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RESEARCH ARTICLE

MALATHION TOXICITY ON FISH - A REVIEW

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ABSTRACT

Fishes are very sensitive to the changes in their aquatic environment. For this reason, they are known as the bio-indicator species to monitor the water pollution. Organophosphate pesticides are widely used amidst various group of pesticide in intensive agricultural practices to protect the crops from various pest and diseases owing to their high insecticidal property, low mammalian toxicity, low persistence and rapid biodegradability in the ecosystem. Pesticide exposure may also fatal to many non-target organisms like fish where it hampers its health through impairment of metabolism, occasionally leading to the death of the fish. Malathion ($C_{10}H_{19}O_6PS_2$), one of the earliest organophosphate insecticides is being extensively used as dust, emulsion, and vapour to control wide variety of insect pests under different conditions. Malathion, one of the most extensively studied pesticides, may induce many significant changes in fish. Present study is aimed to review the toxicological effects on haematological parameters, physical parameters, biochemical parameters, behavioural changes, neurotoxic, histopathological alterations, respiratory responses, bioaccumulation and chromosomal changesin fishes exposed to the organophosphate pesticide Malathion.

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INTRODUCTION

Since the removal of the organochlorine insecticide owing to its ceaseless harmful effect, organophosphate (OP) insecticides have been chosen as the most widely favoured insecticide in today's world to make the crop free from pest for greater productivity. All OPs apparently share the common mechanism of choline esterase inhibition at nerve endings resulting excess acetylcholine in the nerve ending which overstimulates the effector organ. Malathion ($C_{10}H_{19}O_6PS_2$), one of the earliest organophosphate insecticides developed in 1950, was first registered for use in the United States in 1956 by the United States Department of Agriculture (USDA), which is now regulated by the United States Environmental Protection Agency (USEPA, 2006). This pesticide is being extensively used as dust, emulsion, and vapour to control wide variety of insect pests under different conditions. Malathion is one of the most effective organophosphorus insecticides used for the control of pest on greenhouses, nurseries, home and garden vegetables, field crops, fruits and domestic animals.In other words, it is used to control aphids, mites, scale, flies, leafhoppers, leaf miner, thrips, loopers, mealy bugs, spittlebugs, corn earworms, chinch bugs, grasshoppers, armyworms, boll weevils, bollworms, lice, ticks, ants, spiders, and mosquitoes.

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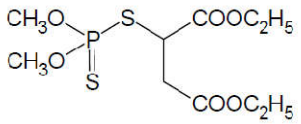
It is applied to alfalfa, clover, pasture and range grasses, non-agricultural land, cereal crops, cotton, sunflower, soybeans, sugar beets, corn, beans, blueberries, stored grain, and inside homes (Malathion Fact Sheet, 2000). International Union of Pure and Applied Chemistry (IUPAC) name for malathion is diethyl 2-dimethoxyphosphinothioylsulfanylbutanedioate. Chemical name is O,O-dimethyl dithiophosphate of diethyl mercaptosuccinate. It is also known as *S*-1,2-bis (ethoxycarbonyl) ethyl O,O-dimethyl phosphorodithioate. Structural formula ofMalathionis-



Malathion can enter into surface water through spray drift, during the time of application, and runoff after rainfall. Malathion undergoes three types of metabolic modifications in animals, namely oxidative, hydrolytic and the elimination of a methyl group catalysed by glutathione S-transferase (GST) (Toxicological Profile on Malathion, 2003). Malathion and its metabolite, malaoxon, is a more potent organophosphate than the parent compound, inhibit the enzyme acetyl cholinesterase and hence, prevent the hydrolysis of the neurotransmitter acetylcholine in the central and peripheral nervous systems. Thus, Inhibition of acetyl cholinesterase results in accumulation and continuous action of the neurotransmitter

acetylcholine at postsynaptic sites. The water solubility of Malathion is 145 mg/L at 20°C, and its vapour pressure is 3.97× 10-5 mm Hg at 30°C. It has an octanol/water partition coefficient (log KOW) of 2.36, and bioconcentration has not been observed in majority of the studies (US EPA, 2015). Bioactivation of Malathion is necessary for it to exert its toxic effect. Bioactivation is primarily mediated by cytochrome P450 enzymes in the liver, which create the active metabolite malaoxon through oxidative sulfuration (Malathion, Technical Fact Sheet, 2010). Although Organophosphorous pesticides (OPs) malathion has rapid biodegradability, susceptible to photolysis as well as hydrolysis and lesser persistency in the environment, with 81 to 94% degradation occurring in various non-sterile soils within ten days, yetit leaves residues in the soil and water for several days after their application, and pose a constant threat to non-target organisms, especially fish (Magare and Patil, 2000). Rauf, 2015 cited Martinez and Leyhe, 2004 and stated that the half-life of malathion is up to 11 days and it degrades into malaoxon which is more toxic than the parent.

