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

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Insect life traits are key factors in mercury accumulation and transfer within the terrestrial food web

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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 µg/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.
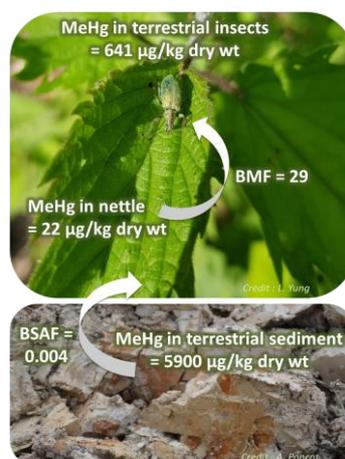
INTRODUCTION

Landfills in the chlor-alkali industries require stabilization of mercury (Hg) to prevent air pollution by evaporated Hg[1]. Revegetation of these sites offers a suitable solution to confine the Hg released from the soil in plant biomass[2], as plants are net sinks that incorporate the atmospheric Hg into leaf tissues[3]. These plantations, mainly from Salicaceous species (*i.e.,* willow), favour the appearance of spontaneous herbaceous cover highly dominated by the common stinging nettle, *Urtica dioïca* L. (Urticaceae)[4]. In natural conditions, this nettle is considered an invertebrate "super-host" providing food for a large diversity of insects with different biology[5] 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 plants[9]. 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 chironomids[25] or dragonflies[26] that move to terrestrial biota

59 can serve as both prey[27] and biovector of contaminants for local terrestrial consumers,

60 including ants or spiders[28,29]. Consequently, the exposition of terrestrial insects is linked to the

61 vicinity to aquatic biota and the Hg bioavailability, conditioned by the physical and chemical

62 factors of these systems (*e.g.*, dissolved organic carbon, quantity of aqueous nutrients)[25,30].

63 For strictly terrestrial habitats, factors such as insect habitats, have been hypothesized to

64 influence Hg accumulation in terrestrial insects[18,31], but no studies examining biological

65 and/or ecological factors affecting Hg concentrations in these organisms exist, as previously

66 performed in lakes and wetlands[25,32].

67   The present study aimed to study Hg uptake and biomagnification through an original

68 and specific soil-plant-terrestrial insect system in a Hg-contaminated area in France and to

69 estimate the extent to which insect-related factors affected Hg uptake. To that end, we used a

70 chlor-alkali landfill revegetated by poplar plantations where the stinging nettle, *Urtica dioïca*

71 L., colonized spontaneously the herbaceous layer, hosting a large diversity of insects. The

72 specific objectives were i) to quantify THg (total Hg) and MeHg in soil, different

73 compartments of nettles and a large number of well-identified insect taxa; ii) to characterize

74 insect taxa by their trophic guilds, feeding guilds, dietary range and habitats at both the larval

75 and adult stages, their relationship with the nettle and their morphological characteristics (*i.e.*,

76 life stage and weight); and iii) to assess the relative importance of these factors on THg and

77 MeHg concentrations in insects at the revegetated landfill.

78

79 **MATERIALS AND METHODS**

80 **Site description**

81   A phytomanagement field trial was implemented in 2011 on a chlor-alkali sediment

82 landfill located in Saint-Symphorien-sur-Saône in the Bourgogne Franche-Comté region

83   (France). The location, history and phytomanagement set-up at the sediment landfill have

84   been described in detail in previous studies[33,22]. Five years after planting, biotic and abiotic

85   environmental conditions have promoted the emergence of a spontaneous herbaceous cover

86   strongly dominated by stinging nettle (*Urtica dioïca* L.). The control site is a natural area

87   located at Courcelle-les-Montbéliard, 140 km east of the chlor-alkali plant, not directly

88   influenced by the point-source.

89   **Sample collection**

90   To ensure the most representative diversity in insect species, the study was carried out

91   over two consecutive years (2017 and 2018). Six poplar plots were selected at the

92   experimental site, three with the Skado cultivar (*Populus trichocarpa* x *P. maximowiczii*) and

93   three with the I-214 cultivar (*P. deltoides* x *P. nigra*). Soil and plant samples were collected in

94   June 2017 and monthly in 2018 from April to September (*i.e.*, during the vegetation period)

95   for the experimental site and in July 2018 for the control site. Three samples of soil and nettle

96   plants were composed of three mixed subsamples taken randomly within each sampling plot.

97   Soil samples consisting of bulk soil were collected from the first 20-cm layer, cleared from

98   roots and other plant materials. Soil and plant samples were placed into plastic and paper

99   bags, respectively, stored into a refrigerated container and shipped to the laboratory, where

100   they were dried at ambient temperature (24 °C) for two weeks before Hg analyses. In parallel,

101   an exhaustive sampling of the entomofauna related to the stinging nettle was carried out in the

102   six studied plots monthly from April to September (*i.e.*, period of insect activity) during the

103   two consecutive years (2017 and 2018). At each plot (chlor-alkali and control sites), insects

104   from six nettle patches of similar size were caught using a sweep net and pooled in a collector

105   tube with absolute ethanol or ethyl-acetate as usually used to assure optimal preservation and

106   identification. All samples were stored at -20°C until identification and Hg analyses.

