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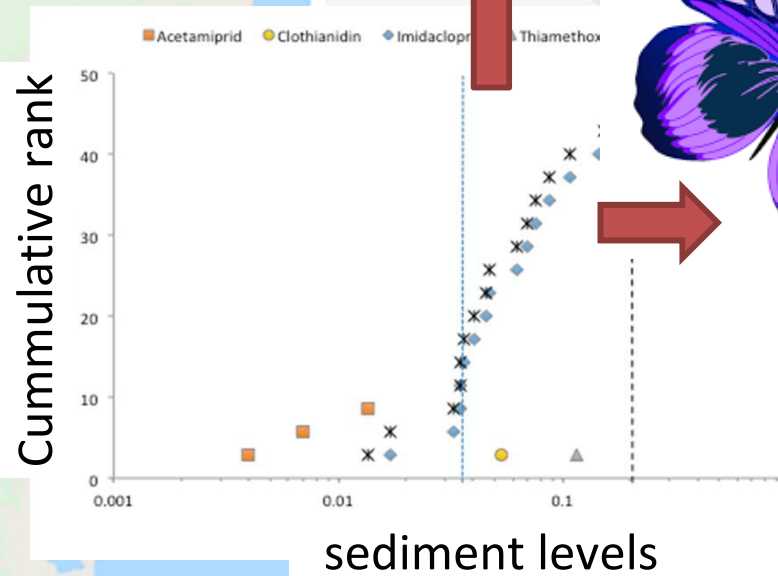
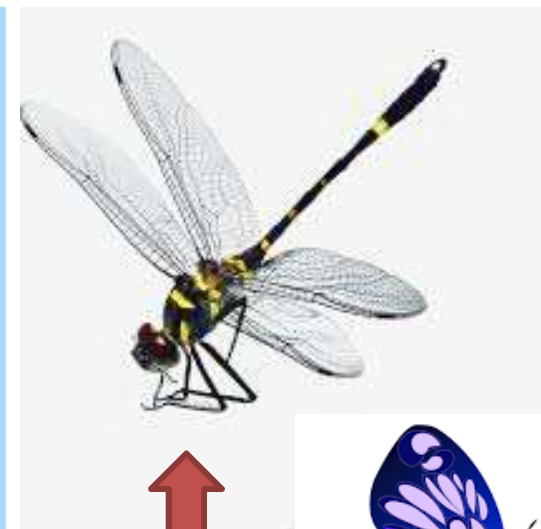
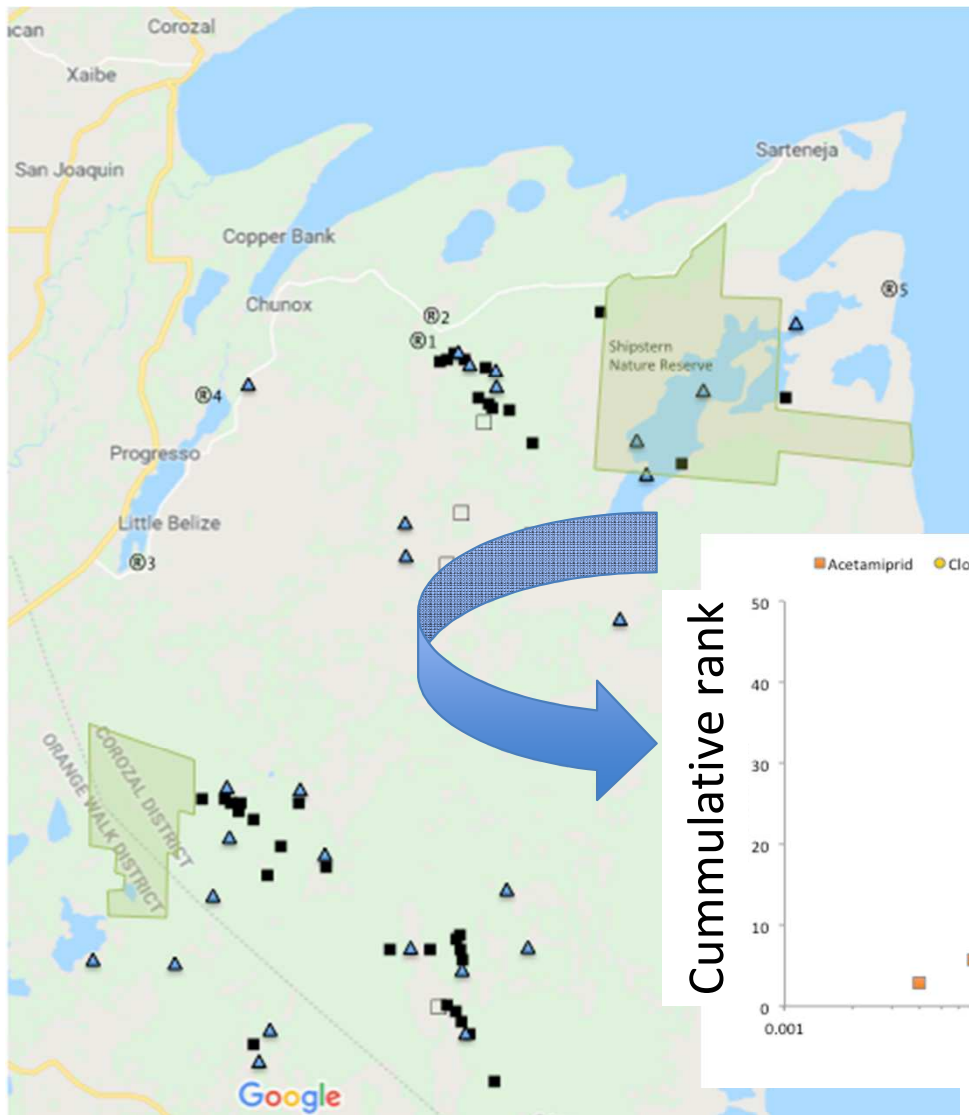
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1 A survey and risk assessment of neonicotinoids in water, soil and sediments of Belize

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18

19 Abstract

20 Usage of neonicotinoids is common in all agricultural regions of the world but data on environmen-
21 tal contamination in tropical regions is scarce. We conducted a survey of five neonicotinoids in soil,
22 water and sediment samples along gradients from crops fields to protected lowland tropical forest,
23 mangroves and wetlands in northern Belize, a region of high biodiversity value. Neonicotinoid fre-
24 quency of detection and concentrations were highest in soil (68%) and lowest in water (12%). Im-
25 idacloprid was the most common residue reaching a maximum of 17.1 ng/g in soil samples. Concen-
26 trations in soils differed among crop types, being highest in melon fields and lowest in banana and
27 sugarcane fields. Residues in soil declined with distance to the planted fields, with clothianidin being
28 detected at 100 m and imidacloprid at more than 10 km from the nearest applied field. About half
29 (47%) of the sediments collected contained residues of at least one compound up to 10 km from the
30 source. Total neonicotinoid concentrations in sediments (range 0.014 to 0.348 ng/g d.w.) were
31 about 10 times lower than in soils from the fields, with imidacloprid being the highest (0.175 ng/g). A
32 probabilistic risk assessment of the residues in the aquatic environment indicates that 31% of sedi-
33 ment samples pose a risk to invertebrate aquatic and benthic organisms by chronic exposure,
34 whereas less than 5% of sediment samples may incur a risk by acute exposure. Current residue levels
35 in water samples do not appear to pose risks to the aquatic fauna. Fugacity modeling of the four
36 main compounds detected suggest that most of the dissipation from the agricultural fields occurs via
37 runoff and leaching through the porous soils of this region. We call for better monitoring of pesticide
38 contamination and invertebrate inventories and finding alternatives to the use of neonicotinoids in
39 agriculture.

