BIOACCUMULATION AND ENZYME ACTIVITY INHIBITION OF PROFENOFOS IN JAPANESE MEDAKA (*Oryzias latipes* TEMMINCK AND SCHLEGEL, 1846)

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ABSTRACT

Assessment of the ecotoxicological effect of pesticides to the aquatic biota may be a prerequisite to adverse effects on the ecosystem. Effects of the organophosphate pesticide (OP) profenofos to acetylcholinesterase enzyme (AChE) activity inhibition were investigated using the brain samples from Japanese medaka (*Oryzias latipes* Temminck and Schlegel, 1846).

River water samples from six different sampling sites along Dalaguete River were tested for pesticide concentration, the result of which was used as the basis for the Range Finding and Exposure Tests. A total of 30 Japanese medaka of approximately of the same weight and length were grouped into five (n=6 per group) for the RFT and were exposed to different concentrations of profenofos at 0.04ppm, 0.20ppm, 1.00ppm and 3.00ppm, respectively. For the exposure test, a total of 96 medaka grouped into four (n=24 per group) were exposed to profenofos (0.04ppm, 0.20ppm and 1.00ppm).

Profenofos caused a positive dose-dependent and exposure-time-dependent relationship on AChE activity inhibition. On Day 2, the highest concentration (T_3=1.00ppm) was already causing significant AChE activity inhibition. On Day 5, all exposure concentrations were already significantly different from Control.

Increasing concentration of profenofos leads to higher bioaccumulation of the pesticide in exposure water. Accumulated profenofos is remarkably greater as its concentration was increased from 0.2ppm (T_2) to 1.0ppm (T_3) reaching point of equilibrium on Day 5. Consequently, the amount of pesticide accumulation declined in the fish tissues with longer period of exposure. It appears that the Japanese medaka has the ability to metabolize the profenofos.

The study involved one-time sampling of river water in Dalaguete River. It is recommended that periodic water sampling be done to establish levels of profenofos and other pesticides present in the river with due consideration for wet and dry seasons. Further studies like degradation and mobility profenofos are also recommended.

Keywords: profenofos, acetylcholinesterase inhibition, bioaccumulation

INTRODUCTION

As a result of massive global uses, pesticides and their degradation products spread through the environment and contaminate water, soil and atmosphere matrices, leading to a consequent potential risk to human populations and the environment. Surface waters located in intensive agriculture areas are more vulnerable to pesticides, which is a major concern

if the water is intended for human consumption/utilization or is supporting aquatic life (Palma *et al*., 2014).

Aquatic organisms may be harmed by pesticides contaminating or reaching the rivers and streams though surface runoff which can be highly lethal to aquatic life. Herbicides reaching bodies of water can cause fish kill when dead plants decay and consume the water's oxygen, suffocating the fishes. Repeated exposure to sublethal doses of some pesticides can cause physiological and behavioral changes that reduce fish populations, such as abandonment of nests and broods, decreased immunity to disease, and decreased predator avoidance (Helfrich *et al.,* 1996).

Barangay Mantalongon and its neighboring barangays in Dalaguete, Cebu is one of the prime producers of vegetables in the province hence, it is dubbed as the "Vegetable Basket of Cebu". In order to meet the demand for farm produce, as well as, to earn higher profit, farmers in the area apply pesticides heavily during the whole duration of the cropping season, especially on brassicas (cabbage and pechay), spring onions, tomatoes and cauliflowers. Surface run offs from the different farms are fed into the major river system, namely, the Dalaguete River (south side of Mantalongon) through different tributaries. The runoff of the river is likewise used by residents for tilapia ponds. Guppies, catfishes and freshwater shrimps also thrive in Dalaguete River. The rampant and increased use of pesticides in the area is an immediate concern and poses a great challenge to the local government of the Municipality of Dalaguete. Based on accounts of residents in Dalaguete, there has been an increase in fetal deaths, premature births and other illnesses that they blamed on the presence of pesticides in the water and vegetables that they ingest. There are still no scientific studies conducted, however, that would probe into these incidents. In fact, most farmers in Mantalongon and other barangays have their own separate plots for vegetables that they use for personal consumption.

