Environmental Pollution 236 (2018) 432–441

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Effects of imidacloprid on the ecology of sub-tropical freshwater microcosms☆

Kizar Ahmed Sumon [a, b], Afifat Khanam Ritika [b], Edwin T.H.M. Peeters [a], Harunur Rashid [b, c], Roel H. Bosma [d], Md. Shahidur Rahman [b], Mst. Kaniz Fatema [b], Paul J. Van den Brink [a, e, *]

[a] Aquatic Ecology and Water Quality Management Group, Wageningen University, P.O. Box 47, 6700 AA Wageningen, The Netherlands
[b] Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh
[c] Science and Math Program, Asian University for Women, Chittagong 4000, Bangladesh
[d] Aquaculture and Fisheries Group, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands
[e] Wageningen Environmental Research (Alterra), P.O. Box 47, 6700 AA Wageningen, The Netherlands

ARTICLE INFO

Article history:
Received 1 November 2017
Received in revised form
29 January 2018
Accepted 29 January 2018

Keywords:
Neonicotinoid
Insecticide
Freshwater ecosystem
Bangladesh

ABSTRACT

The neonicotinoid insecticide imidacloprid is used in Bangladesh for a variety of crop protection purposes. Imidacloprid may contaminate aquatic ecosystems via spray drift, surface runoff and ground water leaching. The present study aimed at assessing the fate and effects of imidacloprid on structural (phytoplankton, zooplankton, macroinvertebrates and periphyton) and functional (organic matter decomposition) endpoints of freshwater, sub-tropical ecosystems in Bangladesh. Imidacloprid was applied weekly to 16 freshwater microcosms (PVC tanks containing 400 L de-chlorinated tap water) at nominal concentrations of 0, 30, 300, 3000 ng/L over a period of 4 weeks. Results indicated that imidacloprid concentrations from the microcosm water column declined rapidly. Univariate and multivariate analysis showed significant effects of imidacloprid on the zooplankton and macroinvertebrate community, some individual phytoplankton taxa, and water quality variables (i.e. DO, alkalinity, ammonia and nitrate), with *Cloeon* sp., *Diaptomus* sp. and *Keratella* sp. being the most affected species, i.e. showing lower abundance values in all treatments compared to the control. The observed high sensitivity of *Cloeon* sp. and *Diaptomus* sp. was confirmed by the results of single species tests. No significant effects were observed on the species composition of the phytoplankton, periphyton biomass and organic matter decomposition for any of the sampling days. Our study indicates that (sub-)tropical aquatic ecosystems can be much more sensitive to imidacloprid compared to temperate ones.

1. Introduction

The shift from traditional to modern and intensive agricultural practices in developing countries like Bangladesh, has led to an increasing use of pesticides over the last decades (Rahman, 2013). Pesticide use in Bangladesh raised from 7350 metric tons in 1992 to 45,172 metric tons in 2010 (Ali et al., 2017). This was partly due to governments' policy to stimulate chemical control measures against insect pests to increase crop production as well as to prevent pre- and post-harvest crop losses (Shahjahan et al., 2017; Sumon et al., 2016).

Imidacloprid ((*E*)-1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylideneamine; CAS No. 138261-41-3) is a neonicotinoid synthetic insecticide and veterinary substance. It was first introduced in the USA in the 1990s to control insect pests and is now registered in about 120 countries for use in more than 140 crops including rice, maize, cotton, potatoes, tomatoes, sugar beets and various greenhouse-grown plants (Jeschke and Nauen, 2008; Morrissey et al., 2015; Lewis et al., 2016).

Imidacloprid may affect non-target aquatic organisms via exposure due to spray drift (Hilz and Vermeer, 2012) and runoff resulting from its' high solubility in water (Armbrust and Peeler, 2002). After entering into water bodies, the dissipation time 50% (DT50) of imidacloprid merely depends on photolysis, however,

☆ This paper has been recommended for acceptance by Charles Wong.

* Corresponding author. Aquatic Ecology and Water Quality Management Group, Wageningen University, P.O. Box 47, 6700 AA Wageningen, The Netherlands.
E-mail address: paul.vandenbrink@wur.nl (P.J. Van den Brink).

https://doi.org/10.1016/j.envpol.2018.01.102

variation in $DT50_{water}$ was observed between different water bodies. For example, the European Food Safety Authority (EFSA) reported $DT50_{water}$ values ranging from 30 to 150 days for three water-sediment studies performed at 22 °C in laboratory in the dark (EFSA, 2008), indicating a likely long-term exposure of imidacloprid to aquatic ecosystem when light conditions are poor. However, imidacloprid was found to dissipate very rapidly in different studies under UV light due to photolysis (e.g. Lavine et al., 2010). Colombo et al. (2013) recorded a DT50 of 1.2 day from the water column monitored for 28 days in field-based microcosms in Germany, whereas a DT50 of 8.2 day was reported in a pond microcosm in Germany (Posthuma-Doodeman, 2008). A DT50 of 1 day was recorded by Thuyet et al. (2011) for a rice paddy system in autumn in Japan. However, imidacloprid has been detected worldwide in surface waters at concentrations ranging from 0.001 to 320 μg/L, the highest of which was found in Netherlands (Morrissey et al., 2015). Imidacloprid has been found in aquatic ecosystems at 3.29 μg/L in the California's agricultural regions in the USA (Starner and Goh, 2012) and up to 11.9 μg/L in Canadian agricultural areas (CCME, 2007). The field monitoring data on imidacloprid is only available for temperate countries, but the systemic study from sub- (tropical) countries is lacking.

During the past years, a large number of studies focusing on the toxicity of imidacloprid to the aquatic environment have been published, partly also due to the debate on the negative relationship between the use of neonicotinoids and non-target beneficial invertebrates, in particular arthropods (EASAC, 2015; Van Dijk et al., 2013; Vijver and Van den Brink, 2014). Both single species laboratory tests (Alexander et al., 2007; Stoughton et al., 2008; Roessink et al., 2013; Cavallaro et al., 2017; Van den Brink et al., 2016) and model ecosystem studies (Hayasaka et al., 2012a; Mohr et al., 2012; Colombo et al., 2013) using imidacloprid, were all conducted in temperate regions. To date no study seem to have been undertaken to investigate the sensitivity of imidacloprid on the aquatic organisms in the sub-tropics and tropics. Van den Brink et al. (2016) found that a reproducing, summer generations of several arthropods were more sensitive to imidacloprid than their non-reproducing, winter generation. Earlier studies demonstrated that higher temperature also might increase the sensitivity of arthropods (Camp and Buchwalter, 2016; Van den Brink et al., 2016). Hence, a difference in sensitivity between tropical and temperate communities to imidacloprid can be hypothesized. To address this knowledge gap, the present study aimed at assessing fate and effects of imidacloprid on the structural (phytoplankton, zooplankton, macroinvertebrates, and periphyton) and functional (organic matter decomposition) endpoints of freshwater ecosystems located in the sub-tropical country Bangladesh.

2. Materials and methods

Most of the materials and methods used for the microcosm experiment have been described by Rico et al. (2014).

2.1. Design of the microcosm study and acute toxicity tests

The present study was conducted in sixteen freshwater microcosms at the Faculty of Fisheries, Bangladesh Agricultural University (Mymensingh, Bangladesh; 24.7434°N, 90.3984°E). The open experimental area was roofed with transparent plastic slates (Fig. S1). Each microcosm comprised of a PVC tank (diameter: 172 cm; total height: 78 cm) which was coated with non-toxic epoxy paint. Each microcosm was initially filled with 4.5 cm of sediment (collected from nearby ponds of Bangladesh Agricultural University campus) and 400 L of tap water (a layer of 56 cm). Microcosm water was allowed to dissipate the possible chlorine residues for one week. Each system was gently aerated to provide some water movement. The systems were stocked with algae and invertebrates collected from same ponds where sediment was collected. These ponds were selected because they were uncontaminated sources (as agricultural activities were not practised near the Bangladesh Agricultural University campus) and were quite biodiverse in terms of algae and invertebrates. Macroinvertebrates were stocked by distributing an equal numbers of each of the taxa into each microcosm, while equal amounts of concentrated plankton in terms of volume were added into each microcosm. The algae and invertebrate communities were allowed to develop themselves over a pre-treatment period of 6 weeks. During the pre-treatment period, every two weeks about 20% of the water volume was exchanged between the microcosms to promote the uniformity in the structure of the communities between the microcosms. As recommended by Daam and Van den Brink (2011), urea (containing 1.4 mg/L nitrogen) and trisodium phosphate (0.18 mg/L phosphorus) were administered every two weeks to the systems during the experimental period.

