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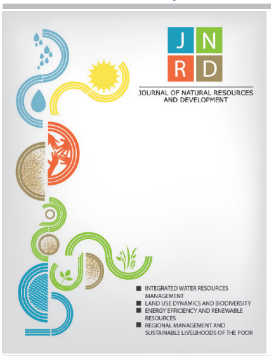

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Purification and characterization of toxic waste in the aquatic environment using common carp, *Cyprinus carpio*

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Abstract

The organophosphorus (OP) pesticide (malathion) is used heavily for many crops such as vegetable and cotton to control serious key insecticide in many areas of Egypt. This study has focused on the effect of malathion on aquatic environment and aquatic organisms. The experimental work was carried out using malathion at different dosage of water lasted 96h and was carried out undertaken laboratory conditions. It evaluated the sensitivity of organic toxic waste and their purification techniques for common carp, *Cyprinus carpio* by determining enzymes activity as biomarker indicators in various organs of the studied fish.

The results showed that exposure to malathion caused a significant increase in enzyme activity and total protein contents in the investigated tissues and inhibition of brain and liver acetylcholinesterase (AChE). Moreover, among the tissues studied, it appeared that the brain, gills and liver were more sensitive to pollution and seemed to be the most appropriate tissues to monitor water pollution by OP pesticides. In this context for environmental biomonitoring, the evaluation of toxic waste purification can be done to assess sensitivity of aquatic organism in recycling water to meet national goals and environmental safety.

Introduction

Natural fresh water are the ultimate recipient of most toxic substances, generated by industrial, agricultural and domestic activities which are released into the environment. Deleterious effects on aquatic ecosystems may result from toxicant exposure, through run-off, from agricultural field or directly through careless application that directly causes the death of an organism (acute effect) or produces sub-lethal chronic effects on the organist that hinder its ability to develop, grow and reproduce in the ecosystem (Eason 2011). Organophosphorus pesticides (OPs) used in agricultural intensively has led to the contamination of aquatic environments and affected the non-target aquatic organisms, particularly fish (Meng and Kang 2008). Therefore, fish have been used as a bioindicator to assess the

environmental impacts of pollution and evaluate the purification of water resources (Arias et al. 2007). Thus, many researchers have studied the monitoring OPs by biomarker measurements and their response to OPs elimination in the aquatic environment (Meng and Kang 2008).

Malathion, is an organophosphorus pesticide (OPs) widely used in agriculture and domestic activities (Abhilash and Singh 2009). Correlation between depressed accumulation levels and manifestation of toxic effects has been reported for malathion and other OPs (Tilak et al. 2004) and (Henry 2006). Many studies have investigated the persistence and accumulation of OP insecticides in brain more than other fish tissues specially in carp tissues (Tilak et al. 2004). Also, the residues of OPs in water of various environments were detected and seem to decline over time (Henry 2006).

Rick and Edwards (2010) have recently shown environmental investigations that used environmental approaches for measuring biological effects and green chemical process to remove toxicity in aquatic environment and to provide valuable results for a decision maker. So-called screening systems that elimination of toxic pesticides from aqueous solutions and waste water using enzymes activity are urgently needed (Weiping et al. 2006). Because of their toxic and hazardous nature, removal of malathion from water and wastewater has been of concern to scientists; treatment options should be efficient, economic and safe (Kiang et al. 2006).

Therefore, the biological treatment seems suitable for the degradation of hazardous aqueous pollutants for removing pesticide residue from contaminated water. On other hand, several reactions catalyzed by enzymes of specific microorganisms take place (Jiang et al. 2006) in Laboratory Scale Activated Sludge Unit (LSASU).

The objective of this study is to assess the sensitivity of fish tissues to exposure to sublethal concentrations of malathion (2 mg malation/l) ½LC_{50} for 96h on common carp, *Cyprinus carpio* and then allowed to recover for 192h. The selected biomarkers are acetylchloinesterase (AChE), alkaline phosphate (ALP), alanine aminotransferases (ALT), asparatate aminotransferases (AST) in fish's brain and liver and total protein contents (T.P.) in liver and muscles resulting from malathion residues in aquatic environment. These measurements have been used as an important bio-indicator to monitor exposure – effect relationships, damage by OP pesticides to increase the predictability of laboratory and field eco-toxicology data.

