Oxidative stress and changes in swimming performances at zebrafish model (*Danio Rerio* H. 1822) produced by acute exposure to deltamethrin

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Abstract

Delamethrin can easy reach in the aquatic ecosystems and affects the life forms. It produces significant damage to the fish community. It is less know how the fish community reacts in the first hours when insecticide deltamethrin reaches their ecosystem and what biochemical mechanisms of protection are activated. The measurements of the behavior parameters from the tests can explain how fish community reacts before lethal and sublethal deltamethrin dosage. This can be similar to a shock wave produced during an ecological catastrophe where the fish community is involved. Which is the fate of the survivors? Can be their swimming performances recovered to assure their surviving? The aim of this study was to assess on zebrafish model the oxidative stress intensity (MDA, CAT, SOD, GPx) triggered by the acute exposure to lethal and sublethal relevant concentrations of delthametrin and swimming performances recovery of the survivors for: total distance swim, swim velocity, active swim, clockwise rotations, counterclockwise rotations and maximum acceleration.

Keywords: Zebrafish, Deltamethrin, Acute exposure, Oxidative stress, Behavior

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Introduction

The rapid expansion of human population requires more and more water, food and other resources for everyday surviving. Man has been developed new ways to increase the food production by reducting the competition with pests (Chromcova *et al.*, 2015, Fiorino *et al.*, 2018, Gulhan *et al.*, 2012, Gulhan *et al.*, 2014, Orun *et al.*, 2013, Velmurugan *et al.*, 2009). The use of the chemical sinthesis compounds is one of the most frequently method applied to control the pests. The synthetic pyretroids were considered to have a high insecticidal activity, a low toxicity in mammals and no residue in the biosphere (He *et al.*, 1989). At least this was mention in the past. In now days the scientific community agreed that synthetic pyretroids are quite dangerous in environment to many vertebrate and invertebrate organisms.

Deltamethrin (DM) [(S)α-cyano-3-phenoxybenzyl-(1R)-cis-3-(2,2-dibromovinyl)-2,2dimethylcyclopropane-carboxylate] is a type II pyrethroid extensively used in agriculture and forestry because of high activity against insect pests (Dinu *et al.*, 2010, Dubey *et al.*, 2013, He *et al.*, 1989, Köprücü *et al.*, 2008). This insecticide once released in environment can easy reach in the aquatic ecosystems and it will do damages in life forms populations. It produces significant damage to the fish community. This was proved in various experiments performed under laboratory conditions with freshwater fish species like: rainbow trout (Aksakal *et al.*, 2010, Velisek *et al.*, 2009), mirror carp (Çalta *et al.*, 2004), *Carassius auratus* L. 1758 (Dinu *et al.*, 2010), common carp (Cengiz, 2006, Köprücü *et al.*, 2004, Svobodová *et al.*, 2003) , Nile tilapia (Dubey *et al.*, 2013, Yildirim *et al.*, 2006), zebrafish (DeMicco *et al.*, 2010, Huang *et al.*, 2014), guppy (Israel Stalin *et al.*, 2008, Güneş *et al.*, 2011, Viran *et al.*, 2003), North African catfish (Datta *et al.*, 2003), liver catfish (Kumar *et al.*, 1999), *Pangasius hypophthalmus* S. 1878 (Hedayati *et al.*, 2014), *Ancistrus multispinis* (Da Silva de Assis *et al.*, 2009). It is less know how the fish community reacts in the first hours when insecticide deltamethrin reaches their ecosystem and what biochemical mechanisms of protection will be activated.

Propolis may serve as an antitoxic agent against pesticide toxicity to aquatic animals. It may improve some biochemical markers associated with oxidative stress in fish brain, after exposure to synthetic compounds (Kakoolaki *et al.*, 2013, Orun *et al.*, 2014, Selcuk *et al.*, 2014).

The zebrafish model (*Danio rerio*) is a very popular model organism that is used in scientific research (behavior neuroscience, ecotoxicology, drug development, genotoxicity, brain disorders and ecology). It can be used to measure the pollutants effects upon the fish community and biodiversity (Bartoskova *et al.*, 2013, Fazio *et al.*,

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2012, Güneş *et al.*, 2011, Hedayati *et al.*, 2014, Plhalova *et al.*, 2018). The measurements of the behavior parameters from the tests can explain how fish community reacts during the lethal and sublethal acute exposure to deltamethrin which has different intensities. This is similar to a shock wave produced during an ecological catastrophe where the fish community is involved. Which is the fate of the survivors? Can be their swimming performances recovered to assure their surviving?

The aim of this study was to assess on zebrafish model the oxidative stress intensity triggered by the acute exposure to lethal and sublethal relevant concentrations of delthametrin and swimming performances recovery of the survivors.

