

Effects of carbofuran and metsulfuron-methyl on the benthic macroinvertebrate community in flooded ricefields

Efeito do carbofurano e metsulfuron-methyl sobre a comunidade de macroinvertebrados bentônicos de lavoura de arroz irrigado

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Abstract: Aim: This study evaluated the effect of the insecticide carbofuran and the herbicide metsulfuron-methyl on the abundance and diversity of benthic macroinvertebrates in a paddy ricefield. **Methods:** To achieve this goal, two pesticide treatments [the insecticide carbofuran (IC) and the herbicide metsulfuron-methyl (HM)] and a control (Co) treatment with no added pesticide were established in an experimental area of the Plant Science Department of the Federal University of Santa Maria. Soil samples were collected in triplicate from each treatment 30 days before and 1, 10, and 51 days after the pesticide application, for macrofauna identification. **Results:** Among the 21 *taxa* identified, Trichoceridae was present only in Co, Hydroptilidae was recorded only in IC, and Corixidae in HM. In Co, the most abundant group was Annelida, while in IC and HM Diptera (Chironomidae) was dominant. Significant differences were observed between Co and IC, in the density of Odontoceridae (Control>IC) and Hydrophilidae (Control<IC), and between Co and HM in the density of Odontoceridae (Control>HM) and Hirudinea (Control<HM). **Conclusion:** In spite of the possible negative effects of the pesticides on the benthic community, the assemblages recovered rapidly.

Keywords: environmental impact, density, benthic community, biomonitoring, pesticides.

Resumo: Objetivo: O objetivo desse estudo foi avaliar o efeito do inseticida carbofurano e do herbicida metsulfuron-methyl na abundância e diversidade de macroinvertebrados bentônicos em campo de arroz irrigado. **Métodos:** Para isto, dois tratamentos com agrotóxicos [o inseticida carbofurano (IC) e o herbicida metsulfuron-methyl (HM)] e um controle (Co), tratamento sem pesticida, foram aplicados em uma área experimental no Departamento de Fitotecnia da Universidade Federal de Santa Maria. Amostras de solo foram coletadas em triplicatas em cada tratamento 30 dias antes (DBA) e 1, 10 e 51 dias após a aplicação dos pesticidas, posteriormente a macrofauna foi identificada. **Resultados:** Vinte e um (21) *taxa* foram identificados, entre esses Trichoceridae esteve presente apenas em Co, enquanto que Hydroptilidae foi coletado apenas em IC, e Corixidae em HM. Em Co e IC, o grupo mais abundante foi Annelida, e em HM foi Diptera (Chironomidae) que prevaleceu. Diferenças significativas foram observadas entre Co e IC, na densidade de Odontoceridae (Co>IC) e Hydrophilidae (Co<IC), e entre Co e HM na densidade de Odontoceridae (Co>HM) e Hirudinea (Co<HM). **Conclusão:** Entretanto, apesar dos possíveis efeitos negativos dos pesticidas sobre a comunidade bentônica verificados nos primeiros dias após a aplicação, verificou-se que de forma geral a comunidade recuperou-se rapidamente.

Palavras-chave: impacto ambiental, densidade, comunidade bentônica, biomonitoramento, agrotóxicos.

1. Introduction

Flooded rice is an important crop in southern Brazil. Ricefields cover an area of about 1 million hectares in the state of Rio Grande do Sul, where 60% of the national rice crop is produced (CONAB, 2006). During the rice-growing season from October to March (austral spring and summer), the flooding system creates immense shallow lakes in these lowland areas. In general, these conditions are ideal for the establishment of many aquatic organisms and others that live associated with them, but some are considered pests in rice crops, such as certain coleopterans (Curculionidae) and dipterans (Chironomidae), among others (Sosbai, 2010; Halwart, 2006).

To maximize rice yields, sustainable management of the crop is needed to control the main pests that limit rice growth and development. It is necessary to use pesticides, including herbicides, fungicides, and insecticides (Nakagome et al., 2006). In recent years, the insecticide carbofuran (2,3-dihydro-2-2 dimethyl-7- benzofuranyl methylcarbamate), commercial brand Furadan 100G[®], has been among the most often used. However, its fate in aquatic systems continues to be almost unknown (NRCC, 1979), and a better understanding of its effects on these ecosystems is necessary (Wayland and Boag, 1995). The herbicide metsulfuron-methyl {methyl 2[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]benzoate}, commercial brand Ally[®], is widely used in rice crops to control broadleaf weeds (Rodrigues and Almeida, 2005). Investigation of these pesticides is of the utmost importance for a better understanding of their dynamics in the environment.

