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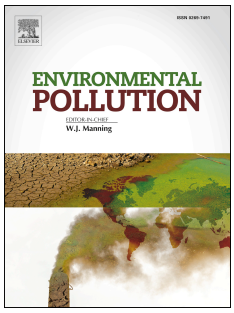

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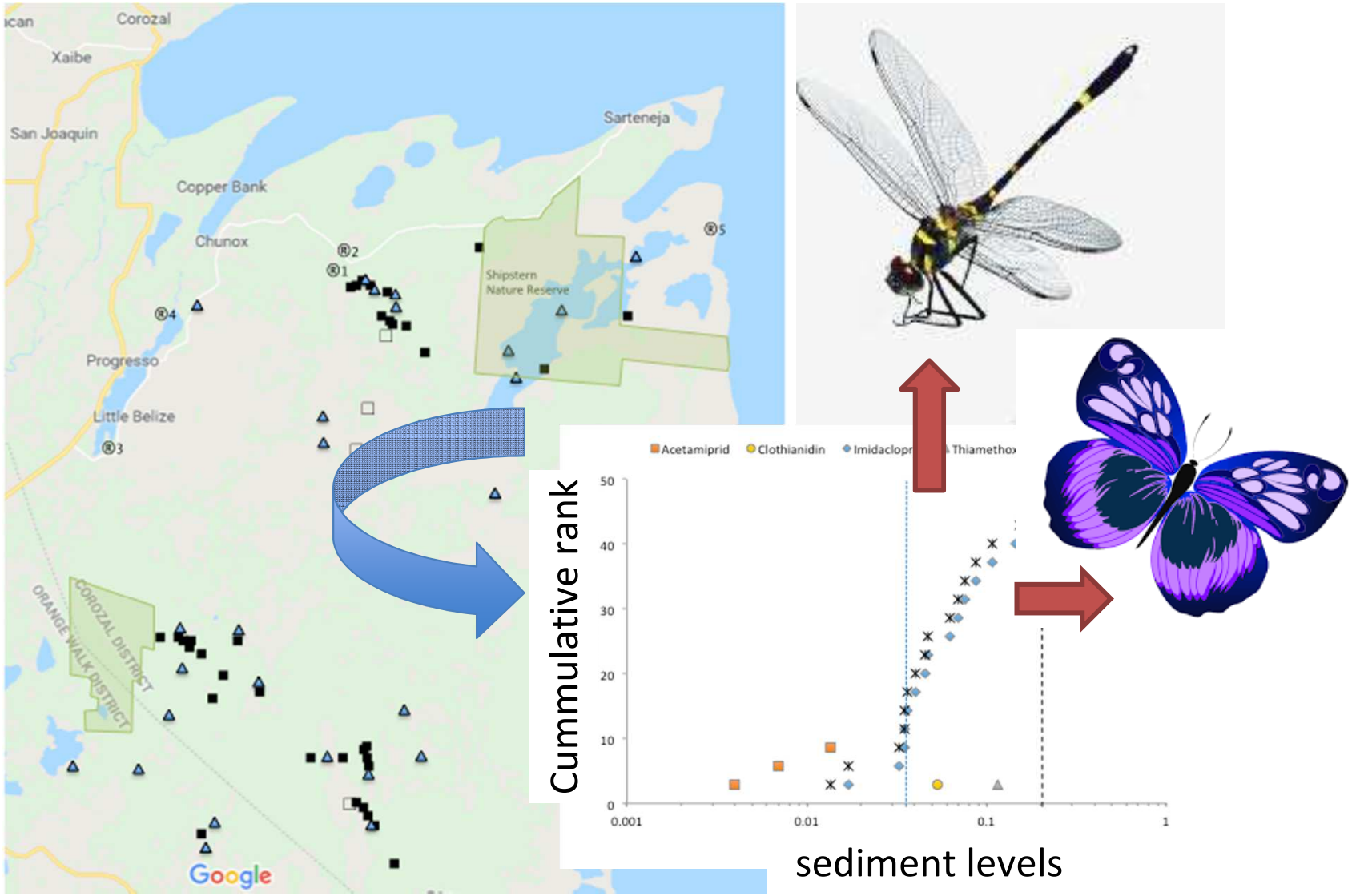
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Chunox
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Progresso
Little Belize
COROZAL DISTRICT
ORANGE WALK DISTRICT
Google
Acetamiprid
Clothianidin
Imidaclopr
Thiamethox
Cummulative rank
50
40
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20
10
0
0.001
0.01
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1
sediment levels

