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

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Abstract

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

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

1. Introduction

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

Investigations revealing the effects of pollutants on the life history of organisms, including longevity, reproductive success, developmental rates, the underlying physiological and genomic impacts, and the cascading consequences on population dynamics, are of considerable interest. Such studies improve our understanding of the ecological effects of pollution on wildlife (Saaristo et al., 2018). So far, a range of works have examined the effects of pesticides (Dewer et al., 2016; Serra et al., 2020; Engell Dahl et al., 2021), heavy metals (Berni et al., 2019; Cosio and Renault, 2020), pharmaceuticals (Prud'homme et al., 2018; Renault et al., 2018) or polymers (de Souza Machado et al., 2018; Kögel et al., 2020) on the biology and physiology of plants and insects. In addition to describing the conspicuous impacts on growth and development, these investigations have revealed the latent effects pollutants can have on the ecology and biology of wildlife. The insidious nature of xenobiotics toxicity includes, for instance, deleterious effects of antibiotics on the muscle structure of flies (Renault et al., 2018), impacts of ibuprofen on the development of mosquitoes (Prud'homme et al., 2018), or cryptic effects of low doses of pesticides on plant metabolism (Serra et al., 2013) and animal behavior (Desneux et al., 2007; Hanlon and Relyea, 2013).

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

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

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

In this work, we examined the transfers of chromium and nickel through a terrestrial trophic web and investigated the potential for bioaccumulation of these pollutants along the trophic chain. To that aim, soil, plants, invertebrates, and vertebrates were sampled in different localities to the south of Cairo (El-Tebbin, Egypt). This region represents 17% of the Egyptian industrial activity (Soliman et al., 2017), with petroleum coke and cement factories, metallurgy and ceramics and a range of other industrial activities (Gomaa et al., 2020). Previous studies in this region have already reported large amounts of heavy metals from soil samples, in particular cadmium, lead, copper and zinc (Soliman et al., 2019; Gomaa et al., 2020). In addition, Soliman et al. (2019) showed that cadmium, copper, lead, and zinc are accumulated in plants and their associated herbivorous insects (grasshoppers and mantids), while these metals tended to be diluted when measured from vertebrates. Yet, the effects of chromium and nickel were not considered by Soliman et al. (2019), despite the relatively high concentrations they can reach in Egyptian soils (Abdel-Sabour and Zohny, 2004; Said et al., 2019). The levels of chromium and nickel released in the environment has increased with industrial activities, and more particularly with the manufacture of stainless steel (Tchounwou et al., 2012). Here, we investigate if heavy metals (chromium hereafter referred to as Cr, and nickel hereafter referred to as Ni) were enriched in terrestrial species from one trophic level to the next and analyze if trophic transfer behavior changes according to soil metal concentrations or distance to the pollution source (industrial area). We hypothesize that high Cr and Ni concentrations will be measured from soil samples collected near industrial activities, while being further absorbed and accumulated by plants. We further expect that these pollutants would be transferred along the trophic web, in particular to herbivorous insects, but also to higher trophic levels. First, we measured the concentrations of Ni and Cr in the soil from seven localities of the El-Tebbin region, starting from the supposedly pollution source, and from gradually distant sites. We assumed that the Ni and Cr values would decrease from the pollution site onwards, providing an in-field dose-response assessment of the effect of the metal concentrations on the food web. In this context, we assumed that the organisms thriving at the proximity of the pollution source would be characterized by the higher Cr and Ni tissue contents for the less mobile species, while species having a larger mobility, and thus a higher foraging range, should be characterized by metal concentrations independent of the sampling distance to the pollution source. As heavy metals are known to affect physiology and life history traits of living organisms, we computed an index of body condition for resident vertebrates, including amphibians, reptiles, birds, and rodents, to get an estimate of individual health and fitness levels in relation to Cr and Ni concentrations in the liver of these animals.

2. Material and methods

2.1. Study area

The studied sites are located in El-Tebbin (Helwan Province, East of the Nile River), an area located at 20 km to the south of Cairo (Egypt) **(Supplementary Material 1)**. This area is one of the largest industrial zones of Egypt, covering 43100 hectares. The main industrial activities that can be found in this zone include ferrous and nonferrous metallurgical work, a petroleum coke factory, chemical and cement industry (Soliman and El-Shazly, 2017). The area is surrounded by agricultural production, in the form of different crops and vegetables including cultures of alfalfa, beans, eggplant, maize, okra, pepper, potato, and squash **(Supplementary Material 1)**. The experimental work presented in this study was conducted in October 2020.

2.2. Sample collection and preparation

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

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

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

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

Vertebrates, amphibians, and reptiles were hand collected from the grass strips along the edges of the seven farmlands (For each locality, the number of sampled animals is detailed in **Supplementary Material 2)** and were killed with chloroform. Rodents were collected in Sherman live traps, baited inside with peanut butter; each collection site was surveyed for two consecutive nights, and traps were checked every morning. Finally, birds were caught by bownet traps, which were containing mice as attractant. We sacrificed birds and small mammals by CO_2 inhalation. Only the minimum number of individuals required for the analysis was collected and sacrificed. All steps were conducted as stated in AVMA guidelines for the euthanasia of animals (Leary et al., 2013) and after approval, ID#: CU 1-F-50-20, from the University of Cairo's animal ethical committee.

2.3. Morphometric analyses and index of body condition of vertebrates

For each individual vertebrate, the body length (BL) of the animal was measured to the nearest 0.01 mm with calipers. For amphibians and reptiles, body length was recorded as the snout-vent length (SVL). Body length was measured from the tip of the bill and the tip of the tail in birds, and from nose to anus in rodents. Body mass (BM) was measured with a portable electronic balance (Model 30064413, Ohaus Scout Pro; accuracy: 0.01g). After measurements, all vertebrates were dissected, and the liver of each animal was weighed, transferred individually into labeled plastic containers, and stored at -20 °C prior to being used for subsequent chemical analyses.

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

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

2.4. Heavy metal analysis

Before starting the assays, the flasks were soaked in 2N nitric acid overnight, and then abundantly rinsed with deionized water. Digestion of plant and animal samples (whole arthropods and livers of vertebrates) was conducted according to the method described by Moor et al. (2001) and Soliman et al. (2019). Briefly, samples (0.5 g each: soil samples, whole leaves for plants, whole individuals for arthropods, or liver for vertebrates) were placed in a 25 mL flask and pre-digested with a volume of 10 mL of 65% HNO_3 for 24 h at room temperature. The suspension was then digested to near dryness on a thermostatically controlled hotplate at 90 °C in a fume cupboard, before the adding of 2 mL of 30% H_2O_2 which aids the digestion of the organic matter. The flask walls were then washed with 10 mL of deionized water. The suspension was filtered through Whatman filter paper (No. 41) in a volumetric flask, diluted to 25 mL and stored in polyethylene bottles at 4 °C until being used for the analyses.

Soil digestion was conducted in a similar way, but samples were allowed to stand for 24 h with 12 mL of a mixture made of 37% HCl: 65% HNO_3 (3:1). Then, volumes of 2.5 mL of 37% HCl and 2.5 mL of 30% H_2O_2 were added to complete the digestion of soil samples. The filtered solutions were diluted in a 50 mL volumetric flask so that the final volume of each sample reached 50 mL. Inductively coupled plasma (ICP-AES-Jobin Yovin ultima2, France) was used to measure the concentrations of Cr and Ni from each sample. Wavelengths and detection limits of the ICP for the measured elements were: 205.552 nm and 0.0041 mg L^{-1} for Cr; 231.604 nm and 0.01 mg L^{-1} for Ni. A reagent process blank and a certified reference material (standard reference materials from the National Institute of Standards and Technology (NIST), USA: NIST 1547, 1577 and 2709 for plant, animal tissues and soil, respectively) were carried out for each analytical batch to ensure the accuracy of the measures. Mean values of three replicates were calculated for each measurement. Chromium and Ni concentrations were expressed as mg kg^{-1} dry mass.

2.5. Data analyses

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

3. Results

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

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

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

A negative relationship was obtained when the concentrations of Cr in the soil, plant, and animals were plotted as a function of the distance to the industrial area **(Fig. 3)**. The correlation was not statistically significant for the house mouse and the Norway rat ($r = -0.100$, $P = 0.873$; $r = 0.029$, $P = 0.957$, respectively). For Ni, the correlation was only significant for the wolf spider ($r = -0.929$, $P < 0.01$). For vertebrates, the analyses revealed that the liver concentrations of the two metals significantly varied with respect to collection site ($F = 3.4$, df = 6, $P < 0.01$ for Cr, $F = 2.9$, df = 6, $P < 0.01$ for Ni). Lower concentrations of Cr and Ni were measured from the vertebrates from the reference site (site G), while the two metals were characterized by increasing amounts in the livers of the specimens that were collected closer to the pollution source (except for sites E and F for Cr amounts, and site E for Ni amounts). This pattern was supported by significant differences among values of the reference site (site G) and site A in the case of Cr ($P < 0.01$), and among the reference site and sites A, C, and D in the case of Ni ($P < 0.050$, 0.05, and 0.01, respectively) **(Tables 2 and 3)**.

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

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

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

Pearson correlation analyses showed significant positive relationships between BCI, and Cr and Ni concentrations measured from amphibian livers (Cr: $r = 0.632$, $p < 0.001$; Ni: $r = 0.693$, $p < 0.001$) and Cr measured from bird livers ($r = 0.533$, $p = 0.013$). In contrast, Cr concentrations measured from reptile and rodent livers were negatively correlated ($r = -0.283$, $p = 0.046$; $r = -0.632$, $p < 0.001$, respectively) with BCI indicating that reptiles and rodent species with higher concentrations of trace elements (Cr and Ni) are characterized by impaired body conditions **(Fig. 4)**.

4. Discussion

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

4.1. The fate of heavy metal pollution in the environment

We found that the soils from the El-Tebbin region are characterized by Ni amounts which always exceeded the threshold values of trace elements reported for Ni (50 mg/kg) from agricultural soils 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 fraction of soil Ni at El-Tebbin (Egypt). Importantly, the phyto-availability of Ni is known to greatly vary with soil characteristics, in particular pH (Kukier at al., 2004; Adamczyk-Szabeal et al. 2015), and Ni values exceeding 30 and 75 mg/kg for acidic and neutral alkaline soils, respectively are considered toxic for the plants (European Directive 86/278/EEC). In this work, we did not measure the pH of the soil; yet all values are higher than maximum admissible Ni concentrations for acidic soils, and three out the seven measured concentrations are close – or even exceed – maximum Ni concentrations for neutral alkaline soils. Even if the Dutch target value (Dutch pollutant standards) is as high as 210 mg/kg) for Ni (Dutch Target and Intervention Values, 2000), it is crucial to bear in mind that toxicity level can greatly vary with plant or animal species, developmental stage, or duration of exposure. As a result, depending on the toxicity risk, the permissible Ni concentration will vary from one region to another, as is the case in Poland where Ni permissible values ranging from 35 mg / kg soil to 300 mg / kg soil (Barałkiewicz and Siepaks, 1999). Finally, the Dutch value reports a Ni target value of 35 mg/kg soil (Dutch Target and Intervention Values, 2000).

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

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

4.2. Trophic transfer of heavy metals from soil to plants

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

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

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

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

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

4.4. Trophic transfer of Ni and Cr to carnivores

The highest metal concentrations across plant and animal samples were recorded from the predatory wolf spider (*Hogna ferox*) which is a ground-dwelling arthropod praying on a range of invertebrates, including springtails and flies. This finding is consistent with the available literature reporting that wolf spiders are particularly successful in surviving in metal-polluted habitats, even showing large bioaccumulation of heavy metals, defining them as macro-concentrators (Peterson et al., 2003; Butt and Aziz, 2016). The exact nature of the diet of *H. ferox* remains under investigations, and wolf spiders are considered generalist predators, with a large part of the prey consisting in Araneae, Diptera and Hemiptera in *Pardosa lugubris* (Edgar 1969), aphids, Collembola and Diptera in *P. agrestis*, *P. amentata* and *P. palustris* (Nyffeler and Benz, 1988). From our results, it can be seen that the concentration of Ni and Cr from the body of the spiders parallels the one recorded from the soil along the pollution gradient we considered but was slightly different from the pattern reported for grasshoppers. The metal body burden in spiders varies with hunting strategy, soil properties, or excretion rate (Hendrickx et al., 2003; Wilczek and Babczyńska 2000). Altogether, this suggests that the grasshoppers may have represented a small proportion of the diet of the spiders, and further studies should assess body metal burden from other terrestrial invertebrates that are potential food items for the wolf spiders to complete our understanding of heavy metal transfers along the food chain.

The transfer of contaminants to carnivores having a higher position in the food web, such as reptiles, has been demonstrated for several metals (reviewed in Britta and Schiesari, 2010), making these animals valuable bioindicators of environmental pollution by heavy metals (Silva et al., 2020). Consistently, amphibians and lizards in our study concentrated more Cr and Ni than the other vertebrates, yet had lower concentrations than the wolf spiders. The Cr values we measured from the livers of the Egyptian toad and the Mascarene grass frog (*Ptychadena mascareniensis*) are higher than those found from the liver of *Rana esculenta* (River Guma, Nigeria) (Shaapera et al., 2013). In amphibians and reptiles, the bioaccumulation of heavy metals mainly occurs during the consumption of contaminated invertebrate preys, as reported in the *Psammodromus algirus* lizards (Márquez-Ferrando et al., 2009). When feeding, the accumulation of cadmium, zinc, and lead in *Acanthodactylus boskianus* also evidenced the transfer of these heavy metals from aquatic to terrestrial food webs (Nasri et al., 2017). Of note, we cannot exclude that the higher metal amounts reported from amphibians and reptiles may have also resulted from accidental ingestion of contaminated soil present on diet items, inhalation, or absorption of the heavy metals through the skin in the case of amphibians (Alford and Richards, 1999).

Ni and Cr concentrations measured from the liver of the house mouse (*Mus musculus*) and the Norway rat (*Rattus norvegicus*) are high when we consider that chronic exposure of rats at Ni amounts higher than 1.3 mg/kg/day reduce the survival of the offspring (Ambrose et al. 1976). The lower BCI of rodents from sites near the industrial complex additionally suggests the lower nutritional status of these animals thriving in contaminated areas. Sánchez-Chardi et al. (2007) observed that the body condition index tended to decrease in adult wood mice, *Apodemus sylvaticus*, from a landfill site. Even if no difference was found in the morphological parameters of the greater white-toothed shrew, *Crocidura russula* (Sánchez-Chardi and Nadal, 2007), Nunes et al. (2001) found that body length was higher in *Mus spretus* mice from a reference site compared to mice inhabiting a metal-contaminated area. Mammals are generally better able to deal with moderate heavy metal contamination in the environment than birds (Outridge and Scheuhammer, 1993). Moreover, trophic position of the mammals in the food chain did not correlate with Ni body concentration. A number of factors may explain this result: (1) metabolic regulation that maintains a constant internal metal concentration, independent of environmental concentrations, and prevents high bioaccumulation in small mammals and birds (e.g., Talmage and Walton, 1991; Goyer, 1997; Ma and Talmage, 2001); (2) organisms at higher trophic levels may exhibit more efficient excretion of heavy metals (Vizzini et al., 2013); and (3) vertebrates with large foraging range likely reflects environmental pollution at a broader spatial scale than the one we considered, and may have not – or less – been exposed to heavy metals when feeding. In particular, large bird predators probably have higher home ranges as compared with smaller vertebrates and invertebrates (McCauley et al., 2015), and may have had the possibility to feed upon arthropods in other non-polluted areas. Finally, we found that in amphibians (Cr and Ni) and birds (Cr only), the individuals who had higher concentrations of trace metals in their liver also had a higher BCI. This puzzling results in difficult to explain but could perhaps be due large natural size variations between the species that make up these taxa (**Table 4**) or higher tolerances for heavy metal concentrations.