Methodology

A simple and versatile laboratory experiment was designed to measure the influences of sub-lethal toxicity levels of malathion, accumulation concentrations in studied tissues and residues degradation in aquatic system. The common carp, *Cyprinus carpio* was selected as a bio-indicator to determine AST, ALT, ALP, AChE enzyme activity and total proteins (T.P.) of a screening system for toxic organic waste in aquatic environment with fish. Contaminated water samples were analyzed and treated to determine malathion residues, its degradation and purification approach of aqueous solution using biological treatment coupling with activated carbon technique and its response to fish. High Liquid Performance Chromatography (HLPC) equipped with UV at 220 wave length was used to detect malathion residues in all environmental samples including water and fish organs (brain, gills, liver and muscles).

Chemicals

Organophosphorus insecticide, malathion (57% E.C.) Kafrel-Zayat Company-Egypt: O-O dimethyl S(1,2-dicarbethoxy-ethyl) phosphorodithioate (IUPAC) (Fig.1).

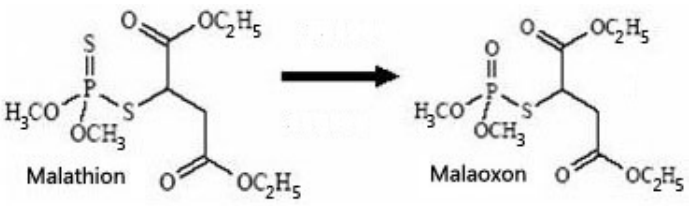

Figure 1. Chemical Structure of Malathion and Malaoxon.

Purification of aqueous solution

A survey was carried out to determine aqueous solution treatment process and disposal management procedure for hazard organic waste. Laboratory Scale Activated Sludge Unit (LSASU) is simply designed as biotechnology coupling with an activated carbon system to remove organic waste (Fig. 2). LSASU simply involves mixing of wastewater and a culture of microorganisms from the incoming sewage and sludge recycle. Sample of mixed liquor (homogenous) and sludge were enriched by bacterial culture of genus Pseudomonas under aerobic conditions.

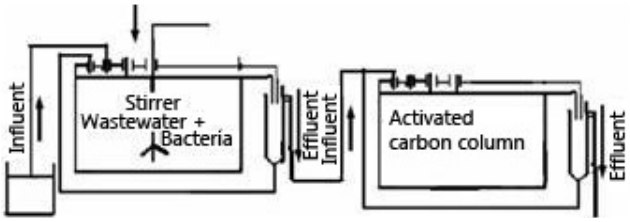

Figure 2. Diagram of laboratory-Scale activated Sludge Unit and Activated Carbon Column.

Fish

The fresh water common carp, *Cyprinus carpio* fish was collected from Abo-saleh Island productions, Beni-suif governorate, Egypt and transported to laboratory in plastic ice-box containing dechlorinated and oxygenated water. Fish were acclimated for one month before beginning the experiment. The physico-chemical characteristics of the test water were measured (APAH 2005) and recorded. Means results were; pH: 7.5 ± 0.07, Temperature: 27 ± 0.05 ºC, dissolved oxygen 6.4 ± 0.024 mg/l, alkalinity 2.43 ± 0.18 meq as $CaCO_3$, Hardness 97.8 ± 2.7 mg/l as $CaCO_3$. The healthy fish samples had average total length (16 - 18.3) cm and average total weight (180-200g) The fish were fed once daily with commercial dry pellets (25%

protein) at a rate of 2% of the body weight (Sprague 1969) before beginning of the experiment and during exposure period and aquaria water was changed once daily for cleaning. Fish were transferred to experimental aerated aquaria (70x50x60 cm^3, 100 liter capacity) at the beginning of the experiment.

Determination of LC_{50} of malathion for common carp

9 concentrations of malathion (6, 5, 4, 3, 2, 1, 0.75, 0.50, and 0.25 mg/l) were prepared in 9 equal-sized aquaria, in addition to one aquarium for the control. 10 fish individuals were transferred for each individual malathion containing aquaria. The experiment was continued for 96h at each malathion concentration and water was changed daily to keep the concentration constant during the exposure period. At the end of the 96h, the mortality percentages were calculated according to probit analysis method (Finney 1971).