107   **Insect identification and characterization**

108   Among the whole insect diversity collected from the entomological survey, in the

109   present study we targeted the most abundant taxa, representing 63% of the total insects

110   collected on nettles at the experimental site during the two years. Prior to the analyses, each

111   taxon was identified to the lowest possible taxonomic level using several references that

112   included some identification keys[35,36], on-line sources, such as insecte.org and/or specialists

113   from the Franche-Comte Natural Spaces Conservatory. Each taxon was classified according

114   to their life traits using published literature (SI Table S1). First, taxa were assigned according

115   to their habitat both at the larval and adult stages, which allowed them to be split into two

116   groups, "the nettle-related group", which included insects hosted by nettles (*i.e.,* insects living

117   on the nettle during their whole life cycle), and the "nettle-unrelated group", which refers to

118   all other insects. We further classified taxa according to their trophic levels (*i.e.,* herbivores,

119   predators I or predators II), their larval and adult feeding guild (*i.e.,* plant parts for herbivores

120   and type of prey for predators) and their dietary range (*i.e.,* insects that are host specific

121   within a family were categorized as "specialists", and those that feed on plants or insects from

122   more than one family were categorized as "generalists"). Finally, to test the influence of the

123   morphological characteristics of insects on THg content, we identified the life stage of each

124   taxon (larvae or adult) and measured the dry weight (mg).

125   **THg and MeHg analyses in soil, plants and insects**

126   Before analyses, dried nettle stems and leaves were separated and ground into a

127   homogenous powder in a MM40 Mixer Mill (Retsch, Eragny sur Oise, France) for 4 min at 30

128   Hz and 7 min at 30 Hz for leaves and stems, respectively. Soil samples were manually

129   homogenized and sieved to <4 mm. Insects euthanized with ethyl acetate were freeze-dried,

130   and those euthanized with absolute ethanol were transferred into new plastic tubes and opened

131   overnight at ambient temperature (24 °C) for the evaporation of ethanol. As the mass of some

132   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 study[37]. 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 HNO_3 leaching / CH_2Cl_2 extraction method, as described by[38]. 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 $CuSO_4$, 5 mL 5 M HNO_3 and 10 mL CH_2Cl_2, 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 H_2O (MilliQ) under 50 mL/min N_2 flow in a water bath at 50°C. MeHg was analysed as described elsewhere[39]. 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:

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

180 According to the information collected from the literature on trophic interactions **(Table S1)**,

181 we calculated the Bio-Magnification Factors (BMF) for nettle-related insects from the

182 experimental site according to the following equation:

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

183 Trophic Magnification Factors (TMF) were determined for both THg and MeHg at the

184 experimental site. TMF considered all taxa along the five level trophic web (*i.e.,* plants,

185 herbivorous insects, predator I, predator II, predator III) except those from the Elateridae

186 species, which were removed from the herbivorous group as they accumulated large quantities

187 of Hg through their life cycle. Hence, TMF were determined from the slope of a regression

188 between the Hg concentrations and trophic level of insects in the food web and calculated as

189 the antilog of the regression slopes (b):

$$Log\ [Hg] = a + bTL$$

190 TMF refers to the average rate of change in contaminant concentrations from diet-to

191 consumer along the trophic web. In TMF, slope values above zero are indicative of

192 biomagnification (*i.e.*, TMF>1)[40].

193

194 **RESULTS**

195 **Characterisation of targeted taxa**

196 The present study covered insects from six orders: Coleoptera, Diptera, Hemiptera,

197 Hymenoptera, Lepidoptera and Mecoptera, belonging to 16 common families within these

198 orders (SI Table S1). The 23 targeted insect taxa were identified up to the family level for

199 100% of them, to the genus level for 96% and to the species level for 74%. The identification

200 of 16 caterpillars could not go beyond the order level. The 23 selected taxa were then

201 classified according to their relationship or lack of relationship with nettle as a host-plant and

202 their life traits (SI Table S1). The nettle-related group included 12 different genera of insects

203 belonging to orders of Hemiptera and Coleoptera and unidentified families of Lepidopteran

204 larvae (SI Table S1). These insects belonged to three different trophic guilds: herbivores,

205 predators I and predators II. Herbivores belonged to taxa feeding on nettle sap, seeds or leaves

206 during their larval or adult stages. Predators I were mainly specialists belonging to the family

207 Coccinellidae and Miridae, which fed almost exclusively on aphids. Predators II were

208 represented by the genus *Nabis,* which included certain species known as generalist predators

209 that hunt herbivorous or other predatory species (*i.e.,* aphids, Lepidopteran or Coleopteran

210 larvae and some Heteropteran and Dipteran adults) on herbaceous layers. The nettle-unrelated

211 group included all the other abundant taxa represented by 8 families (*i.e.,* Formicidae,

212 Panorpidae, Aphrophoridae, Chrysomelidae, Curculionidae, Elateridae, Chironomidae, and

213 Sciomyzidae) and 9 genera. All taxa were sampled on nettles but were not directly related to

214 it. Most of these taxa were characterized by a high mobility and a specific life cycle that

215 occurred at different habitats, such as a larval cycle in the soil, in water or in snails as

216 parasitoids.