5. Conclusions

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

Data availability

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

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CRediT authorship contribution statement

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

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Abdel-Sabour, M., Zohny, E., 2004. Impact of industrial activities on total chromium in alluvial Egyptian soils as determined by neutron activation analysis. J. Radioanal. Nucl. Chem. 260, 233–236. https://doi.org/10.1023/b:jrnc.0000027089.39021.cc.

Abu Salem, H.S., Abu Khatita, A., Abdeen, M.M., Mohamed, E.A., El Kammar, A.M., 2017. Geo-environmental evaluation of Wadi El Raiyan Lakes, Egypt, using remote sensing and trace element techniques. Arab J. Geosci. 10, 224. https://doi.org/10.1007/s12517-017-2991-3.

Adamczyk-Szabela, D., Markiewicz, J., Wolf, W.M., 2015. Heavy metal uptake by herbs. IV. Influence of soil pH on the content of heavy metals in *Valeriana officinalis* L. Water Air Soil Pollut. 226, 106. https://doi.org/10.1007/s11270-015-2360-3.

Adriano, D.C., 2001. Trace elements in terrestrial environments (2nd ed.). New York: Springer.

Alford, R.A., Richards, S.J., 1999. Global amphibian declines: a problem in applied ecology. Annu. Rev. Ecol. Syst. 30, 133–165. https://doi.org/10.1146/annurev.ecolsys.30.1.133.

Ali, H., Khan, E., 2019. Trophic transfer, bioaccumulation, and biomagnification of non-essential hazardous heavy metals and metalloids in food chains/webs—Concepts and implications for wildlife and human health. Hum. Ecol. Risk Assess. 25, 1353–1376. https://doi.org/10.1080/10807039.2018.1469398.

Ambrose, A.M., Larson, P.S., Borzelleca, J.F., Hennigar Jr, G.R., 1976. Long term toxicologic assessment of nickel in rats and dogs. J. Food Sci. Technol. 13, 181–187.

Andrade, L., O'Dwyer, J., O'Neill, E., Hynds, P., 2018. Surface water flooding, groundwater contamination, and enteric disease in developed countries: A scoping review of connections and consequences. Environ. Pollut. 236, 540–549. https://doi.org/10.1016/j.envpol.2018.01.104.

Azam, I., Afsheen, S., Zia, A., Javed, M., Saeed, R., Sarwar, M.K., Munir, B., 2015. Evaluating insects as bioindicators of heavy metal contamination and accumulation near industrial area of Gujrat, Pakistan. Biomed. Res. Int. 2015, 942751. https://doi.org/10.1155/2015/942751.

Barałkiewicz, D., Siepak, J., 1999. Chromium, nickel and cobalt in environmental samples and existing legal norms. Pol. J. Environ. Stud. 8, 201–208.

Bhattacharya, T., Chakraborty, S., Banerjee, D.K., 2010. Heavy metal uptake and its effect on macronutrients, chlorophyll, protein, and peroxidase activity of *Paspalum distichum* grown on sludge-dosed soils. Heavy metal uptake and its effect on *P. distichum*. Environ. Monit. Assess. 169, 15–26. https://doi.org/10.1007/s10661-009-1146-8.

Berni, R., Luyckx, M., Xu, X., Legay, S., Sergeant, K., Hausman, J.F., Lutts, S., Cai, G., Guerriero, G., 2019. Reactive oxygen species and heavy metal stress in plants: Impact on the cell wall and secondary metabolism. Environ. Exp. Bot. 161, 98–106. https://doi.org/10.1016/j.envexpbot.2018.10.017.

Britta, G., Schiesari, L., 2010. The ecotoxicology of metals in reptiles, in: Sparling, D.W., Linder, G., Bishop, C.A., Krest, S. (Eds.), Ecotoxicology of Amphibians and Reptiles. CRC Press, Taylor & Francis Group, Boca Raton, FL, 2nd ed, pp. 337–448. http://dx.doi.org/10.1201/EBK1420064162-c12.

Butt, A., Aziz, N., 2016. Bioaccumulation of heavy metals mixture and its effect on detoxification enzymes of wolf spider, *Pardosa oakleyi*. J. Anim. Plant Sci. 26, 1507–1515.

Butt, A., Qurat-Ul-Ain, Rehman, K., Khan, M.X., Hesselberg, T., 2018. Bioaccumulation of cadmium, lead, and zinc in agriculture-based insect food chains. Environ. Monit. Assess. 190, 698. https://doi.org/10.1007/s10661-018-7051-2.

Cigliano, M.M., Braun, H., Eades, D.C., Otte, D., 2021. Orthoptera Species File. Version 5.0/5.0. [22.01.2021]. Available at: http://Orthoptera.SpeciesFile.org.

Cosio, C., Renault, D., 2020. Effects of cadmium, inorganic mercury and methyl-mercury on the physiology and metabolomic profiles of shoots of the macrophyte *Elodea nuttallii*. Environ. Pollut. 257, 113557. https://doi.org/10.1016/j.envpol.2019.113557.

de Souza Machado, A.A., Kloas, W., Zarfl, C., Hempel, S., Rillig, M.C., 2018. Microplastics as an emerging threat to terrestrial ecosystems. Glob. Change Biol. 24, 1405–1416. https://doi.org/10.1111/gcb.14020.

Desneux, N., Decourtye, A., Delpuech, J.M., 2007. The sublethal effects of pesticides on beneficial arthropods. Annu. Rev. Entomol. 52, 81–106. https://doi.org/10.1146/annurev.ento.52.110405.091440.

Dewer, Y., Pottier, M.A., Lalouette, L., Maria, A., Dacher, M., Belzunces, L.P., Kairo, G., Renault, D., Maibeche, M., Siaussat, D., 2016. Behavioral and metabolic effects of sublethal doses of two insecticides, chlorpyrifos and methomyl, in the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). Environ. Sci. Pollut. Res. 23, 3086–3096. https://doi.org/10.1007/s11356-015-5710-1.

Dirzo, R., Young, H.S., Galetti, M., Ceballos, G., Isaac, N.J.B., Collen, B., 2014. Defaunation in the Anthropocene. Science 345, 401–406. http://dx.doi.org/10.1126/science.1251817.

Dudka, S., Piotrowska, M., Terelak, H., 1996. Transfer of cadmium, lead, and zinc from industrially contaminated soil to crop plants: A field study. Environ. Pollut. 94, 181–188. https://doi.org/10.1016/S0269-7491(96)00069-3.

Dutch Target and Intervention Values (the New Dutch List), 2000. Circular on target values and intervention values for soil remediation. The Hague, the Netherlands: The Ministry of Housing, Spatial Planning and Environment, Department of Soil Protection (VROM). Available at: https://www.esdat.net/environmental%20standards/dutch/annexs_i2000dutch%20environmental%20standards.pdf. [Accessed 26 May 2021]

Edgar, W.D., 1969. Prey and predators of the Wolf spider *Lycosa lugubris*. J. Zool. 159, 405–411. https://doi.org/10.1111/j.1469-7998.1969.tb03897.x

El-Amier, Y., Elhindi, K., El-Hendawy, S., Al-Rashed, S., Abd-ElGawad, A., 2019. Antioxidant system and biomolecules alteration in *Pisum sativum* under heavy metal stress and possible alleviation by 5-aminolevulinic acid. Molecules 24, 4194. https://doi.org/10.3390/molecules24224194.

Engell Dahl, J., Marti, S.L., Colinet, H., Wiegand, C., Holmstrup, M., Renault, D., 2021. Thermal plasticity and sensitivity to insecticides in populations of an invasive beetle: Cyfluthrin increases vulnerability to extreme temperature. Chemosphere 274, 129905. https://doi.org/10.1016/j.chemosphere.2021.129905.

Everhart, J.L., McNear, D. Jr, Peltier, E., van der Lelie, D., Chaney, R.L., Sparks, D.L., 2006. Assessing nickel bioavailability in smelter-contaminated soils. Sci. Total Environ. 367, 732–744. https://doi.org/10.1016/j.scitotenv.2005.12.029.

Fuentes, I., Márquez-Ferrando, R., Pleguezuelos, J.M., Sanpera, C., Santos, X., 2020. Long-term trace element assessment after a mine spill: pollution persistence and bioaccumulation in the trophic web. Environ. Pollut. 267, 115406. https://doi.org/10.1016/j.envpol.2020.115406.

Gomaa, M.M., Melegy, A., Metwaly, H., Hassan, S., 2020. Geochemical and electrical characterization of heavy metals in contaminated soils. Heliyon 6, e04954. https://doi.org/10.1016/j.heliyon.2020.e04954.

Goyer, R.A., 1997. Toxic and essential metal interactions. Annu. Rev. Nutr. 17, 37–50. https://doi.org/10.1146/annurev.nutr.17.1.37.

Grześ, I.M., 2010. Ants and heavy metal pollution – A review. Eur. J. Soil Biol. 46, 350–355. https://doi.org/10.1016/j.ejsobi.2010.09.004.

Hanlon, S.M., Relyea, R., 2013. Sublethal effects of pesticides on predator-prey interactions in amphibians. Copeia 4, 691–698. http://dx.doi.org/10.1643/CE-13-019.

Hasan, M.K., Cheng, Y., Kanwar, M.K., Chu, X.Y., Ahammed, G.J., Qi, Z.Y., 2017. Responses of plant proteins to heavy metal stress — A review. Front. Plant Sci. 8, 1492. https://doi.org/10.3389/fpls.2017.01492.

Heliövaara, K., Väisänen, R., 1990. Concentrations of heavy metals in the food, faeces, adults, and empty cocoons of *Neodiprion sertifer* (Hymenoptera, Diprionidae). Bull. Environ. Contam. Toxicol. 45, 13–18. https://doi.org/10.1007/bf01701822.

Hendrickx, F., Maelfait, J-P., Langenbick, F., 2003. Absence of cadmium excretion and high assimilation result in cadmium biomagnification in a wolf spider. Ecotoxicology and Environmental Safety 55, 287-292.

Horváth, Z., Ptacnik, R., Vad, C.F., Chase, J.M., 2019. Habitat loss over six decades accelerates regional and local biodiversity loss via changing landscape connectance. Ecol. Lett. 22, 1019–1027. https://doi.org/10.1111/ele.13260.

Hu, C., Shui, B., Yang, X., Wang, L., Dong, J., Zhang, X., 2021. Trophic transfer of heavy metals through aquatic food web in a seagrass ecosystem of Swan Lagoon, China. Sci. Total Environ. 762, 143139. https://doi.org/10.1016/j.scitotenv.2020.143139.

Hu, Y., Wang, D., Wei, L., Zhang, X., Song, B., 2014. Bioaccumulation of heavy metals in plant leaves from Yan'an city of the Loess Plateau, China. Ecotoxicol. Environ. Saf. 110, 82–88. https://doi.org/10.1016/j.ecoenv.2014.08.021.

Husk, B., Sanchez, J.S., Leduc, R., Takser, L., Savary, O., Cabana, H., 2019. Pharmaceuticals and pesticides in rural community drinking waters of Quebec, Canada – a regional study on the susceptibility to source contamination. Water Qual. Res. J. 54, 88–103. https://doi.org/10.2166/wqrj.2019.038.

Kapusta, P., Sobczyk, Ł., 2015. Effects of heavy metal pollution from mining and smelting on enchytraeid communities under different land management and soil conditions. Sci. Total Environ. 536, 517–526. https://doi.org/10.1016/j.scitotenv.2015.07.086.

Karadjova, I., Markova, E., 2009. Metal accumulation in insects (Orthoptera, Acrididae) near a copper smelter and copper-flotation factory (Pirdop, Bulgaria). Biotechnol. Biotechnol. Equip. 23:204-207. https://doi.org/10.1080/13102818.2009.10818401.

Kelly, B.C., Ikonomou, M.G., Blair, J.D., Morin, A.E., Gobas, F.A., 2007. Food web-specific biomagnification of persistent organic pollutants. Science 317, 236–239. https://doi.org/10.1126/science.1138275.

Kim, H., Lee, M., Lee, J.H. Kim, K.H., Owens, G., Kim, K.R., 2020. Distribution and extent of heavy metal(loid) contamination in agricultural soils as affected by industrial activity. Appl. Biol. Chem. 63, 31. https://doi.org/10.1186/s13765-020-00517-x.

Kögel, T., Bjorøy, Ø., Toto, B., Bienfait, A.M., Sanden, M., 2020. Micro- and nanoplastic toxicity on aquatic life: Determining factors. Sci. Total Environ. 709, 136050. https://doi.org/10.1016/j.scitotenv.2019.136050.

Kukier, U., Peters, C.A., Chaney, R.L., Angle, J.S., Roseberg, R.L., 2004. The Effect of pH on metal accumulation in two Species. J. Environ. Qual. 33, 2090–2102. https://doi.org/10.2134/jeq2004.2090

Leary, S., Underwood, W., Anthony, R., Cartner, S., Grandin, T., Greenacre, C., et al. 2020. AVMA guidelines for the euthanasia of animals: 2020 edition, Schaumburg, IL. American Veterinary Medical Association. Available at: https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf. [Accessed 18 April 2021]

Lewis, S.L., Maslin, M.A., 2015a. A transparent framework for defining the Anthropocene Epoch. Anthr. Rev. 2, 128–146. https://doi.org/10.1177%2F2053019615588792.

Lewis, S.L., Maslin, M.A., 2015b. Defining the Anthropocene. Nature 519, 171–180. https://doi.org/10.1038/nature14258.

Liao, B., Guo, Z., Probst, A., Probst, J-L., 2005. Soil heavy metal contamination and acid deposition: Experimental approach on two forest soils in Hunan, Southern China. Geoderma 127, 91–103. http://dx.doi.org/10.1016/j.geoderma.2004.11.019.

Ma, W., Talmage, S., 2001. Insectivora, in: Rattner, B.A., Shore, R.F. (Eds.), Ecotoxicology of wild mammals Ecological and Environmental Toxicology Series. John Wiley and Sons Ltd., New York, pp. 123–158.McCauley, D.J., Pinsky M.L., Palumbi, S.R., Estes, J.A., Joyce, F.H., Warner, R.B., 2015. Marine defaunation: Animal loss in the global ocean. Science 347, 1255641.

Marabuto, E., 2014. The Afrotropical Miomantis caffra Saussure 1871 and Miomantis paykullii Stal 1871: first records of alien mantid species in Portugal and Europe, with an updated checklist of Mantodea in Portugal (Insecta: Mantodea). Biodivers. Data J. 12, e4117. http://dx.doi.org/10.3897/BDJ.2.e4117.

Márquez-Ferrando, R., Santos, X., Pleguezuelos, J.M., Ontiveros, D., 2009. Bioaccumulation of heavy metals in the lizard *Psammodromus algirus* after a tailing-dam collapse in Aznalcóllar (Southwest Spain). Arch. Environ. Contam. Toxicol. 56, 276–285. https://doi.org/10.1007/s00244-008-9189-3.

