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Aquatic toxicity of ivermectin in cattle dung assessed using microcosms

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ABSTRACT

Ivermectin (IVM) is a parasiticide widely used for livestock. It is a semisynthetic derivative of avermectin, a macrocyclic lactone produced by *Streptomyces avermitilis*. This drug is only partly metabolized by livestock; considerable amounts of parent drug are excreted mostly via feces. To simulate exposure of aquatic invertebrates and macrophytes to direct excretion of cattle dung into surface waters, a microcosm experiment with IVM spiked in cattle dung was conducted. The objectives of this study were to characterize accumulation of IVM in water, sediment + dung, roots of the floating fern *Salvinia* and the zooplankton *Ceriodaphnia dubia*, the amphipod *Hyalella* and the apple snail *Pomacea*; to determine the effect of this drug spiked in cattle dung on life-history traits of these invertebrates; and to evaluate the influence of IVM on aquatic nutrient cycling. Dung was spiked with IVM to attain concentrations of 1150, 458, 50 and 22 μg kg^{-1} dung fresh weight, approximating those found in cattle dung at days 3, 7, 16 and 29 following subcutaneous injection. Concentrations found in dung during the first week of excretion were lethally toxic to *Ceriodaphnia dubia* and *Hyalella*, whereas no mortality was observed in *Pomacea*. Concentrations of IVM in roots, sediment + dung and *Pomacea* increased significantly from the lowest to the highest treatment level. The effect of this drug on decomposition and release of nutrients from dung would have negative consequences for nutrient cycling in water. Increasing concentrations in sediment + dung with days of the experiment suggested that toxic concentrations would persist for an extended period in the water–sediment system. IVM represents an ecological risk for aquatic ecosystems, underscoring the need for livestock management strategies to limit its entry into water bodies.

1. Introduction

Ivermectin (IVM) has been widely used as veterinary parasiticide for more than two decades (Shoop and Soll, 2002; Õmura, 2008). It is a semisynthetic derivative of avermectin, a macrocyclic lactone produced by *Streptomyces avermitilis* (Campbell et al., 1983). It acts by interfering with glutamate-gated or γ-aminobutyric acid related chloride channels in synapse membranes (Campbell et al., 1983; Duce and Scott, 1985; Cully et al., 1994). Being highly effective against a variety of nematodes and arthropods (insects, ticks and mites), IVM is administered as endo and ecto parasiticide to livestock, such as cattle, pigs, sheep and horses (Strong and Brown, 1987; Shoop et al., 1995; Õmura, 2008). Generally, IVM is only partly metabolized by livestock. As a consequence, considerable amounts of parent drug are excreted, mostly via feces (Halley et al., 1989; Hennessy and Alvinerie, 2002). The high percentage of elimination of the drug via feces causes several environmental problems (Liebig et al., 2010). One of the most important is related to the persistence of IVM in the environment (Kövecses and Marcogliese, 2005). The parasiticide is very persistent in cattle dung with 10–60% of the initially measured IVM concentration still present after 180 days in a field study in Argentina (Suarez et al., 2003). The persistence of this drug in dung could pose a risk for a wide variety of terrestrial insects colonizing and consuming dung pats (Wardhaugh and Beckmann, 1996; Iglesias et al., 2006), potentially limiting the rate of return of nutrients in dung to the soil (Strong and James, 1993; Petney, 1997).

IVM has also been identified as a risk for aquatic ecosystems (Davies et al., 1998), and has been considered of high priority for further environmental monitoring and risk assessment (Boxall et al., 2003). This drug sorbs strongly to soil and has a low potential for leaching (Boxall et al., 2003). As a result, erosion of particulate matter containing IVM and direct excretion by treated pasture animals into water bodies represent the most important routes of IVM entry into the freshwater

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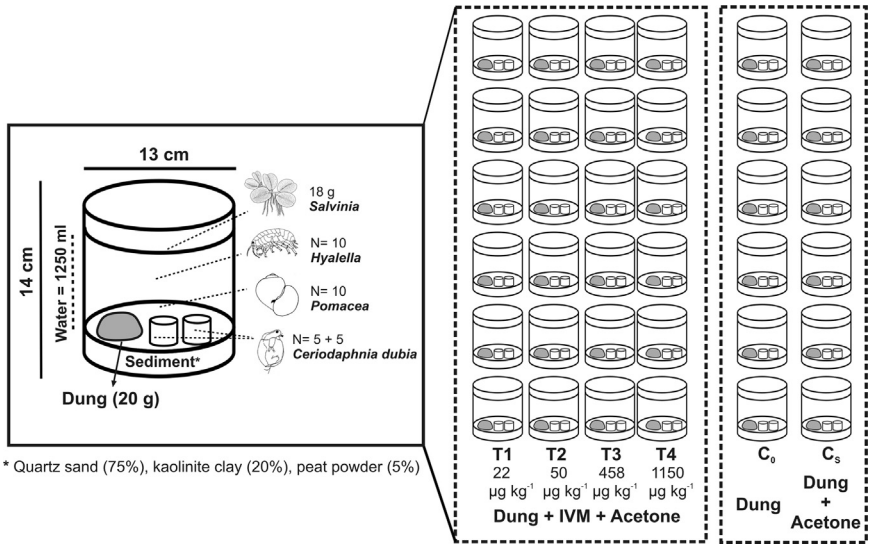

Fig. 1. Experimental design of the water-sediment test system. The four treatments (T1-T4) varied in the concentrations of ivermectin (IVM) in the added dung. IVM-free controls contained either dung alone (C_0) or dung + solvent (C_s).

environment (Kövecses and Marcogliese, 2005).

