counted  
121 every hour (from 9 am to 5 pm), thus allowing the calculation of the mean daily consumption  
122 per bee (daily consumption divided by the mean number of bees alive in the cage).

123

#### 124 **(c) Devaluation assays following antennal stimulation**

125 We tested the ability of bees to perceive metal salts diluted in water using a devaluation assay  
126 that assesses whether repeatedly pairing a previously rewarding odour to contaminated water  
127 delivered to the antennae could lead to the devaluation of this odour, thus meaning that it would  
128 be perceived as aversive [20]. Workers of unknown age were collected from the top of the  
129 frames of eight different hives, cooled on ice, and harnessed in individual plastic holders  
130 allowing free movements of their antennae and mouthparts. We fixed their head to the holder  
131 using a droplet of melted bee wax, fed them 5 µL of sucrose solution (50% w/v) and let them  
132 rest for 3 h in an incubator (dark, 28 ± 1 °C, 60% relative humidity). Before starting the  
133 experiment, we checked for intact proboscis extension reflex (PER) by touching the antennae

134 with a toothpick soaked in 50% (w/v) sucrose solution without subsequent feeding. Bees that  
135 did not exhibit the reflex were discarded.

136 The first phase of the assay started with three trials pairing an odour (pure 1-nonanol) with  
137 a 50% (w/v) sucrose solution reward. The second phase consisted of 10 trials where a  
138 presentation of the trained odorant was followed by the stimulation of the antennae with a metal  
139 solution, or just water for the control group. For the second phase of the assay, we only kept  
140 bees that performed a PER response to 1-nonanol (92% of the bees). The odour was presented  
141 via an automated odour delivery system with a continuous air-stream as described in [41]. For  
142 each trial, the harnessed bee was placed in the conditioning set-up for 15 s to allow  
143 familiarization, then 1-nonanol was released for 6 s. Four seconds after odour onset, the  
144 antennae were stimulated with 50% (w/v) sucrose solution (phase 1), or metal solutions, or  
145 water (phase 2) for 2 s followed by 1 s of feeding with sucrose. The bee was left in the  
146 conditioning setup for 20 s before being removed. Inter-trial interval was 15 min for both  
147 phases. We recorded the proboscis extension response at each trial (extension = 1, no extension  
148 = 0).

149

#### 150 **(d) Devaluation assay following proboscis stimulations**

151 We assessed whether bees were able to perceive metal salts through their proboscis and/or post-  
152 ingestive consequences (i.e. malaise-like state) [23], by testing their potential devaluating effect  
153 when applied to the proboscis. We collected workers of unknown age from the top of the frames  
154 of four different hives, harnessed and fed them with 5  $\mu$ L of sucrose solution (50% w/v) and  
155 left them to rest for 3 h (dark,  $28 \pm 1^\circ\text{C}$ , 60% relative humidity). For 12 trials, bees were  
156 conditioned to associate 1-nonanol with ingestion of the sucrose-contaminated stimulus: after  
157 application of a droplet of 30% (w/v) sucrose onto the antennae to trigger PER, a 0.4  $\mu$ L droplet  
158 of a metal-spiked sucrose solution was delivered to the proboscis. We recorded the proboscis  
159 extension response upon odour delivery for each of the 12 trials (extension = 1, no extension =  
160 0). Here again, we expected that any decrease of response frequency would reveal aversion to  
161 some stimuli. In addition, we collected bees in the same conditions, harnessed and fed them  
162 with 4.8  $\mu$ L (12 times 0.4  $\mu$ L) of each solution and monitored their survival for 150 min (i.e.  
163 the duration of the proboscis response assay).

164

#### 165 **(e) Electrophysiological recordings**

166 We performed electrophysiological recordings on chaetic sensilla [42,43], which can be easily  
167 identified by their external morphology [44] (Figure 3A). We focused on the antennae, the

168 organs concentrating the highest number of taste sensilla [29], and specifically on the tip ventral  
169 zones [45], which are devoid of olfactory sensilla [42].