40 **Capsule:** Neonicotinoid residues found in wetlands of a Neotropical Nature Reserve pose a risk to
41 aquatic invertebrates

42

43 **Keywords:** systemic insecticides; leaching; dissipation; residues; aquatic risk; tropical agriculture

44

45 **Introduction**

46 Neonicotinoids are the most widely used insecticides in the world (Simon-Delso et al., 2015). Not
47 only are they applied to all kinds of crops in temperate and tropical regions but also used in forestry,
48 plant nurseries, gardens, urban parks and as veterinary products for ectoparasite control in pet ani-
49 mals (Sánchez-Bayo, 2018). Their flexibility of use is due to their systemic properties, which allow
50 them to be applied as direct sprays over the crop as well as soil drenches, granules or most com-
51 monly as seed-coatings (Jeschke et al., 2011). The latter applications, while avoiding spray drift, may
52 however cause the contamination of soil in the applied fields and immediate surroundings (Botías et
53 al., 2016; Long and Krupke, 2016). Because of their long persistence in soil and high solubility in wa-
54 ter, neonicotinoid residues tend to leach through the soil profile (Kurwadkar et al., 2014; Wettstein
55 et al., 2016) and move into groundwater aquifers or are transported in runoff to nearby creeks
56 (Chrétien et al., 2017), lakes and eventually into the marine environment (Smith et al., 2012; Gonza-
57 lez-Rey et al., 2015). Thus, the main dissipation routes of these compounds are through leaching and
58 water runoff, both of which contaminate the water systems (Bonmatin et al., 2015). Due to the high
59 toxicity of neonicotinoids towards aquatic arthropods, this contamination poses a great risk to
60 aquatic ecosystems (Morrissey et al., 2015; Basley and Goulson, 2018) and also to water supplies for
61 either livestock or human consumption.

62 In the past few years there have been many surveys of these ubiquitous chemicals in water systems
63 in North America (Main et al., 2014; Hladik and Kolpin, 2016), Brazil (López-Doval et al., 2017), Japan
64 (Yamamoto et al., 2012) and some European countries (Kreuger et al., 2010; Masiá et al., 2015;
65 Münze et al., 2015), but with the exception of Vietnam (Lamers et al., 2011) and subtropical areas of
66 Australia (Wallace et al., 2016), data on tropical regions is scarce. The overall conclusion of these
67 studies is that there is currently a widespread and constant neonicotinoid contamination of aquatic
68 ecosystems over the world, impacting invertebrate organisms (Morrissey et al., 2015) and, there-
69 fore, impairing the ecosystem services they provide and the food chain they support (Chagnon et al.,
70 2015; Sánchez-Bayo et al., 2016). Reports of neonicotinoid residues in soil show a similar bias to-
71 wards temperate regions (Jones et al., 2014; Schaafsma et al., 2015); studies on tropical soils are
72 even scarcer, with only one recent study in cocoa plantations of Ghana (Dankyi et al., 2018). Since

73 environmental conditions in temperate regions are quite different from those in tropical regions,
74 more data on dissipation and possible contamination by neonicotinoids in the latter regions are nec-
75 essary to evaluate their environmental risks.

76 Belize is located on the eastern side of the Yucatan Peninsula, facing the Caribbean Sea and pos-
77 sessed the World's second longest coral reef barrier. Although it has the lowest population density
78 in Central America, its population growth rate is the second highest in the region, demanding thus
79 more agricultural production. In recent decades Belize has undergone rapid development of agricul-
80 ture by clearance of primary lowland forest and usage of intensive cultivation practices, including
81 the use of neonicotinoid insecticides.

82 The Corozal district selected in this study includes one of the largest protected areas in Central
83 America, namely the Shipstern Nature Reserve. This nature reserve is downstream from agricultural
84 areas and comprises a wetland system of mangrove forests, streams, rivers and lagoons which are
85 home to many protected and endangered animals such as the American wood stork (*Mycteria*
86 *americana*) and the endemic black catbird (*Melanoptila glabrirostris*) among some 300 bird species,
87 and a large diversity of insects: 122 butterflies, 49 hawk-moths, 16 silk-moths and 54 species of
88 dragonflies and damselflies among the most conspicuous. Given that populations of agricultural and
89 migratory bird species have been declining over the last few years in North America and European
90 countries (Donald et al., 2001; Murphy, 2003) it is important to identify possible causes for this trend
91 since there is evidence that neonicotinoids impair the migratory ability of birds (Eng et al., 2017).
92 Neonicotinoid residues have been detected in the blood of long-distance migratory honey buzzards
93 (*Pernis apivorus*) in the U.K. (Byholm et al., 2018) as well as in that of Eurasian eagle-owls (*Bubo bu-*
94 *bo*) in Spain (Taliensky-Chamudis et al., 2017), and appear in the feathers of house sparrows (*Passer*
95 *domesticus*) in Switzerland (Humann-Guilleminot et al., 2019). Because local farmers use modern
96 agricultural practices and routinely apply pesticides to their crops, we hypothesized that environ-
97 mental contamination by agricultural pesticides may be increasing to levels potentially incompatible
98 with long-term conservation of biodiversity in nearby protected areas. Hence, we conducted a sur-
99 vey of neonicotinoids in this area of Belize to assess the risk that these insecticides may pose to the
100 environment in this tropical region.

101 **Materials and Methods**

102 *Chemicals*

103 Analytical standards (99.6 – 99.9 % purity) of five neonicotinoids (acetamiprid, clothianidin, im-
104 idacloprid, thiacloprid and thiamethoxam) were purchased from Sigma-Aldrich (Germany). Isotopi-

105 cally labelled neonicotinoids were purchased from CDN Isotopes (Canada) to be used as internal
106 standards. Chromatography grade solvents (acetonitrile, ethyl-acetate, methanol) were purchased
107 from Fisher Scientific (UK). Magnesium sulfate, sodium chloride, trisodium citrate and sodium hy-
108 drogencitrate sesquihydrate were purchased from Sigma-Aldrich. Isolute PSA and ZeoPrep 90 C18
109 bulk phases were purchased at Biotage (Sweden) and ZeoChem (Switzerland), respectively. Ul-
110 trapure water was obtained from a Milli-Q system (Millipore, France) and solvents (acetonitrile) and
111 additives (formic acid, ammonium formate) for LC-MS were obtained from Biosolve (The Nether-
112 lands).

113 *Study area*

114 The Corozal district lies about 100 km north of Belize city. It is a lowland tropical region (altitude is 2-
115 20 m above sea level) where crops such as sugarcane, banana, other fruits and sorghum are grown
116 in two areas: a large area near the towns of Little Belize and Progreso on the western side of the
117 district, and a smaller area on the south, near the border with the Belize district. The largest agricul-
118 tural area drains into the Dead Lagoon to the west and to the Shipstern Nature Reserve on the
119 north-east, which covers 87 km² of tropical forest and wetlands that flow into the Belize Barrier Reef
120 in the Caribbean Sea (Figure 1). The smaller agricultural area to the south drains into the Freshwater
121 Creek to the west. Soils are alkaline over a pediment of limestone of coral origin. The sampled soils
122 were not formally described but can generally be broadly classified as Cambisols (FAO classification)
123 or Inceptisols (USDA classification).

