Insect Life Traits Are Key Factors in Mercury Accumulation and Transfer within the Terrestrial Food Web

Loïc Yung, Coralie Bertheau, David Cazaux, Nicole Regier, Vera Slaveykova, Michel Chalot

► To cite this version:

Loïc Yung, Coralie Bertheau, David Cazaux, Nicole Regier, Vera Slaveykova, et al.. Insect Life Traits Are Key Factors in Mercury Accumulation and Transfer within the Terrestrial Food Web. Environmental Science and Technology, 2019, 53 (19), pp.11122-11132. 10.1021/acs.est.9b04102 . hal-02355645

HAL Id: hal-02355645

<https://hal.science/hal-02355645>

Submitted on 4 Jan 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8
- 9
- 0
- 1
- 2
- 3
- 4
- 5

4
5

7
8

9

0
1
2

4

ABSTRACT

As plants and associated insects are at the bottom of some terrestrial food webs, they are the primary contributors to mercury (Hg) fluxes in ecosystems. In addition to the trophic position of these organisms, factors related to their life traits have been hypothesized to influence their exposure to Hg. This study investigates the transfer of Hg in a soil-nettle-insect system and the insect-related factors affecting their Hg concentrations in a revegetated chlor-alkali landfill. Twenty-three insect species were identified and classified according to their life traits, their relationship with nettle and their morphological characteristics. We observed low total mercury (THg) concentrations in nettles, with only 1% methylmercury (MeHg) being detected, while concentrations ranged from 5 to 3700 $\mu\text{g}/\text{kg}$ dry wt. in insects with a MeHg percentage of up to 75%. The nettle-related insects were primarily exposed to Hg through the food web with significant biomagnification, particularly at the level of secondary predators. Within the nettle-unrelated group, the insect habitat was the most explanatory factor, with the highest enrichment being for the insects that spent part of their cycle in direct contact with Hg sources. Therefore, these insects require special attention because they are an essential vector of Hg transfer for terrestrial top predators.

INTRODUCTION

Landfills in the chlor-alkali industries require stabilization of mercury (Hg) to prevent air pollution by evaporated Hg¹. Revegetation of these sites offers a suitable solution to confine the Hg released from the soil in plant biomass², as plants are net sinks that incorporate the atmospheric Hg into leaf tissues³. These plantations, mainly from Salicaceous species (*i.e.*, willow), favour the appearance of spontaneous herbaceous cover highly dominated by the common stinging nettle, *Urtica dioica* L. (Urticaceae)⁴. In natural conditions, this nettle is considered an invertebrate “super-host” providing food for a large diversity of insects with different biology⁵ and is involved in strong trophic interactions^{6–8}. In this context, herbivorous insects on revegetated landfills are potentially exposed to Hg and, more precisely, to methylmercury (MeHg), the form of Hg that biomagnifies readily from soil and sediment to plants⁹. The accumulation of MeHg in herbivorous insects can cause serious physiological, neurological, behavioural and reproductive disorders for insects or invertebrate predators, birds and mammals through their diet^{10,11}.

Studies and reviews focusing on Hg transfer in the terrestrial food chain and its toxicity on specific groups (*i.e.*, birds, invertebrates, mammals, and microorganisms) have increased since 2010^{12,13}. Some recent works, mainly targeting high trophic levels taxa, concluded that elevated MeHg concentrations in high trophic wildlife are ubiquitous across the world’s ecosystems and that there is a lack of accurate information on the impact of Hg in terrestrial ecosystems^{14–17}. Investigations in Chinese industrial areas showed that Hg level in the soil-plant-insect system increased from plants to herbivorous insects and to predators, showing a biomagnification primarily conditioned by trophic factors^{15,18–21}.

Other factors such as the insect life stages can play a major role in the transfer of Hg through food webs and particularly from aquatic to terrestrial ecosystems^{22–24}. Indeed, emerging aquatic insects such as chironomids²⁵ or dragonflies²⁶ that move to terrestrial biota

can serve as both prey²⁷ and biovector of contaminants for local terrestrial consumers, including ants or spiders^{28,29}. Consequently, the exposition of terrestrial insects is linked to the vicinity to aquatic biota and the Hg bioavailability, conditioned by the physical and chemical factors of these systems (e.g., dissolved organic carbon, quantity of aqueous nutrients)^{25,30}. For strictly terrestrial habitats, factors such as insect habitats, have been hypothesized to influence Hg accumulation in terrestrial insects^{18,31}, but no studies examining biological and/or ecological factors affecting Hg concentrations in these organisms exist, as previously performed in lakes and wetlands^{25,32}.

The present study aimed to study Hg uptake and biomagnification through an original and specific soil-plant-terrestrial insect system in a Hg-contaminated area in France and to estimate the extent to which insect-related factors affected Hg uptake. To that end, we used a chlor-alkali landfill revegetated by poplar plantations where the stinging nettle, *Urtica dioica* L., colonized spontaneously the herbaceous layer, hosting a large diversity of insects. The specific objectives were i) to quantify THg (total Hg) and MeHg in soil, different compartments of nettles and a large number of well-identified insect taxa; ii) to characterize insect taxa by their trophic guilds, feeding guilds, dietary range and habitats at both the larval and adult stages, their relationship with the nettle and their morphological characteristics (i.e., life stage and weight); and iii) to assess the relative importance of these factors on THg and MeHg concentrations in insects at the revegetated landfill.

MATERIALS AND METHODS

Site description

A phytomanagement field trial was implemented in 2011 on a chlor-alkali sediment landfill located in Saint-Symphorien-sur-Saône in the Bourgogne Franche-Comté region

(France). The location, history and phytomanagement set-up at the sediment landfill have been described in detail in previous studies^{33,22}. Five years after planting, biotic and abiotic environmental conditions have promoted the emergence of a spontaneous herbaceous cover strongly dominated by stinging nettle (*Urtica dioica* L.). The control site is a natural area located at Courcelle-les-Montbéliard, 140 km east of the chlor-alkali plant, not directly influenced by the point-source.

Sample collection

To ensure the most representative diversity in insect species, the study was carried out over two consecutive years (2017 and 2018). Six poplar plots were selected at the experimental site, three with the Skado cultivar (*Populus trichocarpa* x *P. maximowiczii*) and three with the I-214 cultivar (*P. deltoides* x *P. nigra*). Soil and plant samples were collected in June 2017 and monthly in 2018 from April to September (*i.e.*, during the vegetation period) for the experimental site and in July 2018 for the control site. Three samples of soil and nettle plants were composed of three mixed subsamples taken randomly within each sampling plot. Soil samples consisting of bulk soil were collected from the first 20-cm layer, cleared from roots and other plant materials. Soil and plant samples were placed into plastic and paper bags, respectively, stored into a refrigerated container and shipped to the laboratory, where they were dried at ambient temperature (24 °C) for two weeks before Hg analyses. In parallel, an exhaustive sampling of the entomofauna related to the stinging nettle was carried out in the six studied plots monthly from April to September (*i.e.*, period of insect activity) during the two consecutive years (2017 and 2018). At each plot (chlor-alkali and control sites), insects from six nettle patches of similar size were caught using a sweep net and pooled in a collector tube with absolute ethanol or ethyl-acetate as usually used to assure optimal preservation and identification. All samples were stored at -20°C until identification and Hg analyses.