170 We immobilized the antennal flagellum with a metal thread stuck with wax and placed  
171 a glass electrode (ext. diameter 10–20  $\mu\text{m}$ ) over a single taste sensillum [29]. We used a silver  
172 wire inserted into the contralateral eye as grounded reference electrode. Electrodes were pulled  
173 from borosilicate glass capillaries, filled with different solutions and stored in a humid chamber  
174 before use. We prepared 30 mM sucrose solutions, contaminated or not with metal pollutant  
175 salts (Table S1) in 1 mM KCl, which ensured the necessary conductivity for recording, and kept  
176 them at 4 °C (1mM KCl was used as the reference). We stimulated taste sensilla in the following  
177 order: 1mM KCl, 30 mM sucrose, and 30 mM sucrose containing increasing concentrations of  
178 metal pollutant salts. In a separate experiment, increasing concentrations of KCl (1 mM, 10  
179 mM, 50 mM and 500 mM) diluted in 30 mM sucrose, were also tested. All stimuli were applied  
180 for 2 s, with an interstimulus interval of 1 min. The recording and reference electrodes were  
181 connected to a preamplifier (TasteProbe - Syntec, Kirchzarten, Germany). The electric signals  
182 were amplified ( $\times 10$ ) using a signal connection interface box (Syntec, Kirchzarten, Germany)  
183 in conjunction with a 100-3000Hz band passfilter. Experiments started when the recording  
184 electrode contacted the sensillum under study, which triggered data acquisition and storage on  
185 a hard disk (sampling rate: 10kHz). We then analysed these data using Autospike (Syntec) and  
186 quantified the number of spikes after stimulus onset.

187

#### 188 **(f) Statistical analysis**

189 We performed all statistical analyses in R [46]. For the choice assay, we analysed the  
190 consumption preference (difference between mean daily consumptions of each food in g/bee)  
191 with linear mixed effect models (LMMs; lme4 package [47]), against zero (no preference). For  
192 the no-choice assay, we analysed the daily consumption (g/bee) with LMMs. Models were fitted  
193 with treatment as a fixed effect and cages nested in hive as a random effect. Models were  
194 followed by pairwise comparisons (multcomp package [48]). We analysed the survival  
195 probability over three days using a Cox regression model [49].

196 For antennal and proboscis response assays, we scored the PER of each bee as a binary  
197 variable (response=1, no response=0), and analysed the mean score (averaged over the trials)  
198 using a binomial generalised linear mixed model (GLMM, lme4 package [47]), with treatment  
199 as fixed effect, trial number as a covariate, individual identity nested in the colony, and trial as  
200 random grouping variable. For proboscis responses, we also applied GLMMs separately for  
201 each trial, with treatment as fixed effect and individual identity nested in the colony, to better

202 capture the temporal dynamics of responses. We analysed the survival probability over 150 min  
203 using a Cox regression model.

204 Electrophysiological data were analysed by comparing frequencies of recorded spikes  
205 using a negative binomial GLMM using Template Model Builder [50], with treatment as a fixed  
206 effect and bee identity as random variable to take into account the repeated measurements per  
207 individual. Models were followed by pairwise comparisons [48].

