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Ecotoxicological Effects of Diazinon Pesticides on Fish *Cyprinus carpio* and Mussel *Sinanodonta woodiana*

A Thesis

Submitted to the Council of the College of Science, University of Babylon in Partial Fulfillment of the Requirements for the Degree of
Doctor of Philosophy in Biology

By

Jaafar Badr Obaid Mohamed Algburi

B.Sc. Ecology and pollution / Faculty of Science/ University of kufa / 2013

M.Sc. Ecology / University of ANU / India / 2016

Supervised by

Prof. Dr. Moayed Jassim Yass Alamari

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بِسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيمِ

﴿ يُؤْتِي الْحِكْمَةَ مَن يَشَاءُ ۚ وَمَن يُؤْتَ الْحِكْمَةَ فَقَدْ أُوتِيَ خَيْرًا كَثِيرًا ﴾

صدق الله العلي العظيم

سورة البقرة / الآية (٢٦٩)

Dedication

To

The spring that never stops giving and support, to my mother who weaves my happiness with strings from her merciful heart filled with love and kindness……

To

Whom he strives to bless comfort and welfare and never stints what he owns to push me in the success way who taught me to promote life stairs wisely and patiently, to my father.

To

Whose love flows in my veins, and my heart always remember them, my wife, brothers, sisters, and my little girl Fatima.

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Jaafar

Supervisor Certification

I certify that this thesis is prepared under my supervision at the Department of Biology / College of Science / University of Babylon, as a partial requirement for the degree Doctor of Philosophy of Science in Biology/ Ecology.

Supervisor

Dr. **Moayed Jassim Yass Alamari**

(Professor)

Department of Biology\College of Science\University of Babylon.

/ /2022

In view of the available recommendation, I forward this thesis for debate by the examining committee.

Dr. Adi Jassim Abd AL Rezzaq

(Asst. Professor)

Head of Biology Department\ College of Science\ University of Babylon

/ /2022

Committee Certification

We, the examining committee, certify that we have read this thesis "**Ecotoxicological Effects of Diazinon Pesticides on Fish *Cyprinus carpio* and Mussel *Sinanodonta woodiana***" and examined the student " **Jaafar Badr Obaid Algburi"** in its contents and that in our opinion; it is adequate for awarding Degree Doctor of Philosophy of Science in Biology/Ecology with **"Excellent"** estimation.

Dr. Jasim Mohamed Salman
Chairman (Professor)
/ /2022

Dr. Mohammed Jawad Salih Al-Haidarey
Member (Professor)
/ /2022

Dr. Adi Jassim Abd AL Rezzaq
Member (Asst. Professor)
/ /2022

Dr. Ali Abdulhamza obaid Al-Fanharawi
Member (Assistant Professor)
/ /2022

Dr. Nuha Falih Kadhim
Member (Professor)
/ /2022

Dr. Moayed Jassim Yass Alamari
Supervisor (professor)
/ /2022

Approved by The Council of The College of Science, University of Babylon.

Prof.Dr. Mohammed Mansour Kadhum Al Kafajijh
(Professor)
Dean of College of Science, University of Babylon
/ /2022

Summary

Ecotoxicology is concerned primarily with the release of toxic pollutants into the environment, their distribution and fate in the biosphere, especially in food chains, qualitative and quantitative measurement of toxic responses in ecosystems.

The present study investigates the adverse effects of exposure to the organic toxicant diazinon pesticide on aquatic organisms, and its potential effects on some physiological parameters and residue in organism tissue. Two aquatic organisms were selected to estimate diazinon toxicity in *Cyprinus carpio* fish and *Sinanodonta woodiana* bivalve.

The LC50 96 - hours for diazinon was estimated for *C. carpio* (total n= 72) were exposed to different concentrations of diazinon (60% EC) (0, 6, 10, and 15 mg/L). The LC50 for 96 h was found to be 9.5 mg /L. The fish are exposed to 25% of the LC50 (2.37 mg/L). After 4, 14 and 28 days, blood samples and muscle sample was collected and biochemical analysis. histopathology, DNA damage, behavioral effect and insecticide residuals were estimated.

The LC50 of *S. woodiana* use (total n= 108) were exposed to different concentrations of diazinon (60% EC), (0, 10, 15, 20, 25 and 30 mg/L). The LC50 for 96 h is found to be 13.8 mg /L. The bivalves are exposed to 1/4 of lC50 (3.45 mg/L). After four, fourteen, and twenty-eight days, bivalves samples were collected, then biochemical analysis, histopathology, DNA damage, behavioral effect and insecticide residuals were estimate.

The behavioral results in this study were observed in fish (damage caudal fin, hyperactivity to hypoactive, Change in skin pigmentation, sinking to the bottom, fish scales falling), while in bivalve, the clams closed their valves tightly

over longer periods of time and open them slightly for a short time, extending the foot and siphons from time to time when exposed to diazinon.

When compare with control group, blood parameters showed a non-significant decreased at ($p < 0.05$) in (RBC and WBC count and PLT) and non-significant increase ($p < 0.05$) in Hb concentrations in fish.

Total protein in fish shows a significant decrease in the 4^{th} and 28^{th} days and a significant increase in the 14^{th} day ($p < 0.05$). In bivalves there is a significant increase on the 28^{th} day.

Antioxidant enzymes (SOD and CAT) showed non-significant changes ($p < 0.05$) of SOD in fish. SOD levels were significant increases in bivalves. In contrast, CAT in both organisms has a significant change ($p < 0.05$) compared with the control group when exposed to diazinon.

In malondialdehyde, there was a non-significant increase ($p < 0.05$) in fish. While, with bivalve is record a significant increases at ($p< 0.05$). There is a significant decrease ($p < 0.05$) in acetylcholinesterase activity in the blood of *C. carpio* and *S. woodiana* extract.

The liver function value is found to have significant increases in AST, ALT and ALP ($p < 0.05$) in fish.

The insecticide residual value in fish was (0.696 ± 0.04) µg/g, and in bivalves it was (0.943 ± 0.077) µg/g. Elevated in treatment groups when compared with control groups.

As for the histological changes of the fish and bivalve, we notice a slight change in the tissues, necrosis in some areas, and damage to the intersections that

preserve the general histological structure of the muscles as well as the submucosal layer of the largest size when compared to the control group.

DNA damage, showed a significant increase ($p < 0.05$) in tail length in bivalves and fish when exposed to diazinon in the 28^{th} day.

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List Of Abbreviations

AChE : acetylcholinesterase
ALK: Alkaline Phosphatase
WBC: white blood cell
RBC : Red blood cell
PL: Platelets count
AS : antioxidant system
CAT: Catalase enzyme
DDT : dichlore diphenyl trichlorethane
DO: Dissolved Oxygen
DMSO: dimethyl sulfoxide
EMS: ethyl methanesulfonate
EPA: Environmental Protection Agency
ERA: Environmental Risk Assessment
GOT: Glutamic Oxaloacetic Transaminase
GPT : Glutamic Pyruvic Transaminase
Hb : Hemoglobin
IMI: Imidacloprid
LC50: Lethal concentration 50%
LMPA: low-melting-point agarose
MC-LR dose: Microcystin-
MCP : Monocrotophos pesticide
MDA : Malondialdehyde
OCPs : organo-chlorine pesticides,
PBFR: poly-brominated flame retardants
PBS: phosphate-buffered saline

PCBF: polychloro-dibenzo furans
PCBs: polychlorinated biphenyls
PCDD : polychloro-dibenzopara dioxins
PCP : pentachlorophenol
PFF : profenofos pesticide
ROS: reactive oxygen species
SOD : Superoxide Dismutase
TBA: thiobarbituric acid
TCA : Trichloroacetic acid
TMX : thiamethoxam neonictinoids insecticide
γ-HCH : gamma-hexachlorocyclohexane
HPLC : High-performance liquid chromatography
DTNB: 5:5-dithiobis-2- nitrobenzoate ion

1. Introduction and Literature Review

1.1. Introduction

Human activities cause extensive damage to different ecosystem (Appannagari, 2017). Environmental pollution is defined as the changes that result from nature or humans that lead to the introduction into the environment and ecosystems of substance or energy that would cause risks to human health, or harm living resources, or damage the structure, or interfere with the legitimate uses of the environment (Özkara *et al*., 2016). Water pollution is mainly affected by mining and industrial drainage processes, and the predominant pollutants from these processes are heavy metals and radionuclides. Water pollution is also strongly associated with agriculture. The irrigated areas are characterized by high concentrations of pesticide residues, nitrogen, ammonia, and phosphorous (Liu *et al*., 2021).

Organic pollutants are chemical compounds that have the ability to remain in nature for a long time because of their ability to resist environmental conditions, biological and chemical degradation and photolysis processes, it has the ability to accumulate in organism's tissue, causing harm to the environment and health (Megharaj *et al*., 2011). Persistent organic pollutants are generally classified into five main types which include poly-chlorinated biphenyls (PCBs), organo-chlorine (OC) pesticides, polychloro-dibenzopara dioxins (PCDD), polychloro-dibenzo furans (PCBF) and poly-brominated flame retardants. Organo-chlorine compounds were banned in the late 1970s because of remaining in the environment and in animal tissues for a long period as well as their toxicity (Ngwa *et al*., 2015). Diazinon it is considered a hazardous insecticide, and belong to the organophosphorous group of pesticides that are most widely used in agricultural

activities, the availability of diazinon in surface and groundwater waters has an impact on different ecosystem (Dehghani *et al*., 2019).

Ecotoxicology is concerned primarily with the release of toxic pollutants into the environment, their distribution and fate in the biosphere, especially in food chains. the qualitative and quantitative measurement of toxic responses in ecosystems and ecosystem components (Boudou & Ribeyre, 1997). It also deal with microorganisms, plants, and animals and man of all species relation to their abiotic environment in an integrated way. Ecotoxicology is environmental toxicology from an ecological perspective. ecotoxicology concentrates on the effects of dispersed and mixed pollutants and their transformation products as well as the toxicological relationships among a wide range of organisms (Kendall *et al*., 2014). Environmental toxicology is concerned with the effects of toxic substances on populations, rather than individuals (Moriarty, 1988). Xenobiotics are hazardous chemicals compound that are foreign to the ecosystem and biological system and are difficult to degrade or recycle. Xenobiotics are found within an organism that are not naturally produced or expected to be present within the organism (Narwal & Gupta, 2017).

The present study investigates to the adverse effects of exposure to diazinon pesticide on aquatic organisms, which is more commonly used in Iraq, and potential effects on some physiological parameters and residue in organism tissue.

1.1.1 Aim of Study

The aim of the study was to assess the ecotoxicological characteristic of some organisms after exposure to diazinon pesticide. This aim includes the following objects:

1. Estimation of the LC50 (Lethal Concentration 50%) concentration of diazinon for *Cyprinus carpio* fish and *Sinanodonta woodiana* bivalve.
2. Study the effect of diazinon on behavioral and morphological characteristics of organisms.
3. Study the effect of diazinon on physiological parameters in studied organisms.
4. Study the bioaccumulation of diazinon in muscle of studied organisms.
5. Study the effect of diazinon pesticide on DNA (comet assay).

1.2 Literature Review

1.2.1 Environmental Effect of Organic Toxicant

Organic toxicants defined as organic chemicals above natural levels with biochemical or physiological modes of action that adversely affect organisms, such as pesticides (Schäfer *et al*., 2016). Toxicants often co-occur with other stressors in the ecosystem such as desiccation and heat stress, leading to potential interactive effects (Alexander *et al*., 2016). Organic toxicants can contribute to local and regional losses of freshwater biodiversity and ecosystem services. Organic toxicants were likely to exert acute lethal and chronic long-term effects on sensitive fish, invertebrate, or algae species (Schäfer *et al*., 2011). Pesticides, tributyltin, polycyclic aromatic hydrocarbons, and brominated flame retardants were the major contributors to the chemical risk (Cruz *et al*., 2015). The risk of potential acute lethal and chronic long-term effects increased with the number of ecotoxicologically relevant chemicals (Malaj *et al*., 2014).

Xenobiotics have been defined as foreign chemicals pollutant to which an organism is exposed that are foreign to the normal metabolism of that organism (Kennedy & Tierney, 2013). Without metabolism, numerous xenobiotics would reach toxic concentrations, most metabolic activities inside the cell require energy, cofactors, and enzymes in order to occur (Croom, 2012). Technological progress, longer life, better access to medicine (for humans and animals), as well as daily use of personal care products and/or pesticides, introducy new substances into the environment, these substances can cause problems and should be studied in detail, including their short- and long-term effects on humans, animals, plants and the ecosystem (air, water, and soil), either as single substances or as a mixture of them (Štefanac *et al*., 2021). The US EPA “Environmental Protection Agency” defines

xenobiotics new substances that have effects on the environmental and health, but whose damage not well understood (EPA, 2021).

The main problem is their physicochemical structures, such as small molecular size, ionizability, water solubility, lipophilicity, polarity, and volatility , which make them difficult to identify, quantify, and remove (Kassinos *et al*., 2011).

The xenobiotics found in different environments and place such as water, soil, air, animals, plants, humans and are classified as pesticides, pharmaceutical compounds, personal care products, illicit drugs, industrial products, and nuclear waste fig (1-1) (Štefanac *et al*., 2021).

Xenobiotics are difficult to degrade because of their complex structures, thus, they can accumulate in living organisms. They accumulate through food chains and food webs. The various xenobiotic compounds were identified which causing adverse effect on our environment (Varsha *et al*., 2011).

Effects of xenobiotics on animals are most likely seen on their reproduction and immune functions. Many pesticides, such as herbicides, fungicides, and rodenticides, are harmful to animals and humans, causing cancer, lung irritation, or neurological disorders. Their effect on the aquatic ecosystem also causes many problems, Veterinary drugs enter the aquatic environment through surface application, runoff, or direct application (Tudi *et al*., 2021).

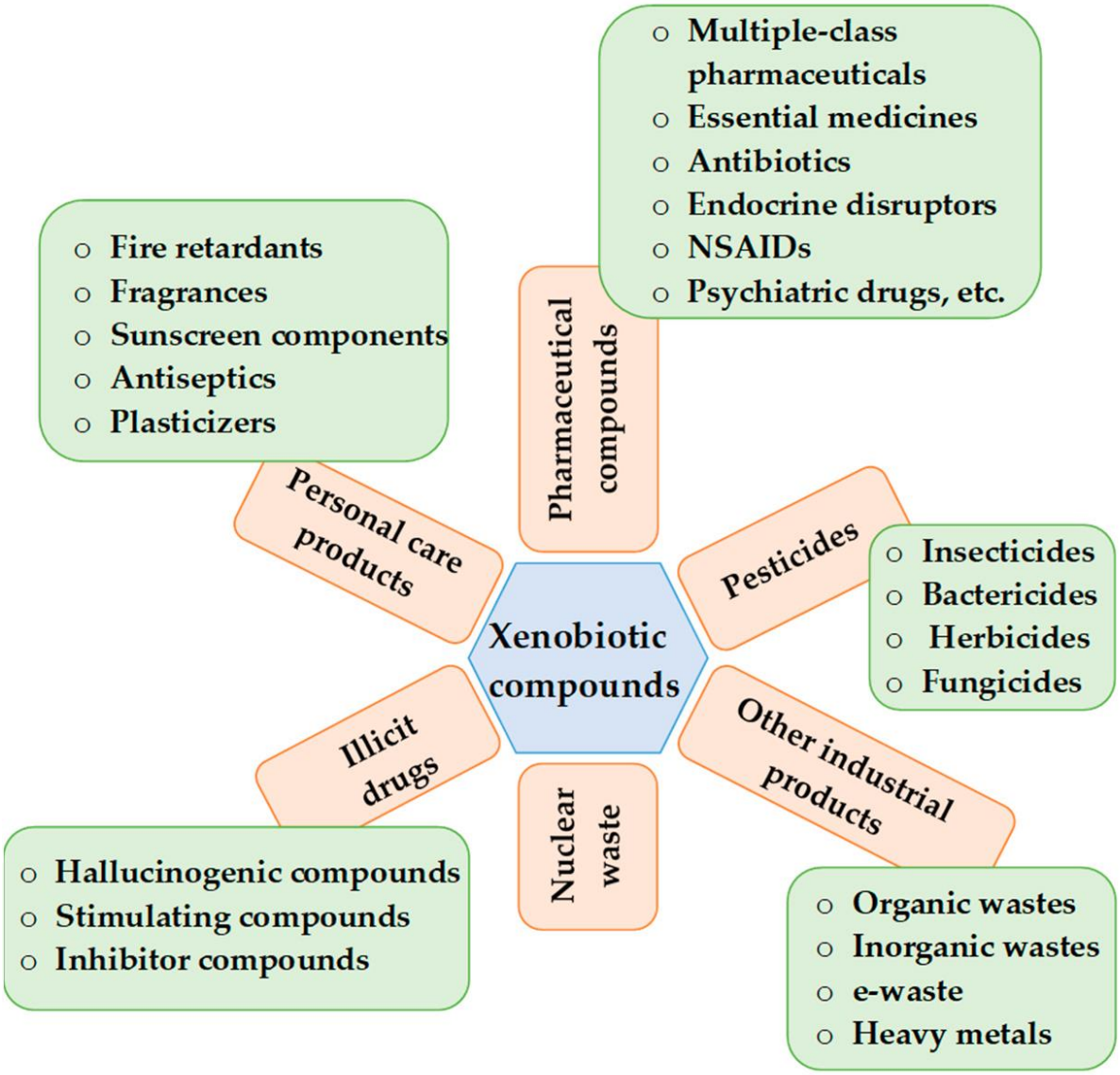

Figure (1-1): Classification of xenobiotic compounds (Kumar & Chopra,.2020)

Organic pesticide in fish and other aquatic organisms, may cause disruption of the reproductive, endocrine system, or they may directly affect gamete development and viability as a result either of their cytotoxicity or by altering the hormonal environment during gamete development (Kime, 1999). Xenobiotic molecules can alter the physiological homeostasis of fish and trigger an oxidative stress through overproduction of reactive oxygen species (ROS), suppressing the antioxidant system (AS) activity. An overwhelming generation of ROS is usually

accompanied by damage to lipids, proteins and DNA structure, which can be associated with mutations, chromosomal aberrations and carcinogenesis (Burgos-Aceves *et al*., 2018).

In essential aquatic invertebrates, such as mussels of the genus *Mytilus*, the organic toxicant accumulate and tolerate at levels higher than those being present into the aquatic environment, because the filter feeding nature. Providing accurate and reliable biological endpoints (e.g. physiological, behavioral, cellular, biochemical and molecular indices) and effect on digestive gland because it consider a target-tissue for the compounds ingested in the organism (Faggio *et al*., 2018).

1.2.2 Toxicity and Health Effects of diazinon

Exposure to organic compounds causes a variety of adverse health effects. Increased levels of the toxic organic compounds in the environment have been associated with human health risks including cancer. Many toxic organic compounds are persistent and are stored in fat tissue, due to their hydrophobic properties, resulting in bioaccumulation. in higher levels in food chains organisms (e.g., humans), there are greater concentrations of these bio-accumulated toxins (Alharbi *et al*., 2018).

The organic and inorganic pollutants have neurotoxicity effect, toxic to juvenile channel catfish (*Ictalurus punctatus*) and accumulation to gill > liver > ovary > muscle (Sharma & Singh, 2021). There are Several studies about organic contamination that highlighted reduced gonad size, change in secondary sexual character, delayed maturity and suppression of sex hormone in fish rainbow trout (*Oncorhynchus mykiss*) and mosquitofish (*Gambusia holbrooki*) (Drèze *et al.*, 2000). The catfish and African sharptooth (*Clarias gariepinus*) exposure to diazinon (organophosphorus pesticide) in different sub-lethal

concentration, the rustle indicate to there are a serious alterations in Kidney, that was found to be the most seriously affected organ compared to gonads and liver (Banik *et al*., 2016).

The health effects reported was, changing Protein level in plasma of fish treated with pesticide but the change was insignificant. Histological changes in the liver, gills and kidney of fishes exposed to diazinon were apparent when compared to control. Hepatocytes necrosis and bleeding were more distinct in the fishes exposed to pesticide. Glomerulus hypertrophy and bleeding in kidneys; and fusion and degeneration of secondary lamellae and epithelial hyperplasia in gills were also observed in the exposed fish (Al-Otaibi *et al*., 2018).

The health effects of organic compounds in bivalves is, inhibits cholinesterase and other enzyme in animal tissues it is report in (*Anodonta cygnea*) after exposed to various concentrations of diazinon (0, 1, 7, 15, 19, 23 mg L-1) in different bivalve tissues (Shiri *et al*., 2014). In other study to evaluated the genotoxic and lethal effects of chemicals to freshwater mussels (*Utterbackia imbecillis*). Many organic compound such as diazinon , glyphosate, carbaryl, commercial formulations of atrazine and copper, have genotoxicity effect on freshwater mussel, the effect was estimated with Comet assay (the alkaline single-cell gel electrophoresis assay) (Conners & Black, 2004).

1.2.3 Bioaccumulation of Organic Toxicant in Aquatic Ecosystems.

Chemicals organic compounds are necessary part of everyday life. They came in different shape of antibiotics, insecticide, fuels, plastic containers, agricultural fertilizers, photocopy materials, and so on. Society cannot be out in its present form without them. Growth in the numbers of chemicals used during recent

decades has been extraordinary (Connell, 1988). The term bioaccumulation is defined as uptake, storage, and accumulation of organic and inorganic contaminants by organisms from their environment (Gray, 2002).

Bioaccumulation is the intake of a chemical and its concentration in the organism by all possible means, including contact, inhalation and ingestion. Bioaccumulation use in environmental risk assessments to estimate the distribution of pollutant transport within food webs (Miller *et al.*, 2020). One difference is that bioaccumulation refers to the build-up of the chemical in the body of one organism while biomagnification refers to the build-up in multiple organisms (Onyango *et al*., 2020). Organic toxins accumulate in various organisms of the aquatic environment. The accumulation process is carried out by daily bioactivities.

The organisms in which pollutants accumulate are various such as aquatic plants, algae, crustaceans, zooplankton, fish, mollusks, etc. (Rahman *et al*., 2012). Organic pollutants get into fish through Gills, skin and feeding. Because the difficulty to remove organic pollutants from the body by metabolism so that the pollutant will accumulate in fish tissue such as lipid and muscle (Fair *et al*., 2018). Mollusca (bivalve) effected by accumulate different organic pollutant in flesh of organism, this pollutants discharge to aquatic system from agriculture activates such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs). in some Studies organochlorine pesticide Aldrin is found in mollusca tissue (Uvayeva *et al*., 2020).

1.2.3.1 Bioaccumulation of diazinon Pesticides in Fish and bivalves

Bioaccumulation of chemicals by aquatic organisms, especially fish, mussels and Daphnia, is an important criterion in risk assessment. There are two uptake

routes in bioaccumulation process in fish : dietary uptake (by ingestion of polluted food particles) and aqueous uptake (of water-borne chemicals) (Streit, 1998). Bioconcentration from water must be considered in context with toxicity, biotic and abiotic degradation and other physical-chemical factors in order to protect the freshwater and marine environments with their organisms (Geyer *et al*., 2000).

The ability to assess bioaccumulation in the invertebrates are important for understanding food-web dynamics and transfer of contaminants throughout the ecosystem. In aquatic toxicology it is important to determine what proportion of the total toxicant is available to an organism in order to adequately characterize the degree to which bioaccumulation and adverse effects can occur (Mackay *et al*., 2001). Other studies estimate bioaccumulation in tissue of different bivalves like *Chamelea gallina, lithophaga, Mytilus galloprovincialis* and *Venus verrucosa*, Concentrations of organic compounds as dichlore diphenyl trichloretane (DDT) and gamma-hexachlorocyclohexane (γ-HCH or lindane) (Deudero *et al*., 2007).

1.2.4 Diazinon

Daizinon (O,O-Diethyl O-[4-methyl-6-(propan-2-yl)pyrimidin-2-yl] phosphorothioate), is an organophosphate pesticide, it was developed in 1952, for widely used in agriculture to protect many crops from a wide range of *Hymenopterans* and *Hemipteran* insects (Saha *et al*., 2018). After application in agriculture, Diazinon easily washes into surface water and ultimately reaches to rivers, ponds and lakes. During and after the treatment with diazinon to control pest, non-target animals are also exposed to the pesticide (Rahman *et al*., 2020). However, diazinon likes most of the pesticides ultimately find their way into surface waters influencing the health of aquatic animals such as fishes and bivalves

(Ribeiro *et al*., 2016). Past studies have report the fast decay of diazinon, however its continual input makes its presence continual in water bodies for long-term and lead to stressful in aquatic organisms (Banaee *et al*., 2013).

Diazinon is persistent in aquatic environment, especially in freshwater (Banks *et al*., 2005). Diazinon is fairly resistant to degradation by sunlight, high temperatures and moisture (Zaruk *et al*., 2001). The hydrolysis half-life of diazinon in water, soil, biota and air is 140, 100, 30 and less than 1 days, respectively. Therefore, toxicity of diazinon to aquatic animals, particularly fishes, is inescapable (Banaee *et al*., 2013).

Aquatic exposure to sub-lethal concentrations of diazinon, it is absorbed through the gills, skin or gastrointestinal tract of fish and is rapidly metabolized and excreted, but it may also be bioconcentrated in several tissues (Lushchak *et al*., 2018). Studies on animals have indicated that the major steps in the metabolic pathway include the hydrolytic and oxidative cleavage of the ester bond leading to the formation of pyrimidinyl derivatives (WHO, 1998).

The toxicity of diazinon can be decreased by the action of carboxylesterase enzyme which catalyzes the hydrolytic degeneration of diazinon and by the action of glutathione S-transferase which catalyzes the formation of excrete-able conjugate (Banaee *et al*., 2013). However, dysfunctions in different biological systems of exposed fishes to diazinon are reported during the detoxification process (Rabie *et al*., 2016). In the recent years, several approaches have been used to monitor the deleterious effects of diazinon poisoning in different fishes and bivalves species (Hemming & Waller, 2004, Far *et al*., 2012, Shiri *et al*., 2014, Rafieepour *et al*., 2019, Saha *et al*., 2021).

Reish & Oshida, (1987) said that concentration in aquaria should be re-concentration because:

1- decreased concentrations due to adsorption on the aquarium walls.
2- decreased concentration due to evaporation.
3- decreased concentrations due to absorption by fish tissue.
4- The accumulation of metabolic waste in the water medium.
5- Microbiology growth.

1.2.5 The organisms used in experiment

1.2.5.1 Common carp (*Cyprinus carpio*)

The common carp (*Cyprinus carpio*) belongs to the family Cyprinidae. In nature, carp live in the middle or lower reaches of a river with slow currents, or in marshes. Their habitats are usually weedy areas with a muddy bottom. Carp fry feed on zooplankton such as rotifers and copepods, but as they grow up they become benthic feeders, feeding on animals and other organic material (Mohale *et al*., 2020).

The cyprinids have been farmed since ancient times and today they are undoubtedly the most important teleost family cultivated on a global scale, the current production figure reaching over 25 million tons per year. The common carp is an important culture species among the cyprinids , next only to silver carp and grass carp, and its production has doubled over the last decades. A large percentage of this is from the Asian region, particularly China. While production of common carp is widely practiced, only about 3 % of the cyprinids are cultivated in intensive systems (Takeuchi *et al*., 2002).

Many studies that use *C. carpio* as bioindicator for oxidative stress (Islas-Flores *et al*., 2014, Cortes-Diaz *et al*., 2017). Ghazanfar *et al*., (2018), study the effect of fipronil and buprofezin insecticides, on common carp (*C. carpio*), and reduce biochemical and genotoxic damage by use Vitamin C. Korkmaz, c., & Dönmez, (2017), they studied change in liver, plasma vitellogenin and gonad tissues of common carp (*C. carpio)* exposer to diazinon.

1.2.5.2 *Sinanodonta woodiana* (Chinese Pond Mussel)

The Chinese pond mussel *Sinanodonta woodiana* is a species native to East and South–East Asia. Some authors have placed this species within the order *Anodonta* though newer taxonomic research has placed it in the order *Sinanodonta* (Bogatov & Sayenko, 2002). The chinese pond mussel (*S. woodiana)* is a benthic filter-feeder that prefers soft-bottomed freshwater habitats and has successfully spread into both tropical and temperate water bodies outside its natural southeast asian range (Douda & Čadková, 2018).

Most of all bivalves families such as *Unionid* provide vital services in freshwater ecosystems by contributing to water purification, nutrient circulation, bottom bioturbation and provision of habitats (Ożgo *et al*., 2021). Asian pond mussels of the genus *Sinanodonta* are hyper-successful invaders. Their expansion is associated with commercial trade in freshwater fish, which, when infested with mussel larvae (glochidia), serve as vectors for their spread. Most notably, members of the Chinese pond mussel *S. woodiana* species complex rapidly expand their range and have already colonized large parts of Europe and Russia, Southeast Asia and Australasia, Central America and the USA (Urbańska *et al.*, 2021).

There are many studies that use *S. woodiana* and other bivalves types in environmental study were, Petrović *et al*., (2011), they used *S. woodiana* as water contamination bioindicators. Arumugam *et al*., (2020), evolution the health risk to humans by checking of toxic elements in *Carassius gibelio* and *S. woodiana*.

1.2.6 Aquatic Organisms Under Organic Pesticides

The widely using of organic toxicant substance have effect on aquatic organisms, there are study to evaluated three Organic pesticides material 2,4-dichlorophenoxyacetic acid (2,4-D), 2-chloro-2,6-diethyl-N-(butoxymethyl) acetanilide (Butachlor) and pentachlorophenol (PCP), for acute toxicity and stress behavior on different type of freshwater fish (*Heteropneustes fossilis*, *Clarias batrachus*, *Channa punctatus*) and mosquito larvae (*Culex pipiens fatigans*). The experiment results show the (*Heteropneustes fossilis*) have high sensitive, while mosquito larvae mostly appeared resistant than fish. The stress signs is observed in aquatic organisms changes in behavior (Farah et al., 2004).

The bivalve under xenobiotics stress have observed many physiological change such as cytotoxic damage to lysosomal integrity, reduced physiological scope for growth and cellular damage are considered as characteristics of the general stress syndrome induced by the toxic action of the xenobiotics (Moore *et al*., 1980).

1.2.6.1 Oxidative Stress and Antioxidant Defense System

There is an evident relationship between the deleterious effects of pesticide toxicity and oxidative stress due to over generation of free radicals. It has been suggested that pesticide toxicity could be caused by the accumulation of

deleterious effects of reactive oxygen species (ROS), throughout the pesticide detoxification. Fish cells possess a defense system for the detoxification of potential harmful ROS (Tejada *et al*., 2007).

The antioxidant enzymes that provide the first line of cellular defense to ROS include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR). However, an imbalance between the activities of cellular antioxidant enzymes and ROS production results in oxidative stress and cellular damage. If the antioxidant system is not able to eliminate or neutralize the excess of ROS, there is an increased risk of oxidative damage (Isik & Celik, 2008). It is well established that waterborne pollutants induce oxidative stress and cellular damage in affected aquatic organisms (Mahboob, 2013).

Oxidative stress has been reported to play an important role in the toxicity of various xenobiotic in fish, including insecticides (Monteiro *et al*., 2006, Vieira *et al*., 2018, Uçkun *et al*., 2021). Monitoring changes in antioxidant enzyme activities could be a useful biomarker in ecotoxicology because an induction of these enzymes in exposed organisms can indicate the existence of oxidative stress (Hemalatha *et al*., 2016, Guo *et al*., 2021).

Superoxide dismutases (SOD) constitute the first line of defense against ROS. $O_2^{-\bullet}$ is produced at any location where an electron transport chain is present, and hence O2 activation may occur in different compartments of the cell (Mishra & Sharma, 2019). This being the case, it is not surprising to find that SOD are present in all subcellular locations. While all compartments of the cell are possible sites for O_2^- formation.

1.2.6.2 Toxicity Effects of Diazinon on Fish and Bivalves Behavior

Environmental pollutants that alter behavior can have major impacts on populations as well as community structures by changing species interactions. The most common symptoms of behavioral abnormalities in fish including neural paralysis, imbalance, abnormal swimming, and paleness (Khan *et al*., 2021). Immediately after exposure to poison, fish are suffering from severe impatience and increasing toxin concentrations with fish swimming in a half circle and the curvature of the spine (Far *et al*., 2012). Similar observations are also reported by (Allison & Hermanutz, 1977, Svoboda *et al*., 2001, Khoshbaver Rostami *et al*., 2006).

One important behavior is the ability to capture prey (Gaworecki & Klaine, 2008). The exposure to diazinon, an organophosphate pesticide, may lead to feeding behavior abnormalities in (hybrid striped bass) (*Morone saxatilis x M. chrysops*) through inhibition of brain acetylcholinesterase (AChE) activity. This can potentially reduce the organism's survival by affecting its ability to find and capture food (Gaworecki *et al*., 2009).

Freshwater mussels (Bivalves) are among the most imperiled aquatic fauna in aquatic environment, and pollution by chemical contaminants has been cited as a major factor contributing to their decline (Cope *et al*., 2021). Shan *et al*., (2020) is study the effect Imidacloprid insecticide on Asian Clam (*C. fluminea*) by assess the potential toxicity effects of adult clams exposed to 20, 200, and 2,000 μg/L imidacloprid for 30 day by determining behaviour, biomarkers of various functions, Imidacloprid significantly inhibited the siphoning and burrowing behavior.

The Behavioral changes in bivalve were observed in the clams closed their valves tightly over longer periods and open them slightly for short time, extended

the foot and siphons from time to time, Mucous secretion or a layer above the water surface when exposed to dimethoate pesticide (organophosphate insecticide) (Hassan & Sheiko, 2013).

1.2.6.3 Histological and Physiological Effects of Diazinon

Histopathology involves the microscopic examination of cells and tissues of an organism and the semi-quantitative determination of histological abnormalities. Pesticides, like other toxicants, induce the generation of free radicals that destruct the vital macromolecules constituents of the cells and make alterations in tissue of organisms (Tchounwou *et al*., 2015).

Macroscopically, signals of toxicity are almost always preceded by changes of the organs, tissues, cellular and molecular levels, important organs like the kidney, liver, gills, stomach, brain muscles and genital organs are mostly damaged (Jothy *et al*., 2011).

Damaging effects of diazinon, on bluegills fish (*Lepomis macrochirus*) was recorded after exposure to different concentration of diazinon the result show, the diazinon concentrations caused various types of changes, such as lifting of the epithelial layer, hyperplasia and necrosis, shortening of the lamellae and frequent epithelial rupture, lamellar fusion, severe hyperplasia and mucous cells hypertrophy, extensive fusion, and clavate lamellae (Maxwell & Dutta, 2005).

Histo-pathological studies in mussels after exposure two types of dinoflagellate (*Alexandrium fundyense*) and (*Prorocentrum minimum*) toxin detected an inflammatory response consisting of degranulation and diapedesis of hemocytes into the alimentary canal and, as the exposure continued, hemocyte migration into the connective tissue surrounding the gonadal follicles *(Galimany et al*., 2008).