Materiala and methods

Test Animals

The adults (6-7 months old) of zebrafish with long fin strain were purchased from different breeders to have a higher genetic diversity (Iasi District, Romania). A total number of 120 specimens (60 males and 60 females) were brought in laboratory, housed in two 90 L aquariums and acclimated for one month. During this time, the water was under constant mechanical and biological filtration. It was changed at every 48 h to avoid the intoxication with harmful nitrogen compounds resulted from degradation of organic matter (eg. ammonia and nitrates). The water parameters were constantly measured with a HI 9828 multiparameter for water analyses from Hanna instruments and in Table 1 are presented the values. The illumination of the aquarium for housing and testing was from white led bands (light intensity was 307.5 LUX) controlled by trigger timers on a 14/10 light/darkness photoperiodic cycle (lights on at 5:00 am and off at 7:00 pm). The fishes were fed with TetraMin Tropical Flakes. Each experimental aquarium had the volume of 7 L, filled up with dechlorinated tap water that was constantly aerated by an air pump. The animals were strictly maintained and treated according to EU Commission Recommendation of 18 June 2007 on guidelines for the accommodation and care of animals used for experimental and other scientific purposes and Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. This experiment has been approved by the Faculty of Biology from "Alexandru Ioan Cuza" University of Iasi, with the registration number 4465/2017.

Table 1:Water parameters (pH, temperature (t°C), Total Dissolved Solids (TDS), Salinity (Sal.), Conductivity and Oxydation Reduction Potential (ORP)) of the housing aquarium and treatment aquarium.

Housing aquarium					
pH	t°C	TDS (mg L^{-1})	Sal.	Conductivity µS cm^{-1}	ORP
7.66±0.2	24±0.5	276	0.27	553	377
Treatment aquarium					
7.65±0.1	23.5±0.5	274	0.27	552	376

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Experimental Design

It was simulated in this study a possible scenario of contamination with deltamethrin in a freshwater ecosystem. In this case we use a homologated insecticide with high quality certificate purchased from market packaged in 2.5 ml vial. The active compound from this insecticide is deltamethrin with the standard certified concentration of 100 g L^{-1}. This was diluted in the treatment aquariums until they were reached the concentrations necessary for this study. Firstly it was studied the scientific literature regarding the concentrations of DM that were used in zebrafish studies. Huang *et al.* (2014) conducted an acute exposure study on adult zebrafish with sublethal concentrations of: 0.15, 1.5, 3.75, 7.5 and 15 μg L^{-1}. The authors studied three behavioral parameters: swimming speed, swimming depth and hyperactivity/surfacing. DeMicco *et al*. (2010) calculated the lethal concentrations (LC) values in zebrafish: LC_{50} was 40 ± 0.08 μg L^{-1}; 16% mortality at 19 μg L^{-1} and 84% mortality at 100 μg L^{-1}.

In our study they were selected three relevant concentrations based on the previous studies: 3.12, 12.5 and 25 μg L^{-1}. These were integrated in the scenario presented in this paper. The acute exposure with these concentrations had 72h length. The test animals were separated in four groups with 10 specimens each (5 males and 5 females): group control, group exposed to 3.12 μg L^{-1}, group exposed to 12.5 μg L^{-1} and group exposed to 25 μg L^{-1}. The acute exposure with these concentrations was repeated for three times to assure the quality control of the statistic tests. The control group simulated a 72 h length exposure to have relevant results about the unharmed fish population.

The behavioral measurements were performed in a multipurpose cross maze that was turned into a T maze. This maze was made from transparent Plexiglas. The infrared light had a source located under it. The images were recorded by an infrared camera connected to a computer and analysed by the software EthoVision XT 11.5 (NOLDUS, Holland) that was previously calibrated for the swimming performance test. The length of each trial was 4 minutes per individual specimen. During this time, each one should explore the area of aquarium. The camera recorded them and the software calculated the raw data for the follow swimming performance variables: total distance swim (cm), swim velocity (cm s^{-1}), active swim (s), clockwise rotations, counterclockwise rotations, maximum acceleration (cm s^{-2}). Each group of fish required 24 h for accommodation with experimental aquarium before exposure. In the next day they were conducted pre-treatment tests to establish the unaffected normal behavior of each group. This test was conducted in triplicates for each group with one day before the treatment. In the next day they were administrated the treatments for each experimental

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aquarium. In the control aquariums it was not administrated DM. The experiment started at 6 am. The behavioral tests were conducted at different hours (h) during DM exposure and treatment simulation: 2 h, 9 h, 24 h, 48 h and 72 h.

The fishes that presented symptoms similar with paralysis, moments before death were removed from experimental aquariums and killed via head impact. This procedure was applied to all surviving fishes at the end of exposure and treatment simulation. They were stored in labelled plastic bags at - 20°C for oxidative stress analyses.