For aquatic organisms, ricefields are an extreme environment with regard to the abiotic conditions (Fernando, 1993), and invertebrates need special adaptations to survive in this environment (Williams, 1987). According to Mesléard et al. (2005), although many studies have demonstrated the negative effects of pesticides on aquatic communities, they deal mainly with certain specific species of chironomids. Studies with macroinvertebrates in rice crops are still insufficient to fully clarify the cost-benefit relationships of using or not using these products.

Benthic macroinvertebrates are one of the main groups of organisms used to evaluate environmental impacts and for biological monitoring in aquatic ecosystems (Goulart and Callisto, 2003). These organisms spend all or part of their life cycles in the sediments of these environments, associated

with different substrates, both organic and inorganic. Therefore, the benthic macroinvertebrate communities express the ecological conditions of aquatic ecosystems in which they live (Morgan et al., 2006). Even though they are not natural wetlands, irrigated ricefields can be suitable for many aquatic organisms, thus contributing to the conservation of biodiversity (Halwart, 2006; Stenert et al., 2009, Machado and Maltchik, 2010). The aim of this study was to evaluate the impacts of the insecticide carbofuran and the herbicide metsulfuron-methyl on the benthic macroinvertebrate community in paddy ricefields. This study is justified by the fact that, even though it is known that pesticides can damage adjacent communities, it is still little understood how these communities, in this case the benthic community, specifically respond to these pesticides.

2. Material and Methods

The study was conducted in an experimental floodplain rice area of the Plant Science Department (Federal University of Santa Maria), during the 2005/06 season. The soil is a planosol with medium texture and flat relief, and the substrate is a recent alluvial deposit (EMBRAPA, 1999); the altitude is 95 m. The climate is humid subtropical, Cfa classification, with an annual mean rainfall of 1616 mm.

The experimental design consisted of three blocks (8 × 6 m each) with three treatments: Control (no treatment), carbofuran (IC) (Furadan 100 G[®], at a dose of 400 g.a.i.ha⁻¹), and metsulfuron-methyl (HM) (Ally[®], at a rate of 2 g.a.i.ha⁻¹), according to technical recommendations (Sosbai, 2005). In each treatment, three soil samples were taken randomly, using a core sampler (0.01 m² in area 0.1 m high). Sampling was carried out 30 days before the pesticide application (DBA), and 1, 10, and 51 days after the pesticide application (DAA). At 30 DBA, the rice seedlings were in the three-leaf growth stage; between 1 and 10 DAA, they were in an intermediate growth stage; and at 51 DAA, the grains were swelling. In the laboratory, the soil samples were washed on a 0.25 mm sieve, and the macroinvertebrates were placed in plastic bottles, stained with Rose Bengal, and after one hour were fixed with absolute ethyl alcohol. The material was identified at the lowest possible taxonomic level using a stereomicroscope and appropriate identification keys (Costa et al., 2006; Fernández and Domínguez, 2001; Pereira et al., 2000) and then stored in 70% ethyl alcohol.

During the experiments, pH was measured with a Hanna pH meter (HI 8424), and temperature and dissolved oxygen (DO) were measured with an YSI oxygen meter (model Y5512). The results for macroinvertebrate densities were submitted to a two-way Analysis of Variance (ANOVA), to evaluate the interaction between treatment and sampling time. The means were compared through a Tukey Test ($p < 0.05$) to determine the differences among the treatments. An ANOSIM was performed to determine whether the community composition was similar between treatments on the four sampling dates. The Bray-Curtis index was used to construct the similarity matrix. For these analyses, the programs PAST 1.82b (Hammer et al., 2001) and BioEstat 5.0 (Ayres et al., 2007) were used.

3. Results

A significant difference was found for DO among the treatments and the sampling dates, whereas pH and temperature only showed a difference among the sampling dates (Table 1). The concentration of dissolved oxygen was low, probably because of the shallow water that was maintained at a depth 10 cm throughout the experiment.