Effects on Hematological parameters

Venkataraman and Sandhya Rani, 2013 studiedacute toxicity and blood profile of freshwater fish, *Clariasbatrachus (Linn.)* exposed to sub-lethal concentration of 0.05, 0.25 and 0.5 ppm malathion after a time interval of 24, 48, 72 and 96 hours. They have calculated the 96 h LC $_{50}$ of malathion as 1 ppm. Both the researchers had noticed that with increase in the concentration and exposure period of malathion, there were significant reductions in Hb (haemoglobin), haematocrit (PCV), RBC (Red Blood Cell Count) and MCV (Mean Corpuscular Volume) levels and increase in WBC (White Blood Cell Count), MCHC (Mean Corpuscular Haemoglobin Concentration) and MCH (Mean Corpuscular Hemoglobin) levels in the malathion treated fish in contrary to the normal values of haematological parameters in control group. According to the records of this study, fishes exposed to 0.5 ppm (maximum concentration) for 96 h exposure of malathion exhibiteddecrease (2.36±0.04 x 106mm3) in RBC, whereas a significant risewas reported in WBC(40.91±0.20 x 103mm3), MCH (16.00±0.43 pg) andMCHC (52.83±1.86%) values. MCHC value reported with a substantial increase from 45.32 ± 0.90%(in 0.5 ppm for 24 h) to 52.83 ± 1.86% (in*/ 0.5 ppm for 96h) in treated fishes. On the other hand, fishes exposed to0. 05 ppm to 0.5 ppm malathion for 24 h to 96 h revealeda minor increase in their colorindex.

According to the researchers, acute anaemia resulting in *C. batrachus* owing to a decrease in the concentration of haemoglobin in blood is due to the effect of toxic malathion on gills, as well as decrease in oxygen. In this investigation, haemolysis was also predicted as one of the causes for reduction in Hb, RBC and PCV values. Increase in WBC count; reflect the occurrence of leucocytosis in the treated fish samples. Both the researcher cited Sudha, 2012 and mentioned probable cause of increase in WBC count firstly as a typical defensive response of the fish against a toxic invasion and secondly asleukaemia.Alterations in MCV, MCHC, MCH were considered responsible reason by the investigators after studying the findings of Shah, 2006 to direct or feedback responses of structural damage to RBC membranes which resulted in haemolysis and impairment in haemoglobin synthesis, stress related release of RBCs from the spleen (Shah, 2006).Finally both the researchers have concluded that malathion was moderately toxic to *C. batrchus*. Ahmad, 2012 in his study the effects of sub-lethal exposure of malathion on haematological parameters of *Clarias gariepinus* observed decreased value of RBC and WBC counts, hemoglobin (Hb) concentration and haematocrit (Ht) values as compared to the control fish after exposed to sublethal doses of malathion. The 96 h LC50 value was calculated to be 8.22 mg/L from which sub-lethal concentrations of 0.5, 1.0 and 2.0 mg/L malathion was exposed to the fishes for 4 weeks.

Effect on Physical Parameters

After determination of LC_{50}as 5.8 ppm by Kabeer *et al.*, 1981, fish in batches of 6 were exposed to sublethal concentration of 2 ppm malathion for 48 h. Based on the data reported by Kabeer *et al.* (1981), a slight but progressive decrease in the body weight of malathion treated fish in due course of timewere observed compared with normal fish which suggested the loss of some constituents of fish body. They have not reported any change in the water content of the normal and malathion exposed fish.