Maxwell, S.L., Fuller, R.A., Brooks, T.M., Watson, J.E., 2016. Biodiversity: the ravages of guns, nets and buldozers. Nature 536, 143–145. https://doi.org/10.1038/536143a.

McGrath, T.J., Ball, A.S., Clarke, B.O., 2017. Critical review of soil contamination by polybrominated diphenyl ethers (PBDEs) and novel brominated flame retardants (NBFRs); concentrations, sources and congener profiles. Environ. Pollut. 230, 741–757. https://doi.org/10.1016/j.envpol.2017.07.009.

Miller, M.E., Hamann, M., Kroon, F.J., 2020. Bioaccumulation and biomagnification of microplastics in marine organisms: A review and meta-analysis of current data. PLoS ONE 15, e0240792. https://doi.org/10.1371/journal.pone.0240792.

Mohamed, A.A., Elmogy, M., Dorrah, M.A., Yousef, H.A., Bassal, T.T., 2013. Antibacterial activity of lysozyme in the desert locust, *Schistocerca gregaria* (Orthoptera: Acrididae). Eur. J. Entomol. 110, 559–565. https://doi.org/10.14411/eje.2013.076.

Moor, C., Lymberopoulou, T., Dietrich, V.J., 2001. Determination of heavy metals in soils, sediments and geological materials by ICP-AES and ICP-MS. Microchim. Acta 136, 123–128. https://doi.org/10.1007/s006040170041.

Nasri, I., Hammouda, A., Hamza, F., Zrig, A., Selmi, S., 2017. Heavy metal accumulation in lizards living near a phosphate treatment plant: possible transfer of contaminants from aquatic to terrestrial food webs. Environ. Sci. Pollut. Res. 24, 12009–12014. https://doi.org/10.1007/s11356-015-5390-x.

Nunes, A.C., Mathias, M.L., Crespo, A.M., 2001. Morphological and haematological parameters in the Algerian mouse (*Mus spretus*) inhabiting an area contaminated with heavy metals. Environ. Pollut. 113, 87–93. https://doi.org/10.1016/s0269-7491(00)00159-7.

Nyffeler, M., Benz, G. 1988. Feeding ecology and predatory importance of wolf spiders (*Pardosa* spp.) (Araneae, Lycosidae) in winter wheat fields. J. Appl. Entomol. 106, 123–134. https://doi.org/10.1111/j.1439-0418.1988.tb00575.x

O'Kelly, B.C., El-Zein, A., Liu, X., Patel, A., Fei, X., Sharma, S., Mohammad, A., Goli, V.S.N.S., Wang, J.J., Li, D., Shi, Y., Xiao, L., Kuntikana, G., Shashank, B.S., Sarris, T.S., Rao, B.H., Mohamed, A.M.O., Paleologos, E.K., Nezhad, M.M., Singh, D.N., 2021. Microplastics in soils: an environmental geotechnics perspective. Environ. Geotech. https://doi.org/10.1680/jenge.20.00179.

Obbard, R.W., 2018. Microplastics in polar regions: The role of long range transport. Curr. Opin. Environ. Sci. Health, 1, 24–29. https://doi.org/10.1016/j.coesh.2017.10.004.

Ololade, I.A., Ashoghon, A.O., Adeyemi, O., 2007. Plants level of chromium and nickel at a refuse site, any positive impact? J. Appl. Sci. 7, 1768–1773. https://dx.doi.org/10.3923/jas.2007.1768.1773.

Outridge, P.M., Scheuhammer, A.M., 1993. Bioaccumulation and toxicology of nickel: implications for wild mammals and birds. Environ. Rev. 1, 172–197. https://doi.org/10.1139/a93-013.

Parihar, J.K., Parihar, P.K., Pakade, Y.B., Katnoria, J.K., 2021. Bioaccumulation potential of indigenous plants for heavy metal phytoremediation in rural areas of Shaheed Bhagat Singh Nagar, Punjab (India). Environ. Sci. Pollut. Res. 28, 2426–2442. https://doi.org/10.1007/s11356-020-10454-3.

Peterson, L., Trivett, V., Baker, A., Aguiar, C., Pollard, A.J., 2003. Spread of metals through an invertebrate food chain as influenced by a plant that hyperaccumulates nickel. Chemoecology 13, 103–108. http://dx.doi.org/10.1007/s00049-003-0234-4.

Picó, Y., Alvarez-Ruiz, R., Alfarhan, A.H., El-Sheikh, M.A., Alshahrani, H.O., Barceló, D., 2020. Pharmaceuticals, pesticides, personal care products and microplastics contamination assessment of Al-Hassa irrigation network (Saudi Arabia) and its shallow lakes. Sci. Total Environ. 701, 135021. https://doi.org/10.1016/j.scitotenv.2019.135021.

Pokhrel, B., Gong, P., Wang, X., Khanal, S. N., Ren, J., Wang, C., Gao, S., Yao, T., 2018. Atmospheric organochlorine pesticides and polychlorinated biphenyls in urban areas of Nepal: spatial variation, sources, temporal trends, and long-range transport potential. Atmos. Chem. Phys. 18, 1325–1336. https://doi.org/10.5194/acp-18-1325-2018.

Prud'homme, S.M., Renault, D., David, J.P., Reynaud, S., 2018. Multiscale approach to deciphering the molecular mechanisms involved in the direct and intergenerational effect of ibuprofen on mosquito *Aedes aegypti*. Environ. Sci. Technol. 52, 7937–7950. https://doi.org/10.1021/acs.est.8b00988.

Radwan, M.A., El-Gendy, K.S., Gad, A.F., 2010. Oxidative stress biomarkers in the digestive gland of *Theba pisana* exposed to heavy metals. Arch. Environ. Contam. Toxicol. 58, 828–835. https://doi.org/10.1007/s00244-009-9380-1.

Rawlins, B.G., Tye, A., Lark, R.M., Hodgkinson, E., Webster, R., O'Donnell, K.E., Smith, B., 2006. Linking historical smelter emissions across Humberside (UK) to enhanced soil metal concentrations using geostatistics and preserved environmental samples. Chin. J. Geochem. 25, 8. https://doi.org/10.1007/BF02839746.

Renault, D., Yousef, H., Mohamed, A.A., 2018. The multilevel antibiotic-induced perturbations to biological systems: Early-life exposure induces long-lasting damages to muscle structure and mitochondrial metabolism in flies. Environ. Pollut. 241, 821–833. https://doi.org/10.1016/j.envpol.2018.06.011.

Ripple, W.J., Wolf, C., Newsome, T.M., Betts, M.G., Ceballos, G., Courchamp, F., Hayward, M.W., Valkenburgh, B., Wallach, A.D., Worm, B., 2019. Are we eating the world's megafauna to extinction? Conserv. Lett. 12, e12627. https://doi.org/10.1111/conl.12627.

Saaristo, M., Brodin, T., Balshine, S., Bertram, M.G., Brooks, B.W., Ehlman, S.M., McCallum, E.S., Sih, A., Sundin, J., Wong, B.B.M., Arnold, K.E., 2018. Direct and indirect effects of chemical contaminants on the behaviour, ecology and evolution of wildlife. Proc. Biol. Sci. 285, 20181297. https://doi.org/10.1098/rspb.2018.1297.

Said, I., Salman, S.A.E., Samy, Y., Awad, S.A., Melegy, A., Hursthouse, A.S., 2019. Environmental factors controlling potentially toxic element behaviour in urban soils, El Tebbin, Egypt. Environ. Monit. Assess. 191, 267. https://doi.org/10.1007/s10661-019-7388-1.

Sánchez-Chardi, A., Nadal, J., 2007. Bioaccumulation of metals and effects of landfill pollution in small mammals. Part I. The greater white-toothed shrew, *Crocidura russula*. Chemosphere 68, 703–711. https://doi.org/10.1016/j.chemosphere.2007.01.042.

Sánchez-Chardi, A., Peñarroja-Matutano, C., Ribeiro, C.A.O., Nadal, J., 2007. Bioaccumulation of metals and effects of a landfill in small mammals. Part II. The wood mouse, *Apodemus sylvaticus*. Chemosphere 70, 101–109. https://doi.org/10.1016/j.chemosphere.2007.06.047.

Serra, A.A, Nuttens, A., Larvor, V., Renault, D., Couée, I., Sulmon, C., Gouesbet, G., 2013. Low environmentally relevant levels of bioactive xenobiotics and associated degradation products cause cryptic perturbations of metabolism and molecular stress responses in *Arabidopsis thaliana*. J. Exp. Bot. 64, 2753–2766. https://doi.org/10.1093/jxb/ert119.

Serra, A.A., Bittebière, A.K., Mony, C., Slimani, K., Pallois, F., Renault, D., Couée, I., Gouesbet, G., Sulmon, C., 2020. Local-scale dynamics of plant-pesticide interactions in a northern Brittany agricultural landscape. Sci. Total Environ. 744, 140772. https://doi.org/10.1016/j.scitotenv.2020.140772.

Shaapera, U., Nnamonu, L., Eneji, I., 2013. Assessment of heavy metals in *Rana esculenta* organs from River Guma, Benue State Nigeria. Am. J. Analyt. Chem. 4, 496–500. http://dx.doi.org/10.4236/ajac.2013.49063.

Sharaf, M.R., Aldawood, A.S., Mohamed, A.A., Hita Garcia, F., 2020. The genus *Lepisiota* Santschi, 1926 of the Arabian Peninsula with the description of a new species, *Lepisiota elbazi* sp. nov. from Oman, an updated species identification key, and assessment of zoogeographic affinities. J. Hymenopt. Res. 76, 127–152. https://doi.org/10.3897/jhr.76.50193.

Silva, J.M., Navoni, J.A., Freire, E.M.X., 2020. Lizards as model organisms to evaluate environmental contamination and biomonitoring. Environ. Monit. Assess. 192, 454. https://doi.org/10.1007/s10661-020-08435-7.

Solgi, E., Parmah, J., 2015. Analysis and assessment of nickel and chromium pollution in soils around Baghejar Chromite Mine of Sabzevar Ophiolite Belt, Northeastern Iran. Trans. Nonferrous Met. Soc. China 25, 2380–2387. https://doi.org/10.1016/S1003-6326(15)63853-5.

Soliman, M., El-Shazly, M., Abd-El-Samie, E., Fayed, H., 2019. Variations in heavy metal concentrations among trophic levels of the food webs in two agroecosystems. Afr. Zool. 54, 21–30. http://dx.doi.org/10.1080/15627020.2019.1583080.

Soliman, M.M., El-Shazly, M.M., 2017. Bioaccumulation of heavy metals by grasshoppers and a mantid along a pollution gradient. Ecol. Balk. 9, 7–21. https://doaj.org/article/eb1ab77348f74154a79b12dc7c1a86ee.

Soliman, M.M., Haggag, A.A., El-Shazly, M.M., 2017. Assessment of grasshopper diversity along a pollution gradient in the Al-Tebbin region, South Cairo, Egypt. J. Entomol. Zool. Stud. 5, 298–306. https://www.entomoljournal.com/archives/?year=2017&vol=5&issue=1&ArticleId=1478

Steinnes, E., Lukina, N., Nikonov, V., Aamlid, D., Røyset, O., 2000. A gradient study of 34 elements in the vicinity of a copper-nickel smelter in the Kola Peninsula. Environ. Monit. Assess. 60, 71–88. https://doi.org/10.1023/A:1006165031985.

Sun, T., Wu, H., Wang, X., Ji, C., Shan, X., Li, F., 2020. Evaluation on the biomagnification or biodilution of trace metals in global marine food webs by meta-analysis. Environ. Pollut. 264, 113856. https://doi.org/10.1016/j.envpol.2019.113856.

Talmage, S.S., Walton, B.T. 1991. Small mammals as monitors of environmental contaminants. Rev. Environ. Contam. Toxicol. 119, 47–145. https://doi.org/10.1007/978-1-4612-3078-6_2.

Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K., Sutton, D.J., 2012. Heavy metal toxicity and the environment. Exp. Suppl. 101, 133–164. https://dx.doi.org/10.1007%2F978-3-7643-8340-4_6.

Tóth, G., Hermann, T., Da Silva, M., Montanarella, L., 2016. Heavy metals in agricultural soils of the European Union with implications for food safety. Environ. Int. 88, 299–309. https://doi.org/10.1016/j.envint.2015.12.017.

Usman, K., Al-Ghouti, M.A., Abu-Dieyeh, M.H., 2019. The assessment of cadmium, chromium, copper, and nickel tolerance and bioaccumulation by shrub plant *Tetraena qataranse*. Sci. Rep. 9, 5658. https://doi.org/10.1038/s41598-019-42029-9.

Vizzini, S., Costa, V., Tramati, C., Gianguzza, P., Mazzola, A., 2013. Trophic transfer of trace elements in an isotopically constructed food chain from a semi-enclosed marine coastal area (Stagnone di Marsala, Sicily, Mediterranean). Arch. Environ. Contam. Toxicol. 65, 642–653. https://doi.org/10.1007/s00244-013-9933-1.

Wilczek, G., Babczyńska, A., 2000. Heavy metals in the gonads and hepatopancreas of spiders (Araneae) from variously polluted areas. Ekol. Bratislava 19, 283-292.

WSC, 2021. World Spider Catalog. Version 22.5. Natural History Museum Bern, online at http://wsc.nmbe.ch [16.01.2021]. Available at: http://doi.org/10.24436/2.

Zhang, H., Zhao, Y., Wang, Z., Liu, Y., 2021. Distribution characteristics, bioaccumulation and trophic transfer of heavy metals in the food web of grassland ecosystems. Chemosphere 278, 130407. https://doi.org/10.1016/j.chemosphere.2021.130407.

Zhang, Y., Gao, T., Kang, S., Sillanpää, M., 2019. Importance of atmospheric transport for microplastics deposited in remote areas. Environ. Pollut. 254, 112953. https://doi.org/10.1016/j.envpol.2019.07.121.

Żmudzki, S., Laskowski, R., 2012. Biodiversity and structure of spider communities along a metal pollution gradient. Ecotoxicology 21, 1523–1532. https://doi.org/10.1007/s10646-012-0906-3.

Zocche, J.J., da Silva, L.A., Damiani, A.P., Mendonça, R.Á., Peres, P.B., dos Santos, C.E., Debastiani, R., Dias, J.F., de Andrade, V.M., Pinho, R.A., 2014. Heavy-metal content and oxidative damage in *Hypsiboas faber*: The impact of coal-mining pollutants on amphibians. Arch. Environ. Contam. Toxicol. 66, 69–77. https://doi.org/10.1007/s00244-013-9949-6.