This experiment was repeated twice and the average LC_{50} value of malathion was found to be 4mg/l. Fig.(3) showed that the mortality percentage of common carp, *Cyprinus carpio* fish exposed to special concentration of malathion in aquatic environment followed the linear equation (-8.2418x + 110.92) with R^2 = 0.98. The recorded LC_{50} for malathion 4 mg/l (50% mortality).

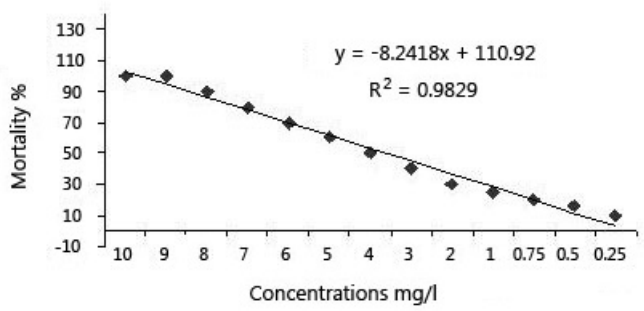

Figure 3. Determination of LC50 of common carp, *Cyprinus carpio* fish exposed to malathion.

Acute experiment for 96h to ½LC_{50} of malathion

10 fish of common carp, *Cyprinus carpio* were transferred to individual aquaria containing 1/2 LC_{50} of malathion (2mg/l) for 96h, in addition to the control. Fish were dissected to collect tissues for each 24h interval during the exposure period and kept at -4^{0}C till complete analysis within few days after sampling.

Experimental design

3 groups (10 fish each) were investigated under laboratory conditions i.e. control group, dealing group and recovery group (Table 1). First group was exposed to dechlorinated and oxygenated tap water, second group was exposed for 1/2 LC_{50} of malathion (2mg/l) for 96h (acute exposure) and the third group in which the treated common carp, *Cyprinus carpio* fish was transferred to malathion free water for 192h.

Table 1. Environmental management

Set	Dealing Conditions
Control group	Fish exposed to de-chlorinated and oxygenated tap water
Dealing group	Fish were exposed to ½LC_{50} malathion - 96hrs
Recovery group	Treated fish transferred to de-chlorinated and oxygenated water for 8 days till complete recovery.
Scarifying	Each fish was dissected for pieces of muscle, liver, gill and brain tissues collection and storage was carried out at - 4°C.

Bioaccumulation analysis

Simple experimental set-up and measure procedure were carried out to assess malathion concentration and its degradation in water samples, easy generation of dose - and time- dependent responses and bioaccumulation in tissues. The methodology is demonstrated with several different dosages of organophosphorus malathion to provide the sensitivity of pesticide. Malathion in water and fish tissue samples was extracted, analyzed using HPLC equipped with UV at 220 wave length and following the procedure outlined according to the method described by (El- Sheamy et al. 1991). The method was applied as following:

Extraction

50 g fresh ground fish flesh was accurately weighed, transferred to a high speed blender and mixed for 8 min with 100 g anhydrous sodium sulfate in the presence of 150 ml petroleum ether. The extract was decanted through 500 ml Buchner funnel (containing 2 12 cm Whatman N-1 filter papers) in a suction flask. The residue in the blender cup was re-extracted with 2 100 ml portions of petroleum ether, blended for 8 min for each portion, decanted through the Buchner funnel, and pooled with the first extract. The obtained extract was poured through a 40 x 25 mm column of anhydrous sodium sulfate, and the eluate was collected in a 500 ml flask and placed in a rotary evaporator, to obtain the fat.

Cleanup

The extracted fat was transferred to a 125 ml separatory funnel with the aid of 15 ml petroleum ether; 30 ml acetonitrile saturated with petroleum ether was added and the mixture was shaken vigorously for 5 min. The layers were allowed to separate and the acetonitrile layer was drained into 1 l separatory containing 650 ml water, 40 ml saturated NaCl solution and 100 ml petroleum ether. Separation technique was repeated 3 times, beginning with the transfer of the extracted fat to the 125 ml separatory funnel.

All the extracts were collected in the 1 l separatory funnel and mixed thoroughly for 30-45 seconds. The layers were allowed to separate and the aqueous layer was drained into another 1 l separatory funnel containing 100 ml petroleum ether was added and the mixture was shaken vigorously for 15 S. After the layers separation, the petroleum

ether layer was combined with the previous one and washed with 2 100 ml portions of water. The petroleum ether layer was drawn off through a 50 x 25 mm column of anhydrous sodium sulphate. The eluted petroleum ether extract was evaporated to 10 ml in a rotary evaporator after which it was transferred to a florisil column prepared as described below.