Aquatic invertebrates are thus more likely to be exposed to IVM by consumption of particulate matter than by bioaccumulation of dissolved IVM. However, only a few studies have addressed IVM exposure of aquatic benthic invertebrates via sediment (Thain et al., 1997; Davies et al., 1998; Allen et al., 2007; Egeler et al., 2010), and only one study has addressed exposure via feces (Schweitzer et al., 2010). In addition, the effect of this drug on decomposition of dung in aquatic systems, and the consequences for nutrient cycling, are completely unknown.

Land use in the Middle Paraná River floodplain in Argentina has changed significantly in recent decades, as the expansion of upland soybean production has forced the relocation of cattle to marginal floodplain sites, increasing the stocking density significantly (PROSAP, 2009; Quintana et al., 2014). Injection of cattle with IVM has been a practical and accessible tool for parasite control in this region. The massive administrations of IVM to cattle and direct contact with seasonally inundated wetlands following injection have raised concerns about the risk of ecotoxicity of the active drug excreted in the floodplain environments.

To examine the potential toxicity of IVM in cattle dung to freshwater invertebrates, a four-species water–sediment microcosm experiment was performed to expose representative invertebrates to cattle dung spiked with IVM. The objectives of this study were to (1) characterize accumulation of IVM in water, sediment + dung, roots of *Salvinia* and planktonic *Ceriodaphnia dubia*, pleustonic *Hyalella* and benthic *Pomacea*; (2) determine the effect of this drug spiked in cattle dung on life-history traits (survival, growth, and reproduction) of these invertebrates; and (3) evaluate the influence of IVM on nutrient cycling in water. We hypothesized that IVM would accumulate in sediments, plants, and invertebrates at concentrations that would be toxic to at least some species, and that it would reduce the rate of natural nutrient regeneration from the dung.

2. Materials and methods

2.1. Test organisms

Each microcosm consisted of a vessel containing water, sediment, a small floating aquatic fern (*Salvinia* sp.) and three invertebrates – the zooplanktonic microcrustacean *Ceriodaphnia dubia* (Crustacea: Branchiopoda), the amphipod *Hyalella* sp. (Crustacea: Amphipoda), and the apple snail *Pomacea* sp. (Mollusca: Gastropoda). These invertebrates were selected as representative of planktonic, pleustonic and benthic taxa of floodplain water bodies along the Middle Paraná River, respectively. *Salvinia* sp. was included as a widely distributed macrophyte in wetlands of this floodplain system. All these taxa were taken from our own stock cultures. Water temperatures averaged from 25 ± 1 °C in the cultures. *C. dubia* were fed with *Chlorella* sp. ad libitum, *Hyalella* sp. with Tetramin® fish food, and snails with romaine lettuce every day before the initiation of the experiments.

2.2. Spiking dung with IVM

Fresh cattle dung used in the experiments was collected near wetlands of the Middle Paraná River system where cattle congregate to sleep. Dung collection was done before the injection of cattle with IVM in order to ensure minimum concentration of this drug. Dung was homogenized and kept refrigerated until the initiation of the experiment.

IVM (CAS-No. 70288-86-7; 94% IVM B1a, 2.8% IVM B1b) was obtained from Sigma-Aldrich GmbH (Taufkirchen, Germany; lot no. 051K1374). Stock solutions and dilutions in cattle dung were prepared with acetone as solvent. Twenty grams of cattle dung was spiked with different concentrations of IVM. The applied nominal IVM concentrations were 1150 (T4), 458 (T3), 50 (T2) and 22 µg kg^{-1}(T1) dung fresh weight, corresponding approximately to those found in cattle dung at days 3, 7, 16 and 29 in studies conducted in Argentina following subcutaneous injection (Lifschitz et al., 2000; Suarez et al., 2003). The IVM solution was added on the surface of dung to obtain the afore mentioned nominal concentrations. Special care was taken to allow the complete absorption of the solution into the dung. Samples were left for 90 min to allow evaporation of the acetone.

2.3. Microcosm set-up

Six test vessels (glass flasks, 13 cm diameter, 14 cm height, 1.45 L volume) were prepared for each treatment (T1, T2, T3 and T4), six for the control (C_0) and six for the solvent control (C_s) (total = 36 test vessels) (Fig. 1). The artificial sediment consisted of kaolinite clay (20%), quartz sand (75%) and peat powder (5%). Individuals of *C. dubia* were placed inside two small flasks (6 cm height, 2 cm diameter, 30 ml volume, covered with a 50 µm mesh), to avoid the loss of these invertebrates and to enable the rapid visual inspection of the individuals in each vessel (Fig. 1). The experiment was carried out under a light regime of 12 h light/12 h dark at constant environmental conditions (continuously gently aerated water, water temperature 25 ± 1 °C), without food addition. During the course of the experiment, evaporated water was replaced by non-chlorinated water. Before the initiation of the experiment, ten *C. dubia* (five in each small flask), ten *Hyalella*, and ten *Pomacea* were introduced in each test vessel (Fig. 1).