For the acute toxicity tests, *Cloeon* sp. and *Diaptomus* sp. were collected from the nearby ponds of Bangladesh Agricultural University campus (see some photos of *Cloeon* sp. and *Diaptomus* sp. in Figs. S2 and S3, respectively). *Cloeon* sp. was transferred in an aerated plastic bucket with a mixture of pond and de-chlorinated test water first and then only in test water to acclimate to the laboratory conditions for at least 3 days at ambient temperature. During the acclimation period, they were fed ad libitum with *Enhydra fluctuans*, *Eichhornia crassipes* and biofilms. *Diaptomus* sp. was stocked in an aerated glass beaker with de-chlorinated test water in the laboratory condition at ambient temperature and fed with algae. After an acclimation period of 3 days, 10 individuals of *Cloeon* sp. were transferred into each of the 21 glass beakers containing 500 mL de-chlorinated tap water (water holding capacity: 750 mL) and 20 individuals of *Diaptomus* sp. were transferred into 21 glass beakers containing 50 mL de-chlorinated tap water (water holding capacity: 100 mL), which were put in the laboratory at ambient temperature and receiving no direct sunlight. An aeration system was introduced in all beakers to provide sufficient oxygen throughout the experimental period of 96 h. Feeding was stopped 24 h before and throughout the exposure period. Both species were exposed to seven different concentrations (0, 3, 10, 30, 100, 300, 3000 ng/L) of imidacloprid including control with triplicate treatment for 96 h separately. Imidacloprid (as Premier with 20% active ingredient, 6% adjuvants and 74% water and produced by the world of Hayleys) was purchased from a local pesticide seller (Mymensingh, Bangladesh). The stock solutions were prepared by dissolving the required weighed amount of imidacloprid in distilled water so a concentration of 200 g/L imidacloprid was achieved. Water quality variables (i.e. dissolved oxygen, temperature, pH and EC) were measured in the lowest and highest treatment, and in the control at 0 h and 96 h of exposure. Mortality and immobility were checked at every 24 h of exposure for *Cloeon* sp. and after 96 h of exposure for *Diaptomus* sp. Individuals were considered immobile when there was no observed movement within 20 s for *Cloeon* sp. and 15 s for *Diaptomus* sp., and dead when there was no observed movement within 3–5 s for both after a tactile stimulation using a Pasteur's capillary pipette (OECD, 2004). Dead individuals were removed immediately from the experimental units. Immobile individuals were kept in the systems because there was a possibility for recovery, and these specimens were used to calculate effect concentration levels based on immobilization. The test was valid when the mortality of the control did not exceed 10% at the end (96 h) of the test (OECD, 2004).

2.2. Application and analysis of imidacloprid

Like in the acute toxicity tests, imidacloprid was applied using the Premier formulation in microcosm experiment. Imidacloprid was applied to each microcosms weekly at either nominal concentrations of 0, 30, 300 or 3000 ng/L over a period of 4 weeks, using four replicates for each treatment. The doses were chosen based on the acute and chronic toxicity of imidacloprid to the most sensitive organisms, mayflies. The lowest concentration (30 ng/L) was based on the 28-d EC10 value of imidacloprid for *Cloeon dipterum* (33 ng/L; Roessink et al., 2013) in the Netherlands. The highest concentration of 3000 ng/L of imidacloprid in both the microcosm experiment and the acute toxicity tests reflected the acute toxicity (96 h-EC50) for the same species (1770 ng/L; Roessink et al., 2013). The four microcosms serving as controls received only aerated tap water. The control and treatments were randomly assigned to the experimental microcosms prior to the first imidacloprid application. Stock solutions of 1 L were prepared for each of the 4 applications by dissolving the weighed amount of imidacloprid with distilled water in a volumetric flask so a concentration of 200 g/L imidacloprid was achieved and the solution was sonicated for 30 min at 45 °C.

The imidacloprid concentrations were analytically verified in microcosm water samples collected from one of the four replicates of all treatments just after application and before the next application. Water samples were collected at 1 h, and 1, 2, 6.9, 7.1, 13.9, 14.1, 20.9, 21.1 and 28 days. For the acute toxicity tests, water samples were collected to measure imidacloprid concentrations from one of the replicates of the control, the lowest and the highest treatment at 0 h and 96 h. Approximately 3 mL water samples were collected using a pipette and kept in a glass vial containing 1 mL of acetonitrile for both experiments. The samples were shaken thoroughly by hand and subsequently preserved in a freezer (−20 °C) until analysis. Imidacloprid concentrations from the water samples were analysed by liquid chromatography-tandem mass spectrometry (LC-MS) as described in Roessink et al. (2013). In this study, a matrix-matched method was used to correct for matrix effects in the instrumental quantification of imdacloprid. The limit of detection (LOD) and the limit of quantification (LOQ) in the microcosm study were 9 ng/L and 29 ng/L, respectively, and in the acute toxicity tests 6 ng/L and 19 ng/L, respectively.

2.3. Invertebrates and algae

The macroinvertebrate community was sampled using two pebble baskets (height: around 30 cm; diameter: around 20 cm) that served as artificial substrates in each microcosm. Each of the two artificial substrates was placed on the sediment's surface and were left for colonization for two weeks. Macroinvertebrates were sampled 7 days before the first imidacloprid application and on days 2, 9, 16 and 23 after the first imidacloprid application. The two artificial substrates present in the same microcosm were sampled alternately. For sampling, one of the substrates was carefully retrieved from the sediment and immediately enfolded by a nylon net. The substrate was carefully shaken in the net to extract the invertebrates from the substrate. In order to sample the pelagic macroinvertebrates, the net was moved through the water column close to one quarter of the microcosm wall. A core sediment sampler (inner diameter: around 8 cm) was used to collect the invertebrates inhabiting the sediment (Chironomid larvae and *Tubifex tubifex*) on day 28 after the first imidacloprid application. All sampled invertebrates were transferred to a white tray, subsequently identified and counted alive, and finally placed back into their original microcosms.

Plankton was sampled on days 7 and 1 before the first imidacloprid application, and on days 2, 9, 16, 23 and 28 after the first imidacloprid application. Two 5 L depth-integrated water samples were collected using a Perspex tube in a plastic bucket and filtered over a net with a mesh size of either 20 μm for phytoplankton or 55 μm for zooplankton, yielding two samples of 100 mL. The samples were preserved in plastic bottles with 10% buffered formalin solution and stored at 4 °C. The individuals present in a sub-sample (1 mL) of the concentrated phytoplankton and zooplankton samples were identified to the lowest practical level with an inverted microscope (Olympus CX 41) and recalculated to numbers of individuals per litre of microcosm water.

The possible effects of imidacloprid on the chlorophyll-a content of the periphyton biomass was evaluated by introducing three series of 3 microscopic glass slides (7.5 cm × 2.5 cm) at 30 cm water depth in each microcosm 7 days before the first imidacloprid application. A glass slide series was retrieved on days 2, 16 and 28 after the first imidacloprid application and attached periphyton was collected by scraping and then the scraped periphyton was transferred to a glass vial containing 0.25 L tap water. The chlorophyll-a in the resulting periphyton - water mixture was measured according to APHA (2005) and the amount of chlorophyll-a per square centimetre of glass slide was determined.

2.4. Water quality variables and organic matter decomposition

Temperature (T), dissolved oxygen (DO), pH, electrical conductivity (EC) were monitored at 8 a.m. on 7 days and 1 day before the first imidacloprid application, and on days 0, 9, 16, 23 and 28 after the first imidacloprid application, using a multimeter (Hach, HQ 40 d). On these days, also total alkalinity levels and ammonia, nitrite, nitrate and total phosphorus concentrations were measured in water samples collected from each microcosm. For this, a depth-integrated water sample of approximately 1 L was collected in each microcosm using a Perspex tube and stored at 4 °C in a plastic bottle in the dark. Alkalinity and nutrient concentrations were determined within 7 days according to APHA (2005).