The Diazinon have Physiological effects in Catfish (*Siluriformes*) on hematological (hemoglobin, total red blood count, total white blood count, and mean corpuscular hemoglobin), growth (condition factor, hepatosomatic index, specific growth rate), biochemical (total serum glucose, total serum protein). There are a decreasing values with increasing toxicant concentration and exposure duration. A significant inverse relationship between variables (mean corpuscular hemoglobin, condition factor, specific growth rate, tri-iodothyronine, thyroxine, and total serum protein) and elevated chronic diazinon exposure concentrations (Saha *et al*., 2021).

The physiological effects of exposure to different biotoxin and xenobiotic in bivalves and led to increase of hemocyte counts, accompanied by changes in the hemocyte subpopulations and alteration of the phagocytic activity in some species (Gorbi *et al*., 2013). On the contrary, some studies report non-significant effects on hemocyte number, morphology, or functions in *Crassostrea virginica*, while others evidenced a strong individual variability in the response in *Ruditapes philippinarum* (Manfrin *et al*., 2012).

1.2.6.4 Other biological observations

Damaging effect of diazinon on aquatic organisms, Some of the visible effects of this chemical are reduction in fish survival, growth and reproduction (Adebayo *et al*., 2013). The toxicity effects of diazinon are due to the inhibition of an enzyme needed for proper nervous system function (Ullah *et al*., 2018). The range of doses that result in toxic effects varies widely with formulation and with the individual species being exposed (Chorus & Welker, 2021).

Toxicity to different species of fish is affected by age, sex, body size, climatic conditions, chemical formulation, and chemistry of the environment among others (Adedeji *et al*., 2009).

1.2.7 Monitoring of organic compounds and risk management

Fish bioaccumulation markers may be applied in order to elucidate the aquatic behavior of environmental contaminants, as bioconcentrators to identify certain substances with low water levels and to assess exposure of aquatic organisms (Galindo *et al*., 2012).

The use of biomonitoring methods in the control strategies for chemical pollution has several advantages over chemical monitoring (Besse *et al*., 2012). Many of the biological measurements form the only way of integrating effects on a large number of individual and interactive processes in aquatic organisms (Kroeker *et al*., 2013). Moreover, biological and biochemical effects may link the bioavailability of the compounds of interest with their concentration at target organs and intrinsic toxicity (Dıaz *et al*., 2004). The limitations of biomonitoring, such as confounding factors that are not related to pollution, should be carefully considered when interpreting biomarker data. Based upon this overview there is little doubt that measurements of bioaccumulation and biomarker responses in fish from contaminated sites offer great promises for providing information that can contribute to environmental monitoring programs designed for various aspects of ERA (Environmental Risk Assessmen) (Van der Oost *et al*., 2003).

Freshwater bivalves, such as *C. fluminea* are widely distributed at the water-sediment interface in freshwater environments (Fan *et al*., 2018), and these organisms filter large volumes of water and can potentially accumulate environmental pollutants (Wright *et al*., 2013). Therefore, *C. fluminea* is considered a primary species for biological monitoring and has been the focus of several toxicological studies (Shan *et al*., 2020).

1.2.8 DNA damage assessment

DNA degradation is very crucial to evaluate the brutality of genotoxic compounds which released into the aquatic ecosystems, the degree of DNA integrity has been proposed as a sensitive sign or effective biomarker for the monitoring of environmental carcinogens, mutagens, and teratogens (Buschini *et al*., 2013). Oxidative stress that are generated by pollution such as pesticide can generate reactive oxygen species ROS in cell and that induced DNA damage (Kelainy *et al*., 2019).

Free radicals, such as superoxide (O2•-) is caused DNA damage that occur via association with DNA lead to form apurinic and apyrimidinic DNA sites , oxidized purines and pyrimidines, single and double strands DNA break and mutations (Kryston *et al*., 2011). DNA damage occur in three basic steps; the first step is formation of adducts with toxic molecule, in second stage is caused secondary modification of DNA such as single and double strand breakage, change in the repair of DNA and base oxidation and crosslink, the third stage cell function is altered cell proliferation and consequently cancer (Ambekar *et al*., 2017).

Khadairi *et al*., (2017) study effect exposure rats microcystin leucine arginine on DNA damage markers such as comet length, tail length, and tail moment were measured with the single cell gel electrophoresis also called comet assay. it found there are significantly increased in DNA damage with increasing MC-LR dose.

Mohamed *et al*., (2020) study the effect of exposure profenofos pesticide (PFF) on common carp fish (*C. carpio*) on DNA damage, The results indicate profenofos pesticide significantly increased the DNA tail length, tail moment, and the level of 8-hydroxy-2′-deoxyguanosine. The studies indicate there are clear alter in DNA molecular marker in fish blood of (*Labeo rohita*) type after expouser to

thiamethoxam neonictinoids insecticide (TMX), the result showed a significantly increased in thiamethoxam treated fish and also in hepatocytes there are significantly increased in DNA damage by comet assay (Hussain *et al*., 2022). The South American fish (*Prochilodus lineatus*) is exposure to four sub- lethal concentration of Imidacloprid (IMI) insecticide to detect the impact on fresh water fish, the result indicate there are DNA damage that evidenced by the comet test (Vieira *et al*., 2018).

Ionizing radiation that release to environment have harmful effect on the aquatic organisms and make damage in DNA, two types of bivalves was exposure to different doses of gamma radiation and different concentrations of ethyl methanesulfonate (EMS) to detect the DNA damage, the result showed a significant increase in DNA damage is observed as indicated by an increase in tail DNA damage at different concentrations of EMS and all the doses of gamma radiation as compared to controls in both bivalve species (Kumar *et al*., 2014). Three sub-lethal concentration of Monocrotophos pesticide (MCP) is apply on estuarine bivalve to evaluate the alterations DNA damage and genotoxicity, the result indicate to significant change in DNA damage with high concentration of Monocrotophos (Dias *et al*., 2021). The bivalves that culture commercially are particularly exposed to pollutants, among them pesticides, to detect the possible transport damaged of DNA through generations, during the gametogenesis period apply diuron herbicide on bivalves, the results obtained by the Comet assay clearly showed a higher level of DNA strand breaks in both the hemocytes and spermatozoa of diuron-exposed genitors (Barranger *et al*., 2014).

2. Materials and Methods

2.1 Chemical and materials

Table (2-1): List of chemicals, which used in the current study.

Chemicals	Supplier	Origin
Acetone	Baker analyzed	Germany
Acetylthiocholine iodide	sigma	USA
Agarose	Promega	USA
Benzene	Himedia	India
Ethanol	Chem-Lab	Belgium
Ethidium Bromide dye	Biotium	USA
Formaldehyde	Sigma	USA
Hydrochloric Acid	Thomas Bake	India
Low Melting Point Agarose	biopass	China
Methanol	Biosolve	France
MnSO4	sigma	USA
Phosphate buffer solution	solarbio	China
Potassium Iodide	BDH	USA
Sodium Chloride	Merck	USA
Sodium hydroxide	Panreac	Spain
Sodium thiosulphate	BDH	USA
Starch	Merck	USA
Sulfuric acid	BDH	USA

2.2 Equipment And Instruments

Table (2-2): List of instruments and equipment and their manufactures.

Instruments	Company	Origin
Aquarium air pump	Sebo aquarium	India
Cooling Centrifuge	Hettich	Germany
Deionization distillate water	Pure water	Taiwan
Electrophoresis apparatus	Cleaver	UK
Eppendorf tubes centerfuge	Fischer	USA
Fluorescence Microscope	ZEISS	Germany
HPLC model	SYKAM	Germany
Incubator	Memmert	Germany
microscope light	Novel	China
Microtome	Biobase	China
Multi meter 340i/SET	WTW	Germany
Oven	Memmert	Germany
Plastic aquarium	Hirad plast	Iran
Refrigerator	Dawlance	Pakistan
rotary evaporator	Heidolph	USA
Sensitive Balance	Sartorius	Germany
Spectrophotometer	EMClab	Germany
Spectrophotometer UV	Emclab	Germany
Ultrasonic / homogenizer	Sonics	USA
Water bath	GFL	Germany

2.3 Study Samples:

2.3.1 Fish Samples

The fish used in the present study, common carp (*Cyprinus carpio Linnaeus 1758*), were obtained from fish farm in Al-Mahaweel city in Babylon Province, Iraq. Samples were transported by using plastic containers to advanced environmental laboratory in Ecology and pollution Department, faculty of Science University of Kufa where experiment was done, in February 2021. The healthy fish were selected, while the abnormal fish were excluded. The fish were acclimated to the laboratory conditions for 14 days in aerated dechlorinated freshwater that changed every 48 hours, and were fed twice daily with commercial fish food (Mostakim *et al*., 2015).

2.3.2 Bivalve samples

The bivalves that used in the present study, was Chinese pond mussel (*Sinanodonta woodiana*), The taxonomy used here follows to Bogan *et al*., (2021), were obtained from the Shatt Al-Hilla River in Babylon Province, Iraq. Samples were transported by using plastic containers to advanced environmental laboratory in Ecology and pollution Department, faculty of Science University of Kufa, where experiment did take place, in July 2021. The healthy bivalves were selected, while the not normal individuls were excluded. The bivalves were acclimated to the laboratory conditions for 14 days in aerated dechlorinated freshwater that changed every 48 hours (Oliveira *et al*., 2018).

The fish and bivalve samples were placed in an ovoid plastic aquarium with a capacity of 100 liter with beige color. The oxygen was maintained continuously for an average of 24 hours daily by aerating the containers with two sources of

oxygen. One was an aquarium pump, which provides dissolved oxygen and filters the water. The second source was a central air pump (air bubbles motors) that distributes air by rubber tubes to all sample containers. Chlorine- free water was used by storing tap water in tanks for 72 hours. The temperature of the water is adjusted by using the air conditioner in the lab. Throughout the experiment, water is changed every 48 hours fig.(2-1).

Figure(2-1): The plastic aquarium that used in experiment

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2.4 Water quality analysis in experiment

2.4.1 Water quality

Water temperature, electrical conductivity, salinity, and pH were measured directly by a portable digital multimeter; Model 340i / SET, WTW made in Germany after titration with standard solutions at (4.1, 7, 10.1) and a special buffer solution.

2.4.2 Dissolved oxygen

Dissolved Oxygen (DO) was measured according to the Azide modification of the Winkler method (APHA, 2012). The results were expressed as mg/l **(appendix1)**

2.5 Laboratory experiments

2.5.1 Determination of the lethal concentration LC_{50} in fish

Healthy and active fish of common carp (*C. carpio*) (n= 72), with total length and weight of fish were 18.5 ± 1.45 cm and 89.8 ± 5.6 g, respectively, and were maintained in continuously aerated aquaria to get acclimatized to laboratory conditions for two weeks under controlled natural illumination (12/12 h, light/dark).

The water in containers was supplied with oxygen continuously (air bubbles motors) and the water was replaced every 48 h. The temperature was kept at 19 ± 2 °C. for the periods of acclimation and experiment, commercial fish food was

given to fishes twice a day, but the fish were deprived of food for 24 h prior to the experiment and throughout the acute toxicity test (Mostakim *et al*., 2015).

The Diazinon with a purity of 60% was purchased from Al-Fares Company and used to prepare test solutions for Diazinon. Fish were exposed to three different concentrations (6, 10, and 15 mg/L) of Diazinon (Al-Otaibi *et al*., 2018) to determine 96 hour LC50 values. Three aquaria were used for each concentration, and each aquarium contained six fish in 70 L of dechlorinated tap water (Svoboda *et al*., 2001). One control group contain the same number of fishes and the same volume of water, but without Diazinon. Before cultivating the fish in the container, Diazinon was added and the water was aerated for an hour for a homogenous distribution of Diazinon in the water. In addition the water in the container was replace every 48 hours.

The death rate was recorded after 24, 48, 72, and 96 h of exposure. Dead fish were removed to avoid possible deterioration of the water quality (Gooley, 2001). The Probit analysis test was used to calculate LC50 values (Finney, 1971). Every 48 hours, the water were changed because Reish & Oshida, (1987) said that concentration in aquaria should be re-concentration.

2.5.2 Determination of the lethal concentration LC50 in bivalve

There were a total of 72 Chinese pond mussel (*S. woodiana*), the length and weight of bivalves were 14.2 ± 0.8 cm and 229 ± 16.2 g, respectively, and were maintained in continuously aerated aquaria to get acclimatized to laboratory conditions for two weeks under controlled natural illumination (12/12 h, light/dark).

Bivalves were exposed to four different concentrations (15, 20, 25 and 30 mg/L) of Diazinon (Hemming & Waller, 2004) to determine 96 hrs LC50 values for the test bivalves. Three aquaria were used for each concentration and each

aquarium contained six bivalve individuals in 70 L of dechlorinated tap water. One control group contained six individuals of bivalves and the same volume of water, but without Diazinon. Before culturing the bivalves in the aquaria, Diazinon was added and the water was aerated for an hour for a homogenous distribution of Diazinon in the water. Also, water in the aquaria was replace every 48 hours (Brahma & Gupta, 2020).

The death rate was recorded after 24, 48, 72, and 96 h of exposure. Dead bivalves were removed to avoid possible deterioration of the water quality (Brahma & Gupta, 2020). The Probit Analysis test was used to calculate LC50 values (Finney, 1971). Replaced concentrations were done every 48 hours (Brahma & Gupta, 2020).

2.5.3 Sub chronic toxicity

The fishes and bivalves were exposed to 2.37 mg/L, 3.45 mg/l of diazinon respectively for 28 days as sub lethal concentration, to estimate chronic toxicity effect by using 36 individuals from both experimental organisms, one concentration of sub lethal concentration, was chosen according to the diazinon 96-hour LC50 value previously determined for fishes (7 mg/L) and the bivalves (24 mg/L).

2.5.4 Blood sample collection and preparation for fish

The blood was taken from the fish different experimental groups at different periods (4, 14 and 28 days). To obtain blood samples, fish were caught gently in a small scoop net and then quickly taken out of the water and held firmly on a bench with a cloth covering the head, and blood samples from each fish were withdrawn

from the caudal vein at the posterior end of the anal fin base by using a pen type disposable vacuum vacutainer needle.

Whole blood withdrawal process took less than one minute per fish which was considered important to avoid stress effects and to minimize an error in normal blood values (Mostakim *et al*., 2015). Blood samples were taken from 3 fish randomly selected from each treatment.

These blood samples were collected in anticoagulated (K3-EDTA) tubes for blood parameters, acetylcholinesterase enzyme, and DNA damage. Another blood sample was collected in a gel tube and centrifuged at 3000 rpm for 5 min in order to separate the blood serum (Yang & Chen, 2003). Then the sample of blood serum was stored at –20 C for CAT, SOD, MDA, total protein, and liver function tests.

2.5.5 Sample preparations for biochemical assays in bivalve

Bivalves extract, was done according to (Doyotte *et al*., 1997), by taking 0.5 g of the bivalves flesh, and adding 2 ml of potassium phosphate buffer (pH = 7) to the powder, after which the bivalves powder cells are homogenized by the ultrasonic device (Homogenizer) for 3 minutes, stopping every 20 seconds. Then the model is discarded at a speed of 12,000 rpm for 30 minutes, after which the filtrate volume is completed to 5 ml with buffer. Stored at –20 C for estimate CAT, SOD, MDA, AChE and total protein.

2.5.6 Behaviorally effect

Observations of behavioral and morphological response of *C. carpio* fish exposed to diaznon were conducted at I through during the chronic toxicity tests. the methods developed by Drummond *et al*., (1986) were used for this study. Five

behavioral and morphological indicators were observed in this study: loss of equilibrium, general activity, startle response, hemorrhage, and deformity (including postural indicators). Each test container was observed for (5-10) minutes.

The following behavioral parameters were selected for monitoring bivalves: (Brahma & Gupta, 2020).

1. locomotor behavior as the number of times movement in the form of gliding and turning occurred during the monitoring period.
2. duration of extensive extensions of foot and siphons together.
3. duration of complete closing of valves without any extension of foot and siphons.

2.6 Biochemical analysis

2.6.1 Hematological tests for fish:

The blood parameters were determined in the fish after each exposure period of diazinon. 2mL blood from each replicate was collected. These blood samples were collected in anticoagulated (K3-EDTA) tubes for hematologic analysis. The hematologic analyses were determined on the same day the blood samples were taken from the fish.

2.6.1.1 Red blood cells (RBCs) and white blood cells (WBCs) count

RBC and WBC were counted according to (Talib & Khurana, 1996), by using a hemocytometer (Neubauer's counting chamber). Hayem's solution for red blood cell count and Turk's solution for white blood cell count.

2.6.1.2 Hemoglobin estimation (Hb)

Drabkin's Reagent was used for the quantitative, colorimetric determination of hemoglobin concentration in whole blood in the spectrophotometer at 540 nm. (Varley, 1954).

2.6.1.3 Platelets count (PL)

The platelets were counted, placing 20 µl of whole blood + EDTA to be examined in a test tube and adding 380 µl of ammonium oxalate solution. Then shake the mixture well and leave it for 5 - 10 minutes until all the RBC and WBC decompose and the platelets appear brightly clear. By using a hemocytometer (Neubauer's counting chamber) to count the platelets of a cell (Talib & Khurana, 1996).

2.6.2 Estimation of total protein in bivalve and fish

The total proteins in bivalves and fishes were determined according to the Lowry method modified by Bradford (1976) by taking 0.5 ml of the previously prepared extract of bivalve and fish serum, adding 2 ml of Biuret solution after mixing it with a preheater that was heated to 30 C° for half an hour and then measured at 555 nm and compared with the standard solution depending on the bovin serum albumin protein at Series of concentrations, which is prepared by dissolving 0.1 g of Bovin with 100 ml of puffer solution, so that the concentration is 100 µg /l fig.(2-2).

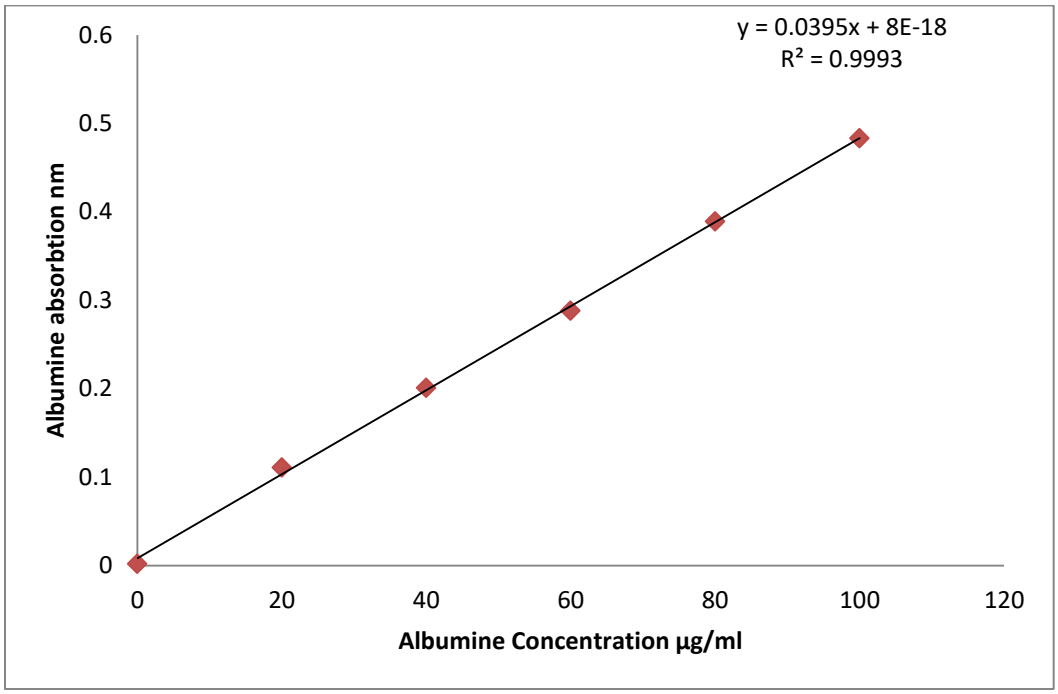


Figure (2.2) Albumine Standar Curve for Bivalves and Fishes

2.6.3 Antioxidants enzymes

2.6.3.1 Determination of superoxide dismutase (SOD)

The superoxide dismutase (SOD) activity was determined according to the method of (Marklund & Marklund, 1974), with modifications. SOD activity was measured according to, the assay of superoxide dismutase is based on the ability of the enzyme to inhibit the autoxidation of pyrogallol in the presence of EDTA. It is estimated by taking 50 microliters of the bivalve extract and fish serum and 2 ml of Tris-buffer and taking the reading at 420 nm after 5 minutes that it takes the second reading (△A0) and then adding 0.2 ml of pyrogallol solution and the reading is taken at 420 nm and then after five minutes, the second reading (△A1) is taken. SOD activitis calculated from the following equation.

SOD activity (µ/mL)= [(△A0 -△A1/△A0)/50%] × Volume of sample

2.6.3.2 Determination of catalase enzyme (CAT)

Catalase (CAT) activity was determined according to the method of Goth, (1991). The assay is based on the reduction in the absorbance of hydrogen peroxide (H_2O_2) at 405 nm. (200 µL of bivalves extract and fish serum were taken and adding 1 ml H_2O_2, incubation in a water bath for 60 min at 37 °C, then adding 1ml ammonium molybdate, then read the absorbance spectrophotometrically at 405 nm, (3 blanks were also run with the sampleing). Calculated activity from the following equation.

$$\textbf{Catalase activity CAT (U/ml)} = \frac{A(\,sample) - A(blank\ 1)}{A(blank\ 2) - A(blank\ 3)} * \mathbf{271}$$

2.6.4 Non- enzymatic antioxidant

2.6.4.1 Determination of malondialdehyde (MDA) in fishes and bivalves.

The principle of the following method was based on the spectrophotometric measurement of the color at 532 nm, and it occurred during the reaction between thiobarbituric acid (TBA) and MDA (Rifai, 2017). The procedure (150 µl of bivalve extract and fish serum were added to the test tube, 1 ml of 17.5% TCA (Trichloroacetic acid) and 0.06% TBA (Thiobarbutric acid) were added to the tube of sample, then mixed well by vortex. After that, the test tubes were incubated in a boiling water bath for 15 minutes, then allowed to cool. Then add 1ml of 70% TCA and let the mixture stand or incubate for 20 minutes. After that, the sample tubes are centrifuged for 15 minutes at 2000 g, which then measures the optical density of the supernatant at a wavelength 532 nm against the reagent blank).

Calculation

$$\textbf{The concentration of MDA} = \frac{A\ sample}{L * \varepsilon} * \textbf{D}$$

Where:
L= light path (1cm)
ε= extinction coefficient ($1.56 * 10^5 M^{-1} CM^{-1}$)

$$\textbf{D} = \frac{1ml\ (volume\ used\ in\ reference)}{0.15\ (volume\ used\ in\ sample)} = \textbf{6.7}$$

2.6.4.2 Determination of acetylcholinesterase activity(ache) in fish and bivalve.

Acetylcholinesterase enzyme activity was determined according to Ellman *et al*. (1961). The principle of the method is the measurement of the rate of production of thiocholine as acetylthiocholine is hydrolyzed. This is accomplished by the continuous reaction of the thiol with the 5:5-dithiobis-2- nitrobenzoate ion.

A fairly stable suspension was formed as a whole. The assay of blood was carried out as follows:

1- A suspension of the blood cells in phosphate buffer (pH 8.0, 0.1 M) was prepared. The most practical dilution was 1 : 600 (e.g. 10µ blood into 6 ml of buffer).
2- Exactly 3.0 ml of the suspension was pipetted into a cuvette.
3- 10µ of 0.1% quinidine sulfate was added to inhibit plasma esterase.
4- 25µ DTNB reagent were added. The cuvette was placed in the photometer.

5- The slit of the photometer was adjusted so that the absorbance (at 412 nm) of the suspension in the cuvette was zero.

6- The substrate, 20µ was added to this cuvette. Changes in absorbance at 412 nm were recorded for at least 6 min.

Calculations:

$$\textbf{Moles substrate hydrolyzed/min per RBC} = 4.41(10^{-14})\frac{\Delta A}{RBC}$$

Where: $4.41(10^{-14})$ = factor for dilution, extinction coeffcient and changes inunits.

$$= \frac{600}{13600} \times \frac{1}{10^6/mm^3} \times \frac{1}{10^6 RBC}$$

Δ**A** = change in absorbance/min

RBC = red cell count (in millions per mm^3)

In bivalves extraction it calculation by the followeng equation.

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

Where:

R= rate in moles substrate hydrolyzed per min per g of tissue.

Δ**A** = change in absorbance/min

$\mathbf{C_o}$ = original concentration of tissue (mg/ml).

2.6.5 Determination of liver functions in serum of fish

2.6.5.1 Assay of aspartate aminotransferase (AST/GOT)

The same assay method described for ALT was used, with the exception that the ALT reagent was replaced with the AST reagent.

AST activity (nm/min) = 1746 × ΔA 340 nm/min

Where:

1746 = Extinction coefficient

ΔA 340 nm/min = change in absorbance per minute for the sample

2.6.5.2 Assay of alanine aminotransferase (ALT/GPT)

The method described by Renner (2007) using Randox kits was used. 50 µl of fishes serum and 500 µl of the ALT reagent were mixed in a test tube, and the initial absorbance at 340 nm was read after 1 minute. The timer was set at the same time, and after 1,2,3 minutes, reading of the absorbance were taken.

ALT activity (nm/min) = 1 746 × ΔA 340 nm/min

Where:

1746= Extinction coefficient

ΔA 340 nm/min = change in absorbance per minute for the sample.

2.6.5.3 Assay of alkaline phosphatase (ALP)

Alkaline phosphatase estimated according to Otto *et al*. (1946) as modified by Wright *et al*. (1972) using Randox kits. In a cuvette, 10 µl of sample was mixed with 500 µl of the reagent. The initial absorbance was read at 405 nm and subsequently over 3 minutes. The mean absorbance per minute was used in the calculation:

$$\textbf{ALP activity (U/l)} = 2742 \times \Delta A \text{ 405 nm/min}$$

Where:

2742 = Extinction coefficient

ΔA 405 nm/ min = change in absorbance per minute for sample.

2.7 Histological examination

The fish and bivalves muscles in treated and control organisms after ending period of exposure of 28 days were killed, quickly dissected, and their flesh was removed, sliced, and fixed in 10% formalin solution. After 24 h, tissues were rinsed three times in 70% ethanol, dehydrated using a graded ethanol series, and then embedded in paraffin wax. Paraffin sections were cut into 5 micrometers thick slices by using a microtome, stained with haematoxylin and eosin, and examined under a light microscope (Sarhan, 2011).

2.8 Estimation the insecticide residues in tissue of organisms

The tissues of the animal bivalves and fishes samples after the ending period of exposure 28 days, were freeze-dried, then powdered and packed in a thimble and desiccated overnight before extraction. The desiccated thimble was extracted for 24 hours using the Soxhlet apparatus and a (Methanol : Benzene) solvent, as stated by (Al-Saad *et al*., 2009).

The analyses were carried out in the laboratories of the Ministry of Science and Technology using High performance liquid chromatography (HPLC) model SYKAM German . The mobile phase was water–acetonitrile (30 : 70 v / v) . The pH of the water was adjusted to 3.0 using 1M acetic acid. The separation column was C18- ODS (25 cm * 4.6 mm) while the UV detection of the wavelengths was 254 nm for Diazinon. A 100 µL sample was injected into the HPLC (Abu-Qare & Abou-Donia, 2001).

2.9 DNA damage (Comet assay)

The method of Singh *et al*. (1988), was applied to determine the DNA damage of bivalves and fish. The comet assay was performed on blood samples collected from the caudal veins of fishes, and haemolymph was collected from bivalves. The blood and haemolymph samples were diluted with 1.0 mL of phosphate-buffered saline (PBS), and then 15 µL of cell suspension (~15,000 cells) with 85 µL of 0.5% low-melting-point agarose (LMPA) were mixed and layered on the pit of the slide, coated with a layer of 200 µL of 1% normal agarose and 100 µL LMPA, respectively.

After gel solidification, the slides were immersed in a lysing solution for 60 minutes at 4 °C containing 2.5M NaCl, 100mM Na2-EDTA, 10mM Tris, pH 10, with 10% DMSO and 1% Triton X-100. To unwind the DNA helix and convert of alkali-labile sites to single-strand breaks, the slides were plunged into a horizontal electrophoresis tank, filled with fresh alkaline electrophoresis buffer (300mM NaOH, 1mM EDTA, and 0.2% DMSO, pH 13.5) at 4 °C for 25 min to unwind the DNA. Subsequently, the electrophoresistank was connected to a power source and adjusted to 60 min. volt 70. To remove excess alkali, the slides were gently washed for 10 min at 4 °C with neutral buffer (0.4M Tris, pH 7.4) and stained with 75 µL ethidium bromide (10–20 µg/mL). The slides were examined with a ZEISS fluorescence microscope.

2.10 Statistical analysis

This study used the analysis of variance (ANOVA), LSD, median, standard deviation, minimum and maximum to find the significance among the study variances by using SPSS statistical program software (version 17), for creation the plots and tables, used Microsoft excel.

3 Results and discussion

3.1 water quality of aquarium

The water quality result was with the following characteristics: pH 8.5±0.3, Temperature 19±2 °C in fish experiment, while in bivalves experiment was 28±2 °C , and dissolved oxygen 6.4±0.45 mg/L, salinity 1.2 ± 0.1 ppt. . Electrical Conductivity 2360 ± 48 µS/cm.

3.2 Lethal concentration (LC50).

3.2.1 Lethal concentration (LC50) in fish

The lethal concentration (LC50) in experiment was calculating for (*C. Carpio*), carried out in 96 hours, and used different concentrations of pesticide (6, 10 and 15 mg/L). The lethal concentration (LC50) for 96 h was computed by the probit method (Finney, 1971), the log of concentration and probit mortality shown in table (3-1).

Table(3-1): The log of concentration and probit mortality for *C. carpio*

Concentration mg/l	Log of concentration	Mortality rates	Probit mortality
0	0	0 %	0
6	0.778	5 %	3.36
10	1	55 %	5.14
15	1.176	100 %	8.7

The LC50 was determined by Graph plotted for log of concentration against probit mortality of *C. carpio* exposed to Diazinon, fig. (3-1), then calculate the LC50 from the equation of straight line and was found to be 7 mg/l.

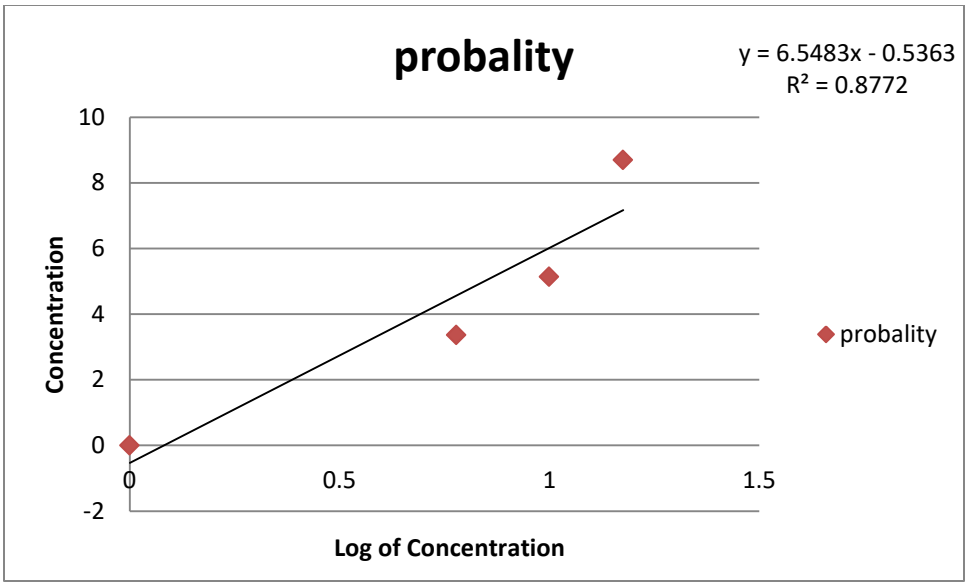


Figure (3-1) Linear regression of toxicity in *C. carpio* exposed to diazinon

The LC50 for 96 hour in the present study was found 7 mg/L for *C. carpio* exposed to diazinon. There is several studies dealing with the effect of diazinon on common carp *C. carpio* such as Svoboda *et al*., (2001) who studied the effect of diazinon on haematological indices of common carp (*C. carpio*) and they found the LC50 was 26.7 mg/L. Another study by Korkmaz & Dönmez, (2017) found that the LC50 of diazinon of *C. carpio* was 9. 76 mg/L. Also previous study on other species of carp that carry out by Haider & Rauf, (2014) about "sub-lethal effects of diazinon on hematological indices and blood biochemical parameters in indian carp, *cirrhinus mrigala*" they found that lC50 for diazinon on *C. mrigala* was 8.15 mg/L. The estimated 96 - hour LC50 for juvenile *Cyprinodon variegatus* exposed to diazinon is 1.4 mg/L (Goodman *et al*., 1979).

The LC50 values in this study and the other studies was belong to the known differences in environmental properties, the water quality used in the experiments that have an effect on the toxicity of the pesticide to the fish, also the period of exposure, fish size, fish age, weight, length and hereditary content (Mitchell *et al*.,

1987). All these factors could change the metabolism of the fish, stability of the pesticide and its presence in the water, dissolved substances in the water that decrease the pesticide bio availability (Murty, 2018).

3.2.2 Lethal concentration (LC50) in bivalve.

The lethal concentration (LC50) in experiment was calculating for *S. woodiana*, it was carried out in 24, 48, 72 and 96 hours, and used different concentrations of diazinon (15, 20, 25 and 30 mg/L). The median lethal concentration (LC50) for 96 h was computed by the probit method (Finney, 1971), the log of concentration and probit mortality shown in table (3-2).

Table(3-2): The log of concentration and probit mortality for *S. woodiana*

Concentration mg/l	Log of concentration	Mortality rates	Probit mortality
0	0	0 %	0
15	1.176	16 %	4.01
20	1.3	33 %	4.56
25	1.397	61 %	5.28
30	1.477	77 %	5.47

The LC50 was determined by Graph plotted for log of concentration against probit mortality of (*S. woodiana*) exposed to diazinon, fig.(3-2), then calculate the LC50 from the equation of straight line and was found to be 24 mg/l.