Individuals of each taxon included in the experiment were selected according to approximate length criteria: *C. dubia* 0.7 ± 0.1 mm, *Hyalella* 3 ± 1 mm and *Pomacea* 10 ± 1 mm (shell diameter). Eighteen grams (wet weight) of *Salvinia* was included in each test vessel, covering 70% of its surface (Fig. 1).

2.4. Responses of invertebrate taxa

Thirty randomly selected individuals of each taxon were randomly taken for length measures before the initiation of the experiment (day 0). Survival, length and reproduction of *C. dubia* were recorded at day 7, whereas for *Hyalella* and *Pomacea*, these parameters were recorded at days 7 and 17. Each surviving *C. dubia* individual was observed under a microscope, and length was determined from the head just above the compound eye to the base of the tail spine. This measure enabled the categorization of the stage of each individual (neonates, adults) in order to determine if reproduction occurred in the experimental units. After 7days of exposure, individuals of *Hyalella* were collected from three replicates of the controls and treatments by passing the sediment and water through a strainer. Length was measured by taking high-resolution photographs of each individual under a stereoscopic microscope and digitizing them using the TPSdig2 program (Rohlf, 2006). On the same day, five individuals of *Pomacea* were removed from each treatment and controls (n = 30 in either one) and shell length was measured according to Boulding and Hay (1993). Mortality was determined when snails failed to maintain the operculum closed. By this day, *Pomacea* had eaten almost half of the initial weight of *Salvinia* included in the experimental units. The remaining *Salvinia* in the test vessel was collected, washed with non-chlorinated water, and roots were separated from others part of the plant and preserved for IVM analysis.

At the end of the experiment (day 17), all individuals of *Hyalella* and *Pomacea* were sieved from the sediment using a 200 μm mesh to determine the number of surviving individuals and their body lengths. After measuring shell length in *Pomacea* at day 7 and 17, snails were placed in distilled water for one day to flush undigested sediment out of the mantle cavity and gut, which would create an inaccuracy in the assessed body burden (King and Davies, 1987; Van Roon, 2000). Snails were frozen and later dissected into three parts (shell, foot, and viscera mass) following adaptation of a method by Gomot-de Vaufleury and Pihan (2002).

2.5. Analysis of IVM concentrations

Samples of dung collected in the field were analyzed for IVM to correct for background concentrations. Target IVM concentrations in samples of sediment + dung, roots of *Salvinia*, and water were collected from three test vessels of controls and treatments at the middle (day 7) and final day of the experiment (day 17). For each control and treatment, whole body samples of *C. dubia* and *Hyalella* and visceral mass of the extracted snails collected on each day were separately pooled and preserved at −20 °C for analyses of IVM accumulation. The extraction of IVM from experimental samples and HPLC analysis were carried out following the technique first described by Lifschitz et al. (2000). Samples were weighed, homogenized and combined with the internal standard compound (abamectin). One milliliter of acetonitrile was added and the preparation was mixed (Multi Tube Vortexer, VWR Scientific Products, West Chester, PA, USA) for15 min. The solvent-sample mixture was centrifuged at 2000*g* for 15 min. The supernatant was then placed on the appropriate rack of an Aspec XL sample processor (Gilson, Villiers Le Bel, France) to perform the solid-phase extraction. The derivatization of MLs was done with 100 μl of a solution of N-methylimidazole (Sigma Chemical, St Louis, MO, USA) in acetonitrile (1:1) and 150 μl of trifluoroacetic anhydride (Sigma Chemical, St Louis, MO, USA) solution in acetonitrile (1:2). After completion of the reaction (< 30 s), an aliquot (100 μl) of this solution was injected directly into the HPLC system. IVM concentrations were determined by HPLC using a Shimadzu 10 A HPLC system with autosampler (Shimadzu Corporation, Kyoto, Japan). HPLC analysis was undertaken using a reverse phase C_{18} column (Kromasil, Eka Chemicals, Bohus, Sweden, 5 μm, 4.6 mm × 250 mm) and an acetic acid 0.2% in water/methanol/acetonitrile (1.6/60/38.4) mobile phase at a flow rate of 1.5 ml/min at 30 °C. IVM was detected with a fluorescence detector (Shimadzu, RF-10 Spectrofluorometric detector, Kyoto, Japan), reading at 365 nm (excitation) and 475 nm (emission wavelength). Percent recovery was estimated as the difference between the target concentration values of IVM spiked in dung and those measured by the HPLC analysis.

2.6. Physico-chemical variables

The pH, conductivity (corrected to 25 °C), dissolved oxygen (all measured with a Hanna meter) and water temperature (standard thermometer) were determined daily during the experiment in all test vessels at the same time of the day. Ten milliliters of subsurface water were obtained from the controls and treatments for nutrient analyses. Water samples were taken before the beginning of the experiment and after two days. Water was immediately filtered through Whatman GF/F glass-fiber filters and refrigerated until determination of dissolved components within 24 h after sampling. Soluble reactive phosphorus (SRP) was determined by the ascorbic acid method (Murphy and Riley, 1962), nitrate + nitrite (NO_3^- + NO_2^-) by reduction of NO_3^- with hydrazine sulfate and subsequent colorimetric determination of NO_2^- (Hilton and Rigg, 1983), and total ammonia (NH_4^+ + NH_3) by the indophenol blue method (Koroleff, 1970). Concentrations of unionized ammonia (NH_3) were estimated from pH and water temperature according to Emerson et al. (1975).

