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Ecotoxicological effects of Vertimec® 18EC on plankton

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Abstract

The use of pesticides to boost crop yields is increasing. However, their adverse effects are unquestionable. One major problem is the fact that they have a broader effect than just on target organisms, being carried by the rainwater, leached or volatilized. Among wide used agricultural chemicals is the acaricide/insecticide abamectin (also known by the trade name Vertimec® 18EC). The present work evaluates the toxicity of Vertimec® 18EC on cladocerans *Daphnia similis* and *Ceriodaphnia dubia* and alga *Pseudokirchneriella subcapitata*. Two land plots were prepared, one contaminated with Vertimec® 18EC and the other used as a control. After simulated rainfall, samples of the runoff water were collected and used in acute and chronic bioassays to verify the effects of the contaminant on planktonic organisms. The results showed high toxicity to the cladocerans. For *D. similis* the 48h EC_{50} was 13.87% in the runoff, corresponding to 5.54 µg L^{-1} of abamectin. For *C. dubia* it was impossible to calculate the inhibition concentration because the effect was lethal, preventing reproduction. No toxic effect was noted for *P. subcapitata*. However, a physical inhibitory effect was observed due to the high concentration of suspended material in the water samples.

Keywords: abamectin, *Ceriodaphnia dubia*, *Daphnia similis*, pesticides, *Pseudokirchneriella subcapitata*, soil-water interaction, toxicity.

Efeitos ecotoxicológicos do agrotóxico Vertimec® 18CE em organismos planctônicos

Resumo

Para maximizar a produção agrícola, a utilização de agrotóxicos se tornou uma prática frequente na agricultura. No entanto, seus efeitos nocivos são inquestionáveis. Um dos maiores problemas consiste no fato de que a maior parte aplicada não atinge os organismos-alvo, sendo carreada pelas águas das chuvas, lixiviada ou volatilizada. Entre os diversos agrotóxicos amplamente utilizados, destaca-se a abamectina, um inseticida e acaricida também conhecido pelo nome comercial de Vertimec® 18CE. Este estudo procurou avaliar a toxicidade do Vertimec® 18CE em *Daphnia similis* e *Ceriodaphnia dubia* (zooplâncton) e *Pseudokirchneriella subcapitata* (fitoplâncton). Para tanto, parcelas de solo foram preparadas, sendo uma contaminada com Vertimec® 18CE e a outra utilizada como controle. Após simulação de chuva, amostras do escoamento superficial foram coletadas e utilizadas em bioensaios agudos e crônicos, verificando-se os efeitos do contaminante nos organismos. Para *D. similis* o valor de CE_{50},48h foi de 13,87% de *runoff,* que corresponde a 5.54 µg L^{-1} de abamectina. Para *C. dubia* não foi possível calcular o valor da concentração de inibição porque o efeito foi letal, impedindo a reprodução do organismo. Para *P. subcapitata* não foi verificado efeito tóxico do produto e sim um efeito físico da turbidez, contribuindo para a redução da densidade algal.

Palavras-chave: abamectina, *Ceriodaphnia dubia, Daphnia similis*, agrotóxicos, *Pseudokirchneriella subcapitata*, interação solo-água, toxicidade.

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INTRODUCTION

Abamectin, a natural product of the fermentation of the bacterium *Streptomyces avermitilis*, was discovered in 1975 by Merck & Co., Inc. It is commonly used as an insecticide and acaricide on fruit and vegetable crops and ornamental plants, or as an animal deworming agent (Fisher & Mrozik, 1992). The commercial preparations contain 80% or more of avermectin B_{1a} and up to 20% of avermectin B_{1b} (Wislocki *et al.*, 1989). They are sold under various brand names, such as Affirm®, Agri-Mek®, Avid®, Dynamec®, Vertimec® and Zephyr®.

Because of its intense and widespread use, Vertimec® 18EC can cause environmental impacts and risks to human health. It is estimated that less than 0.3% of the product applied reaches the target organisms (Pimentel, 1991), while the rest is lost, much of which can reach aquatic ecosystems through surface runoff, leaching and precipitation.

The official toxicological classification of Vertimec® 18EC is class III ("moderately toxic"), but according to the product's informative insert it is considered very dangerous to the environment (class II), highly persistent and extremely toxic to microcrustaceans and fish, affecting non-target organisms (Campbell *et al.,* 1989). The abamectin interact with the glutamate-gated chloride channels and GABA (gamma-aminobutyric acid)-gated chloride channels in arthropods and nematodes causing strong chloride influx, which results in disrupted neural signal transmission (Turner & Schaeffer, 1989; Tišler & Eržen, 2006).