208

### 209 **3. Results**

210

#### 211 **(a) Bees only avoided high concentrations of Pb and Zn in food**

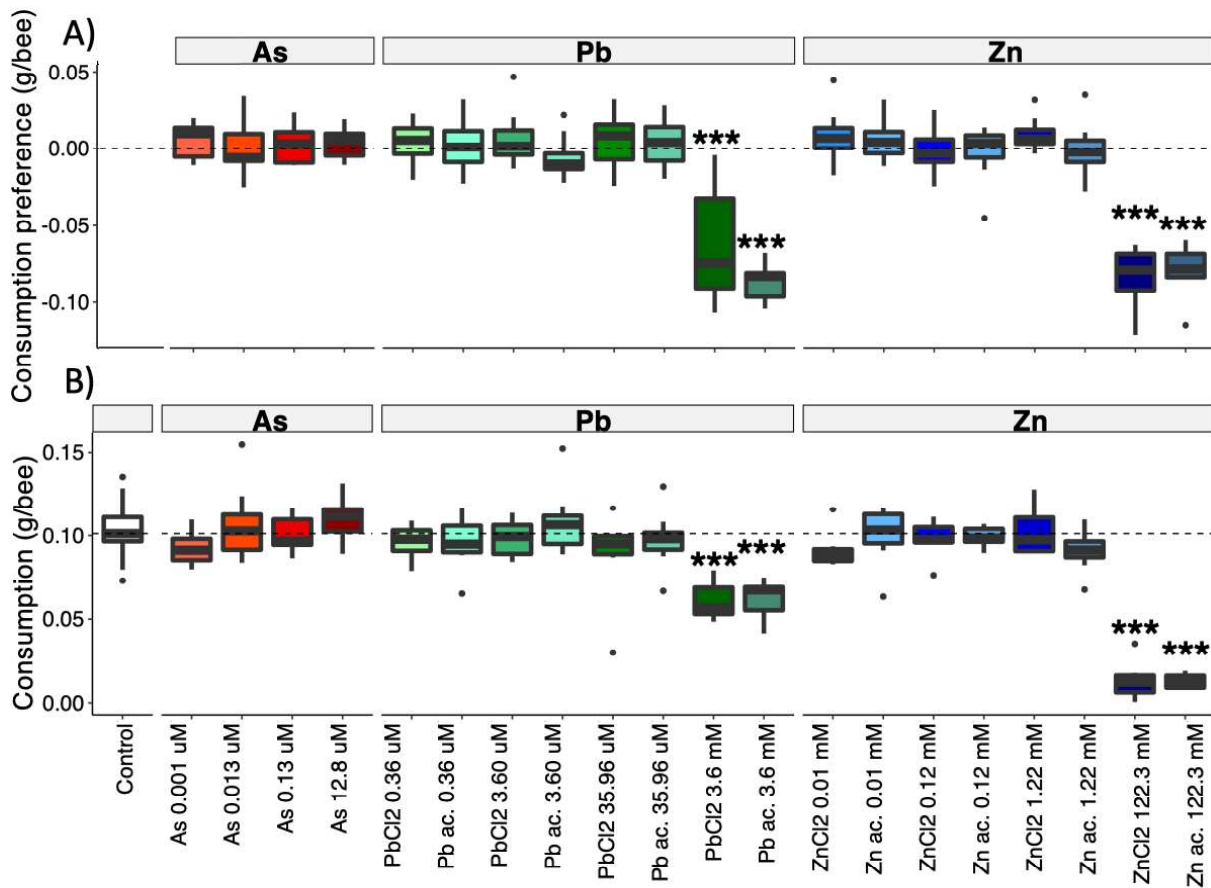
212 The highest concentrations of Zn salts (both chloride and acetate) in food were toxic, inducing  
213 high mortality after 24 h (Cox model:  $p < 0.001$  and  $p = 0.010$  respectively, Figure S1). Therefore,  
214 we compared food consumption across all treatments and for choice and no-choice feeding  
215 assays over the first 24 h only (Figure S2).

216 We first tested whether bees discriminated metals in food when given a choice between  
217 two accessible sucrose solutions, one of which contained one out of four concentrations of As,  
218 Pb or Zn (Figure 1A). None of the As solutions were avoided or preferred when compared to  
219 pure sucrose solution. Similarly, there was no difference in consumption of sucrose solutions  
220 containing low concentrations of Pb and Zn and pure sucrose (Table S2A). However, the  
221 highest concentrations of Pb (3.6 mM, LMM:  $p < 0.001$  for both chloride and acetate) and Zn  
222 (122.3 mM, LMM:  $p < 0.001$  for both salts) were consumed significantly less than pure sucrose.

223 We then tested whether bees would still avoid their consumption of metals in food when  
224 they had no alternative choice (Figure 1B). Bees showed similar consumption of food  
225 containing either As (all concentrations), low concentrations of Pb and Zn, or no metal salts  
226 (control) (LMM:  $p > 0.05$ ). However, they reduced their total food consumption by 40% when  
227 it contained the highest concentration of Pb (3.6 mM, LMM:  $p < 0.001$  for both salts), and by  
228 87% when it contained the highest concentration of Zn (122.3 mM, LMM:  $p < 0.001$  for both  
229 salts) (Table S2B). These effects were independent of the chemical forms (acetate or chloride)  
230 of Pb and Zn (LMM:  $p > 0.05$  for pairwise comparison for each concentration). For all metals,  
231 we found a positive significant correlation between the quantity of metal ingested and the  
232 quantity of metal bioaccumulated in the bees' bodies (As:  $R = 0.91$ ,  $p < 0.001$ ; Pb:  $R = 0.97$ ,  
233  $p < 0.001$ ; Zn:  $R = 0.82$ ,  $p < 0.001$ ; Figure S3), hence validating our exposure protocol.