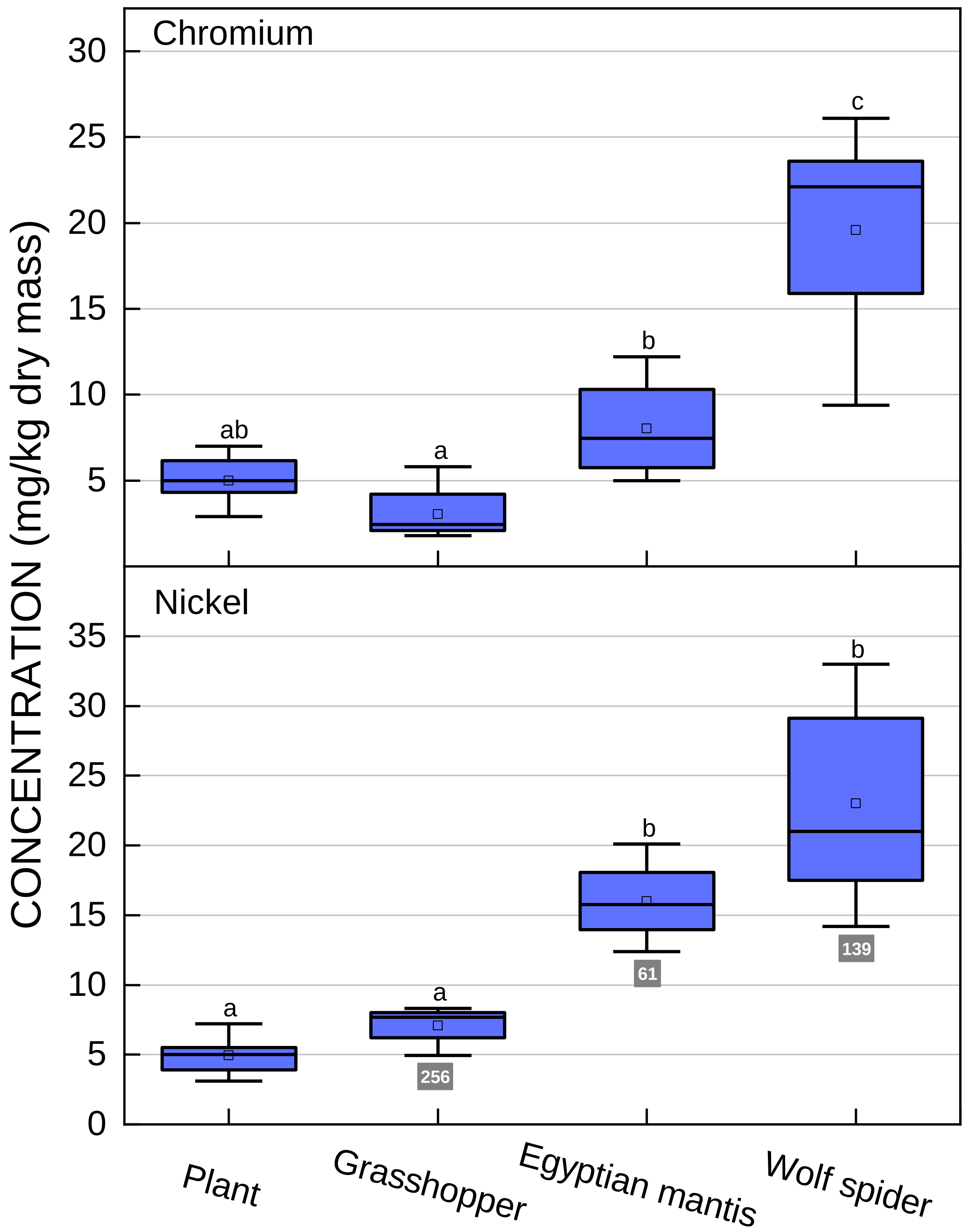


Figure 1. Mean metal concentrations in plants and arthropods from El-Tebbin area. Data are presented as mean ± SD in mg/g dry mass. Bars followed by different letters denote significant differences in metal concentrations (analysis of variance [ANOVA]; Bonferroni test; $P < 0.05$). The number below each 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.

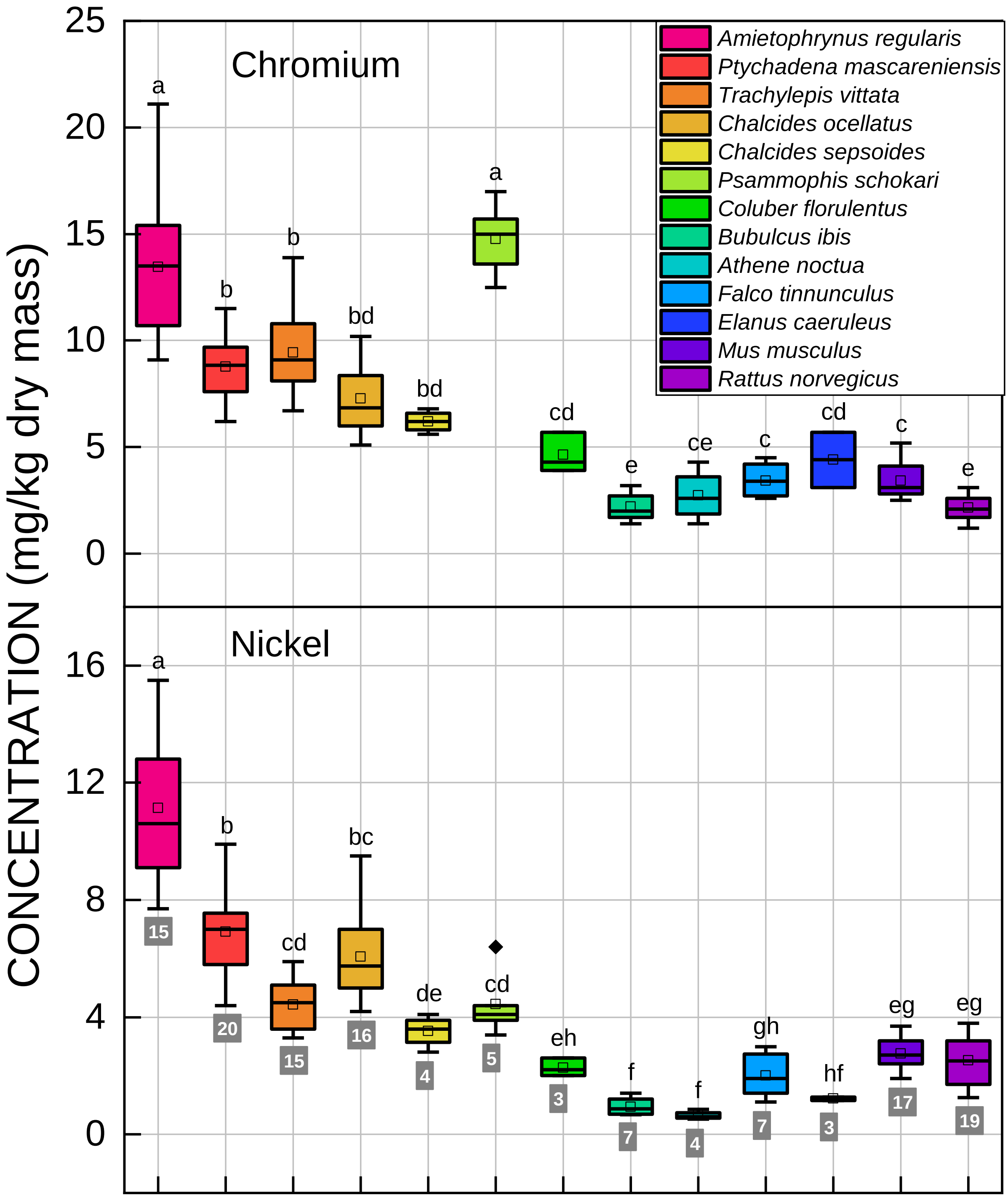


Figure 2. Mean metal concentrations in vertebrates from El-Tebbin area. Data are presented as mean ± SD in mg/ kg dry mass. Boxes followed by different letters denote significant differences in metal concentrations (analysis of variance [ANOVA]; Bonferroni test; $P < 0.05$). The number below each box 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.

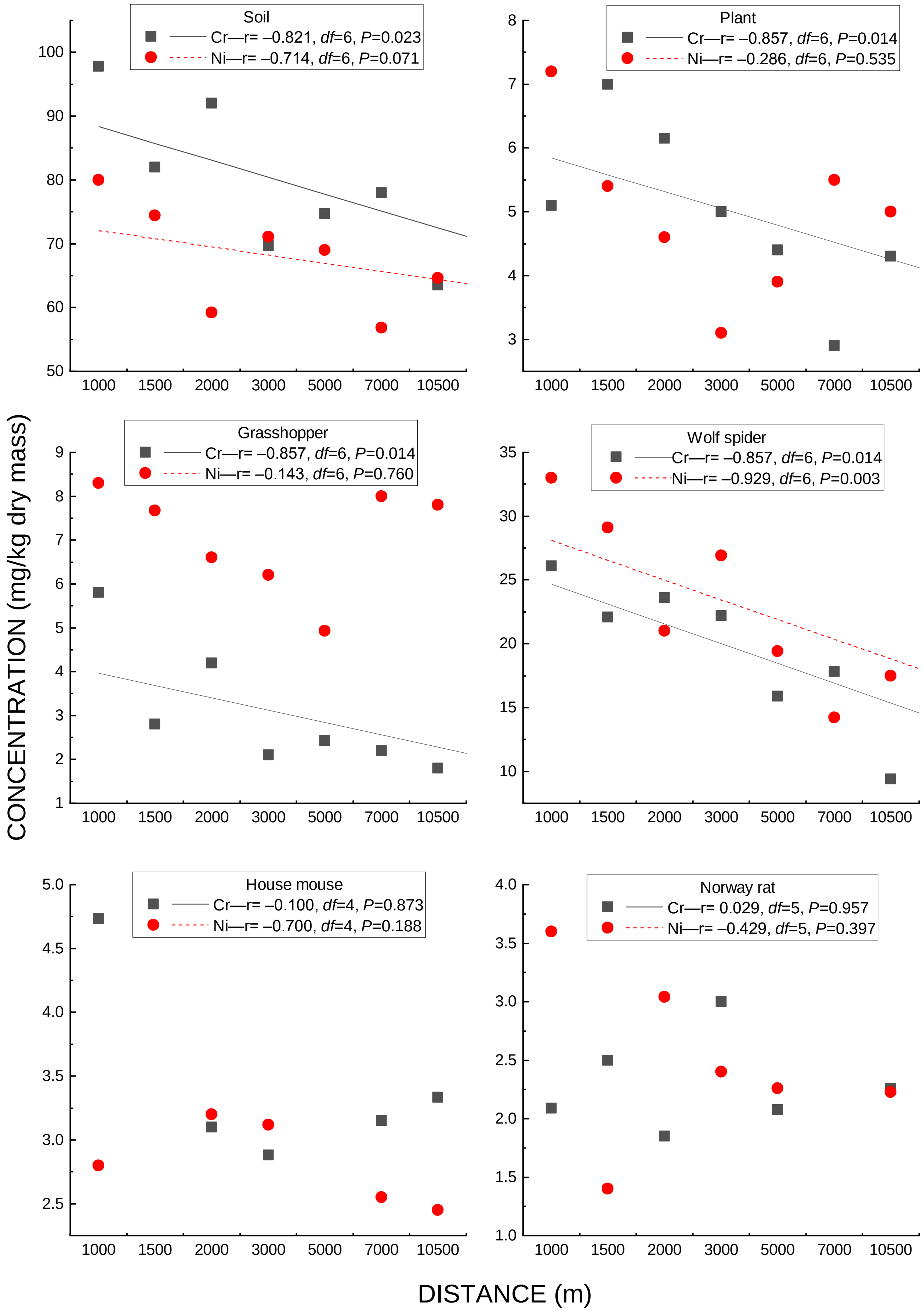

Soil
Cr—r= –0.821, df=6, P=0.023
Ni—r= –0.714, df=6, P=0.071
Plant
Cr—r= –0.857, df=6, P=0.014
Ni—r= –0.286, df=6, P=0.535
Grasshopper
Cr—r= –0.857, df=6, P=0.014
Ni—r= –0.143, df=6, P=0.760
Wolf spider
Cr—r= –0.857, df=6, P=0.014
Ni—r= –0.929, df=6, P=0.003
House mouse
Cr—r= –0.100, df=4, P=0.873
Ni—r= –0.700, df=4, P=0.188
Norway rat
Cr—r= 0.029, df=5, P=0.957
Ni—r= –0.429, df=5, P=0.397
CONCENTRATION (mg/kg dry mass)
DISTANCE (m)
1000
1500
2000
3000
5000
7000
10500

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.

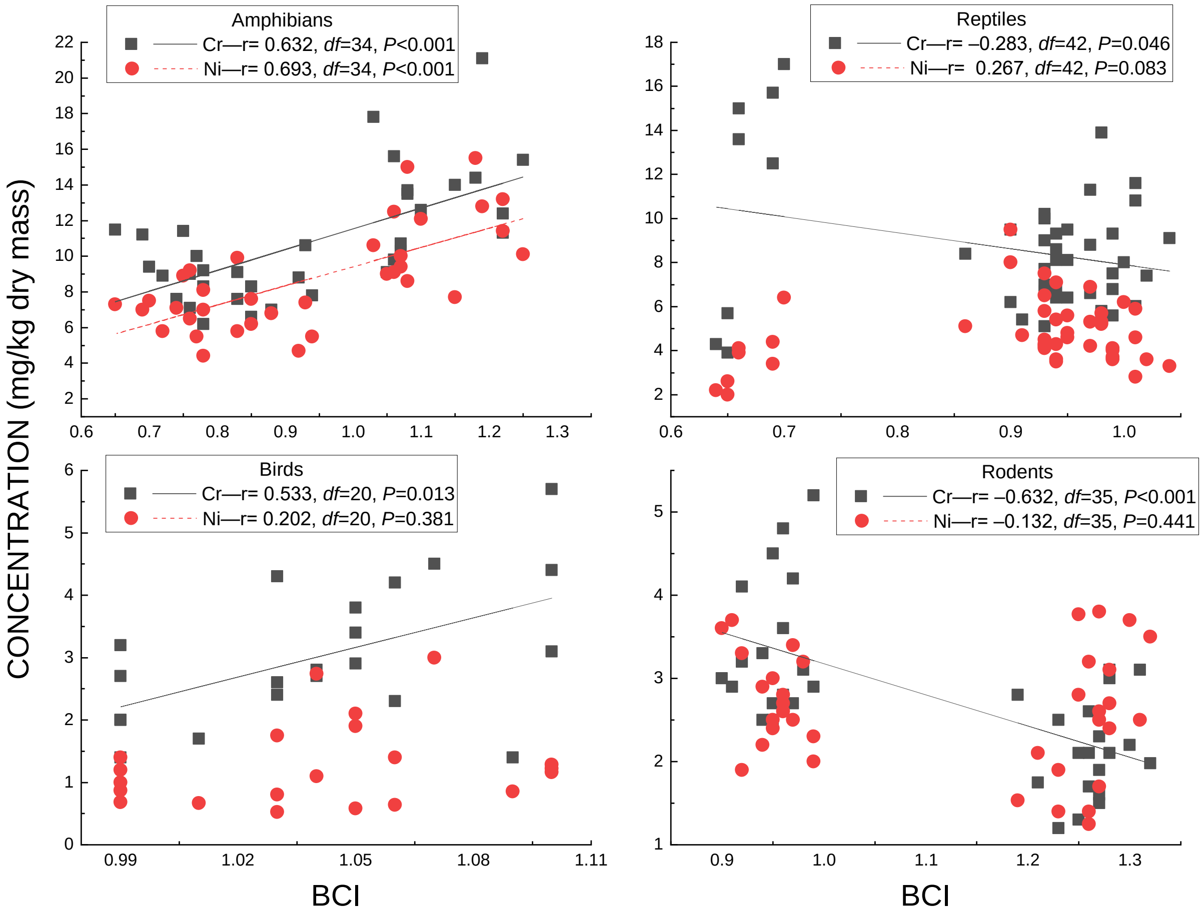


Figure 4. Chromium and Ni concentration in vertebrate livers plotted against body condition index. Values of the Pearson correlation coefficient test (r), degrees of freedom (df), and probability (p) are given for each metal. Species of each taxon group are given in **Supplementary material 2.**

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 ± SD)	
			Cr	Ni
A (El Shobak)	1.0	NA	97.8 ± 9.2	80.0 ± 5.6
B (El Shobak El-Sharqi)	1.5	0.5	82.0 ± 6.4	74.4 ± 4.7
C (El Shurafa)	2.0	0.5	92.0 ± 7.9	59.2 ± 8.1
D (Kafr Turkhan)	3.1	0.6	69.7 ± 6.1	71.1 ± 6.3
E (Ghammazah Al Kubra)	5.0	1.9	74.7 ± 4.2	69.0 ± 7.7
F (Al Ikhsas Al Qiblyyah)	7.0	2.0	78.0 ± 7.6	56.8 ± 2.6
G (Arab Ghammazah Al Kubra)	10.5	3.5	63.5 ± 3.0	64.6 ± 3.8
Threshold values in Agricultural Soils of the European Union**			*100*	*50*

* Taken from (Tóth et al. 2016).