124 Insert Figure 1

125 *Sampling design*

126 Pesticides applied to the crops in both areas are thought to contaminate the creeks and lagoons in
127 the vicinity and could pose a risk to the nature reserve's wetlands, where many migratory water-
128 birds stopover in their route between North and South America. Our sampling design thus aimed to
129 capture the range of likely exposure of soils and water from the cultivated fields and increasing dis-
130 tance to protected areas.

131 We conducted a survey of neonicotinoid residues in the two agricultural areas of the Corozal district.
132 Samples were collected over a one week period, from 14 to 20 of May 2016, before the onset of the
133 wet season and when most crops are sprayed for pest control. To assess the degree of contamina-
134 tion by these insecticides and their possible impact on a broad range of organisms, samples of soil
135 were collected from six planted crop fields and four fallow fields as well as from a range of habitats
136 at various distances: at 5, 20, 100, 500, 2500 and 10000 m from the fields. Water and sediment

137 samples were collected in a similar fashion at various distances: at 2, 50, 250, 2000, 4500, 6500 and
138 10000 m from the sampled agricultural fields. Complementary samples of water and sediment were
139 also taken at 5 locations within the Dead Lagoon, a system of ponds draining into the Shipstern Na-
140 ture Reserve and at the outflow of this wetland into the sea (Fig. 1), which represent reference sites
141 away from the agricultural area.

142 Each soil sample was a composite of 5 subsamples of 10 cm topsoil, taken from the corners and cen-
143 tre of a square plot (about 4 x 4 m) with a bulb-planter after removing the vegetation and plant ma-
144 terial. Samples were kept in sealed plastic bags and placed in a cooler for transportation to a freezer.

145 At each sampling site, two water samples were taken using 50ml centrifuge tubes. Duplicate sedi-
146 ment samples were taken at the same spots as the water samples, by immersing open 50ml centri-
147 fuge tubes in the water with opening facing down, inserting them into the sediment a few cm and
148 then rotating them slowly to a horizontal position while pushing them forward to fill them with sed-
149 iments. The tubes were sealed, leaving some air inside to avoid breakage during freezing. The tubes
150 were then brought to the surface, left to settle and the excess water, if any, discarded. They were
151 placed in a cooler for transportation and stored in a freezer until analysis.

152 All samples were packed in a cooler and stored at -20°C and shipped to the University of Neuchâtel
153 in Switzerland for residue analysis of neonicotinoids.

154 *Extraction and preparation of samples*

155 Extraction of water samples was performed soon after arrival at the laboratory of the Neuchâtel
156 Platform of Analytical Chemistry (NPAC), University of Neuchâtel, as follows: 5 mL water taken into a
157 15 mL Falcon tube and added 20 µL of internal standard solution containing isotopically labelled
158 standards of neonicotinoids (125 ng/mL in acetonitrile) plus 5 mL of ethyl-acetate. The tube was
159 vigorously shaken by hand and the supernatant was collected in a 13x100 mm glass tube. Three ml
160 of ethyl-acetate were added to the first tube and the procedure repeated. The organic phase was
161 then evaporated under vacuum using a centrifugal evaporator (CentriVap, Labconco, USA) at 35°C.
162 The dried residue was re-suspended in 0.5 mL of ultrapure H₂O:methanol (75:25, v/v), vortexed,
163 ultrasonicated, and the sample was filtered through a 13 mm PTFE filter (0.22 µm porosity, BGB Ana-
164 lytik, Switzerland) into an HPLC vial containing a 250 µL conical insert.

165 Preparation and extraction of soil and sediment samples was performed following a protocol
166 adapted from Mitchell et al. (2017) and Humann-Guillemot et al. (2019). Fresh samples were dried
167 for 2-3 weeks at 40°C in an oven, homogenized, sieved through (0.5 mm mesh) and ground to a fine
168 powder with a mortar and a pestle. One g of powder was then weighed in a 15 mL Falcon tube to

169 which 9 mL of ultrapure H₂O:acetonitrile (50:50, v/v) and 20 µL of internal standard solution (125
170 ng/mL in acetonitrile) were added. The samples were then placed in a vertical rotation shaker at 60
171 rpm overnight. Samples were then centrifuged at 4000 rpm for 5 min and the supernatant was col-
172 lected in a new 15 mL Falcon tube. The pellet was re-extracted with 1 ml of ultrapure
173 H₂O:acetonitrile (50:50, v/v) and both supernatants were combined. Extracts were purified by
174 QuEChERS by adding 2 g of magnesium sulfate, 0.5 g of sodium chloride, 0.5 g of trisodium citrate
175 and 0.25 g of sodium hydrogen citrate sesquihydrate to the tubes. The tubes were vigorously shaken
176 by hand for about 2 min until the salt pellet detached from the tube. After centrifugation (4000 rpm
177 for 5 min), the epiphase (ca. 5 mL of ACN) was collected and put in another 15 ml tube containing
178 150 mg of MgSO₄ and 100 mg of both PSA and C18 bulk phases. The tubes were shaken, centrifuged
179 (4000 rpm for 5 min) and the supernatant collected in a 13 x 100 mm glass tube. The glass tubes
180 were evaporated to dryness under vacuum using a centrifugal evaporator (Centrivap, Labconco). The
181 samples were finally reconstituted in 0.5 mL of ultrapure H₂O:methanol (75:25, v/v), filtered through
182 a 13 mm PTFE filter (BGB Analytik) and transferred in an HPLC vial containing a 250 µL conical insert.

183 *Neonicotinoid analysis by LC-MS/MS*

184 The quantification of five neonicotinoids (imidacloprid, clothianidin, thiamethoxam, thiacloprid, ac-
185 etamiprid) was performed by UHPLC-MS/MS following a protocol described in Humann-Guillemot
186 et al. (2019). Briefly, a UPLCTM system (Waters, USA) coupled to a TQ-S triple quadrupole mass spec-
187 trometer (Waters) was employed and the separation was achieved on an Acquity UPLCTM BEH C18
188 column (50 x 2.1mm i.d., 1.7 µm particle size, Waters). Mobile phase A consisted of H₂O + 0.05%
189 formic acid+5mM NH₄FA and mobile phase B of acetonitrile+0.05% formic acid. The following gradi-
190 ent program was developed using a temperature of 25°C and a flow rate of 0.4 mL/min.: 5-33% B in
191 4.0 min, 33-100% B in 1.0 min, holding at 100% B for 2 min and returning to initial conditions at 5% B
192 for 1.0 min. The injection volume was 5 µL. MS/MS detection was performed in positive electrospray
193 ionization using the multiple reaction monitoring (MRM) mode – see Table S1. The system was con-
194 trolled by Masslynx 4.1 (Waters) and data processing was performed using Quanlynx (Waters). Ne-
195 onicotinoids were quantified by internal calibration using calibration solutions prepared in ultrapure
196 H₂O:methanol (75:25, v/v) at 0.005, 0.05, 0.5, 2, 20 and 50 ng/mL, each containing internal stand-
197 ards at a concentration of 5 ng/mL. Linear regressions weighted by 1/x were applied. Limits of quan-
198 tification, determined as 10 times the signal-to-noise ratio for the different neonicotinoids were in
199 the range 2-20 pg/g for soil and sediment (dry weight) and 0.1-1 pg/ml for water (Table 1). Blank
200 samples (i.e. solvent without matrix submitted to the entire extraction procedure) were injected in
201 the UHPLC-MS/MS system to ensure that no contamination occurred during sample preparation.
202 Process efficiency was determined for each analyte by dividing the average peak area of the internal

203 standard in the samples by that of the internal standard in the calibration solutions (spiked at 5
204 ng/mL). Process efficiency takes into account both recovery and matrix effects and is a better indica-
205 tor of the overall method quality than recovery alone (Matuszewski et al., 2003).