Insect identification and characterization

Among the whole insect diversity collected from the entomological survey, in the present study we targeted the most abundant taxa, representing 63% of the total insects collected on nettles at the experimental site during the two years. Prior to the analyses, each taxon was identified to the lowest possible taxonomic level using several references that included some identification keys^{35,36}, on-line sources, such as insecte.org and/or specialists from the Franche-Comte Natural Spaces Conservatory. Each taxon was classified according to their life traits using published literature (SI Table S1). First, taxa were assigned according to their habitat both at the larval and adult stages, which allowed them to be split into two groups, "the nettle-related group", which included insects hosted by nettles (*i.e.*, insects living on the nettle during their whole life cycle), and the "nettle-unrelated group", which refers to all other insects. We further classified taxa according to their trophic levels (*i.e.*, herbivores, predators I or predators II), their larval and adult feeding guild (*i.e.*, plant parts for herbivores and type of prey for predators) and their dietary range (*i.e.*, insects that are host specific within a family were categorized as "specialists", and those that feed on plants or insects from more than one family were categorized as "generalists"). Finally, to test the influence of the morphological characteristics of insects on THg content, we identified the life stage of each taxon (larvae or adult) and measured the dry weight (mg).

THg and MeHg analyses in soil, plants and insects

Before analyses, dried nettle stems and leaves were separated and ground into a homogenous powder in a MM40 Mixer Mill (Retsch, Eragny sur Oise, France) for 4 min at 30 Hz and 7 min at 30 Hz for leaves and stems, respectively. Soil samples were manually homogenized and sieved to <4 mm. Insects euthanized with ethyl acetate were freeze-dried, and those euthanized with absolute ethanol were transferred into new plastic tubes and opened overnight at ambient temperature (24 °C) for the evaporation of ethanol. As the mass of some dried insects was <1 mg, they were carefully weighed using a NewClassic MS analytical

balance (Mettler-Toledo AG, Greifensee, Switzerland) with a five decimal resolution. Preliminary tests showed no significant influence of the washing and preservation methods on THg concentrations measured in insects (SI, Figure S1). Moreover, in trophic interactions, the whole insects are usually ingested by predators, including the potential fraction of Hg adsorbed on the cuticle or internally accumulated through associated microorganisms (*e.g.*, fungi and bacteria). Thus, whole insects were directly analysed without pretreatment to be closer to natural conditions. For the small insects, multiple individuals of the same taxa were pooled together to reach a minimal mass of 1 mg. THg concentrations were measured in the soil, plant and insect samples using an AMA-254 cold vapor atomic absorption (CV-AAS) Hg analyser (Altec Co., Czech Republic), as fully described in a previous study³⁷. The instrument detection limit (DL) and the quantification limit (QL) using this method were 0.006 and 0.07 ng of THg, respectively. For samples with non-detected Hg, we calculated a THg concentration using the QL value.

To ensure that the preservation of different taxa in ethanol did not induce cross-contaminations, ethanol blank samples were regularly analysed. One hundred microliters of ethanol was placed into the sample container and evaporated. No Hg was detected in the ethanol fraction, which shows that Hg was not released into that fraction during storage.

MeHg was extracted from freeze-dried ground material using a HNO₃ leaching / CH₂Cl₂ extraction method, as described by³⁸. Briefly, 0.02 to 0.5 g of insect, nettle or soil material were weighed into a 50-mL centrifuge tube and homogenized into 2 mL 1 M CuSO₄, 5 mL 5 M HNO₃ and 10 mL CH₂Cl₂, shaken for 1 h and centrifuged for 25 min at 3000 rpm. The solvent layer was transferred to a new tube and MeHg back-extracted into 10 mL H₂O (MilliQ) under 50 mL/min N₂ flow in a water bath at 50°C. MeHg was analysed as described elsewhere³⁹. The number of samples are provided in SI Table S2.

Data analysis

Statistical analyses were performed with R software v.3.5.1 (R Development Core Team, 2013). All variables were checked for their homoscedasticity (Levene test) and normal distribution (Shapiro–Wilk test). Non-normal data were log transformed. Data that did not fit a normal distribution after transformation were analysed with nonparametric tests. All statistical tests were considered to be significant at $P < 0.05$. The results are presented as the means with their standard error (SE). Differences in THg concentrations in soil, nettles and insects between sites (experimental vs. controls) were assessed using the Mann-Whitney-Wilcoxon test. In the experimental site, a comparison of THg and MeHg concentrations between soil, nettle, insect families and between nettle-related and nettle-unrelated insects was performed using a Kruskal-Wallis test and a Mann-Whitney-Wilcoxon test, respectively. A redundancy analysis (RDA) was performed on both the nettle-related and nettle-unrelated groups to assess the correlation between THg concentrations and the insect life traits studied using the “rda()” function available in the “vegan” package. The significance and rank of each trait was then tested with the “envfits()” function (Monte Carlo test with 999 permutations). Then, the effect of each significant insect life trait on THg concentrations for each group was investigated with a Kruskal-Wallis test. For the morphological characteristics of insects, a Mann-Whitney-Wilcoxon test was performed to assess whether THg concentrations differed among insect stages. Pearson’s or Spearman’s correlation coefficients were calculated to check for relationships between THg content and weight of insects grouped according to their life traits.

The Biota-Soil Accumulation Factor (BSAF) was calculated for the nettles from the experimental site and the control site according to the following equation:

$$\text{BSAF nettle} = \frac{[\text{THg}] \text{ nettle}}{[\text{THg}] \text{ soil}}$$

According to the information collected from the literature on trophic interactions (**Table S1**), we calculated the Bio-Magnification Factors (BMF) for nettle-related insects from the experimental site according to the following equation:

$$\text{BMF taxa} = \frac{[\text{THg}] \text{ taxa}}{[\text{THg}] \text{ preys}}$$

Trophic Magnification Factors (TMF) were determined for both THg and MeHg at the experimental site. TMF considered all taxa along the five level trophic web (*i.e.*, plants, herbivorous insects, predator I, predator II, predator III) except those from the Elateridae species, which were removed from the herbivorous group as they accumulated large quantities of Hg through their life cycle. Hence, TMF were determined from the slope of a regression between the Hg concentrations and trophic level of insects in the food web and calculated as the antilog of the regression slopes (b):

$$\text{Log [Hg]} = a + b\text{TL}$$

TMF refers to the average rate of change in contaminant concentrations from diet-to consumer along the trophic web. In TMF, slope values above zero are indicative of biomagnification (*i.e.*, $\text{TMF} > 1$)⁴⁰.