Various studies have shown that abamectin is lipophilic, meaning it is relatively insoluble in water. It also has an affinity to form solid particles and it is subject to rapid photodegradation in water ($t_{1/2}$ = 4-21 h) (Halley *et al.*, 1989; Wislocki *et al.*, 1989). According to Van den Heuvel *et al.* (1996), in a study exposing the fish *Lepomis macrochirusas* to abamectin, the possibility of its bioaccumulation in aquatic environments is small. However, Tišler & Eržen (2006), in a study exposing various aquatic organisms to abamectin, classified the product as being highly toxic in aquatic systems. According to them, even small concentrations of the substance can cause adverse effects, since it can be toxic at low concentrations to various aquatic organisms, such as species of the *Daphnia* genus. Montforts *et al.* (2003) also reported ivermectin is highly toxic to 17 species of crustaceans and mollusks, and Garric *et al.* (2007) confirmed the risk posed by ivermectin to *Daphnia magna* and the green alga *Pseudokirchneriella subcapitata*. Furthermore, according to Collier & Pinn (1998), ivermectin presents a significant risk to benthic animals.

Therefore, the aim of the present study was to evaluate the sensitivity of planktonic organisms to runoff from soil contaminated with Vertimec® 18EC, by acute toxicity tests with *Daphnia similis* and chronic toxicity tests with *Ceriodaphnia dubia* and *Pseudokirchneriella subcapitata*.

MATERIAL AND METHODS

Experimental

The tests were conducted at the Center for Water Resources and Applied Ecology (CRHEA), part of São Carlos School of Engineering of the University of São Paulo, located in the municipality of Itirapina, São Paulo. Two land plots were marked out measuring 8 m^2 each. One was contaminated with Vertimec® 18EC at the recommended dose for strawberries of 0.75 mL L^{-1} (representing 0.125 L m^{-2}). The other uncontaminated plot served as control. Then simulated rainfall was applied to the plots. The intensity (19 mm) was based on the average for the month of February over three consecutive years (2005-2007), obtained from the CRHEA weather station.

The runoff samples were collected by placing plastic tarps in depressions previously dug downfield from the plots. Soil samples were also collected to analyze the grain size and organic matter content.

Physical and chemical analyses

The runoff water samples were analyzed for pH (Micronal B374 potentiometer), conductivity (Orion 145A conductivimeter), dissolved oxygen (OD YSI meter), turbidity (Hach DR/2000 spectrophotometer), hardness (titulation with EDTA, according to APHA, 1995), suspended solids (gravimetry, according to ABNT – NBR 10664/1989) and abamectin (liquid chromatography, according to Lanças (2004). The soil samples were analyzed for grain size (ABNT, 1968) and organic matter content (Trindade, 1980).

Toxicity tests

To conduct the toxicity tests, the runoff water samples (contaminated and uncontaminated) were diluted to five graduated concentrations (3.125%, 6.25%, 12.5%, 25% and 50%, besides the 100% sample itself). The testing procedures as well as preparation of the cultivation water followed the standards specified by the Brazilian Association of Technical Standards ABNT (2004) for *Daphnia similis*, ABNT (2005a) for *Ceriodaphnia dubia* and ABNT (2005b) for *Pseudokirchneriella subcapitata*.

Daphnia similis

The tests of acute toxicity to *D. similis* used five organisms in four repetitions, placed in nontoxic plastic cups containing 10 mL of the test solution at each dilution. Each test lasted 48 hours, and after that the number of live organisms was counted (ABNT, 2004).

Ceriodaphnia dubia

The tests of chronic toxicity to *C. dubia* employed one neonate (between 6 and 24 hours old) in each nontoxic plastic

cup, containing 15 mL of the test solution at each dilution, with 10 repetitions. The water was changed every two days. The organisms were fed as specified in the standard and were kept at temperatures ranging from 22 to 25 ºC, with a photoperiod of 12 hours (ABNT, 2005a). This test lasted eight days, long enough for production of the third brood, and the number of neonates produced during the experiment was counted.