Effected on Biochemical Parameters

Venkataramana *et al.*, 2006, investigated the impact of sub lethal concentrations (0.05, 0.25 and 0.5 ppm) of malathion on heart muscle glycogen, protein and cholesterol in freshwater gobiid fish, *Glossogobius giuris* with an exposure period of 24, 48, 72 and 96 hr. They have analysed the changes in the glycogen, protein and cholesterol and found that these parameters werealtered during different intervals of malathion exposure. In this study, it was evident that at 24 hr exposure time, themuscle glycogen content (1.51±0.40 mg/g) was decreased by 47.93%in comparison to the glycogen content (2.90±0.72 mg/g) of control group of fish where as it was increased subsequently at0.25 ppm (3.96±0.85 mg/g) and 0.5 ppm (4.89±0.71 mg/g) exposure to malathion. At 48 hr and 72 hr exposure time to malathion glycogen content was reported to be maximum (3.20±0.58 mg/g and 3.50±0.45 mg/g respectively) at 0.25 ppm malathion in comparison to control group (2.20±0.25 mg/g and 3.30±0.33 mg/g respectively). However, after 96 hr the quantity of muscle glycogen showed a decreasing trend reaching 20.31% and 21.26% at 0.05 ppm malathion (2.51±0.36 mg/g) and 0.5ppm malathion (2.48±0.34 gm/g) exposure respectively.

Dezwaan and Zandee, 1999 and Chaudhari, 2000 reported possible cause of depletion of glycogen content in heart muscle as glycogenolysis, resulting in anaerobic glycolysis to cope up with the adverse condition. Venkataramana *et al.*, 2006 observed decreasing trend of muscle protein exposed to sub lethal doses malathion for 24 hrs exposure time. The trend in terms of percentage decrease was observed to be 28.75 %(14.32±0.98 mg/g), 23.08 % (15.46±1.45 mg/g) and 29.80% (14.11±1.41 mg/g) with 0.05, 0.25 and 0.5 ppm of malathion treatment respectively compared to control (20.10±0.85 mg/g). The researchers had also witnessed slight elevation of heart muscle protein exposed to 0.25 and 0.5 ppm of malathion after 48 and 72 hrs of treatment compared to both the control (22.00±1.58 mg/g and 19.10±0.13 mg/g) group. However, after 96 hrs of exposure to fish with 0.05 and 0.5 ppm of malathion, a declining trend was noticed by the investigators, attaining a minimum percentage of 29.45 (14.32±1.48 mg/g) and 32.66 (13.67±1.27 mg/g) as compared to control (20.03±2.52 mg/g) group of fish.Venkataramana *et al.,* 2006

cited the suggestions put forwarded by Krishnamohan *et al.*, 1985 and Chandravathy and Reddy, 1994 as decline in the muscle protein content may perhaps be due to reduced protein synthesis, increased proteolysis and also owing to utilization for metabolic processes under toxicity. Venkataramana *et al.*, 2006 also reported enhancement of muscle cholesterol level in the fish exposed to 0.05 ppm of sub lethal malathion and found a maximum level of cholesterol (1.01±0.15 mg/g)at 24h exposure time in comparison to control (0.75±0.75 mg/g). Nevertheless, after 48 hr exposure to 0.05 ppm malathion, the muscle cholesterol quantity increased by a percentage of 40.57 (0.97±0.14 mg/g) compared to control (0.69±0.14 mg/g). A similar fluctuating pattern was also observed by the researchersat 72 hr exposure period.

On the other hand, when the researchers exposed fishes to 0.5 ppm, the muscle cholesterol quantity started declining for 24, 48 and 72 hr. Yet, by 96 hr, they have noticed a decreasing trend in heart muscle cholesterol in the fishes and recorded the minimum percentage of 13.58 (0.70±0.15 mg/g), 14.81(0.69±0.19 mg/g) and 22.22 (0.63±0.18 mg/g) in 0.05, 0.25 and 0.5 ppm dose of malathion respectively compared to control (0.81±0.18 mg/g). Finally all the investigators concluded that fishes exposed to sub lethal concentration 0.5 ppm of malathion for 24 to 96 hrexhibited decrease in the muscle cholesterol level in the heart. Patil, and David, 2009 studied the hepatotoxic potential of malathion in the freshwater teleost, *Labeo rohita*, after exposing to a sublethal concentration (0.9 µl/L) of commercial grade malathion (50% Emulsified Concentration) for 5, 15 and 25 days. Calculated LC_{50} for 96 h to the fishes for malathion was reported to be 9.0 µl/L. Sublethal dose for this study was prepared following to Sprague, 1973.Both the scholars had observed that the total, structural and soluble proteins and Acetyl Choline Esterase activity werediminished, however free amino acids, protease activity and Acetyl Choline (Ach) were found to be enhanced in the fishes exposed to sublethal dose of malathion for 5 and 15 days. Nevertheless, both of them surprisingly noticed that fish exposedtomalathion for 25 days showed values nearer to the control fish and observed to be recovering to normalcy.