2.7. Data analyses

Mean accumulation of IVM in each individual of *Pomacea* was calculated as the product of the visceral mass (in grams) and measured concentration of IVM in the viscera. In addition, percent accumulation of IVM in *Pomacea* in each treatment was calculated as the ratio between the total accumulated in the visceral tissue and the nominal IVM added to each treatment in dung.

One-way ANOVA was used to compare accumulated IVM in water, sediment and roots among treatments and between days in each treatment, as well as survival, length and reproduction of *C. dubia, Hyalella* and *Pomacea* between controls and treatments. Data normality was tested with the Kolmogorov–Smirnov goodness-of-fit test, and homogeneity of variance was assessed with Cochran's test. To determine if there was a significant effect of IVM on nutrient cycling, the relation between the peak observed nutrient concentration in the water and the concentration of IVM spiked in dung was examined by Spearman correlation. Only the solvent control (C_s) was considered for this analysis in order to determine the effect of IVM instead of the effect of IVM + acetone. All tests were performed at the 5% level of significance using the R software.

3. Results

3.1. Accumulation of IVM in water, sediment and invertebrates

For all treatment levels, measured IVM concentrations in freshly prepared dung samples were in good agreement with target concentrations. Recovery rates (measured concentrations as % of the target concentrations) ranged from 77% to 100%. IVM was not detected in the control samples (Table 1). Table 2 shows the concentrations measured in water, sediment, roots of *Salvinia*, and invertebrates at days 7 and 17 of the experiment. No IVM was detected in any of the water samples. Accumulation of samples of sediment + dung in each treatment increased with increasing target concentrations of IVM in both days (RHO = 0.70, $P < 0.01$). At the middle of the experiment, IVM was only

Table 1
Measured concentrations of ivermectin (IVM) added to dung samples used in the experiment. ND = not detected.

Target concentration of IVM (μg kg^{-1} dung wet weight)	Measured concentration of IVM	
	(μg kg^{-1} dung wet weight)	Recovery (% of target)
0 (C_0)	ND	–
0 (Cs)	ND	–
22 (T1)	23.1 (6.0)	100
50 (T2)	38.7 (6.0)	77
458 (T3)	358 (19.8)	78
1150 (T4)	918 (20)	80

detected in sediment + dung at the highest treatment level (1150 μg kg^{-1}, Table 2). In addition, at day 17, increasing concentrations in sediment + dung were detected in all treatments except at the lowest concentration. Accumulation of IVM in sediment + dung at the end of the experiment increased significantly, reaching levels 100 times higher at the highest treatment level in relation to day 7 (Table 2). Values of IVM in roots of *Salvinia* increased significantly from the lowest to the highest treatment level (RHO = 0.90, $P < 0.001$).

Among the test invertebrates, IVM was only detected in *Pomacea*, in which concentrations showed a significant positive linear relation with target concentrations of IVM in dung (RHO = 0.86, $P < 0.001$, Table 2). Mean accumulation of IVM in *Pomacea* increased from the lowest to the highest treatment level on both sampling days (Fig. 2A). At the lowest IVM treatment level, accumulation was significantly higher at day 17 in comparison with day 7 (ANOVA, F = 5.1, $P < 0.05$), whereas in the highest treatment (T4), IVM was higher at day 7 than at day 17 (ANOVA, F = 7.9, $P < 0.001$). In each day, significant differences among treatments were found except in T1 (Fig. 2A). In addition, percent accumulation (%IVM) in *Pomacea* for each treatment at days 7 and 17 is shown in Fig. 2B. At the lowest and highest treatment levels, %IVM differed significantly among days 7 and 17 (ANOVA, $P < 0.001$). In T1, %IVM was significantly higher at day 17 in comparison with the first week of the experiment (ANOVA, F = 315, $P < 0.001$), whereas in T4%IVM was significantly higher at day 7 than 17 (ANOVA, F = 21.4, $P < 0.0001$). At the first week of the experiment, comparisons of % IVM showed significant differences between all treatments (ANOVA, $P < 0.001$) except for T1 and T4. In addition, at day 17, %IVM in T1 differed significantly from other treatments (ANOVA, $P < 0.001$) (Fig. 2B).