Oxidative stress analyses

There were measured enzymes involved in the oxidative stress like: superoxide dismutase (SOD), malondialdehyde (MDA), catalase (CAT) and glutathione peroxidise (GPx). Higher or lower activity of these enzymes in the exposed groups compared with the control group represented the biochemical answer deltamethrin toxicity. It was studied in this paper if the selected concentrations can rapidly trigger or not the oxidative stress as biochemical response. The oxidative stress measurements were applied for quantification of pollution on fish community in experimental conditions (Burgos-Aceves *et al.*, 2018, Dinu *et al.*, 2010, Faggio *et al.*, 2016, Fazio *et al.*, 2012, García-Medina *et al.*, 2010, Gobi *et al.*, 2018, Güneş *et al.*, 2011, Lushchak, 2016).

Each frozen fish was weighted and rapidly grinded for oxidative stress analysis. The superoxide dismutase was measured with SOD Assay Kit (Merck, Darmstadt, Germany). The unit of activity for SOD (USOD) was expressed per mg total protein.

The activity of catalase was estimated using the Sinha method (Sinha, 1972), based on colorimetric determination of chromic acetate resulted in the reaction. The wavelength used for determination was set at 570 nm. A unit of measurement (UCAT) represented the enzyme quantity necessary for decomposing of one micromole of hydrogen peroxide in one minute at 20°C and a pH 7. This was expressed per mg total protein.

The glutathione peroxidase activity was spectrophotometric measured at the wavelength of 412 nm. One unit of GPx (UGPx) represented the enzyme quantity necessary to oxidize a µmol GSH per minute. It was measured with Glutathione Assay Kit (Merck, Darmstadt, Germany). This was expressed per mg total protein. The protein concentrations were measured by the Coomassie blue method using bovine serum albumin as a standard (Bradford, 1976).

Malondialdehyde was measured (nM MDA) with Lipid Peroxidation Assay Kit (Merck, Darmstadt, Germany). All the enzymes were spectrophotometric measured with Specord 210 Plus from Analytik Jena, Germany.

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Statistical analysis

The one-way ANOVA test followed by the Tukey HSD test has been performed, after the Shapiro-Wilk test, to demonstrate the significant differences variance of the investigated behavioral variables from initial condition of the subjects (pre-treatment) to the end of the treatment in the case of each group. For oxidative stress were compared the differences between control group and treatments groups at the end of acute exposure (ANOVA test followed by the Tukey HSD). All results were presented for this study as average±SD. The statistical analyses were carried using OriginPro v.9.3 (2016) software designed and created by OriginLab Corporation, USA.

Results and discussions

According to the published literature the concentration of DM 25 μg L^{-1} and 12.5 μg L^{-1} are below LC_{50} (40±0.08 μg L^{-1}) described by DeMicco *et al*., (2010) and $LC_{50\text{-}24h}$ (14.43±1.031 μg L^{-1}) experimented by Huang *et al*., (2014). In our experiment, the mortality observed for DM 25 μg L^{-1} and 12.5 μg L^{-1} was 100% in both cases before 9 h exposure time. It was observed the first case of mortality only after 30 minutes exposure time for the concentration 25 μg L^{-1} and 45 minutes exposure time for 12.5 μg L^{-1}. Between 10-15 minutes before the death, the zebrafish swim close to the water surface with rapid movements up and down. In the next stage, the fish were semi-paralysed in vertical position in aquarium. Every time when they touched the bottom of aquarium, they tried to swim with spasmic moments close to the surface in a corkscrew manner. Moments later it was observed a total paralysis when the fishes lay down to the bottom of aquarium and hyperventilated their gills. From this moment it was just a matter of time till the death. At all death specimens they were observed red spots close to the pectoral and caudal fins. Huang *et al*. (2014) did the follow observations before death of the zebrafish during the acute exposure to DM: rapid gill movement, erratic swimming, swimming in a corkscrew manner, rapid opercular movement and fish remained vertically in water or lay on one side on the bottom of the aquarium moments before death. The authors observed these symptoms for concentrations above 7 μg L^{-1}. In our study we observed these symptoms also for 25% of the population exposed to 3.2 μg L^{-1} of DM after 24 h. The fish with these symptoms were excluded from the trial tests of swimming performances. It resulted that DM from the studied insecticide was more toxic than the regular standard solution used in other studies. This can be explained by presence of additives that increased the toxicity level. Anyway the sublethal concentration was 3.2 μg L^{-1}.

In Fig. 1 are presented the main picture of the results obtained after the swimming performance tests of the zebrafish without lethal symptoms. In the pre-treatment it was observed homogeneity of the groups regarding

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the exploration of the maze area. The modifications started 2 h after acute exposure with the concentrations 25 µg L^{-1} and 12.5 µg L^{-1}.