The freshwater *O. latipes* provided by the Marine Science Education and Research Institute, Faculty of Fisheries, Kagoshima University, Kagoshima, Japan was used as test organism since it was not feasible to rear tilapia for pesticide risk assessment as it will need larger quantity of pesticide. Freshwater *O. latipes,* on the other hand, has been extensively used for toxicity assessment studies and they are smaller in size hence lesser amount of pesticide to be used. *Oryzias latipes* Temminck and Schlegel (1846) is commonly used as a test organism in many ecological and genetic studies (Boosle *et al.,* 2015). It has been used in many studies of basic fish biology and behavior, as well as, toxicological research, and the species has been proposed by the OECD in 1999 as the standard fish for toxicology tests (Khalil *et al.,* 2013).

The study was conducted to determine the bioaccumulation and enzyme activity inhibition of profenofos in Japanese medaka *(Oryzias latipes* Temminck and Schlegel, 1846). It was specifically conducted to determine the bioaccumulation of profenofos in Japanese medaka and in the test water and the bioconcentration factor of the profenofos to the test organisms and to evaluate the possible risk of profenofos using laboratory results based on the AChE activity inhibition and relating results to field concentration.

MATERIALS AND METHOD

Laboratory experiment was generally conducted at the Marine Science Education and Research Institute, Faculty of Fisheries, Kagoshima University, Kagoshima, Japan. River water samples were analyzed for pesticide residue concentration at the Department of Agriculture Bureau of Plant Industry-Pesticide Analytical Laboratory in Diliman, Quezon City. Test organisms, chemicals, reagents and equipment were provided by Kagoshima University.

Sampling Site

The study area is located within the major basin of Mantalongon, Dalaguete specifically in two of its major tributaries. Six sampling sites were identified (Figure 1). Station 1 is located in the Granchina headwater area. The area though a headwater, is still surrounded with few vegetable farms planted to cabbage and other vegetables. This sampling site was chosen as control taking into consideration the assumption that this will have no or least pesticide concentration at all. Succeeding sampling sites were identified with the following criteria; pollutant source (2, 3, 4 and 5) and downstream (6). Site characteristics like ground coordinates, elevation and distance between sampling sites is presented in Table 1.

Table 1. Sampling site characteristics.

Sampling Site Number	Ground Coordinates (based on Universal Transverse Mercator	Elevation (meters above sea level)	Distance from nearest station (km)
Station 1	51 P 549888 1086888	876	(Headwater)
Station 2	51 P 550664 1085577	751	1.25 from Stn 1
Station 3	51 P 550748 1085425	742	0.41 from Stn 2
Station 4	51 P 550313 1085085	771	(another tributary)
Station 5	51 P 550968 1084241	763	1.1 km from Stn 4
Station 6	51 P 551814 10822745	471	3.4 kms from Stn 5 3.75 kms from Stn 3

Sample Collection

Water Samples

Two-liter samples were collected simultaneously from each of the identified sampling sites. The water samples were taken from a flowing part at the sides and at the middle of each sampling point and were composited. The water samples were contained in acid washed amber colored borosilicate glass bottles previously washed with the river water and filled up to the brim andtightly covered with stopper and cooled in ice coolers maintaining 0–4° Ctemperature while it is being transported to the laboratory. The water samples

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were immediately brought to the laboratory for extraction of pesticides prior to analysis. Physico-chemical parameters such as temperature, pH and dissolved oxygen (DO) were determined *in situ* using a digital multi- probe meter (Thermo Scientific, USA) to establish if the sampling sites are favorable for aquatic life. The digital multi-probe meter was calibrated using standards prior to sampling.