RESULTS

Characterisation of targeted taxa

The present study covered insects from six orders: Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera and Mecoptera, belonging to 16 common families within these orders (SI Table S1). The 23 targeted insect taxa were identified up to the family level for 100% of them, to the genus level for 96% and to the species level for 74%. The identification of 16 caterpillars could not go beyond the order level. The 23 selected taxa were then

classified according to their relationship or lack of relationship with nettle as a host-plant and their life traits (SI Table S1). The nettle-related group included 12 different genera of insects belonging to orders of Hemiptera and Coleoptera and unidentified families of Lepidopteran larvae (SI Table S1). These insects belonged to three different trophic guilds: herbivores, predators I and predators II. Herbivores belonged to taxa feeding on nettle sap, seeds or leaves during their larval or adult stages. Predators I were mainly specialists belonging to the family Coccinellidae and Miridae, which fed almost exclusively on aphids. Predators II were represented by the genus *Nabis*, which included certain species known as generalist predators that hunt herbivorous or other predatory species (*i.e.*, aphids, Lepidopteran or Coleopteran larvae and some Heteropteran and Dipteran adults) on herbaceous layers. The nettle-unrelated group included all the other abundant taxa represented by 8 families (*i.e.*, Formicidae, Panorpidae, Aphrophoridae, Chrysomelidae, Curculionidae, Elateridae, Chironomidae, and Sciomyzidae) and 9 genera. All taxa were sampled on nettles but were not directly related to it. Most of these taxa were characterized by a high mobility and a specific life cycle that occurred at different habitats, such as a larval cycle in the soil, in water or in snails as parasitoids.

Hg concentrations in the soil-plant-insect system

THg concentrations of soil, plant and insects were significantly higher at the experimental site than at the control site. The THg concentrations measured in soil samples from the experimental site were in the range of 4382 – 7119 µg/kg and 10-fold higher than THg concentration (570 ± 70 µg/kg) at the adjacent undisturbed forest soil³³. Nettle stems and leaves were significantly more enriched in THg at the experimental site than their counterparts collected at the control site (Table 1). The BSAFs calculated for the experimental site and the control site were 0.004 and 0.011, respectively, suggesting a noticeable dilution at

both sites. With an average THg of $31 \pm 1 \mu\text{g/kg}$, leaves were significantly ($P < 0.01$) more enriched than stems ($11 \pm 1 \mu\text{g/kg}$ THg). A significant enrichment was observed in the nettle leaves during the season, with THg concentrations ranging from $18 \pm 5 \mu\text{g/kg}$ in samples collected in April to $31 \pm 5 \mu\text{g/kg}$ in samples collected in September ($P < 0.01$).

A total of 312 and 159 analyses of THg concentrations was performed on insects from the experimental and control sites, respectively (Table 1). Average THg concentrations for insects collected at the experimental site were significantly higher than those at the control site ($P < 0.001$), and this difference was found more specifically for 12 of the 14 compared families and orders (Table 1). Taxa (Cicadellidae family and Lepidoptera order), for which no significant differences were observed, had THg concentrations ranging from $< \text{QL}$ to $271 \mu\text{g/kg}$ at the experimental site and from $< \text{QL}$ to $109 \mu\text{g/kg}$ at the control site. No THg was detected in Nabidae from the control site, whereas elevated THg concentrations (ranging from 107 to $1805 \mu\text{g/kg}$) were measured for the same taxa collected at the experimental site, and the same phenomenon was observed for Aphididae and Aphrophoridae, although to a lesser extent. For most insects collected at the control site, THg concentrations remained $< 53 \mu\text{g/kg}$, except for the highly mobile flying insects belonging to the Chironomidae and Panorpididae families, which presented the highest background concentrations (Table 1).

Table 1. Total mercury (THg) concentrations measured in nettles and the 16 families and order of insects from the sediment landfill and the control site. n represents the number of samples analysed. All concentrations are expressed in $\mu\text{g/kg}$ dry wt $\pm$ SE. 'NA' = taxa not collected at the control site. Concentrations measured in the sediment landfill were compared to those measured in the control site using the Wilcoxon rank sum test and the two-sample t-test. The results from the statistical comparisons are represented with the following legend: P -

value '***' 0.001 '**' 0.01 '*' 0.05 'NS' not significant. ^a = data from an adjacent undisturbed forest soil²¹

Organism		Experimental site		Control		Significance test: comparison of the means
		Experimental site THg (µg/kg)		Control sites THg (µg/kg)		
		n	Mean ± S.E	n	Mean ± S.E	
Soil		37	5894 ± 111	7 ^a	570 ± 70 ^a	*** ^a
Nettles		187	21.4 ± 1.0	11	6.3 ± 0.9	***
	Stems	95	11.6 ± 0.7	5	3.8 ± 1.1	***
	Leaves	92	31.5 ± 3.4	6	8.4 ± 0.7	***
Insects	Taxa	312	388.5 ± 38.5	159	24.0 ± 3.7	***
	Elateridae	8	3679.8 ± 372.7	19	18.1 ± 4.2	***
	Panorpidae	6	1231.3 ± 261.0	3	178.6 ± 96.2	**
	Formicidae	21	871.6 ± 46.9	16	11.8 ± 3.4	***
	Nabidae	67	617.3 ± 47.2	19	<DL	***
	Chironomidae	32	518.1 ± 29.3	11	131.0 ± 12.7	***
	Sciomyzidae	5	200.8 ± 38.9	NA	NA	NA
	Chrysomelidae	16	63.5 ± 9.4	12	18.0 ± 4.7	***
	Coccinellidae	13	59.8 ± 20.4	17	4.7 ± 1.5	**
	Curculionidae	39	59.3 ± 15.0	15	6.1 ± 2.9	***
	Cicadellidae	21	53.2 ± 9.6	9	52.8 ± 10.4	NS
	Lepidoptera	18	45.2 ± 18.1	8	38.7 ± 10.5	NS
	Rhyparochromidae	17	19.3 ± 4.1	NA	NA	NA
	Aphididae	22	10.1 ± 1.2	7	<DL	***
	Miridae	17	8.9 ± 2.4	13	4.2 ± 3.3	*
	Pentatomidae	3	7.1 ± 2.7	6	0.5 ± 0.3	*
	Aphrophoridae	7	5.7 ± 1.8	4	<DL	**

The 16 studied families from the experimental site were plotted according to their THg concentration (Figure 1).

Figure 1. Mean THg concentrations (full bar, µg/kg dry wt.), including the MeHg fraction (hatched bar, %), plotted for soil and nettles (*Urtica dioica*) and for all the investigated insect taxa from the nettle-unrelated and nettle-related groups. * = The MeHg concentrations were not analysed. Bars with the same letter did not differ significantly from each other using a pairwise Kruskal–Wallis test ($P < 0.05$).

The highest THg concentrations were measured in Elateridae, Panorpidae and Formicidae followed by Chironimidae and Nabidae, with concentrations higher than 500 µg/kg dry wt., which are significantly different from Elateridae (3679.8 ± 372.7 µg/kg). The measured THg concentrations dropped to 200 µg/kg for Sciomyzidae and below 100 µg/kg for each other family. Within the Curculionidae family, THg concentrations were variable,

ranging from < QL to 321 µg/kg, with concentrations over 150 µg/kg for *Phyllobius sp.* and up to 56 µg/kg for the two other considered species (SI Table S1). All other less enriched families had THg concentrations in the same order of magnitude as those measured for nettles. Overall, nettle-unrelated insects showed mean THg concentrations (981 ± 96 µg/kg) six times higher than nettle-related insects (124 ± 14 µg/kg) (Figure 1). Within the nettle-related group, mean THg concentrations ranged from 8.9 ± 2.4 for Miridae to 617.3 ± 47.2 µg/kg for Nabidae. Concerning the nettle-unrelated group, the lowest mean THg concentrations were obtained for Aphrophoridae and Pentatomidae, whereas the highest concentrations were found in Elateridae. However, the THg enrichment seems not to be only related to the relationship with nettle. Indeed, the nettle-related taxon Nabidae had THg concentrations not significantly different from the nettle-unrelated taxa Panorpidae, Formicidae and Chironimidae (Figure 1).