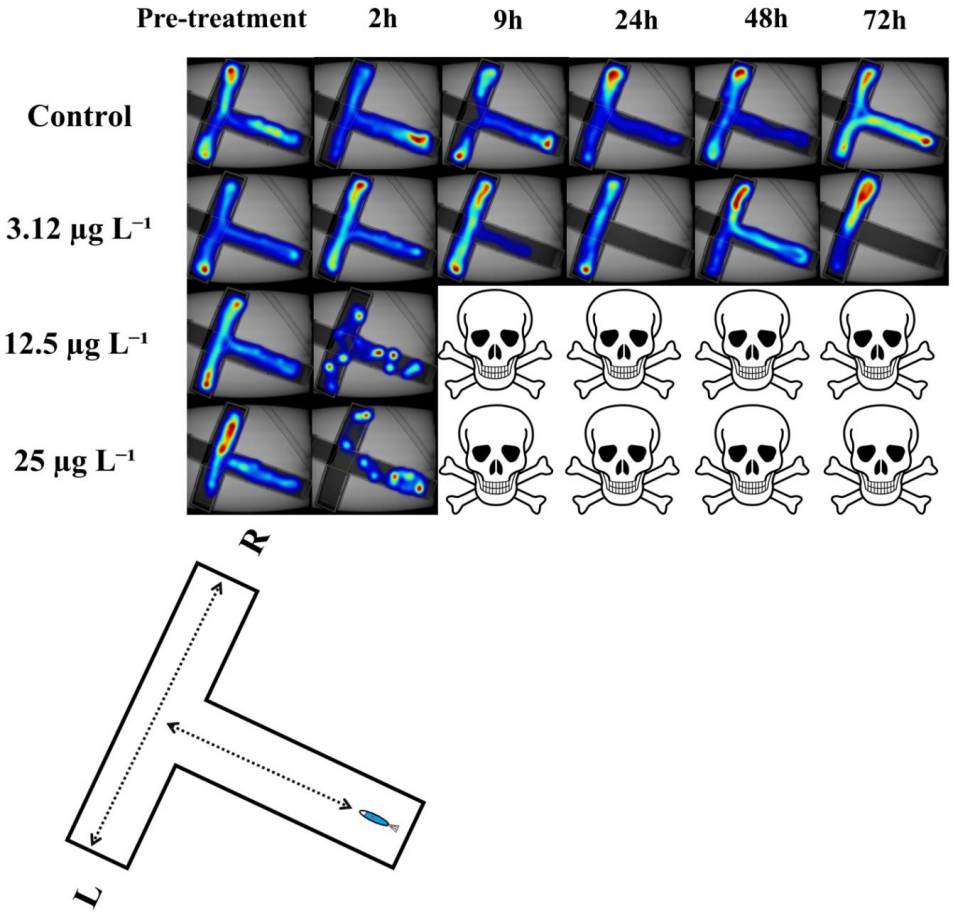

Figure 1: The activity of the groups before and after DM exposure with treatment simulation of the control group.

It was observed same pattern between these two groups. They were observed similar patterns between the control group and exposed group with 3.2 µg L^{-1}.

Swimming performance results

The variable total distance swim shows the capacity of zebrafish to explore the area of the maze. This is similar in a real ecosystem with the capacity of moving away from a pollution source. In the studied groups were observed significant differences (Fig. 2) between the initial condition of the fish and exposure to high concentrations of DM. The group treated with 25 µg L^{-1} had explored in pre-treatment 791.6±264.9 cm per trial and after 2 h exposure time the distance significantly decreased to 337.9±218.6 cm. Similar results were for 12.5 µg L^{-1} where in pre-treatment the distance was 721.3±259.7 cm and after 2 h exposure time significantly decreased to 251.5±137 cm. At the fish without symptoms exposed to 3.2 µg L^{-1} were not observed significant

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differences (p>0.05 ANOVA) between pre-treatment (749.6±270.1 cm) and acute exposure at: 2 h (569.64±352 cm), 9h (644.4±405.7 cm), 24 h (505.2±127.9 cm), 48 h (461.5±163.2 cm) and 72 h (426.8±186.1 cm).