Pseudokirchneriella subcapitata

The tests of chronic toxicity to *P. subcapitata* consisted of exposure of cells at density of 10^5 cells mL^{-1} to the test dilutions, the field control and a laboratory control for 96 hours. They were carried out in triplicate, in 250-mL Erlenmeyer flasks containing 100 mL of the test solution. The flasks were placed on a stirring table at a velocity of 100 to 175 rpm under constant illumination. The temperature was maintained at 20 ± 2 ºC. The glassware used to maintain the algae and in the toxicity tests was previously washed and autoclaved for 20 minutes at 121 ºC (ABNT, 2005b).

To determine the cell density, 2 mL of the sample water was taken from each Erlenmeyer flask after 96 h and the cells were counted under a Carl Zeiss Standard 25 model microscope, using Neubauer chambers. For *P. subcapitata*, changes were also noted in the concentration of chlorophyll-a (Nusch, 1980), along with observation of structural damage and measurement of biovolume (Rocha & Duncan, 1985), in the last case using a Zeiss-Axioskop 2 plus microscope and the Axiovision rel 4.7 program.

The pH, dissolved oxygen level and conductivity of the water samples were measured at the start and end of all the toxicity tests with the zooplankton and phytoplankton.

Statistical analysis

The results of the acute toxicity tests were calculated by the Trimmed Spearman-Karber statistical method and expressed in 48h EC_{50} (Hamilton *et al.*, 1977). Both the results of the chronic toxicity tests and biovolume measurements were subjected to analysis of normality of the data (chi-square test) and homogeneity of variances (Bartlett's test) and then were analyzed by Dunnett's test to compare the means of each treatment against the control, and Tukey's test, which compares all the treatment means against one another. Finally, Fisher's exact test was used to check for a significant difference in the survival between the organisms exposed to the test solutions compared to the control. The Toxstat 3.3 computational program was used to calculate all the test statistics (Gulley *et al.*, 1994).

RESULTS AND DISCUSSION

Soil granulometric analysis and organic matter content

The results of the grain size and organic matter content of the soil in the experimental plots are shown in Table 1. The soil was predominantly sandy (above 60%), with a smaller contribution of silt (15.4%) and clay (1.8%). Therefore, the soil can be considered non-plastic, non-cohesive and easily eroded, facilitating its transport in rainwater runoff. The content of organic matter showed that the soil can be classified as organic.

Table 1 – Granulometric analysis and organic matter content of the soil sample in the experimental plots.

Soil type	Soil fraction (%)
Organic matter (%)	13.4
Coarse sand	2.7
Medium sand	17.3
Fine sand	45.6
Silt	15.4
Clay	1.8

Physical and chemical analyses of the runoff water samples

The results of analyzing the runoff water are shown in Table 2. The water had slightly acidic pH (between 6.0 and 7.0), low conductivity (below 26 µS cm^{-1}) and dissolved oxygen concentration above 6.0 mg L^{-1}. Nevertheless, the levels of turbidity (above 800 NTU) and total solids (above 4000.00 mg L^{-1}) were very high. In the case of total solids, the majority (82%) was represented by the inorganic fraction (fixed solids). The abamectin concentration was zero in the control and 40 µg L^{-1} in the contaminated water.

Because of the high values of turbidity and total solids (and their fractions) in the runoff water samples, we performed measurements of turbidity and suspended solids in each test solution (different dilutions) to be used in the toxicity tests with zooplankton and phytoplankton. The results are in Table 3.

Toxicity tests

Zooplanktonic organisms

In the acute toxicity test with *Daphnia similis*, the sample of 100% uncontaminated runoff (UR) water showed a significant difference to the control according to the Fisher's

Table 2 – Physical and chemical variables of the contaminated (CR) and uncontaminated (UR) runoff water. DO = Dissolved oxygen, TS = total solids, OS = organic solids, FS = fixed solids and ND = not detected.

Sample	*DO (mg L^{-1})*	*pH*	*Hardness (mg L^{-1})*	*Turbidity (NTU)*	*Conductivity (µS cm^{-1})*	*TS (mg L^{-1})*	*OS (mg L^{-1})*	*FS (mg L^{-1})*	*Abamectin (µg L^{-1})*
UR	6.77	6.59	4	930	25.9	4111.09	739.18	3371.91	ND
CR	6.62	6.36	4	890	20.5	4419.10	659.10	3724	40

Table 3 – Turbidity and suspended solids for each test solution. UR (uncontaminated runoff) and CR (contaminated runoff). TS = total solids, FS = fixed solids and OS = organic solids.

