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1 **Trophic transfer of heavy metals along a pollution gradient in a terrestrial agro-industrial**
2 **food web**

3

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22 **Abstract**

23

24 Heavy metal contamination across the food web is a growing concern because of increasing
25 environmental discharges in industrial zones, atmospheric transport, and deposition and erosion
26 during rainfall events. We examined the transfer pathways of chromium (Cr) and nickel (Ni)
27 through a terrestrial trophic web and investigated the potential for their bioaccumulation along
28 the trophic chain. Soil, plants, arthropods, and vertebrates were sampled from different localities
29 in the south of Cairo (El-Tebbin, Egypt) and the amounts of Cr and Ni from these samples were
30 measured. We also computed a body condition index (BCI) for vertebrates to estimate individual
31 health and fitness levels in relation to heavy metal concentrations in the liver. The levels of Cr and
32 Ni varied significantly among the samples. Lower trophic levels showed a tendency for
33 biomagnification, while higher trophic levels showed possible biodilution of the two heavy metals:
34 arthropods, amphibians, and lizards concentrated more Cr and Ni than the other taxonomic
35 groups; conversely birds and small mammals generally showed lower levels of Cr and Ni. A
36 negative relationship was obtained when the concentrations of Cr in the soil, plant, and
37 arthropods, and the concentrations of Ni in the wolf spider were plotted as a function of the
38 distance to the industrial area. A significant inverse relationship was found between the Ni
39 concentration of liver and body length, while body mass had no significant effect. Our study thus
40 highlights the varied effects of heavy metal concentrations across a complex food web at different
41 distances from the pollution source, and the need for further studies of their effect on multiple
42 species in an ecosystem.

43 **Keywords:** Bioaccumulation; Biodilution; Soil; Plant; Arthropod; Vertebrate

44 1. Introduction

45 The Anthropocene era (Lewis and Maslin, 2015a,b) is associated with significant changes in
46 climatic conditions, which, together with the over-consumption of natural resources, will cause
47 irreversible environmental impacts (Ripple et al., 2019). The degradation of habitats, either by
48 direct human exploitation or through anthropogenically induced pollution, is one of the main
49 factors that is currently threatening biodiversity (Maxwell et al., 2016; Horváth et al., 2019). In
50 particular, pollution can have far-reaching consequences, affecting environmental characteristics
51 by contaminating atmosphere, soil (McGrath et al., 2017; O’Kelly et al., 2021), water (Abu Salem
52 et al., 2017; Andrade et al., 2018; Husk et al., 2019), and exposing all living organisms to a large
53 range of potential stressors, including plastics, heavy metals, pesticides, or pharmaceuticals (Picó
54 et al., 2020). Moreover, pollutants can be transported over large distances (Pokhrel et al., 2018;
55 Zhang et al., 2019), meaning that species and environments from less anthropogenically disturbed
56 regions, *i.e.* regions where urbanization, agriculture or industries are less developed (Obbard,
57 2018), are also exposed to pollution. In sum, the major transformations of ecosystems by human
58 activities result in rapid biodiversity erosion, geographic redistribution of species, and biotic
59 homogenization, which, in combination, might result in Earth’s sixth mass extinction (Dirzo et al.,
60 2014).

61 Investigations revealing the effects of pollutants on the life history of organisms, including
62 longevity, reproductive success, developmental rates, the underlying physiological and genomic
63 impacts, and the cascading consequences on population dynamics, are of considerable interest.
64 Such studies improve our understanding of the ecological effects of pollution on wildlife (Saaristo
65 et al., 2018). So far, a range of works have examined the effects of pesticides (Dewer et al., 2016;
66 Serra et al., 2020; Engell Dahl et al., 2021), heavy metals (Berni et al., 2019; Cosio and Renault,
67 2020), pharmaceuticals (Prud’homme et al., 2018; Renault et al., 2018) or polymers (de Souza
68 Machado et al., 2018; Kögel et al., 2020) on the biology and physiology of plants and insects. In

69 addition to describing the conspicuous impacts on growth and development, these investigations
70 have revealed the latent effects pollutants can have on the ecology and biology of wildlife. The
71 insidious nature of xenobiotics toxicity includes, for instance, deleterious effects of antibiotics on
72 the muscle structure of flies (Renault et al., 2018), impacts of ibuprofen on the development of
73 mosquitoes (Prud'homme et al., 2018), or cryptic effects of low doses of pesticides on plant
74 metabolism (Serra et al., 2013) and animal behavior (Desneux et al., 2007; Hanlon and Relyea,
75 2013).

76 When absorbed by living organisms, pollutants can be bioaccumulated, as is the case of
77 several heavy metals. For instance, cadmium, chromium, cobalt, copper, manganese, and zinc can
78 be bioaccumulated in leaves of trees and in several plants (Hu et al., 2014; Parihar et al., 2021).
79 By being bioaccumulated in the tissues of organisms, the pollutants can be further transferred
80 along the food chain, resulting in their biodilution or biomagnification depending on the
81 environmental context and physiochemical features of the contaminated species. For instance,
82 biodilution was reported for arsenic and nickel in marine food webs (Sun et al., 2020), for
83 cadmium, chromium, copper, and lead in a Chinese lagoon (Hu et al., 2021) and in higher trophic
84 level organisms in terrestrial food webs (Zhang et al., 2021). So far, biomagnification across
85 trophic levels has been evidenced for many different pollutants, including persistent organic
86 pollutants (Kelly et al., 2007), polymers and more particularly plastics (Miller et al., 2020), or heavy
87 metals (Ali and Khan, 2019).

88 Heavy metal contamination along food webs is of a growing concern, owing to
89 environmental discharges in industrial zones resulting in high metal concentrations in soil and
90 biota (Dudka et al., 1996), atmospheric transport and deposition, and erosion during rainfall
91 events. Existing research evidenced the large range of impacts of these pollutants in terrestrial
92 organisms, including alteration of enchytraeid communities (Kapusta and Sobczyk, 2015),
93 oxidative stress in the white garden snail (Radwan et al., 2010) and the black smith tree frog

94 [\(Zocche et al., 2014\)](#), alteration of biomolecules in the leguminous plant *Pisum sativum* [\(El-Amier](#)
95 [et al., 2019\)](#) and elicitation of stress response proteins synthesis in plants [\(Hasan et al., 2017\)](#). The
96 diversity of living organisms and trophic guilds exposed to heavy metals [\(Grześ, 2010; Żmudzki](#)
97 [and Laskowski, 2012; Fuentes et al., 2020\)](#) requires additional research efforts to better
98 understand and describe the way heavy metals can affect organisms across trophic levels. It is
99 crucial to consider the effects of heavy metal pollution in a trophic web context, as the pollutants
100 may have little to no effects on primarily exposed species, while their effects may increase along
101 the food chain. This bioaccumulation and/or biomagnification may ultimately place top predators
102 at higher risks.

103 In this work, we examined the transfers of chromium and nickel through a terrestrial
104 trophic web and investigated the potential for bioaccumulation of these pollutants along the
105 trophic chain. To that aim, soil, plants, invertebrates, and vertebrates were sampled in different
106 localities to the south of Cairo (El-Tebbin, Egypt). This region represents 17% of the Egyptian
107 industrial activity [\(Soliman et al., 2017\)](#), with petroleum coke and cement factories, metallurgy
108 and ceramics and a range of other industrial activities [\(Gomaa et al., 2020\)](#). Previous studies in
109 this region have already reported large amounts of heavy metals from soil samples, in particular
110 cadmium, lead, copper and zinc [\(Soliman et al., 2019; Gomaa et al., 2020\)](#). In addition, [Soliman et](#)
111 [al. \(2019\)](#) showed that cadmium, copper, lead, and zinc are accumulated in plants and their
112 associated herbivorous insects (grasshoppers and mantids), while these metals tended to be
113 diluted when measured from vertebrates. Yet, the effects of chromium and nickel were not
114 considered by [Soliman et al. \(2019\)](#), despite the relatively high concentrations they can reach in
115 Egyptian soils [\(Abdel-Sabour and Zohny, 2004; Said et al., 2019\)](#). The levels of chromium and
116 nickel released in the environment has increased with industrial activities, and more particularly
117 with the manufacture of stainless steel [\(Tchounwou et al., 2012\)](#). Here, we investigate if heavy
118 metals (chromium hereafter referred to as Cr, and nickel hereafter referred to as Ni) were

119 enriched in terrestrial species from one trophic level to the next and analyze if trophic transfer
120 behavior changes according to soil metal concentrations or distance to the pollution source
121 (industrial area). We hypothesize that high Cr and Ni concentrations will be measured from soil
122 samples collected near industrial activities, while being further absorbed and accumulated by
123 plants. We further expect that these pollutants would be transferred along the trophic web, in
124 particular to herbivorous insects, but also to higher trophic levels. First, we measured the
125 concentrations of Ni and Cr in the soil from seven localities of the El-Tebbin region, starting from
126 the supposedly pollution source, and from gradually distant sites. We assumed that the Ni and Cr
127 values would decrease from the pollution site onwards, providing an in-field dose-response
128 assessment of the effect of the metal concentrations on the food web. In this context, we
129 assumed that the organisms thriving at the proximity of the pollution source would be
130 characterized by the higher Cr and Ni tissue contents for the less mobile species, while species
131 having a larger mobility, and thus a higher foraging range, should be characterized by metal
132 concentrations independent of the sampling distance to the pollution source. As heavy metals are
133 known to affect physiology and life history traits of living organisms, we computed an index of
134 body condition for resident vertebrates, including amphibians, reptiles, birds, and rodents, to get
135 an estimate of individual health and fitness levels in relation to Cr and Ni concentrations in the
136 liver of these animals.