3.2. Effect of IVM on invertebrates

IVM was highly toxic to *C. dubia* by day 7: survival was 44% at a dung IVM concentration of 22 μg kg^{-1} and 50% at 50 μg kg^{-1}, whereas no survival was detected at the highest concentrations (458, 1150 μg kg^{-1}) (Table 3). No effect of IVM was observed on growth of this taxon; body length was similar in controls and treatments (Table 3). Reproduction of *C. dubia* was detected in the control C_s (ANOVA, $P < 0.05$). No significant difference was detected between treatments (T1, T2) and controls (C_0, C_S) in reproduction of *C. dubia* (F = 1.4, $P > 0.05$) (Table 3). By day 17, survival of *Hyalella* was not affected by IVM at the concentration of 22 μg kg^{-1} in dung but 50% mortality was observed at 50 μg kg^{-1}, and no survival was detected at the highest concentrations (458 and 1150 μg kg^{-1}) (Table 3). No significant effect of IVM was detected on growth of *Hyalella*: length increased over the experiment at the same rate in controls and treatments (ANOVA, F = 80.4, $P < 0.001$) (Table 3). Reproduction of *Hyalella* was not observed. There was no significant effect of IVM on survival, growth or reproduction of *Pomacea* during the experiment (Table 3).

3.3. Nutrient cycling implications

Concentrations of NH_4^+, NH_3, NO_3^- + NO_2^-, and SRP in water showed similar trends in the solvent control Cs and IVM treatments during the experiment (ANOVA, $P > 0.05$) (Fig. 3). Concentrations of NH_4^+ + NH_3 and NH_3 peaked on day 1 (ANOVA, F = 58.9 and 10.4 respectively, $P < 0.001$), remained high until day 4, and decreased thereafter (Fig. 3A and B). The increase in concentration of NO_3^- + NO_2^- delayed relative to NH_4^+ + NH_3 and NH_3, with the highest values on day 4 (ANOVA, F = 5.4, $P < 0.001$) (Fig. 3C). Concentrations of SRP progressively increased from the beginning to day 13 of the experiment (ANOVA, F = 80.9, $P < 0.001$), then decreased slightly (Fig. 3D).

The correlations between IVM concentrations in dung and the peak observed nutrient concentrations in water in the experiment were statistically significant only for NO_3^- + NO_2^- (RHO = −0.76, < 0.001). Water temperature, pH, conductivity and dissolved oxygen remained constant throughout the study across all treatments, with values of 25 ± 1 °C, 7.5 ± 0.3, 500 ± 50 μS cm^{-1}, and 7.2 ± 0.5 mg L^{-1} respectively.

4. Discussion

The results of this work showed that IVM represents a risk for aquatic invertebrates representative of the floodplain of the Middle Paraná River. Concentrations commonly found in dung during the first week of excretion of cattle following injection with IVM produced the complete mortality of *C. dubia* and *Hyalella* populations. In addition, to our knowledge, this is the first study to report accumulation of IVM in *Pomacea* and macrophytes in a water-sediment system, and to show the potential implications for nutrient cycling.

Ceriodaphnia dubia was highly sensitive to IVM in cattle dung. Complete mortality of this taxon was observed at typical concentrations of IVM in dung 3–7 days following cattle injection. The ingestion of fine particulate organic matter derived from dung with IVM, and/or of algae that had accumulated IVM, might have been lethal for this taxon. This result has also been found in other studies (Tišler and Eržen, 2006; Schweitzer et al., 2010), providing additional evidence for a toxic effect of IVM for crustacean zooplankton. Similar to *C. dubia*, the two highest IVM concentrations in dung (458 and 1150 μg kg^{-1}) caused a complete extinction of *Hyalella*. In the Middle Paraná River system, *Hyalella* feeds as a collector-gatherer, consuming mainly vascular-plant detritus and

Table 2
Concentrations of IVM in water, sediment, roots of *Salvinia*, and invertebrates on days 7 and 17 of the experiment. ND= not detected; the detection limit was 0.5 ng g^{-1}.

	Day 7				Day 17			
	22	50	458	1150	22	50	458	1150
Water (ng g^{-1})	ND	ND	ND	ND	ND	ND	ND	ND
Sediment (ng g^{-1})	ND	ND	ND	1.46 (0.9)	ND	3.0 (1.7)	9.2 (0.8)	119.4 (41.3)
Roots of *Salvinia* sp. (ng g^{-1})	ND	2.97 (1.7)	45.2 (2.0)	81.7 (1.5)				
Ceriodaphnia dubia (ng g^{-1})	ND	ND	ND	ND	ND	ND	ND	ND
Hyalella sp. (ng g^{-1})	ND	ND	ND	ND	ND	ND	ND	ND
Pomacea sp. (ng g^{-1})	5.28 (1.53)	8.2 (1.0)	51.3 (6.6)	233 (29.7)	19.3 (0.6)	7.1 (2.3)	61.2 (0.57)	157.8 (45.5)