Litter bags were used to study the effects of the insecticide on organic matter decomposition. The litter bags included 2 g of banana (*Musa*) leaves and three of them were introduced into each microcosm 1 day before the first imidacloprid application. The banana leaves were leached in tap water (2 days) and subsequently dried (40 °C for 48 h) before addition to the litter bags. The litter bags were placed approximate 30 cm below the water surface. On days 2, 16 and 28 after the first imidacloprid application, one of the three litter bags was sampled and the retrieved material was dried (40 °C for 48 h) and weighted. The percentage of organic matter decomposition was calculated by calculating the loss of the initial dry weight over 2, 16 and 28 days.

2.5. Data analyses

No-observed-effect-concentrations (NOECs) were determined for the variables including water quality, all taxa of phytoplankton, zooplankton, macroinvertebrates, periphyton community, and organic matter decomposition data using the Williams test (Williams, 1972; $p < 0.05$) as available in the Community Analysis computer program, version 4.3.05 (Hommen et al., 1994). Prior to the analysis, the abundance data sets were $\ln(Ax + 1)$ transformed. For the determination of A and the rationale behind the transformation is referred to Van den Brink et al. (2000).

The phytoplankton, zooplankton and macroinvertebrate data sets were analysed by the principal response curve (PRC) method using the CANOCO Software package, version 5 (Van den Brink and Ter Braak, 1999; Ter Braak and Šmilauer, 2012). The PRC method is a specific type of redundancy analysis (RDA) that is able to extract the

variation in community composition due to the stressor from the total variation by including the treatment regime and its interaction with time as explanatory variables, and the sampling date as co-variables. The overall significance of the effect of imidacloprid treatment on the variation in community composition ($p \leq 0.05$) was tested by performing 999 Monte Carlo permutations (Van den Brink and Ter Braak, 1999). Each treatment was tested against the control for each sampling date using Monte Carlo permutation tests under the RDA option in order to evaluate the significance of the imidacloprid induced community effects in time.

The LC10, LC50 and LC90 and EC10, EC50 and EC90 values of imidacloprid resulting from the toxicity tests performed with *Cloeon* sp. and *Diaptomus* sp. were determined using log-logistic regression as programmed in the software GenStat 11th (VSN International Ltd., Oxford, UK) according to Rubach et al. (2011).

3. Results and discussion

3.1. Fate of imidacloprid

One hour after each of the four applications, on average, 93% of the applied concentration was found in the highest treatment and on average, 87% was found in the second highest treatment (Fig. 1; Table S1). After 7 days, between 45% and 53% of the applied concentration was present in microcosm water in the highest and second highest treatment, respectively. In the acute toxicity tests 79% of the intended concentration was found in the highest treatment just after imidacloprid application, whereas after 96 h of exposure 47% of the applied concentration was left (Table S2). In our study, the lower dissipation of imidacloprid in the microcosm experiment compared to the acute toxicity tests might be due to UV light absorption by natural organic matter and suspended particulate matter in microcosms which decreases the photodegradation of imidacloprid (Lu et al., 2015). The dissipation was, however, found to be faster in the present sub-tropical study compared to earlier model ecosystem studies (i.e. microcosm and mesocosm studies) and acute studies conducted in temperate regions. For example, Pestana et al. (2009) found 88% of the intended concentrations of imidacloprid after 24 h of exposure in the highest concentration in recirculatory flow-through stream mesocosms at 20 °C in Canada. Van den Brink et al. (2016) measured 94% and 91% of the intended imidacloprid concentration just after application and after 96 h of exposure, respectively in an acute study performed under very low light intensities at 18 °C in Netherlands. The rapid dissipation of imidacloprid in both microcosm and acute studies suggests that the dissipation is higher in the tropics than in temperate region due to higher temperature (28.2 ± 2 °C for microcosm experiment and 27.4 ± 0.6 °C for acute toxicity tests) and photodegradation during the experimental period (Laabs et al., 2007; Chai et al., 2009; Sanchez-Bayo and Hyne, 2011). In the present study, however, we found a build-up of imidacloprid concentrations in later applications in all treatment levels as compared to the first application in microcosm study. For instance, 25% of the intended dose was found after 7 days of first application in the highest treatment while, 65% was present 7 days after the fourth application in the same treatment (Fig. 1; Table S1).

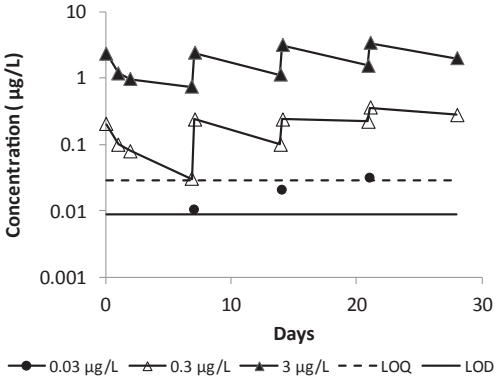

Fig. 1. Dynamics of measured imidacloprid concentrations in microcosm water during the experimental period.

3.2. Invertebrates

The zooplankton community was dominated by Rotifera (6 taxa), followed by Cladocera (4 taxa) and Copepoda (3 taxa) during the experimental period and all of them showed a relatively constant abundance in time (Fig. S4). The PRC showed significant negative effects of imidacloprid on the zooplankton community ($p \leq 0.001$; Fig. 2), with a consistent $NOEC_{community}$ value of 300 ng/L (Table 1 and Table S3). Species weight in the PRC indicated that *Diaptomus* sp. was the taxon most responding to the treatments, followed by nauplius, two Rotifera taxa and three Cladocera taxa (Fig. 2). Univariate analysis indicated that four taxa showed a consistent negative response to the imidacloprid treatment, i.e. with NOECs calculated for at least two consecutive sampling dates (Table 1 and Table S3). Among the 13 taxa identified, *Diaptomus* sp. was the most negatively affected from day 2 after the first imidacloprid application onwards in almost all treatment levels with a consistent NOEC of 300 ng/L, followed by *Keratella* sp., *Sida* sp. and *Brachionus* sp. (Table 1 and Table S3; Figs. 2 and 3; Fig. S4). Our single species toxicity test confirmed the sensitivity of *Diaptomus* sp. when exposed to imidacloprid since an 96-h EC50 of 38.6 ng/L was calculated for this genus (Table 2; Tables S4 and S5). Unfortunately, temperate toxicity values for *Diaptomus* sp. and the three other affected taxa could not be found in the literature and therefore comparison with published data is impossible. One study by Song et al. (1997), however, demonstrated a 48-h LC50 value of 361,230, 000 ng/L for one of the copepods nauplius exposed to imidacloprid, which is several thousand folds higher than we reported for *Diaptomus* sp. In this study, the Cladoceran *Sida* sp. were consistently affected on day 9 (NOEC = <30 ng/L) and 16 (NOEC = 300 ng/L) after the first imidacloprid application. The toxicity data for neonicotinoids towards *Sida* sp. are also not available in the literature for comparison. For Cladocera, the species *Daphnia magna* was tested most often. Earlier temperate studies, however, demonstrated a lower acute sensitivity of *D. magna* to imidacloprid than we reported for *Sida* sp. (i.e. several thousands of nanograms per litre) (Sánchez-Bayo and Goka, 2006; Tišler et al., 2009; Ashauer et al., 2011; Hayasaka et al., 2012b; Daam et al., 2013). A chronic temperate study by Ieromina et al. (2014) also found lower sensitivity of *D. magna* to imidacloprid since an 9-d EC10 and 15-d EC10 (survival endpoint) of 54,160,000 ng/L and 29,630,000 ng/L, respectively was calculated. The higher sensitivity of Cladoceran to imidacloprid in this study compared to earlier acute and chronic studies could partly be explained by the higher temperature in sub-tropics (Sarma et al., 2005). For example, Ieromina et al. (2014) conducted their study at 20 °C while we recorded an average temperature of 28.2 °C during our microcosm experiment. The differences of sensitivity to imidacloprid might also be due to the different species tested in our study as compared to earlier studies (Hayasaka et al., 2012b). However, earlier studies on the toxicity of neonicotinoid insecticides towards microcrustaceans focused on acute effects (96 h or shorter) and only one on chronic effects on a standard test species (i.e., *Daphnia* sp.). Hence, we recommend future acute and chronic studies with more (sub-)tropical crustaceans to get a clearer picture of neonicotinoids