Cleanup by Florisil column

A glass column, 22 mm id, was filled with Florisil (60- 100 mesh, P.R. grade; activated at 675°C for 3 h) to a height of 10 cm and topped with 1 cm anhydrous sodium sulfate. The column was prewetted with 40-50 ml petroleum ether; then the petroleum ether extract from the above (b) step was passed through the column at (5 ml/min). The column was eluted at the same rate using 200 ml eluting solvent (50% diethyl ether in petroleum ether). The eluate was concentrated to a dry film which was dissolved by 2 ml of n-hexane for determination.

Lipid assay

The lipid content of various tissues was determined in an attempt to reduce variability in malathion concentrations in specific tissues by providing an alternative method to sample weight for standardization. Prior to determination of lipid content, glass culturettes were dried in a desecrator until their weight no longer changed. The tissue samples were prepared for lipid extraction by homogenizing approximately 0.1 g of tissue in 2 ml of solvent, 2:1 dichloromethane: methanol (v: v) in a homogenizer. The homogenate was added to a culturette and a folch wash was performed with the addition of 1.5 ml of 0.88% sodium chloride in deionized water. The sample was centrifuged for 8 minutes at 1000 G. The hydrophilic phase was removed with a glass pipette and the lipophilic phase, solvent, was transferred into the pre-weighed, dehydrated culturette. The solvent was evaporated under nitrogen to dryness. The culturette was reweighed and lipid content was calculated on a µg/g basis.

Extraction efficiency was measured in all samples. Malathion, 0.1 µg, was injected into tissues (n=5) in the homogenizer. The samples were extracted and ran on the HPLC-UV for quantification. Extraction efficiency for sets of samples was between 85-110%.

Chromatographic quantification

Malathion burdens were determined using High Performance Liquid Chromatograph Agilent I I 00 Series with work station. The U.V detector set at 220 nm, and the analytical column Zorbax - C18,5 Um (4.6 x 150 mm) was used. The Mobile phase was Acetonitrile - Water at gradient as follows:

Table 2. Analytical conditions

Time / min	Acetonitrile	Water	Flow
0	50	50	0.7
2	30	70	70
5	50	50	50

4 µl of the reconstituted sample was injected in triplicate onto the analytical column. The injection of external standards confirmed the identity of the chromatographic peaks. Detection limits were calculated to be 0.04 µg for malathion and 0.10 µg for malaoxon using curves generated from standards. Standard curves were made from malathion and malaoxon standards, 100 µg/ml in methanol, in dilutions of 100 µg/ml, 75 µg/ml, 50 µg/ml, 25 µg/ml, and 10 µg/ml. All standards were run in triplicate. All tissue burdens were calculated on a ppm or mg/Kg wet weight basis.

There were no detectable levels of analytes in blank samples. The calibration standard is measured every set of group samples for calibration and the resulted concentration of the standard did not exceed than 5% differs of the true value. Performance data were checked by control standard results which fall within designated control limits. The spike recoveries ranged between 80-120%. The sensitivity and recovery for the method were determined using samples spiked with 3 different concentrations (3, 10, 20 mg/kg) of the tested malathion. The results that given in Table 3 had been corrected for percent recovery of the malathion.

Table 3. Recovery and limit of detection of applied method

Pesticide	Spiked conc. µg/kg	Recovery %	Limit of detection µg/kg
Malathion	3	91	1.9
	10	94	10.15
	20	96	20.81

Biomarker measurements

Malathion residues were measured in aquatic environment and investigated organs of common carp. AST, ALT, ALP, AChE were chosen as representative's bio-marker of organic waste effect in aquatic environment to be tested as screening system (Ellman et al. 1961) and (Mukhopadhyay et al. 1982) .

Total proteins (T.P.) of each tissue of the control and treated-malathion were extracted and evaluated (Mukhopadhyay et al. 1982) and (Snedecor and Cochran 1980) respectively. Concentration of each metabolite was expressed as µg/mg of tissue

Statistical analysis

Differences between control and treated-malathion groups were evaluated by an analysis of variance (one-way ANOVA) at a significance level of 0.01 (Snedecor and Cochran 1980). Normality and homogeneity of variances were verified and a parametric one-way analysis (ANOVA) was performed on data. All results are expressed as a mean ± standard error.