217

Hg concentrations in the soil-plant-insect system

219   THg concentrations of soil, plant and insects were significantly higher at the

220 experimental site than at the control site. The THg concentrations measured in soil samples

221 from the experimental site were in the range of 4382 – 7119 μg/kg and 10-fold higher than

222 THg concentration (570 ± 70 μg/kg) at the adjacent undisturbed forest soil [33]. Nettle stems

223 and leaves were significantly more enriched in THg at the experimental site than their

224 counterparts collected at the control site (Table 1). The BSAFs calculated for the experimental

225 site and the control site were 0.004 and 0.011, respectively, suggesting a noticeable dilution at

226 both sites. With an average THg of 31 ± 1 µg/kg, leaves were significantly ($P < 0.01$) more

227 enriched than stems (11 ± 1 µg/kg THg). A significant enrichment was observed in the nettle

228 leaves during the season, with THg concentrations ranging from 18 ± 5 µg/kg in samples

229 collected in April to 31 ± 5 µg/kg in samples collected in September ($P < 0.01$).

230   A total of 312 and 159 analyses of THg concentrations was performed on insects from

231 the experimental and control sites, respectively (Table 1). Average THg concentrations for

232 insects collected at the experimental site were significantly higher than those at the control

233 site ($P < 0.001$), and this difference was found more specifically for 12 of the 14 compared

234 families and orders (Table 1). Taxa (Cicadellidae family and Lepidoptera order), for which no

235 significant differences were observed, had THg concentrations ranging from < QL to 271

236 µg/kg at the experimental site and from < QL to 109 µg/kg at the control site. No THg was

237 detected in Nabidae from the control site, whereas elevated THg concentrations (ranging from

238 107 to 1805 µg/kg) were measured for the same taxa collected at the experimental site, and

239 the same phenomenon was observed for Aphididae and Aphrophoridae, although to a lesser

240 extent. For most insects collected at the control site, THg concentrations remained < 53

241 µg/kg, except for the highly mobile flying insects belonging to the Chironomidae and

242 Panorpidae families, which presented the highest background concentrations (Table 1).

243

244 **Table 1.** Total mercury (THg) concentrations measured in nettles and the 16 families and

245 order of insects from the sediment landfill and the control site. n represents the number of

246 samples analysed. All concentrations are expressed in µg/kg dry wt ± SE. 'NA' = taxa not

247 collected at the control site. Concentrations measured in the sediment landfill were compared

248 to those measured in the control site using the Wilcoxon rank sum test and the two-sample t-

249 test. The results from the statistical comparisons are represented with the following legend: *P*-

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).
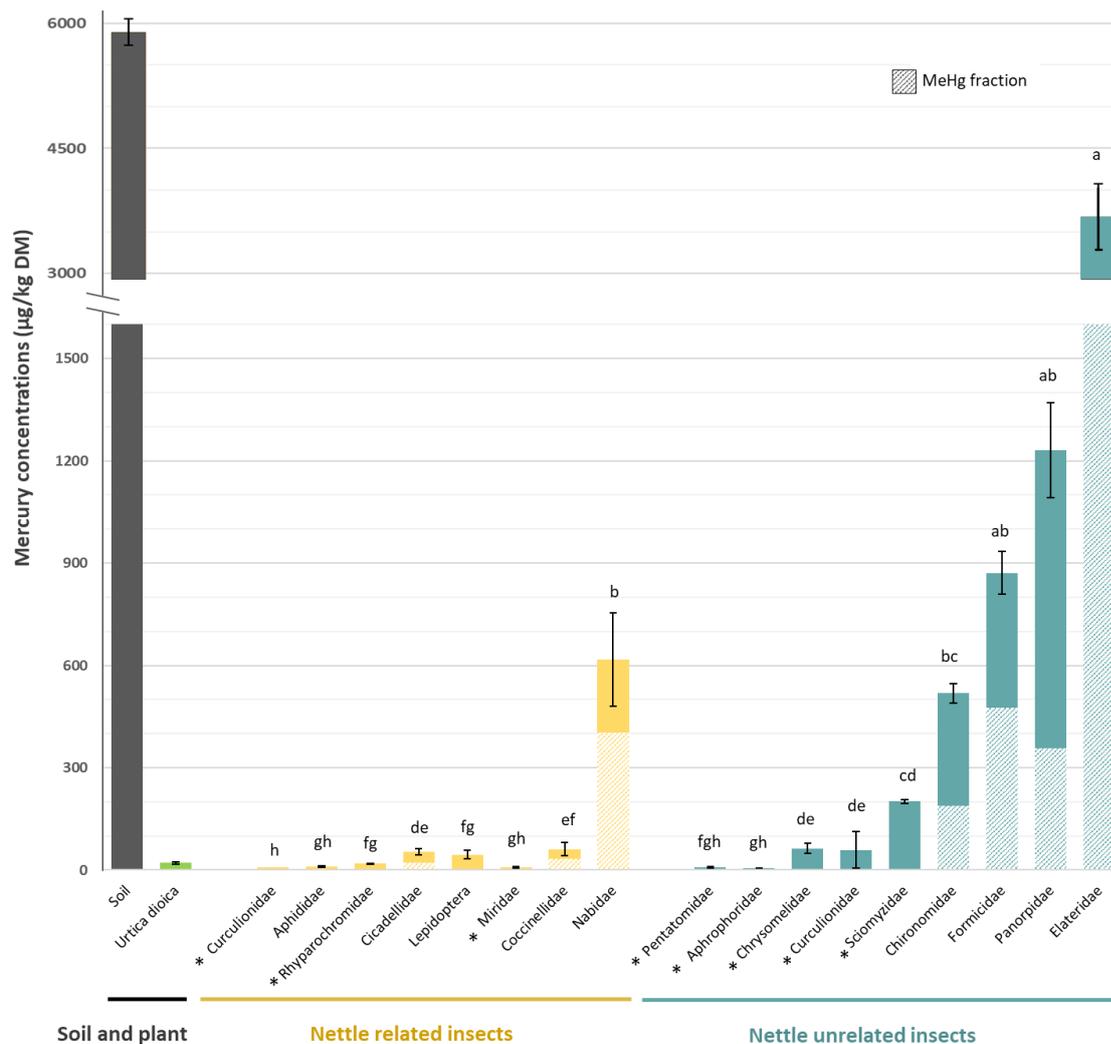
Figure 1. Mean THg concentrations (full bar, µg/kg dry wt.), including the MeHg fraction (hatched bar, %), plotted for soil and nettles (*Urtica dioïca*) and for all the investigated insect taxa from the nettle-unrelated and nettle-related groups. * = The MeHg concentrations were not analysed. Bars with 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 Sciomizidae and below 100 µg/kg for each other family. Within the Curculionidae family, THg concentrations were variable,