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***			
Amietophrynus regularis				0.683	0.222–1.144	0.004**
Ptychadena mascareniensis				0.449	-0.101–0.999	0.108
Trachylepis vittata				0.515	0.016–1.014	0.043*
Chalcides ocellatus				0.432	-0.054–0.918	0.081
Chalcides sepsoides				0.366	-0.149–0.881	0.162
Psammophis schokari				1.062	0.225–1.898	0.013*
Coluber florulentus				0.515	-0.253–1.283	0.186
Bubulcus ibis				0.170	-0.278–0.619	0.453
Athene noctua				0.052	-0.280–0.384	0.758
Falco tinnunculus				0.279	0.008–0.550	0.044*
Elanus caeruleus				0.387	0.168–0.605	0.001**
Mus musculus				0.071	-0.421–0.563	0.775
Rattus norvegicus				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

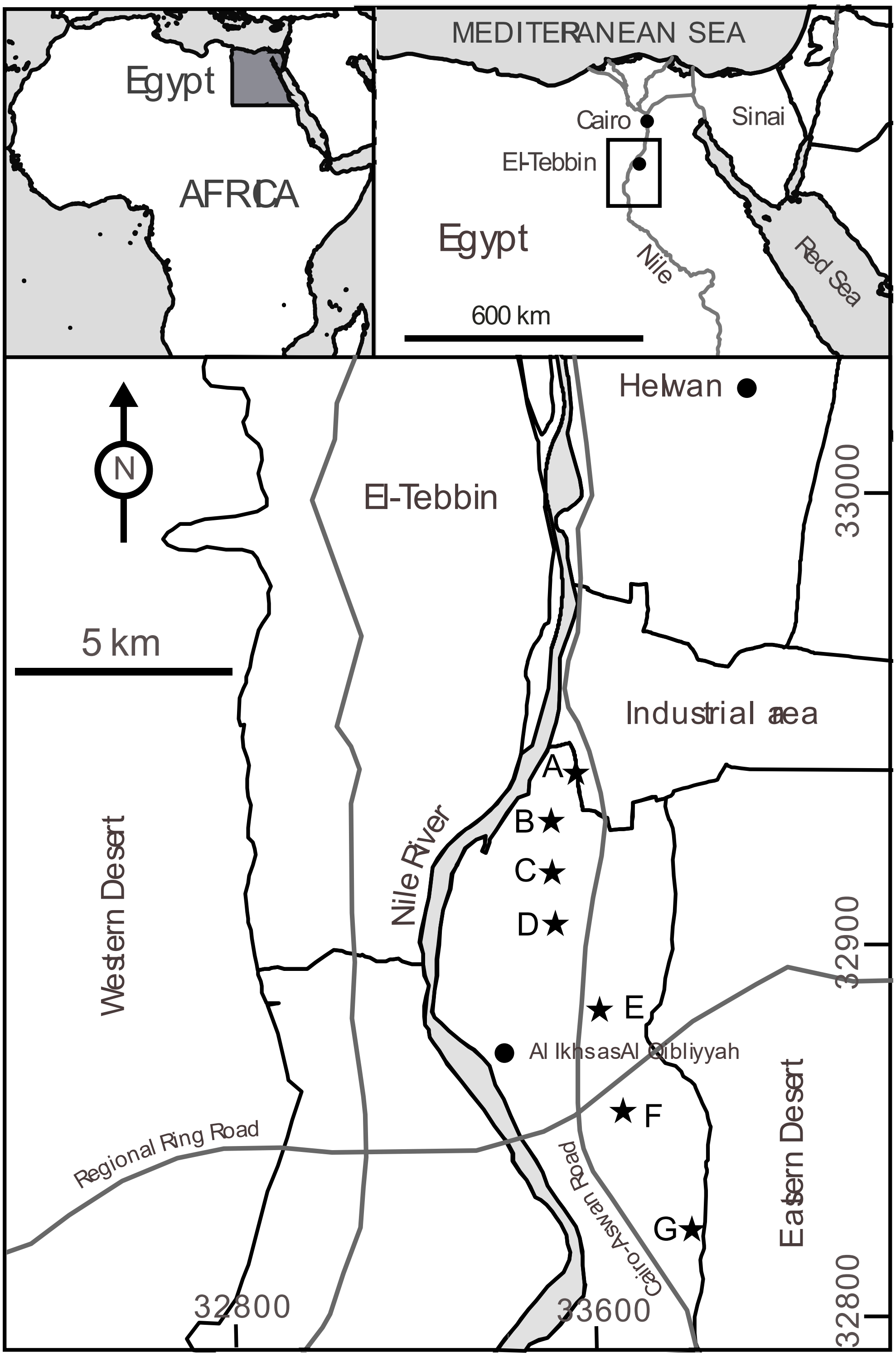
Table 3. Variations in Ni concentrations by vertebrate species, site, and body characteristics (Body mass, body length, and body condition index) with results of the general linear model. B = estimated regression coefficient, P = P-value, significance codes: *** = P < 0.001, ** = P 0.001– 0.01, * = P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

Factor	F	df	*P*	B	95% CI	*P*
Taxon	47.488	12	< 0.001***			
Amietophrynus regularis				0.550	0.069–1.031	0.025*
Ptychadena mascareniensis				0.194	-0.379–0.768	0.504
Trachylepis vittata				0.099	-0.421–0.619	0.707
Chalcides ocellatus				0.271	-0.236–0.777	0.292
Chalcides sepsoides				0.026	-0.511–0.563	0.924
Psammophis schokari				1.158	0.285–2.030	0.010**
Coluber florulentus				0.707	-0.094–1.507	0.083
Bubulcus ibis				0.128	-0.339–0.596	0.588
Athene noctua				-0.463	-0.810–-0.117	0.009**
Falco tinnunculus				0.133	-0.150–0.416	0.353
Elanus caeruleus				-0.100	-0.327–0.128	0.388
Mus musculus				-0.078	-0.591–0.436	0.765
Rattus norvegicus				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	0.795
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

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 (*Amietophrynus regularis*)	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 (*Ptychadena mascareniensis*)	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 (*Trachylepis vittata*)	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 (*Chalcides ocellatus*)	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 (*Chalcides sepsoides*)	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 (*Psammophis schokari*)	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 (*Coluber florulentus*)	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 (*Bubulcus ibis*)	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 (*Athene noctua*)	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 (*Falco tinnunculus*)	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 (*Elanus caeruleus*)	299.3 ± 6.6 (293.4–306.5)	347.3 ± 4.5 (343–352)	1.09 ± 0.0004 (1.1–1.1)	3
House mouse (*Mus musculus*)	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 (*Rattus norvegicus*)	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.

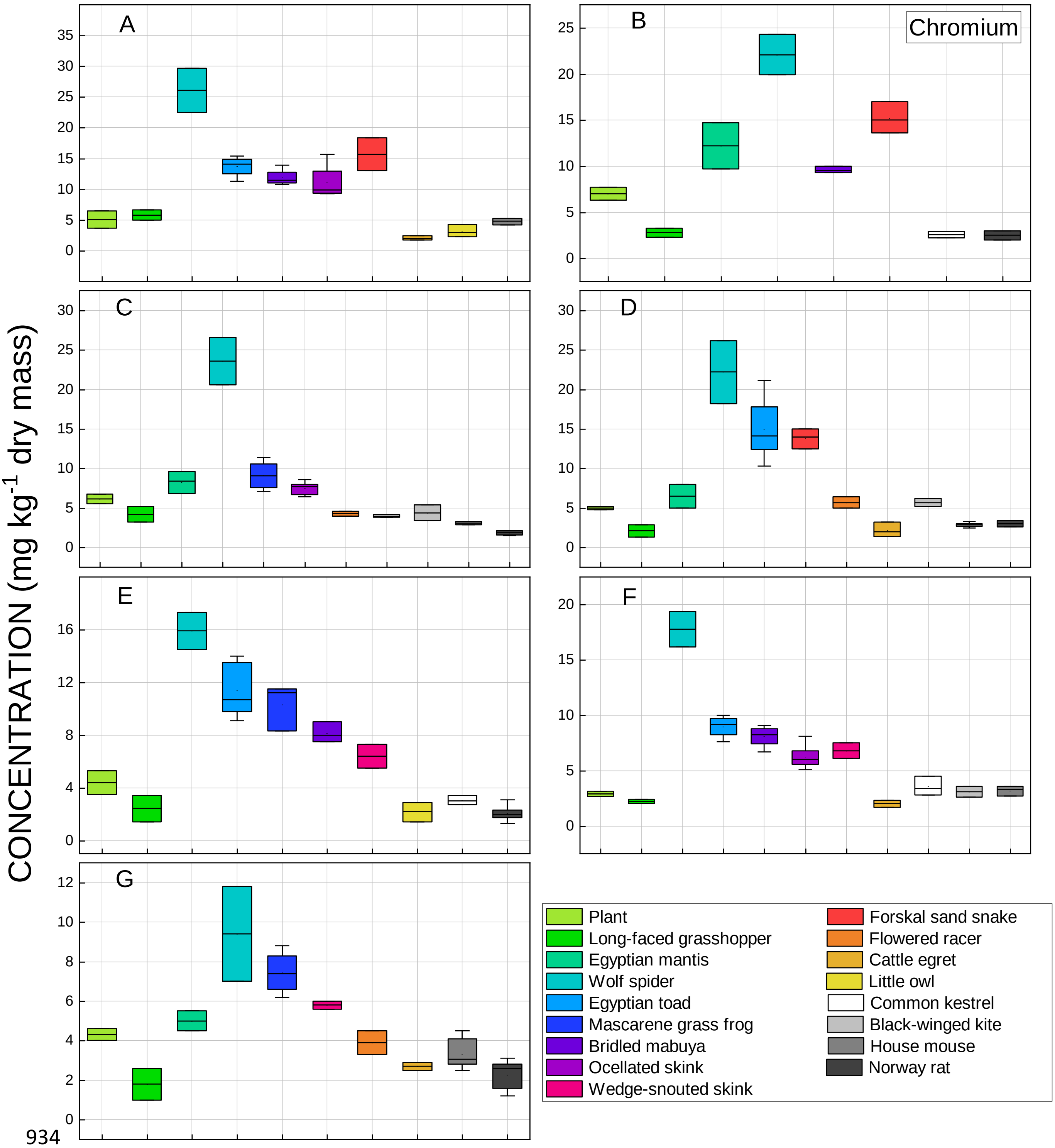


Supplementary Material 1. Map of El-Tebbin in Egypt showing the sites where soil, plants and animals were collected. Each star represents the position of the sampled plots. ArcGIS 10.2 was used to create the location map.

Supplementary Material 2. Presentation of the sampled animal taxa, trophic level, and numbers of animals collected from the seven studied localities (A – F) of El-Tebbin area (Egypt). Trophic level: Herbivore (Herb); Primary carnivore (1° Carn); Secondary carnivore (2° Carn); Top carnivore (Top Carn); Omnivore (Omni)

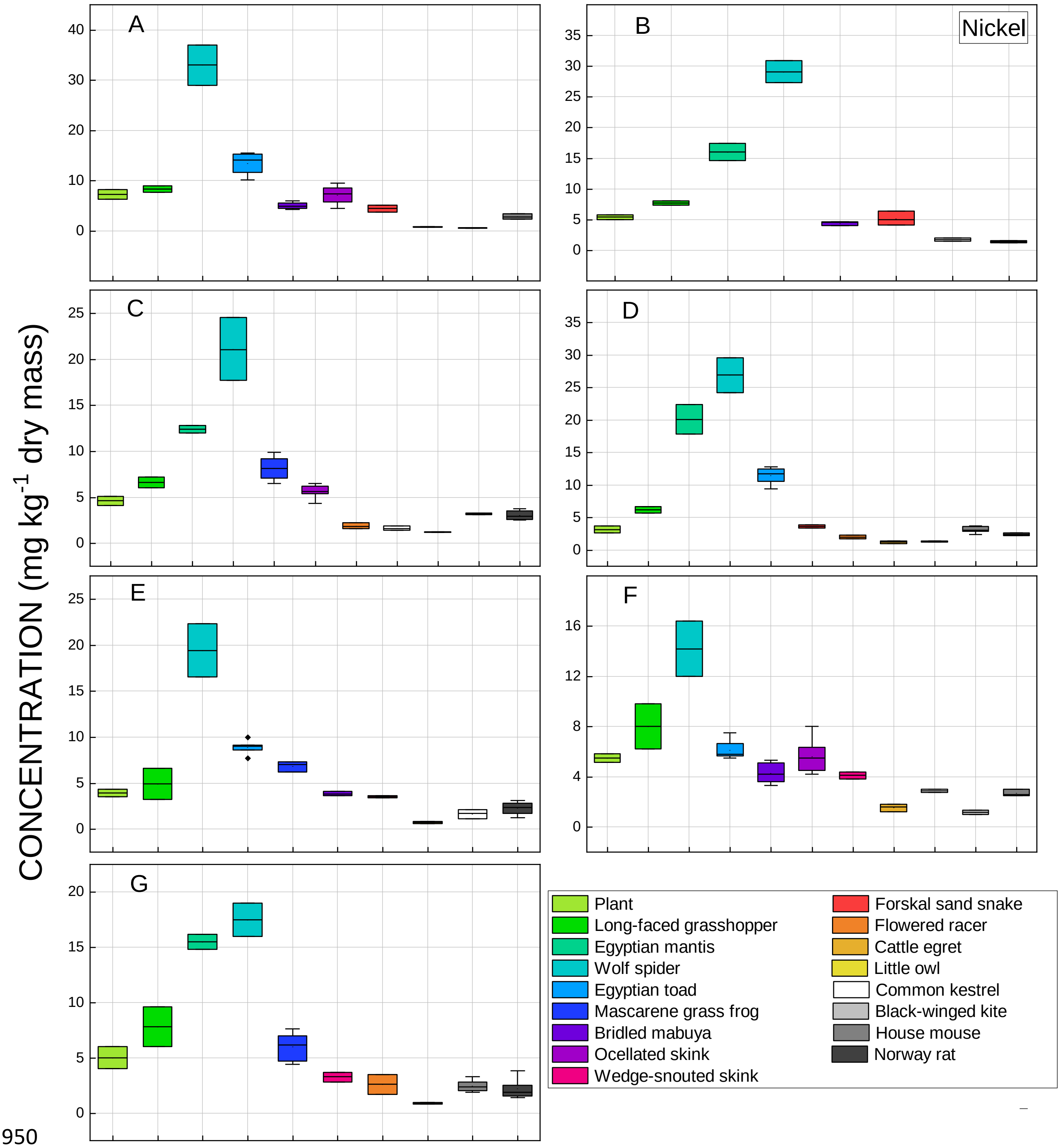
Taxon (Common and Latin names)	Trophic level	Locality							Total
		A	B	C	D	E	F	G	
Arthropods									
Long-faced grasshopper (*Truxalis grandis Klug*)	Herb	29	33	45	38	50	34	27	256
Egyptian mantis (*Miomantis paykullii* Stål)	1° Carn		18	12	11			20	61
Wolf spider (*Hogna ferox* (Lucas))	1° Carn	16	23	18	15	24	20	23	139
Amphibians									
Egyptian toad (*Amietophrynus regularis* (Reuss))	2° Carn	4			6	5			15
Mascarene grass frog (*Ptychadena mascareniensis* (Dumeril & Bibron))	2° Carn			7		3	4	6	20
Reptiles									
Lizards									
Bridled mabuya (*Trachylepis vittata* (Olivier))	2° Carn	4	3			2	6		15
Ocellated skink (*Chalcides ocellatus* (Forskål))	2° Carn	3		5			8		16
Wedge-snouted skink (*Chalcides sepsoides* (Audouin))	2° Carn					1	1	2	4
Snakes									
Forskal sand snake (*Psammophis schokari* (Forskål))	2° Carn	1	2		2				5
Flowered racer (*Coluber florulentus* (Geoffroy Saint-Hilaire))	2° Carn			1	1			1	3
Birds									
Cattle egret (*Bubulcus ibis* (Linnaeus))	2° Carn	3			2		1	1	7
Little owl (*Athene noctua* (Scopoli))	Top Carn	2				2			4
Common kestrel (*Falco tinnunculus* (Linnaeus))	Top Carn		1	2		2	2		7
Black-winged kite (*Elanus caeruleus* (Desfontaines))	Top Carn			1	1		1		3
Small mammals									
House mouse (*Mus musculus* (Linnaeus))	Omni	3		1	5		2	6	17
Norway rat	Omni	2	1	4	1	6		5	19