Results and discussion

Data summary

Various irrigation canals and drains, within crops-growing areas in

spraying seasons received varying levels of malathion exposure from agricultural practices that cause environmental impacts on water quality, water suitability fish as important part of non-target organism in aquatic system and major source for human beings.

The risk of exposure to malathion is due to its effect on immune system of fish and other alterations of its physiological system. The data presented the evaluation of LC_{50} and the potential organic toxic on the common carp, *Cyprinus carpio* fish as well as its effect on enzyme activity that reflect organic waste effects of malathion on health fish using biomarkers measurements and total protein.

Moreover, toxicity of organic waste using laboratory activated sludge unit that includes a culture of active micro-organisms (settled sewage) under aerobic conditions coupled with activated column was applied in a screening system for elimination of malathion contamination in aquatic environment to reduce economic degradation and protect farm's fish under international guidelines to achieve national goals.

Environmental profiling of toxic waste in aquatic environment

1/2 LC_{50} of malathion (2mg/l) was chosen as representative's organic toxic level to be tested as acute effect for 24, 48, 72, 96 hours. Figure 4 demonstrated malathion levels in water samples that declined significantly after 96h from 1.189±0.001 mg/l to 1.086±0.001 mg/l while it was not and non-detected after 8 days of reference concentration.

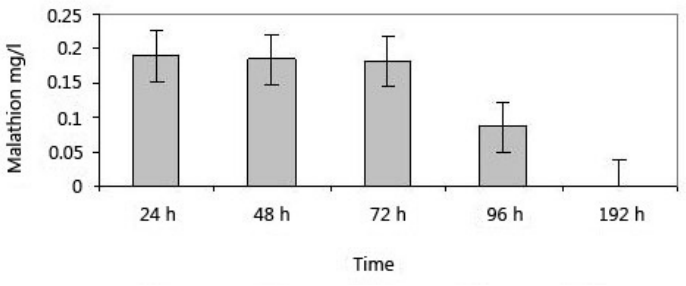

Figure 4. Degradation of Malathion in water samples.

The study showed that malathion persisted in tissues of fish reflecting the general levels of pesticide usage. There was a correlation between the level of malathion degradation in water and corresponding bioaccumulation in fish tissue samples. This means that OPs were degraded and the residue levels were dependent on uptake rate in common carp, *Cyprinus carpio* tissues (Fig.5).

Generally, statistical analysis showed that the brain was the carp tissue in which most Malathion was accumulated. Therefore, the malathion was accumulated in the following order brain > gills> liver > muscles with highly significant elevation ($P<0.05$). This could be attributed to the rapid penetration and binding of insecticide residues in fish tissues since some types of OP pesticides were found within the fat phase in fish depending on the physico-chemical properties (lipophilic) (Pandeya et al. 2011). Further, gills are the main route of pollutants entry, transportation to different tissues took place directly via vascular system. Therefore, the residue levels depending on uptake rate, degree of solubility, stability, octanol-water partition coefficient of OP and /or its rapid conversion to metabolites (Pandeya et al. 2011).

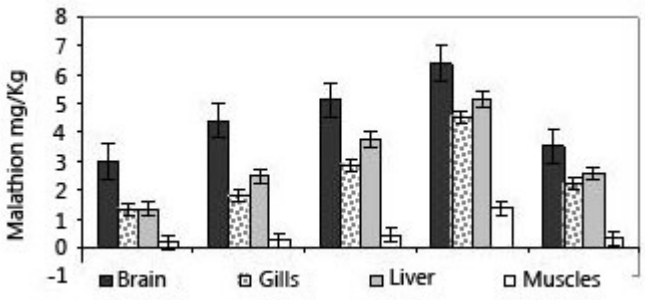

Figure 5. Bio-accumulation of Malathion in Common carp, *Cyprinus carpio* tissues.

Study of aquatic purification and disposal management

The investigated concentrations of malathion for 24h (½ LC_{50}) were selected for on-site treatment before declaring to be safe.

Progress of organic matter decline

The artificial wastewater at experimental 24hours gave a significant volume of wastewater loaded with toxic waste (1.189 mg/l) and other chemical pollutants. The environmental measurements in terms of chemical oxygen demand (COD), biological oxygen demand (BOD_5) were determined for the measurement of total organic load and malathion was measured at end of treatment.