206 Insert Table 1

207 *Data analysis*

208 Soil residues between fallow and planted crop fields were compared by t-test, using residue data
209 within the field and up to 5 m away. As melon, sorghum and banana fields were sampled only once,
210 their data was pooled with that of sugarcane fields to obtain enough data points. Residues of each
211 compound in soil, sediment and water were also compared for different distances away from the
212 field boundary.

213 The evaluation of the possible threats that neonicotinoids may pose to the aquatic environment was
214 done using a probabilistic risk assessment (PRA) method (Solomon et al., 2000). The residue distribu-
215 tion of the individual compounds and their total residual amount in water and sediments was com-
216 pared to the hazard concentration for 5 percent of species (HC5), which constitutes a threshold for
217 protection of the remaining 95% of aquatic species. Based on the species sensitivity distribution for
218 all aquatic species tested with imidacloprid, Morrissey et al. (2015) derived an acute HC5 of 200 ng/L
219 and a chronic HC5 of 35 ng/L. Due to the similarity in toxicity levels among all neonicotinoids, such
220 thresholds can be applied confidently to the other compounds given that their toxicological data are
221 scarce and insufficient. In the absence of regulatory guidelines for neonicotinoid residues in soil,
222 these data could not be assessed here.

223

224 **Results**

225 A total of 40 soil, 34 sediment and 33 water samples were analysed; one water sample was lost dur-
226 ing transportation. Residues of acetamiprid, clothianidin, imidacloprid and thiamethoxam, but not
227 thiacloprid, were found in soil and sediments, with 68 % and 47% samples respectively containing at
228 least one neonicotinoid. Positive soil samples had total residue levels in the range 0.012 to 19.3 ng/g
229 (ppb), whereas sediment samples had lower residue levels in the range 0.014 to 0.348 ng/g. In con-
230 trast, only 12% of water samples were contaminated with at least one neonicotinoid in the range 1
231 to 14 ng/L (ppt). (Table 2). See also Table S2 for individual sample residues.

232 Insert table 2

233 Residues in soil were highest in melon fields, which contained up to 19.3 ng/g of total neonicotinoids
234 (Table 3). Most of the residue was imidacloprid (17.1 ng/g) while the other three compounds con-
235 tributed only 12% of the total load. Sorghum fields contained up to 6.2 ng/g of neonicotinoids, with
236 clothianidin accounting for 75% of the total and the remainder consisting of thiamethoxam and im-
237 idacloprid. Sugarcane fields contained only residues of imidacloprid (1.8 ng/g) while similar levels in
238 banana fields (1.3 ng/g) comprised a mixture of thiamethoxam, clothianidin and imidacloprid. Most
239 clothianidin residues may result from application of this insecticide to the crops, but a certain
240 amount is due to the metabolic degradation of thiamethoxam by microorganisms (Simon-Delso et
241 al., 2015). Fallow fields contained an average of 0.36 ng/g of total neonicotinoids, with levels for the
242 individual compounds similar to those in the planted crops except for imidacloprid, which had resi-
243 dues significantly lower in fallow fields ($p = 0.02$, two-tailed t test).

244 Insert Table 3

245 Acetamiprid and thiamethoxam were only found in the crop fields and its immediate surroundings (5
246 m), whereas small residues of clothianidin (0.013 ng/g) were detected up to 100 m from the nearest
247 treated field and those of imidacloprid (0.028 ± 0.016 ng/g) reached up to 10 km with no discernable
248 decrease in concentration between 100 m and 10 km (Figure 2A). The residue levels of all com-
249 pounds declined with their distance to the source following the typical Gaussian pattern of spray
250 drift (Woods et al., 2001).

251 Insert Figure 2 (A and B)

252 A slightly different pattern of residues with distance from the source was found among sediment
253 samples (Figure 2B). As with soil, acetamiprid, thiamethoxam and clothianidin were found only in
254 the first few metres (3% to 6% of samples), but imidacloprid was present in 44% of samples at con-
255 centrations in the range 0.017 – 0.175 ng/g and distributed all along the aquatic systems up to 10 km
256 away from the agricultural fields. Sediments from the five reference sites contained also residues of
257 the four neonicotinoids even at the outflow of the Shipstern wetland, where acetamiprid was de-
258 tected (Table 4). These results indicate that the dispersion of neonicotinoid residues is widespread
259 and reaches pristine places far from the sources.

260 Insert Table 4

261 Only acetamiprid, imidacloprid and thiacloprid, were detected in 12% of water samples, with no
262 discernible pattern of distribution with distance from the fields. The highest levels corresponded to
263 imidacloprid (8-14 ng/L), followed by one sample that contained thiacloprid at 3 ng/L and 2 samples
264 that had acetamiprid at 1 and 4 ng/L.

265 Our data indicate that the total neonicotinoid load in sediment samples exceeded the chronic
266 threshold for protection of aquatic species in 31% of cases, whereas only 3.5% of them exceed the
267 acute threshold. As for individual compounds, only imidacloprid exceeded the thresholds in 29% and
268 3% of sediment samples respectively. By contrast, none of the water samples reached the protective
269 thresholds (Figures 3A and 3B).

270 Insert Figure 3 (A and B)

271 Discussion

272 This survey provides new evidence that confirms the widespread contamination by neonicotinoids
273 over the world already pointed out by various authors (Hladik et al., 2014; Giorio et al., 2017; Mitch-
274 ell et al., 2017), which has reached even remote tropical regions like Belize and high value protected
275 areas such as Shipstern Nature Reserve. Most of the contamination of soil in Belize appears to be
276 from the use of neonicotinoids in agricultural crops, as demonstrated by the decreasing residue lev-
277 els observed with distance from the crops (Figure 2).

278 Imidacloprid is used on the main crops such as sugarcane to combat the sugarcane borer (*Diatraea*
279 *saccharalis*, Lepidoptera), a pest species native to Central America that is currently controlled only
280 with systemic insecticides. Less clear is the use of imidacloprid in sorghum crops and in banana plan-
281 tations, which are not subject to significant insect damage but rather to fungal diseases. The highest
282 residues of imidacloprid (17.1 ng/g), however, were found in soils planted with melons, together
283 with residues of other neonicotinoids that are probably applied as sprays. As expected, residue lev-
284 els in soils of fallow fields were lower by an order of magnitude on average (Table 3). This is probably
285 due to losses by runoff and leaching, which account for most of the dissipation of these highly solu-
286 ble compounds (Kurwadkar et al., 2014), since half-lives in soils of tropical regions are about 155
287 days (Dankyi et al., 2018), implying that residues remain in the soil for longer than a year.