Sample	TS (mg L^{-1})	FS (mg L^{-1})	OS (mg L^{-1})	Turbidity (NTU)
UR 100%	4111.09	3371.91	739.18	930
UR 50%	900.30	754.00	146.30	550
UR 25%	381.35	321.15	60.20	356
UR 12.5%	155.67	132.90	22.77	166
UR 6.25%	77.93	64.00	13.93	78
UR 3.12%	37.38	26.23	11.15	37
CR 100%	4419.10	3724.00	695.10	890
CR 50%	1163.45	898.40	265.05	400
CR 25%	760.60	633.65	126.95	262
CR 12.5%	262.77	222.43	40.33	128
CR 6.25%	159.43	131.97	27.47	68
CR 3.12%	50.27	39.87	10.40	30

test. In comparing the laboratory control with the runoff samples from soil containing the recommended concentration of Vertimec® 18EC, significant differences were detected starting at a concentration of 12.5% contaminated runoff (CR) water, with greater immobility of the organisms with increasing concentration of contaminated water (Fig. 1). The 48h EC_{50} value obtained for *D. similis* was 13.87% of runoff, corresponding to 5.54 µg L^{-1} of abamectin.

It is important to highlight the inhibitory effects of turbidity on the organisms tested in this study, especially for *C. dubia*, because dilutions of 50% and 100% of water samples from the control plot (UR) also caused inhibitory effects on the test organisms (Fig. 2). The effects of suspended particles on planktonic organisms have been shown in lakes, reservoirs and rivers. Hardy (1980), Lloyd (1985), Hart (1987) and Dejen *et al.* (2004), for example, all demonstrated in various *in situ* studies that increased turbidity interferes in the population dynamics, contributing to decrease the populations of cladocerans.

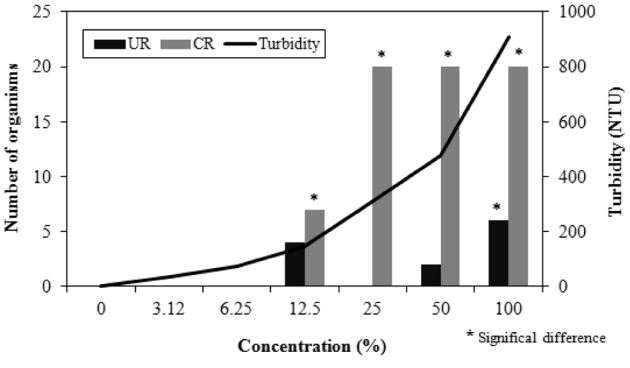

Figure 1 – Comparison of the results of the acute toxicity tests with *Daphnia similis* obtained in the laboratory control with samples of contaminated (CR- Mortality) and uncontaminated (UR- Mortality) runoff water through Fisher's exact test and the relation with the average turbidity of the runoff from the two plots.

The reproduction results from the chronic toxicity tests showed that the species *Ceriodaphnia dubia* was very sensitive to the contaminated runoff samples presenting a significant difference in relation to the control starting with the weakest dilution, 3.125% (about de 1.25 µg L^{-1} of abamectin). For the female mortality results, the significant difference in relation to the control only started at a dilution of 6.25%, corresponding to about 2.5 µg L^{-1} of abamectin (Fig. 3).

The results of the toxicity tests, however, agree with the findings of Tišler & Eržen (2006) and Garric *et al.* (2007). Both research groups recorded toxicity to *D. magna* at very low concentrations, with 48h CE_{50} values of 0.25 µg L^{-1} for abamectin and 5.7 ng L^{-1} for ivermectin, respectively.

The sensitivity range of *D. similis* in the present study is much higher than the values reported in the literature for *D. magna* (Tišler & Eržen, 2006; Garric *et al*., 2007). However, it should be considered that we did not use pure abamectin in the tests, as was the case in the previously cited studies. Also, there are other compounds in the commercial product (Vertimec® 18EC). This fact, along with the turbidity and presence of organic matter, might have interfered in the

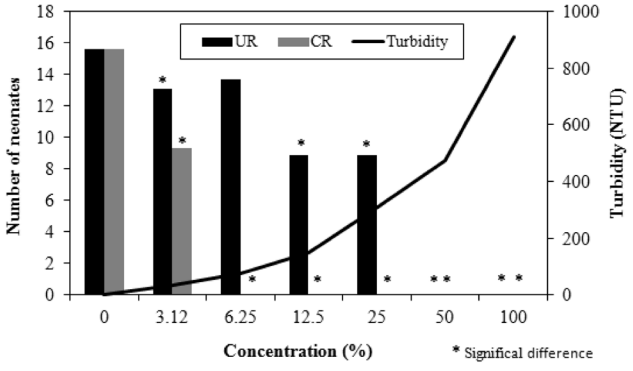

Figure 2 – Comparison of the results of the chronic toxicity tests with *Ceriodaphnia dubia* with samples of contaminated (CR) and uncontaminated (UR) runoff water through the Tukey's test and the relation with the average turbidity of the runoff from the two plots.