Additionally, no significant difference ($P > 0.05$) was found between the lowest mean THg concentrations in the nettle-unrelated group and those in the nettle-related group. Despite high THg concentrations, the percentage of MeHg in the soil was very low ($0.03 \pm 0.002\%$), slightly increased in nettles ($1.2 \pm 0.1\%$) and considerably increased in insects (ranging from 5.6% to 74.2%). Insect MeHg (average 29.2%, a) being significantly different ($P < 0.05$) from nettle MeHg (8.0%, b), and soil MeHg (average 4.5%, b).

The percentages of MeHg were not significantly different between the nettle-related group and the nettle-unrelated group ($P > 0.05$) with mean values of $43 \pm 7\%$ and $49 \pm 5\%$, respectively. Within the nettle-related group, for the 5 studied families, the percentage of MeHg was higher than 25% and reached 65% for Nabidae, except for Lepidoptera, for which MeHg only represented 6% of THg. Elateridae from the nettle-unrelated group exhibited 74% MeHg, representing over 2700 µg/kg. The Panorpidae family presented the most variable MeHg percentages, ranging from 4 to 70%.

Overall factors influencing THg concentrations in insects

The present study clearly demonstrated that insect life traits, such as habitat, trophic guild, feeding guild, and dietary range, at both the larval and adult stages significantly influenced the mean THg concentrations of insects living at the landfill ($P < 0.01$). However, the relative importance of each of these life traits on THg concentrations of insects in the two groups (nettle-related vs. nettle-unrelated insects) was different (Table 2).

Table 2. Hierarchical analysis of factors affecting THg concentrations within the nettle-unrelated and nettle-related groups. P -value '****' 0.001 '***' 0.01 '*' 0.05 'NS' not significant.

	Factors	r ²	Pr(>r)	Significativity
Nettle-related group				
	Trophic guild	0.39	0.001	***
	Feeding guild (adult)	0.39	0.001	***
	Feeding guild (larvae)	0.39	0.001	***
	Dietary range	0.37	0.001	***
	Habitat (larvae)	0.07	0.017	*
	Habitat (adult)	0	1	NS
Nettle-unrelated group				
	Habitat (adult)	0.22	0.001	***
	Feeding guild (larvae)	0.21	0.001	***
	Habitat (larvae)	0.16	0.002	**
	Feeding guild (adult)	0.06	0.115	NS
	Dietary range	0.05	0.037	*
	Trophic guild	0.02	0.475	NS

According to the RDA, THg concentrations in nettle-unrelated insects were mainly influenced by the adult habitat ($P < 0.001$), then by the dietary range at larval stage ($P < 0.001$) and by larval habitat ($P < 0.002$), while the trophic guild and adult feeding guild factors were not critical. Considering adult habitat, THg concentrations followed the

following order: insects living on host plants < insects having their larval cycle in underwater
sediments < insects living a part of their life cycle in terrestrial sediments (**Figure 2A**).

Figure 2. Mean THg concentrations (full bars, µg/kg dry wt.) ± SE and the MeHg fraction (hatched bars, %), measured in classes related to the main factors influencing the Hg enrichment for both the nettle-related group (factor = trophic guild, A) and external group (factor = main habitat, B). *: The MeHg concentrations were not analysed. Bars with the same letter did not differ significantly from each other using a pairwise Kruskal–Wallis test ($P < 0.05$).

The THg concentrations in *Aphrophora alni* (Aphrophoridae), which perform their complete life cycle on plants (especially Salicaceous species), did not exceed 12 µg/kg dry wt. In contrast, taxa with part of their life cycle in soil, such as *Agriotes sp.*, *Phyllobius sp.*, *Chironomus sp.*, *Trypetoptera punctulata*, *Myrmica rubra* and *Panorpa sp.* (SI, Table S1),

had average THg contents higher than 200 µg/kg with a mean percentage of MeHg over 50%. Insects belonging to the genus *Crepidodera*, which also feed on Salicaceae, had the lowest THg content for insects with a part of their life cycle in the soil but still significantly higher than those measured for *Aphrophora alni* ($P < 0.01$) (Figure 1).

For nettle-related insects, feeding guild at larval and adult stages ($P < 0.001$), trophic guild ($P < 0.001$) and then dietary range ($P < 0.001$), accounting individually for more than 37% of the total variability, were the most significant life traits (Table 2). Thus, in the nettle-related food web, the THg contents were in the following order: herbivores < soft-body insect predators < generalist insect predators (Figure 2B), with a percentage of MeHg following the same trend: 24% < 37% < 65%. Herbivorous insects presented no significant THg enrichment compared with nettles, but the mean MeHg concentrations increased from 0.3 µg/kg in nettles to 9.1 µg/kg in herbivorous insects. The biomagnification within the nettle-related group was also observed while considering all the studied taxa (including those from the nettle-unrelated group), with TMF of 2.8 for THg and 6.3 for MeHg.

Each family of insects feeding on nettle sap or nettle seeds had a BMF lower than 1.0, except for leafhoppers (Cicadellidae), which had the highest BMF (4.6) among all herbivorous insects. THg concentrations (µg/kg) for herbivorous insects increased as follows: seed-feeder (13 ± 3 µg/kg) < sap-sucking (22 ± 5 µg/kg) < leaf-feeder (37 ± 10 µg/kg) with no significant differences between these feeding guilds. With regard to defoliating insects, Lepidoptera larvae, which included unidentified taxa with potential generalist feeding specificity, had a BMF of 1.4, whereas that of a specialist species of weevils (Curculionidae) hosted by nettles was 0.6. No significant THg enrichment was obtained for predators I, which included ladybirds (Coccinellidae) and a predatory bug (Miridae), compared to herbivorous insects. Damsel bugs (Nabidae), which were the only studied generalist predators II of the

nettle-related food web, had average concentrations 15 times (THg) and more than 30 times (MeHg) higher than those measured in the lower trophic guild, resulting in a BMF of 4.8.

The influence of the development stage on THg concentrations was studied on two predatory taxa: the ladybirds, *Harmonia axiridis* (Coccinellidae) and the damsel bugs, *Nabis* sp. (Nabidae). THg concentrations measured in the imago ladybirds ($n = 6$) were 10-fold higher ($P < 0.001$) than those measured in larvae ($n = 7$). In contrast, no significant difference was obtained for Nabidae species between the imago (from 148 to 1786 $\mu\text{g/kg}$; $n = 43$) and the larvae (from 108 to 1805 $\mu\text{g/kg}$; $n = 24$). A significant positive correlation between the weight of insects and their THg content was observed when all taxa were pooled ($r_s = 0.62$, $P < 0.001$). The THg content in insects increased proportionally with their weight but differently according to the group studied. We also considered the main life traits, that is, the habitat of nettle-unrelated taxa and the trophic guild for nettle-related taxa. In doing so, the correlation between THg and weight was confirmed for herbivores ($r_s = 0.60$, $P < 0.001$) and predators ($r_s = 0.62$, $P < 0.001$) trophic guilds as well as for underwater sediments ($r_s = 0.44$, $P < 0.01$) and terrestrial sediments ($r_s = 0.81$, $P < 0.001$) habitats (Figure 3).