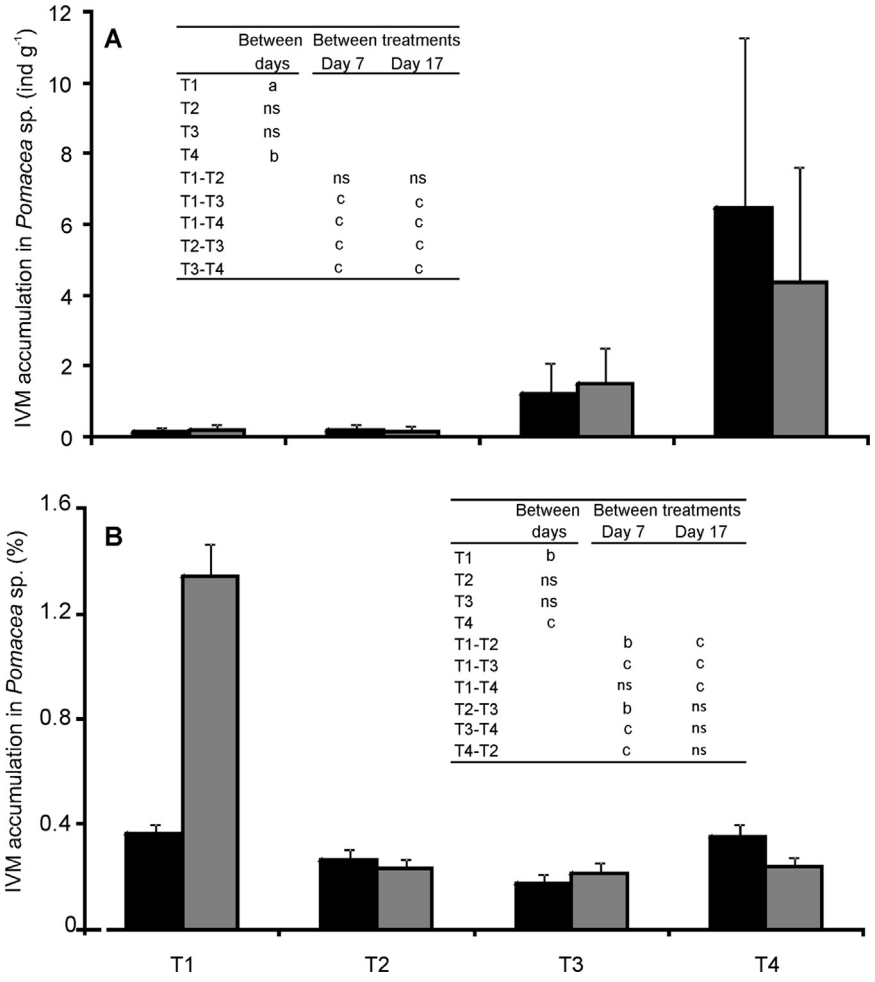

Fig. 2. Mean accumulation of ivermectin (IVM) in *Pomacea* (ng ind^{-1}) (A), and percent accumulation (B) for each treatment at day 7 (black bars) and 17 (grey bars) of the experiment. Results of ANOVA tests between days and between treatments in each day of the experiment are shown. a = $P < 0.05$; b = $P < 0.01$; c = $P < 0.001$.

preferring material with some degree of decomposition due to its higher digestibility (Saigo et al., 2009). Dung enters into aquatic system as a partially decomposed organic matter, constituting a possible food source for gatherer-collectors such as *Hyalella* (Del Rosario et al., 2002; Mesa et al., 2016).

Concentrations of IVM in sediment + dung reported here are similar to those used in the experimental study of Schweitzer et al. (2010), who also spiked dung with IVM. We observed that concentrations in sediment + dung increased with days of the experiment at all treatment levels. Dung containing IVM would be incorporated into sediment through physical disaggregation and decomposition. These observations also suggest that toxic concentrations would persist for an extended period in the water–sediment system. Persistence of IVM in sediment was also observed in other studies of aquatic environments (Sanderson et al., 2007; Boxall et al., 2006), as well as when IVM was experimentally added to sediment (Davies et al., 1998; Egeler et al., 2010), and spiked in dung (Schweitzer et al., 2010).

The high accumulation of IVM in roots of *Salvinia* showed that aquatic plants could play a significant role in removal of IVM from the water column and its transfer to herbivores. Some other studies have documented great potential for aquatic plants to bioaccumulate and metabolize certain lipophilic organic pollutants (Miglioranza et al., 2004; Schreiber et al., 2013). The accumulation of IVM in floating aquatic plants that are readily harvestable suggests that they could be used in phytoremediation of IVM-contaminated water bodies.

Pomacea had a high capacity to accumulate IVM over just 7d of exposure. This taxon has a broad food spectrum (Estebenet, 1995; Cowie and Hayes, 2012). Snail herbivory of algae is well established (e.g. Steinman, 1996), but snails also consume macrophytes (Sheldon, 1987; Newman, 1991). In our experiment, *Pomacea* was observed eating roots and leaves of *Salvinia*, eliminating this macrophyte by day 7 of the experiment. In addition, some snails were observed on the walls of the test vessels feeding on adhering algae, and on the bottom apparently feeding on sediment + dung. These food sources would represent different pathways of absorption of IVM from contaminated organic matter, vascular plants, and algae. In addition, direct consumption of dung is a possible food source for *Pomacea* (Mesa et al., 2016).

Pomacea exposed to the two lowest concentrations of IVM in dung showed low value of accumulation in the visceral mass (Fig. 2A). However, the *Pomacea* in the highest treatment level showed higher accumulation. The accumulation of a contaminant is the difference between intake and elimination, and is mediated by several physiological processes (Streit, 1992; Mackay and Fraser, 2000). The accumulation of a substance is not always proportional to the exposure concentration, as detoxification processes may increase elimination of the substance, thus reducing accumulation. However, detoxification processes may not be completely effective at high exposure concentrations, and the ability to control the accumulation may be compromised. *Pomacea* was able to regulate the internal IVM concentration up to a threshold exposure level beyond which the accumulation was directly proportional to IVM exposure.