Twenty-one *taxa* were identified (Table 2). Of these, Diptera: Trichoceridae was recorded only in Co (30DBA), Trichoptera: Hydroptilidae was present only in IC (30DBA), and Hemiptera: Corixidae was found only in HM (51DAA). Of the other 17 *taxa*, Diptera: Chironomidae was abundant in all treatments and at all sampling times, as were annelids, represented by Oligochaeta. Probably, *taxa* were present that were either sensitive or tolerant to the pesticides tested. Thus, the sensitive *taxa* appeared in the control treatment, while the resistant ones were present in the treatments with pesticides. As an example, tabanid dipterans were present in the control and IC, but were recorded only in the 30DBA sampling, showing sensitivity to the herbicide (Table 2). Predators such as odonates were present in all three treatments during most

of the experiment, thus showing tolerance to the pesticides (Table 2). Some *taxa* were likely favored over others.

The ANOSIM showed differences in the community composition between treatments ($R = 0.278$); however, these differences were not significant ($p > 0.05$) for any treatment or sampling time.

On the first sampling date (30 DBA), there was no significant difference in the density of organisms among treatments. However, in the IC treatment at 1 DAA sampling, the mean density of organisms was lower than in the control ($p < 0.05$). On average, the second sampling showed higher organism densities than the sample before the pesticide application, for the control treatment (Figure 1).

At 10 DAA, the mean density in the control increased again, but did not differ significantly from 1 DAA. However, for HM, the mean density decreased, and showed differences from the control. The IC was also significantly different from the

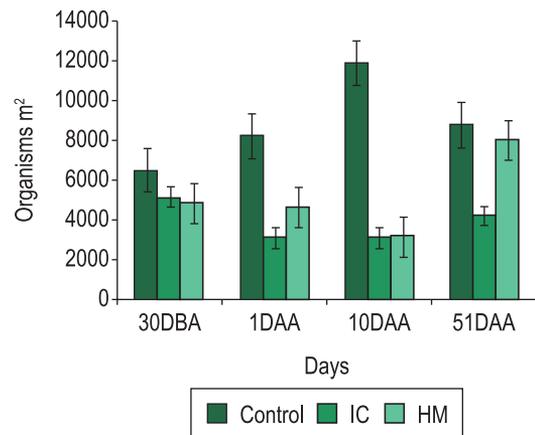


Figure 1. Mean density of organisms found on each sampling date. Control: control treatment without pesticide application; IC: Insecticide treatment; HM: Herbicide treatment. Bars represent the standard deviation. (DBA: days before application; DAA: days after application of the pesticides).

Table 1. Levels of dissolved oxygen (DO), pH, and temperature (T °C) of the rice paddy water at each sampling time, Santa Maria, Rio Grande do Sul, Brazil, 2006.

Sampling Time	DO (mg.L ⁻¹)			pH			T °C		
	Control ¹	IC	HM	Control	IC	HM	Control	IC	HM
30 DBA	2.12 ^A	2.04 ^{ab}	2.04 ^{ab}	6.77 ^a	6.71 ^a	6.79 ^a	23.8 ^c	24.0 ^c	23.6 ^c
1 DAA	2.08 ^A	1.97 ^{ab}	1.94 ^{ab}	6.62 ^a	6.67 ^a	6.69 ^a	26.0 ^b	26.2 ^b	25.8 ^b
10 DAA	1.98 ^A	1.68 ^{cb}	1.7 ^{cb}	6.40 ^c	6.36 ^c	6.35 ^c	27.1 ^a	27.4 ^a	27.0 ^a
51 DAA	2.01 ^A	1.86 ^{bb}	1.87 ^{bb}	6.59 ^b	6.47 ^b	6.47 ^b	25.3 ^b	26.1 ^b	25.7 ^b

¹CT: control treatment; IC: treatment with insecticide and HM: treatment with herbicide; DBA: days before pesticide application; DAA: days after pesticide application. Capital letters represent significant differences among the treatments, and lower-case letters represent significant differences among the sampling times, by Tukey Test ($p < 0.05$).

Table 2. Mean density of organisms per m² in each treatment and at each sampling time. Santa Maria, Rio Grande do Sul, Brazil, 2006.