Figure 3. Relationships between the dry weight (mg) and the THg content (ng) of insects measured in 6 classes, 3 related to the “trophic guild” or 3 related to the “habitat” factors. The results from Pearson’s and Spearman’s correlation tests are presented with the following legend: *P*-value ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘NS’ not significant. The regression equations were calculated, as well as the regression coefficient (R^2) for each class, for which a correlation was obtained.

The first cluster, characterized by a high equation coefficient ($a > 0.3$), comprised most taxa from the nettle-unrelated insects that spent part of their life cycle in direct contact with Hg sources, *i.e.* Elateridae, Curculionidae, Chironomidae, Formicidae and Panorpidae species. This cluster also included nettle-related predators II (*i.e.*, Nabidae species), predators

I (Coccinellidae) and herbivores (Lepidoptera), which accumulated only Hg via biomagnification from the diet. The second cluster, characterized by a lower coefficient in the equation (calculated when significant, $a < 0.1$), mainly included nettle-related herbivorous insects and predators I, as well as insects strictly living on Salicaceous species (*i.e.*, Cerpopidae, Pentatomidae). This cluster also included a specific beetle (*i.e.*, Chrysomelidae), which spent a short period of its life cycle in terrestrial sediments.

DISCUSSION

Our data provided evidence on the terrestrial biomagnification of Hg at the soil-plant-insect food web at a chlor-alkali site. These results reinforce recently published work in which Hg biomagnification not only concerns wetlands but also a wider range of biota, including terrestrial biota⁴¹. As a result of the chlor-alkali process, Hg enrichment at the sediment landfill were similar to those measured at a mining area in Turkey⁴² and the Liaoning Province in China^{19,21} but 10-fold lower than the Wanshan Mercury Mining District in China¹⁴. Mean THg concentration from our soil samples (5.9 mg/kg) was also 20-fold higher than the average value for global agrosystem soils (0.3 mg/kg)⁴³ and 60-fold higher than the mean concentration in natural surface soil (0.1 mg/kg)⁴⁴. Overall, soils in the vicinities of chlor-alkali plants have Hg concentrations ranging from 0.06 to 100 mg/kg⁴⁵. The methylated form of Hg only represented 0.03% of the THg, which is very low compared to the common rate of 1% to 3% for terrestrial sediments⁴⁴. However, similar rates of MeHg in the previously mentioned Wanshan District (from 0.02 to 0.11%) to the rates of our samples were observed⁴⁶.

Low Hg concentrations were measured for the various tissues of nettles spontaneously growing under the studied poplar plantation, with average THg concentrations not exceeding 100 ng/kg for stems and leaves. These concentrations were in the same order of magnitude

than those measured for the Skado poplar cultivar (*i.e.*, 42.5 µg/kg dry wt.)³⁴. The obtained concentrations in nettles were lower than the average THg concentration (206 µg/kg) measured in the shoots of 12 native herbaceous plant species collected in the previously mentioned mining area in Turkey⁴². Hg concentrations in mature leaf tissues above 1-8 mg/kg have been reported to be excessive or toxic to plants⁴⁵ with a potential 10% yield loss in agronomic species⁴⁷. These thresholds reported in the literature are 10 to 80 times higher than those measured in nettle leaves in our study. A recent study conducted on the same site concluded that Hg entry into poplar leaves was exclusively through an atmospheric pathway². If this property also applies to nettle, it may explain the higher Hg concentrations measured in the nettle leaves compared to the stems.

Insects from the revegetated sediment landfill had Hg concentrations ranging from background values to 5500 µg/kg, which is consistent with studies conducted in industrialized areas with comparable Hg concentrations in terrestrial sediments. Lepidopteran larvae, Formicidae, and Chrysomelidae, had THg concentrations comparable to those measured in previous studies^{18,19}. Given the TMF for THg (2.8) and MeHg (6.3) within the 5 trophic levels, our data suggest that Hg biomagnified at the soil-nettle-insect food web (Figure 4). Our results are in agreement with studies that considered a wider range of taxonomic groups, including insects, birds and mammals¹⁴⁻¹⁷. Our study did not include the latter two higher trophic levels but rather focused on a much higher number of insect taxa and a more accurate level of trophic interactions.

The present study covers a wide taxonomic range of insects for which the accuracy of identification enabled the characterization of several life traits. This functional diversity provided us with a decent overview of the functioning ecosystem and enabled us to study these life traits as factors influencing Hg enrichment in insects. Thus, taxa from the experimental site were enriched in Hg to varying degrees, depending on several factors,

including the trophic level, linked to the feeding guild and the feeding specificity. However, the fact that within the nettle-unrelated group, an herbivorous species (Elateridae) had THg concentrations exceeding those of secondary predators, such as ants or Panorpidae, led us to consider other factors, including those conditioning a direct contact with contaminant sources.

Among all of the taxa considered, Elateridae were the most enriched in both Hg and MeHg, with concentrations in the same order of magnitude as those measured in soil. As herbivorous taxa feeding mainly on the poplar cultivars with a comparable enrichment than nettles³⁴, Elateridae should not have been exposed to high amounts of Hg from their diet. These elevated concentrations may be related to direct cuticle contact with the dredged sediments during the larval cycle. As already mentioned for ants¹⁸, Hg in soil can indeed penetrate their cuticle by uptake or passive diffusion when prolonged dermal contact has occurred. Elateridae larvae, also known as wireworms, have spent most of their life in the soil, including 3-4 months for eggs, 2-5 years as larvae, and can overwinter as adults⁴⁸. The other taxa investigated that may be exposed to Hg through prolonged dermal contact during their larval cycle are Panorpidae that have spent 1-2 years in the soil⁴⁹ and Chironomidae spending one to several years in underwater sediments⁵⁰. The landfill we investigated in the present study was indeed constructed in the vicinity of a mitigation pond³⁷. The lower THg concentrations measured in adult Chironomidae compared to Elateridae may be related to a greater loss of Hg during metamorphosis, as proven for some metals, including Hg⁵¹, or to an active detoxification mechanism⁵². Although the THg concentrations in the soil and in Elateridae were in the same order of magnitude, the concentration of MeHg was approximately 2000-fold higher in Elateridae, suggesting the entry of the methylated form of Hg or the potential methylation of inorganic Hg once taken up. The diet of wireworms mainly consists in decomposed plant matter, and its associated fungal and microbial biomass, which are known to be involved in the methylation of Hg in forest floor⁵³. A recent study already

hypothesized that decomposition of the soil organic matter pool introduces additional MeHg to the diets of detritivore invertebrates⁵⁴. Consequently, the high concentration of Hg measured in Elateriade seemed to be related to both their feeding guild and habitat during their larval stage. Further studies on insect traits related to their development intrinsic, such as the duration of the larval cycle, duration and location during overwinter or diapause, are needed to improve our knowledge regarding Hg accumulation in insects.