Patil, and David, 2009 described that the total, structural and soluble proteins were found to be decreased owing to the occurrence ofhigh proteolytic activity, and inefficiency in the protein biosynthesis. Another reason for this decrease inprotein levels was predicted by the investigators as protein degradation. By citing the findings of Hai *et al*., 1995, the author of the paper had mentioned oxidative stress as the reason for degradation which is a typical feature of organophosphate compounds, besides their in hibitory effect on AChE. According to the researchers oxidative stress might bring changes in free radical production which in turn overwhelms the endogenous antioxidant levels causing substantial cell damage and death. Finally the researchers had concluded that exposure to sublethalmalathion affects protein metabolism and the normal neural physiology of the liver. But recovery in 25 days might be a revitalization phenomenon as all organisms make every effort to overcome stress to survive. Recovery phenomenon as stated by the author of the paper was adaptive and even strategic. Ahmad, 2012 studied the effects of sub-lethal exposure of malathion on biochemical composition and haematological parameters of *Clarias gariepinus*. and reported elevated Plasma glucose level following reduced Liver and muscle glycogen level; nevertheless, plasma protein was reported to be decreased in fishes exposed to sublethal malathion. According to the findings of Ahmad, 2012; Alanine amino transferase (ALT), glutamate oxaloacetate transaminase (GOT) and glutamate pyruvic transaminase (GPT) activities increased in the fish exposed to sublethal malathion. But the researcher has noticed insignificant effect of malathion on magnesium and calcium ions as reduction in the concentration of Ca ions was recorded in the fish exposed to high dose of malathion and in the last period of exposure whereas Mg ions remain unchanged. According to Ahmad, 2012 significant hyperglycemia and hypo-proteinaemia was evident in the fish exposed to various doses of malathion. The investigator had witnessed that these alterations were more pronounced in the higher doses and in the last period of exposure. According to Ahmad, 2012 malfunctioning of the haematopoietic system due to Malathion exposure might be leading to reduction in different blood parameters. He believed that the elevated level of glucose reported in the blood of Malathion treated fish could be due to glycogenolysis to meet the increased energy demand. By citing the findings Pickering, 1981 and Winkaler *et al.,* 2007, Ahmad, 2012 stated that stress stimulated rapid secretion of Glucocorticoids and catecholamine hormones from adrenal gland which were known to produce hyperglycemia in animals. Elevation of these hormones could be attributable to boosted gluconeogenesis response of stressed fish in their effort to satisfy their new energy demands. Ahmad, 2012 concluded that higher activities of experimented enzymes registered in his investigation might be attributed to cellular damage caused to liver by malathion.

Roopavathy *et al.*, 2013 studied Toxicity of Malathion on Metabolic Activities in the River Cauvery of Tilapia Fish *(Oreochromis mossambicus) by* exposing sub lethal concentration (0.5 ppm) of malathion for 24, 48, 72 and 96 hr respectively on the fishes (Four groups where another group was kept as control which was free from the pesticide exposure. when the fishes were exposed to malathion (0.5 ppm) the protein and carbohydrate content were found to have decreased in all the experimental tissues namely muscle, gills, liver and kidney. They have cited the findings of Remia *et al.,* 2008 and stated possible reduction of protein content in the tissues as proteolysis and increased metabolism under toxicant stress. Likewise the researchers had cited Thenmozhi *et al.,* 2010, and anticipated the reason for decrease in carbohydrates contents as impairment of carbohydrate metabolism due to toxic effect. Fahmy, 2012 carried out an experimental study to assess the toxic effect of the insecticide malathion on oxygen consumption and some biochemical characteristics (total protein, carbohydrate and cholesterol in liver, muscle, kidney and gills) of the tilapia fish *Oreochromis niloticus* by exposing a sublethal concentration of 0.5 ppm for 24,48,72, and 96 h respectively. The investigator had found that the rate of oxygen consumption and all biochemical parameters were declined on comparison with control during all the exposure periods. The result of the study showed that protein, carbohydrate and the cholesterol content were significantly declined when the fishes were exposed to malathion (0.5 ppm) in all exposure time. The researcher cited the findings of Remia *et al.,* 2008 to enlighten proteolysis and increased metabolism under toxicant stress as the reason for the reduction of protein. Another reason for reduction in protein content was cited from the findings of Venkatramana *et al.*, 2006 as utilization of the proteins to mitigate the energy demand when the fish were under stress. The decrease in carbohydrates contents was reported to result in impairment of