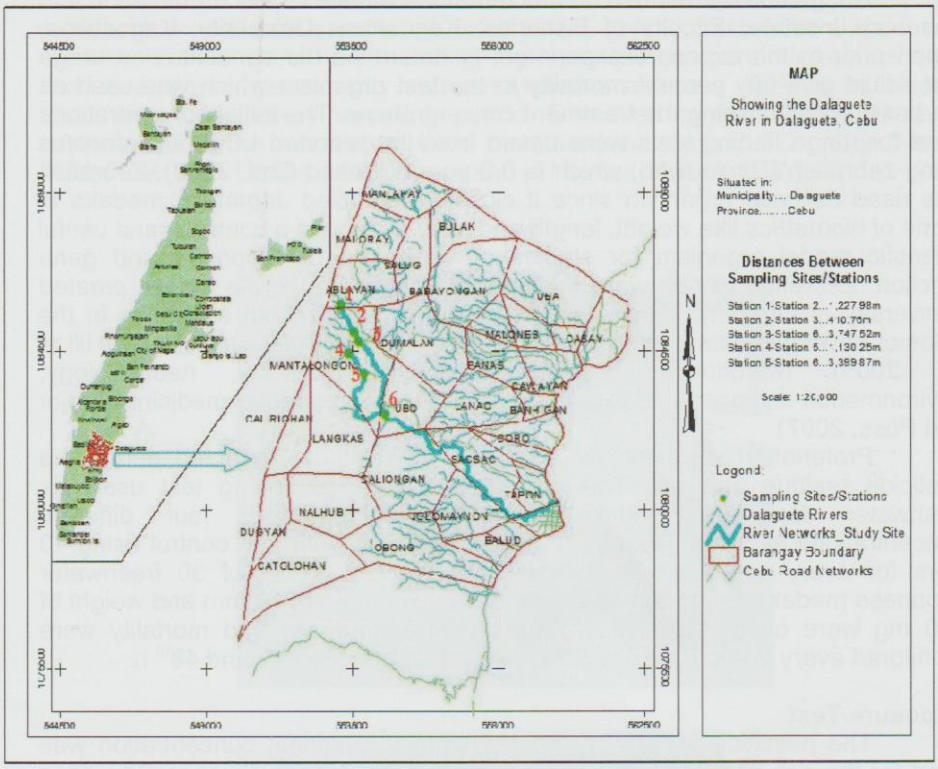

Figure 1. Map showing the location of each sampling sites in Dalaguete River.

Chemicals

Acetylcholine iodide, 5,5–Dithiobis(2-nitrobenzoic acid) and heparin were purchased from Tokyo Chemical Industry Co., Ltd, Japan. Dichloromethane, hexane, and acetone used were all pesticide grades, while silica gel (Wakogel, C100), sodium chloride, disodium hydrogen phosphate twelve hydrate, potassium chloride, 2–phenoxyethanol and potassium dihydrogen phosphate were all analytical grades. These chemicals were purchased from Wako Pure Chemical Industries Ltd., Japan.

Pesticide Analysis in Water

The collected river water samples from the different sampling sites were immediately transported to the Department of Agriculture Bureau of Plant Industry-Pesticide Analytical Laboratory in Diliman, Quezon City.

The pesticide residue concentrations of the samples were analyzed using Gas Chromatography (Agilent Technologies Model HP 6890, USA). The data from the analysis served as basis for the identification of the target pesticide used for the risk assessment.

Range Finding Test

Range finding test was conducted at the Marine Science Education and Research Institute, Faculty of Fisheries, Kagoshima University, Kagoshima, Japan prior to the exposure experiment to determine the concentration range that would give fifty percent mortality to the test organism which was used as the basis in establishing the treatment concentrations. The initial concentrations used for range finding tests were based from the reported LC_{50} of profenofos using zebrafish (*Danio rerio*) which is 0.2 ppm (Min and Cha, 2000). Zebrafish was used as base organism since it closely resembled Japanese medaka in terms of biometrics like weight, length and size. *D. rerio* is a common and useful scientific model organism for studies of vertebrate development and gene function. Zebrafish clones were the first successful vertebrate clones created (University of Oregon). Research with *D. rerio* has yielded advances in the fields of developmental biology, oncology (Xiang *et al*., 2009), toxicology (Hill *et al*., 2005), reproductive studies, teratology, genetics, neurobiology, environmental sciences, stem cell research and regenerative medicine (Major and Poss, 2007)

Profenofos was used since it was the pesticide detected during the pesticide residue analysis. The set up of the range finding test used six freshwater Japanese medaka per concentration with four different concentrations at 3 ppm, 1 ppm, 0.2 ppm, and 0.04 ppm and control using 10 liters for every concentration in static condition. A total of 30 freshwater Japanese medaka fishes with average total body length of 38 mm and weight of 500 mg were used. Dissolved oxygen, temperature, pH and mortality were monitored every 2 hours for 6 hours on Day 0 and on the 24^{th} and 48^{th} h.