288 The presence of neonicotinoids, in particular imidacloprid, in almost half of the sediments collected
289 in the Corozal district suggests a constant movement of residues from the agricultural areas to very
290 distant regions, as illustrated by the fact that even 10 km from the source concentrations did not
291 show any declining trend. This is understandable, since the high water solubility of all these com-
292 pounds makes them prone to transport to adjacent and distant areas through leaching and surface
293 runoff and ultimately find their way into creeks and lagoons. The Corozal district receives annually
294 about 1,200 mm of rainfall, most of it during the wet season that starts late in May and washes off
295 much of the pesticides applied to the crops in the previous month. On the other hand, some im-
296 idacloprid residues found in the sediments of the Dead Lagoon could have their origin in urban run-

297 off from the adjacent towns, since this insecticide is also used for urban pest control and as a flea
298 treatment of pet animals (Stanneck et al., 2012).

299 On average, residue levels of all neonicotinoids in sediments (geometric mean 0.054 ng/g) were
300 about half of the residues found in soils of the crops and their immediate surroundings (geometric
301 mean 0.128 ng/g). Obviously, as the residues spread out over large areas their concentrations de-
302 crease. In addition, a proportion of residues are likely to degrade during their transport in water.
303 Indeed, the combination of alkaline water and intense tropical sunlight favour photolytic degrada-
304 tion of imidacloprid and clothianidin (Lu et al., 2015). In temperate countries, where most surface
305 waters tend to be neutral or even acidic and sunlight is less intense, hydrolysis and photolytic degra-
306 dation of neonicotinoids are reduced. In this regard, it is noteworthy that the only neonicotinoid
307 found in sediments of the Shipstern outflow was acetamiprid, probably because this compound is
308 very stable in water (half-life 420 days) and less amenable to photolysis (half-life 34 days) than the
309 other compounds in the same class (Bonmatin et al., 2015).

310 The scarce and low residue levels found in waters of Belize contrast with those found in temperate
311 regions of America, Europe, Japan and Australia (Hladik and Calhoun, 2012; Yamamoto et al., 2012;
312 Sánchez-Bayo and Hyne, 2014; Ccancapa et al., 2016; Hladik et al., 2018). Two factors may account
313 for this lower waterborne residue levels in this tropical country. First, the intense seasonal rainfall
314 that washes off residues from the soil leads to a large dilution in the nearby creeks, which is further
315 exacerbated when the waterborne residues move into the numerous temporary ponds and lagoons
316 found in the district. Second, the limestone pediment beneath the porous soils allows considerable
317 leaching at any time during the year but particularly during the wet season. This implies that a large
318 amount of residues are dissolved in water within the soil profile, and kept underground until they
319 are released later into the outside environment. Fugacity modeling (level 3) of the four main neon-
320 icotinoids applied under such tropical conditions shows that a large proportion of thiamethoxam and
321 imidacloprid (about 40%) may move down the soil profile, whereas clothianidin and acetamiprid
322 may dissipate more readily in runoff (about 80%) (Figure 4). The dissipation of these insecticides is,
323 therefore, not by degradation but mainly by percolation into the ground or by movement away in
324 the runoff. Whether one way or the other, most of the chemical applied to the crops ends up con-
325 taminating the surrounding environment, whereas the proportion taken up by the plants or degrad-
326 ed is very small.

327 Insert Figure 4

328 Whatever residues may have dissipated with the runoff they would be partitioned into the water
329 phase and the particulate matter associated with the field soil erosion. Eventually, the particulate

330 would drop to the bottom of the creeks and lagoons, leaving only the dissolved residues in the clean
331 waters of this tropical region. Thus, it is not surprising that the water samples collected show con-
332 tamination below the acute and chronic thresholds that are recommended for the protection of
333 aquatic organisms, particularly invertebrates (Figure 3B). However, the residues in the bottom sedi-
334 ments would remain there for a longer time, probably accumulating year after year since the anoxic
335 conditions typically found at shallow depth in sediments would hamper the degradation processes
336 of these persistent compounds. In our survey we found that a significant proportion of sediment
337 samples (31%) pose a risk to more than 5% of the aquatic organisms by chronic exposure to toxic
338 residues. Since insect larvae of mayflies, stoneflies, caddisflies and chironomids as well as freshwater
339 amphipods are particularly sensitive to neonicotinoids (Roessink et al., 2013; van Dijk et al., 2013;
340 Cavallaro et al., 2017), the populations of all these taxa are currently being impacted across the re-
341 gion, likely resulting in population and biodiversity declines. In the Netherlands, the decline of these
342 aquatic invertebrates has caused a decline in many bird species that feed on them (Hallmann et al.,
343 2014). Thus, we can expect that the concentrations of neonicotinoids we measured in Belizean
344 waterways are also having an impact on vertebrates, such as frogs, lizards, bats and birds, not only in
345 the treated agricultural areas but also in protected areas nearby, including the Shipstern Nature
346 Reserve as well as other national parks in the region. Beyond this impact on population and biodi-
347 versity of invertebrate and vertebrate species, such cascade effects may also have a negative impact
348 on essential ecosystem services such as litter decomposition and nutrient cycling in aquatic envi-
349 ronments (Kreutzweiser et al., 2008; Englert et al., 2017).

350 This is the first survey of neonicotinoids in Belizean soils, sediments and water. The frequency of
351 their occurrence and the concentrations we measured reveal a substantial contamination, which is
352 likely impacting on wildlife species that depend on invertebrate food. In the absence of regular mon-
353 itoring of invertebrate populations in Belize, we cannot determine the magnitude of the neonicoti-
354 noids' impact on its ecosystems, but pollution by pesticides, and insecticides in particular, has been
355 shown to be the second major factor in insect declines worldwide (Sánchez-Bayo and Wyckhuys,
356 2019). There is good evidence that neonicotinoids cause such declines among aquatic insects, as
357 demonstrated by mesocosm studies (e.g. Pestana et al., 2009; Rico et al., 2018) and epidemiological
358 studies of dragonflies (Nakanishi et al., 2018). A recent study showed that insect populations de-
359 clined more than 75% in 27 years in protected areas of Germany, which the authors suggest was
360 mostly due to pesticides in the surrounding areas – in the absence of usage data – whereas climate
361 change and other factors had little or no bearing on the declines (Hallmann et al., 2017). In contrast,
362 a similar study from Puerto Rico demonstrates that climate warming is the main driver of major in-
363 sect taxa declines in tropical forests, where pesticides are not sprayed or their use has even de-

364 creased by 80% in recent years (Lister and Garcia, 2018). However, the latter authors probably did
365 not take into account the replacement of spray-insecticides (e.g. organophosphates, pyrethroids,
366 etc.) with seed-coated systemics, since the latter are usually not accounted for as insecticides.
367 Whatever the case, insect declines can be due to different drivers depending on the geographical
368 region because of the differential tolerance of insects to temperature changes: insects of tropical
369 regions may decrease in numbers due to their narrow tolerance to temperature, whereas those of
370 cooler regions have a broader thermal tolerance and warming can even enhance their fitness
371 (Deutsch et al., 2008). Consequently, while pesticides may be the dominant driver of the declines in
372 temperate latitudes, global warming may be more important in tropical regions.