ACCEPTED MANUSCRIPT

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

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Abstract

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

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

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

Introduction

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

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

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

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

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

Materials and Methods

Chemicals

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

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

Study area

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

Insert Figure 1

Sampling design

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

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

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

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

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

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

Extraction and preparation of samples

Extraction of water samples was performed soon after arrival at the laboratory of the Neuchâtel Platform of Analytical Chemistry (NPAC), University of Neuchâtel, as follows: 5 mL water taken into a 15 mL Falcon tube and added 20 µL of internal standard solution containing isotopically labelled standards of neonicotinoids (125 ng/mL in acetonitrile) plus 5 mL of ethyl-acetate. The tube was vigorously shaken by hand and the supernatant was collected in a 13x100 mm glass tube. Three ml of ethyl-acetate were added to the first tube and the procedure repeated. The organic phase was then evaporated under vacuum using a centrifugal evaporator (CentriVap, Labconco, USA) at 35°C. The dried residue was re-suspended in 0.5 mL of ultrapure H_2O:methanol (75:25, v/v), vortexed, ultrasonicated, and the sample was filtered through a 13 mm PTFE filter (0.22 µm porosity, BGB Analytik, Switzerland) into an HPLC vial containing a 250 µL conical insert.

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

which 9 mL of ultrapure H_2O:acetonitrile (50:50, v/v) and 20 µL of internal standard solution (125 ng/mL in acetonitrile) were added. The samples were then placed in a vertical rotation shaker at 60 rpm overnight. Samples were then centrifuged at 4000 rpm for 5 min and the supernatant was collected in a new 15 mL Falcon tube. The pellet was re-extracted with 1 ml of ultrapure H_2O:acetonitrile (50:50, v/v) and both supernatants were combined. Extracts were purified by QuEChERS by adding 2 g of magnesium sulfate, 0.5 g of sodium chloride, 0.5 g of trisodium citrate and 0.25 g of sodium hydrogen citrate sesquihydrate to the tubes. The tubes were vigorously shaken by hand for about 2 min until the salt pellet detached from the tube. After centrifugation (4000 rpm for 5 min), the epiphase (ca. 5 mL of ACN) was collected and put in another 15 ml tube containing 150 mg of $MgSO_4$ and 100 mg of both PSA and C18 bulk phases. The tubes were shaken, centrifuged (4000 rpm for 5 min) and the supernatant collected in a 13 x 100 mm glass tube. The glass tubes were evaporated to dryness under vacuum using a centrifugal evaporator (Centrivap, Labconco). The samples were finally reconstituted in 0.5 mL of ultrapure H_2O:methanol (75:25, v/v), filtered through a 13 mm PTFE filter (BGB Analytik) and transferred in an HPLC vial containing a 250 µL conical insert.