137

138 **2. Material and methods**

139 **2.1. Study area**

140 The studied sites are located in El-Tebbin (Helwan Province, East of the Nile River), an area located
141 at 20 km to the south of Cairo (Egypt) (**Supplementary Material 1**). This area is one of the largest
142 industrial zones of Egypt, covering 43100 hectares. The main industrial activities that can be found
143 in this zone include ferrous and nonferrous metallurgical work, a petroleum coke factory,

144 chemical and cement industry (Soliman and El-Shazly, 2017). The area is surrounded by
145 agricultural production, in the form of different crops and vegetables including cultures of alfalfa,
146 beans, eggplant, maize, okra, pepper, potato, and squash (Supplementary Material 1). The
147 experimental work presented in this study was conducted in October 2020.

148

149 **2.2. Sample collection and preparation**

150 The samples were collected from 5 x 20 m sampling plots in grass strips along the field margins of
151 seven farmlands, which were up to 10 km downwind from the main industrial pollution source.
152 One plot was sampled for each of the seven studied locality, and the distance among the plots
153 ranged from 500 to 3000 m (Supplementary Material 1, Table 1). In order to have the best
154 possible understanding of the effects of pollution on the food web, the sampling design
155 encompassed different collection techniques in order to encompass a variety of taxa having
156 different trophic regimes and mobilities/displacement capacities. To that aim, four topsoil
157 samples (0–15 cm) were taken from each of the seven studied sites. In the laboratory, each soil
158 sample was thoroughly mixed, and 1 kg from each mix was used for the subsequent analyses.

159 Leaves and stems from 10–15 *Paspalum distichum* L. individuals, which is the most
160 abundant wild plant growing at El-Tebbin, were randomly sampled and directly stored in
161 polythene bags. In the laboratory, the collected plant material was washed with deionized water
162 to remove metals stuck at the surface of the leaves and stems.

163 Arthropods were collected from the grass (*P. distichum*) with sweep nets and by hand.
164 The study focused on the long-faced grasshopper (*Truxalis grandis*), the Egyptian mantis
165 (*Miomantis paykullii*) and the wolf spider (*Hogna ferox*) as they were the most abundant
166 arthropods in the plot, and representative of the herbivore and carnivore trophic guilds. In
167 addition, both the spider and mantis prey on grasshopper, and the wolf spider and the Egyptian
168 mantis can potentially prey on each other. The number of sampled arthropods is presented in

169 **Supplementary Material 2** for each of the seven studied locality. The collection was interrupted
170 when the whole surface of the plot was covered by sweep nets and visual inspection. In some
171 localities, the target arthropod species could not be caught, *i.e.* the Egyptian praying mantis in the
172 localities A, E and F (**Supplementary Material 2**). At all studied sites, the sampling took place
173 between 10:00 am and 2:00 pm. The collected individuals were brought back to the laboratory
174 and euthanized by freezing (Mohamed et al., 2013). These collected Palearctic arthropods,
175 following zoogeographic boundaries in Sharaf et al. (2020), were identified after Marabuto (2014),
176 Cigliano et al. (2021), and WSC (2021).

177 Vertebrates, amphibians, and reptiles were hand collected from the grass strips along the
178 edges of the seven farmlands (For each locality, the number of sampled animals is detailed in
179 **Supplementary Material 2**) and were killed with chloroform. Rodents were collected in Sherman
180 live traps, baited inside with peanut butter; each collection site was surveyed for two consecutive
181 nights, and traps were checked every morning. Finally, birds were caught by bownet traps, which
182 were containing mice as attractant. We sacrificed birds and small mammals by CO₂ inhalation.
183 Only the minimum number of individuals required for the analysis was collected and sacrificed.
184 All steps were conducted as stated in AVMA guidelines for the euthanasia of animals (Leary et al.,
185 2013) and after approval, ID#: CU 1-F-50-20, from the University of Cairo's animal ethical
186 committee.

187

188 **2.3. Morphometric analyses and index of body condition of vertebrates**

189 For each individual vertebrate, the body length (BL) of the animal was measured to the nearest
190 0.01 mm with calipers. For amphibians and reptiles, body length was recorded as the snout-vent
191 length (SVL). Body length was measured from the tip of the bill and the tip of the tail in birds, and
192 from nose to anus in rodents. Body mass (BM) was measured with a portable electronic balance
193 (Model 30064413, Ohaus Scout Pro; accuracy: 0.01g). After measurements, all vertebrates were

194 dissected, and the liver of each animal was weighed, transferred individually into labeled plastic
195 containers, and stored at -20 °C prior to being used for subsequent chemical analyses.

196 The index of body condition (BCI) was computed as the ratio of observed to expected
197 values of body mass, as described by [Nunes et al. \(2001\)](#). The expected mass was derived from a
198 linear regression between log BM and log BL. In this relationship, individuals with BCI values
199 greater than 1 are considered in better condition than expected, while the reverse conclusion
200 applies when BCI is lower than 1.

201 Soil, plants, arthropods, and liver samples were oven-dried (60 °C) for 4 days. Then, they
202 were ground to a homogenous powder and kept dry in polythene bags. The number of replicates
203 per site and per species, and for soil samples, ranged from 3 to 6. For arthropods, individuals
204 were pooled for the analysis so that a dry mass of 0.5 g was obtained for each replicate and each
205 species. In the case of the wedge-snouted skink (lizards, *Chalcides sepsoides*), flowered racer
206 (snakes, *Coluber florulentus*), birds (all species), and small mammals (all species), it was very hard
207 to sample a large amount of individuals, which would have additionally impacted their population
208 dynamics. As small sample size (<3 individuals per site) were obtained for these species, two to
209 three replicates were obtained from a single individual to ensure that we would have 3 to 6
210 measures. We acknowledge that this is a low sample size, and the resulting within-individual
211 measurements of Ni and Cr across-individuals as compared with the other group constitute one
212 caveat of our study. Yet, because of the low abundance of these individuals and ethical
213 considerations (number of wildlife specimens that can be captured and handled according to their
214 field abundance and density), it was the only possibility that allowed us to gain information from
215 these groups.

216

217

218 **2.4. Heavy metal analysis**

219 Before starting the assays, the flasks were soaked in 2N nitric acid overnight, and then abundantly
220 rinsed with deionized water. Digestion of plant and animal samples (whole arthropods and livers
221 of vertebrates) was conducted according to the method described by Moor et al. (2001) and
222 Soliman et al. (2019). Briefly, samples (0.5 g each: soil samples, whole leaves for plants, whole
223 individuals for arthropods, or liver for vertebrates) were placed in a 25 mL flask and pre-digested
224 with a volume of 10 mL of 65% HNO₃ for 24 h at room temperature. The suspension was then
225 digested to near dryness on a thermostatically controlled hotplate at 90 °C in a fume cupboard,
226 before the adding of 2 mL of 30% H₂O₂ which aids the digestion of the organic matter. The flask
227 walls were then washed with 10 mL of deionized water. The suspension was filtered through
228 Whatman filter paper (No. 41) in a volumetric flask, diluted to 25 mL and stored in polyethylene
229 bottles at 4 °C until being used for the analyses.

230 Soil digestion was conducted in a similar way, but samples were allowed to stand for 24 h
231 with 12 mL of a mixture made of 37% HCl: 65% HNO₃ (3:1). Then, volumes of 2.5 mL of 37% HCl
232 and 2.5 mL of 30% H₂O₂ were added to complete the digestion of soil samples. The filtered
233 solutions were diluted in a 50 mL volumetric flask so that the final volume of each sample reached
234 50 mL. Inductively coupled plasma (ICP-AES-Jobin Yovin ultima2, France) was used to measure the
235 concentrations of Cr and Ni from each sample. Wavelengths and detection limits of the ICP for
236 the measured elements were: 205.552 nm and 0.0041 mg L⁻¹ for Cr; 231.604 nm and 0.01 mg L⁻¹
237 for Ni. A reagent process blank and a certified reference material (standard reference materials
238 from the National Institute of Standards and Technology (NIST), USA: NIST 1547, 1577 and 2709
239 for plant, animal tissues and soil, respectively) were carried out for each analytical batch to ensure
240 the accuracy of the measures. Mean values of three replicates were calculated for each
241 measurement. Chromium and Ni concentrations were expressed as mg kg⁻¹ dry mass.