Exposure Test

The pesticide present in the field with the highest concentration was used for the risk assessment test conducted at the Marine Science Education and Research Institute, Faculty of Fisheries, Kagoshima University, Kagoshima, Japan. There were four treatments, T_1 (control), T_2 (0.04 ppm), T_3 (0.2 ppm) and T_4 (1.0 ppm). The concentrations of the target pesticide were based on the result of the range finding test wherein the range concentration that resulted to fifty percent mortality for such pesticide to the test animal was determined. Four aquariums (9.5 in x 9 in x 12 in) with 10 liters water were set-up each containing 24 freshwater Japanese medaka, approximately 3–4 cm in length (carrying capacity of approx. 1 g/L). The aerated tap water exposed to activated carbon and the Japanese medaka were provided by the Marine Science Education and Research Institute, Faculty of Fisheries, Kagoshima University, Kagoshima, Japan. The freshwater Japanese medaka have been reared in the laboratory of the Institute. Approximately 1 mL of *Artemia salina* was fed to the fishes every three days. A day prior to pesticide exposure, eight Japanese medaka were sampled to establish baseline data for AChE. This constituted the data for Day 0. The eight fishes were not a part of the 96 fishes used for the exposure test.

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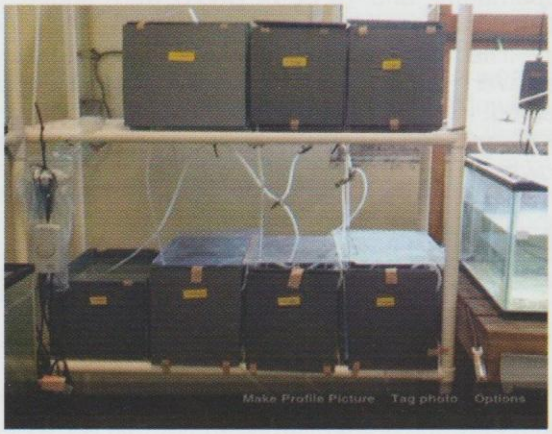
A 10–day exposure was applied. Related study established that concentration of profenofos in zebrafish (*Brachydanio rerio*) maintained an equilibrium until 168 h or 7 days (Min and Cha, 2000). The flow-through exposure method for T_2–T_4 was used. Static condition was followed for Control (Figure 2). Eight Japanese medaka fishes were collected from each aquarium on the 2nd, 5th and 10th day of the exposure period.

Due to the small size of the fishes, fishes were composited to represent a replicate sample. A total of 96 (24x4) freshwater Japanese medaka fishes were used during the risk assessment determination. Oxidative stress, genotoxicity and acetylcholine esterase enzyme inhibition tests were done every sampling period. Dissolved oxygen (DO), pH and temperature of the test waters were monitored daily using DO meter (Navi Model OM – 51, Horiba, Ltd., Japan) and pH meter (Hanna, USA).

A.



B.

Figure 2. Flow-through exposure set. A) with cover b) without cover.

Sampling of Fish for Biochemical Test. Fresh weight, body length and total body length of the fish was determined prior to conducting further tests. This was to establish that the fishes were approximately of the same size and weight. Fishes were anesthetized with 2–phenoxyethanol (0.7 mL/L H_2O).

Acetylcholine Esterase Enzyme Inhibition Test. Brains from two Japanese medaka fishes were collected and combined to compose a replicate sample. The whole brain was extracted and placed in a pre-weighed 1.5 mL microtube (Nippi, Japan). Each sample was homogenized using Power masher II (Nippi, Japan) after the addition of 0.1 *M* phosphate buffer (pH 8.0; 20 mg fresh tissue mL^{-1}). Homogenate was centrifuged at 10,000 g for 15 min at 4°C.

Acetylcholine esterase (AChE) activity was determined using Ellman's reagent (DTNB) and acetylthiocholine (Ellman *et al*., 1961) (Appendix A) modified for a microplate reader (Model 550, Bio-Rad, USA) with a 96–well plate (Khalil *et al*., 2013). In each well, 155 µL of 0.1 M phosphate buffer (pH 8.0), 10 µL of supernatant, and 10 µL of DNTB were added. The plate was incubated at 30°C for 5 min and then 5 µL of substrate (AChI) were added to start the reaction. The enzyme activity was measured using the Bio-rad microplate reader every 2 min for 10 minutes at 412nm. Each enzymatic activity was carried out in triplicate.

The rate of enzymatic activity based on Ellman *et al*. (1961) was calculated as follows:

$$R = \frac{\Delta A}{1.36\,(10^4)} x \frac{1}{(400/3120)C_0} = 5.74\,(10^{-4})\frac{\Delta A}{C_0}$$

Where R = rate, in moles substrate hydrolyzed per min per g of tissue;
ΔA= change in absorbance per min;
C0 = original concentration of protein in tissue (mg/mL).

The protein content of the samples was quantified according to the Bradford dye binding procedure using the Bio-Rad Protein Assay (Bradford, 1976) with bovine serum albumin as standard.