Neonicotinoid analysis by LC-MS/MS

The quantification of five neonicotinoids (imidacloprid, clothianidin, thiamethoxam, thiacloprid, acetamiprid) was performed by UHPLC-MS/MS following a protocol described in Humann-Guilleminot et al. (2019). Briefly, a UPLC™ system (Waters, USA) coupled to a TQ-S triple quadrupole mass spectrometer (Waters) was employed and the separation was achieved on an Acquity UPLC™ BEH C18 column (50 x 2.1mm i.d., 1.7 µm particle size, Waters). Mobile phase A consisted of H_2O + 0.05% formic acid+5mM NH_4FA and mobile phase B of acetonitrile+0.05% formic acid. The following gradient program was developed using a temperature of 25°C and a flow rate of 0.4 mL/min.: 5-33% B in 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 for 1.0 min. The injection volume was 5 µL. MS/MS detection was performed in positive electrospray ionization using the multiple reaction monitoring (MRM) mode – see Table S1. The system was controlled by Masslynx 4.1 (Waters) and data processing was performed using Quanlynx (Waters). Neonicotinoids were quantified by internal calibration using calibration solutions prepared in ultrapure H_2O:methanol (75:25, v/v) at 0.005, 0.05, 0.5, 2, 20 and 50 ng/mL, each containing internal standards at a concentration of 5 ng/mL. Linear regressions weighted by 1/x were applied. Limits of quantification, determined as 10 times the signal-to-noise ratio for the different neonicotinoids were in the range 2-20 pg/g for soil and sediment (dry weight) and 0.1-1 pg/ml for water (Table 1). Blank samples (i.e. solvent without matrix submitted to the entire extraction procedure) were injected in the UHPLC-MS/MS system to ensure that no contamination occurred during sample preparation. Process efficiency was determined for each analyte by dividing the average peak area of the internal

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

Insert Table 1

Data analysis

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

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

Results

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

Insert table 2

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

Insert Table 3

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

Insert Figure 2 (A and B)

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

Insert Table 4

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

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

Insert Figure 3 (A and B)

Discussion

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

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

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

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

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

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

Insert Figure 4

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

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

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

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

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

Contributions

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

Acknowledgements

The authors would like to thank the International Tropical Conservation Fund (ITCF - Netherlands), Stichting Triodos Foundation (The Netherlands), Umwelt Stiftung Greenpeace (Germany), and the M.A.O.C. Gravin van Bylandt Stichting (The Netherlands), for their funding to carry out this survey. We are grateful to Amandine Pillonel for her invaluable help with sample preparation and laboratory 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

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Table 3. Residues of neonicotinoids in soil (mean ± 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 ± 0.042	0.004 ± 0.011
Clothianidin	0.020	1.584	4.691	0.239	<LOQ	0.326 ± 0.728	0.199 ± 0.355
Imidacloprid	0.015	17.122	0.022	0.026	1.802 ± 2.437	2.653* ± 2.415	0.118* ± 0.213
Thiamethoxam	0.002	0.230	1.497	1.038	<LOQ	0.138 ± 0.309	0.035 ± 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