The toxic waste effluent sample for 24h was selected as a test for on-site treatment before disposal (1.189 mg/l) COD concentration (158 mg/l), and BOD_5 (85 mg/l) exceeded the national standards limits (9/2009), showing that wastewater was heavily loaded with organic residues. The alkaline trend side of pH for wastewater fluctuated between "7.5-8.5". There would be 2 sources of malathion removal in the system; one is pH itself and the second is the microorganisms that are major pathway of disappearance of malathion in soil, water, sediment and salt march environments as biologically mediate (Imran et al. 2006).

Furthermore, the aerobic condition: O_2-rich in surface wastewater was maintained to breakdown organic matter and performance of the system especially in removal of COD concentration (158 to 10 mg/l), and BOD_5 (85 to 15mg/l) as function of retention time. Figure 6 illustrated the operating times for integrated aerobic system: 1h-8h have been operated and settling of sludge for 2h that showed the measurements at different detention times in aeration zone reported the optimum operating conditions were 7-8h to complete decomposition.

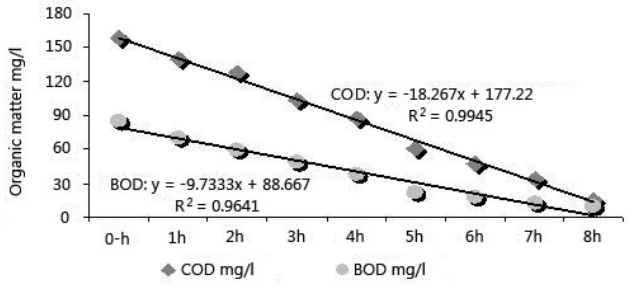

Figure 6. Concentration of organic matter in effluent at different reaction times.

Trend of toxic organic waste degradation

An aquatic experiment was designed to measure the removal of malathion and accumulated concentrations of its residues in aquatic environment under laboratory conditions and malathion degradation in aquatic system to be safe.

Figure 7-8 for chromatography analysis showed a significant improvement of malathion removal rate with time and biodegradation percentage that is used to quantify pollution removal from toxic waste under the given experimental conditions and the degree of water purification from contaminants.

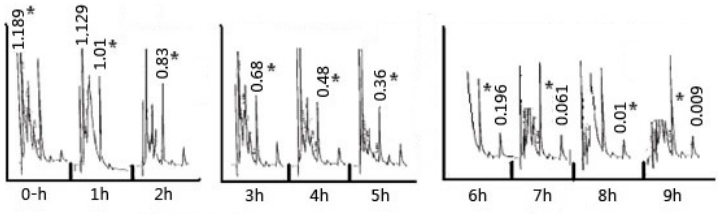

Figure 7. HPLC analysis of Malathion at different times.

The present results of work was conformed with environmental studies that bacterial culture of *Pseudomonas* played important role in pesticides degradation because these bacteria are oxidative, aerobic metabolically versatile, degrade aromatic hydrocarbon, oil, petroleum products and pesticides which used in situ bio-restoration (Balk et al. 2011).

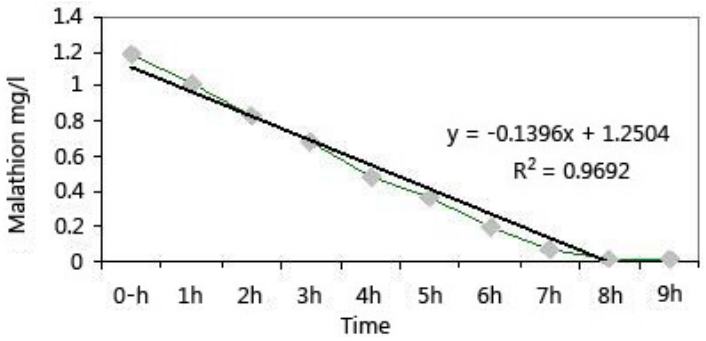

Figure 8. Estimation of Malathion degradation by HPLC at different times.

Moreover, the study coupled the biological treatment with activated carbon column to check and ensure toxic waste purification (1.189 mg/l) to (<0.01 mg/l) that within the Egyptian Environmental Law (9/2009) as shown in Fig. 9. In addition, the treatment performance evaluated as malathion removal percentage and calculated as:
Malathion degradation % = (C0 – C/ C0 X 10), where C_0 and C are initial and final malathion concentrations, respectively.