234





235

236 **Figure 1: Feeding assays. A) Choice experiment.** Consumption preferences (difference in  
237 daily consumption between the two solutions) are plotted. Positive values: preference for the  
238 metal pollutant solution; zero (dotted line): no preference; negative values: preference for the  
239 pure sucrose solution. N = 8 cages of 20 bees per treatment. **B) No-choice experiment.** Daily  
240 food consumption of each solution; the dotted line indicates the median value for control bees  
241 (plain sucrose solution, white). N = 8 cages per treatment and N = 27 cages for control bees. In  
242 both experiments we used three metals (arsenic - red, lead - green, zinc - blue) at four  
243 concentrations each. Box plots show median (horizontal line), 25th to 75th percentiles (box),  
244 smallest and highest values within 1.5\*inter-quartile range of the hinge (error bars), and outliers  
245 (dots). \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ : differences with zero (A) or control bees (B), LMMs  
246 (Table S2).

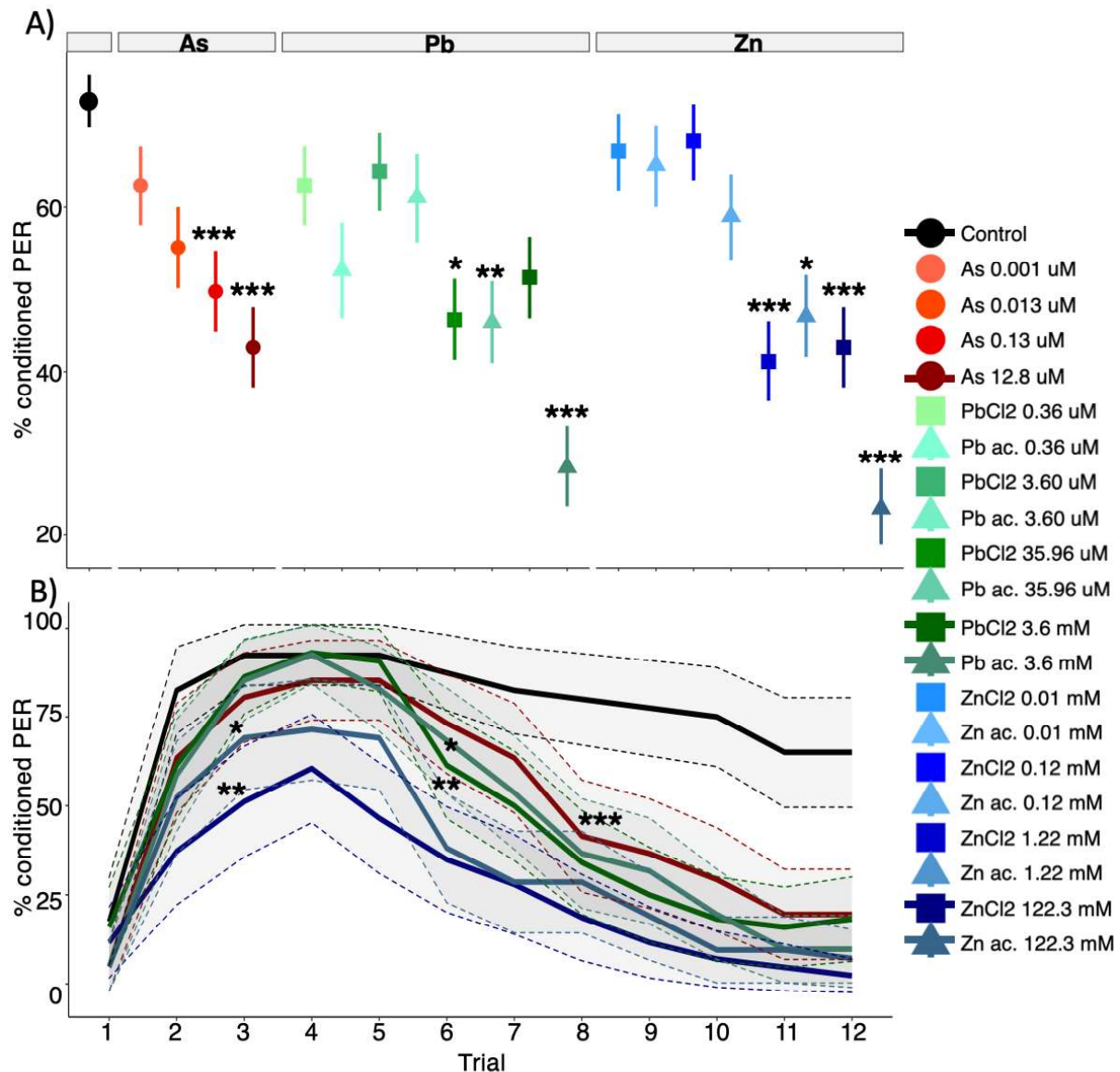
247

248 **(b) Bees perceived only high concentrations of the three metals, with their antennae and**  
249 **proboscis**

250 We tested whether bees were able to perceive metal salts through their antennae in a devaluation  
251 experiment. Since the conditioning odour had been associated with sucrose, we expected a  
252 progressive decrease of the rate of conditioned PER over subsequent unrewarded (water)

253 presentations in all groups. If bees perceive metal salts in water, the decrease of response to the  
254 metal solution should be stronger than with water. Overall, antennal stimulation with solutions  
255 containing metal pollutant salts affected PER responses (Figure 2A, Table S3). The mean PER  
256 rate was significantly reduced for the two highest concentrations of As (12.8  $\mu$ M, 0.13  $\mu$ M), Zn  
257 chloride and acetate (122.3 mM, 1.22 mM), Pb acetate (3.6 mM, 35.96  $\mu$ M) and only for the  