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List of Figures

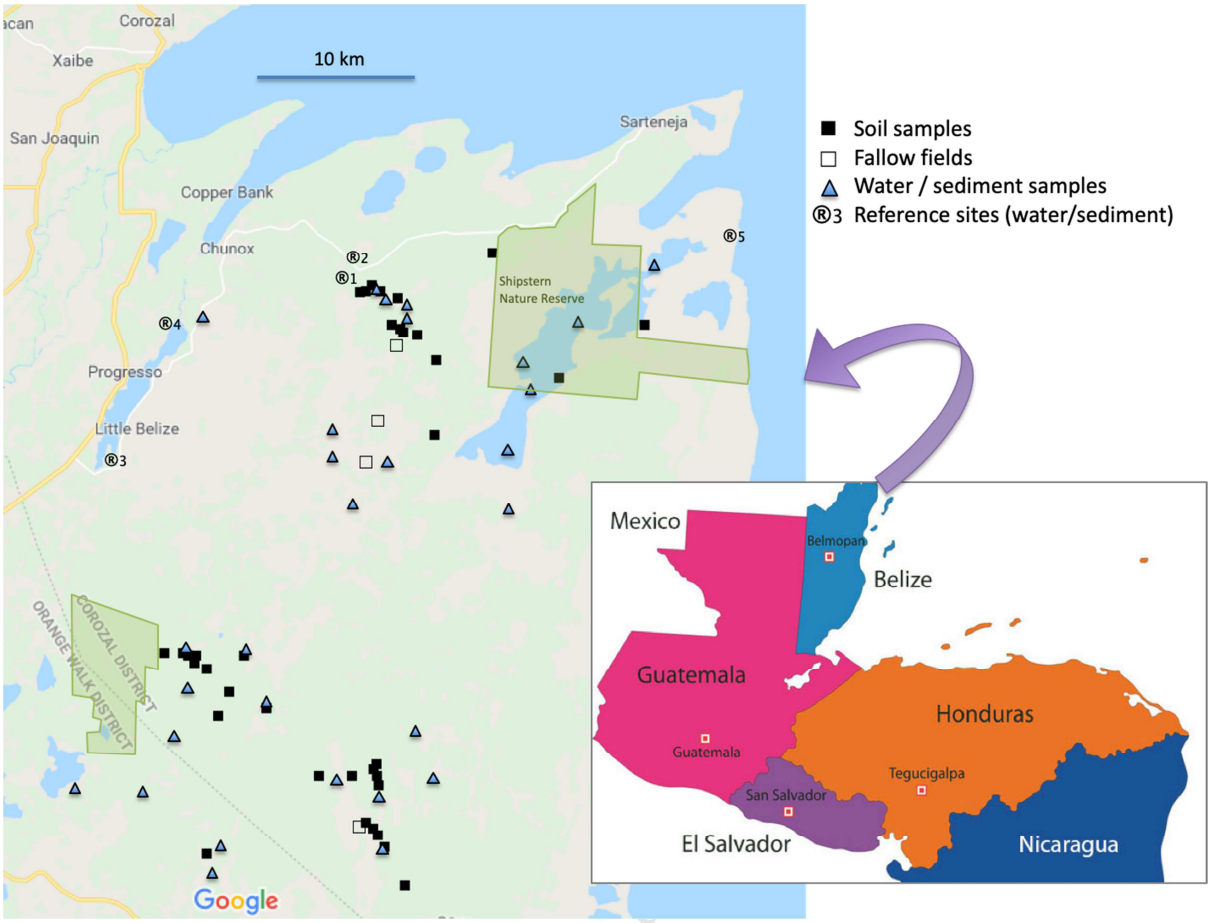

Figure 1. Map of northern Belize indicating the location of the soil, water and sediment samples taken for the study.

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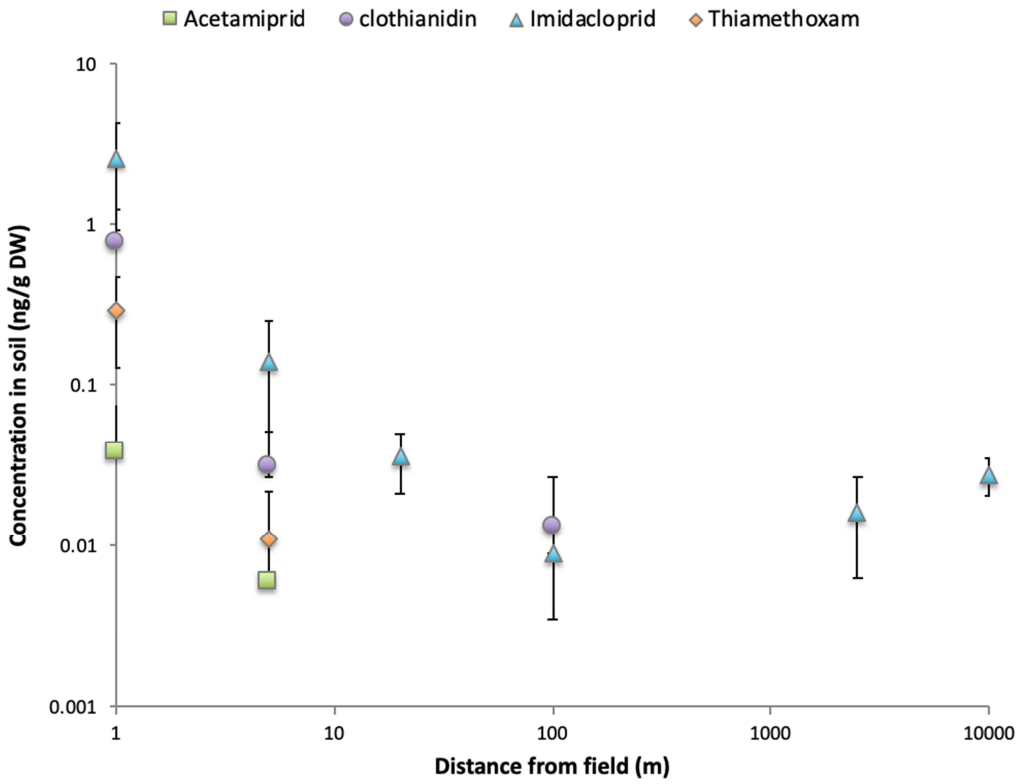
A