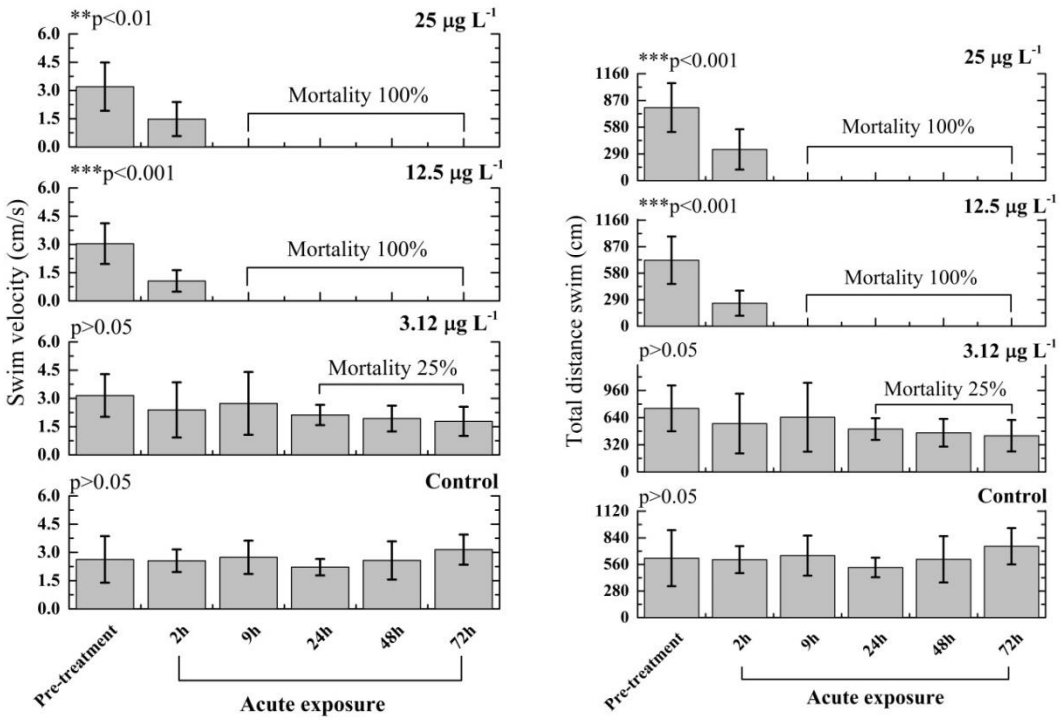

Figure 2: Total distance swim and velocity presented as average±SD with the results of one-way ANOVA test.

For the control group were not observed significant differences between pre-treatment (625.9±294.7 cm) and treatment simulation where at 72 h was measured 751.3±190.6 cm.

The variable swim velocity (Fig. 2) was significant decreased at group exposed to 25 µg L^{-1} where in pre-treatment the zebrafish had 3.2±1.2 cm s^{-2} and at 2h after exposure was 1.06±0.56 cm s^{-2}. Same results were obtained at group exposed to 12.5 µg L^{-1} where in pre-treatment had 3.03±1.07 cm s^{-2} and decreased to 1.06±0.56 cm s^{-2}. The fish group without symptoms exposed to 3.12 µg L^{-1} had no significant difference (p>0.05 ANOVA) between pre-treatment (3.15±1.13 cm s^{-2}) and the end of exposure time at 72h (1.78±0.77 cm s^{-2}). In the control group they were not observed significant differences (p>0.05 ANOVA) between pre-treatment (2.62±1.23 cm s^{-2}) and treatment simulation at 72h (3.14±0.8 cm s^{-2}).

Huang *et al*., (2014) studied same variable during a 360 minutes exposure. The swimming speed in the lowest studied concentration (0.15 µg L^{-1}) was increased from the average 39.6 mm s^{-1}

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to 49.7 mm s^{-1}. It increased in the highest DM concentration (15 μg L^{-1}) from 43.0 mm s^{-1} to 54.0 mm s^{-1} but this happened in the first 60-120 minutes then decreased similar to our results observed at higher concentration. At concentration of 3.75 μg L^{-1} the swimming speed significantly decreased at 180-240 min (22±5.5 mm s^{-1}) during exposure compared with time interval 0-60 min (48.7±3.4 mm s^{-1}). The authors explained that the speed dramatically changed during the DM exposure. In our study at 3.12 μg L^{-1} it was not observed any significant changes in the swimming speed. One of the explanations is represented by testing of the zebrafish that had no symptoms.

The variable active swimming (Fig. 3) was relevant to measure response to DM exposure on activity of zebrafish (active/stationary). For the active swim were measure significant differences for the groups exposure to 25 μg L^{-1} (between pre-treatment with 215.7±35.17 s and 2h with 157.17±57.79 s) and 12.5 μg L^{-1} (between pre-treatment with 216.3±49.2 s and 2h with 159.4±57.7 s).

These results proved that zebrafish become less active similar to a lethargic behaviour at 2h after exposure to these concentrations. In case of the group exposed to 3.12 μg L^{-1} were not measured significant differences between activity in pre-treatment (218.9±46.9 s) and at the end of exposure at 72h (162.9±52.7 s). As for the control group the results of activity were not significant different between pre-treatment (191.5±72.4 s) and the end of simulation at 72h (224.6±35.7 s).