270   ranging from < QL to 321 µg/kg, with concentrations over 150 µg/kg for *Phyllobius sp.* and

271   up to 56 µg/kg for the two other considered species (SI Table S1). All other less enriched

272   families had THg concentrations in the same order of magnitude as those measured for

273   nettles. Overall, nettle-unrelated insects showed mean THg concentrations (981 ± 96 µg/kg)

274   six times higher than nettle-related insects (124 ± 14 µg/kg) (Figure 1). Within the nettle-

275   related group, mean THg concentrations ranged from 8.9 ± 2.4 for Miridae to 617.3 ± 47.2

276   µg/kg for Nabidae. Concerning the nettle-unrelated group, the lowest mean THg

277   concentrations were obtained for Aphrophoridae and Pentatomidae, whereas the highest

278   concentrations were found in Elateridae. However, the THg enrichment seems not to be only

279   related to the relationship with nettle. Indeed, the nettle-related taxon Nabidae had THg

280   concentrations not significantly different from the nettle-unrelated taxa Panorpidae,

281   Formicidae and Chironimidae (Figure 1).

282   Additionally, no significant difference ($P > 0.05$) was found between the lowest mean

283   THg concentrations in the nettle-unrelated group and those in the nettle-related group. Despite

284   high THg concentrations, the percentage of MeHg in the soil was very low (0.03 ± 0.002%),

285   slightly increased in nettles (1.2 ± 0.1%) and considerably increased in insects (ranging from

286   5.6% to 74.2%). Insect MeHg (average 29.2%, a) being significantly different *($P < 0.05$)*

287   from nettle MeHg (8.0%, b), and soil MeHg (average 4.5%, b).

288   The percentages of MeHg were not significantly different between the nettle-related

289   group and the nettle-unrelated group ($P > 0.05$) with mean values of 43 ± 7% and 49 ± 5%,

290   respectively. Within the nettle-related group, for the 5 studied families, the percentage of