Taxa	Control ¹				IC				HM			
	30 DBA	1 DAA	10 DAA	51 DAA	30 DBA	1 DAA	10 DAA	51 DAA	30 DBA	1 DAA	10 DAA	51 DAA
ARTHROPODA												
INSECTA												
Coleoptera												
Curculionidae	0	266.66	133.33	0	0	100	0	0	0	0	66.66	0
Hydrophilidae	33.33	33.33	0	33.33	66.66	133.33	33.33	66.66	33.33	0	0	100
Diptera												
Ceratopogonidae	500	800	1200	466.66	1066.7	266.66	100	233.33	833.33	466.7	66.66	1266.66
Chironomidae	2100	1866.66	1566.66	1400	2033.3	700	566.66	1666.7	2000	1033	1233.3	2933.33
Tabanidae	0	33.33	66.66	66.66	33.33	0	33.33	0	66.66	0	0	0
Trichoceridae	66.66	0	0	0	0	0	0	0	0	0	0	0
Ephemeroptera												
Leptohyphidae	33.33	0	0	0	66.66	66.66	0	300	33.33	100	0	0
Hemiptera												
Corixidae	0	0	0	0	0	0	0	0	0	0	0	100
Odonata												
Aeshnidae	0	133.33	33.33	333.33	66.66	66.66	0	200	300	0	33.33	0
Coenagrionidae	0	0	0	0	66.66	66.66	0	0	266.66	166.7	100	0
Gomphidae	0	33.33	0	0	100	33.33	0	0	33.33	33.33	33.33	0
Perilestidae	33.33	0	0	0	0	0	0	0	66.66	0	0	0
Trichoptera												
Odontoceridae	1066.7	1733.33	1833.33	866.66	366.66	766.66	933.33	666.66	333.33	833.3	366.66	566.66
Hydroptilidae	0	0	0	0	33.33	0	0	0	0	0	0	0
CRUSTACEA												
Ostracoda	166.66	66.66	166.66	433.33	0	0	0	33.33	0	100	33.33	100
ANNELIDA												
Hirudinea	366.66	466.66	333.33	333.33	333.33	200	166.66	100	66.66	33.33	266.66	100
Oligochaeta	400	1866.66	4900	2666.66	466.66	133.33	500	600	266.66	1500	466.66	2466.66
MOLLUSCA												
Basommatophora												
Planorbidae	0	66.66	66.66	100	0	0	0	33.33	33.33	33.33	33.33	0
Bivalvia	33.33	433.33	733.33	1100	0	33.33	0	0	0	0	133.33	0
Gastropoda												
Architaenioglossa												
Ampullariidae												
<i>Pomacea</i> sp.	766.66	133.33	633.33	566.66	66.66	233.33	100	333.33	200	66.66	166.66	233.33
NEMATODA	1000	333.33	300	466.66	433.33	333.33	66.66	33.33	333.33	333.3	200	200
	6566.7	8260.66	12940.9	8833.28	5199.9	3133.3	2500	4266.6	4866.6	4700	3066.6	8066.84

¹Control: control treatment without pesticide application; IC: treatment with insecticide; HM: treatment with herbicide; DBA: days before pesticide application; DAA: days after pesticide application.

control treatment, although there was no increase in the mean density.

On DDA 51 the density increased in the IC and the HM. However, even with this increase, the density of organisms was lower than the IC and HM, probably because the CI treatment is more toxic to aquatic invertebrates in general.

At the family level, Odontoceridae showed significant differences between the control and insecticide treatments. These differences occurred on the first and 10DAA, but did not repeat on day 51, and also between the control and the herbicide treatments (Table 2). Two families

belonging to Coleoptera were found: Curculionidae and Hydrophilidae; but only the latter showed significantly higher abundances in IC than in the control. Although the density decreased on 1DAA in the treatments with pesticides, the density had recovered by 10DDA. In the control treatment, this family only appeared up to the second sampling, and the restructuring of the population seen during the treatment with insecticide did not occur in the control treatment (Table 2). The subclass Hirudinea differed significantly in density between the control and herbicide treatments (Table 2).

4. Discussion

The influence of pesticides in reducing water quality was also reported by Molozzi et al. (2006), who evaluated irrigation and drainage water from ricefields. In rice crop areas, the concentration of dissolved oxygen (DO) tends to be low due to the height of the water, usually between 5 and 15 cm. Other studies in the same area reported DO concentrations from 0.6 to 2.2 mg.L⁻¹ (Golombieski et al., 2008), 2.4 to 4.6 mg.L⁻¹ (Reimche et al., 2008) and 2.5 to 11.90 mg.L⁻¹ (Baumart and Santos, 2010). In this study, DO ranged between 1.7 and 2.12 mg.L⁻¹; however, because this difference was recorded before the pesticide application, probably the oxygen levels were not associated with these treatments. The low DO availability may have, however, restricted the colonization of the area by certain *taxa* that could be present if the DO concentrations were close to saturation, such as reported by Baumart and Santos (2010), who reported a higher diversity of *taxa*, when only control treatments are compared.