The variable maximum acceleration was not affected by DM exposure in all studied cases (Fig. 3). They were not resulted significant differences for all exposed groups. The zebrafish still had the capacity to swim fast on short distance. The values of this variable were for the studied groups: 25 μg L^{-1} (between pre-treatment with 54.4±13.06 cm s^2 and 2h with 50.5±2.02 cm s^2), 12.5 μg L^{-1} (between pre-treatment with 54.9±9 cm s^2 and 2h with 50.9±4.6 cm s^2), 3.12 μg L^{-1} (between pre-treatment with 53.9±2.6 cm s^2 and 72h with 50.6±1.5 cm s^2) and control group (between pre-treatment with 53.3±3.6 cm s^2 and 72h with 52.3±1.22 cm s^2).

The number of clockwise and counter clockwise rotations is related with the decisions of fishes during the trial tests of changing their direction in exploration (Fig. 4). Same as in case of the other variables the clockwise rotations significantly decreased at the groups exposed to 25 μg L^{-1} DM (between pre-treatment with 5.25±4.2 and 2h with 1.87±1.12) and 12.5 μg L^{-1} (between pre-treatment with 4.73±3.53 and 2h with 2±1.6). For the group exposed with 3.2 μg L^{-1} were not measured significant differences for clockwise rotations between pre-treatment (5.7±3.3) and 72h (1.3±1.9). Same results were for the control group between pre-treatment (3.7±2.4) and 72h (5.7±3.1). There were significant differences in the groups exposed to 25

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µg L^{-1} DM (between pre-treatment with 5.13±2.2 and 2h with 3±3) and 12.5 µg L^{-1} (between pre-treatment with 6.7±4.3 and 2h with 1.6±0.9) where this variable significantly decreased. The group exposed with 3.2 µg L^{-1} and the control had not significant variations of this variable.

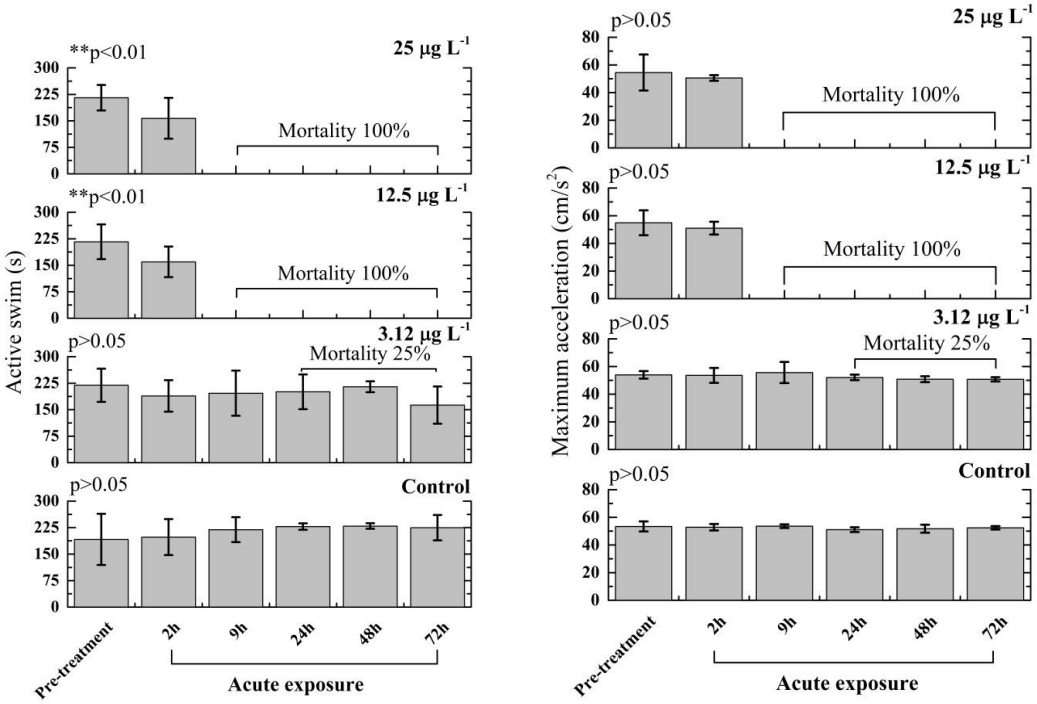

Figure 3: Active swimming and stationary time presented as average±SD with the results of one-way ANOVA test.