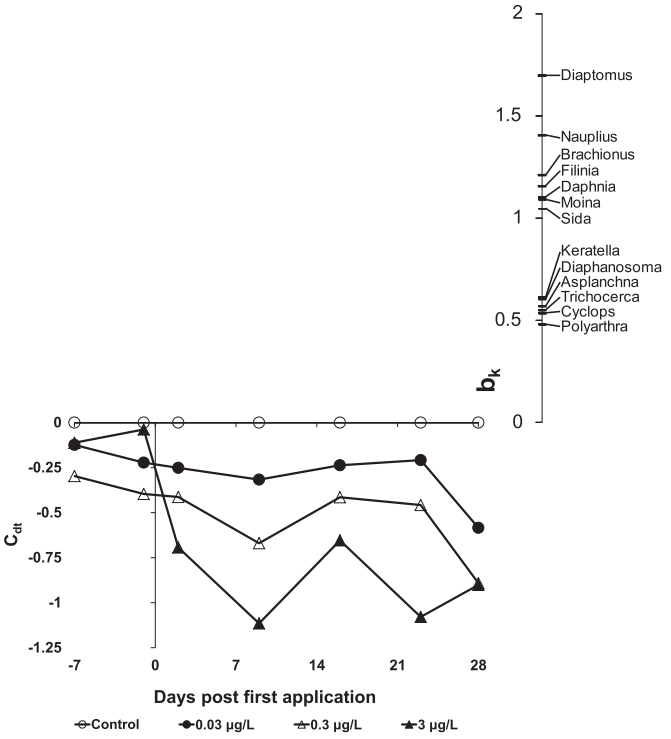

Fig. 2. PRC resulting from the analysis of the zooplankton data set, indicating the effects of imidacloprid on the zooplankton community. Of all variance, 7% could be attributed to sampling date; this is displayed on the horizontal axis. 20% percent of all variance could be attributed to treatment. Of this variance, 49% is displayed on the vertical axis. The lines represent the course of the treatment levels in time. The species weight (b_k) can be interpreted as the affinity of the taxon with the PRC. The Monte Carlo permutation test indicated that a significant part of the variance explained by treatment is displayed in the diagram ($p \leq 0.001$). The second PRC was not significant.

toxicity towards tropical freshwater ecosystems, as we cannot fully explain why in our experiment *Diaptomus* sp. is so sensitive as compared to temperate crustaceans.

In the present study, 10 macroinvertebrate taxa were identified belonging to three different taxonomic groups: Insecta (6 taxa), Mollusca (3 taxa) and Annelida (1 taxon). The results of the PRC showed significant effects of imidacloprid on the macroinvertebrate community ($p = 0.002$; Fig. 4), with a consistent $NOEC_{community}$ value of 300 ng/L (Table 1 and Table S6). The species weights in the PRC indicated that *Cloeon* sp. was the taxon most strongly responding to the treatments i.e. showing lower abundance values in all treatments compared to the control (Figs. 3 and 4). The univariate analysis showed consistent significant negative effects of imidacloprid on two insect species, as well as on *Tubifex tubifex* and Chironomid larvae, which were only sampled once (Table 1 and Table S6). Among 10 identified taxa, *Cloeon* sp. was the most affected taxon (NOEC < 30 ng/L on day 2 and 9), followed by *Notonecta* sp., who also showed a consistent response to the treatments (Table 1 and Table S6; Fig. S5). The single species toxicity test confirmed the high sensitivity of *Cloeon* sp. towards imidacloprid since an 96-h EC50 and LC50 of 5.48 and 23.8 ng/L, respectively, was calculated for this genus (Table 2; Tables S4 and S5). The results of our study are in accordance with the previous study by Roessink et al. (2013) in the sense that *Cloeon* sp. was the most sensitive taxa among the studied invertebrates in both studies. In our study, however, effects were found at much lower concentrations since they reported the 96-h and 28-d EC50 values of 1000 ng/L and 130 ng/L, respectively for *Cloeon dipterum,* which are about two orders of magnitude higher than the 96-h EC50 reported in our study. Alexander et al. (2007) reported a 96-h LC50 value of 650 ng/L for one of the mayfly species *Epeorus longimanus*, which is again about 27 folds higher than the value we reported for *Cloeon* sp. The higher sensitivity of *Cloen* sp. to imidacoprid in our study can partly be explained by differences in temperature as Van den Brink et al. (2016) showed an increase in the sensitivity of *Cloeon dipterum* due to increased temperature. They reported that the 96-h EC50 and LC50 values of imidacloprid for *Cloeon dipterum* were 1.7 and 4.2 folds lower, respectively at 18 °C compared to 10 °C. The higher temperature in the sub-tropics might modify the toxicity of imidacloprid through the elevation of metabolic rates of *Cloeon* sp., which leads to increased uptake rates of imidacloprid

Table 1
The No Observed Effect Concentrations (NOECs) for phytoplankton, zooplankton, macroinvertebrates and water quality endpoints expressed in terms of nominal single-dose of imidacloprid concentrations (ng/L) measured on each sampling day (Williams test; $p \leq 0.05$). Only individual taxa or parameters that showed treatment-related effect on at least two successive sampling days are included. See Tables S3 and S6–S8 for the results for all species and parameters.

Endpoint	Sampling days						
	−7	−1	0–2	9	16	23	28
Zooplankton							
Community	>	>	>	300	>	300	30
Diaptomus sp.	>	>	300 (−)	300 (−)	<30 (−)	300 (−)	<30 (−)
Brachionus sp.	>	>	>	>	>	300 (−)	30 (−)
Keratella sp.	>	>	<30 (−)	<30 (−)	>	<30 (−)	>
Sida sp.	>	>	>	<30 (−)	300 (−)	>	>
Macroinvertebrates							
Community	>	NM	300	300	300	>	NM
Cloeon sp.	>	NM	<30 (−)	<30 (−)	300 (−)	30 (−)	NM
Notonecta sp.	>	NM	30 (−)	300 (−)	300 (−)	>	NM
Chironomid larvae	NM	NM	NM	NM	NM	NM	300 (−)
Tubifex tubifex	NM	NM	NM	NM	NM	NM	300 (−)
Phytoplankton							
Community	>	>	>	>	>	>	>
Scenedesmus sp.	>	>	>	>	300 (−)	300 (−)	>
Tetraedon sp.	>	>	>	>	<30 (−)	30 (−)	>
Water quality							
Dissolved oxygen	>	>	>	<30 (−)	30 (−)	<30 (−)	30 (−)
Alkalinity	>	>	>	<30 (−)	300 (−)	>	<30 (−)
Ammonia	>	>	>	>	>	300 (+)	300 (+)
Nitrate	300 (−)	>	30 (−)	300 (−)	300 (−)	300 (−)	>

> = no significant effect (NOEC ≥ 3000 ng/L); NM = not measured; significant decrease (−) compared to control.

Table 2
The acute toxicity levels of imidacloprid for *Cloeon* sp. and *Diaptomus* sp. expressed as 96-h L(E)C10, L(E)C50 and L(E)C90 values in ng/L. The control mortality and immobilization were both 7% for *Cloeon* sp. and 5% and 13%, respectively for *Diaptomus* sp.