Bioaccumulation of Pesticide

Bioaccumulation of pesticides in the test organisms and the test waters were determined during the 0, 2nd, 5th and 10th day of the exposure period.

Test Water

Analysis of pesticide concentration in the test water was done following the procedure of Añasco *et al,*. (2010).

Extraction of pesticides was performed within 24 h upon arrival in the laboratory. A solid phase extraction was conducted using C18 Sep-Pak Cartridges. The C18 Sep-Pak cartridges were conditioned by gradually adding first 10 mL of acetone (1 drop/second) to the glass syringe fitted with

the cartridge. This was followed by 10 mL of deionized distilled water (1 drop/ second). Fifty mL of the water sample (1 drop/second) was then allowed to pass gradually through the glass syringe with cartridge. The eluent was allowed to stand for 10 mins. The cartridges were then centrifuged for 10 mins at 3000 rpm at 4°C. The cartridges were then fitted into a new set of glass syringes and were gradually eluted with 20 mL acetone (1drop/second). The eluents were concentrated to 1 mL by gentle flow of nitrogen gas. 100 µL of 1.0 ppm anthracene-d10 was added to the sample as internal standard.

Samples were finally injected into a gas chromatograph (Agilent Technologies 6890N, USA) with mass spectrometry detector (Agilent Technologies 5973N) in splitless mode. Analytical conditions were as follows: (1) injection volume, 2 µL; (2) injector temperature, 250°C; (3) detector temperature, 280°C; (4) oven temperature, initially at 60°C (held for 1 min) then ramped to 180° C at 20°C/min (held for 5 min) then finally increased to 290°C at 3°C/min (held for 3 min); and (5) column, DB-5MS (length, 30 m; internal diameter, 0.25 mm; film thickness, 0.25 µm; J and W Scientific, USA).

Test Organisms

The remaining body tissues of the fish samples were freeze dried for the determination of residual pesticide concentration following the method of Añasco, *et al,*. (2010) with some modifications. Dried fish tissues were cut into small pieces. Needed amount for each sample was placed in 50 mL centrifuge tubes. 10 mL hexane-acetone solvent mixture (1:1) was added. The mixture was subjected to ultrasonic extraction for 15 mins then centrifuged at 3000 rpm for 10 mins at 4°C. Supernatant liquid was collected in another 50 mL centrifuge bottle. The precipitate was then added with 10 mL hexane-acetone solvent mixture (1:1) and the process was repeated. The two batches of supernatant liquids were combined 20 mL deionized distilled water was then added to the supernatant liquid. The centrifuge tube was shaken for 10 mins using a shaker at 8.5 speed, then centrifuged for 10 mins at 3000 rpm at 4°C. The supernatant liquid was then collected to which 10 mL hexane was added. The resulting mixture was then shaken for 10 mins at 8.5 speed, then centrifuged at 3000 rpm for 3 mins at 4°C. The supernatant liquid was collected and the precipitate was subjected to the same process. The final supernatant liquid was passed through a glass funnel with quartz wool and anhydrous $NaSO_4$ to remove excess water. The filtrate was concentrated to 1 mL by a gentle stream of nitrogen gas. Clean-up was performed with florisil containing 3% moisture. Clean-up columns were conditioned with 10 mL hexane. The sample was passed through the column and then eluted with 10 mL hexane followed by 20 mL 10% acetone in hexane. The eluate was concentrated to 1 mL by a gentle stream of nitrogen gas. 100 µL of 1.0 ppm anthracene-d10 was added to the sample as internal standard.

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Bioconcentration Factor

The bioconcentration factor of the identified pesticide in the test organisms was computed from concentration of the pesticide in the fish tissues at steady state over the concentration of the chemical in water during the exposure period.

$$BCF = \frac{\text{pesticide concentration in fish tissue at steady state}}{\text{pesticide concentration in test water}}$$

Statistical Analysis

Analysis of Variance (ANOVA) was used to test the significance between treatments and between days of sampling. Student-Newman-Keuls' or S-N-K Test was used to further test if significant difference among treatments exists. The Spearman's rank order correlation or Spearman's rho was used to compute for the correlation between AChE activity inhibition and bioaccumulation data.