carbohydrate metabolism due to toxic effect (Thenmozhi *et al.,* 2010). According to the researcher the possible cause of reduction of carbohydrate content in the fish might be the active glycogenolysis and glycolytic pathway to provide excess energy in stress condition. The decrease in cholesterol was found to be high in gills and less in muscles. The reduced cholesterol level may be due to the inhibition of cholesterol biosynthesis in the liver or due to reduced absorption of dietary cholesterol (Fahmy, 2012). However, it was reported that the decline of cholesterol may be due to utilization of fatty deposits instead of glucose for energy purpose (Kanagaraj *et al.,* 1993). Magar and Dube, 2013 investigated effect of Sub Lethal Concentration of Malathion on Metabolic Profiles and Histological Studies in Heart Tissue of *Channa punctatus* and found that the fresh water exposed to sub lethal concentration of malathion showed reduction in the level of protein in cardiac muscle of treated group of fish during 96 hours (64.20) compared with control (76.03) whereas total lipid content declined (40.93) during 96 hours compared with control (50.10). Glycogen content in cardiac muscle also reported to be decreased (8.56) during 96 hours compared with control (14.15). In a study carried out by Borthakur, 2006 to investigate the effect of sublethal malathion on three members of hepatic MFO enzyme system namely cytochrome P450, AHH, and XOD in *Heteropneustes fossilis,* a freshwater siluroid fish, reported significantly elevated ($P<0.05$) hepatic cytochrome P450 activity within 24 hours of 0.1 ppm malathion exposure and progressively increased to more than 20% over control within 15th day of exposure. It was followed by a steep rise in activity upto the 25th day ($P<0.001$). similar response was reported to 0.2 ppm malathion exposed group but a highly significant decrease ($P<0.05$) of cytochrome P450 activity was observed in the last five days of the exposure to 0.2 ppm malathion compared to that of the 0.1 ppm exposed group.

Behavioral Changes due to malathion exposure

Behavior is regarded as a convincing tool in ecotoxicology [Drummond and Russom, 1990; Cohn and MacPhail, 1996). In toxic environments, fish showed loss of equilibrium, irregular, erratic and darting swimming movements and reason forthese changes was reported to be inhibition of the enzyme acetylcholinesterase (AChE) activity which resulted accumulation of acetylcholine in cholinergic synapses triggering hyperstimulation in the fishes (Mushigeri and David, 2005). Naserabad *et al.*, 2015 investigated acute toxicity and behavioral changes of the Gold Fish (*Carassius auratus*) exposed to Malathion and Hinosan in due course of finding LC_{50}. The value of LC_{50} calculated by the researchers for 96h duration for malathionwas found to be 4.71 mg/L. They have noticed that fishes that were exposed to malathion started exhibiting behavioralchanges and clinical symptoms at 8 mg/L after 5 hours, but these changes and symptoms appeared after 2 hours when exposed to 16 mg/L malathion. According to the investigators behavioural changes included irregular, erratic and darting swimming movements of the fishes; hyper excitability, bruise in the caudal section, loss of equilibrium and sinking of the fishes to the bottom.