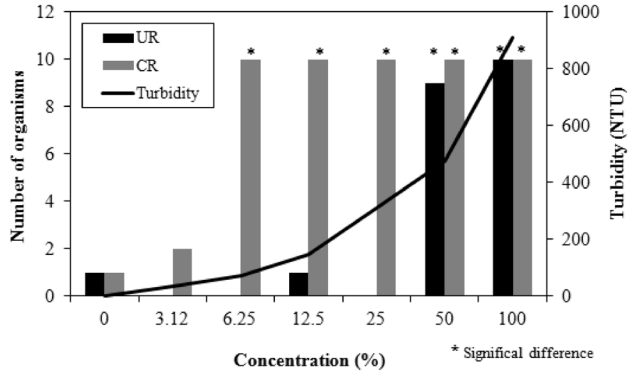

Figure 3 – Comparison of the mortality results of *Ceriodaphnia dubia* obtained with samples of contaminated (CR- Mortality) and uncontaminated (UR- Mortality) runoff water, through Fisher's exact test, and the relation with the average turbidity of the runoff from the two land plots.

product's bioavailability, making it less toxic to the organisms. In this respect, various studies (McCarthy, 1983; Chiou *et al.*, 1979; Wu *et al.*, 2003) have reported reduced bioavailability of hydrophobic organic contaminants in water with high turbidity, caused by the sorption of pollutants to aggregates of inorganic particles with organic carbon.

Pseudokirchneriella subcapitata

In the chronic toxicity test with the alga *Pseudokirchneriella subcapitata*, there were statistically significant differences at all concentrations when comparing the runoff samples from the uncontaminated plot with those from the laboratory, indicating the alga's high sensitivity to turbidity. There were also significant differences between the laboratory control and all contaminated samples, except at runoff water dilution of 6.25% (Fig. 4).

Comparison of the results obtained in the uncontaminated plot (UR) with the contaminated one (CR) at the same dilution showed no significant difference, indicating that the reduced density did not occur because of the product, but rather because of the water turbidity, since it ranged from 30 to 930 NTU. Therefore, it was not possible to calculate the algal growth inhibition concentration value.

The adverse effects of high turbidity on photosynthesizing organisms are well known and often described in the literature (Meyer & Heritage, 1941; Sorenson et al., 1977; Marzolf & Arruda, 1980; Lloyd, 1985; McCubbin *et al.*, 1990). Because high turbidity prevents light from penetrating the water, it impairs photosynthesis.

Various studies submitting algae to abamectin and/or ivermectin have shown that these organisms are less sensitive to the product than are species of zooplankton. In a study with ivermectin, Garric *et al.* (2007) obtained a 72h IC_{50} value of 4 mg L^{-1} for *P. subcapitata* and lowest observed effect concentration (LOEC) of 1.25 mg L^{-1}. In studies conducted with abamectin, Tišler & Eržen (2006) reported a 72h IC_{50} value of 4.4 mg L^{-1} for the Chlorophyceae *Scenedesmus subspicatus* and Ma *et al.* (2002) obtained 96h IC_{50} values of 9.89 mg L^{-1} and 7.31 mg L^{-1} for *Scenedesmus obliqnus* and *C. pyrenoidosa*, respectively.

Figure 5 shows the comparison of the concentrations of chlorophyll-a in the contaminated runoff (CR) water samples against those in the water from the control plot (UR). The effects are similar, confirming the results of the toxicity tests. Therefore, it was not possible to detect any effect of Vertimec® 18EC. The lower chlorophyll-a concentration was only due to the increased turbidity.

The results of biovolume (Fig. 6) and total organic carbon content (Fig. 7) only indicated significant differences in relation to the laboratory control at a concentration of 50% of the runoff from the contaminated plot. These cells had lower volume (an average of 0.38 µm³) and hence lower organic carbon content (0.043 pg cel^{-1}) than those from the control, which had an average volume of 0.9 µm³ and a total organic carbon content of 0.11 pg cel^{-1}. Also, at a dilution of 3.12% the biovolume and total organic carbon content values were higher than in the laboratory control water.