Within the nettle-related food web, the THg concentrations increased as follows: nettles < herbivores < predator specialists < predator generalists, with a gap between predator specialists (including Coccinellidae) and predator generalists, suggesting a likely entry of Hg from another source at this level. Nabidae, which were the only predator generalist in the nettle-related insects, only hunt on the herbaceous layer⁵⁵ with rare contact with the soil surface. Adult Chironomidae, which most likely grew as larvae in the adjacent contaminated pond³⁷ where they became contaminated by Hg, were abundant on nettles. In this context, Chironomidae were potential prey for Nabidae and, consequently, biovectors from aquatic to terrestrial environments. A previous study showed that Chironomidae, among emerging insects, were the main contributors to the transfer of biomass and polyunsaturated fatty acids from aquatic to terrestrial ecosystems, representing a superior food quality for terrestrial consumers²². With comparable THg concentrations (Figure 1) and similar relationships between THg and biomasses (Figure 3), Chironomidae probably played the role of the biovector of Hg from aquatic sediments to Nabidae (Figure 4). Thus, we demonstrated that the feeding specificity of insects might also be considered an explanatory factor, as generalist insects are exposed to high amounts of Hg while feeding on emerging insects. These results were consistent with those for predatory spiders^{24,29,56}, which are also generalist species and fed on Hg-enriched Chironomidae.

Figure 4: THg concentrations in the nettle-related food web (yellow pyramid) and the implication of nettle-unrelated taxa in the flow of THg (blue boxes). THg concentrations (µg/kg dry wt.) are specified under each name of taxa (mean ± SE), as well as the percentage of THg as MeHg in bold red.

Our findings suggest that insects (*e.g.*, Elateridae and Chironomidae) that had direct contact with terrestrial or underwater sediments constitute the main entry source of Hg within the terrestrial nettle-insect food web (Figure 4). These insects may constitute direct food for higher trophic levels (birds, mammals) or food for generalist predator insects. These latter (*i.e.*, Nabidae) are likely to play a role in the export of significant amounts of Hg at higher trophic levels and become another major vector in the transfer of Hg, as recently demonstrated in temperate forest environment⁵⁴. Other insect features of interest, such as

dispersal capacity, vulnerability to predation, and nutritional value, would, however, need to be considered to fully assess the role of insects within Hg dissemination in Hg-contaminated areas.

ASSOCIATED CONTENT

Supporting information.

Figure S1. Results from preliminary tests consisting of evaluating the influence of insect preservation methods on the THg concentrations measured in *Nabis sp.* (A) and *Myrmica rubra* (B) preserved in ethanol or directly ice-dried after collection.

Table S1. Ecological characteristics of each targeted taxa, including their link with nettle, habitat, trophic guild in the nettle-based food web, feeding guild and dietary range according to the literature

Table S2. Details of the MeHg analyses

AUTHOR INFORMATION

Corresponding Author

E-mail contact: michel.chalot@univ-fcomte.fr

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the funding provided by ADEME, France, under grant No. 1772C0018, PHYTOFIBER Project, and the Fondation de France and ADEME for their financial support to LY under a PhD grant.

REFERENCES

- (1) Rhee, S.-W. Control of mercury emissions: policies, technologies, and future trends
<https://www.dovepress.com/control-of-mercury-emissions-policies-technologies-and-future-trends-peer-reviewed-fulltext-article-EECT> (accessed Apr 8, 2019).
<https://doi.org/10.2147/EECT.S73403>.
- (2) Assad, M.; Parelle, J.; Cazaux, D.; Gimbert, F.; Chalot, M.; Tatin-Froux, F. Mercury uptake into poplar leaves. *Chemosphere* **2016**, *146*, 1–7.
<https://doi.org/10.1016/j.chemosphere.2015.11.103>.
- (3) Stamenkovic, J.; Gustin, M. S. Nonstomatal versus stomatal uptake of atmospheric mercury. *Environ. Sci. Technol.* **2009**, *43* (5), 1367–1372. <https://doi.org/10.1021/es801583a>.
- (4) Cronk, Q.; Hidalgo, O.; Pellicer, J.; Percy, D.; Leitch, I. Salix transect of Europe: variation in ploidy and genome size in willow-associated common nettle, *Urtica dioica* L. sens. lat., from Greece to arctic Norway. *Biodivers. Data J.* **2016**, *4*, e10003. <https://doi.org/10.3897/BDJ.4.e10003>.
- (5) Davis, B. N. K. The European distribution of insects on stinging nettles, *Urtica dioica* L.: a field survey. *Bolletino Zool.* **1989**, *56* (4), 321–326. <https://doi.org/10.1080/11250008909355658>.
- (6) Alhmedi, A.; Haubruge, E.; Bodson, B.; Francis, F. Aphidophagous guilds on nettle (*Urtica dioica*) strips close to fields of green pea, rape and wheat. *Insect Sci.* **2007**, *14* (5), 419–424.
<https://doi.org/10.1111/j.1744-7917.2007.00169.x>.
- (7) Alhmedi, A.; Haubruge, E.; D’Hoedt, S.; Francis, F. Quantitative food webs of herbivore and related beneficial community in non-crop and crop habitats. *Biol. Control* **2011**, *58* (2), 103–112. <https://doi.org/10.1016/j.biocontrol.2011.04.005>.
- (8) James, D. G.; Lauby, G.; Seymour, L.; Buckley, K. Beneficial insects associated with stinging nettle, *Urtica dioica* Linnaeus, in Central Washington state. *Pan-Pac. Entomol.* **2015**, *91* (1), 82–90.

- 532 (9) Kidd, K.; Clayden, M.; Jardine, T. Bioaccumulation and biomagnification of mercury through food
533 webs. In *Environmental Chemistry and Toxicology of Mercury*; John Wiley & Sons, Ltd, 2011;
534 pp 453–499. <https://doi.org/10.1002/9781118146644.ch14>.
- 535 (10) Rahman, Z.; Singh, V. P. The relative impact of toxic heavy metals (THMs) (arsenic (As), cadmium
536 (Cd), chromium (Cr)(VI), mercury (Hg), and lead (Pb)) on the total environment: an overview.
537 *Environ. Monit. Assess.* **2019**, *191* (7), 419. <https://doi.org/10.1007/s10661-019-7528-7>.
- 538 (11) Nabi, S. *Toxic effects of mercury*; Springer India, 2014.
- 539 (12) Evers, D. The effects of methylmercury on wildlife: a comprehensive review and approach for
540 interpretation. In *Encyclopedia of the Anthropocene*; Elsevier, 2018; pp 181–194.
541 <https://doi.org/10.1016/B978-0-12-809665-9.09985-7>.
- 542 (13) Mahbub, K. R.; Krishnan, K.; Naidu, R.; Andrews, S.; Megharaj, M. Mercury toxicity to terrestrial
543 biota. *Ecol. Indic.* **2017**, *74*, 451–462. <https://doi.org/10.1016/j.ecolind.2016.12.004>.
- 544 (14) Abeysinghe, K. S.; Qiu, G.; Goodale, E.; Anderson, C. W. N.; Bishop, K.; Evers, D. C.; Goodale, M.
545 W.; Hintelmann, H.; Liu, S.; Mammides, C.; Quan, R. C.; Wang, J.; Wu, P.; Xu, X. H.; Yang, X. D.;
546 Feng, X. Mercury flow through an asian rice-based food web. *Environ. Pollut.* **2017**, *229*, 219–
547 228. <https://doi.org/10.1016/j.envpol.2017.05.067>.
- 548 (15) Rimmer, C. C.; Miller, E. K.; McFarland, K. P.; Taylor, R. J.; Faccio, S. D. Mercury bioaccumulation
549 and trophic transfer in the terrestrial food web of a montane forest. *Ecotoxicology* **2010**, *19*
550 (4), 697–709. <https://doi.org/10.1007/s10646-009-0443-x>.
- 551 (16) Ullrich, S. M.; Ilyushchenko, M. A.; Tanton, T. W.; Uskov, G. A. Mercury contamination in the
552 vicinity of a derelict chlor-alkali plant. *Sci. Total Environ.* **2007**, *381* (1–3), 290–306.
553 <https://doi.org/10.1016/j.scitotenv.2007.02.020>.
- 554 (17) Zhilong, M.; Qiang, W.; Zhongsheng, Z.; Xuehong, Z. Mercury distribution along the food chain of
555 a wetland ecosystem at Sanjiang Plain, Northeast China. *Bull. Environ. Contam. Toxicol.* **2017**,
556 *98* (2), 162–166. <https://doi.org/10.1007/s00128-016-2005-2>.