258 second highest concentration of Pb chloride (3.6 mM). We found no overall effect of the  
259 chemical form (acetate or chloride) of Pb or Zn (Binomial GLMM:  $p>0.05$  for pairwise  
260 comparisons for each concentration). Therefore, bees perceived the highest concentrations of  
261 each metal salt through their antennae and reduced their appetitive response.

262 We assessed whether bees were able to perceive metal salts through their proboscis  
263 and/or post-ingestive consequences (i.e. malaise-like state) using another devaluation  
264 paradigm, in which bees were trained to associate an odour with a sucrose presentation on the  
265 antennae (to induce PER) followed by delivery of a lower concentration of sucrose to their  
266 proboscis. Metals were diluted in sucrose instead of water to ensure their ingestion. Bees that  
267 received sucrose containing metals to the proboscis reduced their PER response more than  
268 controls (Figure 2B). Zn-treated bees showed significantly lower levels of PER as early as the  
269 3<sup>rd</sup> trial (Binomial GLMM:  $p=0.001$  for Zn chloride,  $p=0.036$  for Zn acetate). By contrast, the  
270 response levels of Pb and As groups initially reached a maximum similar to the controls. These  
271 response levels decreased from the 6<sup>th</sup> trial onwards with Pb (Binomial GLMM:  $p=0.009$  for  
272 chloride,  $p=0.044$  Pb acetate), and from the 8<sup>th</sup> trial onwards for As (Binomial GLMM:  
273  $p<0.001$ ). These effects were independent of the chemical forms of Zn and Pb (Tukey HSD:  
274  $p>0.05$ ). Thus, bees seemed to evaluate negatively all three metals, through their proboscis  
275 and/or post-ingestive effects, as eventually responded to all contaminated solutions by  
276 markedly decreased PER rates (GLMM: mean PER response:  $p<0.001$  for all treatments). The  
277 ingested volumes of metal pollutant solutions were not sufficient to impact their survival over  
278 the duration of the experiment (Figure S4).