373 Terrestrial and aquatic biodiversity is higher in tropical regions than at higher latitudes, and Belize
374 stands out as a country where human impact was until recently lower than more densely populated
375 countries in the Mesoamerican region. Agriculture intensification driven by increased human popu-
376 lation density and current agricultural practices have resulted in massive increases in pesticide use.
377 The available evidence on impact of neonicotinoids on wildlife and ecosystems from better studied
378 regions and the results of this survey should suffice to serve as a warning sign that we should not be
379 ignored (Dudley et al., 2017). We therefore encourage farmers and policy makers to consider alter-
380 natives to the prophylactic use of neonicotinoid pesticides and raising to the challenge of developing
381 a truly sustainable agriculture (Pretty and Bharucha, 2014).

382 **Contributions**

383 **J-MB** and **MBvL** designed the study and contributed to write the paper; **DN** designed and carried out
384 the sampling; **HM** organized the field operations; **EM** and **OSS** contributed to write the paper; **GG**
385 analysed the samples; **FS-B** wrote the draft and conducted the risk assessment.

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391 analyses.

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Table 1. Performance of the method, with process efficiencies and limits of quantitation (LOQ) of the target neonicotinoids in soil and water matrices. See Table S1 for further details on calibration equations and chromatograms.

Analyte	Soil / sediments		Water	
	Process efficiency	LOQ (pg/g)	Process efficiency	LOQ (pg/ml)
Acetamiprid	62%	2	94%	0.1
Clothianidin	55%	20	92%	1.0
Imidacloprid	74%	15	96%	0.5
Thiacloprid	60%	2	91%	0.1
Thiamethoxam	66%	5	82%	0.2

Table 2. Summary of residues of neonicotinoids in soil, sediment and water samples.

Compound	Soil (ng/g d.w.) (n=40)			Sediment (ng/g d.w.) (n=34)			Water (ng/L) (n= 33)		
	Detections (%)	Average	Max	Detections (%)	Average	Max	Detections (%)	Average	Max
Acetamiprid	3 (7.5)	0.136	0.377	3 (8.8)	0.008	0.008	2 (6.1)	0.0025	0.004
Clothianidin	9 (22.5)	0.886	4.691	1 (2.9)	0.053	0.053	0 (-)	nd	nd
Imidacloprid	26 (65.0)	1.056	17.122	15 (44.1)	0.068	0.175	2 (6.1)	0.011	0.014
Thiacloprid	0 (-)	nd	nd	0 (-)	nd	nd	1 (3.0)	0.003	0.003
Thiamethoxam	7 (17.5)	0.430	1.497	1 (2.9)	0.116	0.116	0 (-)	nd	nd
All compounds	27 (67.5)	0.971	19.312	16 (47.1)	0.036	0.348	4 (12.1)	0.001	0.014

nd = not detected

Table 3. Residues of neonicotinoids in soil (mean \pm SD, ng/g d.w.) of planted and fallow fields.

Compounds	LOQ	Melon	Sorghum	Banana	Sugarcane	All crops	Fallow field
Acetamiprid	0.002	0.377	<LOQ	<LOQ	<LOQ	0.019 \pm 0.042	0.004 \pm 0.011
Clothianidin	0.020	1.584	4.691	0.239	<LOQ	0.326 \pm 0.728	0.199 \pm 0.355
Imidacloprid	0.015	17.122	0.022	0.026	1.802 \pm 2.437	2.653* \pm 2.415	0.118* \pm 0.213
Thiamethoxam	0.002	0.230	1.497	1.038	<LOQ	0.138 \pm 0.309	0.035 \pm 0.059
Total		19.312	6.210	1.303	1.802	3.135	0.357

LOQ = limit of quantitation

*p = 0.02 (t-test)

Table 4. Residues of neonicotinoids in sediments (ng/g d.w.) of reference sites – see Figure 1.

Location	Description	Acetamiprid	Clothianidin	Imidacloprid	Thiamethoxam
Ref1	Inlet of pond	<LOQ	<LOQ	0.033	<LOQ
Ref2	Outlet of pond	<LOQ	<LOQ	0.046	<LOQ
Ref3	Dead lagoon inlet	0.004	0.053	0.175	0.116
Ref4	Dead lagoon outlet	<LOQ	<LOQ	0.035	<LOQ
Ref5	Outflow Shipstern wetland	0.014	<LOQ	<LOQ	<LOQ