- 557 (18) Zhang, Z.; Song, X.; Wang, Q.; Lu, X. Mercury bioaccumulation and prediction in terrestrial
558 insects from soil in Huludao City, Northeast China. *Bull. Environ. Contam. Toxicol.* **2012**, *89* (1),
559 107–112. <https://doi.org/10.1007/s00128-012-0649-0>.
- 560 (19) Zhang, Z.-S.; Lu, X.-G.; Wang, Q.-C.; Zheng, D.-M. Mercury, Cadmium and lead biogeochemistry
561 in the soil–plant–insect system in Huludao City. *Bull. Environ. Contam. Toxicol.* **2009**, *83* (2),
562 255–259. <https://doi.org/10.1007/s00128-009-9688-6>.
- 563 (20) Zheng, D.; Liu, X.; Jin, D.; Li, H.; Li, X. Mercury bioaccumulation in arthropods from typical
564 community habitats in a zinc-smelting area. *Environ. Geochem. Health* **2018**, *40* (4), 1329–
565 1337. <https://doi.org/10.1007/s10653-017-0059-7>.
- 566 (21) Zheng, D.-M.; Wang, Q.-C.; Zhang, Z.-S.; Zheng, N.; Zhang, X.-W. Bioaccumulation of total and
567 methyl mercury by arthropods. *Bull. Environ. Contam. Toxicol.* **2008**, *81* (1), 95–100.
568 <https://doi.org/10.1007/s00128-008-9393-x>.
- 569 (22) Martin-Creuzburg, D.; Kowarik, C.; Straile, D. Cross-ecosystem fluxes: export of polyunsaturated
570 fatty acids from aquatic to terrestrial ecosystems via emerging insects. *Sci. Total Environ.*
571 **2017**, *577*, 174–182. <https://doi.org/10.1016/j.scitotenv.2016.10.156>.
- 572 (23) Blais, J. M.; Macdonald, R. W.; Mackay, D.; Webster, E.; Harvey, C.; Smol, J. P. Biologically
573 mediated transport of contaminants to aquatic systems. *Environ. Sci. Technol.* **2007**, *41* (4),
574 1075–1084. <https://doi.org/10.1021/es061314a>.
- 575 (24) Cristol, D. A.; Brasso, R. L.; Condon, A. M.; Fovargue, R. E.; Friedman, S. L.; Hallinger, K. K.;
576 Monroe, A. P.; White, A. E. The movement of aquatic mercury through terrestrial food webs.
577 *Science* **2008**, *320* (5874), 335–335. <https://doi.org/10.1126/science.1154082>.
- 578 (25) Clayden, M. G.; Kidd, K. A.; Chételat, J.; Hall, B. D.; Garcia, E. Environmental, geographic and
579 trophic influences on methylmercury concentrations in macroinvertebrates from lakes and
580 wetlands across Canada. *Ecotoxicology* **2014**, *23* (2), 273–284.
581 <https://doi.org/10.1007/s10646-013-1171-9>.

- 582 (26) Buckland-Nicks, A.; Hillier, K. N.; Avery, T. S.; O'Driscoll, N. J. Mercury bioaccumulation in
583 dragonflies (Odonata: Anisoptera): examination of life stages and body regions. *Environ.*
584 *Toxicol. Chem.* **2014**, *33* (9), 2047–2054. <https://doi.org/10.1002/etc.2653>.
- 585 (27) Baxter, C. V.; Fausch, K. D.; Saunders, W. C. Tangled webs: reciprocal flows of invertebrate prey
586 link streams and riparian zones. *Freshw. Biol.* **2005**, *50* (2), 201–220.
587 <https://doi.org/10.1111/j.1365-2427.2004.01328.x>.
- 588 (28) Akamatsu, F.; Toda, H. Aquatic subsidies transport anthropogenic nitrogen to riparian spiders.
589 *Environ. Pollut. Barking Essex 1987* **2011**, *159* (5), 1390–1397.
590 <https://doi.org/10.1016/j.envpol.2011.01.005>.
- 591 (29) Alberts, J. M.; Sullivan, S. M. P. Factors influencing aquatic-to-terrestrial contaminant transport
592 to terrestrial arthropod consumers in a multiuse river system. *Environ. Pollut.* **2016**, *213*, 53–
593 62. <https://doi.org/10.1016/j.envpol.2016.02.003>.
- 594 (30) Chaves-Ulloa, R.; Taylor, B. W.; Broadley, H. J.; Cottingham, K. L.; Baer, N. A.; Weathers, K. C.;
595 Ewing, H. A.; Chen, C. Y. Dissolved organic carbon modulates mercury concentrations in insect
596 subsidies from streams to terrestrial consumers. *Ecol. Appl.* **2016**, *26* (6), 1771–1784.
597 <https://doi.org/10.1890/15-0025.1>.
- 598 (31) Ortiz, C.; Weiss-Penzias, P. S.; Fork, S.; Flegal, A. R. Total and monomethyl mercury in terrestrial
599 arthropods from the central California coast. *Bull. Environ. Contam. Toxicol.* **2015**, *94* (4), 425–
600 430. <https://doi.org/10.1007/s00128-014-1448-6>.
- 601 (32) Parkman, H.; Meili, M. Mercury in macroinvertebrates from Swedish forest lakes: influence of
602 lake type, habitat, life cycle, and food quality. *Can. J. Fish. Aquat. Sci.* **1993**, *50* (3), 521–534.
603 <https://doi.org/10.1139/f93-061>.
- 604 (33) Zappelini, C.; Karimi, B.; Foulon, J.; Lacercat-Didier, L.; Maillard, F.; Valot, B.; Blaudez, D.; Cazaux,
605 D.; Gilbert, Yergeau, D.; Greer, C.; Chalot, M. Diversity and complexity of microbial
606 communities from a chlor-alkali tailings dump. *Soil Biol. Biochem.* **2015**, *90*, 101–110.
607 <https://doi.org/10.1016/j.soilbio.2015.08.008>.