Over time, invertebrates in ricefields under cultivation are expected to increase in absolute abundance and richness, because pesticides tend to degrade in the environment, with a decrease in their toxic effect (Schulz & Liess, 1999). As seen in other studies (Schulz & Liess, 1999; Suhling et al., 2000; Baumart and Santos, 2010), with the end of the crop cycle, some *taxa* recolonized the area, and other groups already present increased in abundance.

For the benthic macroinvertebrate assemblage, in the first sampling (prior to the application of the pesticides) there were no differences among the treatment fields, which was expected. However, the changes seen in the subsequent collections reflected the effects of these products, mainly in the sampling on the first day after the use of pesticides, when the organisms may have suffered from so-called instantaneous stress.

According to Tatum (2004), the most commonly used herbicides, including metsulfuron, are soluble in water and rapidly degrade once they enter the environment. According to the same author, they are neither persistent nor do they bioaccumulate, because modern herbicides have been designed only to act on plants, showing a low level of direct toxicity to animals, such as insects, fish, amphibians, or others, if used correctly. The study by Golombieski (2006), confirmed these data, showing that 12 hours after metsulfuron-methyl was applied; it was not possible to detect it in the water of irrigated rice.

Nevertheless, although it was not identified in the water, from the results obtained in the present study it is possible to infer that this product does influence aquatic invertebrates. Because we are working with benthic organisms, analyses of the accumulation of the product in the sediment would be necessary, but this information is not yet available.

In the subclass Hirudinea, which was recorded in all three treatments, there was a significant difference between the control treatment and the treatment with herbicide. Although these organisms are tolerant to low dissolved-oxygen concentrations, we believe that this factor did not increase the assemblage of hirudineans in HM, because once the effect of the herbicide had passed the population declined, returning to the numbers found in the first, pesticide-free sampling. During their exposure to the herbicide, these organisms probably increased in abundance due to favorable conditions for their development. In studies in Danish rivers (Friberg et al., 2003), where high concentrations of pesticide metabolites were recorded, high densities of Hirudinea were also reported in the spots with a higher concentration of agrottoxins, which may indicate that these organisms are resistant to these compounds. According to Bacey and Spurlock (2007), these favorable conditions may reflect a greater quantity of organic matter, coming from decomposition of organisms affected by the herbicide or from the absence of some competitor or predator.

Michael et al. (1991), in studies in Florida, also reported that metsulfuron does not produce significant or persistent contamination of surface water, and expressed some doubts about its persistence in the soil. Li et al. (2005) reported that this product can damage rice crops, because it affects plant growth, mainly the morphology of the roots, which become smaller and fewer.

According to Nakagome et al. (2006), carbofuran is highly toxic to aquatic organisms, partly because of its low solubility in water, which requires more time for its degradation. Carbofuran is so highly toxic that its active ingredient can be detected in water up to 17 days after its use, in a 0.013 mg.L⁻¹ concentration (Golombieski, 2006). According to Vyas et al. (1998) this insecticide can decrease the efficiency of the enzyme acetylcholinesterase by about 80% in the brain of birds, which may cause high mortality. This pesticide is highly lipophilic, and accumulates in the fat and mainly in the liver of organisms that ingest it. This is lethal to many animals, including vertebrates, which feed directly

on contaminated seeds, on the grains of the product itself, and also on invertebrates from the treated area. This latter group includes the target *taxa* (acarines, insects, and nematodes) as well as the non-target ones (microcrustaceans, ephemeropterans, plecopterans, odonates) that do not damage the rice crop.

In this study, carbofuran caused a general reduction in the macroinvertebrate assemblage. However, over time, the density of this community recovered. The most critical time for these assemblages was seen on the 10th day, when almost all *taxa* were considerably reduced. This may have occurred due to scarcity of food for the groups, mainly the predators, in addition to poisoning by the active ingredient of the product, considering that the use of pesticides reduces the availability of food to benthic macroinvertebrates, alters the structure of the algae community, and may also have a toxic effect on these resources (Gagneten, 2002).

Because this was a field experiment, many variables may have influenced the results. However, considering that the entire macrobenthos community was analyzed, it is possible to infer that the use of pesticides had some consequences for the benthic communities, directly through the effects of their active ingredients or indirectly through reduction of oxygen, food, etc. Despite these effects, much of the community recovered with time, returning to the point before the pesticide application. This may occur either because the pesticides do not persist for long in the soil, or because their molecules are absorbed by the soil and are not available to the organisms. The results support the conclusion that other studies, focusing on specific taxonomic groups, are of extreme importance to evaluate the action of these pesticides more directly.

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