242

243 **2.5. Data analyses**

244 Chromium and Ni concentrations from plants, arthropods, and vertebrates were log-transformed.
245 Homogeneity of variances (Levene, F-test) and normality (Shapiro–Wilk test) of the data were
246 tested prior to statistical analyses. One-way analysis of variance (ANOVA) was computed to
247 investigate if there were differences in the concentrations of Cr or Ni among the studied taxa;
248 ANOVAs were followed by post-hoc comparisons between taxa. As multiple comparisons were
249 performed, the Bonferroni-Holm method was used. A general linear mixed model was used to
250 determine the factors (species, BM, BL, and BCI) that could be associated with different Cr and Ni
251 concentrations in the livers of dissected vertebrates. The collection site of the samples was added
252 as a random factor in our analyses. Pearson’s correlation coefficients (r) values were calculated
253 to determine whether the correlation between metal concentration in livers of vertebrates and
254 BCI was statistically significant. Correlation analysis of Cr and Ni concentrations in soil, plant, and
255 animal species (long-faced grasshopper, wolf spider, house mouse, and Norway rat) and the
256 distance from the industrial area was considered. All statistical tests were carried out using the
257 IBM SPSS Statistics Version 22 (IBM Corp. Armonk, NY, USA). Significant differences were accepted
258 at $p < 0.05$.

259

260 **3. Results**

261 **3.1. Chromium and nickel in soil, plants, and animals**

262 Concentrations of Cr and Ni in the soil, plants, arthropods, and vertebrates collected from the
263 seven localities of El-Tebbin (Egypt) are presented in **Table 1**, **Figures 1** and **2**, respectively (also
264 see **Supplementary Material 3 and 4**). When compared with the proposed threshold value of Ni
265 toxicity to plants in the European Union ([Adriano 2001](#), [Tóth et al., 2016](#); threshold value for Ni:
266 50 mg/kg soil), the soil samples we collected from El-Tebbin exhibited 1.3–1.6 times higher Ni
267 concentrations (**Table 1**). Conversely, the measured Cr concentrations were below the threshold
268 values (threshold value for Cr: 100 mg/kg soil) in the seven studied sites of El-Tebbin (**Table 1**). In

269 general, levels of soil Cr and Ni decreased with distance to the industrial area (**Table 1, Figures 3**
270 **and 4**), from 97.8 ± 9.2 to 63.5 ± 3.0 mg/kg soil, and from 80.0 ± 5.6 mg/kg soil for Cr and Ni,
271 respectively.

272 Significant differences were reported for the levels of Cr ($F = 111.483$, $df = 17$, $p < 0.0001$)
273 and Ni ($F = 149.748$, $df = 17$, $p < 0.0001$) measured from soil, plants, and animal species (**Table 1,**
274 **Figs. 1 and 2, Supplementary Material 3 and 4**). In general, the highest amounts of Cr and Ni were
275 measured from the wolf spider (*Hogna ferox*) for arthropods. For vertebrates, the concentrations
276 of Cr and Ni significantly varied with respect to species ($F = 37.7$, $df = 12$, $P < 0.001$ for Cr, $F = 47.5$,
277 $df = 12$, $P < 0.001$ for Ni). The highest amounts of Cr and Ni were found in the Egyptian toad
278 (*Amietophrynus regularis*), and high amounts of Cr were also measured from the forskal sand
279 snake (*Psammophis schokari*). The lowest concentrations of Cr were detected in the little owl
280 (*Athene noctua*), in the cattle egret (*Bubulcus ibis*) and in rats (*Rattus norvegicus*), and the lowest
281 concentrations of Ni were found from the little owl and the cattle egret (**Figs. 1 and 2, Tables 2**
282 **and 3, Supplementary Material 3 and 4**). Overall, the species ranked according to their Cr
283 concentration as follows: wolf spiders *Hogna ferox* (Lucas), 19.6 ± 5.6 mg Cr /kg > Forskal sand
284 snake, *Psammophis schokari* (Forskål), 14.7 ± 1.7 Cr mg/kg > Egyptian toad, *Amietophrynus*
285 *regularis* (Reuss), 13.4 ± 3.2 mg Cr/kg (**Figs. 1 and 2, Supplementary Material 3**). For Ni
286 concentrations, the species ranked as follows: wolf spider (23.0 ± 6.8 mg/kg) > Egyptian mantis
287 (*Miomantis paykullii* Stål) (16.0 ± 3.1 mg/kg) > Egyptian toad (11.1 ± 2.3 mg/kg) (**Figs. 1 and 2,**
288 **Supplementary Material 4**).

289 A negative relationship was obtained when the concentrations of Cr in the soil, plant, and
290 animals were plotted as a function of the distance to the industrial area (**Fig. 3**). The correlation
291 was not statistically significant for the house mouse and the Norway rat ($r = -0.100$, $P = 0.873$; r
292 $= 0.029$, $P = 0.957$, respectively). For Ni, the correlation was only significant for the wolf spider (r
293 $= -0.929$, $P < 0.01$). For vertebrates, the analyses revealed that the liver concentrations of the two

294 metals significantly varied with respect to collection site ($F = 3.4$, $df = 6$, $P < 0.01$ for Cr, $F = 2.9$, df
295 $= 6$, $P < 0.01$ for Ni). Lower concentrations of Cr and Ni were measured from the vertebrates from
296 the reference site (site G), while the two metals were characterized by increasing amounts in the
297 livers of the specimens that were collected closer to the pollution source (except for sites E and F
298 for Cr amounts, and site E for Ni amounts). This pattern was supported by significant differences
299 among values of the reference site (site G) and site A in the case of Cr ($P < 0.01$), and among the
300 reference site and sites A, C, and D in the case of Ni ($P < 0.050$, 0.05 , and 0.01 , respectively) (**Tables**
301 **2 and 3**).

302

303 **3.2. Accumulation of chromium and nickel in the liver of vertebrates, and its relationship with** 304 **body length, size, and body condition index**

305 To assess the potential existence of a relationship among the body mass, body length, and BCI of
306 the studied species, and the concentrations of Ni and Cr, we restricted this part of the work to
307 vertebrates whose livers could be dissected and processed. The descriptive statistics for body
308 measurements of vertebrate species collected from the seven studied localities of El-Tebbin area
309 are given in **Table 4**.

310 Body mass (**Tables 2 and 3**) had no significant effect on the measured metal
311 concentrations from vertebrate livers ($P > 0.05$). However, a significant inverse relationship was
312 found between Ni concentration of liver and body length ($F = 7.5$, $P < 0.01$) (**Tables 2 and 3**).

313 Pearson correlation analyses showed significant positive relationships between BCI, and
314 Cr and Ni concentrations measured from amphibian livers (Cr: $r = 0.632$, $p < 0.001$; Ni: $r = 0.693$,
315 $p < 0.001$) and Cr measured from bird livers ($r = 0.533$, $p = 0.013$). In contrast, Cr concentrations
316 measured from reptile and rodent livers were negatively correlated ($r = -0.283$, $p = 0.046$; $r = -$
317 0.632 , $p < 0.001$, respectively) with BCI indicating that reptiles and rodent species with higher

318 concentrations of trace elements (Cr and Ni) are characterized by impaired body conditions (**Fig.**
319 **4**).

320

321 **4. Discussion**

322 In this comprehensive study, we examined the level of soil pollution by the heavy metals Cr and
323 Ni as a function of the distance to the main pollution source, and the cascading effects this
324 pollution can have on the bioaccumulation of these two metals in the flora and fauna at different
325 trophic levels. We found evidence of heavy metal contamination in all species tested in this
326 complex food web, with biomagnification at lower trophic level (**Supplementary Material 5-8**),
327 primarily among invertebrates – with maximum values being measured from the wolf spiders
328 known as generalist predators. Possible biodilution was then reported at higher trophic levels
329 among vertebrates (**Supplementary Material 5-8**). In addition, our results suggest that the
330 concentrations of heavy metal in the tissue of almost all organisms tested decrease with distance
331 from the pollution source.