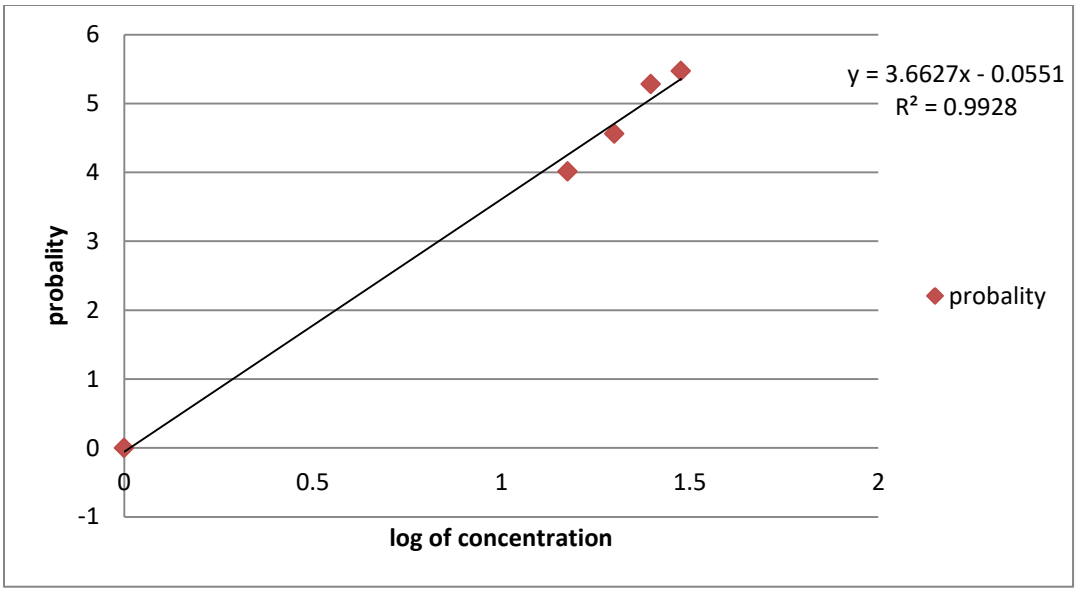


Figure (3-2) Linear regression of toxicity in *S. woodiana* exposed to diazinon

There are different studies dealing with the effect of diazinon on *C. fluminea*, such as, the study that carry out by Hemming & Waller, (2004), that studied the diazinon toxicity to the *C. fluminea* was estimated to be 4.067 mg/l. Other bivalves species study that carry out by Conners & Black, (2004), that study the effect different chemicals on freshwater bivalve that use diazinon with purity 22.4%, it found the LC50 for diazinon on freshwater bivalves *Utterbackia imbecillis* was 19.4 mg/L.

The ability of clams to isolate themselves from their immediate environment may, in part explain the high diazinon resistance and the non-monotonic response observed as the concentrations increased by large amounts. Additionally, the unknown pesticide carriers (also known as "inert" organic solvents) in which these active components (diazinon) are supplied may have had an impact on the outcomes of the toxicity testing (Hemming & Waller, 2004).

3.3 Behaviorally effect

3.3.1 Behaviorally effect in fish:

Observations of behavioral and morphological response of *C. carpio* fish which exposed to diaznon were conducted at I through during the chronic toxicity tests. the methods developed by Drummond *et al*., (1986) were used for this study. Five behavioral and morphological indicators were observed in this study: loss of equilibrium, general activity, startle response, hemorrhage, and deformity (including postural indicators). Each test container was observed for (5-10) minutes.

Table (3.3): Diagnostic Behavioral Effects of diazinon on *C. carpio*

Behavioural and Morphological Symptoms	Diagnosis
Deformities	damage caudal fin
General activity	- hyperactive to hypoactive - Change in skin pigmentation - sinking to the bottom - fish scales falling
Hemorrhage	None
Loss of equilibrium	Yes
Startle response	underreactive

Figure (3-3) Deformities (damage caudal fin) in *C. carpio* exposed to diazinon

Figure (3-4) Change in skin pigmentation in *C. carpio* exposed to diazinon

The damaged caudal fin was recorded by Rashid *et al*., (2012), they study effect of diazinon toxicity on embryonic and larval development stages in catfish. There are many studies that note the deformed, caudal fin damage in early stages development in *C. carpio* with imidacloprid insecticide and other insecticides (Islam *et al*., 2019a). The other behavioral effects such as (less general activity, Change in skin pigmentation and loss of equilibrium) it was observed that in fingerling European catfish that exposure to diazinon (Köprücü *et al*., 2006).

The above symptoms may be due to inhibition of acetylcholinesterase (AChE) activity leading to accumulation of acetylcholine (ACh) in cholinergic synapses ensuing hyperstimulation. Since, inhibition of AChE activity is a typical characteristic of organophosphate compounds (Halappa & David, 2009; Sharbidre & Sopanrao Patode, 2012).

3.3.2 Behaviorally effect in bivalve:

Bivalves have some control over the frequency and duration of such an interaction , providing the initial toxic insult is not acute. Mortality occurs under these conditions when bivalves cannot completely isolate themselves (Hemming & Waller, 2004).

The following behavioral parameters were selected for monitoring: (Brahma & Gupta, 2020)

(1) locomotor behavior as the number of times movement in the form of gliding and turning occurred during the monitoring period.

(2) duration of extensive extensions of foot and siphons together

(3) duration of complete closing of valves without any extension of foot and siphons

The result in our study were observed behavioral changes in bivalve, the clams closed their valves tightly over longer periods and open them slightly for short time, extended the foot and siphons from time to time, when exposed to diazinon pesticide.

The ability of mussels to isolate themselves from their immediate environment may , in part , explain the high resistance and the non-monotonic response as the exposure concentrations increased by large amounts. Additionally, the high diazinon concentrations *S. woodiana* succumbed to are un likely to be present in the environment unless extreme circumstances exist. The ability of *S.*

woodiana to incur such high levels of diazinon insult may have been partially explained by the ability to isolate themselves from the unsuitable environment through valve closure.

3.4 Biochemical analysis

3.4.1 Hematological parameters

3.4.1.1 Red blood cell (RBC) count

The result of present study had been showed significant decreased at ($P<0.05$) in red blood count (RBC) of *C. carpio* in 4th day of experiment during acute exposure period to sub-lethal concentration of diazinon. While there are no significant change between control and treatments groups in 14,28 days of exposure at ($P<0.05$). The maximum value of RBC was (1.41±0.36) 10^6/mm^3 during 14th day with treatment group, while the minimum was (0.42±0.2) 10^6/mm^3 during 4th day with the same group figure (3-5).

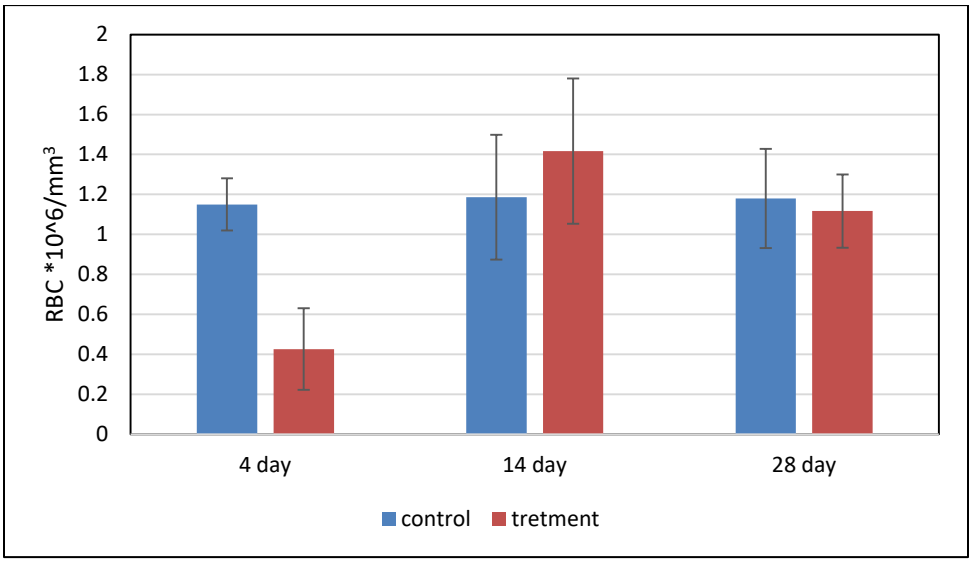


Figure (3-5):The RBC of *Cyprinus carpio* after exposure to sub-lethal concentration of diazinon

The results of present study were agree with the observations of Banaei *et al*., (2008), that observed a significantly lower RBC in *C. carpio* that had been exposed to sub-lethal diazinon concentrations. Decreased erythrocyte count in *C. carpio* after acute exposure to diazinon were also reported by Svoboda *et al.*, (2001). Other effective substances of organophosphorus pesticides also induce changes which give evidence for decreased hematopoiesis followed by anemia induction in fish (Gunasekaran & Vellaichamy, 2019).

The results of present study also observed non significantly increase in RBC count in 14^{th} days it agree with Ahmad, (2011). The adaptation in exposed fish led to elevation in RBC to avoid the surrounding environment stress. It is documented that under stress condition, fish become hyper active perhaps to get out of the stressful medium and would require an increased amount of oxygen to meet their energy requirement (Southamani *et al*., 2015). In such hypoxic condition, there is a stress-mediated synthesis of more haemoglobin and release of new erythrocytes from the erythropoietic organs to improve the oxygen carrying capacity of blood (Martos-Sitcha *et al*., 2019).

3.4.1.2 White blood cell (WBC) count

The total leukocytes count showed no significant change in *c. carpio* fish after exposure to sub-lethal concentration of diazinon through the experimental period at ($P<0.05$). The maximum value of WBC was (78.39±14.6) $10^3/mm^3$ during 14^{th} day with treatment group, while the minimum was (39.27±22.29) $10^3/mm^3$ during 4^{th} day with the same group figure (3-6). The total WBC decreases was recorded in common carp (*C. carpio)* after exposure to diazinon by (Svoboda, *et al*., 2001, Banaei *et al*., 2008, Ahmad, 2011). In contrast the study result disagree with Al-Ghanim, (2013) and Haider & Rauf, (2014).

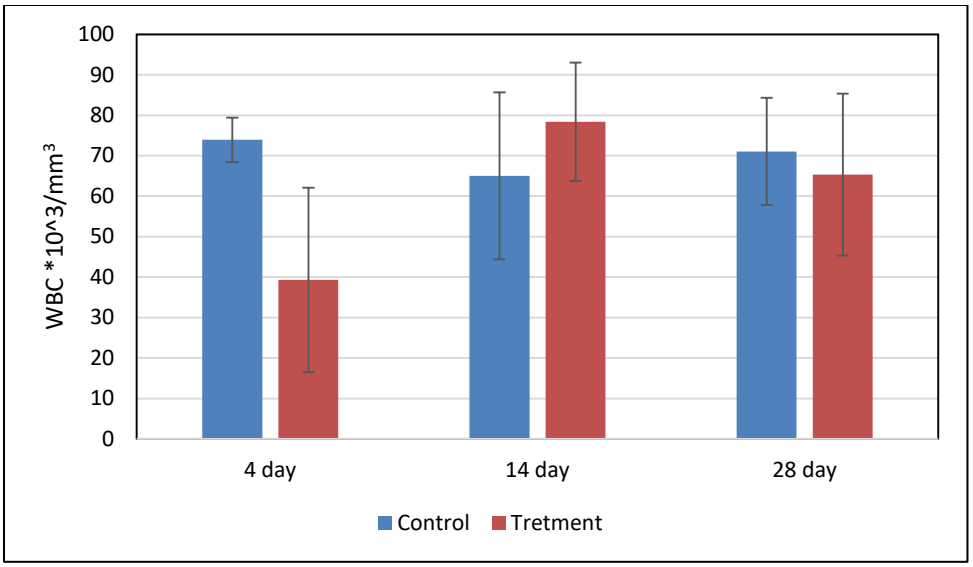


Figure (3-6):The WBC Of *C. carpio* after exposure to sub-lethal concentration of diazinon

The total leukocyte count was increased, which might be due to malfunctioning of the haematopoietic system caused by exposure to diazinon (Far *et al.*, 2012). Changes in the leukocyte system manifest in the form of leukocytosis with heterophilia and lymphopenia, which are characteristics of leukocytic response in animals exhibiting stress (Ahmad *et al.*, 2021). A decreased non-specific immunity in fish can be expected after acute exposure to organophosphorous pesticides due to decreased leukocyte count, lymphopenia and granulocytosis. These changes in differential leukocyte count also give evidence for decreased level of nonspecific immunity in fish after acute exposure to toxic substances (Svoboda, *et al.*, 2001).

3.4.1.3 Hemoglobin estimation (Hb)

The Diazinon did not appear any significant effect at ($P<0.05$) on Hb value compare between the control and treatment groups, there are slight lowering in value in 4^{th} day of experiment with treatment group and slightly elevated in the

treatment value in 28th and 14th day of exposure. The maximum value of was 10.9±1.7 g/dL during 14th day with treatment group, while the minimum was 7.26±3.4 g/dL during 4th day with the same group (Fig.3-7). The current study results were in agreement with the observations of (Svoboda, *et al*., 2001, Banaei *et al*., 2008, Haider & Rauf, 2014). In contrast the study result disagree with (Al-Otaibi *et al*., 2018).

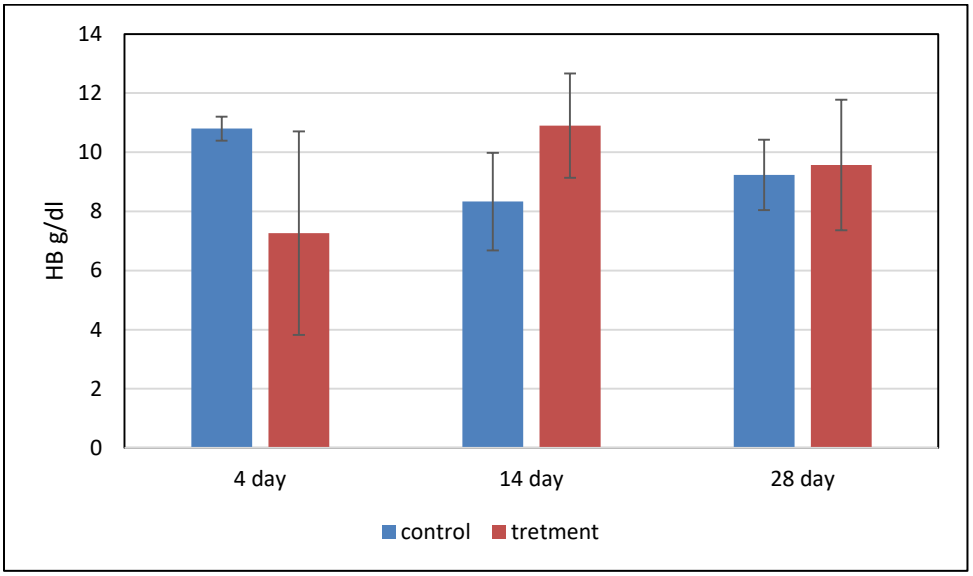


Figure (3-7): The Hemoglobin (HB) Of *Cyprinus carpio* after exposure to sub-lethal concentration of diazinon

When fish are exposed to fixed concentrations of the toxin, the resistance of fish over time is dwindling. The impact of toxin in fish will decrease (Al-Ghanim, 2013). It explain the rise hemoglobin value after 14th day of exposure. Long time of exposure to diazinon increasing the Hb and other hematological parameters (Haider & Rauf, 2014). Adedeji *et al*., (2009) also reported decreased RBC and Hb count after diazinon exposure in giant sturgeon (*Huso huso*) and African catfish, (*C. gariepinus)*. Organophosphorous pesticides stimulate alteration whose give index for decreased haematopoiesis followed by anemia induction in fish. It regards e.g. changes in erythrocyte profile induced by acute effect of dichlorvos in *Clarias batrachus* (Benarji & Rajendranath, 1990).

3.4.1.4 platelet count (PLT)

In the present study, non-significant decrease was observed in platelet count through experiment period in the treatments when compared to the control (p<0.05). The maximum value of platelet was (50±4)10^3/mm^3 during 4_{th} day with control group, while the minimum was (16±9.2)10^3/mm^3 during 14^{th} day with the treatment group (Fig.3-8). The existing study results agreement with the observations of present Gunasekaran & Vellaichamy, (2019).

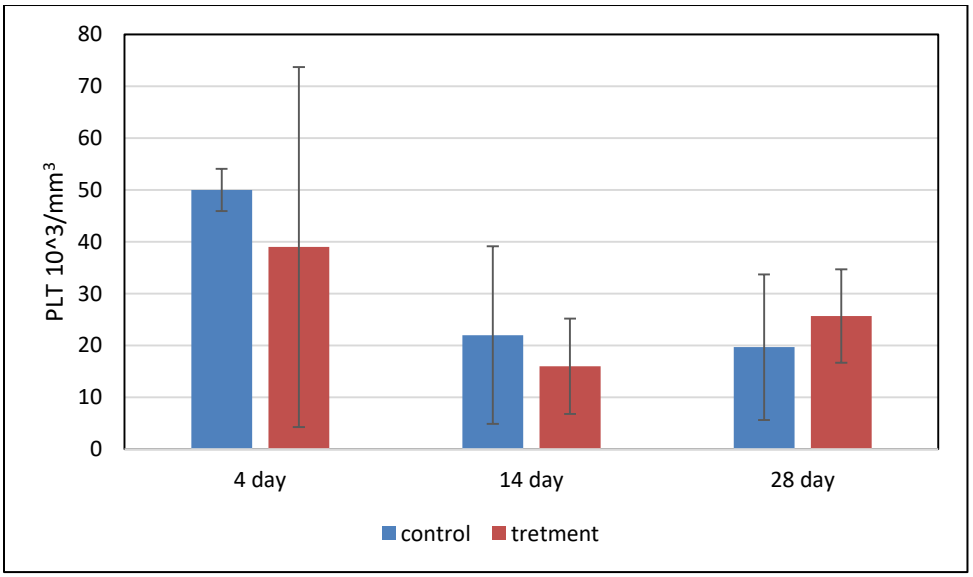


Figure (3-8): The platelet count (plt) of *c. carpio* fish after exposure to sub-lethal concentration of diazinon

Thrombocytopenia was observed in fish exposed to glyphosate or malathion, as evidenced by significant reductions in RBCs (Hassan *et al*., 2022). Thrombocytopenia occurs as a result of a bone marrow disorder such as leukemia or a problems with the immune system (Burgos-Aceves *et al*., 2019).

3.4.2 Total protein

3.4.2.1 Total protein in fish

The maximum total protein in fish was (48.8±0.08) µg/ml at 14th day in treatment group, while the minimum was (25.2±0.01) µg/ml during 28th day compare with control group fig.(3-9). The current study revealed that diazinon exposure resulted in a significant decrease at ($p<0.05$) in 4th and 28th day of total protein contents in serum of (*C. carpio*). The decrease of total protein in common carp was reported by (Lusková *et al*., 2002, Haider & Rauf, 2014).

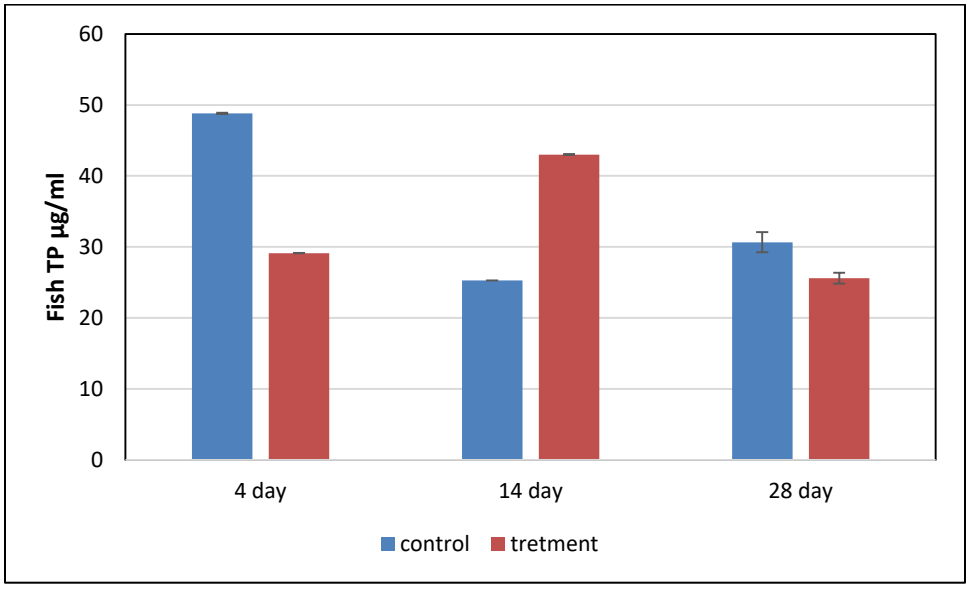


Figure (3-9): The total protein (TP) Of *C. carpio* after exposure to sub-lethal concentration of diazinon

Many reasons drive to decrease total protein contain in common carp exposer to diazinon, may be attributed to disfunction and destruction of hepatocytes caused by diazinon toxicity or malnutrition (Banaei *et al*., 2008, Haider & Rauf, 2014). Decreasing total protein result from loss protein through the intestine and kidney, failure of haemopoiesis is a characteristic indicator of kidney damage which increased renal excretion of blood protein (Akter, 2020).

3.4.2.2 Total protein in bivalve

The maximum total protein in bivalves was (11.8±0.6) µg/ml at 28th day in treatment group, while the minimum was (9.4 ± 0.27) µg/ml during 4th day with treatment group compare with control that record (6.6 ± 0.38) µg/ml in 28th day (fig 3-10). The present study record that diazinon exposure have a significant change at ($p<0.05$) during last day in total protein concentration of (*S. woodiana*).

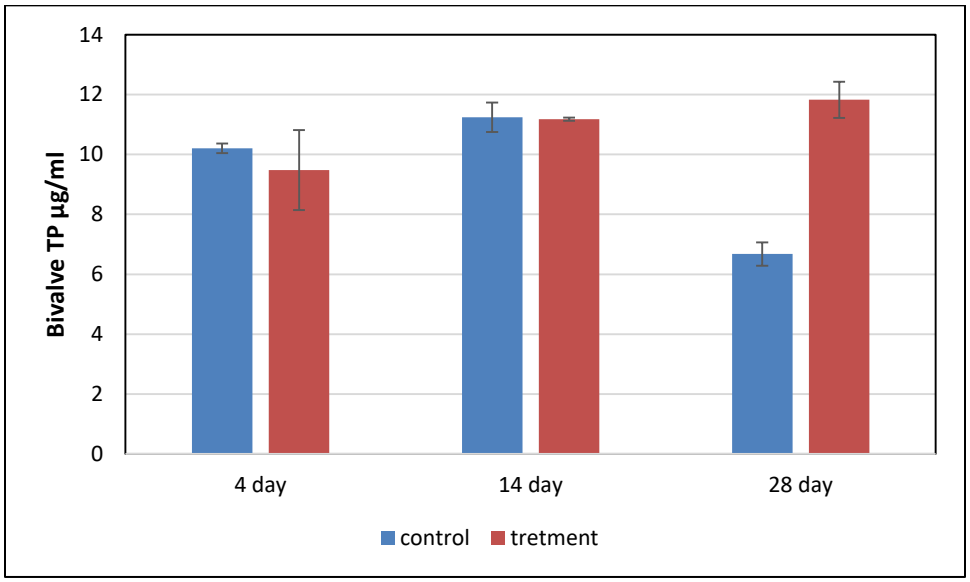


Figure (3-8): The total protein (TP) Of *S. woodiana*, bivalves after exposure to sub-lethal concentration of diazinon

The variation in total protein level between treatment and control group through experiment period in bivalves that under environmental stress, explained that by a decrease or increase of general metabolic activity (Géret *et al*., 2002).

3.4.3 Antioxidants Enzymes

3.4.3.1 Superoxide Dismutase (SOD)

3.4.3.1.1 Superoxide Dismutase (SOD) in fish

The maximum SOD activity in fish was (31.4 ± 2.9) µg/ml at 28th day in treatment group, while the minimum was (22.54 ± 4.43) µg/ml during 4th day in treatment group fig.(3-11). The study result show a non-significant different at ($p<0.05$) in SOD activity in serum of (*C. carpio*). The decrease in (4th and 14th) day and increase in 28th day.

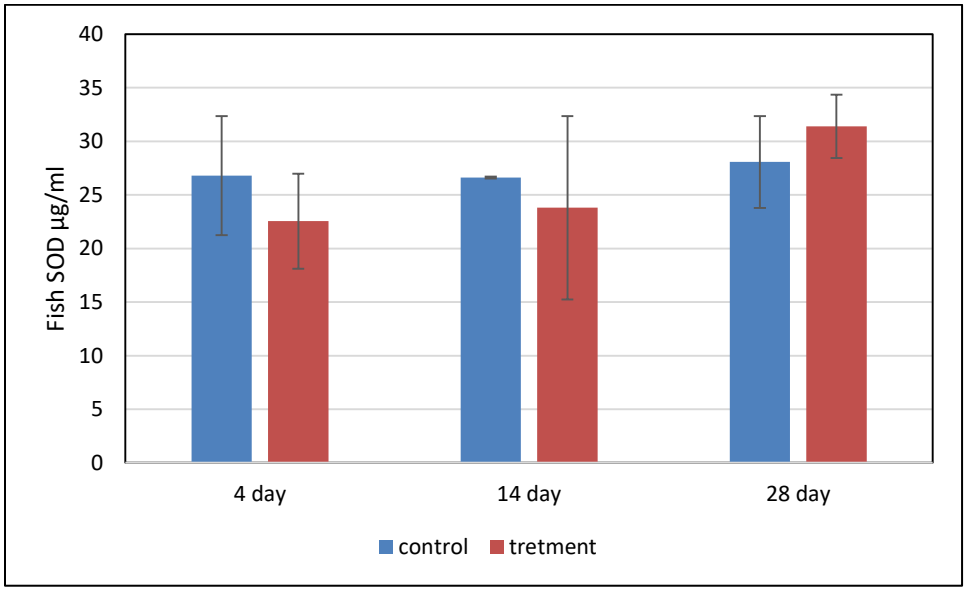


Figure (3-11): SOD activity Of *C. carpio* Fish After Exposure To Sub-Lethal Concentration Of Diazinon

The increase SOD activity in (*C. carpio*) after exposure to diazinon was report by (Banaee *et al*., 2013, Brontowiyono *et al*., 2022). The synthesis of oxidant-antioxidant molecules is balanced within the cell (Yonar, 2019). If oxidant production is increased due to stressful conditions, the cell avoids the harmful effect of cumulative oxidant molecules by overconsumption of antioxidant

compounds (Barkallah *et al.*, 2019). SOD is responsible for removal of superoxide radicals (Winterbourn, 2020). The increased SOD activities in fishes exposed to diazinon might be biochemical responses to over production of superoxide radicals (Nwani *et al.*, 2015).

3.4.3.1.2 Superoxide Dismutase (SOD) in bivalve

The maximum SOD activity in bivalve was (37.14 ± 2.33) µg/ml at 28th day, while the minimum was (20.66 ± 0.94) µg/ml during 4th day in treatment group fig.(3-12). The study result show that in a significant increase at ($p<0.05$) in SOD activity in (*S. woodiana*). The increase SOD activity in *Corbicula fluminea* exposure to imidacloprid was report by (Shan *et al.*, 2020).

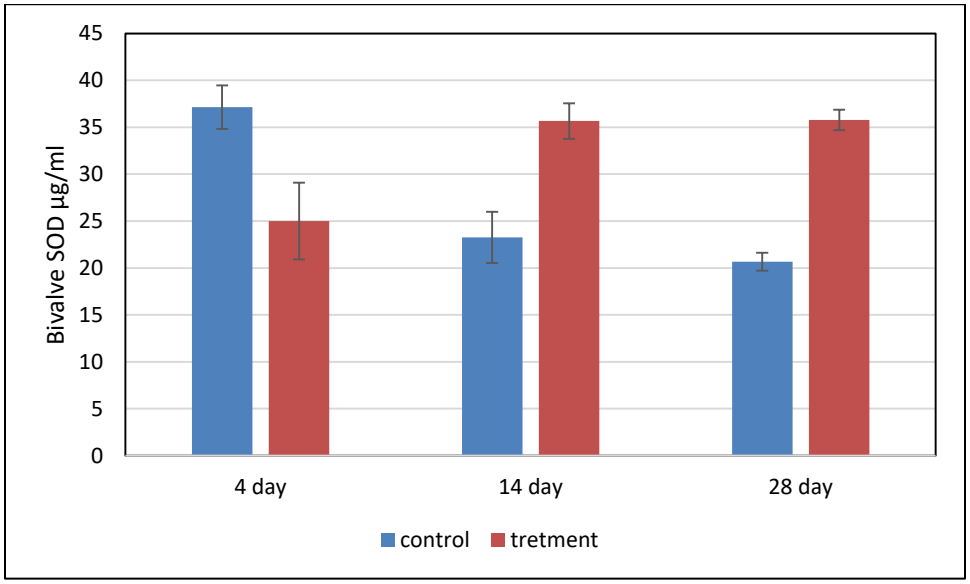


Figure (3-12): Superoxide Dismutase (SOD) Of *S. woodiana*, Bivalves After Exposure To Sub-Lethal Concentration Of Diazinon

Superoxide dismutase (SOD) is an important enzyme for detoxification of reactive oxygen species in all the organisms. SOD plays an important role in protecting cells against the oxidative damage of free radicals by catalyzing the conversion of superoxide anion to oxygen (O2) and hydrogen peroxide (H2O2),

which is then catalyzed either by CAT or Glutathione peroxidase enzyme (Ighodaro & Akinloye, 2018).

During Sub-Lethal Concentration exposure Of Diazinon in *S. woodiana*, SOD activities increasing which indicated generation of superoxide radical and hydrogen peroxide after the exposure to diazinon in bivalves, this reason it observed in asian clams (*C. fluminea*) exposured to cadmium (Wang *et al*., 2021).

3.4.3.2 Catalase Enzyme Activity (CAT)

3.4.3.2.1 Catalase Enzyme Activity in fish

The maximum CAT activity in fish was (209.56 ± 0.62) U/ml at 28th day in treatment group, while the minimum was (149.87 ± 2.37) U/ml during 4th day with treatment group fig.(3-13) compare with control group. The study result show that in a significant increase at ($p<0.05$) in CAT activity in serum of (*C. carpio*). The increasing in CAT activity was report by (Banaee *et al*., 2013). CAT catalyses the conversion of H_2O_2 into O2 and H2O, reducing the generation of hydroxyl radicals (-OH), thereby protecting the antioxidant system (Ighodaro & Akinloye, 2018).

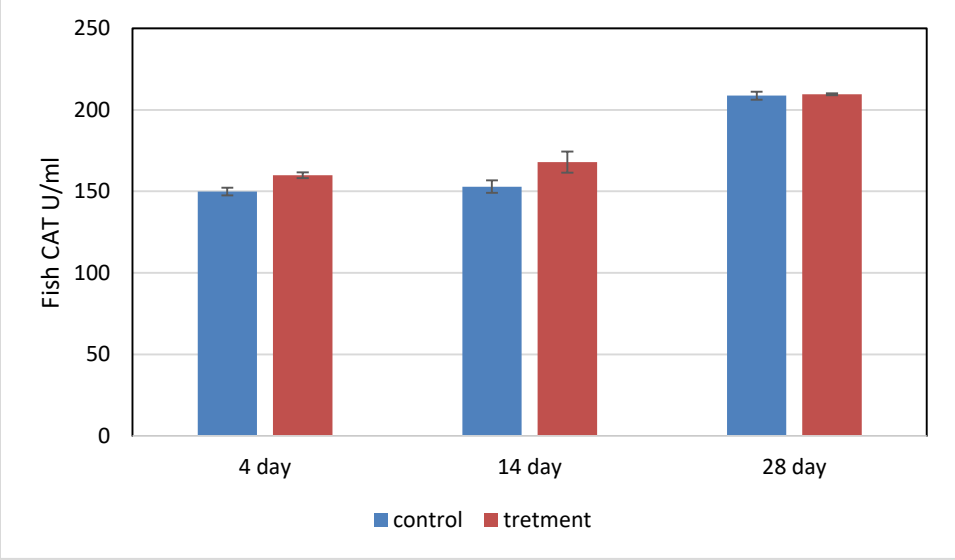


Figure (3-13): Catalase Activity (CAT) Of *C. carpio* Fish After Exposure To Sub-Lethal Concentration Of Diazinon

Catalase maintained elevated activities during all the treatment period. CAT catalyzes the decomposition of hydrogen peroxide to water and oxygen. The increased CAT activities in hepatocytes of fishes exposed to diazinon might be biochemical responses to over production of superoxide radicals and H_2O_2 (Banaee *et al*., 2013). It has been shown that the CAT activity may be related to H_2O_2 production in a xenobiotic detoxification process (Kaur & Jindal, 2017). Monteiro *et al*., (2006) was report the elevated of CAT in fresh water fish after exposure to organophosphorus insecticide.

3.4.3.2.2 Catalase Enzyme Activity in bivalves

The maximum CAT activity in bivalve was (41.57 ± 1.29) U/ml at 4^{th} day in treatment group, while the minimum was (27.77 ± 0.69) U/ml during 28^{th} day with treatment group fig.(3-14). The control group record the maximum in 14^{th} day while the minimum was in the 4^{th} day. The study result show that in a significant different at ($p<0.05$) in CAT activity in (*S. woodiana*). The result appear increase in CAT activity in 4^{th} day, while in (14^{th} and 28^{th}) day of exposure the result show a decrease in CAT activity compare between the control and treatment groups.

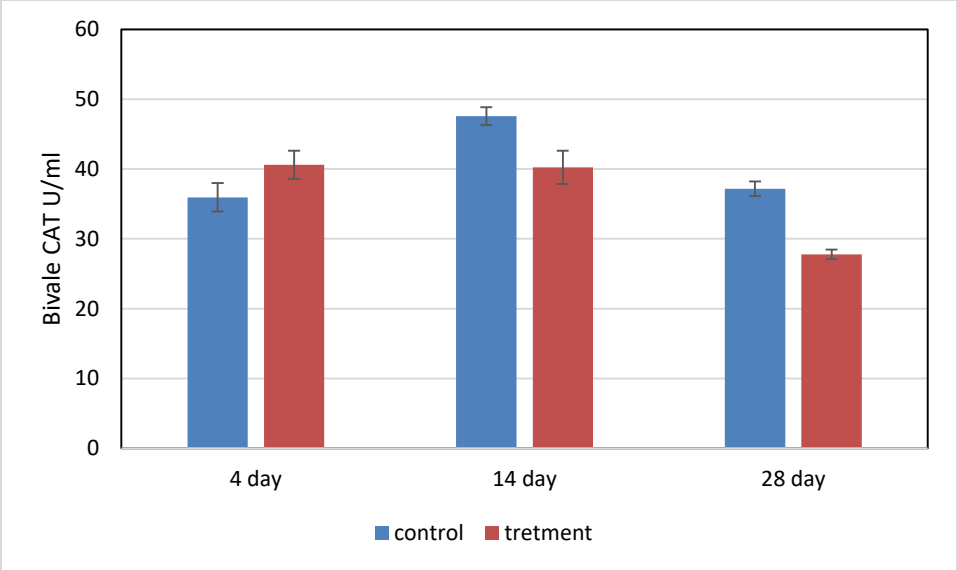


Figure (3-12): Catalase Activity (CAT) Of *Sinanodonta woodiana,* Bivalves After Exposure To Sub-Lethal Concentration Of Diazinon

The increasing in CAT activity with 4th day and lowering in concentration in 14th and 28th day, this variation in CAT due to organisms adaptation to environmental stress (Woo *et al*., 2013). CAT exerts its antioxidant function through removing hydrogen peroxide radicals, It has been shown that the CAT activity may be related to H_2O_2 production in a xenobiotic detoxification process (Bhagat *et al*., 2016).