Oxidative stress results

The scientific literature was focused on deltamethrin effects produced on the oxidative stress on molluscs, fish and rat model. Köprücü *et al.* (2008) acute exposed (1, 24, 48, 72 and 96 h) the freshwater mussel *Unio elongatulus eucirrus* to DM with different concentrations: 25, 50, 100, 200, 400, 800 and 1,600 µg L^{-1}. The study concluded that glutathione and catalase levels in digestive gland and gill of freshwater mussel decreased as a response to DM toxicity.

Dinu *et al*. (2010) studied the toxicological effects of a single dose of DM (2µg L^{-1}) on *Carassius auratus gibelio* for 14 days. Their study had been concluded that DM induced oxidative stress in liver and intestine. Dubey *et al.* (2013) proved on their study focused on rat model that in the group treated with delthametrin, the activity of SOD and CAT significantly decreased.

In our study it was measured the oxidative stress (an imbalance between the production of free radicals and the body's antioxidant defence system) was

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triggered or not by the acute exposure to DM at zebrafish in 72 h (Fig. 5). It explained in this scenario if the fish community was capable or not to defend on the studied concentrations. The fish with intoxication symptoms were collected moments before death and the survivors were sacrificed at the end of the experiment.

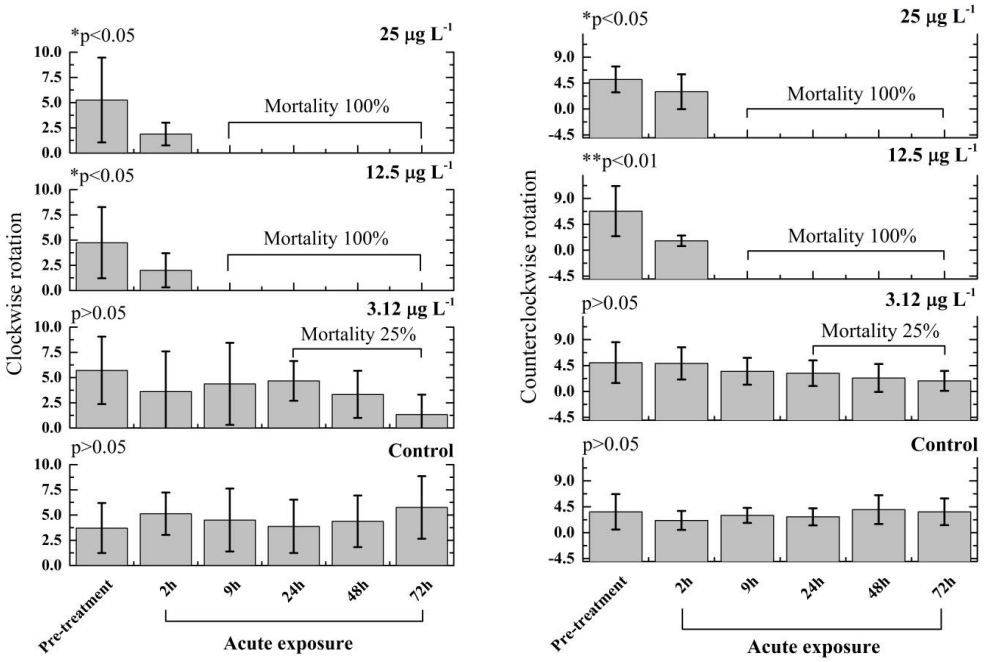

Figure 4: Clockwise and counterclockwise rotations presented as average±SD with the results of one-way ANOVA test.

Malondialdehyde activity significantly increased (*p<0.05) in the group exposed with 25 µg L^{-1} (303.07±16.03 nM MDA) and 12.5 µg L^{-1}(281.6±14.88 nM MDA), based on Tukey HSD results, compared with control group (224.7±25.94 nM MDA). There were not significant differences between the group exposed to 3.12 µg L^{-1} (232.39±61.9 nM MDA) and control group. MDA is an important reactive metabolite and a marker of LPO. It represents one of the most frequent reactions caused by free radical attack on biological structures (Dubey *et al.*, 2013).

Superoxide dismutase activity significantly varied between the studied groups (**p<0.01 ANOVA). In our studied it was a different pattern in case of the groups exposed to lethal concentrations. The group exposed to 25 µg L^{-1} had the lowest values (0.22±0.04 USOD/mg protein) and was significant lower than control (0.35±0.06 USOD/mg protein), group treated with 3.12 µg L^{-1} (0.43±0.01 USOD/mg protein) and group treated with 12.5 µg L^{-1} (0.7±0.04 USOD/mg

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protein). This suggested a significant oxidative stress caused on the zebrafish body in a short period of time. Its body is fighting against the DM toxicity. On the other hand, the group treated with 12.5 µg L^{-1} had values of SOD significant higher than control group suggesting that at this concentration the antioxidant biochemical equipment of the fish has problems.