Species name	96-h LC10 With 95% confidence limits	96-h LC50 With 95% confidence limits	96-h LC90 With 95% confidence limits	96-h EC10 With 95% confidence limits	96-h EC50 With 95% confidence limits	96-h EC90 With 95% confidence limits
Cloeon sp.	0.109 (0.005–2.17)	23.8 (8.15–69.6)	5230 (531–51400)	0.0556 (0.00256–1.21)	5.48 (1.72–17.5)	541 (100–2920)
Diaptomus sp.	1.21 (0.054–27)	6540 (743–57700)	35,448,000 (65,000 –19,454,979,000)	1.43 (0.404–5.05)	38.6 (21.6–68.9)	1040 (422–2580)

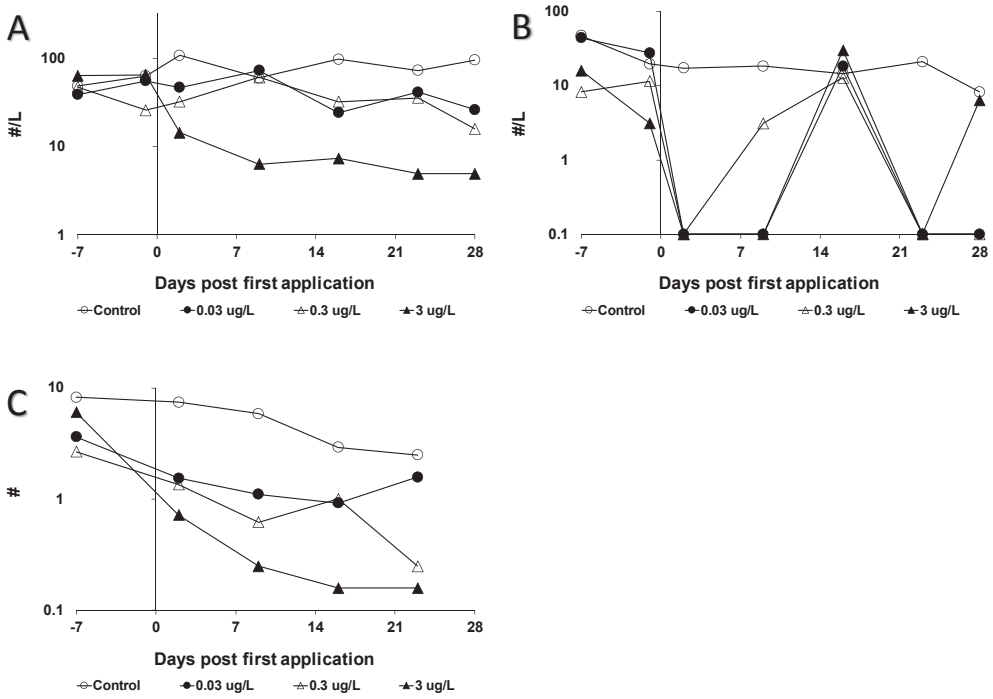

Fig. 3. The population dynamics of the zooplankton taxa *Diaptomus* sp. (A) and *Keratella* sp. (B) and the macroinvertebrate taxon *Cloeon* sp. (C) under the four imidacloprid concentrations.

and thus could partly explain the higher sensitivity (Camp and Buchwalter, 2016). Moreover, the species of *Cloeon* sp. we used in our study continuously reproduces which could be another reason of their high sensitivity to imidacloprid. An earlier study by Van den Brink et al. (2016) found that the reproducing, summer generations of *Cloeon dipterum* (28-d EC50 = 130 ng/L) were approximately five times more sensitive to imidacloprid than their non-reproducing, winter generations (28-d EC50 = 680 ng/L). The sensitivity differences between summer and winter generations of aquatic insects towards toxicants might depend on the differences in their physiologies and life histories, with concomitant implications for sensitivity to toxicants (Kwok et al., 2007). For example, based on metabolic principle, it has been hypothesized that tropical aquatic insects might be more sensitive to toxicants than their temperate counterparts (Castillo et al., 1997). The higher sensitivity of *Cloeon* sp. in our study can also be explained by the differences in use of different formulations or technical grade of imidacloprid in earlier studies, as the formulated product can enhance the bioavailability and toxicity to target organisms (Malev et al., 2012). For instance, Stoughton et al. (2008) reported the 96-h LC50 value (654,300 ng/L) of technical-grade imidacloprid for *Hyalella azteca*, which is approximately four times higher than the 96-h value (174,400 ng/L) of commercial formulation Admire (240 g/L) for the same species; thus indicating Admire is more toxic than the technical-grade imidacloprid. All these differences between temperate and tropical circumstances and species, can, however, not fully explain why the tropical *Cloeon* sp. is so much more sensitive to imidacloprid compared to its temperate counterpart.

The second most sensitive taxon after *Cloeon* sp. tested in our study was *Notonecta* sp., which was negatively affected from day 2 after the first imidacloprid application onwards for three consecutive sampling dates with a consistent NOEC value of 300 ng/L (Table 1). The present study showed, however, higher sensitivity of *Notonecta* sp. to imidacloprid than that was reported by Roessink et al. (2013) because they calculated an 96-h EC10 of 3000 ng/L, which is about ten times higher than we reported the NOEC value for this genus. Kobashi et al. (2017) demonstrated no treatment-related significant effects of imidacloprid (at 157,000 ng/L) on *Notonecta triguttata* in their rice mesocosm study in Japan. The higher sensitivity of *Notonecta* sp. to imidacloprid in this study compared to earlier temperate studies could be explained by the higher temperature in sub-tropics (Camp and Buchwalter, 2016).

3.3. Primary producers

A total of 32 different phytoplankton taxa were identified in the present study belonging to five major taxonomic groups: Chlorophyceae (12 taxa), Bacillariophyceae (10 taxa), Cyanophyceae (7

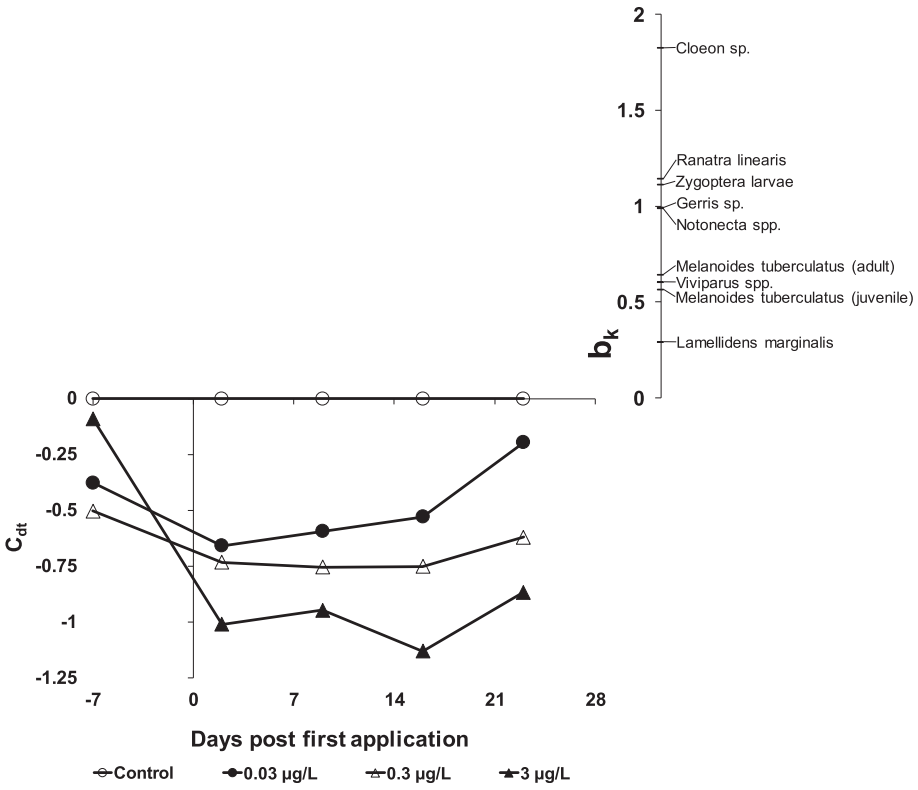

Fig. 4. PRC resulting from the analysis of the macroinvertebrate data set, indicating the effects of imidacloprid on the macroinvertebrate community. Of all variance, 22% could be attributed to sampling date; this is displayed on the horizontal axis. 14% percent of all variance could be attributed to treatment. Of this variance, 74% is displayed on the vertical axis. The lines represent the course of the treatment levels in time. The species weight (b_k) can be interpreted as the affinity of the taxon with the PRC. The Monte Carlo permutation test indicated that a significant part of the variance explained by treatment is displayed in the diagram ($p = 0.002$). The second PRC was not significant.

taxa), Euglenophyceae (2 taxa) and Desmidiaceae (1 taxon). The most abundant taxa in decreasing order were *Ankistrodesmus* sp., followed by *Microcystis* sp., *Fragillaria* sp., *Oscillatoria* sp., *Ulothrix* sp., and *Tetraedon* sp. during the experimental period. The PRC did not reveal significant effects of imidacloprid on the phytoplankton community ($p = 0.718$). However, univariate analysis showed significant effects of imidacloprid on certain phytoplankton taxa (15 out of 32) (Table S7; Fig. S6). Among 15 significant taxa, however, only two taxa (*Scenedesmus* sp. and *Tetraodon* sp.) were negatively affected for two consecutive sampling days (Table 1). *Scenedesmus* sp. had lower abundance values on day 16 and 23 in the highest treatment level (NOEC of 300 ng/L for both sampling days) (Table 1 and Table S7; Fig. S6A) and *Tetraedon* sp. had lower abundance values on day 16 in all treatment levels (NOEC < 30 ng/L) and on day 23 in the second highest and highest treatment level (NOEC of 30 ng/L) (Table 1 and Table S7; Fig. S6B).