3.4.4 Non-enzymatic antioxidant

3.4.4.1 Malondialdehyde (MDA)

3.4.4.1.1 Malondialdehyde in fish

The maximum MDA activity in fish was (1.76 ± 1.01) µmol/L at 14th day, while the minimum was (0.64 ± 0.09) µmol/L during 28th day with treatment group compare with control group that maximum in 14th day and minimum in 28th day fig.(3-15). The study result show that a non-significant increase at ($p<0.05$) in

MDA activity in serum of (*C. carpio*). The increasing in MDA activity was report by (Al-Ghanim, 2014), also increasing MDA was report in (*C. carpio*) after exposure to lufenuron and flonicamid insecticide by (Ghelichpour *et al*., 2020).

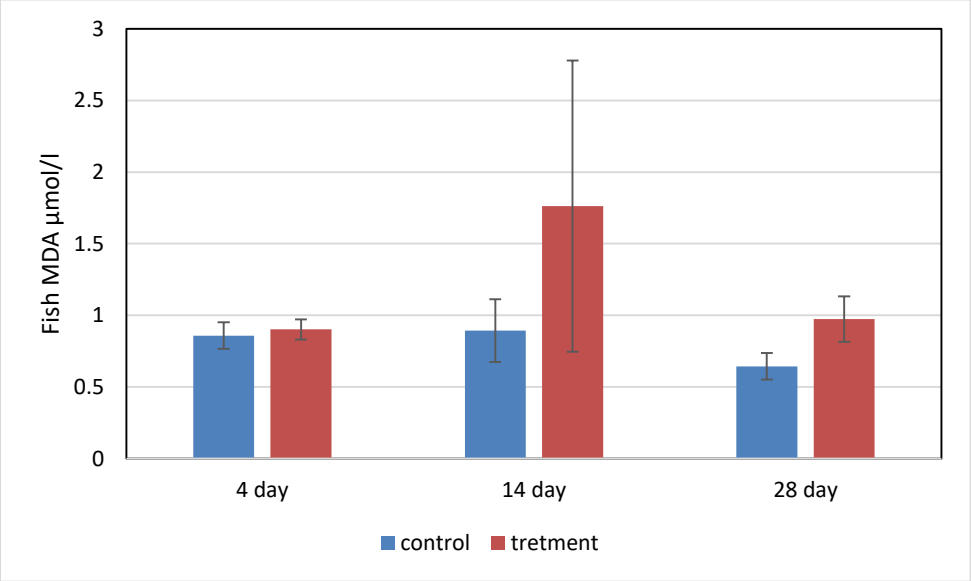


Figure (3-15): Malondialdehyde Activity (MDA) Of *Cyprinus carpio* Fish After Exposure To Sub-Lethal Concentration Of Diazinon

One of the most damaging effects of free radicals and their products in cells is the peroxidation of membrane lipids of which MDA is an indicator (Amin & Hashem, 2012). MDA is the final product of lipid peroxidation and a sensitive diagnostic index of oxidative injury in cells. Lipid peroxidation is one of the major mechanisms involved in the oxidative cell injury (Al-Ghanim, 2014).

MDA is a major oxidation product of peroxidized polyunsaturated fatty acids and increased MDA content is an important indicator of lipid peroxidation (Rajeshkumar *et al*., 2017). However, when a low level of stress is applied, an adaptive response takes place in the cells. This adaptation may be associated with de novo protein synthesis, or might be due to the activities of various removal and repair enzymes (Zhao *et al*., 2020).

3.4.4.1.2 Malondialdehyde in bivalve

The maximum MDA activity in bivalve was (0.91 ± 0.02) µmol/L at 28th day in treatment group, while the minimum was (0.45 ± 0.02) µmol/L during 4th day with treatment group fig.(3-16). The control group record the maximum (0.74 ± 0.01) µmol/L at 28th and minimum (0.35 ± 0.02) µmol/L during 4th compare with treatment. The study result show that in a significant increase at ($p<0.05$) in MDA activity in (*S. woodiana*). The result appear decrease in MDA activity in 4th day, while in (14th and 28th) day of exposure the result show increase in MDA activity compare with control groups.

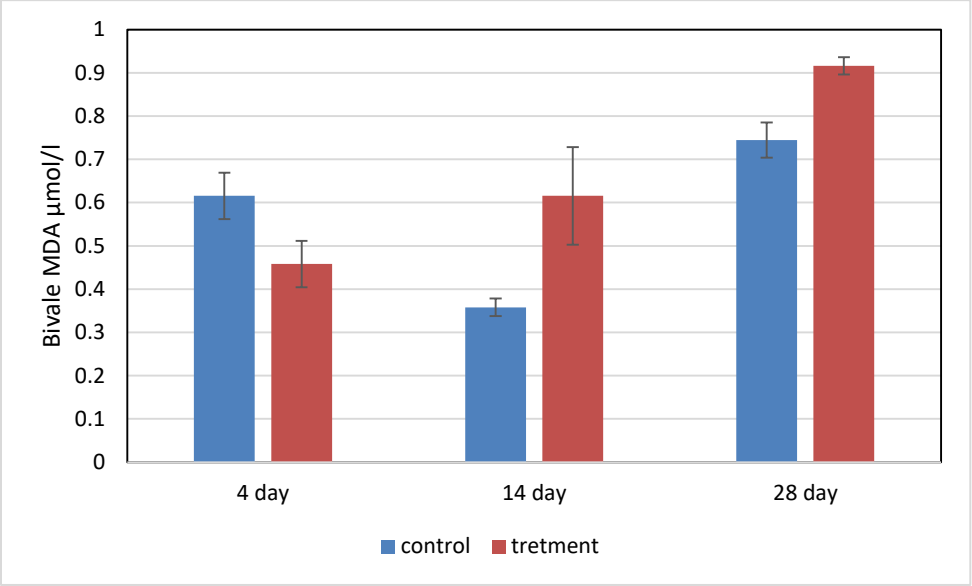


Figure (3-16): Malondialdehyde Activity (MDA) Of *Sinanodonta woodiana*, Bivalves After Exposure To Sub-Lethal Concentration Of Diazinon

The increase in MDA activity was report in freshwater mussels after exposure to organophosphates pesticide by Al-Fanharawi *et al*., (2019). Köprücü *et al*., (2010) was record the elevated in MDA activity in freshwater bivalve when exposure to cypermethrin insecticide. MDA is a primary product of lipid peroxidation, which is cytotoxic and can damage membranes (Mousavi *et al*.,

2020). This might be a severe oxidative stress response to excessive amounts of H2O2 and $O_2^{\bullet-}$.

The increase in MDA content indicated that the body suffered oxidative damage (Chen *et al*., 2020). When a cell or a tissue is not able to prevent oxidative damage, there is an increase of lipid peroxidation, measured as an increase in MDA level (Al-Fanharawi *et al*., 2019).

3.4.4.2 Acetylcholinesterase activity (AChE)

3.4.4.2.1 AChE activity in fish

The maximum Acetylcholinesterase activity in fish was (2.01 ± 0.12)*10^{-9} µmol/min per RBC at 4th day in treatment group, while the minimum was (0.23 ± 0.06)*10^{-9} µmol/min per RBC during 28th day with treatment group fig.(3-17). Compare with control group the maximum in 4th day and the minimum in 28th day. The study result show that a significant decrease at ($p<0.05$) in AChE activity in blood of (*C. carpio*).

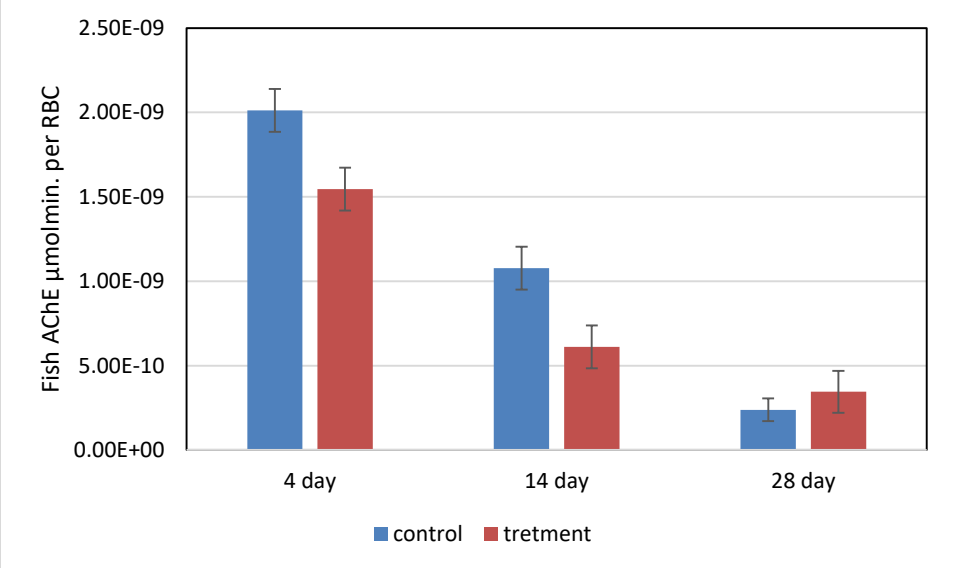


Figure (3-17): Acetylcholinesterase Activity (AChE) Of *C. carpio* Fish After Exposure To Sub-Lethal Concentration Of Diazinon

The decreasing in AChE activity in common carp exposure to diazinon was documentation by (Haider & Rauf, 2014). Banaee *et al*., (2011) was record lowering in AChE activity in rainbow trout fish exposure to diazinon.

Diazinon induced suppressed activity of AChE results in accumulation of acetylcholine at neural and neuromotor regions that causes hyper excitability and ultimately influences fish behavior (Guzman *et al*., 2020). The decreased activity of AChE and altered behavioral response of *C. cyprinus* in present study supports the findings of previous studies. damage to red blood cells and anemia are other factors reducing AChE (Hatami *et al*., 2019).

The AChE inhibition that induced by neurotoxicity by organophosphate, may also associated with the hyperactivity in fishes.

3.4.4.2.2 AChE Activity in Bivalve

The maximum Acetylcholinesterase activity in bivalve was (1.64 ± 0.01) µmol/L per min. during 4^{th} day in treatment group, while the minimum was (0.028 ± 0.004) µmol/L per min during 28^{th} day with same group compare with control group fig.(3-18). The study result show that a significant decrease at ($p<0.05$) in AChE activity in (*S. woodiana*).

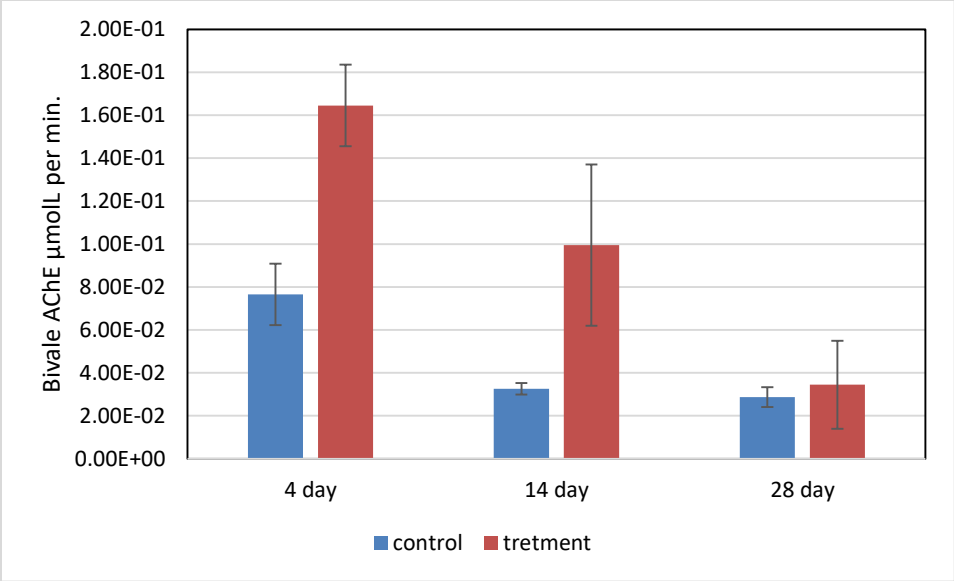


Figure (3-18): Acetylcholinesterase Activity (AChE) Of *S. woodiana*, Bivalves After Exposure To Sub-Lethal Concentration Of Diazinon

The decreasing in AChE activity in bivalves exposure to diazinon was documentation by (Bouldin *et al*., 2007). AchE is responsible for hydrolysis of Neurotransmitter acetylcholine into choline and acetic acid. The AchE plays crucial role in nerve conduction processes at the neuromuscular junction. The inhibition of this enzyme is directly connected to the toxicity of organophosphate insecticide. AchE activity is widely used as biomarker of neurotoxicity in invertebrates (Rakhi *et al*., 2013).

AchE enzyme has many essential functions like cilliary activity of gill epithelium, cilliary activity for transport of suspended particles, valve opening and closing etc. (Corsi *et al*., 2007). The decreasing in AChE activity belong to the effect synaptic junctions in muscle tissue and foot tissues muscular part by organophosphate insecticide exposure because it susceptible (Jebali *et al*., 2013).

3.5 Liver Function

3.5.1 Aspartate Aminotransferase (AST/GOT)

The maximum AST activity in fish was (798 ± 11.22) U/L during all the time of experiment day with 28th day in treatment group, while the minimum was (231 ± 84.43) U/L during 14th day with treatment group fig.(3-19). The study result show that a significant increase at ($p<0.05$) in AST activity in serum of (*C. carpio*).

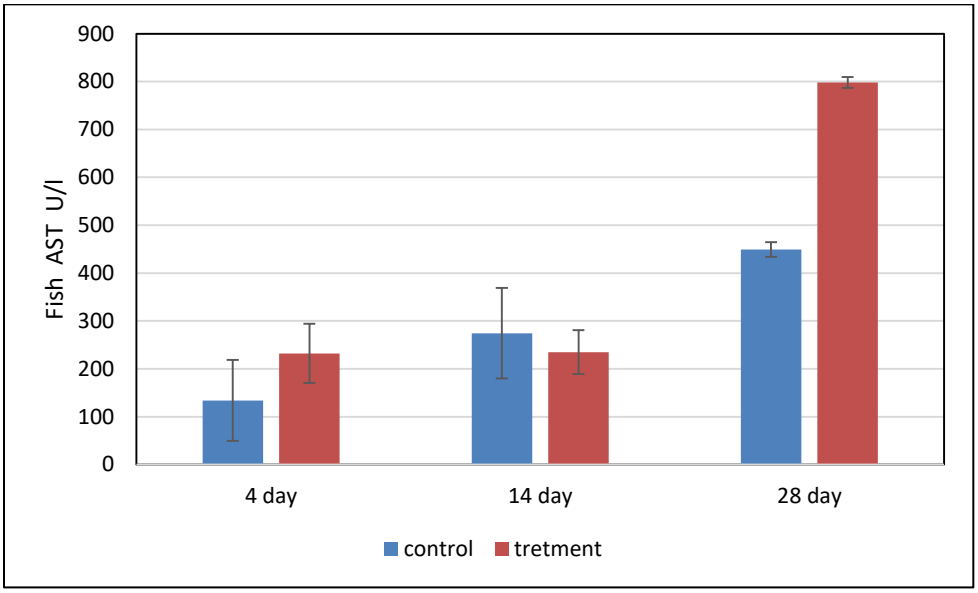


Figure (3-19): Aspartate Aminotransferase (AST) Of *Cyprinus carpio* Fish After Exposure To Sub-Lethal Concentration Of Diazinon

3.5.2 Alanine Aminotransferase (ALT/GPT)

The maximum ALT activity in fish was (92.66 ± 2.86) U/L during all the time of experiment day with 28th day in treatment group, while the minimum was (8 ± 1.63) U/L during 4th day with same group fig.(3-20). The study result show that a significant increase at ($p<0.05$) in ALT activity in serum of (*C. carpio*).

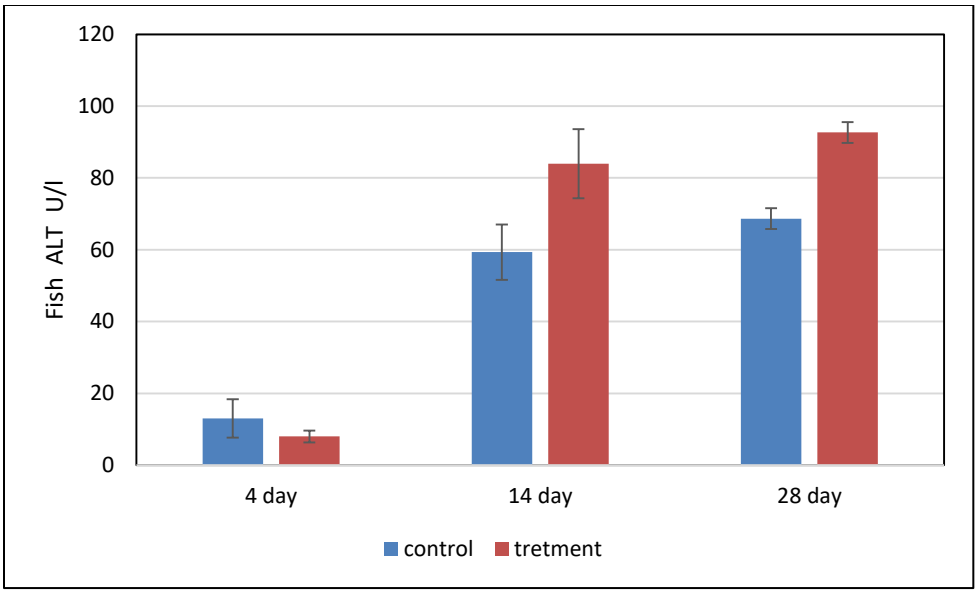


Figure (3-20): Alanine Aminotransferase (ALT) Of *Cyprinus carpio* Fish After Exposure To Sub-Lethal Concentration Of Diazinon

3.5.3 Alkaline phosphatase (ALP)

The maximum ALP activity in fish was (84.33 ± 4.98) U/L at 28th day in treatment group, while the minimum was (17 ± 5.88) U/L during 4th day with same group fig.(3-21). The study result show that a significant increase at ($p<0.05$) in ALP activity in serum of (*C. carpio*).

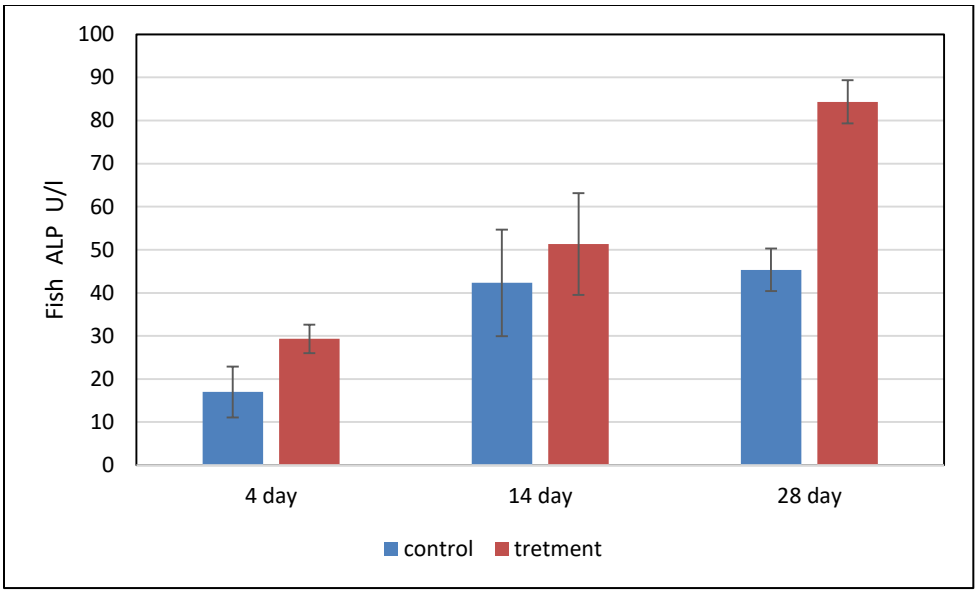


Figure (3-21): Alkaline phosphatase (ALP) Of *Cyprinus carpio* Fish After Exposure To Sub-Lethal Concentration Of Diazinon

The increase in AST,ALT,ALP activity in common carp exposure to sub lethal concentration of diazinon was documented by (Banaei *et al*., 2008, Ahmad, 2011, Haider & Rauf, 2014). Also there are many study record alter in AST, ALT, ALP activity in different types of fish under different insecticide and environmental stress (Al-Ghanim, 2012, Al-Otaibi *et al*., 2018, Hassan *et al*., 2022). In the current study, the increased activities of AST and ALT in the plasma of *C. carpio* indicate that long-term exposure to diazinon caused tissue damage in fish. Increase in the plasma activities of AST and ALT has also been reported in *C. mrigala*, exposed to diazinon (Haider & Rauf, 2014).

Alteration in the activity of these enzymes in the fish due to the exposure of pollutants could be a sensitive indicator of cellular damage (Javed *et al*., 2016). Any increase in these enzymes after exposure to pollutants is a sensitive indicator of cellular damage (Ahmad, 2011). Therefore, higher activities of these enzymes registered in this investigation may be ascribed to damage caused by diazinon to liver.

3.6 Histopathological Effect

Following exposure to 2.37 mg/L of diazinon for 28 days in *C. carpio* fish. The muscle tissues exhibited typical histological structure in control groups, (Figs. 3-22 A1 and A2). A typical skeletal muscle, showing different muscle features such as the muscle Perimysium and Fascicle. Also show a typical striated muscle, showing are organized in small bundles and small bundles are wrapped as big bundles. The largest bundle with definite wrapping and definite shape is called a piece of striated muscle. Whereas, in treatment group extensive damages in the muscle tissues of *C. carpio* fish were noticed. showing degenerative and loss the histological regulation with many necrosis zones as well as disappear the muscle features such as the muscle perimysium and fascicle fig. (3-23). In contrast, the histological examination in untreated bivalve group showing the normal histological structure of mussel fig. (3-24), whereas, in treated group with diazinon of S. woodiana bivalves showing the degenerative of the histological structure of mussel fig.(3-25).

Histopathological alterations after the exposure to diazinon in various fish species are described by several authors. Hamm *et al*., (1998) reported an increased occurrence of retinal cells necrosis in medaka. Dutta *et al*., (1993) described the gill alterations in bluegill sunfish, *Lepomis macrochirus* (lifting of the epithelial layer, hyperplasia and necrosis, shortening of the lamellae and frequent epithelial rupture, lamellar fusion and mucous cells hypertrophy). Sub lethal concentrations of diazinon caused serious damage to gut wall in a freshwater teleost *Channa punctatus* (Islam *et al*., 2019b).

The histopathological deformities in the muscle tissues of fish could act as a sensitive indicator for deciding signs of pollutants stress (Bharti & Rasool, 2021).

Moreover, according to the Tchounwou *et al*., (2015), pesticides, like other toxicants, induce the generation of free radicals that destruct the vital macromolecules constituents of the cells and therefore, the alterations in fish muscle or bivalves, are the only reflections of the toxic effect of contaminants. Several biomonitoring programs have used the histological changes observed in different fish organs as biomarkers for ecological quality of aquatic ecosystems (Vieira *et al*., 2019).

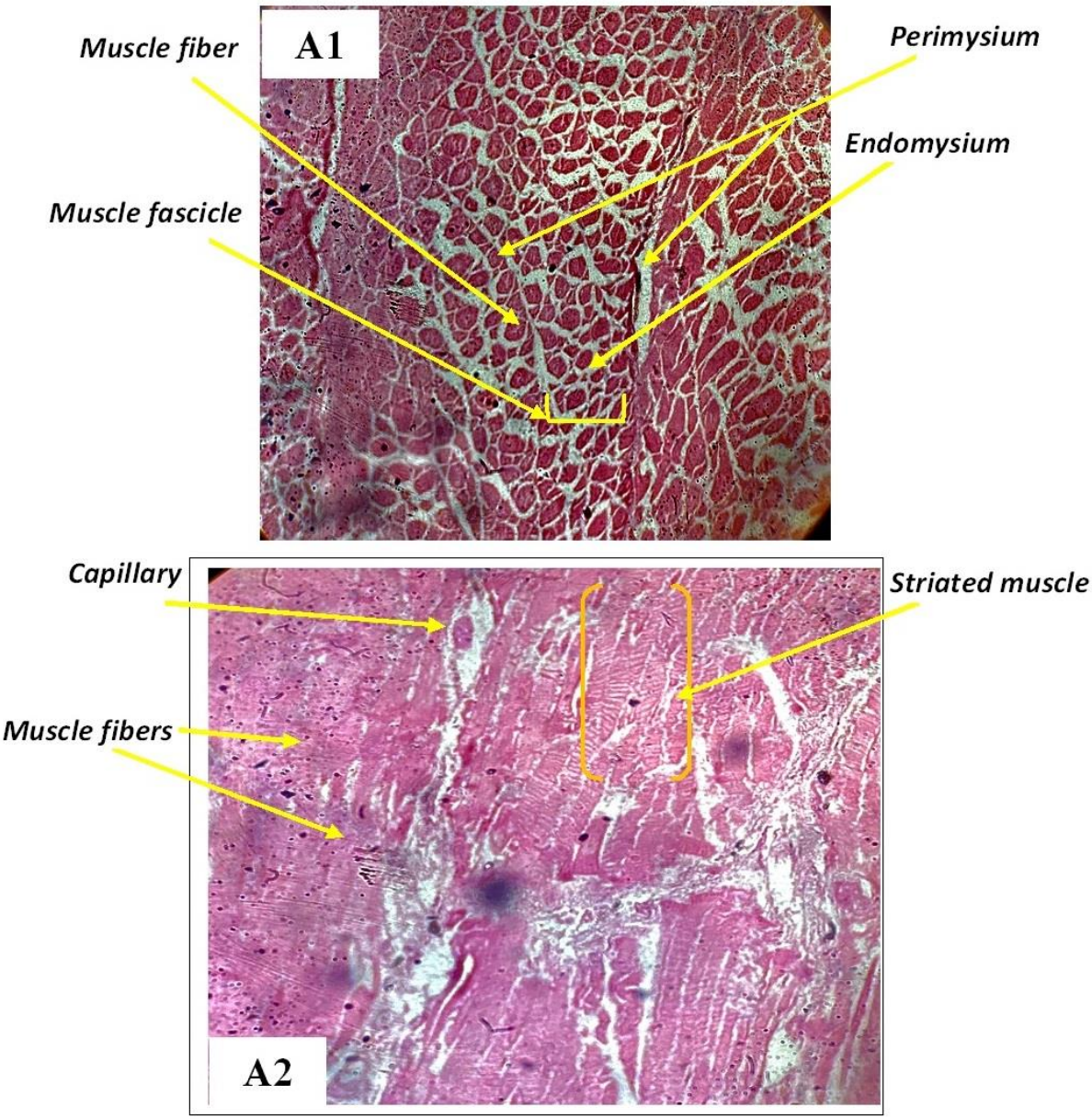


Figure (3-22): Cross Section in Muscle Tissue in Control Group of *C. carpio* Fish

Fig (3-22 A1): A photomicrograph of a cross section of a typical skeletal muscle of a *C. carpio* showing different muscle features such as the muscle Perimysium and Fascicle as analyzed in the study (H&E)

Fig (3.22 A2): A photomicrograph of a cross section of a typical striated muscle of a *C. carpio* showing are organized in small bundles and small bundles are wrapped as big bundles. The largest bundle with definite wrapping and definite shape is called a piece of striated muscle (H&E)

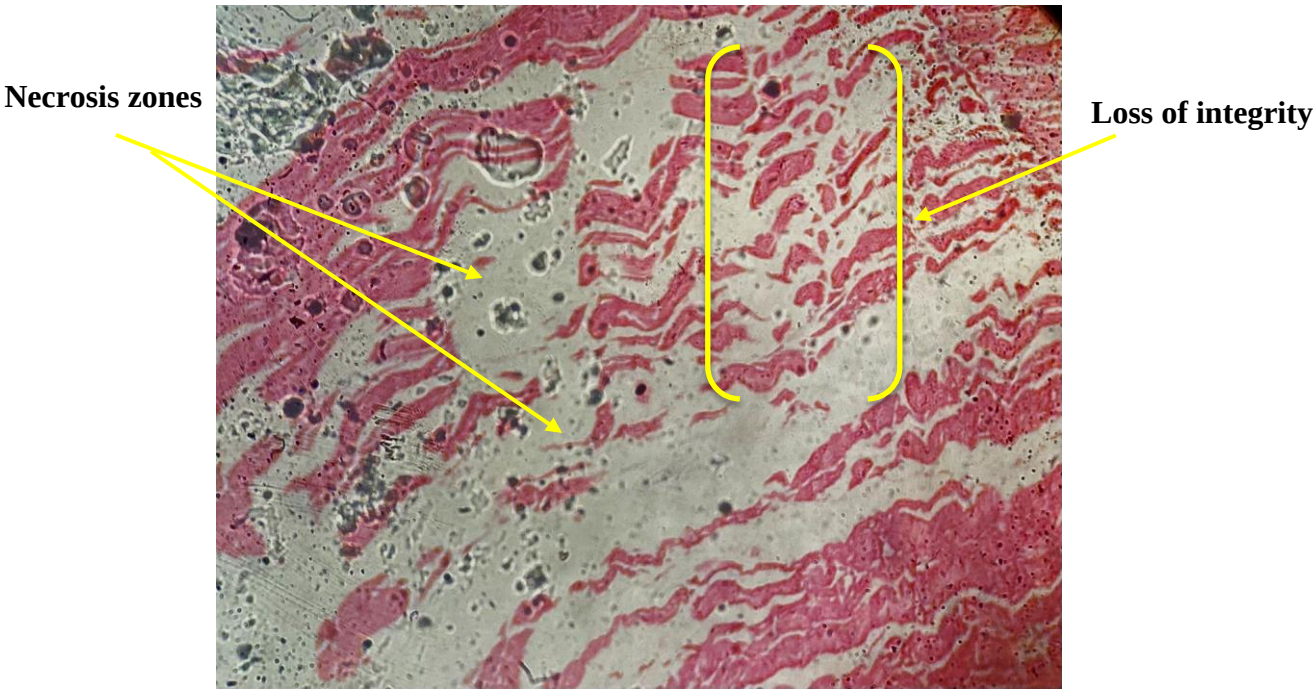


Figure (3-23): Histological Alteration in Muscle Tissue of a Treated Group of *C. carpio* Fish After Exposure to Sub-Lethal Concentration of Diazinon. Showing Degenerative and Loss The Histological Regulation With Many Necrosis Zones As Well As Disappear The Muscle Features Such As The Muscle Perimysium and Fascicle As Analyzed in The Study (H&E)

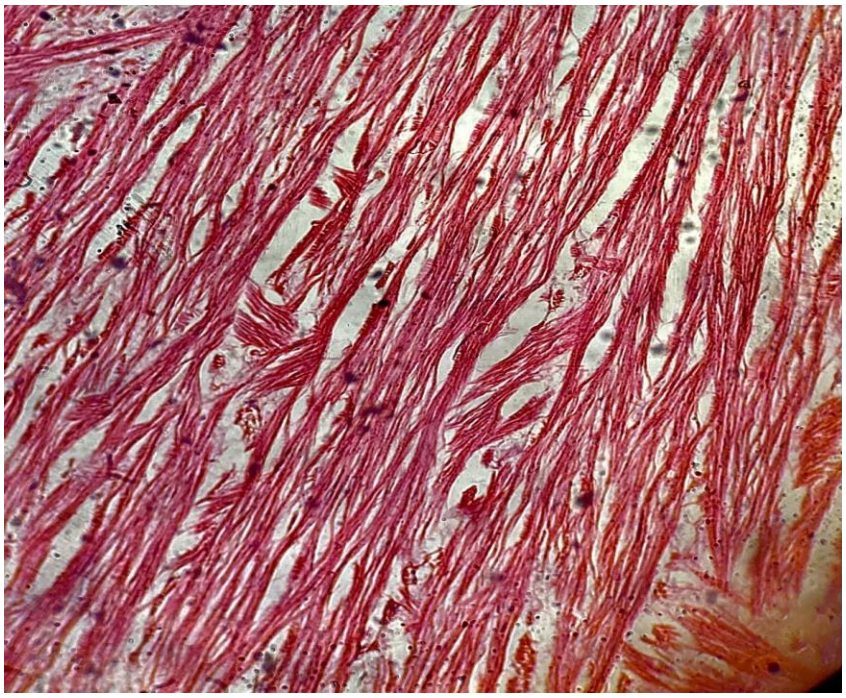


Fig (3-24) A Cross Section of a Control Group of *S. woodiana* Bivalve Showing The Normal Histological Structure of Mussel (h&e)

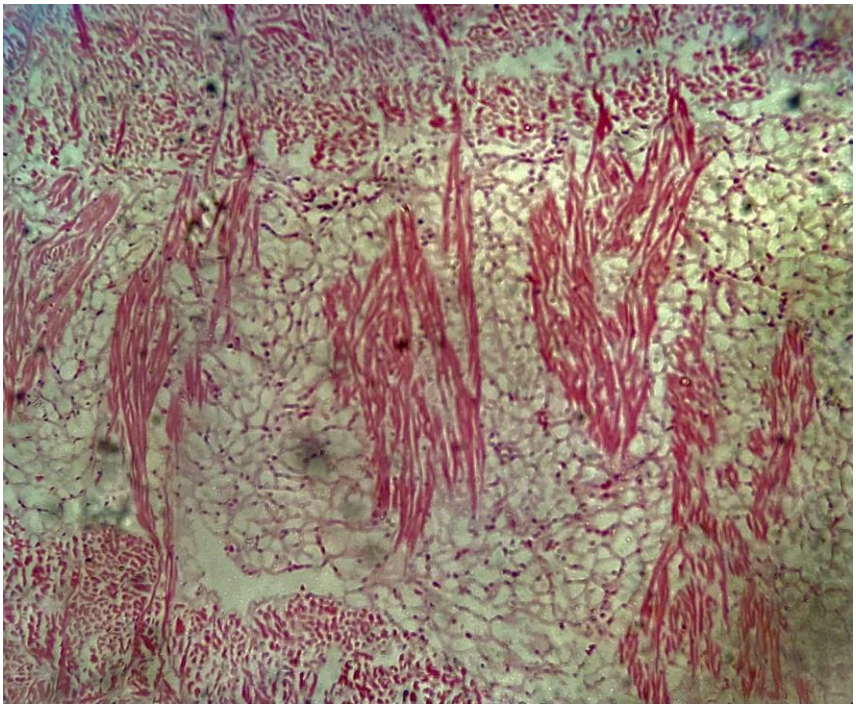


Fig (3-25) A Cross Section of a Treated Group of *S. woodiana* Bivalve After Exposure to Sub-Lethal Concentration of Diazinon, Showing The Degenerative of The Histological Structure of Mussel (H&E).

a. The Insecticide residual concentration in organisms

The insecticide residues samples were collected in last day of experimental in both organism, and estimate in HPLC. The obtained results was as in appear in fig. (3-24). The study result show that a significant difference at ($p<0.05$) in bivalves and fish. The insecticide residual value in fish was (0.696 ± 0.04) µg/g in treatment group, and concentration residual in control group was (0.012 ± 0.004) µg/g. While insecticide residual concentration in bivalve was (0.943 ± 0.077) µg/g in treatment group, and concentration residual in control group was (0.075 ± 0.012) µg/g.