332

333 **4.1. The fate of heavy metal pollution in the environment**

334 We found that the soils from the El-Tebbin region are characterized by Ni amounts which always
335 exceeded the threshold values of trace elements reported for Ni (50 mg/kg) from agricultural soils
336 of the European Union ([Tóth et al., 2016](#)), this value. Similar Ni values were reported by [Said et al. \(2019\)](#), who additionally demonstrated the anthropogenic contribution in the available
337 fraction of soil Ni at El-Tebbin (Egypt). Importantly, the phyto-availability of Ni is known to greatly
338 vary with soil characteristics, in particular pH ([Kukier et al., 2004](#); [Adamczyk-Szabeal et al. 2015](#)),
339 and Ni values exceeding 30 and 75 mg/kg for acidic and neutral alkaline soils, respectively are
340 considered toxic for the plants (European Directive 86/278/EEC). In this work, we did not measure
341 the pH of the soil; yet all values are higher than maximum admissible Ni concentrations for acidic
342

343 soils, and three out the seven measured concentrations are close – or even exceed – maximum
344 Ni concentrations for neutral alkaline soils. Even if the Dutch target value (Dutch pollutant
345 standards) is as high as 210 mg/kg) for Ni ([Dutch Target and Intervention Values, 2000](#)), it is crucial
346 to bear in mind that toxicity level can greatly vary with plant or animal species, developmental
347 stage, or duration of exposure. As a result, depending on the toxicity risk, the permissible Ni
348 concentration will vary from one region to another, as is the case in Poland where Ni permissible
349 values ranging from 35 mg / kg soil to 300 mg / kg soil ([Barańkiewicz and Siepaks, 1999](#)). Finally,
350 the Dutch value reports a Ni target value of 35 mg/kg soil ([Dutch Target and Intervention Values,](#)
351 [2000](#)).

352 The seven El-Tebbin localities are also characterized by relatively high concentrations of
353 Cr, and the concentrations are also decreased when the distance from the main pollution source
354 was increased. The existing Cr values from unpolluted Egyptian soils are in the range 9.7-24.9
355 mg/kg ([Abdel-Sabour and Zohny, 2004](#)), while highly polluted soils had Cr concentrations ranging
356 from 69 mg / kg to 108 mg / kg, strongly driven by soil composition ([Said et al., 2019](#)). These Cr
357 values are close to the target value of 100 mg / kg proposed in the Dutch values ([Dutch Target](#)
358 [and Intervention Values, 2000](#)).

359 Often, a significant proportion of the environmentally-released metals is deposited in the
360 vicinity of the emission source ([Steinnes et al., 2000](#); [Solgi and Parmah, 2015](#); [Kim et al., 2020](#)).
361 However, a non-negligible fraction could have been transported over longer distances, of up to
362 24 km in the case of Pb and Sn ([Rawlins et al., 2006](#)). Here, we report that a contamination
363 gradient exists for Ni and Cr from the pollution source onwards, with soil values of the two metals
364 remaining at high levels at the more distant studied sites, thus introducing high toxicity risks for
365 the wildlife. As expected, Ni and Cr values progressively decreased from the pollution site
366 onwards, thus providing in-field evaluation of the heavy metal doses on the trophic transfer
367 behavior.

368

369 **4.2. Trophic transfer of heavy metals from soil to plants**

370 Low concentrations of Ni and Cr were measured from the leaves and stems of *P. distichum* (Ni:
371 4.9, Cr: 5.0 mg / kg) as compared with those found in the soil. This is not surprising, as heavy metal
372 uptake by *P. distichum* is far higher in belowground organs, 9-18 and 16-40 times higher in roots
373 than in shoots for Ni and Cr, respectively (Bhattacharya et al. 2010). Similarly, Usman et al. (2019)
374 reported little accumulation of Ni and Cr in the shoot of the shrub plant *Tetraena gataranse* grown
375 on soils polluted by heavy metals, while roots heavily accumulate Ni and Cr at amounts that
376 correlate with metal concentrations present in the soil (Usman et al. 2019). Little (Ni) or even
377 nearly undetectable (Cr) amounts have been reported from shoots of *P. distichum* experimentally
378 harvested on unpolluted soils (Bhattacharya et al. 2010), while the amounts of these two metals
379 were doped when the plants were grown on heavy metal-enriched sludges. In our study, Cr
380 amounts reported for leaves are very high considering that there is little translocation of this
381 metal to aboveground plant tissues (Bhattacharya et al. 2010).

382 While Ni and Cr leave concentrations had a tendency to decline until a distance of 2 km
383 far from the pollution source, following the pattern we reported for the concentration of these
384 two metals in the soil, the relationship was less clear when the distance to the pollution source
385 was increased further. It is known that the transport of Ni from soil to plant roots is improved at
386 lower soil pH (Everhart et al., 2006). A similar pattern has been reported by Ololade et al. (2007)
387 who reported that the uptake of Cr and Ni was higher in the roots of the four studied plant species
388 sampled from acidic soils. In the present work, even if acidic deposition is often increased in soils
389 contaminated by heavy metals (Liao et al. 2005), we have no information on soil pH, which might
390 have been variable among the studied localities, and may have blurred the signal.

391

392 **4.3. The fate of Ni and Cr: from plants to herbivores**

393 Of the three arthropod species investigated, the lowest metal concentrations were measured
394 from the herbivorous long-faced grasshopper (*Truxalis grandis*). Yet, specimens of *T. grandis*
395 tended to have slightly higher amounts of Ni and Cr than those measured from the leaves of *P.*
396 *distichum* on which they feed. Consistently, [Peterson et al. \(2003\)](#) found that Ni concentrations
397 were higher in grasshoppers thriving in localities where Ni-hyperaccumulator plants were
398 dominant. The grasshopper *Aiolopus thalassinus* feeding on berseem plants (*Trifolium*
399 *alexandrinum*) in agricultural habitats in Pakistan had a higher bioaccumulation of heavy metals
400 than phloem feeding aphids (*Sitobion avenae*) ([Butt et al., 2018](#)). This suggests that grasshoppers
401 may bioaccumulate heavy metals when feeding on contaminated leaves of *P. distichum*, with
402 bioaccumulation being lowered when the distance of the pollution source was increased.

403 A continuous decrease in metal levels (Fe, Cu, Ni and Cd) has been noted in the European
404 pine sawfly and its larval food plants as a function of increasing distance from factories ([Heliövaara](#)
405 [and Väisänen \(1990\)](#)). Likewise, [Azam et al. \(2015\)](#) observed higher metal concentrations (Cd, Cr,
406 Cu, Ni, and Zn) in terrestrial insects (dragonfly, grasshopper, and butterfly) collected from sites
407 closer to the industrial zone of Gujrat (Pakistan) than those from a control site. [Karadjova and](#)
408 [Markova \(2009\)](#) reported higher metal concentrations (Cd, Co, Cu, Fe, Mn, Ni and Pb) in acridid
409 grasshoppers collected from sites in Bulgaria that were closer to a copper smelter and copper-
410 flotation factory than those from a control site.

411

412 **4.4. Trophic transfer of Ni and Cr to carnivores**

413 The highest metal concentrations across plant and animal samples were recorded from the
414 predatory wolf spider (*Hogna ferox*) which is a ground-dwelling arthropod preying on a range of
415 invertebrates, including springtails and flies. This finding is consistent with the available literature
416 reporting that wolf spiders are particularly successful in surviving in metal-polluted habitats, even
417 showing large bioaccumulation of heavy metals, defining them as macro-concentrators ([Peterson](#)

418 [et al., 2003](#); [Butt and Aziz, 2016](#)). The exact nature of the diet of *H. ferox* remains under
419 investigations, and wolf spiders are considered generalist predators, with a large part of the prey
420 consisting in Araneae, Diptera and Hemiptera in *Pardosa lugubris* ([Edgar 1969](#)), aphids,
421 Collembola and Diptera in *P. agrestis*, *P. amentata* and *P. palustris* ([Nyffeler and Benz, 1988](#)). From
422 our results, it can be seen that the concentration of Ni and Cr from the body of the spiders
423 parallels the one recorded from the soil along the pollution gradient we considered but was
424 slightly different from the pattern reported for grasshoppers. The metal body burden in spiders
425 varies with hunting strategy, soil properties, or excretion rate ([Hendrickx et al., 2003](#); [Wilczek and](#)
426 [Babczyńska 2000](#)). Altogether, this suggests that the grasshoppers may have represented a small
427 proportion of the diet of the spiders, and further studies should assess body metal burden from
428 other terrestrial invertebrates that are potential food items for the wolf spiders to complete our
429 understanding of heavy metal transfers along the food chain.