The chlorophyll-a density in periphyton biomass increased in all treated microcosms including the controls on day 16 after the first imidacloprid application but decreased slightly on day 28 (Fig. 5A). However, the results of the univariate analysis did not show any significant effects of imidacloprid on periphyton biomass for any of the sampling days (NOECs $\geq$ 3000 ng/L).

The results of this study indicates that the majority of the primary producers were tolerant to imidacloprid. This could be explained by the fact that the primary producers are not sensitive to neonicotinic imidacloprid based on their known insecticidal type of action (Daam et al., 2013; Anderson et al., 2015). Furthermore, we noticed a bloom of floating algae and macrophytes (*Lemna minor*) in all microcosms including control in the present study which we, unfortunately, did not quantify. On average, 75% surface area of microcosms was covered with primary producers in the highest concentrations of imidacloprid, while on average, 40% area was covered in control microcosms (visual observation). Toxicity data for neonicotinoids towards primary producers, such as algae and macrophytes is limited, however, the available data indicate EC50 values larger than 1000,000 ng/L (Tišler et al., 2009; Malev et al., 2012; Bayer CropScience, 2013; Daam et al., 2013).

3.4. Water quality variables

The daily average water temperature in microcosms gradually increased during the experimental period from 27.9 °C, 1 h after first application, to 31.7 °C on day 28 after the first application (Fig. S7A). However, a decrease to 24.3 °C was observed on day 16 after the first application. The latter day coincided with cloudy weather while the other days were not cloudy. The average DO between replicates measured in the microcosm water during the experimental period ranged between 4.35 mg/L and 8.33 mg/L. DO concentrations decreased significantly on day 9 and onwards after the first application with a consistent NOEC value of 30 ng/L. The lowest average DO (4.35 mg/L) was measured on day 28 in the highest treatment level (Table 1; Fig. 6A). The pH, EC, phosphate and nitrite showed no consistent response to the treatment (Table S8; Fig. S7). A significant decrease was observed for alkalinity levels for two consecutive sampling days on day 9 and 16 for almost all treatment levels (Table 1; Fig. 6B). Average ammonia concentrations in the experimental microcosms ranged between 0.4 mg/L (pre-treatment period) and 2.5 mg/L (on day 28 after the first application). Ammonia concentrations increased significantly for the two consecutive sampling days on day 23 and 28 in the highest

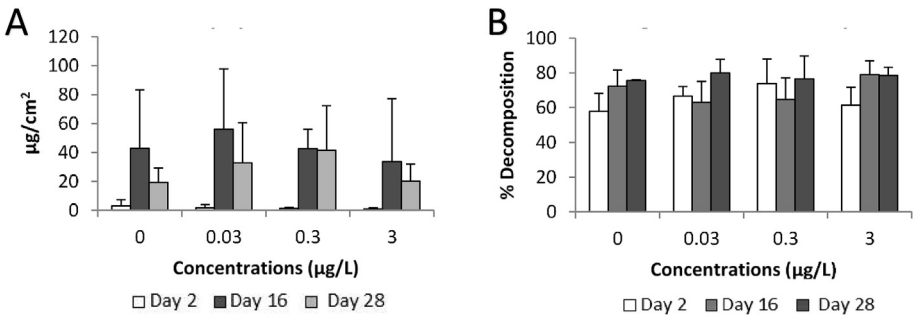

Fig. 5. Chlorophyll-a in periphyton (A) and organic matter decomposition of banana (Musa) leaves (B) on day 2, 16, and 28 after first imidacloprid application (mean ± standard deviation) (NOEC ≥ 3 µg/L).

treatment level with a NOEC of 300 ng/L (Table 1; Fig. 6C). Nitrate concentrations decreased consistently in the highest treatment level at all sampling days except on day 28 with a NOEC of 300 ng/L. The highest nitrate concentration (1.7 mg/L) was measured on day 28 in the second highest treatment level (300 ng/L) (Table 1; Fig. 6D).

In the present study, the effects found on water quality variables exposed to imidacloprid concentrations were indirect. Dissolved oxygen was consistently affected from day 9 after the first imidacloprid exposure onwards. This reduced dissolved oxygen level in microcosm water could be explained by reduced photosynthesis in the water column due to a bloom of floating algae and macrophytes. In our study, we observed that the majority of macro- and micro-crustaceans were negatively affected on day 9 after the first imidacloprid application. Reduced grazing of these invertebrates and nutrient-rich environment in microcosms (e.g. ammonia, phosphate and nitrite were significantly increased for different sampling days) might have led to a bloom of floating algae and macrophytes (own observations) which hindered the light penetration into cosms and thus affected the photosynthesis. The reduced light penetration induced by floating algae and macrophytes might have reduced the photolysis of imidacloprid, thus increasing the exposure of macro- and micro-crustaceans to imidacloprid.

3.5. Organic matter decomposition

The decomposition rates of banana (*Musa*) leaves (mean ± SD) in the control microcosms were 58 ± 10%, 72 ± 9% and 76 ± 0.5% on day 2, 16 and 28, respectively after the first imidacloprid application (Fig. 5B). In this study, the decomposition of banana leaves increased gradually with an increasing exposure period. The results of the univariate analysis, however, did not show any treatment-related significant effects of imidacloprid on the decomposition of banana leaves for any of the sampling days (NOECs ≥ 3000 ng/L) (Fig. 5B). The results of this study is line with earlier microcosm and

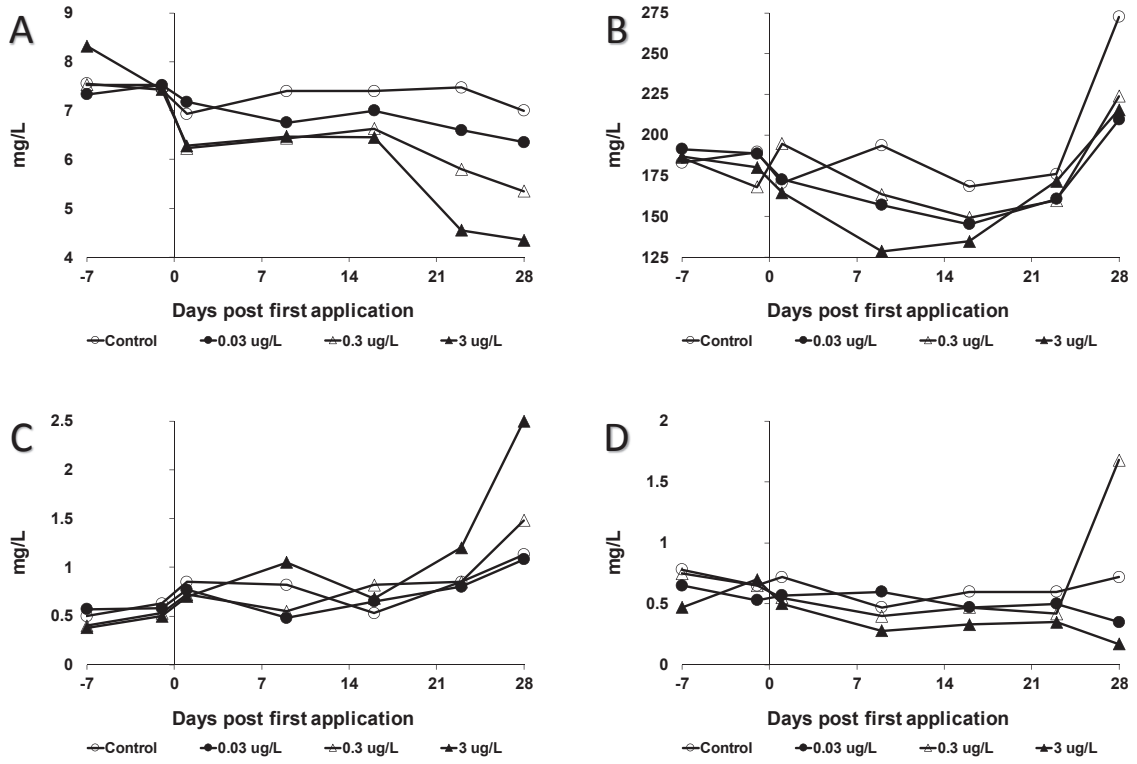

Fig. 6. The dynamics of the water quality parameters DO (A), alkalinity (B), ammonia (C) and nitrate (D) measured during the experimental period.

mesocosm studies in the sense that they did not find treatment-related significant effects of imidacloprid on the microbial decomposition of different leaves used in their studies (Kreutzweiser et al., 2008; Pestana et al., 2009; Böttger et al., 2013).