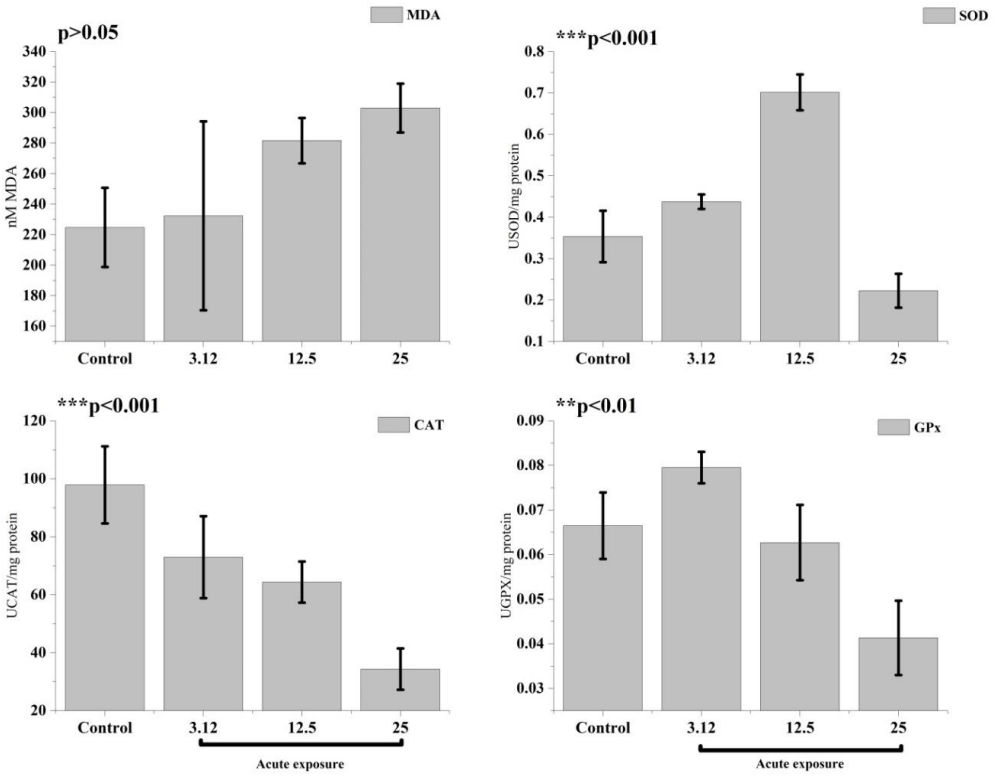

Figure 5: The analysis of the average±SD with the results of one-way ANOVA test.

Between the group treated with the lowest concentration and control there were not significant differences but the trend suggests that these DM concentrations had antioxidant effect similar with the group 12.5 µg L^{-1}. Stress conditions result in the decrease in antioxidant enzyme activity because to their excessive utilization (Dubey *et al.*, 2013).

The catalase activity showed an excessive utilization of the antioxidant enzymes promoting the oxidative stress that increased with the concentration of DM dissolved in water. Fish biochemical system is able to fight from the first moments against the DM acute toxicity. The variations of CAT activity were significant lower between control group (97.9±13.2 UCAT/mg protein)<3.12 µg L^{-1}(72.9±10.3 UCAT/mg protein); 12.5 µg L^{-1}(64.3±3.2 UCAT/mg protein)<25 µg L^{-1} (34.3±7.1 UCAT/mg protein).

Glutathione peroxidase was significant lower at the group exposed to 25 µg L^{-1} (0.04±0.008 UGPx/mg protein) compared with the others. Between the other groups did not result significant differences concerning the

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activity of this enzyme: control (0.06±0.007 UGPx/mg protein), 3.12 μg L^{-1} (0.07±0.003 UGPx/mg protein) and 12.5 μg L^{-1} (0.06±0.008 UGPx/mg protein).

Based on these results DM trigger the oxidative stress based on the activity of these enzymes. It is possible that at the zebrafish that survived at the lowest concentration of DM, the oxidative stress was decreasing as a slow recovery against its toxicity.

Conclusions

The concentrations of deltamethrin used in this study were more toxic than similar used in other studies. This can be explained by other chemicals additives added in the insecticide that increased its toxicity. The scenario presented in our study showed that the fishes which survived behind the shock wave of DM toxicity are still capable to same swimming performances as the control group. This may help them to swim away from the pollution source and to repopulate the habitat after this danger passed. Further experiments are necessary to measure the damages on fish population that survived on acute exposure to DM regarding complex cognitive processes like memory, aggressive behaviour and anxiety. The toxicity of this compound was related with the oxidative stress. In case of lethal concentrations it was triggered fast to keep for a longer time the body functions. For sublethal concentrations, it was also activated the oxidative stress but with a lover intensity suggesting a possible recovery of the organism.

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