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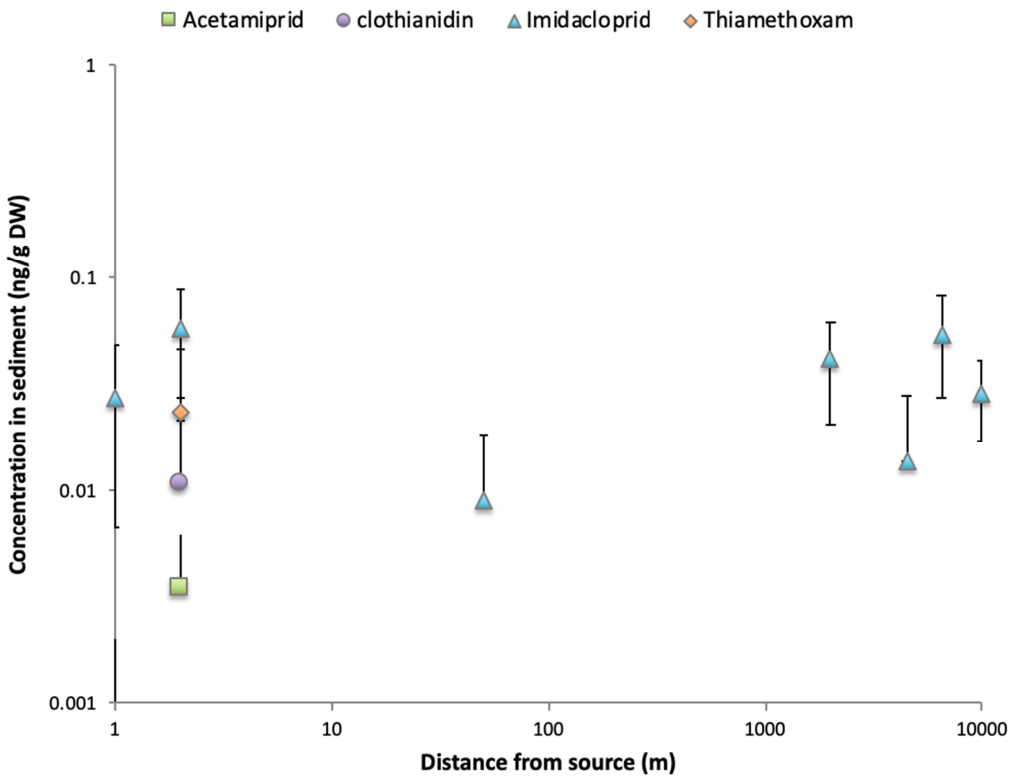
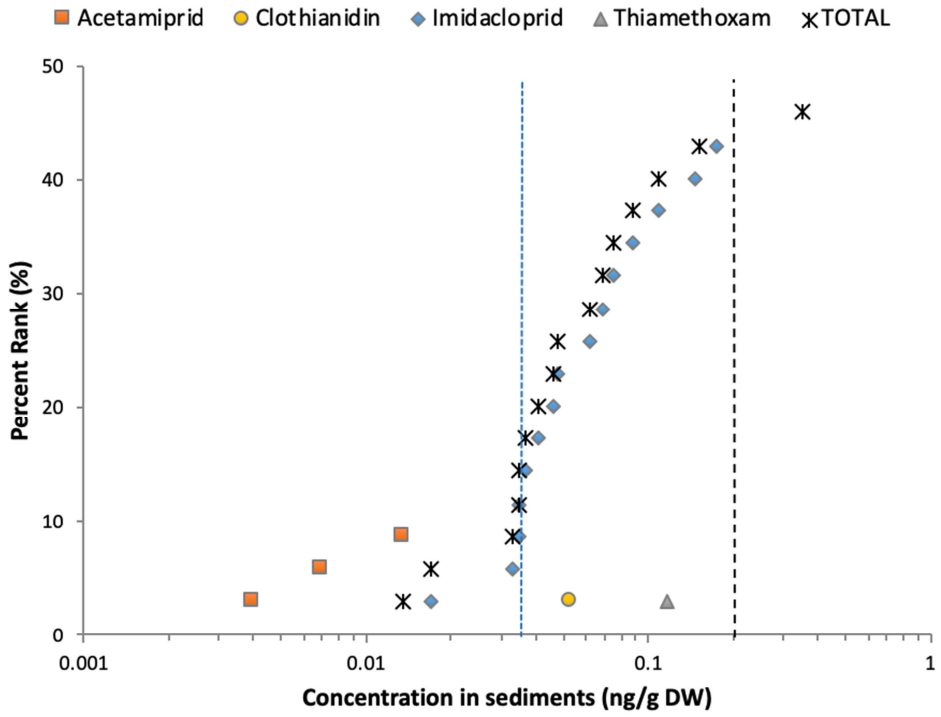