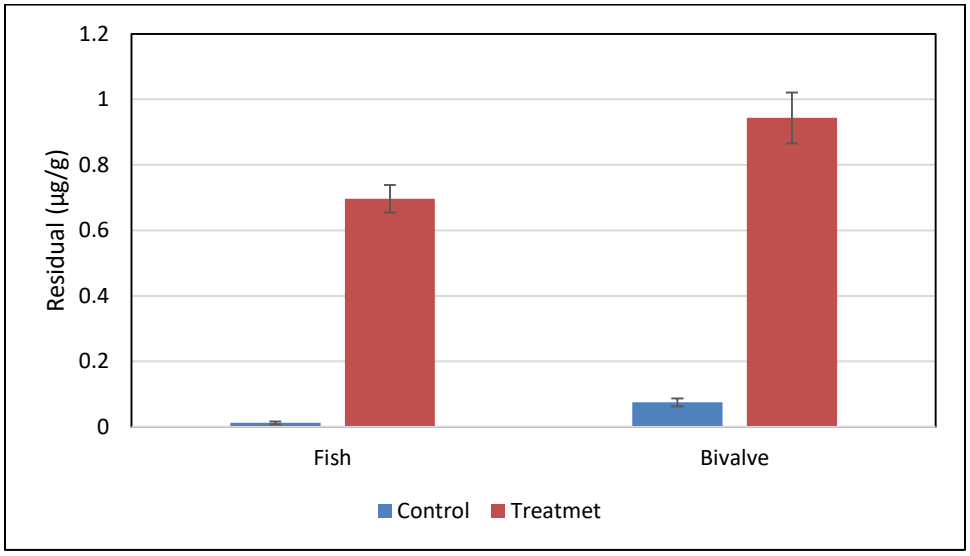


Figure (3-26): Insecticide Residual Of Fish and Bivalve After Exposure To Sub-Lethal Concentration Of Diazinon

The toxicity of pollutants depends generally on size and species of fish and bivalves as well as duration of exposure.

The toxic level of diazinon related to the disparities in susceptibility and tolerance linked to it's accumulation, biotransformation and excretion. The toxic potential of pesticides mainly depends upon the accumulation, the chemistry of

compounds forming the pesticide and response of animals exposed to compound (Pourgholam *et al*., 2013).

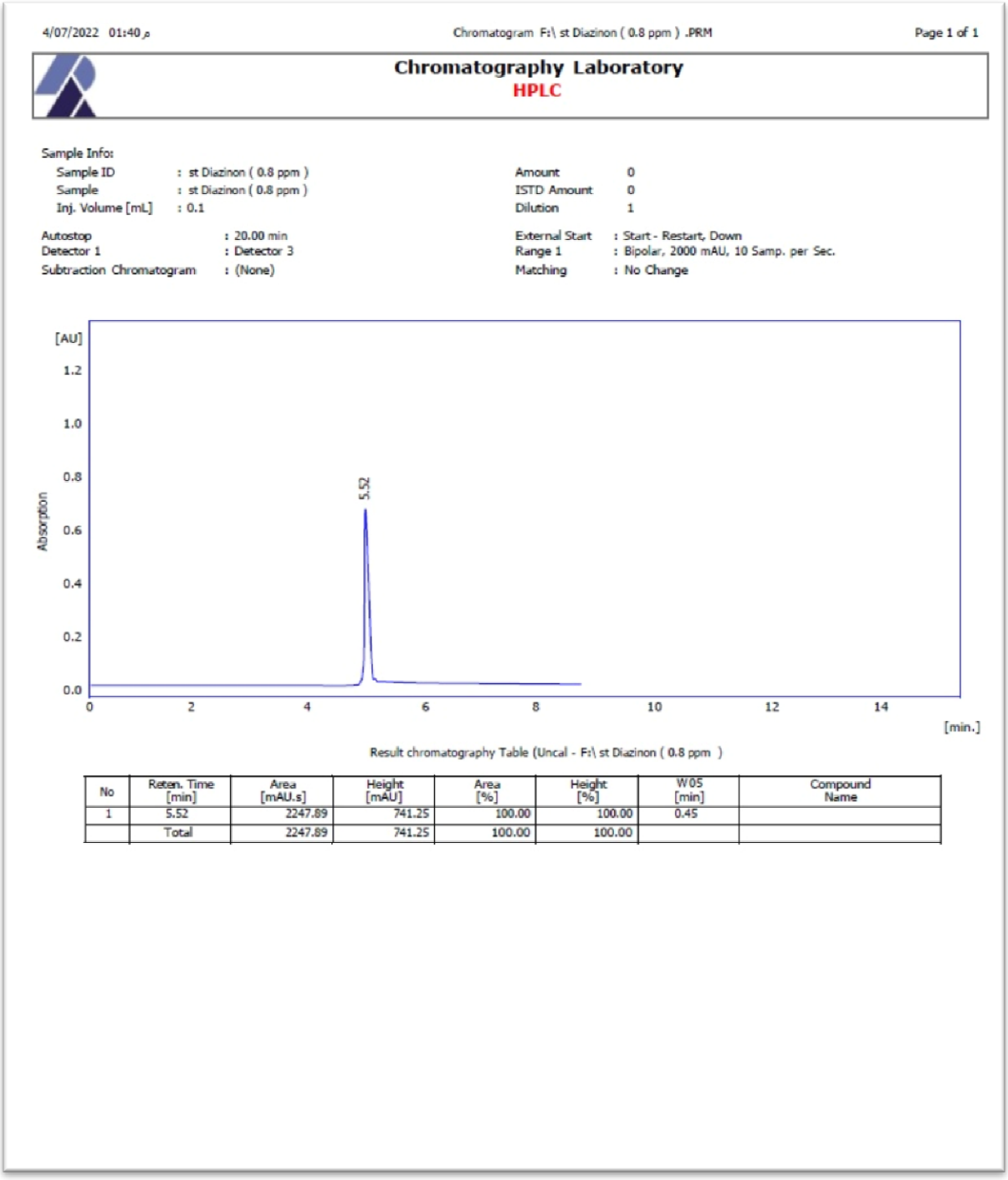


Figure (3-27): HPLC Analysis of diazinon standard

3.8 Comet assay test

The discovery of DNA damage by using single-cell gel is very interested in explaining the double and single-strand breaks, alkali labile sites, imperfect repair sites, cross-links and repair in individual cells (Azqueta & Collins, 2013). Exposure to organophosphorus insecticide may improve the genetic system frequency in aquatic organisms and damage to DNA among organisms who are exposed to the pollutant (Alvim & dos Reis Martinez, 2019).

Bivalves in particular, receive special attention both as sentinel and toxicity-testing subjects, and biomonitoring programs involving aquatic ecosystems (Gajski *et al*., 2019). Mussels and clams have become one of the most important targets to detection genotoxicants, using the comet assay owing to their worldwide distribution and known sensitivity to pollutants (De Lapuente *et al*., 2015).

There are significant change in fish (LSD; $p \leq 0.05$) in low there was decrease while, high comet percentage there was significant increase (Fig. 3-28) of treatment compared with control, while there was no significant differences in medium comet percentage, between control and treatment. In contrast the bivalves result show a significant change (LSD; $p \leq 0.05$) in low, there are a significant decrease comet percentage (Fig. 3-29) of exposure compared with control, while there was significant increase differences in medium and high comet percentage between control and treatment.

In the present study tail length (TL) were used as parameters to assess the DNA damage. Exposed groups were found to have a significantly higher value of these parameter as compared to control showing the genotoxic effect of diazinon.

Fish results were agreed with Khafaga *et al.*, (2020) studied the effect cypermethrin on DNA damage, biochemical changes, oxidative stress, histopathological alterations in Common carp (*C. carpio*). dos Santos & Martinez, (2014) study effect of exposure to atrazine and Roundup® herbicide in Asian clams (*C. fluminea*) and it genotoxic effect, the result show a significant increase in the occurrence of DNA damage.

Different researchers observed an increase in DNA damage in different fish species after exposure to various organic compounds (Ullah *et al*., 2016, Rajan & Anandan, 2017). Kolarević *et al*., (2016) study the genotoxic pollution by measuring of DNA damage in the haemocytes of freshwater mussels *S. woodiana* under environmental stress.

DNA lesions and cytogenetic abnormalities in lymphocytes are surrogate endpoints in surrogate cells that are thought to indicate genetic changes involved in target tissue carcinogenesis.

Individual susceptibility variables such as genetic variation influencing genomic stability (DNA repair, folate metabolism) and xenobiotic metabolism may also explain the outcomes of genotoxic endpoints (Di *et al*., 2017). Alavinia *et al*., (2019) evaluated the damage of DNA in the whole blood of common carp exposure to organophosphate insecticide using the comet assay, and they concluded that high comet assay of DNA damage in blood were in treatment compared with controls groups.

When organisms exposure to diazinon, that lead to DNA damage (tail length increase), may be due to insufficient produce of antioxidant defense systems to scavenge the reactive oxygen species that are generated by diazinon

which lead to find their way across nuclear membrane indicating DNA strand breakage (Khadairi *et al*., 2017).

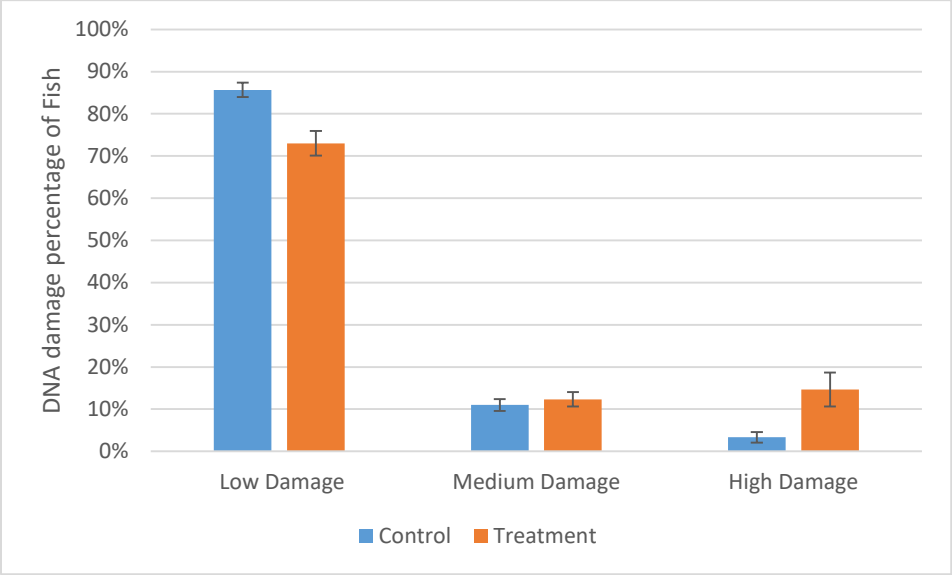


Figure (3-28): DNA damage(commet assay) Of *Cyprinus carpio* Fish After Exposure To Sub-Lethal Concentration Of Diazinon

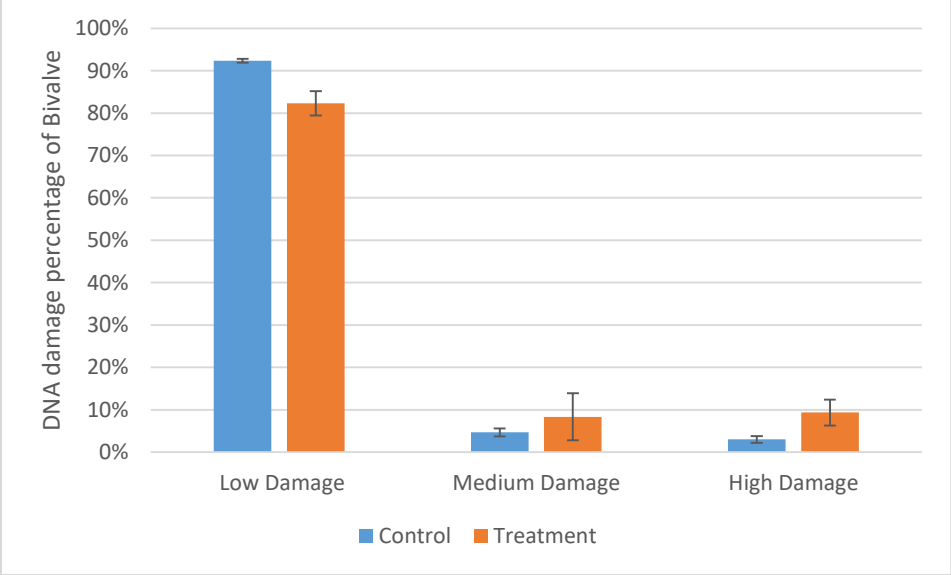


Figure (3-29): DNA damage (comet assay) Of *Sinanodonta woodiana,* Bivalves After Exposure To Sub-Lethal Concentration Of Diazinon

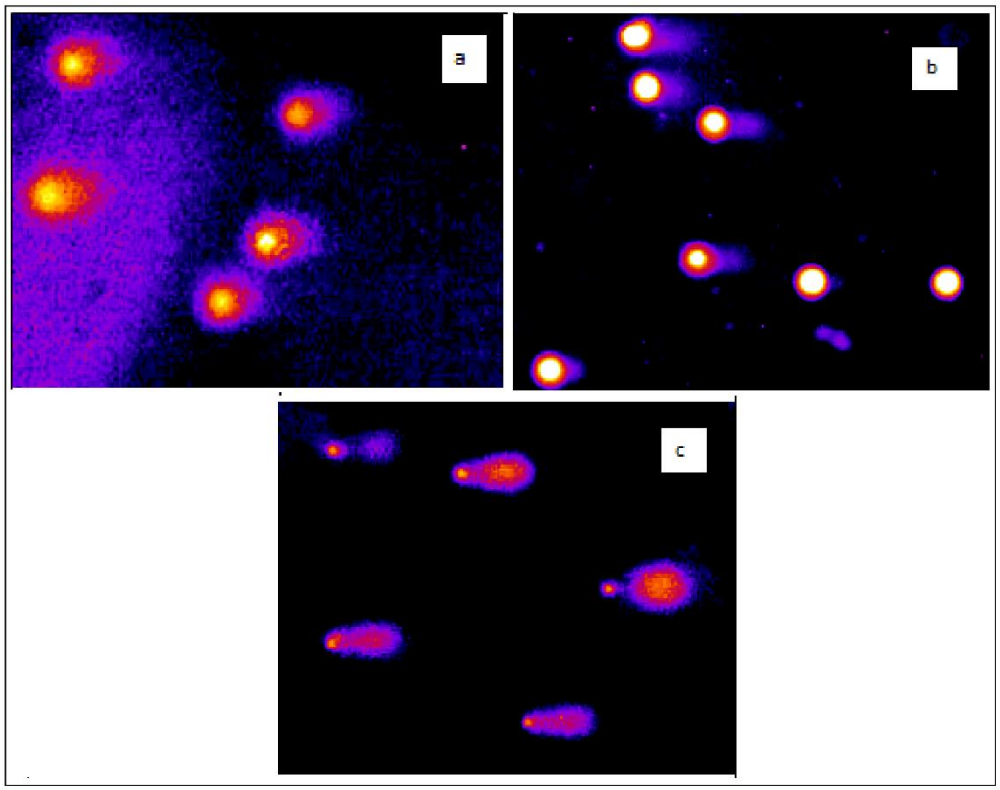


Fig.(3-30): Comet image analysis by comet score software, scoring categories for comet assay (a: normal to low DNA damage, b: medium DNA damage, c: high DNA damage.

• Conclusions

1- The *C. carpio* fish influenced by diazinon more than *S. woodiana* bivalves.
2- Exposure to low concentrations of diazinon results a non-significant hematological alterations in fish and behavioral changes in both organisms.
3- The sub lethal exposure to diazinon causes hepatic damage, which in turn, decrease fish health.
4- Diazinon stimulate oxidative stress in *C. carpio* and *S. woodiana*.
5- Diazinon cause DNA damage in *C. carpio* and *S. woodiana*.
6- Most of all the biochemical markers changes in both *C. carpio* and *S. woodiana.*
7- Histological alterations in *C. carpio* and *S. woodiana* induced by Diazinon.
8- The *C. carpio* fish have high ability to remove toxic residues compare with *S. woodiana* bivalves..

• Recommendations

1- Study effect different insecticides through the food chain.
2- Study of insecticides effects in freshwater river, lake and water purification plant.
3- Using antioxidant non enzyme, to show, its protective role against insecticide in fish and laboratory animals.
4- Study the modification, in gene expression that effected by Diazinon in fish, and laboratory animals.

References

Abu-Qare, A. W., & Abou-Donia, M. B. (2001). Determination of diazinon, chlorpyrifos, and their metabolites in rat plasma and urine by high-performance liquid chromatography. Journal of Chromatographic Science, 39(5), 200–204.

Adebayo, I. A., Akin-Obasola, B. J., & Bajulaye, O. M. (2013). Toxicological effect of diazinon on African catfish (Clarias anguillaris). IOSR Journal of Environmental Science. Toxicology and Food Technology, 3(1), 64-71.

Adedeji, O. B., Adeyemo, O. K., & Agbede, S. A. (2009). Effects of diazinon on blood parameters in the African catfish (Clarias gariepinus). African Journal of Biotechnology, 8(16), 3940.

Ahmad, Z, Al-Balawi, H. F. A., Al-Ghanim, K. A., Al-Misned, F., & Mahboob, S. (2021). Risk assessment of malathion on health indicators of catfish: Food and water security prospective research. Journal of King Saud University-Science, 33(2), 101294.

Ahmad, Zubair. (2011). Acute toxicity and haematological changes in common carp (Cyprinus carpio) caused by diazinon exposure. African Journal of Biotechnology, 10(63), 13852–13859. https://doi.org/10.5897/ajb11.1247

Akter, S. (2020). effects of commonly used herbicide pretilachlor on reproduction, organ specific histopathology, haematological parameters and plasma enzymes activity in common carp (cyprinus carpio). chattogram veterinary and animal sciences university chattogram-4225, Bangladesh, thesis.

Alavinia, S. J., Mirvaghefi, A., Farahmand, H., Rafiee, G., Alavinia, S. J., Shiry, N., & Moodi, S. (2019). DNA damage, acetylcholinesterase activity, and hematological responses in rainbow trout exposed to the organophosphate malathion. Ecotoxicology and Environmental Safety, 182, 109311.

Alexander, A. C., Culp, J. M., Baird, D. J., & Cessna, A. J. (2016). Nutrient–insecticide interactions decouple density-dependent predation pressure in aquatic insects. *Freshwater Biology*, *61*(12), 2090-2101.

Al-Fanharawi, A. A., Rabee, A. M., & Al-Mamoori, A. M. J. (2019). Multi-biomarker responses after exposure to organophosphates chlorpyrifos in the freshwater mussels Unio tigridis and snails Viviparous benglensis. Human and Ecological Risk Assessment: An International Journal, 25(5), 1137–1156.

Al-Ghanim, K. A. (2012). Acute toxicity and effects of sub-lethal malathion exposure on biochemical and haematological parameters of Oreochromis niloticus. Sci Res Essays, 7(16), 1674–1680.

Al-Ghanim, K. A. (2013). Study on the effect of diazinon on haematological indices in common carp (Cyprinus carpio L.). Life Science Journal, 10(10), 327–332.

Al-Ghanim, K. A. (2014). Effect of an organophosphate insecticide diazinon on the activity of acetylcholinesterase and lipid peroxidation of a common carp, cyprinus carpio L. Pakistan Journal of Zoology, 46(1), 161–166.

Alharbi, O. M., Khattab, R. A., & Ali, I. (2018). Health and environmental effects of persistent organic pollutants. Journal of Molecular Liquids, 263, 442-453.

Allison, D. T., & Hermanutz, R. O. (1977). *Toxicity of diazinon to brook trout and fathead minnows*. Environmental Protection Agency, Office of Research and Development, Environmental Research Laboratory 3-77.

Al-Otaibi, A. M., Al-Balawi, H. F. A., Ahmad, Z., & Suliman, E. M. (2018). Toxicity bioassay and sub-lethal effects of diazinon on blood profile and histology of liver, gills and kidney of catfish, Clarias gariepinus. Brazilian Journal of Biology, 79, 326–336.

Al-Saad, H. T., Farid, W. A., & Al-Adhub, A. H. Y. (2009). Distribution and seasonal variations of hydrocarbons in the molluscs of Shatt Al-Arab river. Mesop. J. Mar. Sci, 24(2), 102-111.

Alvim, T. T., & dos Reis Martinez, C. B. (2019). Genotoxic and oxidative damage in the freshwater teleost Prochilodus lineatus exposed to the insecticides lambda-cyhalothrin and imidacloprid alone and in combination. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 842, 85–93.

Ambekar, S. S., Hattur, S. S., & Bule, P. B. (2017). DNA: Damage and repair mechanisms in humans. *group*, *1*, 7.

Amin, K. A., & Hashem, K. S. (2012). Deltamethrin-induced oxidative stress and biochemical changes in tissues and blood of catfish (Clarias gariepinus): antioxidant defense and role of alpha-tocopherol. BMC Veterinary Research, 8(1), 1–8.

APHA,. (2012). Standard Methods for examination of water and wastewater. American public health association, water environment federation, washigton, DC, USA .

Appannagari, R. R. (2017). Environmental pollution causes and consequences: a study. *North Asian International Research Journal of Social Science and Humanities*, *3*(8), 151-161.

Arumugam, A., Li, J., Krishnamurthy, P., Jia, Z. X., Leng, Z., Ramasamy, N., & Du, D. (2020). Investigation of toxic elements in Carassius gibelio and Sinanodonta woodiana and its health risk to humans. Environmental Science and Pollution Research, 27(16), 19955–19969.

Azqueta, A., & Collins, A. R. (2013). The essential comet assay: a comprehensive guide to measuring DNA damage and repair. Archives of Toxicology, 87(6), 949–968.

Banaee, M, Sureda, A., Mirvaghefi, A. R., & Ahmadi, K. (2011). Effects of diazinon on biochemical parameters of blood in rainbow trout (Oncorhynchus mykiss). Pesticide Biochemistry and Physiology, 99(1), 1–6.

Banaee, M., Sureda, A., Mirvaghefi, A. R., & Ahmadi, K. (2013). Biochemical and histological changes in the liver tissue of rainbow trout (Oncorhynchus mykiss) exposed to sub-lethal concentrations of diazinon. Fish Physiology and Biochemistry, 39(3), 489–501.

Banaei, M., MIR, V. A. R., Rafei, G. R., & Majazi, A. B. (2008). Effect of sub-lethal diazinon concentrations on blood plasma biochemistry. International Journal Of Environmental Research, 2(3) 189-198.

Banik, U., Rahman, M. M., Khanam, T., & Mollah, M. F. A. (2016). Histopathological changes in the gonads, liver, and kidney of Glossogobius giuris exposed to sub-lethal concentration of diazinon. *Progressive Agriculture*, *27*(4), 530-538.

Banks, K. E., Hunter, D. H., & Wachal, D. J. (2005). Diazinon in surface waters before and after a federally-mandated ban. Science of the Total Environment, 350(1–3), 86–93.

Barkallah, M., Ben Atitallah, A., Hentati, F., Dammak, M., Hadrich, B., Fendri, I., Ayadi, M. A., Michaud, P., & Abdelkafi, S. (2019). Effect of Spirulina platensis biomass with high polysaccharides content on quality attributes of common Carp (Cyprinus carpio) and Common Barbel (Barbus barbus) fish burgers. Applied Sciences, 9(11), 2197.

Barranger, A., Akcha, F., Rouxel, J., Brizard, R., Maurouard, E., Pallud, M., Menard, D., Tapie, N., Budzinski, H., & Burgeot, T. (2014). Study of genetic damage in the Japanese oyster induced by an environmentally-relevant exposure to diuron: evidence of vertical transmission of DNA damage. Aquatic Toxicology, 146, 93–104.

Benarji, G., & Rajendranath, T. (1990). Haematological changes induced by an organophosphorus insecticide in a freshwater fish Clarias batrachus(Linnaeus). Tropical Freshwater Biology. Benin City, 2(2), 197–202.

Besse, J. P., Geffard, O., & Coquery, M. (2012). Relevance and applicability of active biomonitoring in continental waters under the Water Framework Directive. *TrAC Trends in Analytical Chemistry*, *36*, 113-127.

Bhagat, J., Ingole, B. S., & Singh, N. (2016). Glutathione S-transferase, catalase, superoxide dismutase, glutathione peroxidase, and lipid peroxidation as biomarkers of oxidative stress in snails: A review. Invertebrate Survival Journal, 13(1), 336–349.

Bharti, S., & Rasool, F. (2021). Analysis of the biochemical and histopathological impact of a mild dose of commercial malathion on Channa punctatus (Bloch) fish. Toxicology Reports, 8, 443–455.

Bogan, A. E., Al-Fanharawi, A. A., & Lopes-Lima, M. (2021). First record of Sinanodonta woodiana and report for freshwater bivalves from Iraq (Mollusca: Bivalvia: Unionidae). *Ecologica Montenegrina*, *46*, 52-60.

Bogatov, V. V, & Sayenko, E. M. (2002). On the structure and systematic position of the genus Sinanodonta (Bivalvia, Unionidae). Bulletin of the Russian Far East Malacological Society, 7, 85–93.

Boudou, A., & Ribeyre, F. (1997). Aquatic ecotoxicology: from the ecosystem to the cellular and molecular levels. *Environmental health perspectives*, *105*(suppl 1), 21-35.

Bouldin, J. L., Farris, J. L., Moore, M. T., Smith, S., & Cooper, C. M. (2007). Assessment of diazinon toxicity in sediment and water of constructed wetlands using deployed Corbicula fluminea and laboratory testing. Archives of Environmental Contamination and Toxicology, 53(2), 174–182.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72(1–2), 248–254.

Brahma, N., & Gupta, A. (2020). Acute toxicity of lead in fresh water bivalves Lamellidens jenkinsianus obesa and Parreysia (Parreysia) corrugata with evaluation of sublethal effects on acetylcholinesterase and catalase activity, lipid peroxidation, and behavior. Ecotoxicology and Environmental Safety, 189(June), 109939. https://doi.org/10.1016/j.ecoenv.2019.109939

Brontowiyono, W., Jasim, S. A., Mahmoud, M. Z., Thangavelu, L., Izzat, S. E., Yasin, G., Mohammad, H. J., Mustafa, Y. F., & Balvardi, M. (2022). Dietary (Macro-Algae, Sargassaceae) extract improved antioxidant defense system in diazionon-exposed common carp. Annals of Animal Science.

Burgos-Aceves, M. A., Cohen, A., Smith, Y., & Faggio, C. (2018). MicroRNAs and their role on fish oxidative stress during xenobiotic environmental exposures. Ecotoxicology and Environmental Safety, 148, 995–1000.

Burgos-Aceves, M. A., Lionetti, L., & Faggio, C. (2019). Multidisciplinary haematology as prognostic device in environmental and xenobiotic stress-induced response in fish. Science of the Total Environment, 670, 1170–1183.

Buschini, A., Villarini, M., Feretti, D., Mussi, F., Dominici, L., Zerbini, I., Moretti, M., Ceretti, E., Bonfiglioli, R., & Carrieri, M. (2013). Multicentre study for the evaluation of mutagenic/carcinogenic risk in nurses exposed to antineoplastic drugs: assessment of DNA damage. Occupational and Environmental Medicine, 70(11), 789–794.

Chen, Y., Liu, H., Huang, H., Ma, Y., Wang, R., Hu, Y., Zheng, X., Chen, C., & Tang, H. (2020). Squid ink polysaccharides protect human fibroblast against oxidative stress by regulating NADPH oxidase and connexin43. Frontiers in Pharmacology, 10, 1574.

Chorus, I., & Welker, M. (2021). *Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management* (p. 858). Taylor & Francis.

Connell, D. W. (1988). Bioaccumulation behavior of persistent organic chemicals with aquatic organisms. Reviews of Environmental Contamination and Toxicology, 117–154.

Conners, D. E., & Black, M. C. (2004). Evaluation of lethality and genotoxicity in the freshwater mussel Utterbackia imbecillis (Bivalvia: Unionidae) exposed singly and in combination to chemicals used in lawn care. Archives of Environmental Contamination and Toxicology, 46(3), 362–371.

Cope, W. G., Bergeron, C. M., Archambault, J. M., Jones, J. W., Beaty, B., Lazaro, P. R., Shea, D., Callihan, J. L., & Rogers, J. J. (2021). Understanding the influence of multiple pollutant stressors on the decline of freshwater mussels in a biodiversity hotspot. Science of the Total Environment, 773, 144757.

Corsi, I., Pastore, A. M., Lodde, A., Palmerini, E., Castagnolo, L., & Focardi, S. (2007). Potential role of cholinesterases in the invasive capacity of the freshwater bivalve, Anodonta woodiana (Bivalvia: Unionacea): a comparative study with the indigenous species of the genus, Anodonta sp. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 145(3), 413–419.

Cortes-Diaz, M. J. A., Rodríguez-Flores, J., Castañeda-Peñalvo, G., Galar-Martínez, M., Islas-Flores, H., Dublán-García, O., & Gómez-Oliván, L. M. (2017). Sublethal effects induced by captopril on Cyprinus carpio as determined by oxidative stress biomarkers. Science of the Total Environment, 605, 811–823.

Croom, E. (2012). Metabolism of xenobiotics of human environments. Progress in Molecular Biology and Translational Science, 112, 31–88.

Cruz, R., Cunha, S. C., & Casal, S. (2015). Brominated flame retardants and seafood safety: a review. *Environment International*, *77*, 116-131.

De Lapuente, J., Lourenço, J., Mendo, S. A., Borràs, M., Martins, M. G., Costa, P. M., & Pacheco, M. (2015). The Comet Assay and its applications in the field of ecotoxicology: a mature tool that continues to expand its perspectives. Frontiers in Genetics, 6, 180.

Dehghani, M. H., Kamalian, S., Shayeghi, M., Yousefi, M., Heidarinejad, Z., Agarwal, S., & Gupta, V. K. (2019). High-performance removal of diazinon pesticide from water using multi-walled carbon nanotubes. Microchemical Journal, 145, 486–491.

Deudero, S., Box, A., March, D., Valencia, J. M., Grau, A. M., Tintore, J., Calvo, M., & Caixach, J. (2007). Organic compounds temporal trends at some invertebrate species from the Balearics, Western Mediterranean. Chemosphere, 68(9), 1650–1659.

Di, Y., Aminot, Y., Schroeder, D. C., Readman, J. W., & Jha, A. N. (2017). Integrated biological responses and tissue-specific expression of p53 and ras genes in marine mussels following exposure to benzo (α) pyrene and C60 fullerenes, either alone or in combination. Mutagenesis, 32(1), 77–90.

Dias, R., D'Costa, A., Praveen Kumar, M. K., & Shyama, S. K. (2021). DNA damage and biochemical responses in estuarine bivalve Donax incarnatus (Gmelin, 1791) exposed to sub-lethal concentrations of an organophosphate pesticide monocrotophos. Environmental Monitoring and Assessment, 193(6), 1–12.

Dıaz, M. L., Blasco, J., Sales, D., & DelValls, T. A. (2004). Biomarkers as tools to assess sediment quality: laboratory and field surveys. *TrAC Trends in Analytical Chemistry*, *23*(10-11), 807-818.

Dos Santos, K. C., & Martinez, C. B. R. (2014). Genotoxic and biochemical effects of atrazine and Roundup®, alone and in combination, on the Asian clam Corbicula fluminea. Ecotoxicology and Environmental Safety, 100, 7–14.

Douda, K., & Čadková, Z. (2018). Water clearance efficiency indicates potential filter-feeding interactions between invasive Sinanodonta woodiana and native freshwater mussels. Biological Invasions, 20(5), 1093–1098.

Doyotte, A., Cossu, C., Jacquin, M. C., Babut, M., & Vasseur, P. (1997). Antioxidant enzymes, glutathione and lipid peroxidation as relevant biomarkers of experimental or field exposure in the gills and the digestive gland of the freshwater bivalve Unio tumidus. Aquatic Toxicology, 39(2), 93–110. https://doi.org/10.1016/S0166-445X(97)00024-6

Drèze, V., Monod, G., Cravedi, J. P., Biagianti-Risbourg, S., & Le Gac, F. (2000). Effects of 4-nonylphenol on sex differentiation and puberty in mosquitofish (Gambusia holbrooki). *Ecotoxicology*, *9*(1), 93-103.

Drummond, R. A., Russom, C. L., Geiger, D. L., & Defoe, D. L. (1986). Behavioral and morphological changes in fathead minnow (Pimephales promelas) as diagnostic endpoints for screening chemicals according to mode of action. Aquatic Toxicology and Environmental Fate, 9, 415–435.

Dutta, H. M., Richmonds, C. R., & Zeno, T. (1993). Effects of diazinon on the gills of bluegill sunfish Lepomis macrochirus. Journal of Environmental

Pathology, Toxicology and Oncology: Official Organ of the International Society for Environmental Toxicology and Cancer, 12(4), 219–227.

Ellman, G. L., Courtney, K. D., Andres Jr, V., & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology, 7(2), 88–95.

EPA, VEAL, L. (2021). United States environmental protection agency.

Faggio, C., Tsarpali, V., & Dailianis, S. (2018). Mussel digestive gland as a model tissue for assessing xenobiotics: an overview. Science of the Total Environment, 636, 220–229.

Fair, P. A., White, N. D., Wolf, B., Arnott, S. A., Kannan, K., Karthikraj, R., & Vena, J. E. (2018). Persistent organic pollutants in fish from Charleston Harbor and tributaries, South Carolina, United States: A risk assessment. Environmental Research, 167(August), 598–613. https://doi.org/10.1016/j.envres.2018.08.001

Fan, X., Wang, C., Wang, P., Hu, B., & Wang, X. (2018). TiO2 nanoparticles in sediments: Effect on the bioavailability of heavy metals in the freshwater bivalve Corbicula fluminea. Journal of Hazardous Materials, 342, 41–50.