(*Rattus norvegicus* (Berkenhout))



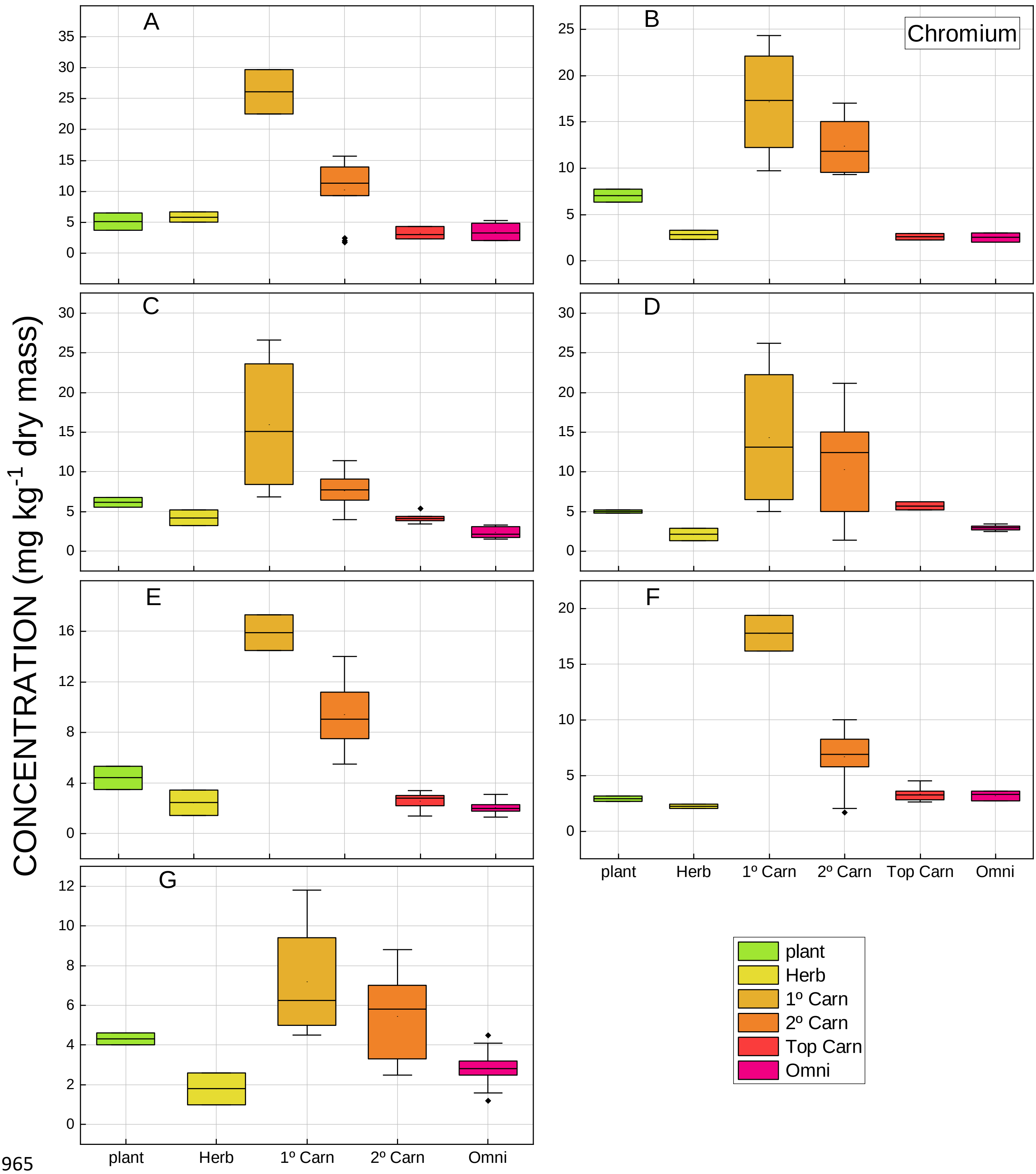
Supplementary material 3. Boxplot presentation for Chromium concentration measured from the flora and fauna at different trophic levels sampled from seven localities of the El-Tebbin region (Egypt), as function of the distance to the main pollution source (from site A to site G). For arthropods, individuals were pooled for the analysis so that a dry mass of 0.5 g was obtained for each replicate and each species. In the case of the wedge-snouted skink (lizards, *Chalcides sepsoides)*, flowered racer (snakes, *Coluber florulentus)*, birds (all species), and small mammals (all species), it was very hard to sample a large amount of individuals, which would have additionally impacted their population dynamics. As small sample size (<3 individuals per site) were obtained for these species, two (when two individuals were collected, Supplementary material 2) to three replicates (when a single individual was collected, Supplementary material 2) were obtained from a single individual to ensure that we would have 3 to 6 measures.

A: El Shobak, **B:** El Shobak, **C:** El-Sharqi, **C:** El Shurafa, **D:** Kafr Turkhan, **E:** Ghammazah Al Kubra, **F:** Al Ikhsas Al Qiblyyah, and **G:** Arab Ghammazah Al Kubra.

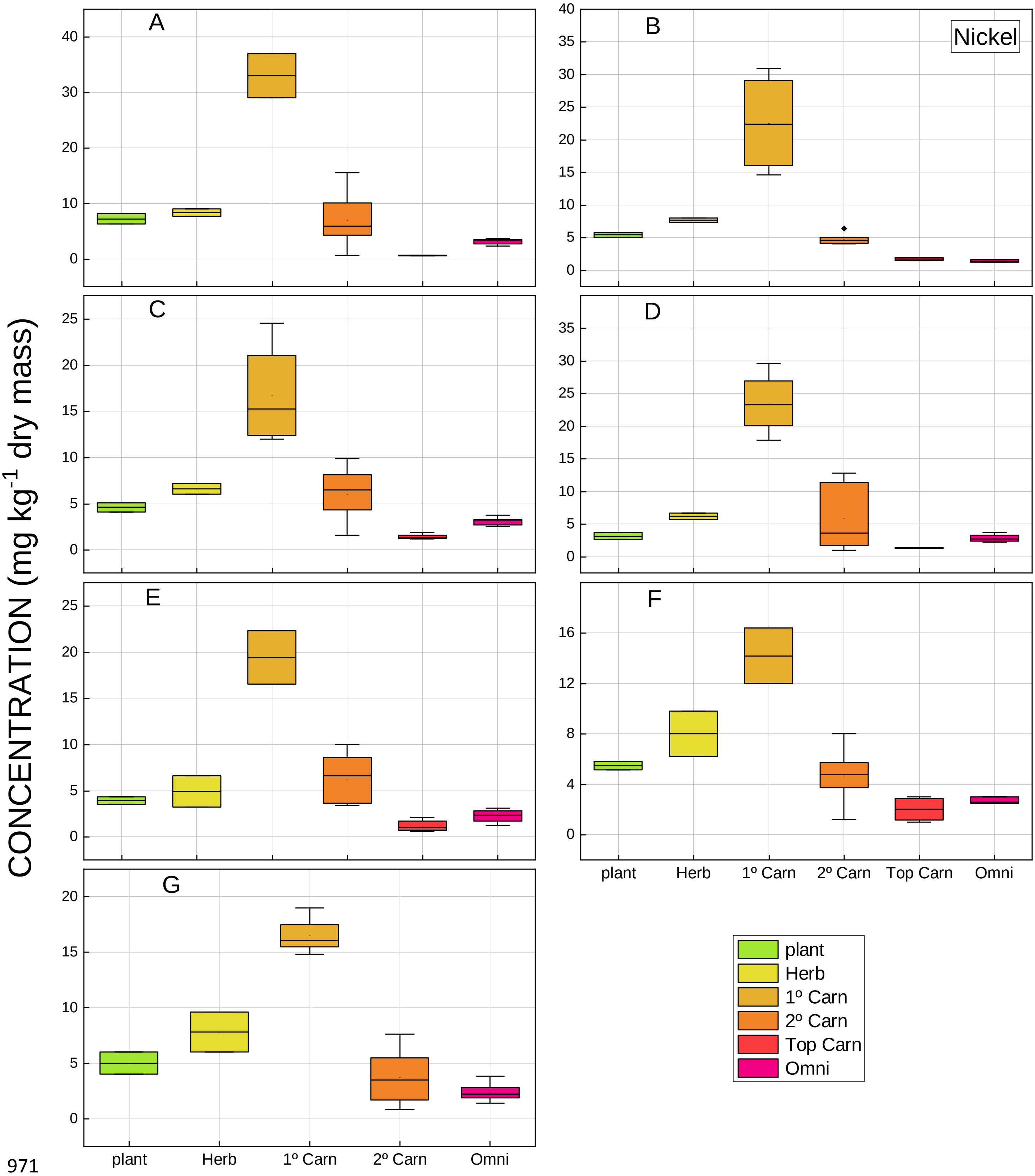


Supplementary material 4. Boxplot presentation for Nickel concentration measured from the flora and fauna at different trophic levels sampled from seven localities of the El-Tebbin region (Egypt), as function of the distance to the main pollution source (from site A to site G). For arthropods, individuals were pooled for the analysis so that a dry mass of 0.5 g was obtained for each replicate and each species. In the case of the wedge-snouted skink (lizards, *Chalcides sepsoides)*, flowered racer (snakes, *Coluber florulentus)*, birds (all species), and small mammals (all species), it was very hard to sample a large amount of individuals, which would have additionally impacted their population dynamics. As small sample size (<3 individuals per site) were obtained for these species, two (when two individuals were collected, Supplementary material 2) to three replicates (when a single individual was collected, Supplementary material 2) were obtained from a single individual to ensure that we would have 3 to 6 measures.

A: El Shobak, **B:** El Shobak, **C:** El-Sharqi, **C:** El Shurafa, **D:** Kafr Turkhan, **E:** Ghammazah Al Kubra, **F:** Al Ikhsas Al Qiblyyah, and **G:** Arab Ghammazah Al Kubra.



Supplementary material 4. Boxplot presentation for Chromium concentration measured from the flora and fauna at different trophic levels sampled from seven localities of the El-Tebbin region (Egypt), as function of the distance to the main pollution source (from site A to site G). **A:** El Shobak, **B:** El Shobak, **C:** El-Sharqi, **C:** El Shurafa, **D:** Kafr Turkhan, **E:** Ghammazah Al Kubra, **F:** Al Ikhsas Al Qiblyyah, and **G:** Arab Ghammazah Al Kubra.