430 The transfer of contaminants to carnivores having a higher position in the food web, such
431 as reptiles, has been demonstrated for several metals (reviewed in [Britta and Schiesari, 2010](#)),
432 making these animals valuable bioindicators of environmental pollution by heavy metals ([Silva et](#)
433 [al., 2020](#)). Consistently, amphibians and lizards in our study concentrated more Cr and Ni than the
434 other vertebrates, yet had lower concentrations than the wolf spiders. The Cr values we measured
435 from the livers of the Egyptian toad and the Mascarene grass frog (*Ptychadena mascareniensis*)
436 are higher than those found from the liver of *Rana esculenta* (River Guma, Nigeria) ([Shaapera et](#)
437 [al., 2013](#)). In amphibians and reptiles, the bioaccumulation of heavy metals mainly occurs during
438 the consumption of contaminated invertebrate preys, as reported in the *Psammodromus algirus*
439 lizards ([Márquez-Ferrando et al., 2009](#)). When feeding, the accumulation of cadmium, zinc, and
440 lead in *Acanthodactylus boskianus* also evidenced the transfer of these heavy metals from aquatic
441 to terrestrial food webs ([Nasri et al., 2017](#)). Of note, we cannot exclude that the higher metal
442 amounts reported from amphibians and reptiles may have also resulted from accidental ingestion

443 of contaminated soil present on diet items, inhalation, or absorption of the heavy metals through
444 the skin in the case of amphibians (Alford and Richards, 1999).

445 Ni and Cr concentrations measured from the liver of the house mouse (*Mus musculus*)
446 and the Norway rat (*Rattus norvegicus*) are high when we consider that chronic exposure of rats
447 at Ni amounts higher than 1.3 mg/kg/day reduce the survival of the offspring (Ambrose et al.
448 1976). The lower BCI of rodents from sites near the industrial complex additionally suggests the
449 lower nutritional status of these animals thriving in contaminated areas. Sánchez-Chardi et al.
450 (2007) observed that the body condition index tended to decrease in adult wood mice, *Apodemus*
451 *sylvaticus*, from a landfill site. Even if no difference was found in the morphological parameters
452 of the greater white-toothed shrew, *Crocidura russula* (Sánchez-Chardi and Nadal, 2007), Nunes
453 et al. (2001) found that body length was higher in *Mus spretus* mice from a reference site
454 compared to mice inhabiting a metal-contaminated area. Mammals are generally better able to
455 deal with moderate heavy metal contamination in the environment than birds (Outridge and
456 Scheuhammer, 1993). Moreover, trophic position of the mammals in the food chain did not
457 correlate with Ni body concentration. A number of factors may explain this result: (1) metabolic
458 regulation that maintains a constant internal metal concentration, independent of environmental
459 concentrations, and prevents high bioaccumulation in small mammals and birds (e.g., Talmage
460 and Walton, 1991; Goyer, 1997; Ma and Talmage, 2001); (2) organisms at higher trophic levels
461 may exhibit more efficient excretion of heavy metals (Vizzini et al., 2013); and (3) vertebrates with
462 large foraging range likely reflects environmental pollution at a broader spatial scale than the one
463 we considered, and may have not – or less – been exposed to heavy metals when feeding. In
464 particular, large bird predators probably have higher home ranges as compared with smaller
465 vertebrates and invertebrates (McCauley et al., 2015), and may have had the possibility to feed
466 upon arthropods in other non-polluted areas. Finally, we found that in amphibians (Cr and Ni) and
467 birds (Cr only), the individuals who had higher concentrations of trace metals in their liver also

468 had a higher BCI. This puzzling results in difficult to explain but could perhaps be due large natural
469 size variations between the species that make up these taxa (**Table 4**) or higher tolerances for
470 heavy metal concentrations.

471

472 **5. Conclusions**

473 We have provided evidence of heavy metal contamination in a complex trophic chain, from plants
474 to arthropods to vertebrates, along a heavy metal pollution gradient. In plant-arthropod food
475 webs, we report clear evidence of heavy metal bioaccumulation, with higher concentrations in
476 the apex predators (the Egyptian mantis and a wolf spider) than in the plant or in the grasshopper.
477 Possible biodilution of the measured heavy metals was then reported at higher trophic levels
478 among vertebrates. In addition, our results suggest that the concentrations of heavy metal in the
479 tissue of almost all organisms tested decrease with distance from the pollution source. In sum,
480 our comprehensive study illustrates the impact heavy metal pollution has at all trophic levels and
481 revealed differences in accumulation patterns of Cr and Ni between lower and higher trophic
482 levels. Future investigations should examine assimilation and excretion of these pollutants at the
483 individual level to better describe the metal transfer pathways in this agro-industrial
484 environment.

485

486 **Data availability**

487 All data generated or analyzed during this study are included in this article.

488

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494

495 **CRedit authorship contribution statement**

496 **Mustafa M. Soliman:** Conceptualization, Methodology, Investigation, Data analysis, Data
497 curation, Writing – original draft. **Thomas Hesselberg:** Supervision, Data analysis, Writing –
498 review & editing. **Amr A. Mohamed:** Conceptualization, Resources, Methodology, Formal
499 analysis, Writing – original draft, Writing – review & editing. **David Renault:** Supervision,
500 Resources, Writing – original draft, Writing – review & editing.

501

502 **Declaration of competing interest**

503 The authors declare that they have no known competing financial interests or personal
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Table 1. Chromium and Ni concentrations in the soil samples collected at each studied site along with distances (km) of the seven sites: (a) with the industrial area (pollution source) and (b) among each other

Study sites	Distance from the pollution source	Distance with the previous studied site	Heavy metals (mg/kg dry mass; mean \pm SD)	
			Cr	Ni
A (El Shobak)	1.0	NA	97.8 \pm 9.2	80.0 \pm 5.6
B (El Shobak El-Sharqi)	1.5	0.5	82.0 \pm 6.4	74.4 \pm 4.7
C (El Shurafa)	2.0	0.5	92.0 \pm 7.9	59.2 \pm 8.1
D (Kafr Turkhan)	3.1	0.6	69.7 \pm 6.1	71.1 \pm 6.3
E (Ghammazah Al Kubra)	5.0	1.9	74.7 \pm 4.2	69.0 \pm 7.7
F (Al Ikhsas Al Qiblyyah)	7.0	2.0	78.0 \pm 7.6	56.8 \pm 2.6
G (Arab Ghammazah Al Kubra)	10.5	3.5	63.5 \pm 3.0	64.6 \pm 3.8
Threshold values in Agricultural Soils of the European Union**			100	50

* Taken from (Tóth et al. 2016).

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Table 2. Variations in Cr concentrations by vertebrate species, site, and body characteristics (Body length, body size and body condition index) with results of the general linear model. B = estimated regression coefficient, P = P-value, significance codes: *** = P < 0.001, ** = P 0.001– 0.01, * = P > 0.01

Factor	F	df	P	B	95% CI	P
Taxon	37.687	12	< 0.001***			
<i>Amietophrynus regularis</i>				0.683	0.222–1.144	0.004**
<i>Ptychadena mascareniensis</i>				0.449	-0.101–0.999	0.108
<i>Trachylepis vittata</i>				0.515	0.016–1.014	0.043*
<i>Chalcides ocellatus</i>				0.432	-0.054–0.918	0.081
<i>Chalcides sepsoides</i>				0.366	-0.149–0.881	0.162
<i>Psammophis schokari</i>				1.062	0.225–1.898	0.013*
<i>Coluber florulentus</i>				0.515	-0.253–1.283	0.186
<i>Bubulcus ibis</i>				0.170	-0.278–0.619	0.453
<i>Athene noctua</i>				0.052	-0.280–0.384	0.758
<i>Falco tinnunculus</i>				0.279	0.008–0.550	0.044*
<i>Elanus caeruleus</i>				0.387	0.168–0.605	0.001**
<i>Mus musculus</i>				0.071	-0.421–0.563	0.775
<i>Rattus norvegicus</i>				Ref	–	–
Site	3.391	6	0.004**			
A				0.098	0.029–0.167	0.006**
B				0.026	-0.073–0.125	0.605
C				0.013	-0.051–0.076	0.697
D				0.058	-0.012–0.128	0.102
E				-0.011	-0.079–0.056	0.740
F				-0.027	-0.094–0.039	0.413
G				Ref	–	–
Body mass	0.044	1	0.833	0.000	-0.002–0.002	0.833
Body length	1.136	1	0.289	-0.001	-0.002–0.001	0.289
BCI	1.128	1	0.290	-0.224	-0.642–0.194	0.290

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Table 3. Variations in Ni concentrations by vertebrate species, site, and body characteristics (Body mass, body length, and body condition index) with results of the general linear model. B = estimated regression coefficient, P = P-value, significance codes: *** = P < 0.001, ** = P 0.001– 0.01, * = P > 0.01