Based on the results of this study and considering cattle dung as the source of contamination, a hypothetical pathway of bioaccumulation of IVM in aquatic invertebrates is shown in Fig. 4. IVM enters water bodies with dung and remains in the sediment for a long time (Schweitzer et al., 2010). Some fraction of the IVM gradually becomes dissolved in the overlying water. Since IVM is highly hydrophobic, it is rapidly

Table 3
Survival, mean length (SD) and reproduction of *Ceriodaphnia dubia*, *Hyalella* sp. and *Pomacea* sp. on days 7 and 17 of the experiment at the different concentrations of ivermectin (IVM) in cattle dung.

Concentration of IVM (μg kg^{-1})	*Ceriodaphnia dubia*					
		Length (mm)		Reproduction	Abundance	
	Survival (%)	Day 0	Day 7		Day 0	Day 7
0 (C_0)	100	0.72 (0.13)	0.77 (0.15)	yes	5	8.8 (8.7)
0 (Cs)	100		0.8 (0.07)	yes		16.2 (9.9)*
22 (T1)	44 (46)		0.77 (0.17)	yes		12.8 (24)
50 (T2)	50 (54)		0.83 (0.13)	yes		7.3 (9.9)
458 (T3)	0					
1150 (T4)	0					
Concentration of IVM (μg kg^{-1})	*Hyalella* sp.					
	Survival (%)	Length (mm)			Reproduction	
		Day 0	Day 7	Day 17		
0 (C_0)	100	3.6 (0.5)	4.6 (0.3)	5.3 (0.4)***	–	
0 (Cs)	100		4.9 (0.9)	6.1 (0.4)***	–	
22 (T1)	100		5.03 (0.5)	5.6 (0.8)***	–	
50 (T2)	50 (45)		4.6 (0.6)	5.4 (0.9)***	–	
458 (T3)	0		4.7 (0.5)		–	
1150 (T4)	0		4.7 (0.3)		–	
Concentration of IVM (μg kg^{-1})	*Pomacea* sp.					
	Survival (%)	Length (mm)			Reproduction	
		Day 0	Day 7	Day 17		
0 (C_0)	100	10.3 (0.3)	10.2 (1.2)	10.8 (0.6)	–	
0 (Cs)	100	10.2 (0.4)	11.6 (0.8)	10.5 (0.9)	–	
22 (T1)	100	10.5 (0.4)	11.0 (0.9)	11.1 (0.4)	–	
50 (T2)	100	10.1 (0.2)	11.8 (1.9)	10.9 (1.0)	–	
458 (T3)	100	10.0 (0.4)	11.0 (1.6)	10.6 (0.5)	–	
1150 (T4)	100	10.1 (0.3)	11.7 (0.9)	10.6 (0.3)	–	

ANOVA test, **P < 0.01.
* P < 0.05.
*** P < 0.001.

removed from the aqueous phase, and could be accumulated in macrophytes, algae or particulate organic matter present in the water column (Tišler and KožuhEržen, 2006; Liebig et al., 2010). Aquatic invertebrates could incorporate IVM by feeding on macrophytes, algae and particulate organic matter that had accumulated this compound.

IVM evidently did not produce any appreciable effect on ammonification of organic nitrogenous compounds, as also observed by Schweitzer et al. (2010). Concentrations of total ammonia peaked during the initial phase of the experiment in both controls and treatments (Fig. 3A). The total ammonia analysis measures the sum of NH_4^+ + NH_3 although concentrations of unionized NH_3—the potentially toxic form—were clearly below values reported to diminish survival of the studied taxa (Borgmann, 1994; Bailey et al., 2001; Sarma et al., 2003). The addition of dung, which has a nitrogen content of 0.2–0.5% (Cook et al., 1996), was the likely source of these high concentrations due to decomposition and ammonification of organic nitrogenous compounds.

The presence of IVM in dung affected nitrogen transformations. The peak in $NO_3^- + NO_2^-$ concentrations observed in our experiments subsequent to the NH_4^+ + NH_3 peak, likely reflects nitrification of the released ammonium. There was evidently a lower rate of nitrification in the high IVM treatments (Fig. 3C), suggesting suppression of nitrifying bacteria by IVM. Suppression of nitrification would profoundly affect nitrogen cycling because NH_4^+ is far less mobile in soils and sediments than NO_3^- and these forms are differentially available for assimilation by plants and microorganisms (Wetzel, 2001). Although the nitrification rate would have been decreased by IVM, NO_3^- + NO_2^- concentrations were very high in all treatments. In this respect, the continuous water aeration during the experiment could have favored oxidation reactions. In floodplain lakes, IVM inputs and oxygen depletion could have an additive or synergistic effect on nitrification limitation (Stenstrom and Poduska, 1980; Mayora et al., 2013).

There is no information in the literature about IVM effects on nitrifying bacteria, but several other drugs used in veterinary medicine have adverse effects on nitrification (Halling-Sørensen, 2001; Ollivier et al., 2010). The combined effect of IVM on nitrifying bacteria and its toxicity to collector-gatherer invertebrates could effectively result in delayed decomposition of dung and release of nutrients, with consequences for nutrient cycling in aquatic systems. Similar effects have been found in terrestrial systems, where IVM reduced rates of recycling of soil nutrients (Suarez et al., 2003; Iglesias et al., 2011).