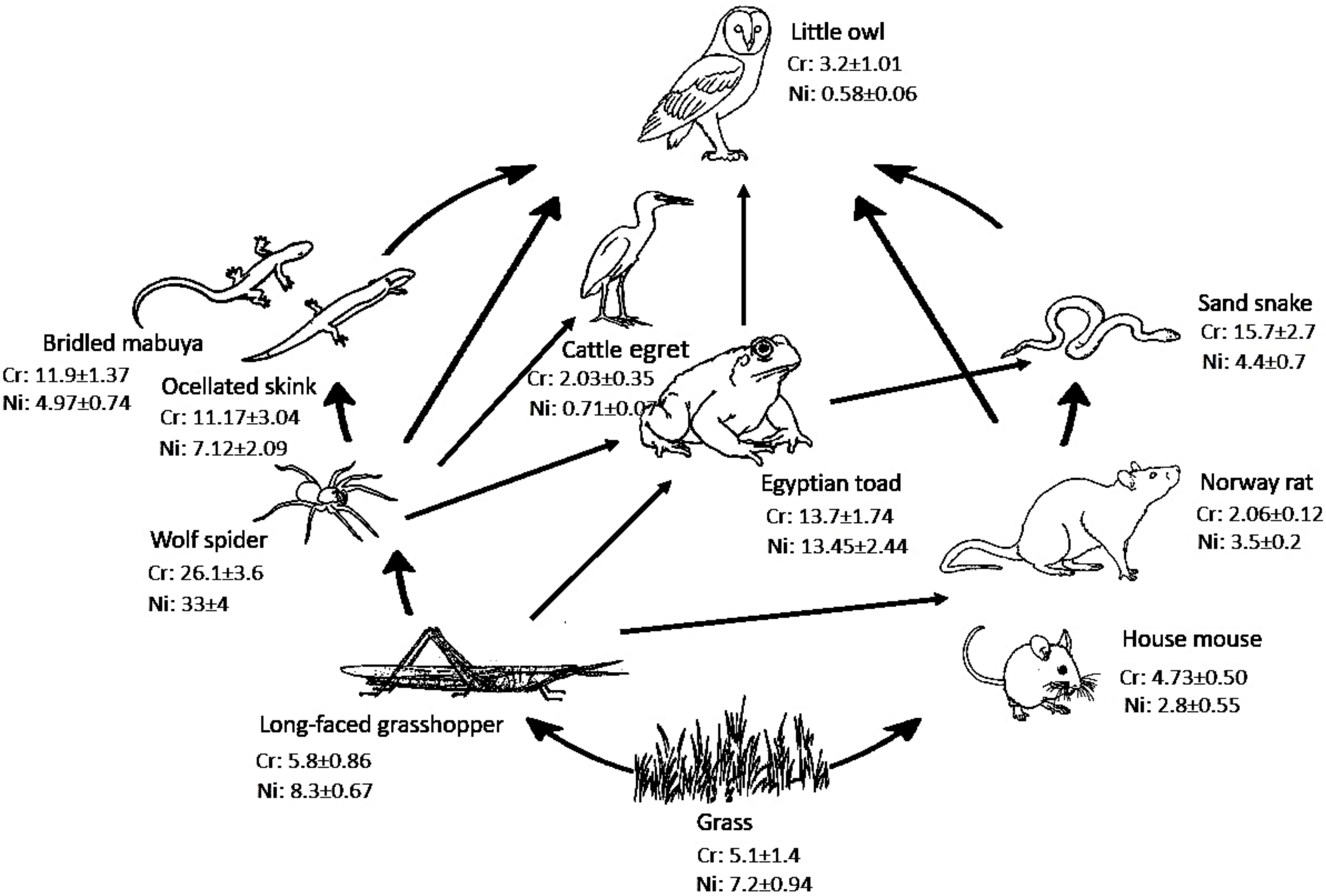


971

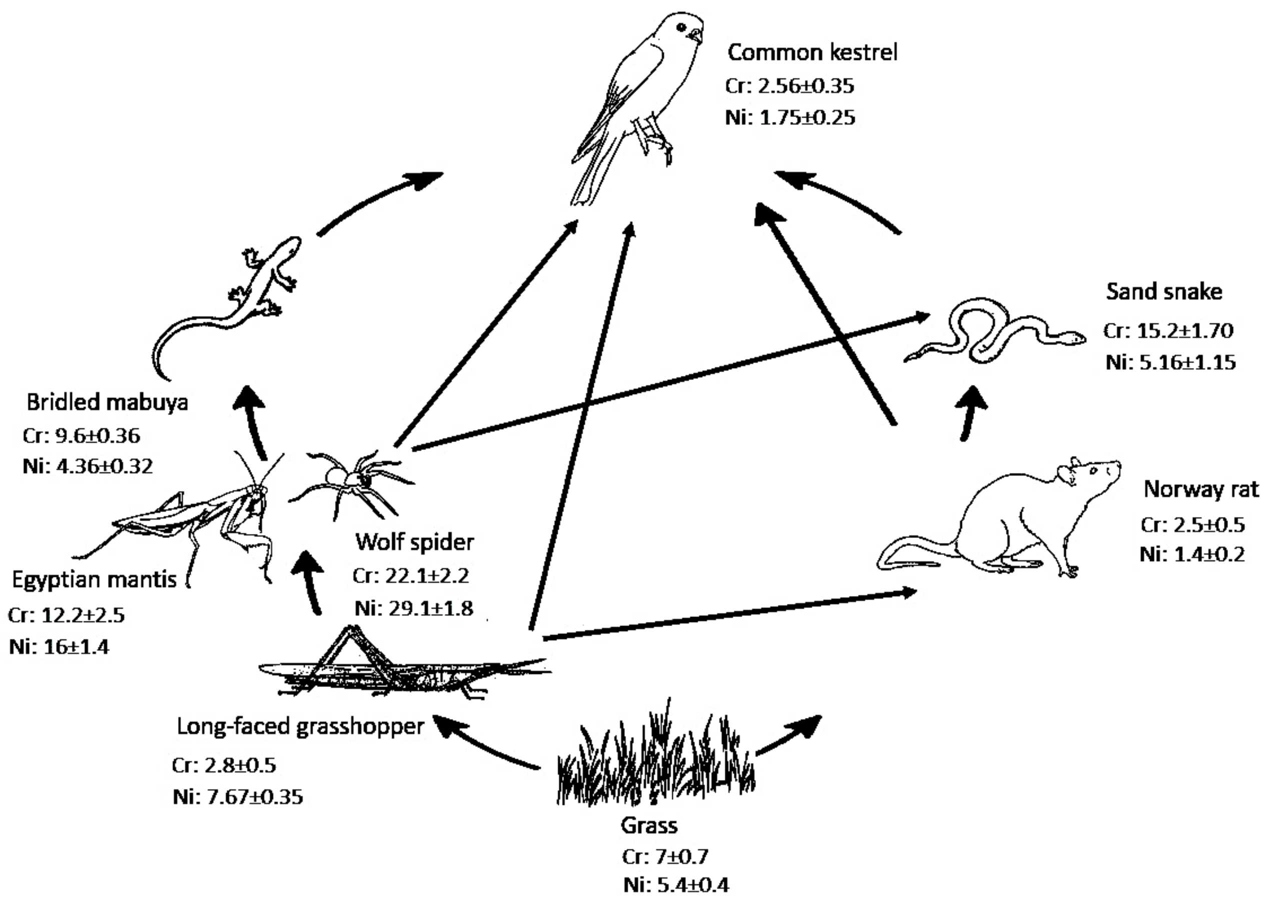
972 **Supplementary Material 6.** Boxplot presentation for Nickel concentration measured from the
973 flora and fauna at different trophic levels sampled from seven localities of the El-Tebbin region
974 (Egypt), as function of the distance to the main pollution source (from site A to site G).

A: El Shobak, **B:** El Shobak, **C:** El-Sharqi, **C:** El Shurafa, **D:** Kafr Turkhan, **E:** Ghammazah Al Kubra, **F:** Al Ikhsas Al Qiblyyah, and **G:** Arab Ghammazah Al Kubra.

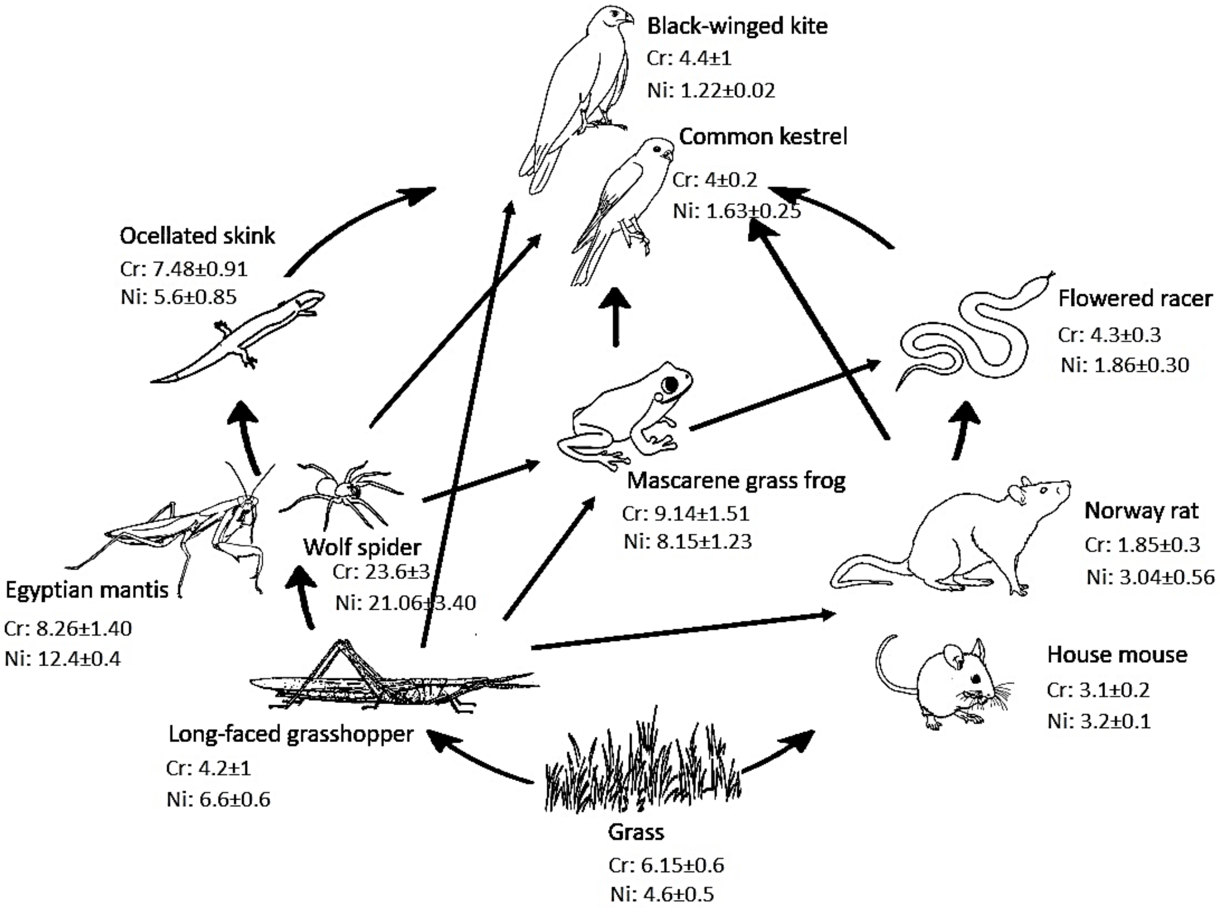
A


Little owl
Cr: 3.2±1.01
Ni: 0.58±0.06
Cattle egret
Cr: 2.03±0.35
Ni: 0.71±0.07
Sand snake
Cr: 15.7±2.7
Ni: 4.4±0.7
Bridled mabuya
Cr: 11.9±1.37
Ni: 4.97±0.74
Ocellated skink
Cr: 11.17±3.04
Ni: 7.12±2.09
Egyptian toad
Cr: 13.7±1.74
Ni: 13.45±2.44
Norway rat
Cr: 2.06±0.12
Ni: 3.5±0.2
Wolf spider
Cr: 26.1±3.6
Ni: 33±4
House mouse
Cr: 4.73±0.50
Ni: 2.8±0.55
Long-faced grasshopper
Cr: 5.8±0.86
Ni: 8.3±0.67
Grass
Cr: 5.1±1.4
Ni: 7.2±0.94

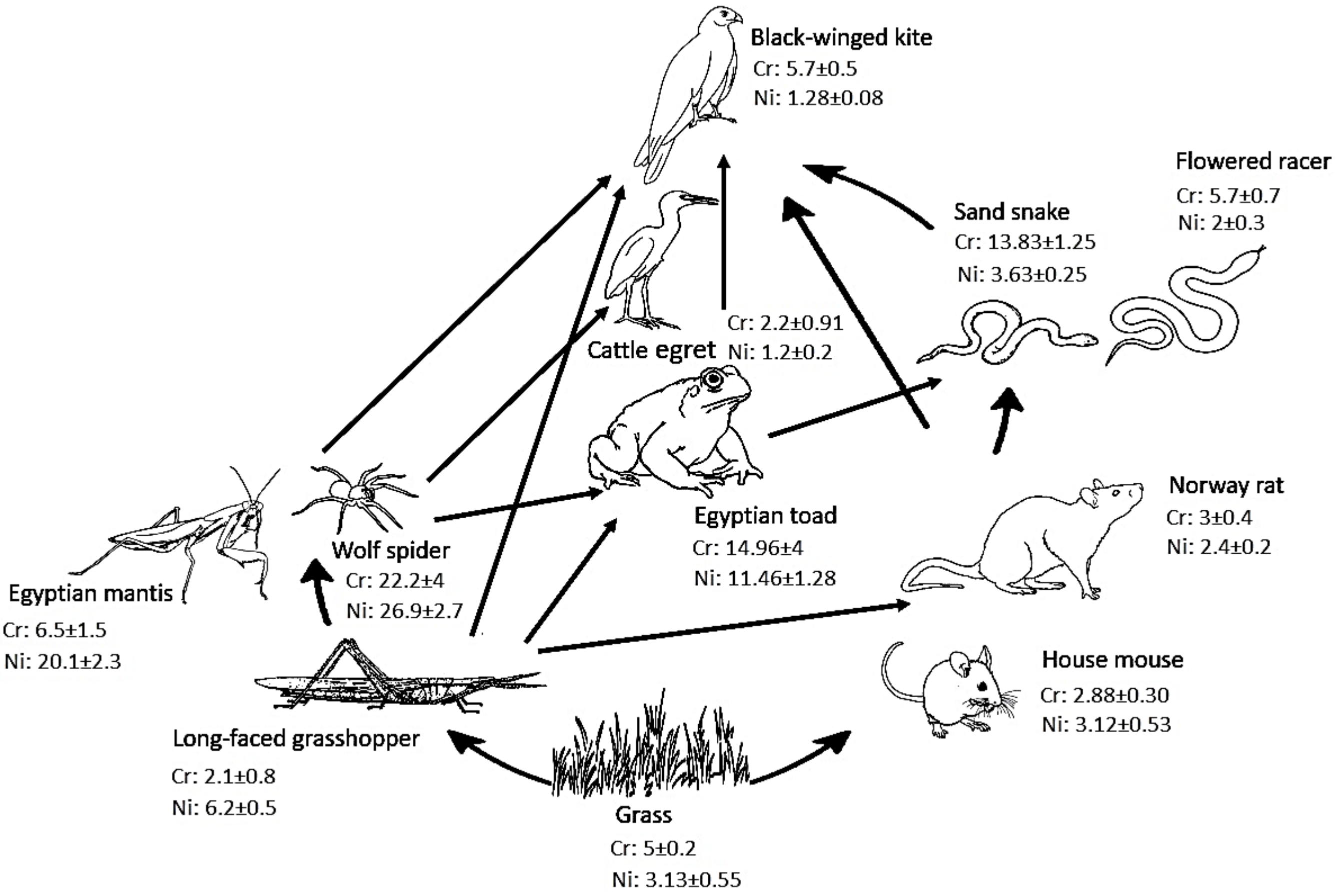
B


Common kestrel
Cr: 2.56±0.35
Ni: 1.75±0.25
Sand snake
Cr: 15.2±1.70
Ni: 5.16±1.15
Bridled mabuya
Cr: 9.6±0.36
Ni: 4.36±0.32
Norway rat
Cr: 2.5±0.5
Ni: 1.4±0.2
Wolf spider
Cr: 22.1±2.2
Ni: 29.1±1.8
Egyptian mantis
Cr: 12.2±2.5
Ni: 16±1.4
Long-faced grasshopper
Cr: 2.8±0.5
Ni: 7.67±0.35
Grass
Cr: 7±0.7
Ni: 5.4±0.4