Factor	F	df	P	B	95% CI	P
Taxon	47.488	12	< 0.001***			
<i>Amietophrynus regularis</i>				0.550	0.069–1.031	0.025*
<i>Ptychadena mascareniensis</i>				0.194	-0.379–0.768	0.504
<i>Trachylepis vittata</i>				0.099	-0.421–0.619	0.707
<i>Chalcides ocellatus</i>				0.271	-0.236–0.777	0.292
<i>Chalcides sepsoides</i>				0.026	-0.511–0.563	0.924
<i>Psammophis schokari</i>				1.158	0.285–2.030	0.010**
<i>Coluber florulentus</i>				0.707	-0.094–1.507	0.083
<i>Bubulcus ibis</i>				0.128	-0.339–0.596	0.588
<i>Athene noctua</i>				-0.463	-0.810–-0.117	0.009**
<i>Falco tinnunculus</i>				0.133	-0.150–0.416	0.353
<i>Elanus caeruleus</i>				-0.100	-0.327–0.128	0.388
<i>Mus musculus</i>				-0.078	-0.591–0.436	0.765
<i>Rattus norvegicus</i>				Ref	–	–
Site	2.869	6	0.012*			
A				0.080	0.008–0.152	0.030*
B				0.017	-0.086–0.120	0.746
C				0.076	0.009–0.142	0.027*
D				0.095	0.022–0.168	0.011*
E				-0.009	-0.080–0.061	0.790
F				0.044	-0.025–0.113	0.213
G				Ref	–	–
Body mass	0.929	1	0.337	0.001	-0.001–0.003	0.337
Body length	7.523	1	0.007**	-0.002	-0.004–-0.001	0.007**
Body condition index	2.250	1	0.136	-0.330	-0.766–0.106	0.136

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Table 4. Descriptive statistics for body characteristics measured from the vertebrate species collected from the seven studied localities of El-Tebbin area (Egypt).

Species	Morphological parameters			n
	Body mass (g)	Body Length (mm)	BCI	
Egyptian toad (<i>Amietophrynus regularis</i>)	31.6 ± 2.3 (27.7–36)	81.7 ± 11.7 (62.3–100)	1.1 ± 0.07 (1.03–1.25)	15
Mascarene grass frog (<i>Ptychadena</i>)	5.4 ± 1.0 (4–7.3)	41.6 ± 5.8 (32.9–52.1)	0.79 ± 0.08 (0.65–0.94)	20
Bridled mabuya (<i>Trachylepis vittata</i>)	15.0 ± 1.7 (12.1–18.1)	65.8 ± 5.5 (56–74.9)	0.97 ± 0.04 (0.86–1.04)	15
Ocellated skink (<i>Chalcides ocellatus</i>)	25.1 ± 4.5 (17.2–32.5)	101.5 ± 12.8 (85.1–127)	0.94 ± 0.03 (0.90–1.0)	16
Wedge-snouted skink (<i>Chalcides sepsoides</i>)	13.6 ± 1.8 (11.8–15.9)	59.4 ± 3.0 (55.8–62.9)	0.98 ± 0.03 (0.94–1.0)	4
Forskal sand snake (<i>Psammophis schokari</i>)	59.7 ± 4.7 (52.8–64.8)	610.0 ± 41.6 (569–661)	0.68 ± 0.02 (0.66–0.70)	5
Flowered racer (<i>Coluber florulentus</i>)	43.2 ± 2.9 (41.1–46.5)	531.3 ± 21.7 (515–556)	0.647 ± 0.006 (0.64–0.65)	3
Cattle egret (<i>Bubulcus ibis</i>)	335.7 ± 30.3 (295.3–380.8)	533.4 ± 21.7 (508–560)	0.995 ± 0.01 (0.99–1.03)	7
Little owl (<i>Athene noctua</i>)	123.3 ± 12.9 (110.2–140.9)	221.2 ± 5.0 (214.2–226)	1.05 ± 0.02 (1.03–1.09)	4
Common kestrel (<i>Falco tinnunculus</i>)	193.6 ± 10.3 (175.6–207.6)	308.1 ± 10.5 (294–321)	1.04 ± 0.01 (1.03–1.07)	7
Black-winged kite (<i>Elanus caeruleus</i>)	299.3 ± 6.6 (293.4–306.5)	347.3 ± 4.5 (343–352)	1.09 ± 0.0004 (1.09–1.1)	3
House mouse (<i>Mus musculus</i>)	21.8 ± 2.4 (17.5–26.2)	89.7 ± 8.2 (76.9–100.7)	0.95 ± 0.02 (0.90–0.99)	17
Norway rat (<i>Rattus norvegicus</i>)	281.9 ± 15.1 (255.9–305.1)	210.8 ± 19.1 (174.3–260.1)	1.26 ± 0.03 (1.2–1.3)	19

Data are presented as mean ± SD (range), n; number of individuals.
For each species, the values presented in the brackets correspond to the lowest and highest measured metal concentration.

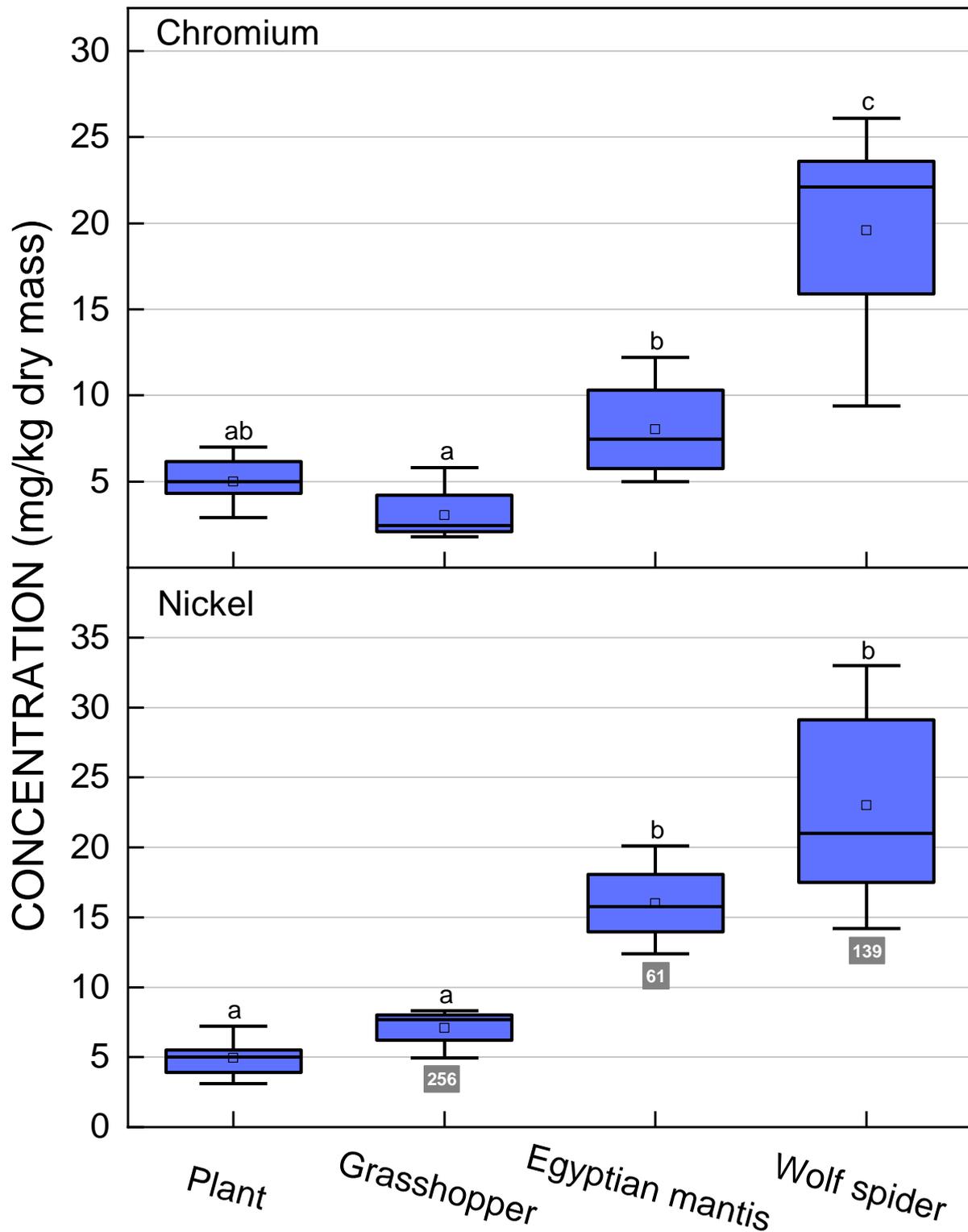
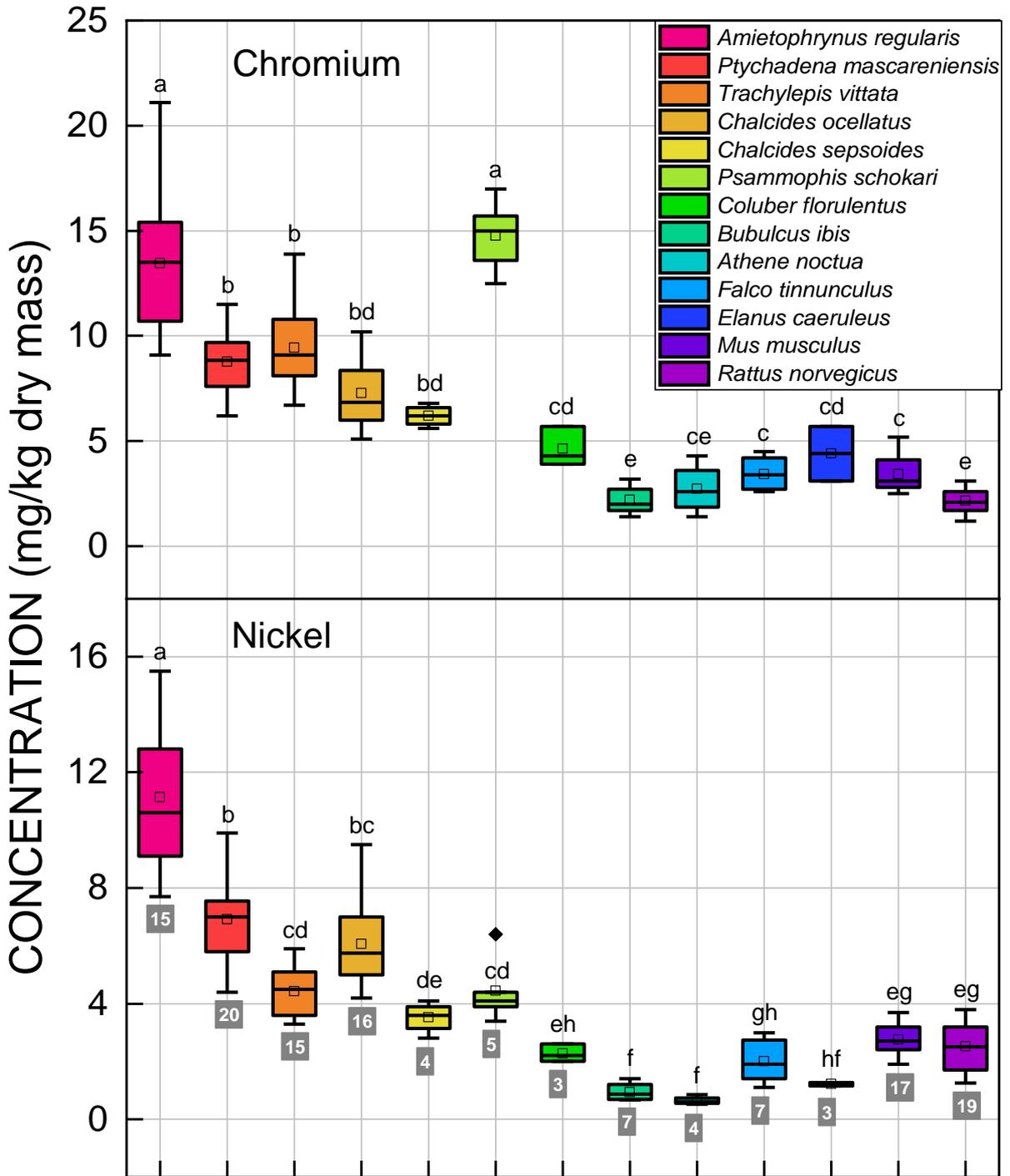