Therefore, the results of this study indicate that abamectin is highly toxic to aquatic organisms, even at low concentrations, so it can cause imbalances in water bodies.

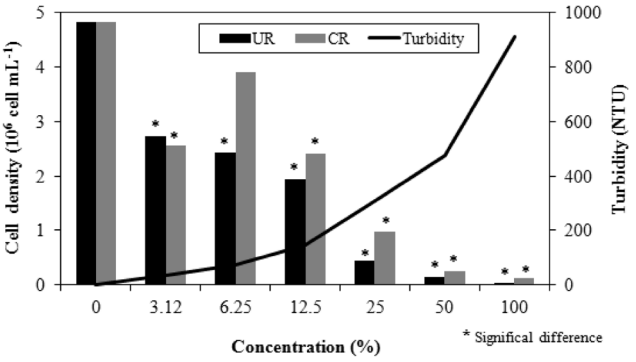

Figure 4 – Comparison of the results of the chronic toxicity tests with *Pseudokirchneriella subcapitata* obtained in the laboratory control with the samples of contaminated (CR) and uncontaminated (UR) runoff water through Dunnett's test and the relation with the average turbidity of the runoff from the two land plots.

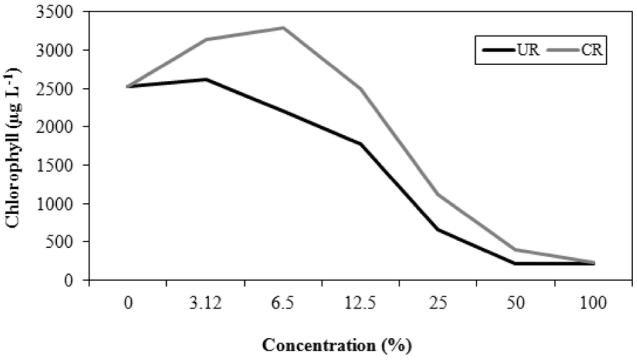

Figure 5 – Comparative analysis of the chlorophyll-a concentrations obtained in the runoff samples from the contaminated (CR) and uncontaminated (UR) land plots.

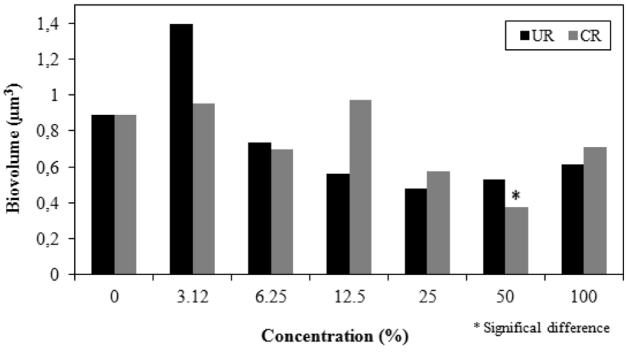

Figure 6 – Comparison of the average biovolume measures of the *Pseudokirchneriella subcapitata* cells obtained in the laboratory control with the samples of contaminated (CR) and uncontaminated (UR) runoff water, through Dunnett's test.

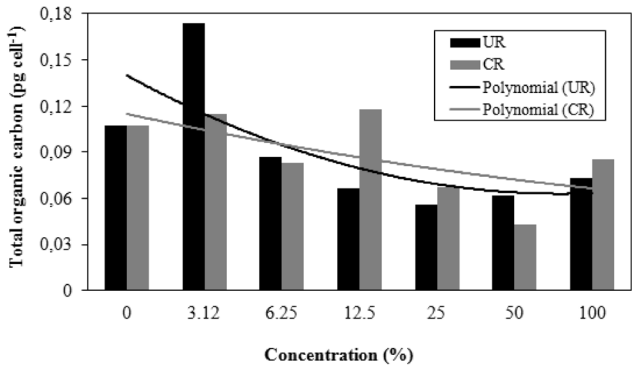

Figure 7 – Comparison of the results of the average carbon content measures of the *Pseudokirchneriella subcapitata* cells obtained in the samples of contaminated (CR) and uncontaminated (UR) runoff water, through Dunnett's test, with the polynomial trend lines.

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