983 **C**

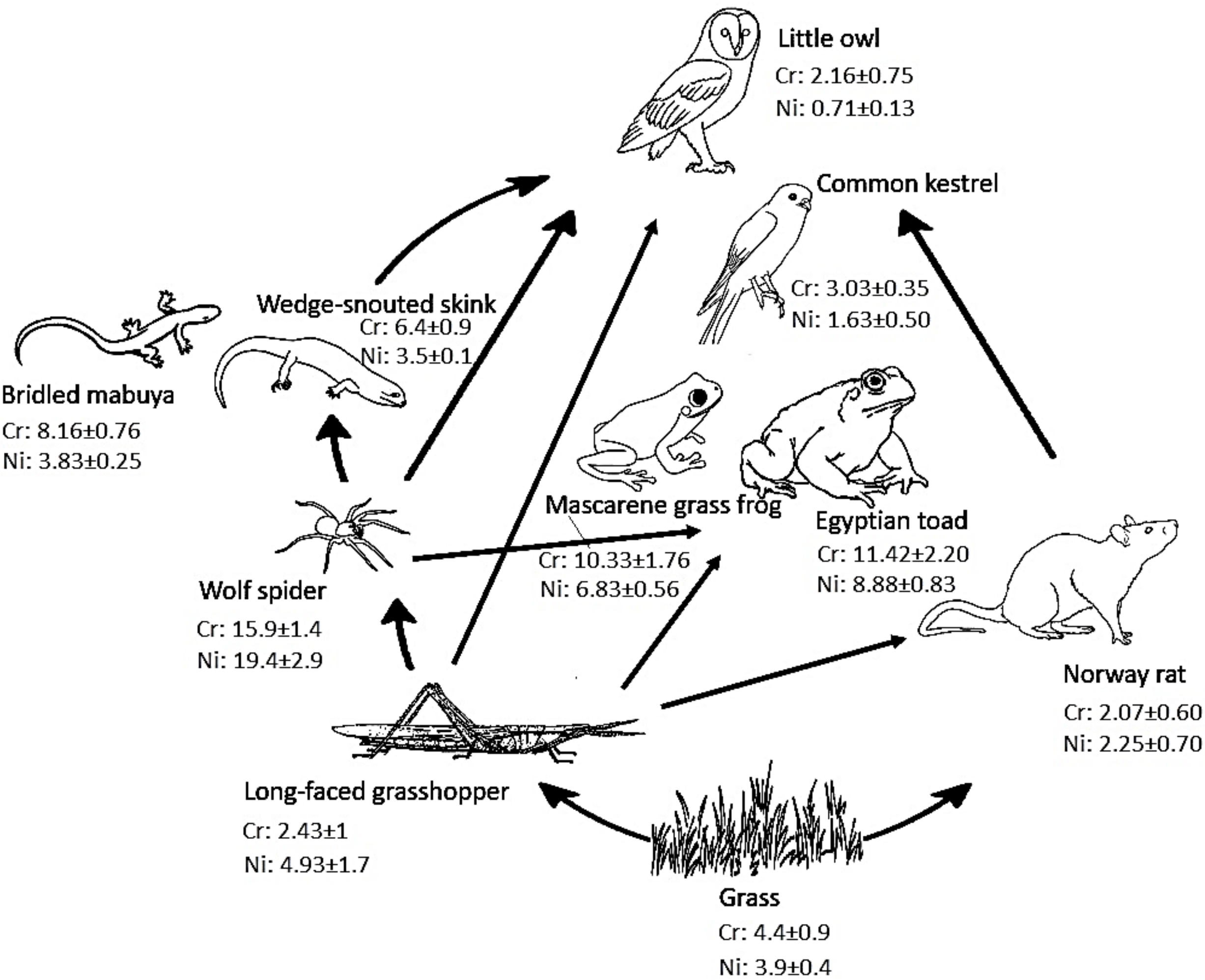

Black-winged kite
Cr: 4.4±1
Ni: 1.22±0.02
Common kestrel
Cr: 4±0.2
Ni: 1.63±0.25
Ocellated skink
Cr: 7.48±0.91
Ni: 5.6±0.85
Flowered racer
Cr: 4.3±0.3
Ni: 1.86±0.30
Mascarene grass frog
Cr: 9.14±1.51
Ni: 8.15±1.23
Norway rat
Cr: 1.85±0.3
Ni: 3.04±0.56
Wolf spider
Cr: 23.6±3
Ni: 21.06±3.40
Egyptian mantis
Cr: 8.26±1.40
Ni: 12.4±0.4
House mouse
Cr: 3.1±0.2
Ni: 3.2±0.1
Long-faced grasshopper
Cr: 4.2±1
Ni: 6.6±0.6
Grass
Cr: 6.15±0.6
Ni: 4.6±0.5

985 **D**

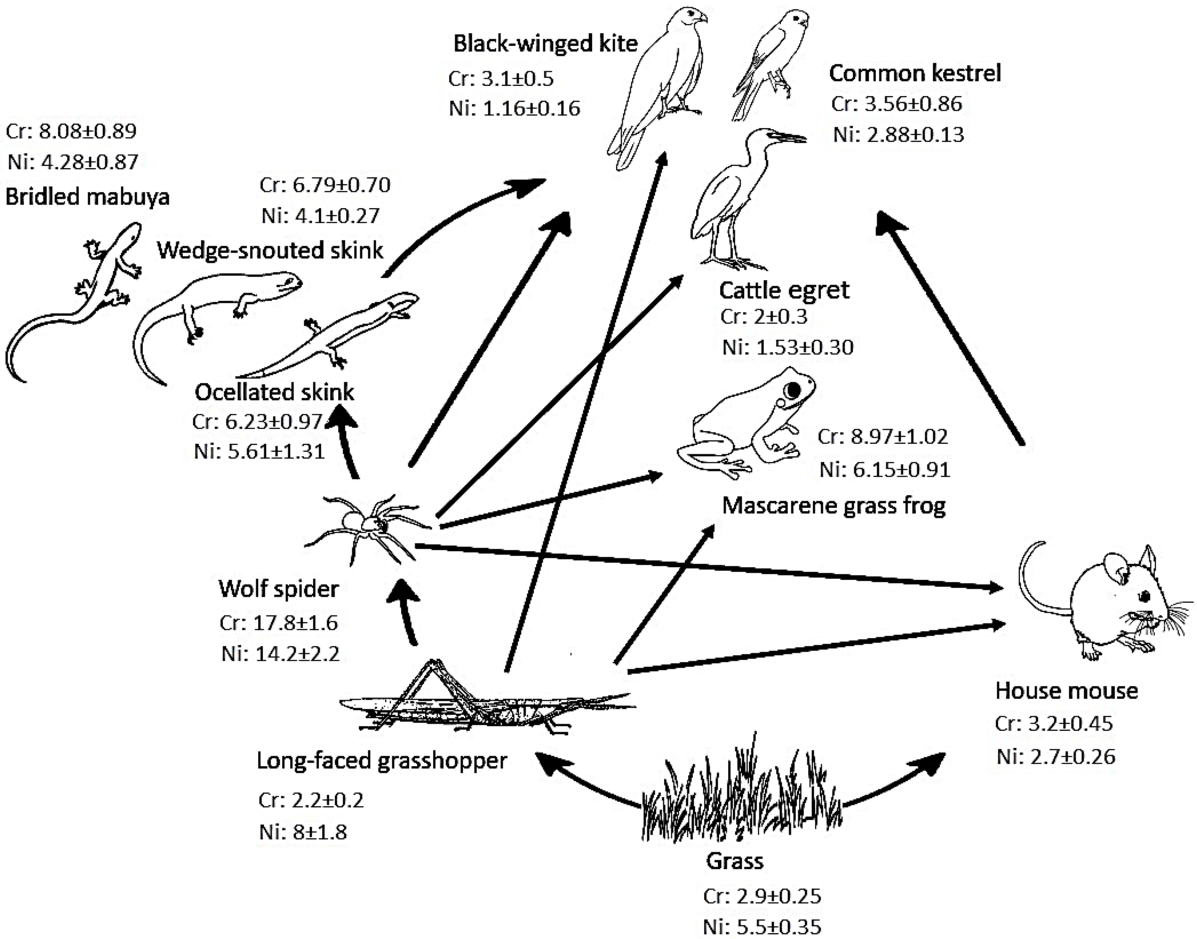

Black-winged kite
Cr: 5.7±0.5
Ni: 1.28±0.08
Flowered racer
Cr: 5.7±0.7
Ni: 2±0.3
Sand snake
Cr: 13.83±1.25
Ni: 3.63±0.25
Cr: 2.2±0.91
Ni: 1.2±0.2
Cattle egret
Norway rat
Cr: 3±0.4
Ni: 2.4±0.2
Egyptian toad
Cr: 14.96±4
Ni: 11.46±1.28
Wolf spider
Cr: 22.2±4
Ni: 26.9±2.7
Egyptian mantis
Cr: 6.5±1.5
Ni: 20.1±2.3
House mouse
Cr: 2.88±0.30
Ni: 3.12±0.53
Long-faced grasshopper
Cr: 2.1±0.8
Ni: 6.2±0.5
Grass
Cr: 5±0.2
Ni: 3.13±0.55

987

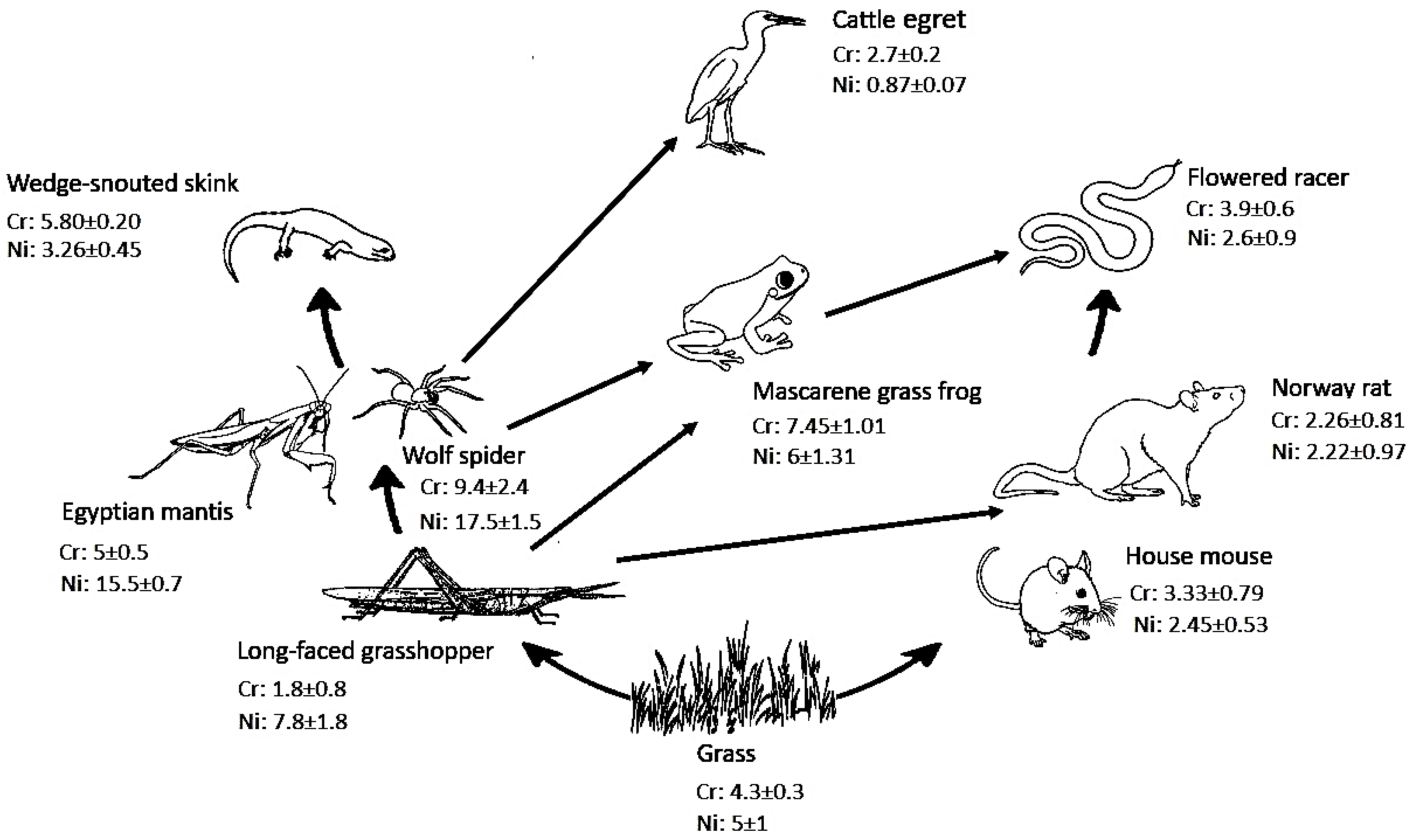
988 **E**


Little owl
Cr: 2.16±0.75
Ni: 0.71±0.13
Common kestrel
Cr: 3.03±0.35
Ni: 1.63±0.50
Wedge-snouted skink
Cr: 6.4±0.9
Ni: 3.5±0.1
Bridled mabuya
Cr: 8.16±0.76
Ni: 3.83±0.25
Mascarene grass frog
Cr: 10.33±1.76
Ni: 6.83±0.56
Egyptian toad
Cr: 11.42±2.20
Ni: 8.88±0.83
Wolf spider
Cr: 15.9±1.4
Ni: 19.4±2.9
Norway rat
Cr: 2.07±0.60
Ni: 2.25±0.70
Long-faced grasshopper
Cr: 2.43±1
Ni: 4.93±1.7
Grass
Cr: 4.4±0.9
Ni: 3.9±0.4

989
990 **F**


Black-winged kite
Cr: 3.1±0.5
Ni: 1.16±0.16
Common kestrel
Cr: 3.56±0.86
Ni: 2.88±0.13
Cr: 8.08±0.89
Ni: 4.28±0.87
Bridled mabuya
Cr: 6.79±0.70
Ni: 4.1±0.27
Wedge-snouted skink
Cattle egret
Cr: 2±0.3
Ni: 1.53±0.30
Ocellated skink
Cr: 6.23±0.97
Ni: 5.61±1.31
Cr: 8.97±1.02
Ni: 6.15±0.91
Mascarene grass frog
Wolf spider
Cr: 17.8±1.6
Ni: 14.2±2.2
House mouse
Cr: 3.2±0.45
Ni: 2.7±0.26
Long-faced grasshopper
Cr: 2.2±0.2
Ni: 8±1.8
Grass
Cr: 2.9±0.25
Ni: 5.5±0.35

G



Supplementary material S7. Putative trophic interactions among the species in the trophic chain (flora and fauna), collected from the seven localities along a pollution gradient (sites A–G, with site A being the pollution source) in El-Tebbin area (Egygpt). Chromium (Cr) and Nickel (Ni) concentrations mesured from the species are presented as mean ± SD in mg/kg dry mass.

A: El Shobak, **B:** El Shobak, **C:** El-Sharqi, **C:** El Shurafa, **D:** Kafr Turkhan, **E:** Ghammazah Al Kubra, **F:** Al Ikhsas Al Qiblyyah, and **G:** Arab Ghammazah Al Kubra.

Supplementary Material 8. Ratio of Cr and Ni concentrations in soil, plant and animals from the polluted site (A: El Shobak) to gradually distant sites (D: Kafr Turkhan and G: Arab Ghammazah Al Kubra).

Taxon	**Cr**		**Ni**	
	Ratio A/D	**Ratio A/G**	**Ratio A/D**	**Ratio A/G**
Soil	1.4	1.5	1.1	1.3
Plant	1.0	1.2	2.3	7.2
Long-faced grasshopper	2.8	3.2	1.3	4.6
Wolf spider	1.2	2.8	1.2	22.0
Egyptian toad	0.91	–	1.1	–
Forskal sand snake	–	–	1.2	–
Cattle egret	0.92	0.75	0.59	10.2
House mouse	1.6	1.4	0.89	5.3
Norway rat	0.68	0.91	1.5	3.6