4. Conclusions

This is the first study assessing the effects of 4 weekly applications of imidacloprid on the freshwater ecosystem under semi-field conditions in sub-tropics. In this study, imidacloprid concentrations between 30 and 3000 ng/L demonstrated significant effects on water quality variables, certain phytoplankton taxa, and on communities of zooplankton and macroinvertebrates. The study revealed toxic effects of imidacloprid on an (sub-)tropical freshwater ecosystem at much lower concentrations than found for temperate systems. Whether these differences in sensitivity holds true for all (sub-)tropical aquatic ecosystems remains to be investigated. This study generates safe environmental values of imidacloprid for the individual taxa and community levels of some endpoints through the derivation of NOECs. For certain taxa, the present study found low levels of NOECs (<30 ng/L) indicating that the standard of imidacloprid (30 ng/L) used in Europe (Vijver and Van den Brink, 2014) might not protect freshwater communities in Bangladesh. We recommend further long-term studies with (sub-)tropical aquatic species and ecosystems to get insight into the comparative toxicity of imidacloprid using the data obtained from this study with those previously obtained in temperate regions.

Acknowledgements

The study was financially supported by the NUFFIC-NICHE-BGD-156 project. We would like to express the gratitude to National Food Safety Laboratory (NFSL), Mohakhali, Dhaka-1212, Bangladesh for analytical verification of imidacloprid. The authors are indebted to Tajmine Naher and Sagiya Sharmin for their kind help in water quality variables analysis and to Rakibul Islam, Most. Farzana Yesmin, Sampa Saha and Sharmin Sultana for macroinvertebrates sampling and counting. We also thank Md. Alal Uddin for his kind cooperation in phytoplankton and zooplankton analyses.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2018.01.102.

References

Alexander, A.C., Culp, J.M., Liber, K., Cessna, A.J., 2007. Effects of insecticide exposure on feeding inhibition in mayflies and oligochaetes. Environ. Toxicol. Chem. 26 (8), 1726–1732.

Ali, M.H., Sumon, K.A., Sultana, M., Rashid, H., 2017. Toxicity of cypermethrin on the embryo and larvae of gangetic mystus, Mystus cavasius. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-017-9399-1.

American Public Health Association (APHA), 2005. Standard Methods for the Examination of Water and Waste Water, twenty-first ed. American Water Works Association and Federal Water Pollution Control Administration, Washington DC, USA, p. 1193.

Anderson, J.C., Dubetz, C., Palace, V.P., 2015. Neonicotinoids in the Canadian aquatic environment: a literature review on current use products with a focus on fate, exposure, and biological effects. Sci. Total Environ. 505, 409–422.

Armbrust, K.L., Peeler, H.B., 2002. Effects of formulation on the run-off of imidacloprid from turf. Pest Manag. Sci. 58 (7), 702–706.

Ashauer, R., Hintermeister, A., Potthoff, E., Escher, B.I., 2011. Acute toxicity of organic chemicals to *Gammarus pulex* correlates with sensitivity of *Daphnia magna* across most modes of action. Aquat. Toxicol. 103, 38–45.

Bayer CropScience, 2013. Confidor® 200 SC Insecticide Safety Data Sheet. Available online: https://www.crop.bayer.com.au/-/media/bcs-inter/ws_australia/use-our-products/product-import-files/148/7219.pdf. (Accessed 6 October 2017).

Böttger, R., Feibicke, M., Schaller, J., Dudel, G., 2013. Effects of low-dosed imidacloprid pulses on the functional role of the caged amphipod *Gammarus roeseli* in stream mesocosms. Ecotoxicol. Environ. Saf. 93, 93–100.

Camp, A.A., Buchwalter, D.B., 2016. Can't take the heat: temperature-enhanced toxicity in the mayfly *Isonychia bicolor* exposed to the neonicotinoid insecticide imidacloprid. Aquat. Toxicol. 178, 49–57.

Castillo, L.E., Fruz, E.D.L., Ruepert, C., 1997. Ecotoxicology and pesticides in tropical aquatic ecosystems of central America. Environ. Toxicol. Chem. 16, 41–51.

Cavallaro, M.C., Morrissey, C.A., Headley, J.V., Peru, K.M., Liber, K., 2017. Comparative chronic toxicity of imidacloprid, clothianidin, and thiamethoxam to *Chironomus dilutus* and estimation of toxic equivalency factors. Environ. Toxicol. Chem. 36, 372–382.

CCME, 2007. Canadian Water Quality Guidelines: Imidacloprid. Scientific Supporting Document. Canadian Council of Ministers of the Environment, Winnipeg.

Chai, L.K., Mohd-Tahir, N., Bruun Hansen, H.C., 2009. Dissipation of acephate, chlorpyrifos, cypermethrin and their metabolites in a humid-tropical vegetable production system. Pest Manag. Sci. 65 (2), 189–196.

Colombo, V., Mohr, S., Berghahn, R., Pettigrove, V.J., 2013. Structural changes in a macrozoobenthos assemblage after imidacloprid pulses in aquatic field-based microcosms. Arch. Environ. Contam. Toxicol. 65, 683–692.

Daam, M.A., Pereira, A.C.S., Silva, E., Caetano, L., Cerejeira, M.J., 2013. Preliminary aquatic risk assessment of imidacloprid after application in an experimental rice plot. Ecotoxicol. Environ. Saf. 97, 78–85.

Daam, M.A., Van den Brink, P.J., 2011. Conducting model ecosystem studies in tropical climate zones: lessons learned from Thailand and way forward. Environ. Pollut. 159, 940–946.

European Academies Science Advisory Council (EASAC), 2015. Ecosystem Services, Agriculture and Neonicotinoids. EASAC policy report 26, ISBN 978-3-8047-3437-1.

European Food and Safety Authority (EFSA), 2008. Conclusion regarding the peer review of the pesticide risk assessment of the active substance imidacloprid. EFSA Sci. Rep. 148, 1–120.

Hayasaka, D., Korenaga, T., Suzuki, K., Saito, F., Sánchez-Bayo, F., Goka, K., 2012a. Cumulative ecological impacts of two successive annual treatments of imidacloprid and fipronil on aquatic communities of paddy mesocosms. Ecotoxicol. Environ. Saf. 80, 355–362.

Hayasaka, D., Korenaga, T., Suzuki, K., Sánchez-Bayo, F., Goka, K., 2012b. Differences in susceptibility of five cladoceran species to two systemic insecticides, imidacloprid and fipronil. Ecotoxicology 21, 421–427.

Hilz, B.E., Vermeer, A.W.P., 2012. Effects of formulation on spray drift: a case study for commercial imidacloprid products. Asp. Appl. Biol. 114, 445–450.

Hommen, U., Düllmer, U., Vith, D., 1994. A computer program to evaluate plankton data from freshwater field tests. In: Hill, I.R., Heimbach, F., Leeuwangh, P., Matthiesen, P. (Eds.), Freshwater Field Tests for Hazard Assessment of Chemicals. Lewis Publishers, Baca Raton, USA, pp. 503–513.

Ieromina, O., Peijnenburg, W.J.G.M., de Snoo, G., Müller, J., Knepper, T.P., Vijver, M.G., 2014. Impact of imidacloprid on Daphnia magna under different food quality regimes. Environ. Toxicol. Chem. 33, 621–631.

Jeschke, P., Nauen, R., 2008. Neonicotinoids-from zero to hero in insecticide chemistry. Pest Manag. Sci. 64, 1084–1098.