Figure 2. Residues of neonicotinoids in soil (A) and sediment (B) with distance to the planted fields.

A



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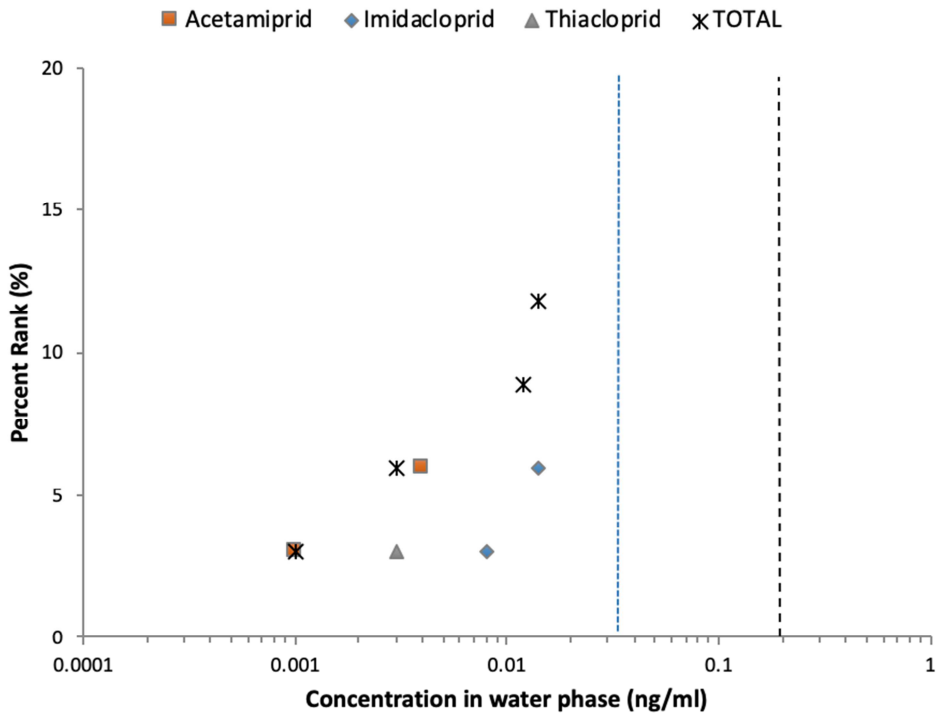

Figure 3. Residues of neonicotinoids is sediments (A) and water phase (B) of creeks, ponds and wetlands of Belize. The dotted blue line marks the chronic threshold for protecting 95% of aquatic species (35 ng/L) and the stippled black line the acute threshold (200 ng/L) for the same species.