RESULTS AND DISCUSSION

Pesticide Concentration in River Water

The pesticide profenofos was the only pesticide detected from the six identified sampling sites along the Dalaguete River with a concentration of 481 µg/L (Table 2). Profenofos with an IUPAC name of 4-bromo-2-chloro-1-[ethoxy (propylsulfanyl)phosphoryl]oxybenzene) is the active ingredient of the commonly used pesticide SelectronÒ in Mantalongon and its neighboring barangays. Profenofos is a broad-spectrum organophosphate pesticide which is used widely for agricultural and household purposes in India (Rao *et al*., 2003), Australia (Kumar and Chapman, 1998), Korea (Min and Cha, 2000), Pakistan and Egypt (Pandey *et al*., 2011). It is very toxic to fish and published 96-h median lethal concentration (LC50) values to three species namely; crucian carp (*Carassius carassius*), rainbow trout (*Oncorhynchus mykiss*) and bluegill (*Lepomis machochirus*) varied from 80 to 300 µg/L (Worthing and Walker, 1987). Physico-chemical parameters for the different sites ranged between 7.1–7.4, 6.8–7.8 ppm and 28.8ºC–30ºC for pH, DO and temperature, respectively.

Table 2. Pesticide residue in the river water samples.

Sampling Site Number	Pesticide Concentration (µg/L)
Station 1	<LOQ
Station 2	Profenofos = 481.0
Station 3	<LOQ
Station 4	<LOQ
Station 5	<LOQ
Station 6	<LOQ

Note:

1. Results were obtained by Gas Chromatography (Agilent Technologies HP 6890, USA).
2. The Limit of Quantification (LOQ) for organophosphates, organochlorines and pyrethroids is 10.0 µg/L..

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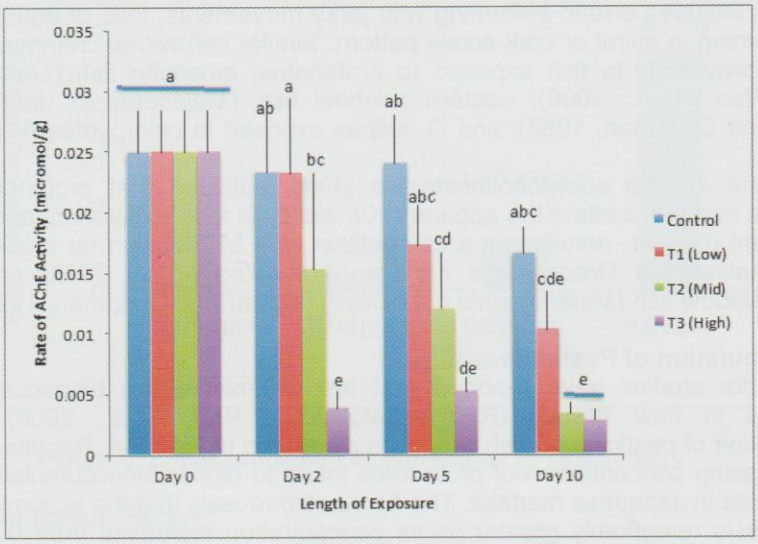
AChE Inhibition Test

Acetylcholinesterase (AChE) activity was significantly inhibited as pesticide concentration was increased (Figure 3). In Days 2 and 5, T_3 (1.00ppm) AChE activity was significantly lower than the rest of the treatments, however, on Day 5 T_1 (0.04 ppm) and T_2 (0.20 ppm) were not significantly different from each other but significantly different from T3 and Control. On the last day of sampling, T_2 and T_3 were not significantly different from each other but significantly different from T_1 and Control.



* Different letters are significantly different.

Figure 3. AChE Activity on *O. latipes* exposed to different concentrations of profenofos.

The findings are in agreement with the results obtained by other researchers where the AChE activity inhibition is dose-dependent when Japanese medaka was exposed to chlorpyrifos (Khalil *et al*., 2013) and diazinon (Park *et al*., 2005); and mosquito fish (*Gambusia affinis*) exposed to diazinon (Kavitha and Rao, 2008).

Inhibition might be due to binding of the actual toxic agents in profenofos and other OPs to *O*–serine in the esteratic site of acetylcholinesterase (Karczmar, 1970). Profenofos induced significant inhibitory effects on the AChE activity of the freshwater shrimps *Paratya australiensis* (Abdullah *et al*., 1994) and *Oreochromis mossambicus* (Rao *et al*., 2003).