Figure 1. Mean metal concentrations in plants and arthropods from El-Tebbin area. Data are presented as mean \pm SD in mg/g dry mass. Bars followed by different letters denote significant differences in metal concentrations (analysis of variance [ANOVA]; Bonferroni test; $P < 0.05$). The number below each box indicates the number of samples. Metal concentrations are presented as means across sites A to G. The detailed Ni and Cr concentrations, for each site and each species, are presented in Supplementary materials 3-4 and 7.

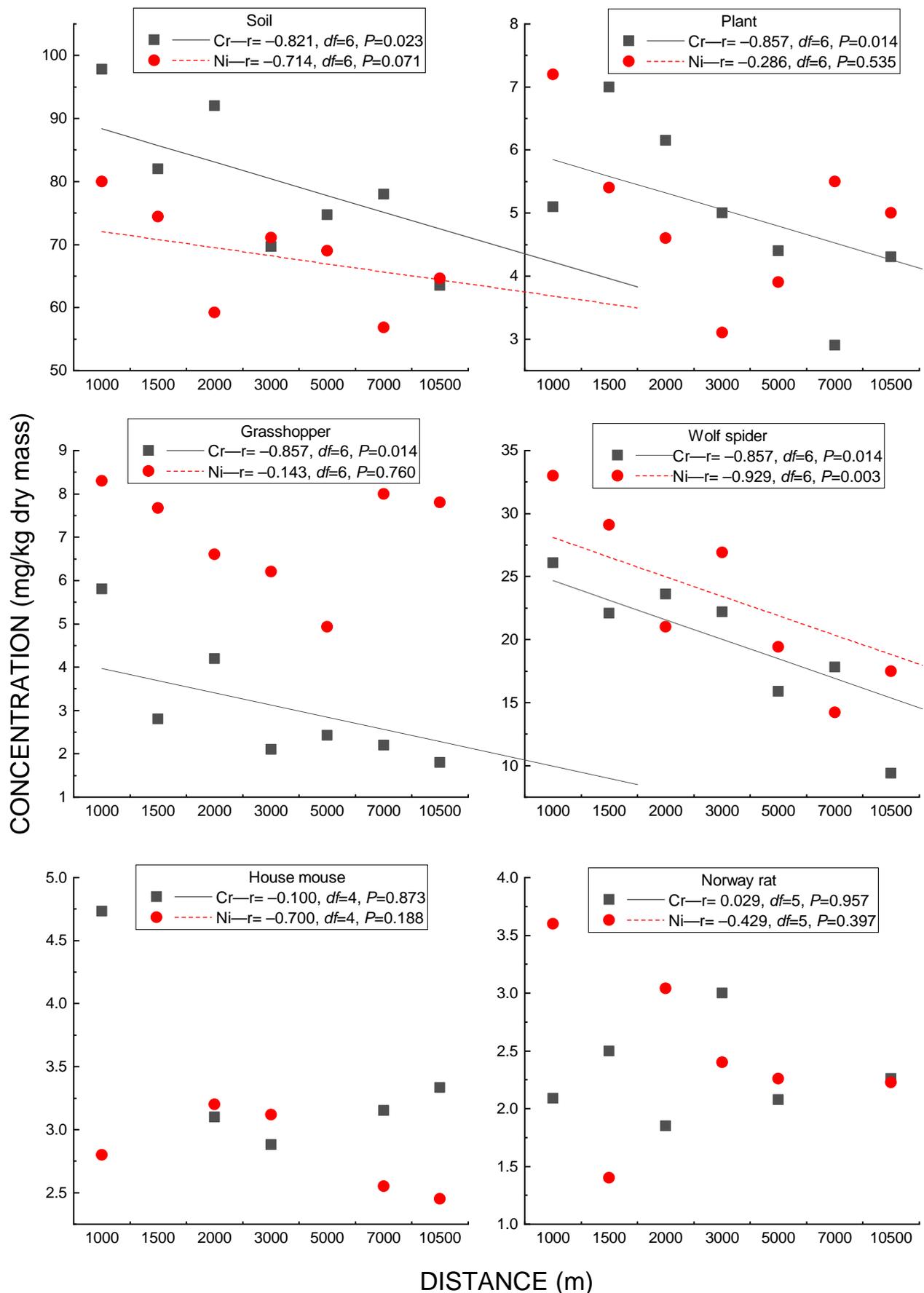
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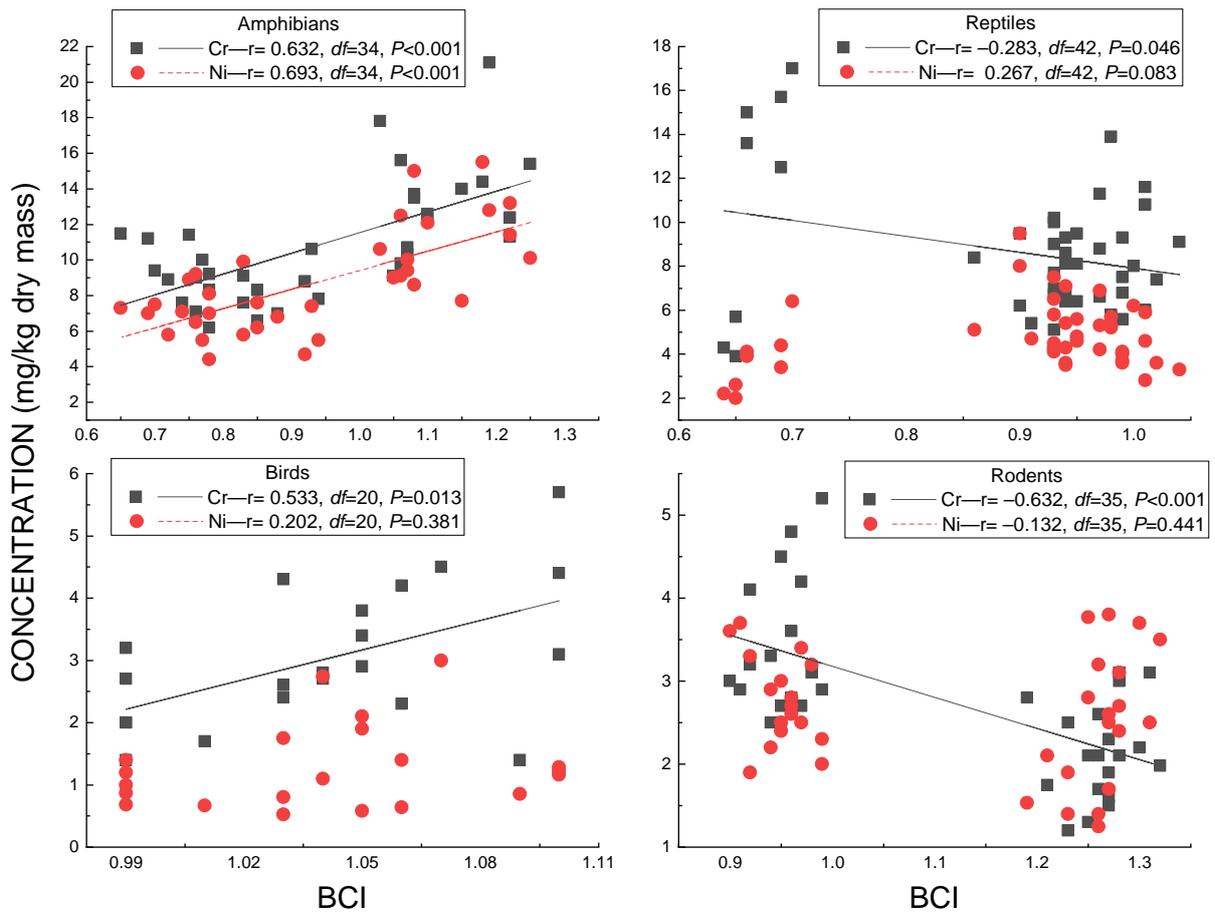
Figure 2. Mean metal concentrations in vertebrates from El-Tebbin area. Data are presented as mean \pm SD in mg/ kg dry mass. Boxes followed by different letters denote significant differences in metal concentrations (analysis of variance [ANOVA]; Bonferroni test; $P < 0.05$). The number below each box indicates the number of samples. Metal concentrations are presented as means across sites A to G. The detailed Ni and Cr concentrations, for each site and each species, are presented in Supplementary materials 3-4 and 7.