279

280 **Figure 2: Devaluating effects of metal salts.** Mean conditioned proboscis extension response  
 281 (PER) and 95% confidence intervals (bars in A, shaded in B) across devaluation trials, for each  
 282 treatment. **A) Application on the antennae.** For lead and zinc, chemical forms are shown by  
 283 the mean point shape, square for chloride (Cl<sub>2</sub>) and triangle for acetate (C<sub>4</sub>H<sub>6</sub>O<sub>4</sub>). N = 35-41  
 284 bees/treatment. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001: binomial GLMM, compared to controls (N =  
 285 79)). **B) Application on the proboscis.** N = 40-42 bees/treatment. \*p<0.05, \*\*p<0.01,  
 286 \*\*\*p<0.001: differences with controls (N=40), displayed only for the first trial showing  
 287 significant differences (binomial GLMM).

288

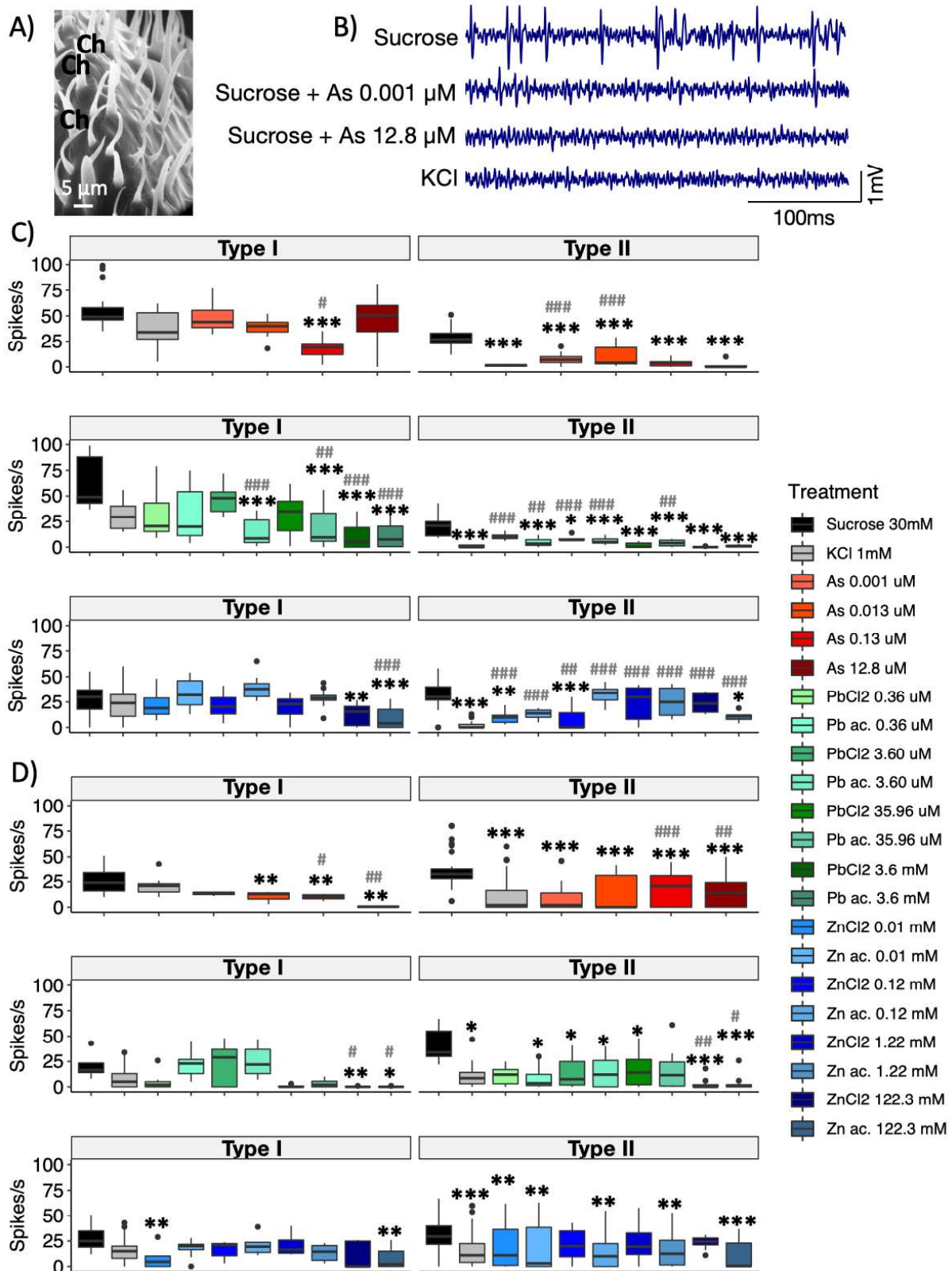
### 289 (c) Highly concentrated metals inhibit sucrose-evoked activity in taste receptors