Far, M. S., Roodsari, H. V., Zamini, A., Mirrasooli, E., & Kazemi, R. (2012). The Effects of Diazinon on Behavior and Some Hematological Parameters of Fry Rainbow Trout (Oncorhynchus mykiss). World Journal of Fish and Marine Sciences, 4(4), 369–375.

Farah, M. A., Ateeq, B., Ali, M. N., Sabir, R., & Ahmad, W. (2004). Studies on lethal concentrations and toxicity stress of some xenobiotics on aquatic organisms. Chemosphere, 55(2), 257–265.

Finney, D. J. (1971). Statistical logic in the monitoring of reactions to therapeutic drugs. Methods of Information in Medicine, 10(04), 237–245.

Gajski, G., Žegura, B., Ladeira, C., Pourrut, B., Del Bo, C., Novak, M., Sramkova, M., Milić, M., Gutzkow, K. B., & Costa, S. (2019). The comet assay in animal models: From bugs to whales–(Part 1 Invertebrates). Mutation Research/Reviews in Mutation Research, 779, 82–113.

Galimany, E., Sunila, I., Hégaret, H., Ramón, M., & Wikfors, G. H. (2008). Pathology and immune response of the blue mussel (Mytilus edulis L.) after an exposure to the harmful dinoflagellate Prorocentrum minimum. Harmful Algae, 7(5), 630–638.

Galindo, M. D., Jurado, J. A., García, M., de Canales, M. L. G., Oliva, M., López, F., ... & Espada, E. (2012). Trace metal accumulation in tissues of sole (Solea senegalensis) and the relationships with the abiotic environment. *International Journal of Environmental Analytical Chemistry*, *92*(9), 1072-1092.

Gaworecki, K. M., & Klaine, S. J. (2008). Behavioral and biochemical responses of hybrid striped bass during and after fluoxetine exposure. Aquatic Toxicology, 88(4), 207–213.

Gaworecki, K. M., Roberts, A. P., Ellis, N., Sowers, A. D., & Klaine, S. J. (2009). Biochemical and behavioral effects of diazinon exposure in hybrid striped bass. Environmental Toxicology and Chemistry, 28(1), 105–112. https://doi.org/10.1897/08-001.1

Géret, F., Jouan, A., Turpin, V., Bebianno, M. J., & Cosson, R. P. (2002). Influence of metal exposure on metallothionein synthesis and lipid

peroxidation in two bivalve mollusks: the oyster (Crassostrea gigas) and the mussel (Mytilus edulis). Aquatic Living Resources, 15(1), 61–66.

Geyer, H. J., Rimkus, G. G., Scheunert, I., Kaune, A., Schramm, K.-W., Kettrup, A., Zeeman, M., Muir, D. C. G., Hansen, L. G., & Mackay, D. (2000). Bioaccumulation and occurrence of endocrine-disrupting chemicals (EDCs), persistent organic pollutants (POPs), and other organic compounds in fish and other organisms including humans. In Bioaccumulation–New Aspects and Developments (pp. 1–166). Springer.

Ghazanfar, M., Shahid, S., & Qureshi, I. Z. (2018). Vitamin C attenuates biochemical and genotoxic damage in common carp (Cyprinus carpio) upon joint exposure to combined toxic doses of fipronil and buprofezin insecticides. Aquatic Toxicology, 196, 43–52.

Ghelichpour, M., Taheri Mirghaed, A., & Zargar, A. (2020). The response of lufenuron- And flonicamid-exposed Cyprinus carpio to saltwater challenge: Study on ion-regulation and stress genes expression and plasma antioxidant characteristics. Aquaculture Research, 51(12), 4829–4837. https://doi.org/10.1111/are.14773

Goodman, L. R., Hansen, D. J., Coppage, D. L., Moore, J. C., & Matthews, E. (1979). Diazinon (R): chronic toxicity to, and brain acetylcholinesterase inhibition in, the sheepshead minnow, Cyprinodon variegatus. Transactions of the American Fisheries Society, 108(5), 479–488.

Gooley, G. J. (2001). Feasibility of aquaculture in dairy manufacturing wastewater to enhance environmental performance and offset costs. Department of Natural Resources & Environment.

Gorbi, S., Avio, G. C., Benedetti, M., Totti, C., Accoroni, S., Pichierri, S., ... & Regoli, F. (2013). Effects of harmful dinoflagellate Ostreopsis cf. ovata exposure on immunological, histological and oxidative responses of mussels Mytilus galloprovincialis. *Fish & shellfish immunology*, *35*(3), 941-950.

Goth, L. (1991). A simple method for determination of serum catalase activity and revision of reference range. Clinica Chimica Acta, 196(2–3), 143–151.

Gray, J. S. (2002). Biomagnification in marine systems: the perspective of an ecologist. *Marine Pollution Bulletin*, *45*(1-12), 46-52.

Grimalt, J. O., & Olivé, J. (1993). Source input elucidation in aquatic systems by factor and principal component analysis of molecular marker data. Analytica Chimica Acta, 278(1), 159–176. https://doi.org/10.1016/0003-2670(93)80094-2

Gunasekaran, S., & Vellaichamy, K. A. (2019). Haematological status of common carp, Cyprinus carpio L. exposed to sublethal dose of organophosphorus pesticide, monocrotophos.

Guo, B., Feng, D., Xu, Z., Qi, P., & Yan, X. (2021). Acute benzo [a] pyrene exposure induced oxidative stress, neurotoxicity and epigenetic change in blood clam Tegillarca granosa. Scientific Reports, 11(1), 1–13.

Guzman, L., Besa, G., Linares, D., González, L., Pont, C., Bartolini, M., Haigis, A.-C., Legradi, J., Muñoz-Torrero, D., & Gómez-Catalán, J. (2020). Evaluation of the effects of acetylcholinesterase inhibitors in the zebrafish touch-evoked response: quantitative vs. qualitative assessment. Environmental Sciences Europe, 32(1), 1–12.

Haider, M. J., & Rauf, A. (2014). Sub-lethal effects of diazinon on hematological indices and blood biochemical parameters in Indian carp, Cirrhinus mrigala (Hamilton). Brazilian Archives of Biology and Technology, 57(6), 947–953. https://doi.org/10.1590/S1516-8913201402086

Halappa, R., & David, M. (2009). Behavioral responses of the freshwater fish, Cyprinus carpio (Linnaeus) following sublethal exposure to chlorpyrifos. Turkish Journal of Fisheries and Aquatic Sciences, 9(2).

Hamm, J. T., Wilson, B. W., & Hinton, D. E. (1998). Organophosphate-induced acetylcholinesterase inhibition and embryonic retinal cell necrosis in vivo in the teleost (Oryzias latipes). Neurotoxicology, 19(6), 853–869.

Hassan, M. A., Hozien, S. T., Abdel Wahab, M. M., & Hassan, A. M. (2022). Ameliorative effect of selenium yeast supplementation on the physio-pathological impacts ofchronic exposure to glyphosate and or malathion in Oreochromis niloticus. BMC Veterinary Research, 18(1), 1–19. https://doi.org/10.1186/s12917-022-03261-0

Hassan, N. A. S., & Sheiko, E. Y. (2013). effect of dimetoate insecticide toxicity on the gills of bivalve pitar rudis as bioindicator (histological study). Banat's Journal of Biotechnology, 4(8), 5.

Hatami, M., Banaee, M., & Haghi, B. N. (2019). Sub-lethal toxicity of chlorpyrifos alone and in combination with polyethylene glycol to common carp (Cyprinus carpio). Chemosphere, 219, 981–988.

Hemalatha, D., Amala, A., Rangasamy, B., Nataraj, B., & Ramesh, M. (2016). Sublethal toxicity of quinalphos on oxidative stress and antioxidant

responses in a freshwater fish Cyprinus carpio. Environmental Toxicology, 31(11), 1399–1406.

Hemming, J. M., & Waller, W. T. (2004). Diazinon and chlorpyrifos toxicity to the freshwater Asiatic clam, Corbicula fluminea Muller, and the estuarine hooked mussel, Ischadium recurvum Rafinesque. Florida Scientist, 1–8.

Hussain, R., Ghaffar, A., Abbas, G., Jabeen, G., Khan, I., Abbas, R. Z., Noreen, S., Iqbal, Z., Chaudhary, I. R., & Ishaq, H. M. (2022). Thiamethoxam at sublethal concentrations induces histopathological, serum biochemical alterations and DNA damage in fish (Labeo rohita). Toxin Reviews, 41(1), 154–164.

Ighodaro, O. M., & Akinloye, O. A. (2018). First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria Journal of Medicine, 54(4), 287–293.

Isik, I., & Celik, I. (2008). Acute effects of methyl parathion and diazinon as inducers for oxidative stress on certain biomarkers in various tissues of rainbowtrout (Oncorhynchus mykiss). Pesticide Biochemistry and Physiology, 92(1), 38–42.

Islam, M. A., Hossen, M. S., Sumon, K. A., & Rahman, M. M. (2019a). Acute toxicity of imidacloprid on the developmental stages of common carp Cyprinus carpio. Toxicology and Environmental Health Sciences, 11(3), 244–251.

Islam, M. S., Haque, M. D. M., Uddin, M. D. N., & Hasanuzzaman, M. D. (2019b). Histopathology in the fish Channa punctatus, Heteropneustes fossilis and Anabas testudineus exposed to diazinon.

Islas-Flores, H., Gómez-Oliván, L. M., Galar-Martínez, M., García-Medina, S., Neri-Cruz, N., & Dublán-García, O. (2014). Effect of ibuprofen exposure on blood, gill, liver, and brain on common carp (Cyprinus carpio) using oxidative stress biomarkers. Environmental Science and Pollution Research, 21(7), 5157–5166.

Javed, M., Ahmad, I., Ahmad, A., Usmani, N., & Ahmad, M. (2016). Studies on the alterations in haematological indices, micronuclei induction and pathological marker enzyme activities in Channa punctatus (spotted snakehead) perciformes, channidae exposed to thermal power plant effluent. SpringerPlus, 5(1), 1–9.

Jebali, J., Khedher, S. Ben, Sabbagh, M., Kamel, N., Banni, M., & Boussetta, H. (2013). Cholinesterase activity as biomarker of neurotoxicity: utility in the assessment of aquatic environment contamination. Revista de Gestão Costeira Integrada-Journal of Integrated Coastal Zone Management, 13(4), 525–537.

Jothy, S. L., Zakaria, Z., Chen, Y., Lau, Y. L., Latha, L. Y., & Sasidharan, S. (2011). Acute oral toxicity of methanolic seed extract of Cassia fistula in mice. Molecules, 16(6), 5268–5282.

Kassinos, F., D., Meric, S., & Nikolaou, A. (2011). Pharmaceutical residues in environmental waters and wastewater: current state of knowledge and future research. *Analytical and bioanalytical chemistry*, *399*(1), 251-275.

Kaur, M., & Jindal, R. (2017). Oxidative stress response in liver, kidney and gills of Ctenopharyngodon idellus (Cuvier & Valenciennes) exposed to chlorpyrifos. MOJ Biology and Medicine, 1(4), 103–112.

Kelainy, E. G., Ibrahim Laila, I. M., & Ibrahim, S. R. (2019). The effect of ferulic acid against lead-induced oxidative stress and DNA damage in kidney and testes of rats. Environmental Science and Pollution Research, 26(31), 31675–31684.

Kendall, R. J., Kendall, R. J., Anderson, T. A., Baker, R. J., Bens, C. M., & Carr, J. A. (2014). University of Nebraska - Lincoln ECOTOXICOLOGY. February 2001.

Kennedy, C. J., & Tierney, K. B. (2013). Xenobiotic protection/resistance mechanisms in organisms. In *Environmental Toxicology* (pp. 689-721). Springer, New York, NY.

Khadairi, M. M., Al-Amari, M. J. Y., & Al-Mamoori, A. M. J. (2017). The biochemical alteration and DNA damage in rats (Rattus rattus) after chronic intraperitoneally injection to purified microcystin-LR from Anabaena circinalis. Asian Journal of Pharmaceutical and Clinical Research, 10(11), 277–283. https://doi.org/10.22159/ajpcr.2017.v10i11.20565

Khafaga, A. F., Naiel, M. A. E., Dawood, M. A. O., & Abdel-Latif, H. M. R. (2020). Dietary Origanum vulgare essential oil attenuates cypermethrin-induced biochemical changes, oxidative stress, histopathological alterations, apoptosis, and reduces DNA damage in Common carp (Cyprinus carpio). Aquatic Toxicology, 228, 105624.

Khan, S., Balkhi, M. H., Abubakr, A., Qayoom, I., Bhat, B. A., Bhat, F. A., & Asmi, O. (2021). Dimethoate Induced Behavioural Anomalies in Juvenile Carps, Cyprinus carpio var. communis. Int. J. Curr. Microbiol. App. Sci, 10(03), 1621-1639.

Khoshbaver Rostami, H. A., Soltani, M., & Hassan, M. D. (2006). Some Hematological and biochemical changes in blood serum of Beluga (Huso huso) after chronic exposure to diazinon. Iranian Journal of Fisheries Sciences, 5(2), 53–66.

Kime, D. E. (1999). A strategy for assessing the effects of xenobiotics on fish reproduction. Science of the Total Environment, 225(1–2), 3–11.

Kolarević, S., Kračun-Kolarević, M., Kostić, J., Slobodnik, J., Liška, I., Gačić, Z., Paunović, M., Knežević-Vukčević, J., & Vuković-Gačić, B. (2016). Assessment of the genotoxic potential along the Danube River by application of the comet assay on haemocytes of freshwater mussels: The Joint Danube Survey 3. Science of the Total Environment, 540, 377–385.

Köprücü, K., Yonar, S. M., & Şeker, E. (2010). Effects of cypermethrin on antioxidant status, oxidative stress biomarkers, behavior, and mortality in the freshwater mussel Unio elongatulus eucirrus. Fisheries Science, 76(6), 1007–1013.

Köprücü, S. Ş., Köprücü, K., Ural, M. Ş., Ispir, Ü., & Pala, M. (2006). Acute toxicity of organophosphorous pesticide diazinon and its effects on behavior and some hematological parameters of fingerling European catfish (Silurus glanis L.). Pesticide Biochemistry and Physiology, 86(2), 99–105. https://doi.org/10.1016/j.pestbp.2006.02.001

Korkmaz, C., & Dönmez, A. E. (2017). Effects of diazinon on 17β-estradiol, plasma vitellogenin and liver and gonad tissues of common carp (Cyprinus carpio, l., 1758). Turkish Journal of Fisheries and Aquatic Sciences, 17(3), 629–640.

Kroeker, K. J., Kordas, R. L., Crim, R., Hendriks, I. E., Ramajo, L., Singh, G. S., ... & Gattuso, J. P. (2013). Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Global change biology*, *19*(6), 1884-1896.

Kryston, T. B., Georgiev, A. B., Pissis, P., & Georgakilas, A. G. (2011). Role of oxidative stress and DNA damage in human carcinogenesis. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 711(1–2), 193–201.

Kumar, D., & Chopra, S. (2020). Xenobiotic Compounds in the Environment: their Fate, Transport and Removal. Patron in Chief, 96.

Kumar, M. K. P., Shyama, S. K., Sonaye, B. S., Naik, U. R., Kadam, S. B., Bipin, P. D., D'costa, A., & Chaubey, R. C. (2014). Evaluation of γ-radiation-induced DNA damage in two species of bivalves and their relative sensitivity using comet assay. Aquatic Toxicology, 150, 1–8.

Liu, Y., Wang, P., Gojenko, B., Yu, J., Wei, L., Luo, D., & Xiao, T. (2021). A review of water pollution arising from agriculture and mining activities in Central Asia: Facts, causes and effects. Environmental Pollution, 291, 118209.

Lushchak, V. I., Matviishyn, T. M., Husak, V. V, Storey, J. M., & Storey, K. B. (2018). Pesticide toxicity: a mechanistic approach. EXCLI Journal, 17, 1101.

Lusková, V., Svoboda, M., & Kolářová, J. (2002). The effect of diazinon on blood plasma biochemistry in carp (Cyprinus carpio L.). Acta Veterinaria Brno, 71(1), 117–123. https://doi.org/10.2754/avb200271010117

Mackay, D., McCarty, L. S., & MacLeod, M. (2001). On the validity of classifying chemicals for persistence, bioaccumulation, toxicity, and potential for long-range transport. *Environmental Toxicology and Chemistry: An International Journal*, *20*(7), 1491-1498.

Mahboob, S. (2013). Environmental pollution of heavy metals as a cause of oxidative stress in fish: a review. Life Sci. J, 10, 336–347.

Malaj, E., von der Ohe, P. C., Grote, M., Kühne, R., Mondy, C. P., Usseglio-Polatera, P., ... & Schäfer, R. B. (2014). Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. *Proceedings of the National Academy of Sciences*, *111*(26), 9549-9554.

Manfrin, C., De Moro, G., Torboli, V., Venier, P., Pallavicini, A., & Gerdol, M. (2012). Physiological and molecular responses of bivalves to toxic dinoflagellates. Invertebrate Survival Journal, 9(2), 184–199.

Marklund, S., & Marklund, G. (1974). Involvement of the Superoxide Anion Radical in the Autoxidation of Pyrogallol and a Convenient Assay for Superoxide Dismutase. European Journal of Biochemistry, 47(3), 469–474. https://doi.org/10.1111/j.1432-1033.1974.tb03714.x

Martos-Sitcha, J. A., Simó-Mirabet, P., de Las Heras, V., Calduch-Giner, J. À., & Pérez-Sánchez, J. (2019). Tissue-specific orchestration of gilthead sea bream resilience to hypoxia and high stocking density. Frontiers in Physiology, 10, 840.

Maxwell, L. B., & Dutta, H. M. (2005). Diazinon-induced endocrine disruption in bluegill sunfish, Lepomis macrochirus. *Ecotoxicology and environmental safety*, *60*(1), 21-27.

Megharaj, M., Ramakrishnan, B., Venkateswarlu, K., Sethunathan, N., & Naidu, R. (2011). Bioremediation approaches for organic pollutants: a critical perspective. *Environment international*, *37*(8), 1362-1375.

Miller, M. E., Hamann, M., & Kroon, F. J. (2020). Bioaccumulation and biomagnification of microplastics in marine organisms: A review and meta-analysis of current data. PLoS ONE, 15(10 October), 1–25. https://doi.org/10.1371/journal.pone.0240792

Mishra, P., & Sharma, P. (2019). Superoxide Dismutases (SODs) and their role in regulating abiotic stress induced oxidative stress in plants. *Reactive Oxygen, Nitrogen and Sulfur Species in Plants: Production, Metabolism, Signaling and Defense Mechanisms*, 53-88.

Mitchell, D. G., Chapman, P. M., & Long, T. J. (1987). Acute toxicity of Roundup® and Rodeo® herbicides to rainbow trout, chinook, and coho salmon. Bulletin of Environmental Contamination and Toxicology, 39(6), 1028–1035.

Mohale, H. P., Sarang, N., & Desai, A. Y. (2020). The Common Carp and its Culture System Management. Academic Publications, Delhi, India.

Mohamed, A. A.-R., Rahman, A. N. A., Mohammed, H. H., Ebraheim, L. L. M., Abo-ElMaaty, A. M. A., Ali, S. A., & Elhady, W. M. (2020). Neurobehavioral, apoptotic, and DNA damaging effects of sub-chronic profenofos exposure on the brain tissue of Cyprinus carpio L.: Antagonistic role of Geranium essential oil. Aquatic Toxicology, 224, 105493.

Monteiro, D. A., De Almeida, J. A., Rantin, F. T., & Kalinin, A. L. (2006). Oxidative stress biomarkers in the freshwater characid fish, Brycon cephalus, exposed to organophosphorus insecticide Folisuper 600 (methyl parathion). Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 143(2), 141–149.

Moore, M. N., Livingstone, D. R., Donkin, P., Bayne, B. L., Widdows, J., & Lowe, D. M. (1980). Mixed function oxygenases and xenobiotic detoxication/toxication systems in bivalve molluscs. Helgoländer Meeresuntersuchungen, 33(1), 278–291.

Moriarty, F. (1988). Ecotoxicology. Human Toxicology, 7(5), 437–441.

Mostakim, G. M., Zahangir, M. M., Mishu, M. M., Rahman, M. K., & Islam, M. S. (2015). Alteration of Blood Parameters and Histoarchitecture of Liver and Kidney of Silver Barb after Chronic Exposure to Quinalphos. Journal of Toxicology, 2015. https://doi.org/10.1155/2015/415984

Mousavi, S. R., Jafari, M., Rezaei, S., Agha-Alinejad, H., & Sobhani, V. (2020). Evaluation of the effects of different intensities of forced running wheel exercise on oxidative stress biomarkers in muscle, liver and serum of untrained rats. Lab Animal, 49(4), 119–125.

Murty, A. S. (2018). Toxicity of pesticides to fish. CRC press.

Narwal, S. K., & Gupta, R. (2017). Biodegradation of xenobiotic compounds: an overview. Handbook of Research on Inventive Bioremediation Techniques, 186–212.

Ngwa, E. N., Kengne, A. P., Tiedeu-Atogho, B., Mofo-Mato, E. P., & Sobngwi, E. (2015). Persistent organic pollutants as risk factors for type 2 diabetes. Diabetology and Metabolic Syndrome, 7(1). https://doi.org/10.1186/s13098-015-0031-6

Nikolova, I., & Georgieva, N. (2013). Banat□s Journal of Biotechnology OM NeemAzal–T/S AND Pyrethrum AND THEIR EFFECT ON THE PEA PESTS AND SEED QUALITY. 8, 4738–12. https://doi.org/10.7904/2068

Nwani, C. D., Ifo, C. T., Nwamba, H. O., Ejere, V. C., Onyishi, G. C., Oluah, S. N., Ikwuagwu, O. E., & Odo, G. E. (2015). Oxidative stress and biochemical responses in the tissues of African catfish Clarias gariepinus juvenile following exposure to primextra herbicide. Drug and Chemical Toxicology, 38(3), 278–285.

Oliveira, M., Frihling, B. E. F., Velasques, J., Magalhães Filho, F. J. C., Cavalheri, P. S., & Migliolo, L. (2020). Pharmaceuticals residues and xenobiotics contaminants: Occurrence, analytical techniques and sustainable alternatives for wastewater treatment. Science of The Total Environment, 705, 135568.

Oliveira, P., Barboza, L. G. A., Branco, V., Figueiredo, N., Carvalho, C., & Guilhermino, L. (2018). Effects of microplastics and mercury in the freshwater bivalve Corbicula fluminea (Müller, 1774): filtration rate, biochemical biomarkers and mercury bioconcentration. *Ecotoxicology and environmental safety*, *164*, 155-163.

Onyango, D. M., Orina, P. S., Ramkat, R. C., Kowenje, C., Githukia, C. M., Lusweti, D., & Lung'ayia, H. B. O. (2020). Review of current state of knowledge of microcystin and its impacts on fish in Lake Victoria. Lakes & Reservoirs: Research & Management, 25(3), 350–361.

Otto, A., Oliver, H., & Jane, M. (1946). A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. Journal of Biological Chemistry, 164(3), 321–329.

Ożgo, M., Urbańska, M., Marzec, M., Kamocki, A., Andrzejewski, W., Golski, J., Lewandowski, K., & Geist, J. (2021). Lake-stream transition zones support hotspots of freshwater ecosystem services: Evidence from a 35-year study on unionid mussels. Science of the Total Environment, 774, 145114.

Özkara, A., Akyıl, D., & Konuk, M. (2016). Pesticides, environmental pollution, and health. In Environmental health risk-hazardous factors to living species. IntechOpen.

Paoletti, F., Aldinucci, D., Mocali, A., & Caparrini, A. (1986). A sensitive spectrophotometric method for the determination of superoxide dismutase activity in tissue extracts. Analytical Biochemistry, 154(2), 536–541. https://doi.org/10.1016/0003-2697(86)90026-6

Petrović, A., Bursić, V., Vuković, G., Marinković, D., Ivanović, I., Konstantinović, B., & Puvača, N. (2011). The Use Of Invasive Species As Water Contamination Bioindicators: Sinanodonta Woodiana Case.

Pourgholam, R., Hassan, M. D., Kakoolaki, S., Khoshbavar Rostami, H. A., Mokarrami Rostami, A., & Pourgholam, M. A. (2013). Some hematological and biochemical changes in blood serum of Grass carp (Ctenopharyngodon

idella) vaccinated with Aeromonas hydrophila following exposure to sublethal concentration of diazinon.

Rabie, M., Asri, Y., & Ahmadi, K. (2016). Effect of Milk thistle plant, Vitis vinifera extract on immune system of rainbow trout (Oncorhynchus mykiss) challenge by diazinon. International Journal of Aquatic Biology, 4(3), 208–214.

Rafieepour, A., Hajirezaee, S., & Rahimi, R. (2019). Moderating effects of dietary oregano extract (Origanum vulgare) on the toxicity induced by organophosphate pesticide, diazinon in rainbow trout, Oncorhynchus mykiss: metabolic hormones, histology and growth parameters. Turkish Journal of Fisheries and Aquatic Sciences, 20(3), 207–219.

Rahman, M. A., Hasegawa, H., & Lim, R. P. (2012). Bioaccumulation, biotransformation and trophic transfer of arsenic in the aquatic food chain. Environmental Research, 116, 118–135.

Rahman, M. M., Awal, M. A., & Misbahuddin, M. (2020). Pesticide application and contamination of soil and drinking water. Drinking Water Contaminants in Bangladesh, 90–131.

Rajan, A. P., & Anandan, S. (2017). Investigation of carcinogenic and mutagenic property of food colour using catfish Clarias Batrachus by using alkaline single-cell gel electrophoresis (comet) assay and micronucleus assay. Int. J. Med. Res. Pharmacol. Sci, 4(7), 29–34.

Rajeshkumar, S., Liu, Y., Ma, J., Duan, H. Y., & Li, X. (2017). Effects of exposure to multiple heavy metals on biochemical and histopathological

alterations in common carp, Cyprinus carpio L. Fish & Shellfish Immunology, 70, 461–472.

Rakhi, S. F., Reza, A. H. M. M., Hossen, M. S., & Hossain, Z. (2013). Alterations in histopathological features and brain acetylcholinesterase activity in stinging catfish Heteropneustes fossilis exposed to polluted river water. International Aquatic Research, 5(1), 1–18.

Rashid, H., Bhowmik, L. R., Kabir, M. F., Taslima, K., Ali, M. R., Alam, M. J., Rokeya, U. K., & Paul, S. P. (2012). Toxicity of Diazinon on embryonic and larval development of stinging catfish, Heteropneustes fossilis. Proceedings of 3rd International Conference on Environmental Aspects of Bangladesh, 47–50.

Reish, D. L., & Oshida, P. S. (1987). Manual of methods in aquatic environment research (Vol. 247). Food & Agriculture Org.

Renner, T. (2007). Quantities, units and symbols in physical chemistry. Royal Society of Chemistry.

Ribeiro, C., Ribeiro, A. R., & Tiritan, M. E. (2016). Occurrence of persistent organic pollutants in sediments and biota from Portugal versus European incidence: a critical overview. Journal of Environmental Science and Health, Part B, 51(3), 143–153.

Rifai, N. (2017). Tietz textbook of clinical chemistry and molecular diagnostics. Elsevier Health Sciences.

Saha, S, Mukherjee, D., & Saha, N. C. (2018). Studies on acute toxicity and behavioral responses of Heteropneustes fossilis (Linn.) exposed to Diazinon. Res. Rev.: A J. Toxicol., 8(1), 9–13.

Saha, Shubhajit, Chukwuka, A. V., Mukherjee, D., Patnaik, L., Nayak, S., Dhara, K., Saha, N. C., & Faggio, C. (2021). Chronic effects of diazinon® exposures using integrated biomarker responses in freshwater walking catfish, clarias batrachus. Applied Sciences (Switzerland), 11(22), 1–20. https://doi.org/10.3390/app112210902

Sarhan, Z. Y. A.-S. (2011). Histological and Biochemical Effects of Diazinon on Liver and Kidney of Rabbits. Life Science Journal, 8(4), 1183–1189.

Schäfer, R. B., Kühn, B., Malaj, E., König, A., & Gergs, R. (2016). Contribution of organic toxicants to multiple stress in river ecosystems. *Freshwater Biology*, *61*(12), 2116-2128.

Schäfer, R. B., van den Brink, P. J., & Liess, M. (2011). Impacts of pesticides on freshwater ecosystems. Ecological impacts of toxic chemicals, 2011, 111-137.

Shan, Y., Yan, S., Hong, X., Zha, J., & Qin, J. (2020). Effect of imidacloprid on the behavior, antioxidant system, multixenobiotic resistance, and histopathology of Asian freshwater clams (Corbicula fluminea). Aquatic Toxicology, 218, 105333. https://doi.org/10.1016/j.aquatox.2019.105333

Sharbidre, A. A., & Sopanrao Patode, P. (2012). Behavioural changes and acetylcholinesterase activity in guppy fish (Poecilia reticulata) exposed to chlorpyrifos. Nat Environ Pollut Technol, 11(3), 487–492.

Sharma, P., & Singh, S. P. (2021). Pollutants characterization and toxicity assessment of pulp and paper industry sludge for safe environmental disposal. In Emerging Treatment Technologies for Waste Management (pp. 207–223). Springer.

Shiri, N., Gholami, S. J., & Poorbagher, H. (2014). Evaluation of short and long-term effects of diazinon on cholinesterase activity in different tissues of freshwater bivalve Anodonta cygnea (Bivalvia: Unionacea). Journal of Natural Environment, 66(4).

Singh, N. P., McCoy, M. T., Tice, R. R., & Schneider, E. L. (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. Experimental Cell Research, 175(1), 184–191.

Southamani, C., Shanthi, G., & Deivasigamani, M. (2015). Hematological response in three Indian major carps in relation to supplementary feeding. International Journal of Fisheries and Aquatic Studies, 3(2), 287–294.

Štefanac, T., Grgas, D., & Landeka Dragičević, T. (2021). Xenobiotics—Division and Methods of Detection: A Review. Journal of Xenobiotics, 11(4), 130–141. https://doi.org/10.3390/jox11040009

Streit, B. (1998). Bioaccumulation of contaminants in fish. Fish Ecotoxicology, 353–387.

Suarez-Lopez, J. R., Himes, J. H., Jacobs Jr, D. R., Alexander, B. H., & Gunnar, M. R. (2013). Acetylcholinesterase activity and neurodevelopment in boys and girls. *Pediatrics*, *132*(6), e1649-e1658.

Svoboda, M., Luskova, V., Drastichova, J., & Žlabek, V. (2001). The effect of diazinon on haematological indices of common carp (Cyprinus carpio L.). Acta Veterinaria Brno, 70(4), 457–465.

Takeuchi, T., Satoh, S., & Kiron, V. (2002). Common carp, Cyprinus carpio. Nutrient Requirements and Feeding of Finfish for Aquaculture. New York: CABI Publishing, 245–261.

Talib, V. H., & Khurana, S. R. (1996). A handbook of medical laboratory technology. 5th, edu. CBS Publ., New Delhi.

Tchounwou, P. B., Patlolla, A. K., Yedjou, C. G., & Moore, P. D. (2015). Environmental exposure and health effects associated with malathion toxicity. Toxicity and Hazard of Agrochemicals, 51, 2145–2149.

Tejada, S., Sureda, A., Roca, C., Gamundi, A., & Esteban, S. (2007). Antioxidant response and oxidative damage in brain cortex after high dose of pilocarpine. Brain Research Bulletin, 71(4), 372–375.

Tudi, M., Daniel Ruan, H., Wang, L., Lyu, J., Sadler, R., Connell, D., ... & Phung, D. T. (2021). Agriculture development, pesticide application and its impact on the environment. *International journal of environmental research and public health*, *18*(3), 1112.

Uçkun, M., Yoloğlu, E., Uçkun, A. A., & Öz, Ö. B. (2021). Acute Toxicity of Insecticide Thiamethoxam to Crayfish (Astacus leptodactylus): Alterations in Oxidative Stress Markers, ATPases and Cholinesterase. Acta Chimica Slovenica, 68(3), 521–531.

Ullah, S., Begum, M., Dhama, K., Ahmad, S., Hassan, S., & Alam, I. (2016). Malathion induced DNA damage in freshwater fish, Labeo rohita (Hamilton, 1822) using alkaline single cell gel electrophoresis. Asian Journal of Animal and Veterinary Advances, 11(2), 98–105.

Ullah, S., Zuberi, A., Alagawany, M., Farag, M. R., Dadar, M., Karthik, K., ... & Iqbal, H. M. (2018). Cypermethrin induced toxicities in fish and adverse health outcomes: its prevention and control measure adaptation. *Journal of Environmental Management*, *206*, 863-871.

Urbańska, M., Kamocki, A., Kirschenstein, M., & Ożgo, M. (2021). The Chinese pond mussel Sinanodonta woodiana demographically outperforms European native mussels. Scientific Reports, 11(1), 1–11.

Uvayeva, O., Vakaliuk, T., & Kostromin, D. (2020). Environmental monitoring and recommendations on decreasing the levels of pesticide pollution in Zhytomyr region of Ukraine. E3S Web of Conferences, 166. https://doi.org/10.1051/e3sconf/202016601004

Vajargah Forouhar, M., Mohamadi Yalsuyi, A., Hedayati, A., & Faggio, C. (2018). Histopathological lesions and toxicity in common carp (Cyprinus carpio L. 1758) induced by copper nanoparticles. Microscopy Research and Technique, 81(7), 724–729.

Van der Oost, R., Beyer, J., & Vermeulen, N. P. E. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environmental Toxicology and Pharmacology, 13(2), 57–149.

Varley, H. (1954). Practical clinical biochemistry. Practical Clinical Biochemistry.

Varsha, Y. M., Naga Deepthi, C. H., & Chenna, S. (2011). An emphasis on xenobiotic degradation in environmental cleanup. *J Bioremed Biodegrad S*, *11*(001).

Vieira, C. E. D., Costa, P. G., Caldas, S. S., Tesser, M. E., Risso, W. E., Escarrone, A. L. V., Primel, E. G., Bianchini, A., & dos Reis Martinez, C. B. (2019). An integrated approach in subtropical agro-ecosystems: Active biomonitoring, environmental contaminants, bioaccumulation, and multiple biomarkers in fish. Science of the Total Environment, 666, 508–524.

Vieira, C. E. D., Pérez, M. R., Acayaba, R. D., Raimundo, C. C. M., & dos Reis Martinez, C. B. (2018). DNA damage and oxidative stress induced by imidacloprid exposure in different tissues of the Neotropical fish Prochilodus lineatus. Chemosphere, 195, 125–134.