List of Figures

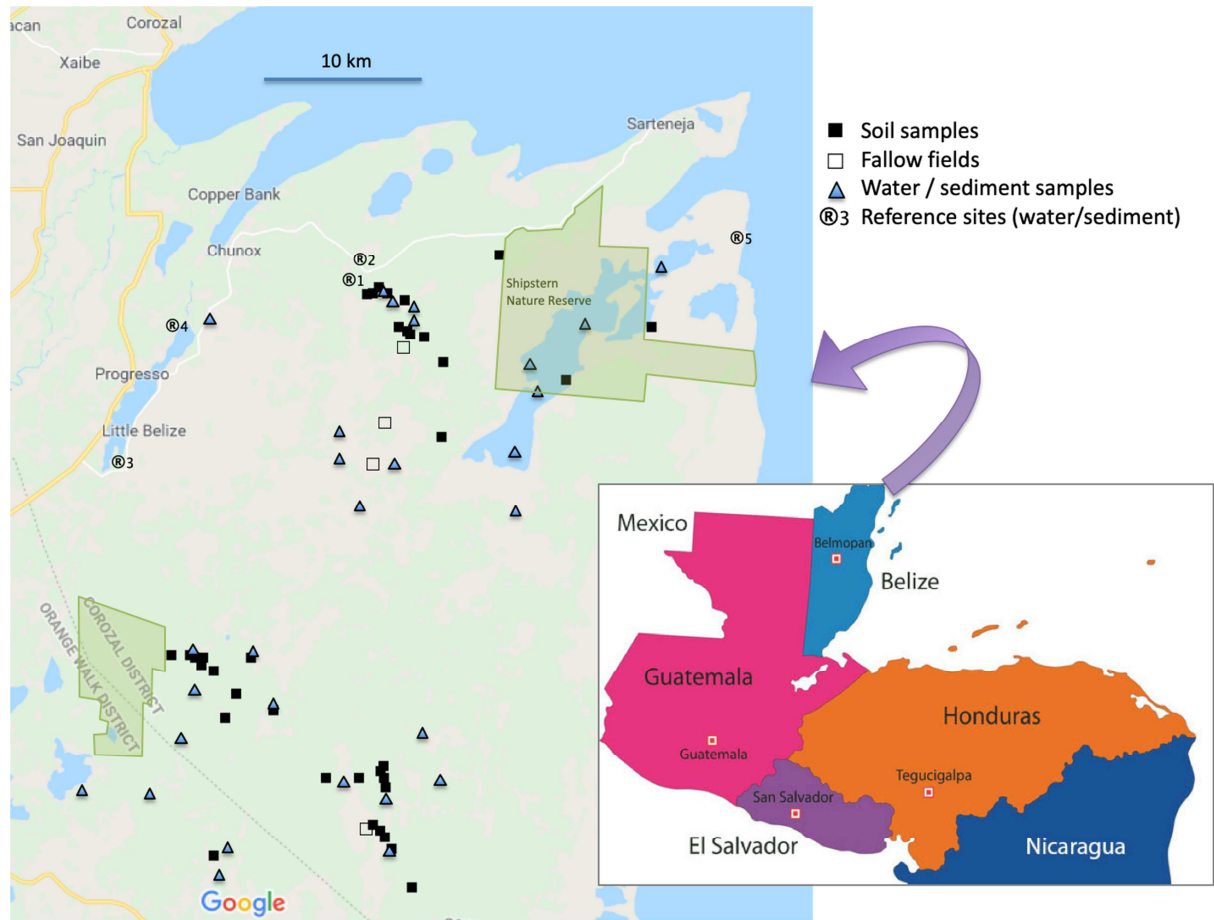
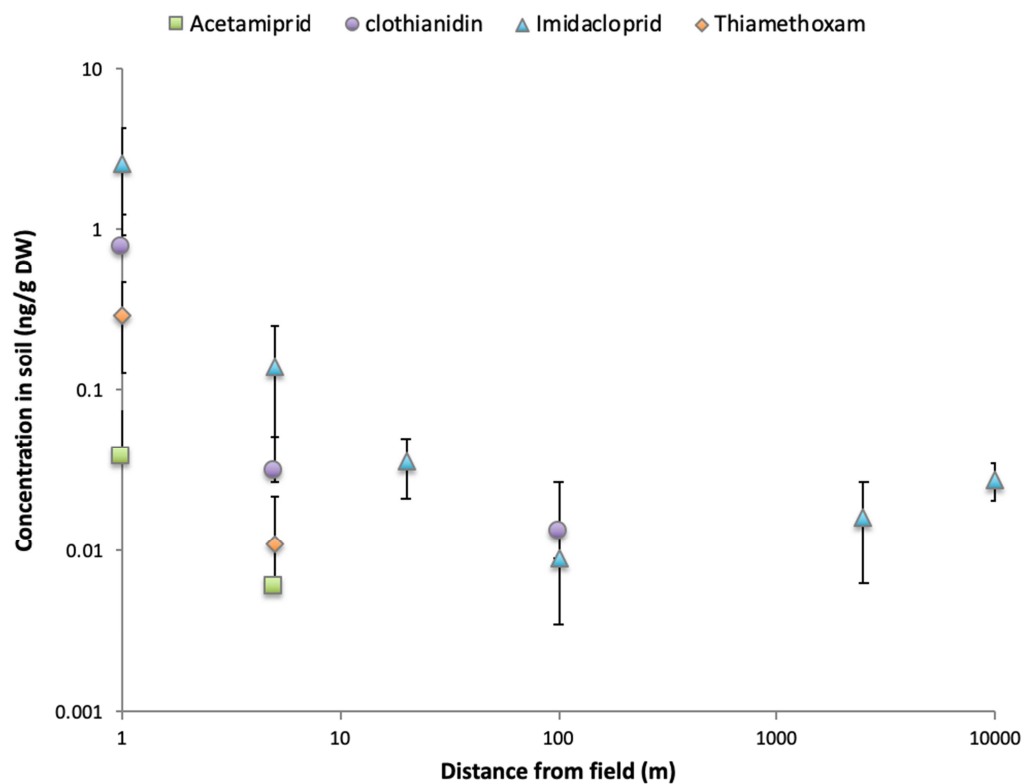


Figure 1. Map of northern Belize indicating the location of the soil, water and sediment samples taken for the study.

A



B

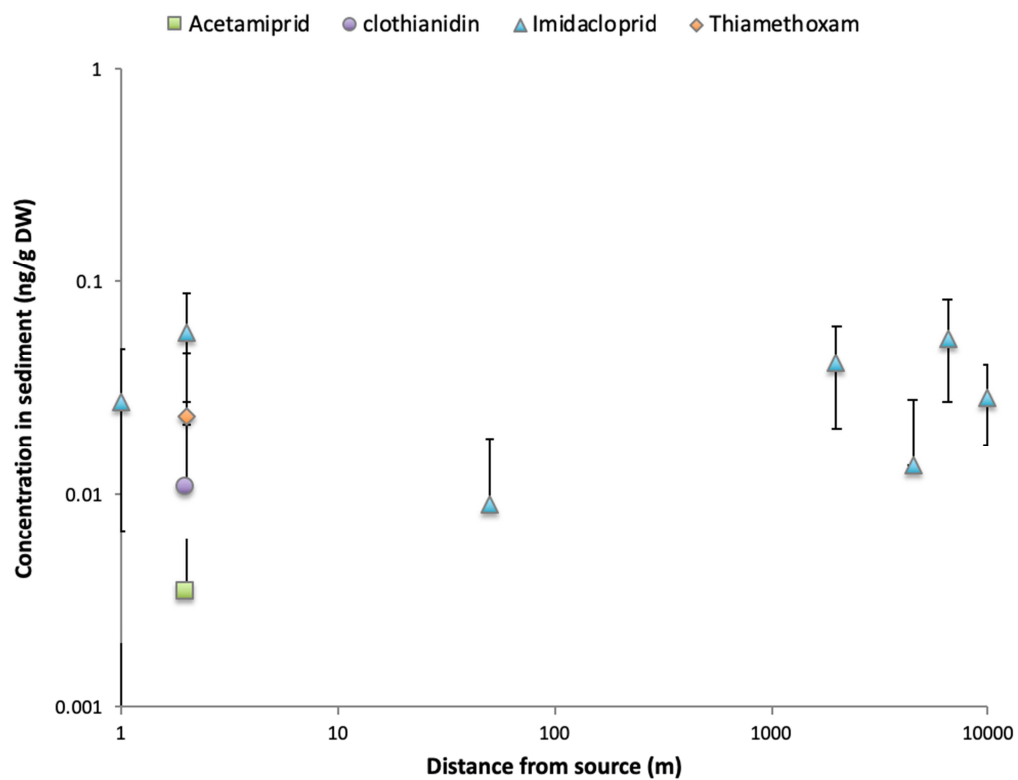
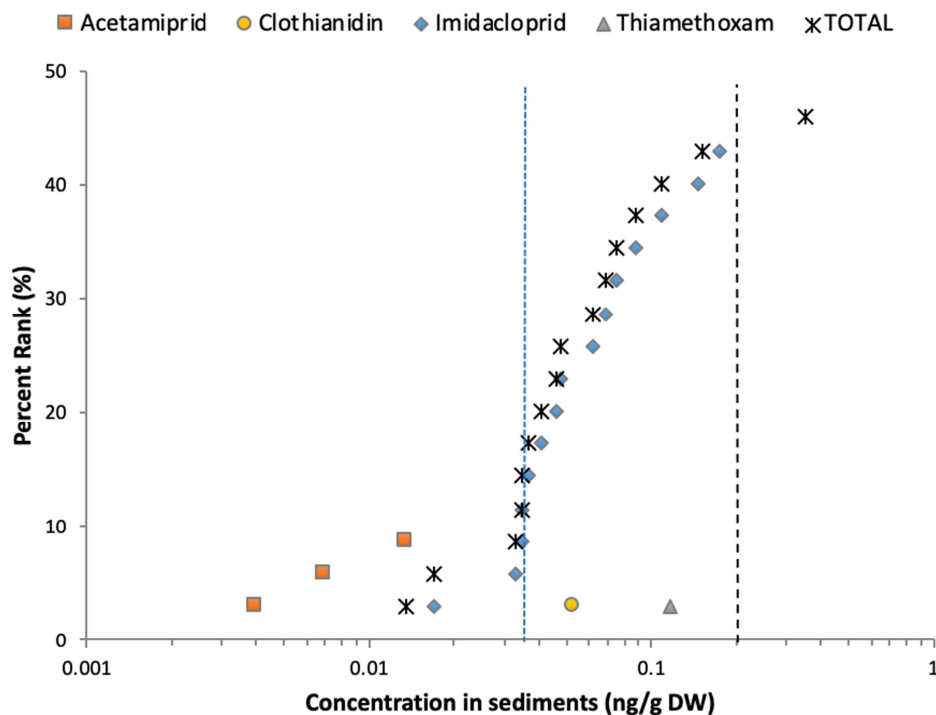


Figure 2. Residues of neonicotinoids in soil (A) and sediment (B) with distance to the planted fields.