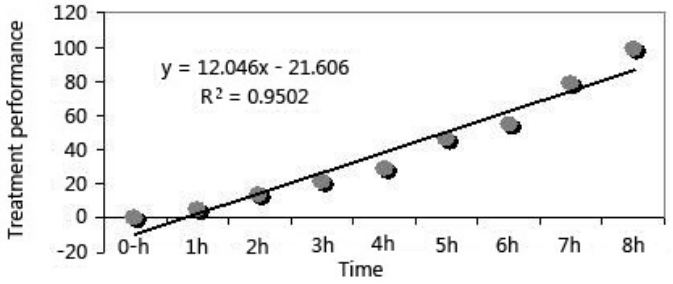

Figure 9. Performance degradation of Malathion– activated carbon.

These data agree with some studies that assessed the activated charcoal is the most commonly used adsorbent for removing pesticide residue from contaminated water to avoid toxic and hazardous nature that could be dangerous to the ecological balance (Christodoulatos et al. 1997) and (Jiang et al. 2006). The out activated column is collected and adjusted as physico-chemical characteristics of the used test water in experimental design. This treated water is used to check the sensitivity of malathion removal in water and fish tissue for 24h. The gills and muscles tissues were selected to represent toxic waste effect that are in direct contact with the malathion (<0.01 mg/l) and assess sensitivity of aquatic organism in recycling water to meet national goals and safe economic environment. The analysis showed that malathion concentration was within FAO/WHO limits for pesticides in fish (FAO/WHO 1997).

Bio-indicator consequence

Quantification of proteins in tissue

Protein contents of 2 tissues of common carp, *Cyprinus carpio* control and malathion-treated (muscles and liver) are presented in Fig. 10. Malathion exposure increased significantly ($p<0.01$) total protein concentrations in all studied tissues that were (3.42±0.008, 3.91±0.03, 4.09±0.05, 4.75±0.04, 4.57±0.07)µg/g for muscles and (4.3±0.1, 4.07±0.01, 4.43±0.06, 6.64±0.3, 4.8±0.03) g/g for liver. The increase of total protein contents is due to activation of protein synthesis involved in defensive mechanisms such as production of metallothionein (Paris et al. 2002). Proteins content would therefore depend on the balance between induction of defenses and their inhibition by impact of pollutants toxicity (Dautremepuits et al. 2004).

Biomarker measurements

The environmental risk assessment and eco-toxicology involved the use of biomarkers designed to highlight an early pollution (Ramon

et al. 2007). To assess the impact of neurotoxic compounds on fish, we had evaluated AChE activity which was used as a biomarker of exposure to nerve agents such as organophosphours pesticides (Rendón-von et al. 2005).

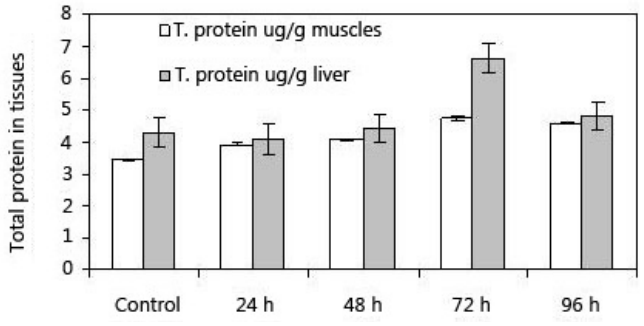

Figure 10. Total protein in various fish tissues.

Figure 11 clarified that AChE activity after malathion exposure in the pieces (brain: 9.6±1.47, 28.06±2.3, 51.88±1.3, 66.08±2.1, 174.6±1.3 ug/g and liver: 18.57±0.02, 28.8±0.01, 41.2±0.02, 47.2±0.005, 81.8±0.006ug/g), that reveals an inhibition during acute period of exposure with a dose dependant on the studied tissues. This decrease was more apparent in the brain > liver as shown in Fig.5. This is due to malaxon (a major metabolite of malathion) which and it was the main inhibitor of AChE (Rick and Edwards 2010) the enzyme that cleaves the neuro transmitter acetylcholine, thereby interfering with proper neurotransmission in cholinergic synapses and neuromuscular junctions. This could interfere with vital functions and also as indication of hepatic disorders (Chandrasekra and Pathiratne 2005). The study suggested that brain AChE activity was depressed by about 70% or greater in fish. This may lead to risk danger of death from the AChE-inhibiting poisoning (Safia et al. 2010).