- 608 (34) Durand, A.; Maillard, F.; Foulon, J.; Gweon, H. S.; Valot, B.; Chalot, M. Environmental
609 metabarcoding reveals contrasting belowground and aboveground fungal communities from
610 poplar at a Hg phytomanagement site. *Microb. Ecol.* **2017**. [https://doi.org/10.1007/s00248-](https://doi.org/10.1007/s00248-017-0984-0)
611 017-0984-0.
- 612 (35) Giustina, W. della; Bonfils, J.; Le Quesne, W. J. *Homoptères Cicadellidae*; Faune de France;
613 Institut national de la recherche agronomique: Paris, 1989.
- 614 (36) Péricart, J. *Hémiptères Nabidae d'Europe Occidentale et Du Maghreb*; Faune de France;
615 Fédération française des sociétés de sciences naturelles: Paris, 1987.
- 616 (37) Maillard, F.; Girardclos, O.; Assad, M.; Zappelini, C.; Pérez Mena, J. M.; Yung, L.; Guyeux, C.;
617 Chrétien, S.; Bigham, G.; Cosio, C.; Chalot, M. Dendrochemical assessment of mercury releases
618 from a pond and dredged-sediment landfill impacted by a chlor-alkali plant. *Environ. Res.*
619 **2016**, *148*, 122–126. <https://doi.org/10.1016/j.envres.2016.03.034>.
- 620 (38) Liu, B.; Yan, H.; Wang, C.; Li, Q.; Guédron, S.; Spangenberg, J. E.; Feng, X.; Dominik, J. Insights
621 into low fish mercury bioaccumulation in a mercury-contaminated reservoir, Guizhou, China.
622 *Environ. Pollut.* **2012**, *160*, 109–117. <https://doi.org/10.1016/j.envpol.2011.09.023>.
- 623 (39) Bloom, N. Determination of picogram levels of methylmercury by aqueous phase ethylation,
624 followed by cryogenic gas chromatography with cold vapour atomic fluorescence detection.
625 *Can. J. Fish. Aquat. Sci.* **1989**, *46* (7), 1131–1140. <https://doi.org/10.1139/f89-147>.
- 626 (40) Borgå, K.; Kidd, K. A.; Muir, D. C.; Berglund, O.; Conder, J. M.; Gobas, F. A.; Kucklick, J.; Malm, O.;
627 Powell, D. E. Trophic magnification factors: considerations of ecology, ecosystems, and study
628 design. *Integr. Environ. Assess. Manag.* **2012**, *8* (1), 64–84. <https://doi.org/10.1002/ieam.244>.
- 629 (41) Rodenhouse, N. L.; Lowe, W. H.; Gebauer, R. L. E.; McFarland, K. P.; Bank, M. S. Mercury
630 bioaccumulation in temperate forest food webs associated with headwater streams. *Sci. Total*
631 *Environ.* **2019**, *665*, 1125–1134. <https://doi.org/10.1016/j.scitotenv.2019.02.151>.

- 632 (42) Sasmaz, M.; Akgül, B.; Yıldırım, D.; Sasmaz, A. Mercury uptake and phytotoxicity in terrestrial
633 plants grown naturally in the Gumuskoy (Kutahya) mining area, Turkey. *Int. J.*
634 *Phytoremediation* **2016**, *18* (1), 69–76. <https://doi.org/10.1080/15226514.2015.1058334>.
- 635 (43) He, Z. L.; Yang, X. E.; Stoffella, P. J. Trace elements in agroecosystems and impacts on the
636 environment. *J. Trace Elem. Med. Biol.* **2005**, *19* (2), 125–140.
637 <https://doi.org/10.1016/j.jtemb.2005.02.010>.
- 638 (44) Kabata-Pendias, A. *Trace elements in soils and plants*, 4th ed.; CRC Press: Boca Raton, 2011.
- 639 (45) Kabata-Pendias, A.; Mukherjee, A. B. *Trace elements from soil to human: With 209 Tables*;
640 Springer: Berlin, 2007.
- 641 (46) Meng, B.; Feng, X.; Qiu, G.; Wang, D.; Liang, P.; Li, P.; Shang, L. Inorganic mercury accumulation
642 in rice (*Oryza sativa* L.). *Environ. Toxicol. Chem.* **2012**, *31* (9), 2093–2098.
643 <https://doi.org/10.1002/etc.1913>.
- 644 (47) Macnicol, R. D.; Beckett, P. H. T. Critical tissue concentrations of potentially toxic elements.
645 *Plant Soil* **1985**, *85* (1), 107–129. <https://doi.org/10.1007/BF02197805>.
- 646 (48) Stolpe Nordin, E. Life cycle of *Agriotes* wireworms and their effect in maize cultivation. **2017**.
- 647 (49) Byers, G. W.; Thornhill, R. Biology of the Mecoptera. *Annu. Rev. Entomol.* **1983**, *28* (1), 203–228.
648 <https://doi.org/10.1146/annurev.en.28.010183.001223>.
- 649 (50) Pinder, L. C. V. Biology of freshwater Chironomidae. *Annu. Rev. Entomol.* **1986**, *31* (1), 1–23.
- 650 (51) Kraus, J. M.; Walters, D. M.; Wesner, J. S.; Stricker, C. A.; Schmidt, T. S.; Zuellig, R. E.
651 Metamorphosis alters contaminants and chemical tracers in insects: implications for food
652 webs. *Environ. Sci. Technol.* **2014**, *48* (18), 10957–10965. <https://doi.org/10.1021/es502970b>.
- 653 (52) Beauvais-Flück, R.; Gimbert, F.; Méhault, O.; Cosio, C. Trophic fate of inorganic and methyl-
654 mercury in a macrophyte-chironomid food chain. *J. Hazard. Mater.* **2017**, *338*, 140–147.
655 <https://doi.org/10.1016/j.jhazmat.2017.05.028>.

- 656 (53) Beckert, W. F.; Moghissi, A. A.; Au, F. H. F.; Bretthauer, E. W.; Mcfarlane, J. C. Formation of
657 methylmercury in a terrestrial environment. *Nature* **1974**, *249* (5458), 674–675.
658 <https://doi.org/10.1038/249674a0>.
- 659 (54) Tsz-Ki Tsui, M.; Liu, S.; Brasso, R. L.; Blum, J. D.; Kwon, S. Y.; Ulus, Y.; Nollet, Y. H.; Balogh, S. J.;
660 Eggert, S. L.; Finlay, J. C. Controls of methylmercury bioaccumulation in forest floor food webs.
661 *Environ. Sci. Technol.* **2019**, *53* (5), 2434–2440. <https://doi.org/10.1021/acs.est.8b06053>.
- 662 (55) Swanson, D. A Synopsis of the damsel bugs (Heteroptera: Nabidae) of Michigan. *Gt. Lakes*
663 *Entomol.* **2012**, *45*, 40–55.
- 664 (56) Gann, G. L.; Powell, C. H.; Chumchal, M. M.; Drenner, R. W. Hg-contaminated terrestrial spiders
665 pose a potential risk to songbirds at Caddo lake (Texas/Louisiana, USA). *Environ. Toxicol.*
666 *Chem.* **2015**, *34* (2), 303–306. <https://doi.org/10.1002/etc.2796>.
667