291   MeHg was higher than 25% and reached 65% for Nabidae, except for Lepidoptera, for which

292   MeHg only represented 6% of THg. Elateridae from the nettle-unrelated group exhibited 74%

293   MeHg, representing over 2700 µg/kg. The Panorpidae family presented the most variable

294   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	r2	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**).
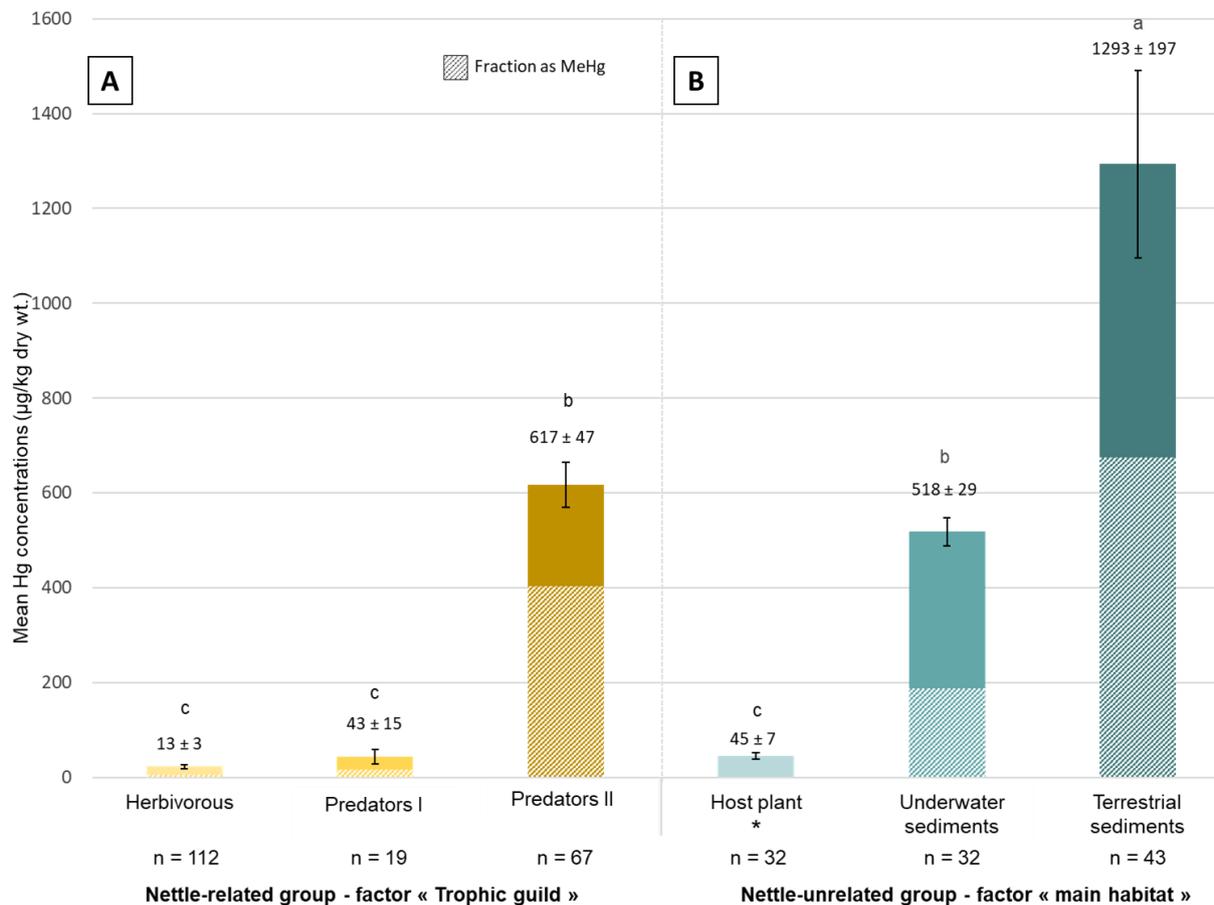
Figure 2. Mean THg concentrations (full bars, µg/kg dry wt.) ± SE and the MeHg fraction

(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),

16

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

346 nettle-related food web, had average concentrations 15 times (THg) and more than 30 times

347 (MeHg) higher than those measured in the lower trophic guild, resulting in a BMF of 4.8.

348   The influence of the development stage on THg concentrations was studied on two

349 predatory taxa: the ladybirds, *Harmonia axiridis (*Coccinellidae*)* and the damsel bugs, *Nabis*

350 *sp. (*Nabidae*)*. THg concentrations measured in the imago ladybirds (n = 6) were 10-fold

351 higher ($P < 0.001$) than those measured in larvae (n = 7). In contrast, no significant difference

352 was obtained for Nabidae species between the imago (from 148 to 1786 µg/kg; n = 43) and

353 the larvae (from 108 to 1805 µg/kg; n = 24). A significant positive correlation between the

354 weight of insects and their THg content was observed when all taxa were pooled ($r_s = 0.62$, *P*

355 < 0.001). The THg content in insects increased proportionally with their weight but

356 differently according to the group studied. We also considered the main life traits, that is, the

357 habitat of nettle-unrelated taxa and the trophic guild for nettle-related taxa. In doing so, the

358 correlation between THg and weight was confirmed for herbivores ($r_s = 0.60$, $P < 0.001$) and

359 predators ($r_s = 0.62$, $P < 0.001$) trophic guilds as well as for underwater sediments ($r_s = 0.44$,

360 $P < 0.01$) and terrestrial sediments ($r_s = 0.81$, $P < 0.001$) habitats (Figure 3).