A



B

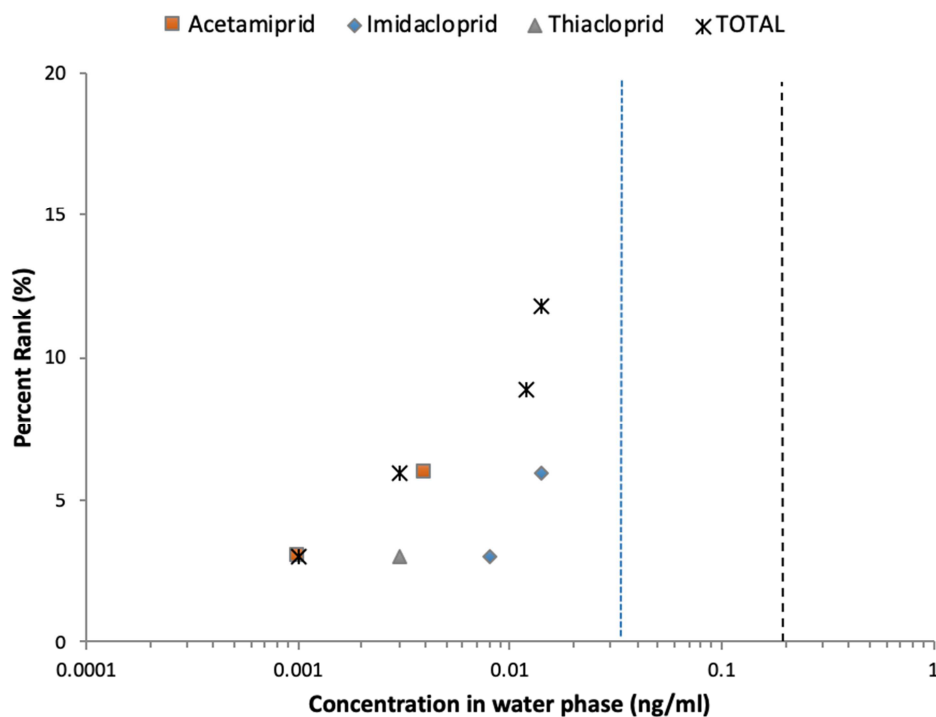


Figure 3. Residues of neonicotinoids in sediments (A) and water phase (B) of creeks, ponds and wetlands of Belize. The dotted blue line marks the chronic threshold for protecting 95% of aquatic species (35 ng/L) and the dashed black line the acute threshold (200 ng/L) for the same species.

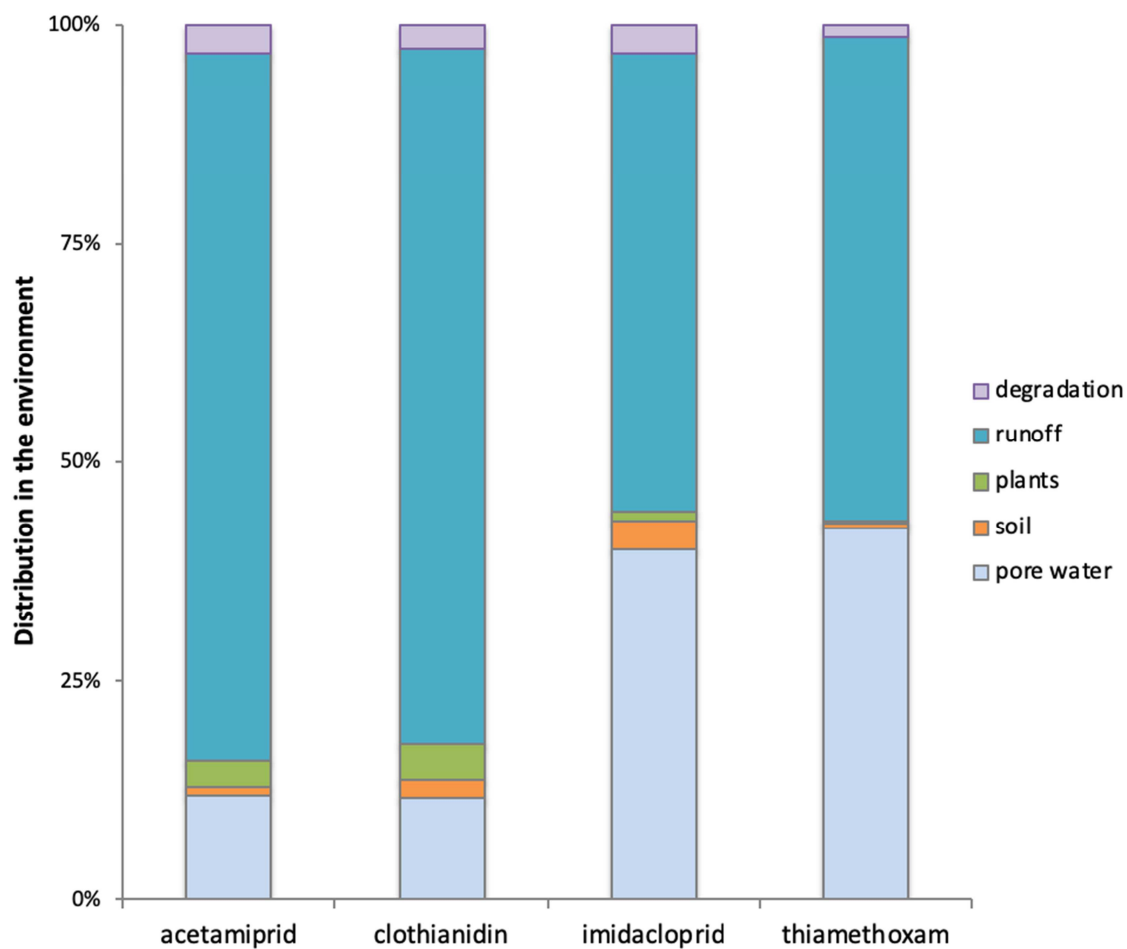


Figure 4. Distribution of four neonicotinoids applied to crops in Belize as determined by Fugacity model III.

A survey and risk assessment of neonicotinoids in Belize**Highlights**

- We measured neonicotinoids in soil, sediment and water in northern Belize
- Neonicotinoid residue levels were highest in soils of planted crops
- Frequency of residues declined from soil (68%) to sediment (47%) and water (12%)
- Concentrations in soil and sediment decreased with distance to field
- 31% of sediment samples may pose a risk to aquatic invertebrates by chronic exposure