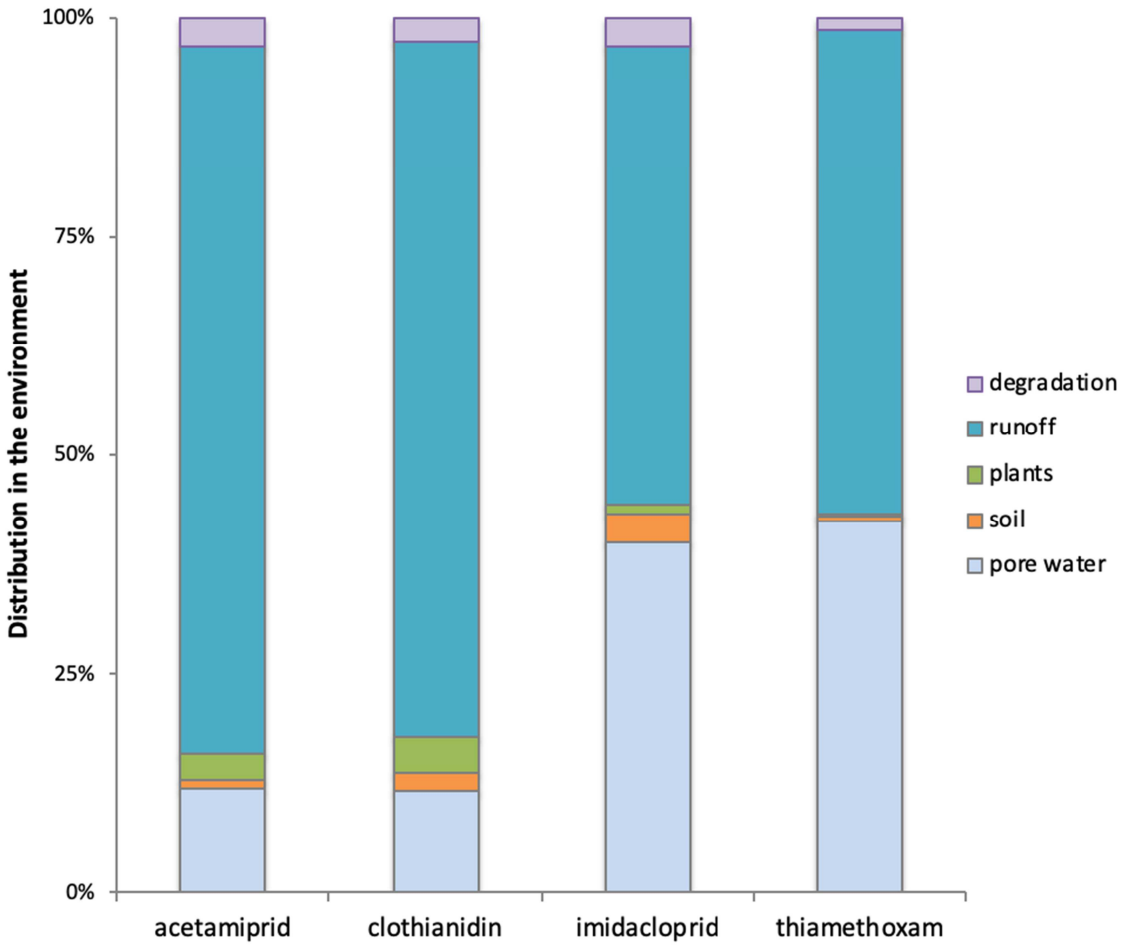

Figure 4. Distribution of four neonicotinoids applied to crops in Belize as determined by Fugacity model III.

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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

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