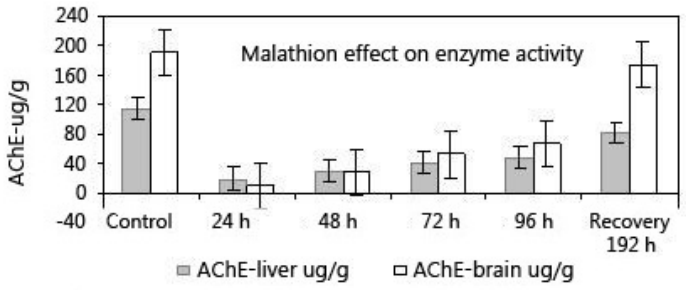

Figure 11. Acetylcholinesterase (AChE) activity in various fish tissues.

Furthermore, ALT, AST and ALP enzymes are considered key enzymes in fish that can be used as response indicators to chemical pollution and to diagnose the impact of sub-lethal toxicity of environmental contamination in fish (Kory and 1993).

Figure 12-A,B showed that ALT (Liver: 17.3±0.4, 37.9±0.8, 44.4±1.5, 50.3±0.6, 73.8±1.3 ug/g and muscles: 14.03±0.38, 33.9±1.07, 47.3±1.19, 53.3±1.15, 61.1±0.91), AST (Liver: 43.5 ±1.08, 63.3±1.7, 79.06±2.09, 96.6±1.3, 125.1±1.7 ug/g and muscles: 40.4±0.36, 46.34±1.25, 61.6±0.57, 84.9±1.08, 93.6±1.14 ug/g) and ALP (Liver: 9.506±0.5, 20.9±1.05, 30.2±1.1, 36.6±1.4, 45.1±1.4 ug/g and muscles: 8.21±0.23, 25.5±1.32, 32.6±0.8, 45.4±1.28, 54.08±0.75 ug/g) increased gradually due to exposure at exposed to malathion in liver than muscles that conformed with recent studied (Oruc and Usta 2007). The increase in the activity of enzymes (AST,ALT&ALP) may be due to liver damage (Ozmen et al. 2008) and (Vandana et al. 2008) and also may be due to high production of oxaloacetic acid and pyruvate and glutaric acid which in turn channeled into citric acid cycle to meet the increase energy demand during stress condition.

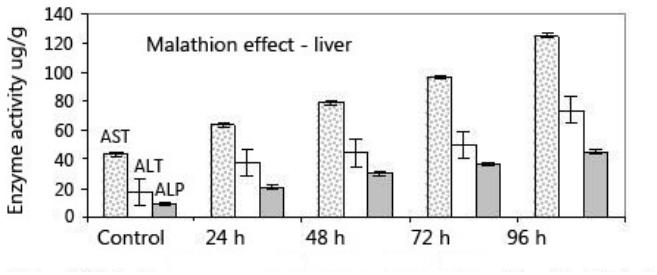

Figure 12-A. Enzyme activity of liver in studied fish.

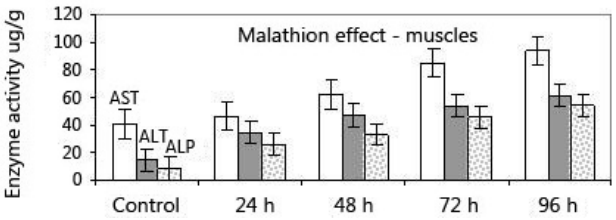

Figure 12-B. Enzyme activity of muscles in studied fish.

Conclusion

The studied fish was selected to determine LC_{50} of malathion, acute (½ LC_{50}) for 96h, characterization of toxic waste (malathion) effect on aquatic organism in environment using HLPC equipped with UV at 220 wave length and enzyme activity (AChE, AST, ALT, ALP activities) as biomarkers causing un-safety for fish populations. The results clarified that enzymes in tissues were affected by malathion at a low concentration, during an acute period and its toxicity increased dependently on dose. Furthermore, the results clarified that T.P depended on the balance between induction of defenses and inhibition with malathion toxicity impact. It can be deduced from the aforementioned results that LSASU in aquatic environment to reduce economic degradation and protect farm's fish under international guidelines to achieve national goals.

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