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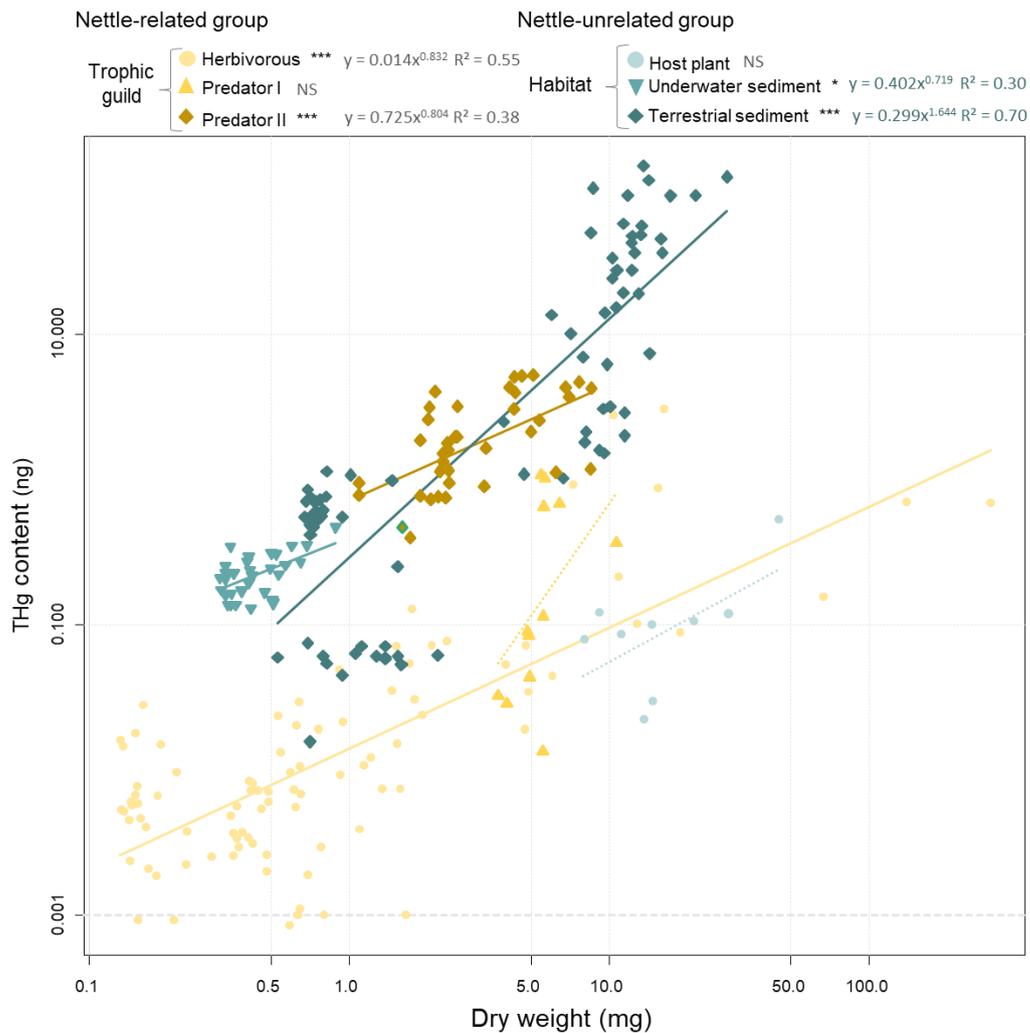
Figure 3. Relationships between the dry weight (mg) and the THg content (ng) of insects measured in 6 classes, 3 related to the "trophic guild" or 3 related to the "habitat" factors. The results from Pearson's and Spearman's correlation tests are presented with the following legend: *P*-value '***' 0.001 '**' 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

373 I (Coccinellidae) and herbivores (Lepidoptera), which accumulated only Hg *via*

374 biomagnification from the diet. The second cluster, characterized by a lower coefficient in the

375 equation (calculated when significant, a < 0.1), mainly included nettle-related herbivorous

376 insects and predators I, as well as insects strictly living on Salicaceous species (*i.e.,*

377 Cerpopidae*,* Pentatomidae). This cluster also included a specific beetle (*i.e.,* Chrysomelidae),

378 which spent a short period of its life cycle in terrestrial sediments.

379

380 **DISCUSSION**

381 Our data provided evidence on the terrestrial biomagnification of Hg at the soil-plant-

382 insect food web at a chlor-alkali site. These results reinforce recently published work in which

383 Hg biomagnification not only concerns wetlands but also a wider range of biota, including

384 terrestrial biota[41]. As a result of the chlor-alkali process, Hg enrichment at the sediment

385 landfill were similar to those measured at a mining area in Turkey[42] and the Liaoning

386 Province in China[19,21] but 10-fold lower than the Wanshan Mercury Mining District in

387 China[14]. Mean THg concentration from our soil samples (5.9 mg/kg) was also 20-fold higher

388 than the average value for global agrosystem soils (0.3 mg/kg)[43] and 60-fold higher than the

389 mean concentration in natural surface soil (0.1 mg/kg)[44]. Overall, soils in the vicinities of

390 chlor-alkali plants have Hg concentrations ranging from 0.06 to 100 mg/kg[45]. The methylated

391 form of Hg only represented 0.03% of the THg, which is very low compared to the common

392 rate of 1% to 3% for terrestrial sediments[44]. However, similar rates of MeHg in the previously

393 mentioned Wanshan District (from 0.02 to 0.11%) to the rates of our samples were

394 observed[46].