Previous research findings on the irreversibility of the inhibition of AChE by OPs were confirmed in the study, where there is no significant difference onthe acetylcholinesterase activity in T_2 (0.20 ppm) and T_3 (1.00 ppm) from Day 2 to Day 10 . It can be inferred that the inhibition of AChE activity on Day 2 was statistically similar to the AChE activities on Days 5 and 10. Worth noting is that T_2, the concentration lower than the actual field pesticide concentration already exhibited significant inhibition as early as Day 2.

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The inhibition of acetylcholinesterase by organophosphorous compounds belong to the irreversible type (Tusanova *et al.,* 1999). Studies of Rao *et al.* (2003) and Kumar and Chapman (1998) determined that prolonged exposure to organophosphate pesticides increases acetylcholinesterase inhibition. Whereas the activity of phosphorylated AChE can be recovered by action of nucleophilic reactivators, these reactivators have no effect on dealkylated AChE (Puu *et al.*, 1986).

Profenofos-exposed fish demonstrated reactions to light stimulus and they swam in circles and knocked at the edges of the aquarium. Other abnormal behaviors included erratic swimming with jerky movements, loss of equilibrium and swimming in spiral or cork screw pattern. Similar behavioral changes were reported previously to fish exposed to profenofos; mosquito fish (*Gambusia affinis*) (Rao *et al.*, 2006); eastern rainbow fish (*Melanotaenia duboulayi*) (Kumar and Chapman, 1998); and *O. latipes* exposed to chlorpyrifos (Khalil *et al.*, 2013).

The *in vitro* acetylcholinesterase study indicated that profenofos is neurotoxic and that it alters the apparent *Km* widely in a concentration–and time –dependent manner, resulting in a competitive type of inhibition for studies on the euryhaline fish *Oreochromis mossambicus* (Rao *et al.*, 2003) and for eastern rainbow fish (*Melanotaenia duboulayti*) (Kumar and Chapman, 1998).

Bioaccumulation of Pesticide

Prior studies have reported that test organisms can bioaccumulate profenofos in their tissues (Rao *et al.,* 2003; Rao *et al.,* 2006). The accumulation of pesticide on fish tissues is presented in Figure 4. Results show that increasing concentration of profenofos leads to higher bioaccumulation of the pesticide in Japanese medaka. The figure also reveals that the accumulated profenofos is remarkably greater as its concentration increases from 0.2ppm (T_2) to 1.0ppm (T_3) reaching point of equilibrium on Day 5.

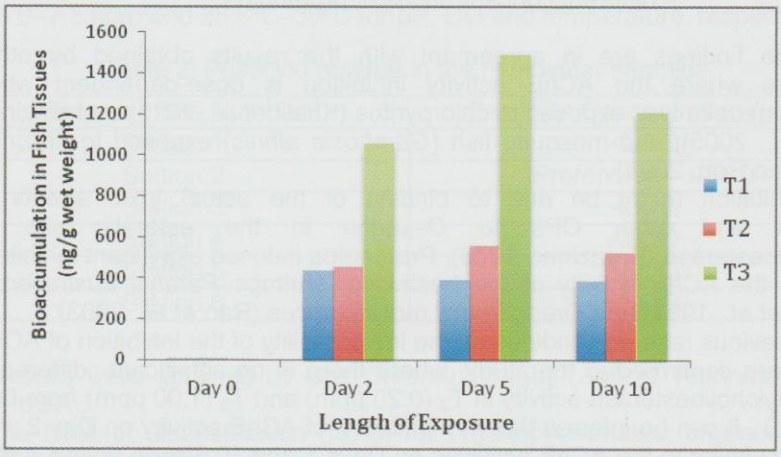

Figure 4. Bioaccumulation of Profenofos in Fish Tissues.

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Consequently, the amount of pesticide accumulation declined in the fish tissues with longer period of exposure. It appears that the Japanese medaka has the ability to metabolize the profenofos. These results are complemented by the data presented in Figure 5 where the amount of profenofos in water increases due to the depuration ability of the fish (Pena-Llopes *et al*., 2003); the relatively low water solubility and relatively slow depuration of profenofos (0.028 g/L at 20°C) and high lipophilicity (log Kow = 1.7) (Montgomery, 1996; Min and Cha, 2000).