Kobashi, K., Harada, T., Adachi, Y., Mori, M., Ihara, M., Hayasaka, D., 2017. Comparative ecotoxicity of imidacloprid and dinotefuran to aquatic insects in rice mesocosms. Ecotoxicol. Environ. Saf. 138, 122–129.

Kreutzweiser, D.P., Good, K.P., Chartrand, D.T., Scarr, T.A., Thompson, D.G., 2008. Toxicity of the systemic insecticide, imidacloprid, to forest stream insects and microbial communities. Bull. Environ. Contam. Toxicol. 80 (3), 211–214.

Kwok, K.W.H., Leung, K.M.Y., Lui, G.S.G., Chu, V.K.H., Lam, P.K.S., Morritt, D., Maltby, L., Brock, T.C.M., Van den Brink, P.J., Warne, M.S.J., Crane, M., 2007. Comparison of tropical and temperate freshwater animal species' acute sensitivities to chemicals: implications for deriving safe extrapolation factors. Integr. Environ. Assess. Manag. 3 (1), 49–67.

Laabs, V., Wehrhan, A., Pinto, A., Dores, E., Amelung, W., 2007. Pesticide fate in tropical wetlands of Brazil: an aquatic microcosm study under semi-field conditions. Chemosphere 67, 975–989.

Lavine, B.K., Ding, T., Jacobs, D., 2010. LC-PDA-MS studies of the photochemical degradation of imidacloprid. Anal. Lett. 43, 1812–1821.

Lewis, K.A., Tzilivakis, J., Warner, D.J., Green, A., 2016. An international database for pesticide risk assessments and management. Hum. Ecol. Risk Assess. 22, 1050–1064.

Lu, Z., Challis, J.K., Wong, C.S., 2015. Quantum yields for direct photolysis of neonicotinoid insecticides in water: implications for exposure to nontarget aquatic organisms. Environ. Sci. Technol. Lett. 2, 188–192.

Malev, O., Sauerborn Klobučar, R., Fabbretti, E., Trebše, P., 2012. Comparative toxicity of imidacloprid and its transformation product 6-chloronicotinic acid to non-target aquatic organisms: microalgae *Desmodesmus subspicatus* and amphipod *Gammarus fossarum*. Pestic. Biochem. Physiol. 104, 178–186.

Mohr, S., Berghahn, R., Schmiediche, R., Hubner, V., Loth, S., Feibicke, M., Mailahn, W., Wogram, J., 2012. Macroinvertebrate community response to repeated short-term pulses of the insecticide imidacloprid. Aquat. Toxicol. 110, 25–36.

Morrissey, C.A., Mineau, P., Devries, J.H., Sanchez-Bayo, F., Liess, M., Cavallaro, M.C., Liber, K., 2015. Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: a review. Environ. Int. 74, 291–303.

Organisation for Economic Co-operation and Development (OECD), 2004. OECD Guideline for Testing of Chemicals: *Daphnia* sp. Acute Immobilisation Test. No. 202. Paris, France.

Pestana, J.L.T., Alexander, A.C., Culp, J.M., Baird, D.J., Cessna, A.J., Soares, A.M.V.M.,

2009. Structural and functional responses of benthic invertebrates to imidacloprid in outdoor stream mesocosms. Environ. Pollut. 157, 2328–2334.
Posthuma-Doodeman, C.J.A.M., 2008. Environmental Risk Limits for Imidacloprid. Letter report 601716018/2008. National Institute for Public Health and the Environment, Bilthoven, The Netherlands.
Rahman, S., 2013. Pesticide consumption and productivity and the potential of IPM in Bangladesh. Sci. Total Environ. 445, 48–56.
Rico, A., Dimitrov, M.R., Van Wijngaarden, R.P.A., Satapornvanit, K., Smidt, H., Van den Brink, P.J., 2014. Effects of the antibiotic enrofloxacin on the ecology of tropical eutrophic freshwater microcosms. Aquat. Toxicol. 147, 92–104.
Roessink, I., Merga, L.B., Zweers, H.J., Van den Brink, P.J., 2013. The neonicotinoid imidacloprid shows high chronic toxicity to mayfly nymphs. Environ. Toxicol. Chem. 32, 1096–1100.
Rubach, M.N., Crum, S.J.H., Van den Brink, P.J., 2011. Variability in the dynamics of mortality and immobility responses of freshwater arthropods exposed to chlorpyrifos. Arch. Environ. Contam. Toxicol. 60, 708–721.
Sánchez-Bayo, F., Goka, K., 2006. Influence of light in acute toxicity bioassays of imidacloprid and zinc pyrithione to zooplankton crustaceans. Aquat. Toxicol. 78, 262–271.
Sanchez-Bayo, F., Hyne, R.V., 2011. Comparison of environmental risks of pesticides between tropical and nontropical regions. Integr. Environ. Assess. Manag. 7, 577–586.
Sarma, S.S.S., Nandini, S., Gulati, R.D., 2005. Life history strategies of cladocerans: comparisons of tropical and temperate taxa. Hydrobiologia 542 (1), 315–333.
Shahjahan, M., Kabir, M.F., Sumon, K.A., Bhowmik, L.R., Rashid, H., 2017. Toxicity of organophosphorous pesticide sumithion on larval stages of stinging catfish *Heteropneustes fossilis*. Chin. J. Oceanol. Limnol. 35, 109–114.
Song, M.Y., Stark, J.D., Brown, J.J., 1997. Comparative toxicity of four insecticides, including imidacloprid and tebufenozide to four aquatic arthropods. Environ. Toxicol. Chem. 16, 2494–2500.
Starner, K., Goh, K.S., 2012. Detections of the neonicotinoid insecticide imidacloprid in surface waters of three agricultural regions of California, USA, 2010–2011. Bull. Environ. Contam. Toxicol. 88, 316–321.
Stoughton, S.J., Liber, K., Culp, J., Cessna, A., 2008. Acute and chronic toxicity of imidacloprid to the aquatic invertebrates *Chironomus tentans* and *Hyalella azteca* under constant- and pulse exposure conditions. Arch. Environ. Contam. Toxicol. 54, 662–673.
Sumon, K.A., Rico, A., Ter Horst, M.M.S., Van den Brink, P.J., Haque, M.M., Rashid, H., 2016. Risk assessment of pesticides used in rice-prawn concurrent systems in Bangladesh. Sci. Total Environ. 568, 498–506.
Ter Braak, C.J.F., Šmilauer, P., 2012. Canoco Reference Manual and User's Guide: Software for Ordination (Version 5.0). Microcomputer Power, Ithaca, NY, USA, p. 496.
Thuyet, D.Q., Watanabe, H., Yamazaki, K., Takagi, K., 2011. Photodegradation of imidacloprid and fipronil in rice-paddy water. Bull. Environ. Contam. Toxicol. 86, 548–553.
Tišler, T., Jemec, A., Mozetič, B., Trebše, P., 2009. Hazard identification of imidacloprid to aquatic environment. Chemosphere 76, 907–914.
Van den Brink, P.J., Hattink, J., Bransen, F., Van Donk, E., Brock, T.C.M., 2000. Impact of the fungicide carbendazim in freshwater microcosms. II. Zooplankton, primary producers and final conclusions. Aquat. Toxicol. 48, 251–264.
Van den Brink, P.J., Ter Braak, C.J.F., 1999. Principal response curves: analysis of time dependant multivariate responses of a biological community to stress. Environ. Toxicol. Chem. 18, 138–148.
Van den Brink, P.J., Van Smeden, J.M., Bekele, R.S., Dierick, W., De Gelder, D.M., Noteboom, M., Roessink, I., 2016. Acute and chronic toxicity of neonicotinoids to nymphs of a may fly species and some notes on seasonal differences. Environ. Toxicol. Chem. 35, 128–133.
Van Dijk, T.C., Van Staalduinen, M.A., Van der Sluijs, J.P., 2013. Macro-invertebrate decline in surface water polluted with imidacloprid. PLoS One 8 (5), e62374.
Vijver, M.G., Van den Brink, P.J., 2014. Macro-invertebrate decline in surface water polluted with imidacloprid: a rebuttal and some new analyses. PLoS One 9 (2), e89837.
Williams, D.A., 1972. The comparison of several dose levels with zero dose control. Biometrics 28, 519–531.