290 We finally performed electrophysiological recordings to investigate the mechanisms by which  
 291 bees detect metal salts, focusing on neurons in antennal gustatory sensilla (Figures 3A-B),

292 which are mostly tuned to detect sugars and salts [29]. Irrespective of responses to metal  
293 pollutant salts, we identified two main response profiles that lead us to distinguish two  
294 functional categories of sensilla: those responding to both sucrose and KCl (Type I, 722  
295 recordings) and those responding to sucrose only (Type II, 953 recordings).

296 We recorded electrophysiological responses to ascending concentrations of each metal  
297 pollutant salt diluted in 30% mM sucrose (Figure 3C). Some sensilla responded equally to both  
298 sucrose and KCl (Type I sensilla), but showed a drop in spike frequency in response to high  
299 metal concentrations. This is a specific response to metal salts since adding a nutrient salt such  
300 as KCl to sucrose had the opposite effect (Figure S5). Other sensilla responded much more to  
301 sucrose than to KCl (Type II sensilla) and showed a similar reduction in their activity in  
302 response to all metal pollutant salts, when compared to pure sucrose. Overall, the chemical form  
303 of Pb or Zn had no effect (GLMM:  $p > 0.05$ ; except for 3.60  $\mu\text{M}$  Pb on type I sensilla:  $p < 0.001$ ;  
304 and 0.12m Zn on type II sensilla:  $p < 0.001$ ). Thus, the presence of metal pollutant salts at high  
305 levels in sucrose solution could be detected by antennal gustatory neurons, which reduced their  
306 activity.

307 We then asked whether metal salts could be detected independently of the presence of  
308 sucrose, and thus used water solutions as stimuli (Figure 3D). Type I sensilla responded to low  
309 concentrations of all metal salts similarly to KCl or sucrose. By contrast, they reduced their  
310 spike frequency when stimulated with high metal concentrations, as compared to both KCl and  
311 sucrose. Type II sensilla failed to show marked activity in response to most metal solutions, as  
312 they did for KCl. Thus, metal salts did not trigger a specific response pattern by themselves,  
313 but rather reduced sucrose-triggered responses when added at high concentration to the sucrose  
314 solution.



315

316 **Figure 3: Electrophysiological recordings of the gustatory neurons from the antennae. A)**

317 Scanning electron microscope picture of the surface of the antenna showing chaetic sensilla

318 (Ch) chosen for recording. **B)** Examples of spike trains recorded from a type II sensilla in

319 response to various stimuli. Note the decreased spike frequency induced by the presence of As

320 in the sucrose solution. **C, D**) Boxplots of the spiking responses to sucrose (black), KCl (grey),  
321 and increasing concentrations of arsenic (red), lead (green) and zinc (blue), for a type I sensilla  
322 (responding to both KCl and sucrose, left) or a type II (responding to sucrose only, right). **C**)  
323 Stimulation with metal salts diluted in sucrose solution (As: N = 4; Pb: N = 5; Zn: N = 6). **D**)  
324 Stimulation with metal salts diluted in water (As: N = 4; Pb: N = 5; Zn: N = 6). (#/\*p<0.05,  
325 \*\*p<0.01, \*\*\*p<0.001: differences with sucrose (star) or KCl (hash) (pairwise comparisons  
326 following GLMM).

327

#### 328 **4. Discussion**

329 Pollinators are impacted by metal pollutants [5,18,51,52]. Here we showed that bees have only  
330 a limited capacity to detect and avoid these poisons in food. Honey bees perceived very high  
331 (unrealistic) concentrations, of Pb and Zn through their proboscis and antennae, and avoided  
332 ingesting them. Sucrose containing concentrated As was detected, but still consumed. Lower,  
333 yet harmful, field-realistic concentrations of the metal pollutants were neither avoided nor  
334 detected in our experimental conditions. Electrophysiological recordings from gustatory  
335 neurons confirmed that bees can only taste a limited concentration range of these metal  
336 pollutants.