The results presented here indicate that IVM should be considered a contaminant of high concern due to its potential to affect the survival of aquatic invertebrates as well as its effects on nutrient cycling. Bioaccumulation and biomagnification of IVM in food webs require more study but the observed accumulation of IVM in sediment, *Salvinia* and *Pomacea* is concerning because aquatic vascular plants and snails constitute a trophic source for a myriad of invertebrates, fishes, birds and mammals. This study showed that *Pomacea* is a strong IVM accumulator. *Pomacea* could serve as an IVM biomonitor because this snail is sedentary, easily collected, has enough mass for analysis, and is well suited to experimental assays (Bryan et al., 1980; Phipps et al., 1993).

The significant increase in cattle densities in the Middle Paraná River floodplain during recent decades combined with the increased use of IVM pose a potential threat to aquatic food webs that demands further study. We suggest field studies to develop livestock management strategies to limit the risk of environmental impacts. It may be appropriate, for example, to recommend that producers keep treated cattle away from waterbodies for at least a week following treatment to reduce the risk to the aquatic system.

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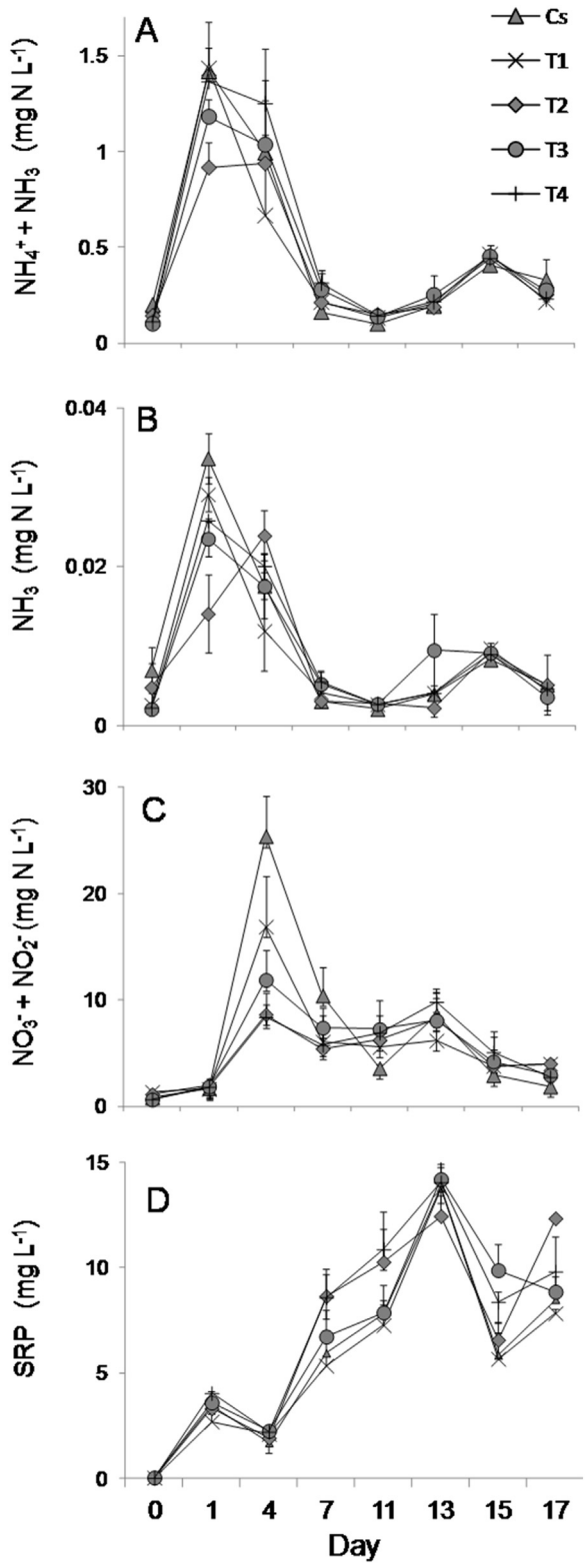

Fig. 3. Concentrations (mg N L^{-1}) of ammonium + ammonia (A), unionized ammonia (B), nitrate + nitrite (C), and soluble reactive phosphorus (C) in the solvent control (Cs) and treatments during the experiment. Vertical bars are standard errors based on three replicates.

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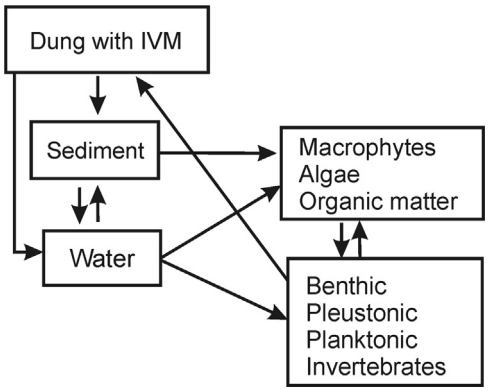

Fig. 4. Hypothetical pathway of accumulation of ivermectin (IVM) in aquatic food webs after its introduction via cattle dung into a water-sediment system.

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