Wang, Z., Kong, F., Fu, L., Li, Y., Li, M., & Yu, Z. (2021). Responses of Asian clams (Corbicula fluminea) to low concentration cadmium stress: Whether the depuration phase restores physiological characteristics. Environmental Pollution, 284, 117182.

WHO, World Health Organization. (1998). Diazinon-Environmental Health Criteria 198.

Winterbourn, C. C. (2020). Biological chemistry of superoxide radicals. ChemTexts, 6(1), 1–13.

Woo, S., Denis, V., Won, H., Shin, K., Lee, G., Lee, T.-K., & Yum, S. (2013). Expressions of oxidative stress-related genes and antioxidant enzyme activities in Mytilus galloprovincialis (Bivalvia, Mollusca) exposed to hypoxia. Zoological Studies, 52(1), 1–8.

Wright, P. J., Leathwood, P. D., & Plummer, D. T. (1972). Enzymes in rat urine: alkaline phosphatase. Enzymologia, 42(4), 317–327.

Wright, S. L., Thompson, R. C., & Galloway, T. S. (2013). The physical impacts of microplastics on marine organisms: a review. Environmental Pollution, 178, 483–492.

Yang, J. L., & Chen, H. C. (2003). Serum metabolic enzyme activities and hepatocyte ultrastructure of common carp after gallium exposure. Zoological Studies, 42(3), 455–461.

Yonar, S. M. (2019). Growth performance, haematological changes, immune response, antioxidant activity and disease resistance in rainbow trout (Oncorhynchus mykiss) fed diet supplemented with ellagic acid. Fish & Shellfish Immunology, 95, 391–398.

Yousefi, M., Vatnikov, Y. A., Kulikov, E. V., Plushikov, V. G., Drukovsky, S. G., Hoseinifar, S. H., & Van Doan, H. (2020). The protective effects of dietary garlic on common carp (Cyprinus carpio) exposed to ambient ammonia toxicity. Aquaculture, 526, 735400.

Zaruk, D., Comba, M., Struger, J., & Young, S. (2001). Comparison of immunoassay with a conventional method for the determination of Diazinon® in surface waters. Analytica Chimica Acta, 444(1), 163–168.

Zhao, L., Luo, J., Liu, Q., Du, J., Yang, H., Li, B., Zhou, Y., & Yang, S. (2020). Different diets can affect the digestion and immunity of common carp (Cyprinus carpio) according to enzyme activity assay and transcriptome sequencing. Aquaculture, 523, 735176.

appendix:

Prepared according to as following:

A-Preparing reagents.

- Manganese Sulfate ($MnSO_4$): dissolved 480g from $MnSO_4.4H_2O$ in distilled water, filtered, and diluted to 1 litter.
- Alkali-Iodide-Azide reagent: It is prepared by dissolving 500 g NaOH and 135 g NaI in a small amount of distilled water and dilute to 1 L.
- Concentrated Sulfuric acid (H_2SO_4):
- Sodium Thiosulfate ($Na_2S_2O_3$): It is prepared from Dissolve 6.205g from $Na_2S_2O_3.5H_2O$ in distilled water and add 0.4g solid NaOH and dilute to liter.
- Starch solution: Dissolved 2 g laboratory grade soluble starch and 0.2g Salicylic acid as a preventive, in 100 ml hot distilled water.

B- Test: The test was carried out as per the following steps:

- Fill Winkler's vial with water sample and close tightly,
- Add 1 ml of Manganese Sulfate ($MnSO_4$) and alkali-iodide-azide reagent to the solution,
- Add 2 ml of H_2SO_4 to the solution,
- Take 50 ml of the last solution and added 2 ml of Starch solution to it until a blue color is formed, then titrate with Sodium Thiosulfate solution until becomes colorless (where 1 ml of thiosulfate used = 1 mg/l from DO is present in the sample). the DO concentration was calculated according to the following equation in (mg/l):

$$DO\ (mg/L) = V \times M / 0.025$$

whereas :

V = mL $Na_2S_2O_3$ solution used to titrate the sample.

M = molarity of $Na_2S_2O_3$ titrant (0.025 N).

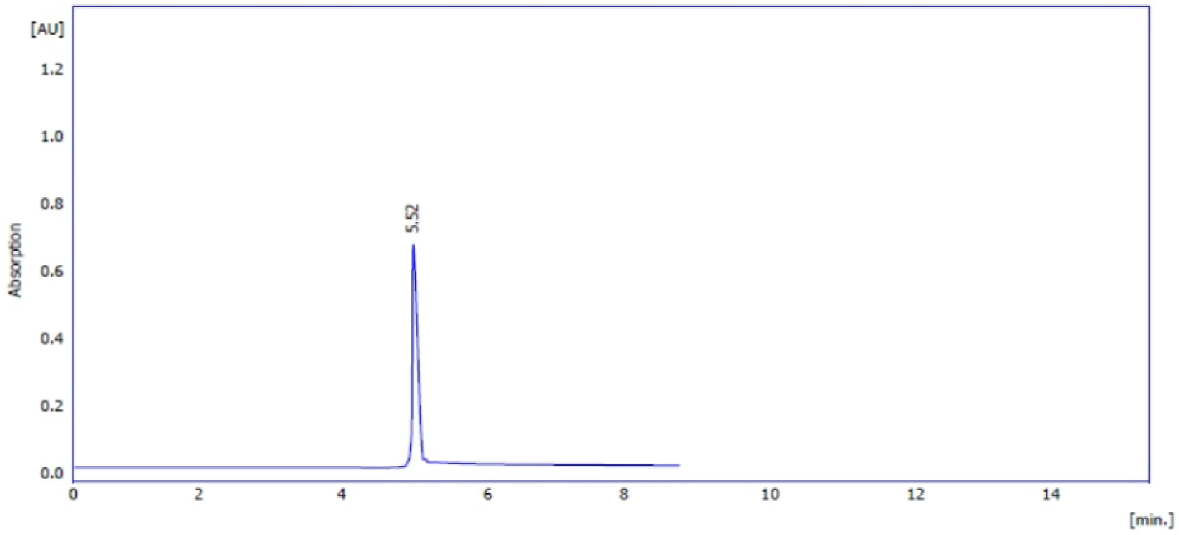
4/07/2022 01:40 م Chromatogram F:\ st Diazinon (0.8 ppm) .PRM Page 1 of 1

Chromatography Laboratory
HPLC

Sample Info:

Sample ID	: st Diazinon (0.8 ppm)	Amount	0
Sample	: st Diazinon (0.8 ppm)	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ st Diazinon (0.8 ppm)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	5.52	2247.89	741.25	100.00	100.00	0.45	
	Total	2247.89	741.25	100.00	100.00		

Figure (): HPLC Analysis of diazinon standard

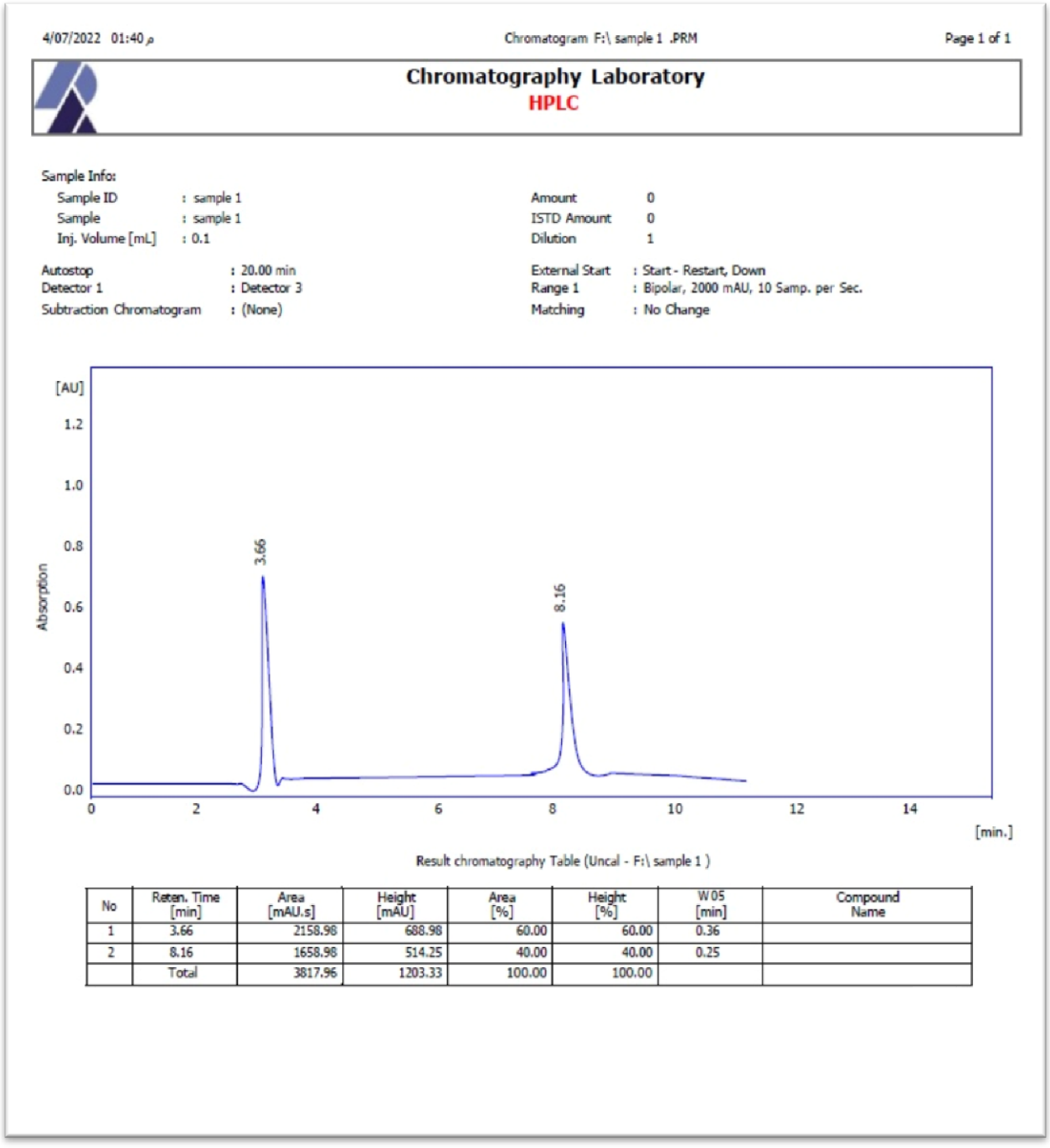


Figure (): HPLC Analysis Of Control Fish after Experimental End

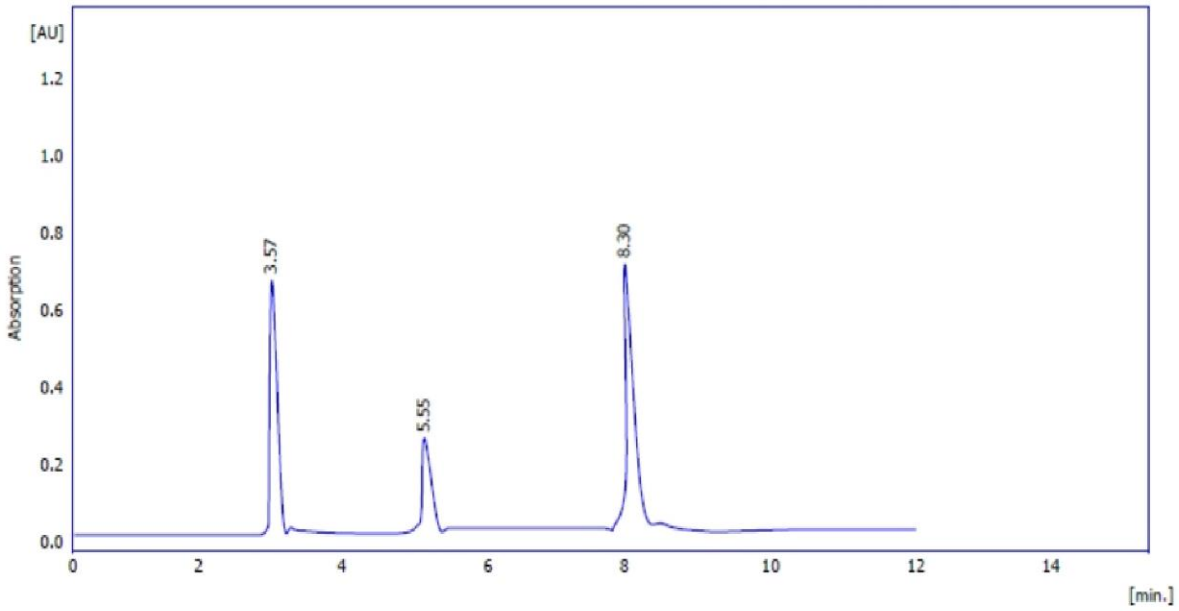
4/07/2022 01:40 م Chromatogram F:\ sample 5 .PRM Page 1 of 1

Chromatography Laboratory
HPLC

Sample Info:

Sample ID	: sample 5	Amount	0
Sample	: sample 5	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ sample 5)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.57	2541.89	612.55	38.22	38.95	0.34	
2	5.55	90.25	214.55	19.25	19.30	0.12	
2	8.30	1415.89	550.19	39.58	38.11	0.24	
	Total	4048.59	1377.18	100.00	100.00		

Figure (): HPLC Analysis Of Treatment Fish after Experimental End

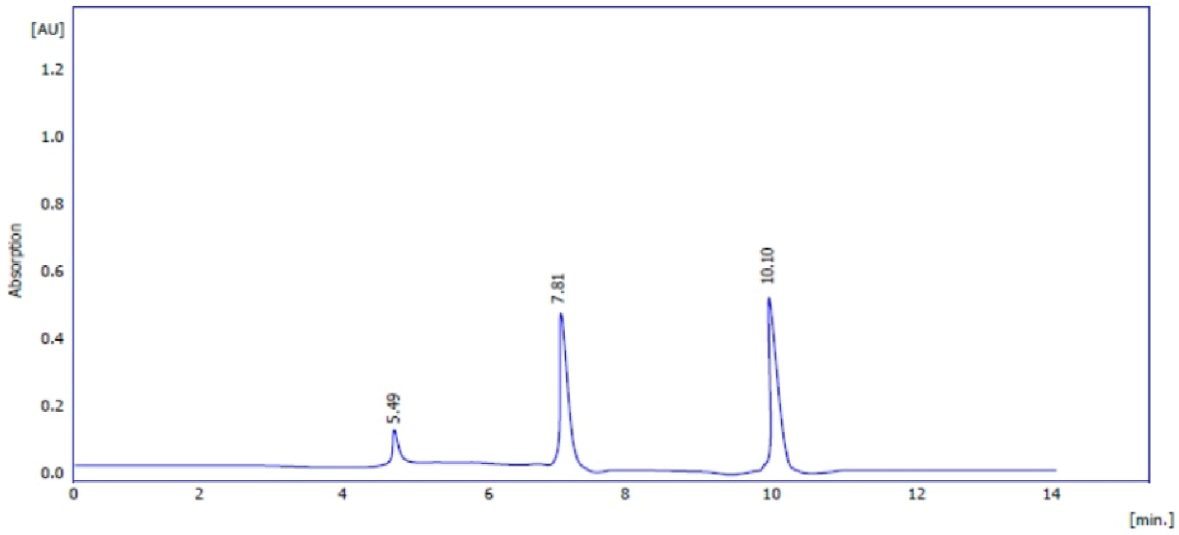
4/07/2022 01:40 م Chromatogram F:\ sample 7 .PRM Page 1 of 1

Chromatography Laboratory
HPLC

Sample Info:

Sample ID	: sample 7	Amount	0
Sample	: sample 7	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1

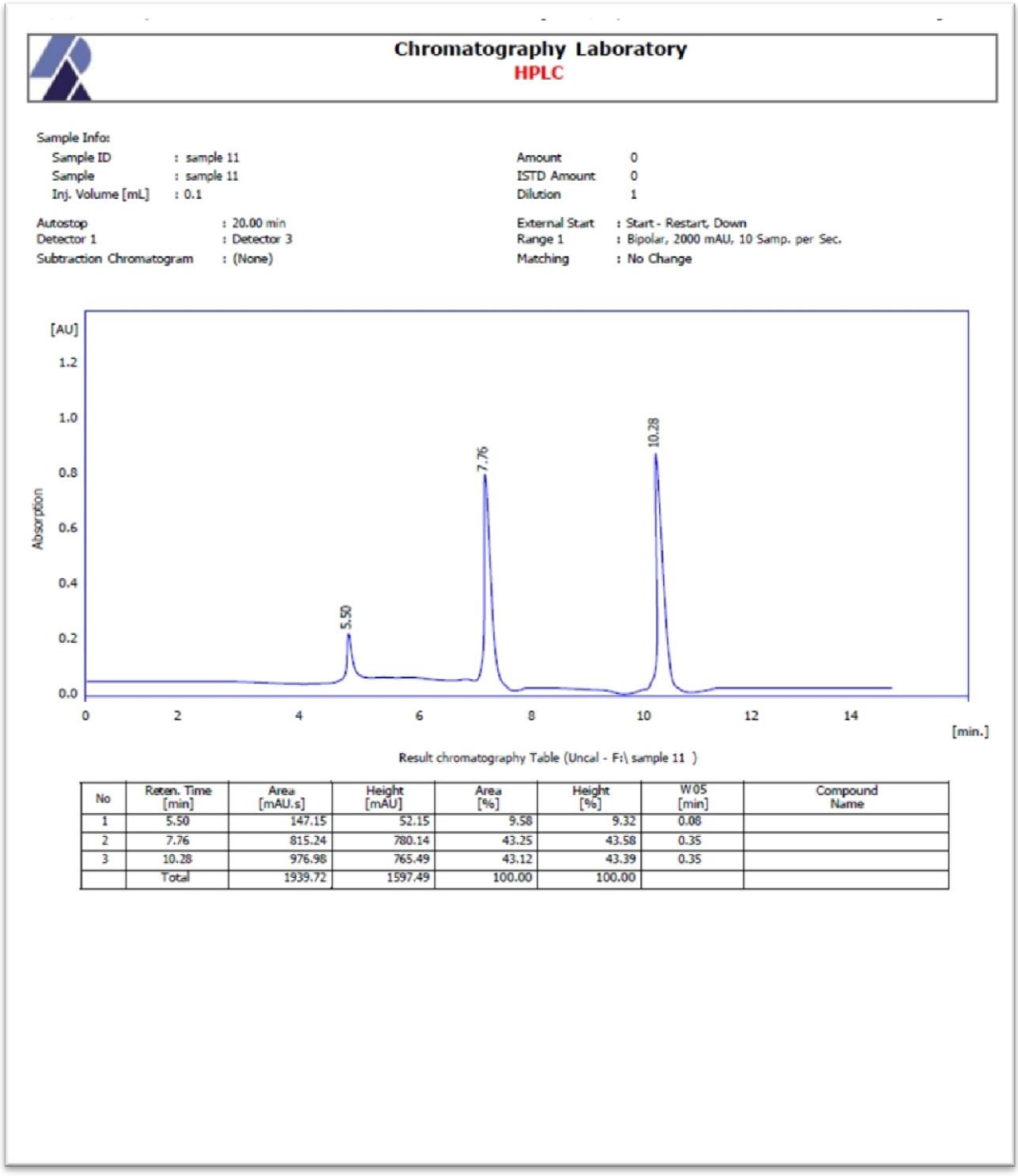
Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ sample 7)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	5.49	13.15	40.55	3.58	3.89	0.05	
2	7.81	658.99	598.47	46.58	46.58	0.35	
3	10.10	902.58	590.89	46.22	46.39	0.35	
	Total	1574.18	1229.91	100.00	100.00		

Figure (): HPLC Analysis Of Control Bivalves after Experimental End



Result chromatography Table (Uncal - F:\ sample 11)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	5.50	147.15	52.15	9.58	9.32	0.08	
2	7.76	815.24	780.14	43.25	43.58	0.35	
3	10.28	976.98	765.49	43.12	43.39	0.35	
	Total	1939.72	1597.49	100.00	100.00		

Figure (): HPLC Analysis Of Treatment Bivalves after Experimental End

ANOVA

WBC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2868.918	5	573.784	1.300	.327
Within Groups	5297.490	12	441.458		
Total	8166.408	17			

Multiple Comparisons

Dependent Variable:

LSD

(I) Groups		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
C 4day	T 4day	34.65167	17.15532	.066	-2.7266	72.0299
	C 14day	8.90167	17.15532	.613	-28.4766	46.2799
	T 14 day	-4.47167	17.15532	.799	-41.8499	32.9066
	C 28day	2.86500	17.15532	.870	-34.5132	40.2432
	T 28day	8.57500	17.15532	.626	-28.8032	45.9532
T 4day	C 4day	-34.65167	17.15532	.066	-72.0299	2.7266
	C 14day	-25.75000	17.15532	.159	-63.1282	11.6282
	T 14 day	-39.12333*	17.15532	.042	-76.5016	-1.7451
	C 28day	-31.78667	17.15532	.089	-69.1649	5.5916
	T 28day	-26.07667	17.15532	.154	-63.4549	11.3016
C 14day	C 4day	-8.90167	17.15532	.613	-46.2799	28.4766
	T 4day	25.75000	17.15532	.159	-11.6282	63.1282
	T 14 day	-13.37333	17.15532	.451	-50.7516	24.0049
	C 28day	-6.03667	17.15532	.731	-43.4149	31.3416
	T 28day	-.32667	17.15532	.985	-37.7049	37.0516
T 14 day	C 4day	4.47167	17.15532	.799	-32.9066	41.8499
	T 4day	39.12333*	17.15532	.042	1.7451	76.5016
	C 14day	13.37333	17.15532	.451	-24.0049	50.7516
	C 28day	7.33667	17.15532	.676	-30.0416	44.7149
	T 28day	13.04667	17.15532	.462	-24.3316	50.4249
C 28day	C 4day	-2.86500	17.15532	.870	-40.2432	34.5132
	T 4day	31.78667	17.15532	.089	-5.5916	69.1649

	C 14day	6.03667	17.15532	.731	-31.3416	43.4149
	T 14 day	-7.33667	17.15532	.676	-44.7149	30.0416
	T 28day	5.71000	17.15532	.745	-31.6682	43.0882
T 28day	C 4day	-8.57500	17.15532	.626	-45.9532	28.8032
	T 4day	26.07667	17.15532	.154	-11.3016	63.4549
	C 14day	.32667	17.15532	.985	-37.0516	37.7049
	T 14 day	-13.04667	17.15532	.462	-50.4249	24.3316
	C 28day	-5.71000	17.15532	.745	-43.0882	31.6682

*. The mean difference is significant at the 0.05 level.

ANOVA

RBC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.703	5	.341	3.556	.033
Within Groups	1.150	12	.096		
Total	2.853	17			

Multiple Comparisons

Dependent Variable:

LSD

(I) Groups		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
C 4day	T 4day	.72333*	.25273	.014	.1727	1.2740
	C 14day	-.03667	.25273	.887	-.5873	.5140
	T 14 day	-.26667	.25273	.312	-.8173	.2840
	C 28day	-.03000	.25273	.907	-.5806	.5206
	T 28day	.03333	.25273	.897	-.5173	.5840
T 4day	C 4day	-.72333-*	.25273	.014	-1.2740	-.1727
	C 14day	-.76000-*	.25273	.011	-1.3106	-.2094
	T 14 day	-.99000-*	.25273	.002	-1.5406	-.4394
	C 28day	-.75333-*	.25273	.011	-1.3040	-.2027
	T 28day	-.69000-*	.25273	.018	-1.2406	-.1394
C 14day	C 4day	.03667	.25273	.887	-.5140	.5873
	T 4day	.76000*	.25273	.011	.2094	1.3106

	T 14 day	-.23000	.25273	.381	-.7806	.3206
	C 28day	.00667	.25273	.979	-.5440	.5573
	T 28day	.07000	.25273	.787	-.4806	.6206
T 14 day	C 4day	.26667	.25273	.312	-.2840	.8173
	T 4day	.99000*	.25273	.002	.4394	1.5406
	C 14day	.23000	.25273	.381	-.3206	.7806
	C 28day	.23667	.25273	.368	-.3140	.7873
	T 28day	.30000	.25273	.258	-.2506	.8506
C 28day	C 4day	.03000	.25273	.907	-.5206	.5806
	T 4day	.75333*	.25273	.011	.2027	1.3040
	C 14day	-.00667	.25273	.979	-.5573	.5440
	T 14 day	-.23667	.25273	.368	-.7873	.3140
	T 28day	.06333	.25273	.806	-.4873	.6140
T 28day	C 4day	-.03333	.25273	.897	-.5840	.5173
	T 4day	.69000*	.25273	.018	.1394	1.2406
	C 14day	-.07000	.25273	.787	-.6206	.4806
	T 14 day	-.30000	.25273	.258	-.8506	.2506
	C 28day	-.06333	.25273	.806	-.6140	.4873

*. The mean difference is significant at the 0.05 level.

ANOVA

HGB

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	29.818	5	5.964	.988	.464
Within Groups	72.447	12	6.037		
Total	102.265	17			

Multiple Comparisons

Dependent Variable:

LSD

(I) Groups		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
C 4day	T 4day	3.53333	2.00619	.104	-.8378	7.9045
	C 14day	2.46667	2.00619	.242	-1.9045	6.8378
	T 14 day	-.10000	2.00619	.961	-4.4711	4.2711

	C 28day	1.56667	2.00619	.450	-2.8045	5.9378
	T 28day	1.23333	2.00619	.550	-3.1378	5.6045
T 4day	C 4day	-3.53333	2.00619	.104	-7.9045	.8378
	C 14day	-1.06667	2.00619	.605	-5.4378	3.3045
	T 14 day	-3.63333	2.00619	.095	-8.0045	.7378
	C 28day	-1.96667	2.00619	.346	-6.3378	2.4045
	T 28day	-2.30000	2.00619	.274	-6.6711	2.0711
C 14day	C 4day	-2.46667	2.00619	.242	-6.8378	1.9045
	T 4day	1.06667	2.00619	.605	-3.3045	5.4378
	T 14 day	-2.56667	2.00619	.225	-6.9378	1.8045
	C 28day	-.90000	2.00619	.662	-5.2711	3.4711
	T 28day	-1.23333	2.00619	.550	-5.6045	3.1378
T 14 day	C 4day	.10000	2.00619	.961	-4.2711	4.4711
	T 4day	3.63333	2.00619	.095	-.7378	8.0045
	C 14day	2.56667	2.00619	.225	-1.8045	6.9378
	C 28day	1.66667	2.00619	.422	-2.7045	6.0378
	T 28day	1.33333	2.00619	.519	-3.0378	5.7045
C 28day	C 4day	-1.56667	2.00619	.450	-5.9378	2.8045
	T 4day	1.96667	2.00619	.346	-2.4045	6.3378
	C 14day	.90000	2.00619	.662	-3.4711	5.2711
	T 14 day	-1.66667	2.00619	.422	-6.0378	2.7045
	T 28day	-.33333	2.00619	.871	-4.7045	4.0378
T 28day	C 4day	-1.23333	2.00619	.550	-5.6045	3.1378
	T 4day	2.30000	2.00619	.274	-2.0711	6.6711
	C 14day	1.23333	2.00619	.550	-3.1378	5.6045
	T 14 day	-1.33333	2.00619	.519	-5.7045	3.0378
	C 28day	.33333	2.00619	.871	-4.0378	4.7045

ANOVA

PLT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2570.278	5	514.056	1.095	.412
Within Groups	5635.333	12	469.611		

Total	8205.611	17			

Multiple Comparisons

Dependent Variable:

LSD

(I) Groups		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
C 4day	T 4day	11.00000	17.69390	.546	-27.5517	49.5517
	C 14day	28.00000	17.69390	.140	-10.5517	66.5517
	T 14 day	34.00000	17.69390	.079	-4.5517	72.5517
	C 28day	30.33333	17.69390	.112	-8.2184	68.8850
	T 28day	24.33333	17.69390	.194	-14.2184	62.8850
T 4day	C 4day	-11.00000	17.69390	.546	-49.5517	27.5517
	C 14day	17.00000	17.69390	.356	-21.5517	55.5517
	T 14 day	23.00000	17.69390	.218	-15.5517	61.5517
	C 28day	19.33333	17.69390	.296	-19.2184	57.8850
	T 28day	13.33333	17.69390	.466	-25.2184	51.8850
C 14day	C 4day	-28.00000	17.69390	.140	-66.5517	10.5517
	T 4day	-17.00000	17.69390	.356	-55.5517	21.5517
	T 14 day	6.00000	17.69390	.740	-32.5517	44.5517
	C 28day	2.33333	17.69390	.897	-36.2184	40.8850
	T 28day	-3.66667	17.69390	.839	-42.2184	34.8850
T 14 day	C 4day	-34.00000	17.69390	.079	-72.5517	4.5517
	T 4day	-23.00000	17.69390	.218	-61.5517	15.5517
	C 14day	-6.00000	17.69390	.740	-44.5517	32.5517
	C 28day	-3.66667	17.69390	.839	-42.2184	34.8850
	T 28day	-9.66667	17.69390	.595	-48.2184	28.8850
C 28day	C 4day	-30.33333	17.69390	.112	-68.8850	8.2184
	T 4day	-19.33333	17.69390	.296	-57.8850	19.2184
	C 14day	-2.33333	17.69390	.897	-40.8850	36.2184
	T 14 day	3.66667	17.69390	.839	-34.8850	42.2184
	T 28day	-6.00000	17.69390	.740	-44.5517	32.5517
T 28day	C 4day	-24.33333	17.69390	.194	-62.8850	14.2184
	T 4day	-13.33333	17.69390	.466	-51.8850	25.2184
	C 14day	3.66667	17.69390	.839	-34.8850	42.2184
	T 14 day	9.66667	17.69390	.595	-28.8850	48.2184
	C 28day	6.00000	17.69390	.740	-32.5517	44.5517

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
SODF	Between Groups	148.276	5	29.655	.787	.578
	Within Groups	451.983	12	37.665		
	Total	600.259	17			
SODB	Between Groups	818.860	5	163.772	18.582	.000
	Within Groups	105.764	12	8.814		
	Total	924.624	17			

Multiple Comparisons

LSD

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
SODF	C 4day	T 4day	4.25238	5.01101	.413	-6.6657	15.1704
		C 14day	.18889	5.01101	.971	-10.7292	11.1069
		T 14 day	2.99365	5.01101	.561	-7.9244	13.9117
		C 28day	-1.26349	5.01101	.805	-12.1815	9.6546
		T 28day	-4.60000	5.01101	.377	-15.5180	6.3180
	T 4day	C 4day	-4.25238	5.01101	.413	-15.1704	6.6657
		C 14day	-4.06349	5.01101	.433	-14.9815	6.8546
		T 14 day	-1.25873	5.01101	.806	-12.1768	9.6593
		C 28day	-5.51587	5.01101	.293	-16.4339	5.4022
		T 28day	-8.85238	5.01101	.103	-19.7704	2.0657
	C 14day	C 4day	-.18889	5.01101	.971	-11.1069	10.7292
		T 4day	4.06349	5.01101	.433	-6.8546	14.9815
		T 14 day	2.80476	5.01101	.586	-8.1133	13.7228
		C 28day	-1.45238	5.01101	.777	-12.3704	9.4657
		T 28day	-4.78889	5.01101	.358	-15.7069	6.1292
	T 14 day	C 4day	-2.99365	5.01101	.561	-13.9117	7.9244
		T 4day	1.25873	5.01101	.806	-9.6593	12.1768
		C 14day	-2.80476	5.01101	.586	-13.7228	8.1133
		C 28day	-4.25714	5.01101	.412	-15.1752	6.6609
		T 28day	-7.59365	5.01101	.156	-18.5117	3.3244
	C 28day	C 4day	1.26349	5.01101	.805	-9.6546	12.1815

		T 4day	5.51587	5.01101	.293	-5.4022	16.4339
		C 14day	1.45238	5.01101	.777	-9.4657	12.3704
		T 14 day	4.25714	5.01101	.412	-6.6609	15.1752
		T 28day	-3.33651	5.01101	.518	-14.2546	7.5815
	T 28day	C 4day	4.60000	5.01101	.377	-6.3180	15.5180
		T 4day	8.85238	5.01101	.103	-2.0657	19.7704
		C 14day	4.78889	5.01101	.358	-6.1292	15.7069
		T 14 day	7.59365	5.01101	.156	-3.3244	18.5117
		C 28day	3.33651	5.01101	.518	-7.5815	14.2546
SODB	C 4day	T 4day	12.14190*	2.42400	.000	6.8605	17.4234
		C 14day	13.87968*	2.42400	.000	8.5982	19.1611
		T 14 day	1.47746	2.42400	.554	-3.8040	6.7589
		C 28day	16.47524*	2.42400	.000	11.1938	21.7567
		T 28day	1.36413	2.42400	.584	-3.9173	6.6456
	T 4day	C 4day	-12.14190-*	2.42400	.000	-17.4234	-6.8605
		C 14day	1.73778	2.42400	.487	-3.5437	7.0192
		T 14 day	-10.66444-*	2.42400	.001	-15.9459	-5.3830
		C 28day	4.33333	2.42400	.099	-.9481	9.6148
		T 28day	-10.77778-*	2.42400	.001	-16.0592	-5.4963
	C 14day	C 4day	-13.87968-*	2.42400	.000	-19.1611	-8.5982
		T 4day	-1.73778	2.42400	.487	-7.0192	3.5437
		T 14 day	-12.40222-*	2.42400	.000	-17.6837	-7.1208
		C 28day	2.59556	2.42400	.305	-2.6859	7.8770
		T 28day	-12.51556-*	2.42400	.000	-17.7970	-7.2341
	T 14 day	C 4day	-1.47746	2.42400	.554	-6.7589	3.8040
		T 4day	10.66444*	2.42400	.001	5.3830	15.9459
		C 14day	12.40222*	2.42400	.000	7.1208	17.6837
		C 28day	14.99778*	2.42400	.000	9.7163	20.2792
		T 28day	-.11333	2.42400	.963	-5.3948	5.1681
	C 28day	C 4day	-16.47524-*	2.42400	.000	-21.7567	-11.1938
		T 4day	-4.33333	2.42400	.099	-9.6148	.9481
		C 14day	-2.59556	2.42400	.305	-7.8770	2.6859
		T 14 day	-	2.42400	.000	-20.2792	-9.7163

		T 28day	14.99778-* -15.11111-*	2.42400	.000	-20.3926	-9.8297
	T 28day	C 4day	-1.36413	2.42400	.584	-6.6456	3.9173
		T 4day	10.77778*	2.42400	.001	5.4963	16.0592
		C 14day	12.51556*	2.42400	.000	7.2341	17.7970
		T 14 day	.11333	2.42400	.963	-5.1681	5.3948
		C 28day	15.11111*	2.42400	.000	9.8297	20.3926