337 Bees avoided Zn and Pb (but not As) at high concentrations above most environmental  
338 levels, even in the absence of alternative food sources. This observation is consistent with  
339 previous reports of decreased food consumption following exposure to high Zn or Pb levels  
340 [15,18,53]. However, honey bees ingested sucrose solutions containing all three metal salts at  
341 concentrations similar to those found in nectar [54,55]. While Zn at low concentrations is an  
342 essential micronutrient, Pb and As can be highly toxic [36]. The absence of any behavioural  
343 responses to these solutions at field relevant concentrations thus suggests honey bees are  
344 incapable of discriminating between essential and toxic metals. This is consistent with studies  
345 reporting indiscriminate visits on metal-contaminated flowers [19,31,56]. At these realistic  
346 concentrations, metal pollutants alter the development [15], learning and memory [51,52], the  
347 metabolism [16,17] and antioxidative responses [57].

348 Stimulations of gustatory organs with metal solutions showed high concentrations were  
349 perceived through the antennae and the proboscis. This devaluating effect occurred with  
350 antennal stimulation only. It is thus independent of post-ingestive effects such as those observed  
351 with other toxic substances [20,23,24]. While methodological differences make difficult a  
352 direct comparison of devaluation responses with and without ingestion, they did not seem to be

353 much stronger when metals were delivered to the proboscis and ingested, indicating that post-  
354 ingestion effects (if any) would have been minimal.

355         The detection of metals by taste receptors was sufficient to reduce appetitive behaviour.  
356 The decreased responsiveness to repeated stimulations with contaminated sucrose on taste  
357 receptors likely results from a mismatch between expected and obtained rewards, possibly  
358 because peripheral detection of metals actively inhibited appetitive behaviour and/or because  
359 sucrose-sensitive taste receptors were inhibited. Both mechanisms have been involved in the  
360 feeding suppression triggered by plant-derived deterrents [26], but electrophysiological data  
361 was lacking to confirm the implication of either process in these and previous behavioural  
362 effects of metal pollutants [18,53]. Here, concentrated Pb, As and Zn decreased sucrose-evoked  
363 spike frequencies in bee taste receptors' response to sucrose. Importantly, this effect was due  
364 to the metals themselves as it was observed irrespective of the salts used (acetate or chloride),  
365 and in a different concentration range as for common salts (e.g. KCl). By contrast, we found no  
366 clear evidence of specific detection systems, consistently with the limited molecular repertoire  
367 of gustatory receptors in this species [58]. Thus, reduced neural activity might result from non-  
368 specific toxic effects, such as oxidative stress and ion channel dysfunction [59,60]. While the  
369 exact mechanism remains to be determined, very high metal concentrations rarely encountered  
370 (even in polluted environments) can trigger rejection of feeding sites that would be toxic at  
371 short term, as already observed for naturally deterrent compounds (e.g. bitter substances)  
372 [21,61]. However, since it is not observed for field-relevant doses of metals, it is unlikely to  
373 have been selected, contrary to the anti-feeding action of many phytochemicals acting as a plant  
374 defence mechanism against phytophagous insects [26].

375

#### 376         **4. Conclusion**

377         Our study echoes to the recent findings that bees cannot detect harmful insecticides  
378 through taste [40,62]. It calls for further research to better characterize the response of bees to  
379 heavy metal pollutants, including to combinations of different elements [56]. Since metal  
380 pollutants are undetected and consumed by bees, low amounts can bioaccumulate, which may  
381 lead to long-term detrimental effects on individuals and colony health [63]. Evidence of hazards  
382 of heavy metals on terrestrial wildlife worryingly accumulate [2]. It has become an urgent issue  
383 to account for such effects in order to adjust permissible levels of environmental metal pollution  
384 accordingly [2].

385

386

387

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## 399 **Author contribution**

400 CM, MGBS, AE, ABB, ML and JMD designed the study. CM, MGBS, LL, OB, GM, JS, GLR  
401 and DB collected the data. CM analysed the data and wrote the first draft of the manuscript. All  
402 authors reviewed the manuscript.

## 403 **Competing interest statement**

404 The authors declare no competing interests.

## 405 **Data availability**

406 Raw data are available in Dataset S1.

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