395 Low Hg concentrations were measured for the various tissues of nettles spontaneously

396 growing under the studied poplar plantation, with average THg concentrations not exceeding

397 100 ng/kg for stems and leaves. These concentrations were in the same order of magnitude

398 than those measured for the Skado poplar cultivar (*i.e.,* 42.5 µg/kg dry wt.)[34]. The obtained

399 concentrations in nettles were lower than the average THg concentration (206 µg/kg)

400 measured in the shoots of 12 native herbaceous plant species collected in the previously

401 mentioned mining area in Turkey[42]. Hg concentrations in mature leaf tissues above 1-8 mg/kg

402 have been reported to be excessive or toxic to plants[45] with a potential 10% yield loss in

403 agronomic species[47]. These thresholds reported in the literature are 10 to 80 times higher than

404 those measured in nettle leaves in our study. A recent study conducted on the same site

405 concluded that Hg entry into poplar leaves was exclusively through an atmospheric pathway[2].

406 If this property also applies to nettle, it may explain the higher Hg concentrations measured in

407 the nettle leaves compared to the stems.

408   Insects from the revegetated sediment landfill had Hg concentrations ranging from

409 background values to 5500 µg/kg, which is consistent with studies conducted in industrialized

410 areas with comparable Hg concentrations in terrestrial sediments. Lepidopteran larvae,

411 Formicidae*,* and Chrysomelidae*,* had THg concentrations comparable to those measured in

412 previous studies[18,19]. Given the TMF for THg (2.8) and MeHg (6.3) within the 5 trophic

413 levels, our data suggest that Hg biomagnified at the soil-nettle-insect food web (Figure 4).

414 Our results are in agreement with studies that considered a wider range of taxonomic groups,

415 including insects, birds and mammals[14–17]. Our study did not include the latter two higher

416 trophic levels but rather focused on a much higher number of insect taxa and a more accurate

417 level of trophic interactions.

418   The present study covers a wide taxonomic range of insects for which the accuracy of

419 identification enabled the characterization of several life traits. This functional diversity

420 provided us with a decent overview of the functioning ecosystem and enabled us to study

421 these life traits as factors influencing Hg enrichment in insects. Thus, taxa from the

422 experimental site were enriched in Hg to varying degrees, depending on several factors,

423 including the trophic level, linked to the feeding guild and the feeding specificity. However,

424 the fact that within the nettle-unrelated group, an herbivorous species (Elateridae) had THg

425 concentrations exceeding those of secondary predators, such as ants or Panorpidae, led us to

426 consider other factors, including those conditioning a direct contact with contaminant sources.

427   Among all of the taxa considered, Elateridae were the most enriched in both Hg and

428 MeHg, with concentrations in the same order of magnitude as those measured in soil. As

429 herbivorous taxa feeding mainly on the poplar cultivars with a comparable enrichment than

430 nettles[34], Elateridae should not have been exposed to high amounts of Hg from their diet.

431 These elevated concentrations may be related to direct cuticle contact with the dredged

432 sediments during the larval cycle. As already mentioned for ants[18], Hg in soil can indeed

433 penetrate their cuticle by uptake or passive diffusion when prolonged dermal contact has

434 occurred. Elateridae larvae, also known as wireworms, have spent most of their life in the soil,

435 including 3-4 months for eggs, 2-5 years as larvae, and can overwinter as adults[48]. The other

436 taxa investigated that may be exposed to Hg through prolonged dermal contact during their

437 larval cycle are Panorpidae that have spent 1-2 years in the soil[49] and Chironomidae spending

438 one to several years in underwater sediments[50]. The landfill we investigated in the present

439 study was indeed constructed in the vicinity of a mitigation pond[37]. The lower THg

440 concentrations measured in adult Chironomidae compared to Elateridae may be related to a

441 greater loss of Hg during metamorphosis, as proven for some metals, including Hg[51], or to an

442 active detoxification mechanism[52]. Although the THg concentrations in the soil and in

443 Elateridae were in the same order of magnitude, the concentration of MeHg was

444 approximately 2000-fold higher in Elateridae, suggesting the entry of the methylated form of

445 Hg or the potential methylation of inorganic Hg once taken up. The diet of wireworms mainly

446 consists in decomposed plant matter, and its associated fungal and microbial biomass, which

447 are known to be involved in the methylation of Hg in forest floor[53]. A recent study already

448 hypothesized that decomposition of the soil organic matter pool introduces additional MeHg

449 to the diets of detritivore invertebrates[54]. Consequently, the high concentration of Hg

450 measured in Elateriade seemed to be related to both their feeding guild and habitat during

451 their larval stage. Further studies on insect traits related to their development intrinsic, such as

452 the duration of the larval cycle, duration and location during overwinter or diapause, are

453 needed to improve our knowledge regarding Hg accumulation in insects.