*. The mean difference is significant at the 0.05 level.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
TPF	Between Groups	1445.586	5	289.117	440.618	.000
	Within Groups	7.874	12	.656		
	Total	1453.460	17			
TPB	Between Groups	52.647	5	10.529	16.406	.000
	Within Groups	7.701	12	.642		
	Total	60.348	17			

Multiple Comparisons

LSD

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
TPF	C 4day	T 4day	19.69620*	.66139	.000	18.2551	21.1373
		C 14day	23.53586*	.66139	.000	22.0948	24.9769
		T 14 day	5.79747*	.66139	.000	4.3564	7.2385
		C 28day	18.15190*	.66139	.000	16.7108	19.5930
		T 28day	23.20675*	.66139	.000	21.7657	24.6478
	T 4day	C 4day	-19.69620-*	.66139	.000	-21.1373	-18.2551
		C 14day	3.83966*	.66139	.000	2.3986	5.2807
		T 14 day	-13.89873-*	.66139	.000	-15.3398	-12.4577
		C 28day	-1.54430-*	.66139	.038	-2.9854	-.1033

		T 28day	3.51055*	.66139	.000	2.0695	4.9516
	C 14day	C 4day	-23.53586-*	.66139	.000	-24.9769	-22.0948
		T 4day	-3.83966-*	.66139	.000	-5.2807	-2.3986
		T 14 day	-17.73840-*	.66139	.000	-19.1794	-16.2973
		C 28day	-5.38397-*	.66139	.000	-6.8250	-3.9429
		T 28day	-.32911	.66139	.628	-1.7702	1.1119
	T 14 day	C 4day	-5.79747-*	.66139	.000	-7.2385	-4.3564
		T 4day	13.89873*	.66139	.000	12.4577	15.3398
		C 14day	17.73840*	.66139	.000	16.2973	19.1794
		C 28day	12.35443*	.66139	.000	10.9134	13.7955
		T 28day	17.40928*	.66139	.000	15.9682	18.8503
	C 28day	C 4day	-18.15190-*	.66139	.000	-19.5930	-16.7108
		T 4day	1.54430*	.66139	.038	.1033	2.9854
		C 14day	5.38397*	.66139	.000	3.9429	6.8250
		T 14 day	-12.35443-*	.66139	.000	-13.7955	-10.9134
		T 28day	5.05485*	.66139	.000	3.6138	6.4959
	T 28day	C 4day	-23.20675-*	.66139	.000	-24.6478	-21.7657
		T 4day	-3.51055-*	.66139	.000	-4.9516	-2.0695
		C 14day	.32911	.66139	.628	-1.1119	1.7702
		T 14 day	-17.40928-*	.66139	.000	-18.8503	-15.9682
		C 28day	-5.05485-*	.66139	.000	-6.4959	-3.6138
TPB	C 4day	T 4day	.72574	.65410	.289	-.6994	2.1509
		C 14day	-1.03797	.65410	.139	-2.4631	.3872
		T 14 day	-.97046	.65410	.164	-2.3956	.4547
		C 28day	3.52743*	.65410	.000	2.1023	4.9526
		T 28day	-1.62025-*	.65410	.029	-3.0454	-.1951
	T 4day	C 4day	-.72574	.65410	.289	-2.1509	.6994
		C 14day	-1.76371-*	.65410	.019	-3.1889	-.3385
		T 14 day	-1.69620-*	.65410	.024	-3.1214	-.2710
		C 28day	2.80169*	.65410	.001	1.3765	4.2269
		T 28day	-2.34599-*	.65410	.004	-3.7712	-.9208
	C 14day	C 4day	1.03797	.65410	.139	-.3872	2.4631
		T 4day	1.76371*	.65410	.019	.3385	3.1889
		T 14 day	.06751	.65410	.920	-1.3577	1.4927
		C 28day	4.56540*	.65410	.000	3.1402	5.9906
		T 28day	-.58228	.65410	.391	-2.0074	.8429
	T 14 day	C 4day	.97046	.65410	.164	-.4547	2.3956
		T 4day	1.69620*	.65410	.024	.2710	3.1214
		C 14day	-.06751	.65410	.920	-1.4927	1.3577
		C 28day	4.49789*	.65410	.000	3.0727	5.9231

		T 28day	-.64979	.65410	.340	-2.0750	.7754
	C 28day	C 4day	-3.52743-*	.65410	.000	-4.9526	-2.1023
		T 4day	-2.80169-*	.65410	.001	-4.2269	-1.3765
		C 14day	-4.56540-*	.65410	.000	-5.9906	-3.1402
		T 14 day	-4.49789-*	.65410	.000	-5.9231	-3.0727
		T 28day	-5.14768-*	.65410	.000	-6.5728	-3.7225
	T 28day	C 4day	1.62025*	.65410	.029	.1951	3.0454
		T 4day	2.34599*	.65410	.004	.9208	3.7712
		C 14day	.58228	.65410	.391	-.8429	2.0074
		T 14 day	.64979	.65410	.340	-.7754	2.0750
		C 28day	5.14768*	.65410	.000	3.7225	6.5728

*. The mean difference is significant at the 0.05 level.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
CATF	Between Groups	11183.398	5	2236.680	124.699	.000
	Within Groups	215.240	12	17.937		
	Total	11398.638	17			
CATB	Between Groups	637.652	5	127.530	29.644	.000
	Within Groups	51.625	12	4.302		
	Total	689.278	17			

Multiple Comparisons

LSD

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
CATF	C 4day	T 4day	-9.99313-*	3.45800	.014	-17.5275	-2.4588
		C 14day	-3.04875	3.45800	.395	-10.5831	4.4856
		T 14 day	-18.10431-*	3.45800	.000	-25.6386	-10.5700
		C 28day	-58.82958-*	3.45800	.000	-66.3639	-51.2952
		T 28day	-59.69528-*	3.45800	.000	-67.2296	-52.1609
	T 4day	C 4day	9.99313*	3.45800	.014	2.4588	17.5275
		C 14day	6.94438	3.45800	.068	-.5900	14.4787
		T 14 day	-8.11118-*	3.45800	.037	-15.6455	-.5768
		C 28day	-48.83646-*	3.45800	.000	-56.3708	-41.3021
		T 28day	-49.70215-*	3.45800	.000	-57.2365	-42.1678
	C 14day	C 4day	3.04875	3.45800	.395	-4.4856	10.5831

		T 4day	-6.94438	3.45800	.068	-14.4787	.5900
		T 14 day	-15.05556-*	3.45800	.001	-22.5899	-7.5212
		C 28day	-55.78083-*	3.45800	.000	-63.3152	-48.2465
		T 28day	-56.64653-*	3.45800	.000	-64.1809	-49.1122
	T 14 day	C 4day	18.10431*	3.45800	.000	10.5700	25.6386
		T 4day	8.11118*	3.45800	.037	.5768	15.6455
		C 14day	15.05556*	3.45800	.001	7.5212	22.5899
		C 28day	-40.72528-*	3.45800	.000	-48.2596	-33.1909
		T 28day	-41.59097-*	3.45800	.000	-49.1253	-34.0566
	C 28day	C 4day	58.82958*	3.45800	.000	51.2952	66.3639
		T 4day	48.83646*	3.45800	.000	41.3021	56.3708
		C 14day	55.78083*	3.45800	.000	48.2465	63.3152
		T 14 day	40.72528*	3.45800	.000	33.1909	48.2596
		T 28day	-.86569	3.45800	.807	-8.4000	6.6686
	T 28day	C 4day	59.69528*	3.45800	.000	52.1609	67.2296
		T 4day	49.70215*	3.45800	.000	42.1678	57.2365
		C 14day	56.64653*	3.45800	.000	49.1122	64.1809
		T 14 day	41.59097*	3.45800	.000	34.0566	49.1253
		C 28day	.86569	3.45800	.807	-6.6686	8.4000
CATB	C 4day	T 4day	-4.64840-*	1.69354	.018	-8.3383	-.9585
		C 14day	-11.63042-*	1.69354	.000	-15.3203	-7.9405
		T 14 day	-4.29083-*	1.69354	.026	-7.9807	-.6009
		C 28day	-1.22326	1.69354	.484	-4.9132	2.4666
		T 28day	8.16764*	1.69354	.000	4.4777	11.8575
	T 4day	C 4day	4.64840*	1.69354	.018	.9585	8.3383
		C 14day	-6.98201-*	1.69354	.001	-10.6719	-3.2921
		T 14 day	.35757	1.69354	.836	-3.3323	4.0475
		C 28day	3.42514	1.69354	.066	-.2648	7.1150
		T 28day	12.81604*	1.69354	.000	9.1261	16.5060
	C 14day	C 4day	11.63042*	1.69354	.000	7.9405	15.3203
		T 4day	6.98201*	1.69354	.001	3.2921	10.6719
		T 14 day	7.33958*	1.69354	.001	3.6497	11.0295
		C 28day	10.40715*	1.69354	.000	6.7172	14.0971
		T 28day	19.79806*	1.69354	.000	16.1081	23.4880
	T 14 day	C 4day	4.29083*	1.69354	.026	.6009	7.9807
		T 4day	-.35757	1.69354	.836	-4.0475	3.3323
		C 14day	-7.33958-*	1.69354	.001	-11.0295	-3.6497
		C 28day	3.06757	1.69354	.095	-.6223	6.7575
		T 28day	12.45847*	1.69354	.000	8.7686	16.1484
	C 28day	C 4day	1.22326	1.69354	.484	-2.4666	4.9132

		T 4day	-3.42514	1.69354	.066	-7.1150	.2648
		C 14day	-10.40715-*	1.69354	.000	-14.0971	-6.7172
		T 14 day	-3.06757	1.69354	.095	-6.7575	.6223
		T 28day	9.39090*	1.69354	.000	5.7010	13.0808
	T 28day	C 4day	-8.16764-*	1.69354	.000	-11.8575	-4.4777
		T 4day	-12.81604-*	1.69354	.000	-16.5060	-9.1261
		C 14day	-19.79806-*	1.69354	.000	-23.4880	-16.1081
		T 14 day	-12.45847-*	1.69354	.000	-16.1484	-8.7686
		C 28day	-9.39090-*	1.69354	.000	-13.0808	-5.7010

*. The mean difference is significant at the 0.05 level.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
MDAF	Between Groups	86.694	5	17.339	1.095	.412
	Within Groups	190.016	12	15.835		
	Total	276.710	17			
MDAB	Between Groups	.594	5	.119	22.749	.000
	Within Groups	.063	12	.005		
	Total	.657	17			

Multiple Comparisons

LSD

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
MDAF	C 4day	T 4day	.14316	3.24907	.966	-6.9360	7.2223
		C 14day	-2.09017	3.24907	.532	-9.1693	4.9889
		T 14 day	-6.01282	3.24907	.089	-13.0919	1.0663
		C 28day	-.60128	3.24907	.856	-7.6804	6.4778
		T 28day	.07158	3.24907	.983	-7.0075	7.1507
	T 4day	C 4day	-.14316	3.24907	.966	-7.2223	6.9360
		C 14day	-2.23333	3.24907	.505	-9.3125	4.8458
		T 14 day	-6.15598	3.24907	.082	-13.2351	.9231
		C 28day	-.74444	3.24907	.823	-7.8236	6.3347
		T 28day	-.07158	3.24907	.983	-7.1507	7.0075

	C 14day	C 4day	2.09017	3.24907	.532	-4.9889	9.1693
		T 4day	2.23333	3.24907	.505	-4.8458	9.3125
		T 14 day	-3.92265	3.24907	.251	-11.0018	3.1565
		C 28day	1.48889	3.24907	.655	-5.5902	8.5680
		T 28day	2.16175	3.24907	.518	-4.9174	9.2409
	T 14 day	C 4day	6.01282	3.24907	.089	-1.0663	13.0919
		T 4day	6.15598	3.24907	.082	-.9231	13.2351
		C 14day	3.92265	3.24907	.251	-3.1565	11.0018
		C 28day	5.41154	3.24907	.122	-1.6676	12.4907
		T 28day	6.08440	3.24907	.086	-.9947	13.1635
	C 28day	C 4day	.60128	3.24907	.856	-6.4778	7.6804
		T 4day	.74444	3.24907	.823	-6.3347	7.8236
		C 14day	-1.48889	3.24907	.655	-8.5680	5.5902
		T 14 day	-5.41154	3.24907	.122	-12.4907	1.6676
		T 28day	.67286	3.24907	.839	-6.4063	7.7520
	T 28day	C 4day	-.07158	3.24907	.983	-7.1507	7.0075
		T 4day	.07158	3.24907	.983	-7.0075	7.1507
		C 14day	-2.16175	3.24907	.518	-9.2409	4.9174
		T 14 day	-6.08440	3.24907	.086	-13.1635	.9947
		C 28day	-.67286	3.24907	.839	-7.7520	6.4063
MDAB	C 4day	T 4day	.15748*	.05903	.020	.0289	.2861
		C 14day	.25769*	.05903	.001	.1291	.3863
		T 14 day	.00000	.05903	1.000	-.1286	.1286
		C 28day	-.12885-*	.05903	.050	-.2575	-.0002
		T 28day	-.30064-*	.05903	.000	-.4293	-.1720
	T 4day	C 4day	-.15748-*	.05903	.020	-.2861	-.0289
		C 14day	.10021	.05903	.115	-.0284	.2288
		T 14 day	-.15748-*	.05903	.020	-.2861	-.0289
		C 28day	-.28632-*	.05903	.000	-.4149	-.1577
		T 28day	-.45812-*	.05903	.000	-.5867	-.3295
	C 14day	C 4day	-.25769-*	.05903	.001	-.3863	-.1291
		T 4day	-.10021	.05903	.115	-.2288	.0284
		T 14 day	-.25769-*	.05903	.001	-.3863	-.1291
		C 28day	-.38654-*	.05903	.000	-.5151	-.2579
		T 28day	-.55833-*	.05903	.000	-.6869	-.4297
	T 14 day	C 4day	.00000	.05903	1.000	-.1286	.1286
		T 4day	.15748*	.05903	.020	.0289	.2861
		C 14day	.25769*	.05903	.001	.1291	.3863
		C 28day	-.12885-*	.05903	.050	-.2575	-.0002
		T 28day	-.30064-*	.05903	.000	-.4293	-.1720

	C 28day	C 4day	.12885*	.05903	.050	.0002	.2575
		T 4day	.28632*	.05903	.000	.1577	.4149
		C 14day	.38654*	.05903	.000	.2579	.5151
		T 14 day	.12885*	.05903	.050	.0002	.2575
		T 28day	-.17179-*	.05903	.013	-.3004	-.0432
	T 28day	C 4day	.30064*	.05903	.000	.1720	.4293
		T 4day	.45812*	.05903	.000	.3295	.5867
		C 14day	.55833*	.05903	.000	.4297	.6869
		T 14 day	.30064*	.05903	.000	.1720	.4293
		C 28day	.17179*	.05903	.013	.0432	.3004

*. The mean difference is significant at the 0.05 level.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
AcetlF	Between Groups	.000	5	.000	70.526	.000
	Within Groups	.000	12	.000		
	Total	.000	17			
AcetlB	Between Groups	.043	5	.009	14.045	.000
	Within Groups	.007	12	.001		
	Total	.050	17			

Multiple Comparisons

LSD

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
AcetlF	C 4day	T 4day	.00000*	.00000	.002	.0000	.0000
		C 14day	.00000*	.00000	.000	.0000	.0000
		T 14 day	.00000*	.00000	.000	.0000	.0000
		C 28day	.00000*	.00000	.000	.0000	.0000
		T 28day	.00000*	.00000	.000	.0000	.0000
	T 4day	C 4day	.00000*	.00000	.002	.0000	.0000
		C 14day	.00000*	.00000	.002	.0000	.0000
		T 14 day	.00000*	.00000	.000	.0000	.0000
		C 28day	.00000*	.00000	.000	.0000	.0000
		T 28day	.00000*	.00000	.000	.0000	.0000
	C 14day	C 4day	.00000*	.00000	.000	.0000	.0000
		T 4day	.00000*	.00000	.002	.0000	.0000

		T 14 day	.00000*	.00000	.002	.0000	.0000
		C 28day	.00000*	.00000	.000	.0000	.0000
		T 28day	.00000*	.00000	.000	.0000	.0000
	T 14 day	C 4day	.00000*	.00000	.000	.0000	.0000
		T 4day	.00000*	.00000	.000	.0000	.0000
		C 14day	.00000*	.00000	.002	.0000	.0000
		C 28day	.00000*	.00000	.008	.0000	.0000
		T 28day	.00000*	.00000	.045	.0000	.0000
	C 28day	C 4day	.00000*	.00000	.000	.0000	.0000
		T 4day	.00000*	.00000	.000	.0000	.0000
		C 14day	.00000*	.00000	.000	.0000	.0000
		T 14 day	.00000*	.00000	.008	.0000	.0000
		T 28day	.00000	.00000	.385	.0000	.0000
	T 28day	C 4day	.00000*	.00000	.000	.0000	.0000
		T 4day	.00000*	.00000	.000	.0000	.0000
		C 14day	.00000*	.00000	.000	.0000	.0000
		T 14 day	.00000*	.00000	.045	.0000	.0000
		C 28day	.00000	.00000	.385	.0000	.0000
AcetlB	C 4day	T 4day	-.08801-*	.02010	.001	-.1318	-.0442
		C 14day	.04401*	.02010	.049	.0002	.0878
		T 14 day	-.02296	.02010	.276	-.0667	.0208
		C 28day	.04783*	.02010	.035	.0040	.0916
		T 28day	.04209	.02010	.058	-.0017	.0859
	T 4day	C 4day	.08801*	.02010	.001	.0442	.1318
		C 14day	.13202*	.02010	.000	.0882	.1758
		T 14 day	.06505*	.02010	.007	.0213	.1088
		C 28day	.13585*	.02010	.000	.0921	.1796
		T 28day	.13011*	.02010	.000	.0863	.1739
	C 14day	C 4day	-.04401-*	.02010	.049	-.0878	-.0002
		T 4day	-.13202-*	.02010	.000	-.1758	-.0882
		T 14 day	-.06697-*	.02010	.006	-.1108	-.0232
		C 28day	.00383	.02010	.852	-.0400	.0476
		T 28day	-.00191	.02010	.926	-.0457	.0419
	T 14 day	C 4day	.02296	.02010	.276	-.0208	.0667
		T 4day	-.06505-*	.02010	.007	-.1088	-.0213
		C 14day	.06697*	.02010	.006	.0232	.1108
		C 28day	.07079*	.02010	.004	.0270	.1146
		T 28day	.06505*	.02010	.007	.0213	.1088
	C 28day	C 4day	-.04783-*	.02010	.035	-.0916	-.0040
		T 4day	-.13585-*	.02010	.000	-.1796	-.0921

		C 14day	-.00383	.02010	.852	-.0476	.0400
		T 14 day	-.07079-*	.02010	.004	-.1146	-.0270
		T 28day	-.00574	.02010	.780	-.0495	.0380
	T 28day	C 4day	-.04209	.02010	.058	-.0859	.0017
		T 4day	-.13011-*	.02010	.000	-.1739	-.0863
		C 14day	.00191	.02010	.926	-.0419	.0457
		T 14 day	-.06505-*	.02010	.007	-.1088	-.0213
		C 28day	.00574	.02010	.780	-.0380	.0495

*. The mean difference is significant at the 0.05 level.

Residuals

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.904	3	.635	212.984	.000
Within Groups	.024	8	.003		
Total	1.928	11			

Multiple Comparisons

Dependent Variable:

LSD

(I) Factors		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control fish	Treatment Fish	-.68400-*	.04458	.000	-.7868	-.5812
	Control Bivalve	-.06233	.04458	.200	-.1651	.0405
	Treatment Bivalve	-.93067-*	.04458	.000	-1.0335	-.8279
Treatment Fish	Control fish	.68400*	.04458	.000	.5812	.7868
	Control Bivalve	.62167*	.04458	.000	.5189	.7245
	Treatment Bivalve	-.24667-*	.04458	.001	-.3495	-.1439
Control Bivalve	Control fish	.06233	.04458	.200	-.0405	.1651
	Treatment Fish	-.62167-*	.04458	.000	-.7245	-.5189
	Treatment Bivalve	-.86833-*	.04458	.000	-.9711	-.7655
Treatment Bivalve	Control fish	.93067*	.04458	.000	.8279	1.0335

	Treatment Fish	.24667*	.04458	.001	.1439	.3495
	Control Bivalve	.86833*	.04458	.000	.7655	.9711

*. The mean difference is significant at the 0.05 level.

ANOVA

ALK.PHOS

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7871.611	5	1574.322	16.239	.000
Within Groups	1163.333	12	96.944		
Total	9034.944	17			

Multiple Comparisons

Dependent Variable: ALK.PHOS

LSD

		Mean Difference			95% Confidence Interval	
(I) Groups	(J) Groups	(I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
C 4day	T 4day	-12.33333-	8.03926	.151	-29.8494-	5.1827
	C 14day	-25.33333-*	8.03926	.008	-42.8494-	-7.8173-
	T 14 day	-34.33333-*	8.03926	.001	-51.8494-	-16.8173-
	C 28day	-28.33333-*	8.03926	.004	-45.8494-	-10.8173-
	T 28day	-67.33333-*	8.03926	.000	-84.8494-	-49.8173-
T 4day	C 4day	12.33333	8.03926	.151	-5.1827-	29.8494
	C 14day	-13.00000-	8.03926	.132	-30.5160-	4.5160
	T 14 day	-22.00000-*	8.03926	.018	-39.5160-	-4.4840-
	C 28day	-16.00000-	8.03926	.070	-33.5160-	1.5160
	T 28day	-55.00000-*	8.03926	.000	-72.5160-	-37.4840-
C 14day	C 4day	25.33333*	8.03926	.008	7.8173	42.8494
	T 4day	13.00000	8.03926	.132	-4.5160-	30.5160
	T 14 day	-9.00000-	8.03926	.285	-26.5160-	8.5160
	C 28day	-3.00000-	8.03926	.716	-20.5160-	14.5160
	T 28day	-42.00000-*	8.03926	.000	-59.5160-	-24.4840-
T 14 day	C 4day	34.33333*	8.03926	.001	16.8173	51.8494
	T 4day	22.00000*	8.03926	.018	4.4840	39.5160
	C 14day	9.00000	8.03926	.285	-8.5160-	26.5160
	C 28day	6.00000	8.03926	.470	-11.5160-	23.5160

	T 28day	-33.00000-*	8.03926	.001	-50.5160-	-15.4840-
C 28day	C 4day	28.33333*	8.03926	.004	10.8173	45.8494
	T 4day	16.00000	8.03926	.070	-1.5160-	33.5160
	C 14day	3.00000	8.03926	.716	-14.5160-	20.5160
	T 14 day	-6.00000-	8.03926	.470	-23.5160-	11.5160
	T 28day	-39.00000-*	8.03926	.000	-56.5160-	-21.4840-
T 28day	C 4day	67.33333*	8.03926	.000	49.8173	84.8494
	T 4day	55.00000*	8.03926	.000	37.4840	72.5160
	C 14day	42.00000*	8.03926	.000	24.4840	59.5160
	T 14 day	33.00000*	8.03926	.001	15.4840	50.5160
	C 28day	39.00000*	8.03926	.000	21.4840	56.5160

*. The mean difference is significant at the 0.05 level.

ANOVA

GOT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	########	5	########	31.115	.000
Within Groups	67090.000	12	5590.833		
Total	########	17			

Multiple Comparisons

Dependent Variable:

LSD

(I) Groups		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
C 4day	T 4day	-98.33333	61.05098	.133	-231.3520	34.6853
	C 14day	-140.33333-*	61.05098	.040	-273.3520	-7.3147
	T 14 day	########	61.05098	.124	-234.0187	32.0187
	C 28day	-315.33333-*	61.05098	.000	-448.3520	-182.3147
	T 28day	-664.00000-*	61.05098	.000	-797.0187	-530.9813
T 4day	C 4day	98.33333	61.05098	.133	-34.6853	231.3520
	C 14day	-42.00000	61.05098	.505	-175.0187	91.0187
	T 14 day	-2.66667	61.05098	.966	-135.6853	130.3520
	C 28day	-217.00000-*	61.05098	.004	-	-83.9813

					350.0187	
	T 28day	-565.66667-*	61.05098	.000	-698.6853	-432.6480
C 14day	C 4day	140.33333*	61.05098	.040	7.3147	273.3520
	T 4day	42.00000	61.05098	.505	-91.0187	175.0187
	T 14 day	39.33333	61.05098	.532	-93.6853	172.3520
	C 28day	-175.00000-*	61.05098	.014	-308.0187	-41.9813
	T 28day	-523.66667-*	61.05098	.000	-656.6853	-390.6480
T 14 day	C 4day	101.00000	61.05098	.124	-32.0187	234.0187
	T 4day	2.66667	61.05098	.966	-130.3520	135.6853
	C 14day	-39.33333	61.05098	.532	-172.3520	93.6853
	C 28day	-214.33333-*	61.05098	.004	-347.3520	-81.3147
	T 28day	-563.00000-*	61.05098	.000	-696.0187	-429.9813
C 28day	C 4day	315.33333*	61.05098	.000	182.3147	448.3520
	T 4day	217.00000*	61.05098	.004	83.9813	350.0187
	C 14day	175.00000*	61.05098	.014	41.9813	308.0187
	T 14 day	214.33333*	61.05098	.004	81.3147	347.3520
	T 28day	-348.66667-*	61.05098	.000	-481.6853	-215.6480
T 28day	C 4day	664.00000*	61.05098	.000	530.9813	797.0187
	T 4day	565.66667*	61.05098	.000	432.6480	698.6853
	C 14day	523.66667*	61.05098	.000	390.6480	656.6853
	T 14 day	563.00000*	61.05098	.000	429.9813	696.0187
	C 28day	348.66667*	61.05098	.000	215.6480	481.6853

*. The mean difference is significant at the 0.05 level.

ANOVA

GPT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	19305.611	5	3861.122	77.222	.000
Within Groups	600.000	12	50.000		
Total	19905.611	17			

Multiple Comparisons

Dependent Variable:

LSD

(I) Groups		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
C 4day	T 4day	5.00000	5.77350	.403	-7.5794	17.5794
	C 14day	-46.33333*	5.77350	.000	-58.9127	-33.7540
	T 14 day	-71.00000*	5.77350	.000	-83.5794	-58.4206
	C 28day	-55.66667*	5.77350	.000	-68.2460	-43.0873
	T 28day	-79.66667*	5.77350	.000	-92.2460	-67.0873
T 4day	C 4day	-5.00000	5.77350	.403	-17.5794	7.5794
	C 14day	-51.33333*	5.77350	.000	-63.9127	-38.7540
	T 14 day	-76.00000*	5.77350	.000	-88.5794	-63.4206
	C 28day	-60.66667*	5.77350	.000	-73.2460	-48.0873
	T 28day	-84.66667*	5.77350	.000	-97.2460	-72.0873
C 14day	C 4day	46.33333*	5.77350	.000	33.7540	58.9127
	T 4day	51.33333*	5.77350	.000	38.7540	63.9127
	T 14 day	-24.66667*	5.77350	.001	-37.2460	-12.0873
	C 28day	-9.33333	5.77350	.132	-21.9127	3.2460
	T 28day	-33.33333*	5.77350	.000	-45.9127	-20.7540
T 14 day	C 4day	71.00000*	5.77350	.000	58.4206	83.5794
	T 4day	76.00000*	5.77350	.000	63.4206	88.5794
	C 14day	24.66667*	5.77350	.001	12.0873	37.2460
	C 28day	15.33333*	5.77350	.021	2.7540	27.9127
	T 28day	-8.66667	5.77350	.159	-21.2460	3.9127
C 28day	C 4day	55.66667*	5.77350	.000	43.0873	68.2460
	T 4day	60.66667*	5.77350	.000	48.0873	73.2460
	C 14day	9.33333	5.77350	.132	-3.2460	21.9127
	T 14 day	-15.33333*	5.77350	.021	-27.9127	-2.7540
	T 28day	-24.00000*	5.77350	.001	-36.5794	-11.4206

T 28day	C 4day	79.66667*	5.77350	.000	67.0873	92.2460
	T 4day	84.66667*	5.77350	.000	72.0873	97.2460
	C 14day	33.33333*	5.77350	.000	20.7540	45.9127
	T 14 day	8.66667	5.77350	.159	-3.9127	21.2460
	C 28day	24.00000*	5.77350	.001	11.4206	36.5794

*. The mean difference is significant at the 0.05 level.

الخلاصة:

يهتم علم السموم البيئية في المقام الأول بإطلاق الملوثات السامة في البيئة ، وتوزيعها ومصيرها في المحيط الحيوي وخاصة في سلاسل الغذاء، والتقدير النوعي والكمي للاستجابات السامة في النظم البيئية ومكونات النظام البيئي.

تبحث الدراسة الحالية في الآثار الضارة للتعرض لمبيد الديازينون العضوي السام لكائنات البيئة المائية والتاثيرات في بعض الخصائص الفسيولوجية ومتبقياتة في أنسجة الكائنات الحية. تم اختيار نوعين من كائنات البيئة المائية لتقدير سمية الديازينون، هما سمك الكارب الشائع *Cyprinus carpio* و ذوات الصدفتين من العائلة الرخوية *Sinanodonta woodiana*.

تم تقدير التركيز نصف القاتل لمدة 96 ساعة (LC50) لمبيد الديازينون لسمك *C. carpio* حيث تم استخدام إجمالي 72 فرد من الاسماك، تم تعريضهم لتركيزات مختلفة من الديازينون (0 ، 6، 10، 15 ملغرام/ لتر) ذات نقاوة (60 %) ، حيث وجدت قيمة (LC50) لمدة 96 ساعة لتكون 9.5 ملغرام / لتر. تم تعريض الأسماك لمدة 28 يوم (التعريض المزمن) لتراكيز واحد قيمتة 25% من تركيز الجرعة القاتلة لنصف العدد (2.37 ملغرام/ لتر). التأثير السلوكي تم دراستة في بداية التجربة. بعد (4 ، 14 و 28) يوم تم جمع عينات الدم للتحليلات البايوكيميائية ، في اليوم الاخير من التجربة جمعت عينات لتقدير التأثيرات النسيجية و التركيز المتبقي من المبيد في العضلات، الموشرات الجزيئية (تلف الحمض النووي) اجريت في اليوم الاخير من التجربة.

بالنسبة لمحار *S. woodiana* تم حساب LC50 حيث استخدم 108 فرد من المحار وتم تعريضها لتركيزات مختلفة من الديازينون (0، 10، 15، 20، 25، 30 ملغلرام/ لتر) (ذات نقاوة 60٪). حيث كانت قيمة (LC50) لمدة 96 ساعة هي 13.8 ملغرام/ لتر. تم تعريض ذوات الصدفتين ل 25% من تركيز الجرعة القاتلة لنصف العدد (3.45 ملغرام/ لتر)، التأثير السلوكي تم دراستة في بداية التجربة. بعد (4 ، 14 و 28) يومًا تم جمع عينات ذوات الصدفتين، لتحليل الموشرات البايوكيميائية، وفي اليوم الاخير تم دراسة التغيرات النسيجية ، الموشرات الجزيئية (تلف الحمض النووي) وتقدير التركيز المتبقي من المبيد.

لوحظت التأثيرات السلوكية والمظهرية في دراستنا، في الأسماك هناك (تلف الزعنفة الذيلية ، فرط النشاط إلى قلة النشاط ، تغير في تصبغ الجلد ، الغرق في القاع ، سقوط قشور الأسماك). بينما في المحار

ذات الصدفتين (أغلق المحار صماماته بإحكام لفترات أطول وفتحها قليلاً لفترة قصيرة، وتمدد القدم والسيفون من وقت لآخر) عند التعرض لمبيد الديازينون.

أظهرت النتائج في معاملات دم اسماك الكارب انخفاض غير معنوي عند ($p < 0.05$) في قيمة (عدد كرات الدم الحمراء والكريات البيضاء و الصفيحات الدموية) وزيادة غير معنوية عند ($p < 0.05$) في (تركيزات الهيموغلوبين) مقارنة مع مجموعة السيطرة.

أظهر قيمة البروتين الكلي في الأسماك انخفاضًا معنويًا في اليوم الرابع والثامن والعشرين ، وزيادة معنوية في اليوم الرابع عشر ($p < 0.05$). في القواقع اظهرت النتائج قيمة البروتين الكلي، هناك زيادة كبيرة في اليوم الثامن والعشرين.

أظهرت نتائج الدراسة الحالية لقيم انزيمات المضادة للأكسدة SOD انخفاض غير معنوي عند ($p < 0.05$) لأسماك الكارب مقارنة بمعامل السيطرة. بينما يوجد ارتفاع غير معنوي في قيم SOD في القواقع. اما فعالية انزيم CAT سجلت ارتفاع في كلا الكائنين لها تغير كبير عند ($p < 0.05$) مقارنة بمجموعة التحكم عند التعرض لمبيد للديازينون.

كانت نتيجة انزيم مالونديالديهيد فيها زيادة غير معنوية عند ($P < 0.05$) في الأسماك. بينما سجل مع القواقع زيادة معنوية عند ($p < 0.05$). هناك انخفاض معنوي عند ($p < 0.05$) في نشاط انزيم Acetylcholinesterase في دم *C. carpio* و *S. woodiana*.

الدراسة الحالية سجلت زيادة معنوية في قيم وظائف الكبد في AST، ALT، ALP عند ($p < 0.05$).

كانت قيمة المتبقي من المبيد في الأسماك (0.696 ± 0.04) ميكروغرام / غرام ، وفي القواقع (0.943 ± 0.077) ميكروغرام / غرام. هناك ارتفاق في القيم في مجاميع المعالجة مقارنة مع مجموعة السيطرة.

بالنسبة للتغيرات النسيجية للأسماك والمحار، نلاحظ تغيرًا في الأنسجة ونخرًا في بعض المناطق وتلفًا في التقاطعات التي تحافظ على التركيب النسيجي العام للعضلات وكذلك الطبقة تحت المخاطية الأكبر حجمًا عند المقارنة لمجموعة السيطرة.

الموشرات الجزيئية (تلف الحمض النووي) ، أظهرت النتيجة زيادة معنوية عند (p 0.05) في طول الذيل في ذوات الصدفتين والأسماك عند التعرض للديازينون في اليوم الثامن والعشرين.

جمهورية العراق
وزارة التعليم العالي و البحث العلمي
جامعة بابل / كلية العلوم
قسم علوم الحياة

التأثيرات السمية البيئية لمبيد الديازينون على سمك *Cyprinus carpio* ومحار *Sinanodonta woodiana*

أطروحة مقدمة

الى مجلس كلية العلوم/ جامعة بابل كجزء من متطلبات نيل درجة الدكتوراه فلسفة في

العلوم / علوم الحياة

من قبل

جعفر بدر عبيد محمد الجبوري

بكالوريوس علوم البيئة والتلوث /جامعة الكوفة 2013
ماجستير علوم البيئة /جامعة نكرجونا - الهند 2016

بأشراف

أ.د. مؤيد جاسم ياس العماري

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