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Figure 3. Chromium and Ni concentrations in soil, plants, and some animal species against distance from the industrial area. Values of the Spearman rank-order correlation coefficient test (rs), degrees of freedom (df), and probability (p) are given for each metal.

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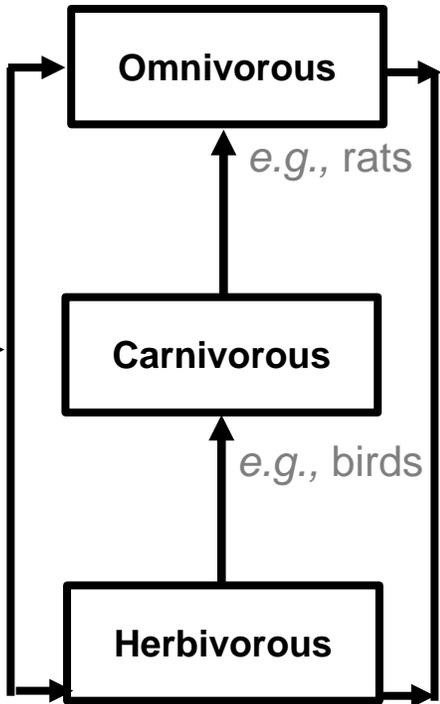
Figure 4. Chromium and Ni concentration in vertebrate livers plotted against body condition index. Values of the Pearson correlation coefficient test (r), degrees of freedom (df), and probability (p) are given for each metal. Species of each taxon group are given in **Supplementary material 2**.

Potential sources

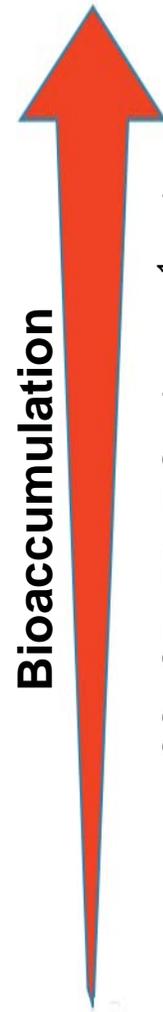
Paints
Cock
Still industry
Burned fuel
Fertilizers
Pesticides

Cr & Ni polluted soils

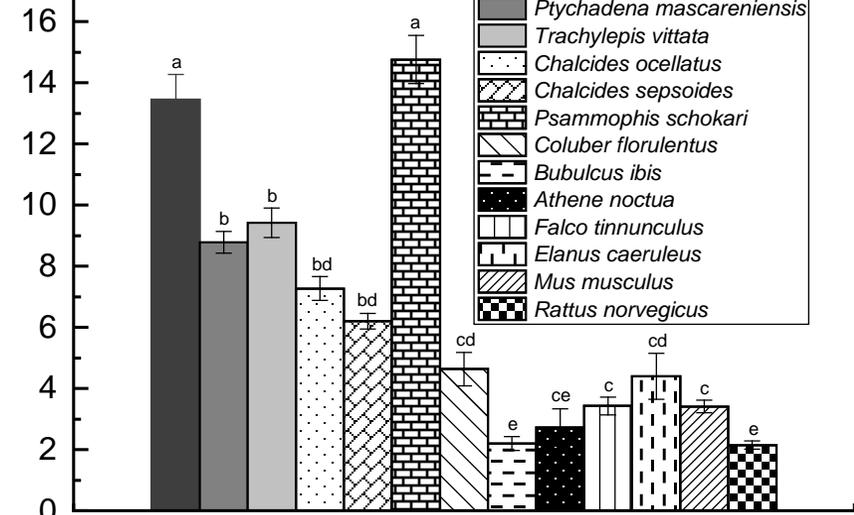
Plants



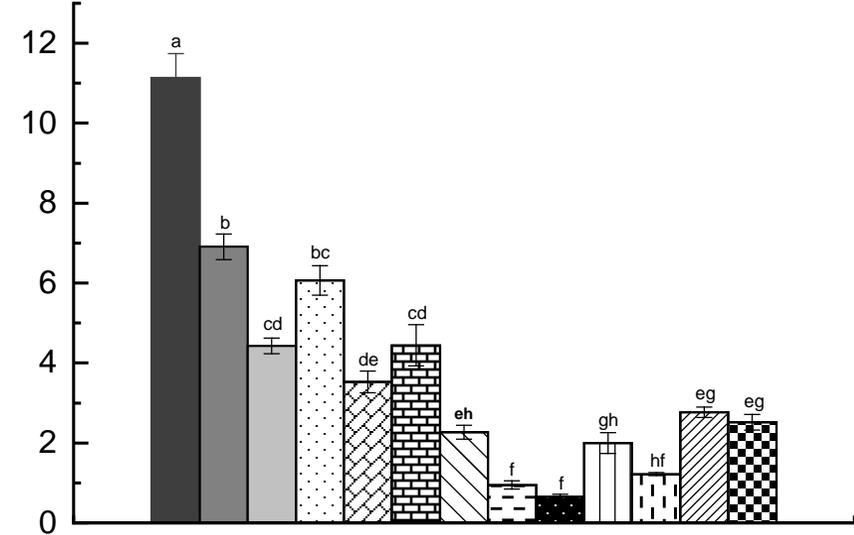
Trophic levels



Chromium



Nickel



Highlights	1
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• The level of soil pollution by the heavy metals Cr and Ni as a function of the distance to the main pollution source.	3 4
• Differences in accumulation patterns and possible sources of metals between lower and upper trophic levels.	5 6
• Heavy metal contamination in all species tested in this complex food web, with biomagnification at lower trophic	7 8
• Possible biodilution was then reported at higher trophic levels among vertebrates.	9
• Concentrations of heavy metal generally decreased in the trophic chain with distance from the pollution source.	10 11
	12