454   Within the nettle-related food web, the THg concentrations increased as follows:

455 nettles < herbivores < predator specialists < predator generalists, with a gap between predator

456 specialists (including Coccinellidae) and predator generalists, suggesting a likely entry of Hg

457 from another source at this level. Nabidae, which were the only predator generalist in the

458 nettle-related insects, only hunt on the herbaceous layer[55] with rare contact with the soil

459 surface. Adult Chironomidae, which most likely grew as larvae in the adjacent contaminated

460 pond[37] where they became contaminated by Hg, were abundant on nettles. In this context,

461 Chironomidae were potential prey for Nabidae and, consequently, biovectors from aquatic to

462 terrestrial environments. A previous study showed that Chironomidae, among emerging

463 insects, were the main contributors to the transfer of biomass and polyunsaturated fatty acids

464 from aquatic to terrestrial ecosystems, representing a superior food quality for terrestrial

465 consumers[22]. With comparable THg concentrations (Figure 1) and similar relationships

466 between THg and biomasses (Figure 3), Chironomidae probably played the role of the

467 biovector of Hg from aquatic sediments to Nabidae (Figure 4). Thus, we demonstrated that

468 the feeding specificity of insects might also be considered an explanatory factor, as generalist

469 insects are exposed to high amounts of Hg while feeding on emerging insects. These results

470 were consistent with those for predatory spiders[24,29,56], which are also generalist species and

471 fed on Hg-enriched Chironomidae.

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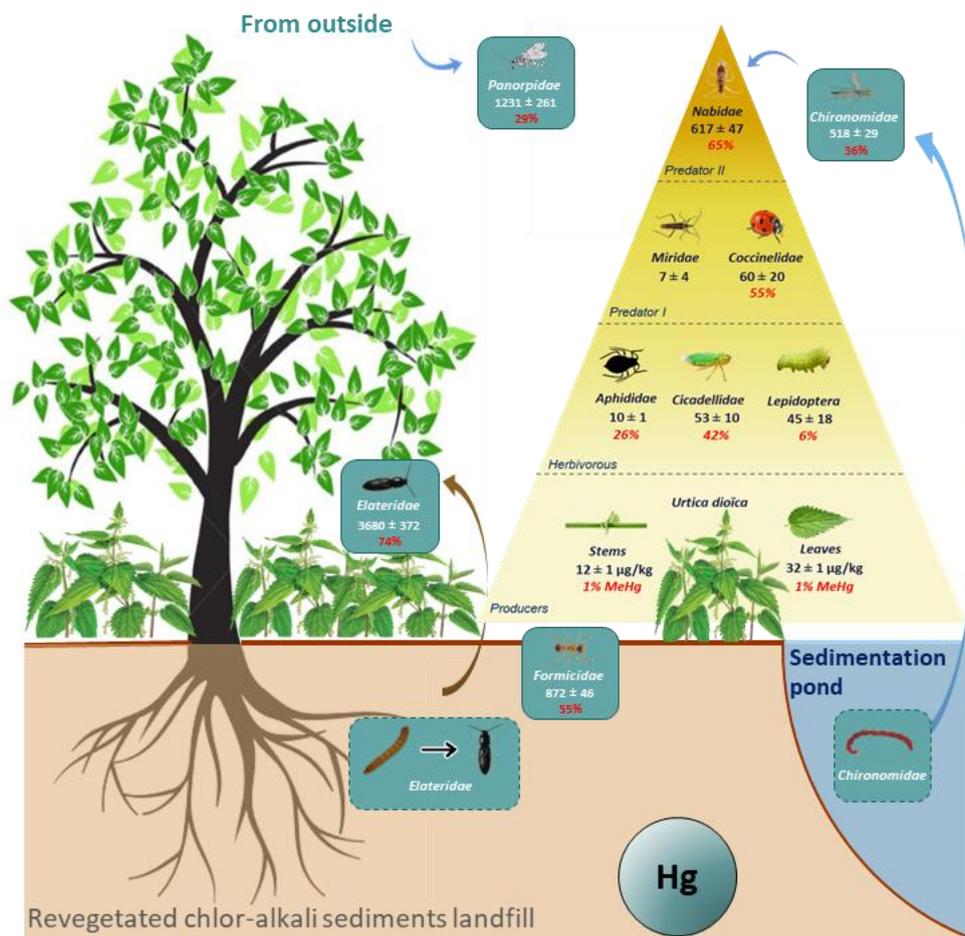
Figure 4: THg concentrations in the nettle-related food web (yellow pyramid) and the implication of nettle-unrelated taxa in the flow of THg (blue boxes). THg concentrations (µg/kg dry wt.) are specified under each name of taxa (mean ± SE), as well as the percentage of THg as MeHg in bold red.

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 environment[54]. Other insect features of interest, such as

484 dispersal capacity, vulnerability to predation, and nutritional value, would, however, need to

485 be considered to fully assess the role of insects within Hg dissemination in Hg-contaminated

486 areas.

487 **ASSOCIATED CONTENT**

488 **Supporting information.**

489 **Figure S1.** Results from preliminary tests consisting of evaluating the influence of insect

490 preservation methods on the THg concentrations measured in *Nabis sp.* (A) and *Myrmica*

491 *rubra* (B) preserved in ethanol or directly ice-dried after collection.

492 **Table S1**. Ecological characteristics of each targeted taxa, including their link with nettle,

493 habitat, trophic guild in the nettle-based food web, feeding guild and dietary range according

494 to the literature

495 **Table S2**. Details of the MeHg analyses

496

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501 Notes

502 The authors declare no competing financial interest.

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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-

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