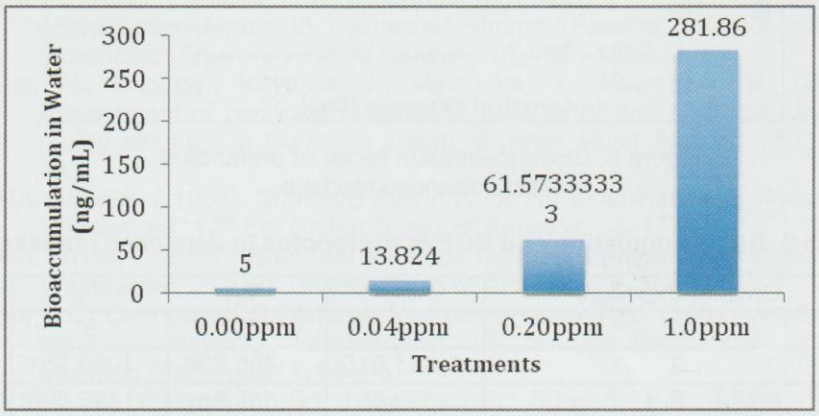

Figure 5. Bioaccumulation of Profenofos in Test Waters.

Bioconcentration Factor

The average BCF values of profenofos decreased as the pesticide concentration increases which is expected because even though bioaccumulation increased significantly as concentration was increased, the amount bioaccumulated in the test waters also increased. A bioconcentration factor greater than 1 is indicative of a hydrophobic or lipophilic chemical. It is an indicator of how probable a chemical is to bioaccumulate (Landis *et al.*, 2011). These chemicals have high lipid affinities and will concentrate in tissues with high lipid content instead of in an aqueous environment. As can be seen in Table 3, all BCF values for the three treatments were all greater than 1 implying that profenofos has more affinity to bioaccumulate in the fish tissues than in the water.

Relationship between AChE activity inhibition and Bioaccumulation

Correlation analysis using Spearman's rank-order correlation were done to determine the monotonic relationship of the results for AChE activity inhibition and bioaccumulation of profenofos in Japanese medaka. On Day 2 it was found out that there was no correlation between AChE activity inhibition and bioaccumulation of profenofos in the fish tissues. However, on Days 5 and 10 there existed a strong negative correlation between the two parameters ($p<0.01$). This means that the degree of decrease in AChE activity was proportional to the degree of increase of the bioaccumulation of profenofos in fish tissues.

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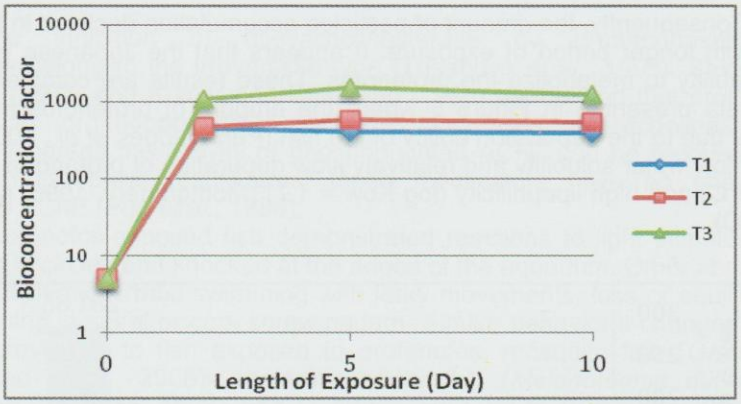

Figure 6. Bioconcentration factor of profenofos against Japanese medaka.

Table 3. Bioaccumulation and BCF of profenofos in Japanese medaka.

Days of Exposure	T_1	T_2	T_3
0	5	5	5
2	441.515	455.826	1060.551
5	393.651	558.881	1496.096
10	385.522	524.057	1210.217
Average (Fish) ng/g wet weight	406.896	512.921	1255.621
Average (Water) ng/mL	13.824	61.573	281.860
BCF	29.434	8.330	4.455

CONCLUSIONS

This study demonstrated that profenofos has a significant effect on AChE activity inhibition in the brain of Japanese medaka. The effect was dose-dependent and exposure-time dependent. An increase in inhibition was observed the longer the exposure period and the higher the concentration of profenofos. There was a strong negative correlation between the enzyme activity inhibition and that of the bioaccumulation of profenofos in the tissues of Japanese medaka. Evaluating the toxicity risk posed by profenofos in terms of the two biomarkers, it can be concluded that profenofos in the actual field condition poses risk to the aquatic organisms present in Dalaguete River since laboratory concentrations which were lower than the field concentration already caused significant effect on the AChE Activity inhibition. However, more detailed studies are required to establish its effect on other aquatic organisms specifically those thriving in Dalaguete river like guppies, tilapia, catfish and shrimps. Further studies on degradation and fate and transport of profenofos are also recommended.

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