Naserabad *et al*., 2015 also calculated and reported the value of MAC (Maximum Allowable Concentration) for malathion at mean temperature 24±1 C^{o} as 0.471 mg/l, andLOEC (lowest observable effect concentration, which represents the initial toxicity threshold of a chemical) as 4 mg/lwhereas NOEC (No Observed Effect Concentration, which represent the concentration of toxicant that will not cause any effect) as 2 mg/l. Salwa and Ella, 2008 conducted an empirical study on Toxicity of malathion and its effect on the activity of acetylcholinesterase in various tissues of the grass carp, *Ctenopharyngodon idella* Val. The Acute toxicity (LC50 values) reported by them for 24 h, 48h, 72h, and 96h were 3.728 mg/l, 2.838 mg/l, 2.444 mg/l, and 2.138 mg/l respectively for grass carp. The safe concentrations of malathion was 0.0513 mg/l. The fish showed typical changes in behaviour when exposed to various concentrations of malathion. They have observed that the fishes experienced progressive lethargicity, loss of equilibrium, difficulty in respiration, exhibited convulsions, dashing against the wall of the experimental aquaria and short unpredictable bumpy body movements, settling to bottom before death. Light coloured skin was observed by the on the fishes exposed to malathion with an excessive amount of mucus secretion over the body. However, Control fish behaved normally according to their observation. According to the researchers, the fish exposed to malathion showed intense opercular movements due to hypoxic stress accompanied by a progressive inhibitory effect of this compound on the respiratory mechanism. Mount and Brungs, 1967 had recommended that the safe level of malathion for fishes can beat some point between 1/15 and 1/45 of the 96h LC_{50} concentration.

The toxicity of sub-lethal carbaryl and malathion to *Clarius batrachus* was studied in a static renewal bioassay for 48 hrs and 96 hrs by Yogesh *et al.,* 2009. The LC50 value of carbaryl for 48 hours was 13.24 ppm and 5.248 ppm respectively and for malathion 48 hours and 96 hours was 0.31 ppm and 0.25 ppm respectively. The acute toxicity was measured in terms of physical, morphological and behavioural changes for these doses of LC_{50}. The increase in opercular movement and bottom to upward movement of the fish to overcome on hypoxic condition was observed in the 48 hrs and 96 hrs dose. Chronic toxicity was observed exposing the dose of 0.05 ppm malathion for 45 days and it was observed that body weight decreased in chronic dose.Resting at bottom, excess secretion of mucus, colour change, the loss of equilibrium of fish were few behavioural change observed during chronic exposure period. The results revealed that malathion is more toxic to *Clarius batrachus* than carbaryl. Rauf, 2015 in his investigation on the acute toxicity of malathion as an aquatic pollutant on the behavior and hematological indices in Indian carp (*Cirrhinus mrigala*) reported LC_{50} values of malathion for 1, 24, 48, 72 and 96 hrson the test fish as 14.55 mg/L, 12.48 mg/L, 11.56 mg/L, 10.85 mg/L and 9.32 mg/L, respectively. During 96 hrsexposure to 9.32 mg/L of malathion, behavioral abnormalities such as hyperactivity, cough, convulsions, erratic swimming, loss of balance, rapid opercular movements, gill mucous secretion, surfacing and gulping of air were observed by him in the test fish. The hematological changes in exposed fish after 96 hrs exposure to malathion included a significant decrease in erythrocyte count, hemoglobin content, hematocrit, leukocyte count and a significant increase in neutrophils count as compared to the control fish.

Neurotoxic Effects

Salwaand Ella, 2008 found a significant decrease in acetylcholinesterase activity up to 72h in the brain, muscle, gill and liver tissues of the fishes exposed to malathion. As per the findings of the researchers, acetylcholinesterase activity was

reported to be inhibited maximum at 24 and 48 h intervals. The increased trend of the inhibition of acetylcholinesterase activity from 12 to 24 and 48 h and decreased trend from 48 h to 96 h suggested that inhibition of the enzyme activity by malathion was dependent on the duration of exposure. The investigators had assumed that the effect of malathion decreases after 48h probably due to its degradation.The differential inhibition (descending order) of acetyl cholinesterase activity in the four tested tissues (brain> muscle> gill>liver) may be due to the presence of isozymes with different affinities for the substrate and the inhibitor.

Histopathological Alterations

Rosety *et al.,* 2005 aimed to chronologically assess antioxidant response and histopathological changes in gills of gilthead seabream *Sparus aurata L* after acute exposure to malathion in order to assess their potential role as early-warning bio-indicators before irreversible damage occurs. Significant modifications in gill tissues exposed to a sublethal concentration of malathion (0.4 mg/l) for 24, 48, 72 and 96 h. were observed by the investigators on all of the studied detoxification enzymes (superoxide dismutase (SOD), catalase (CAT) and gluthathione peroxidase (GPX) ($p<0.05$). the researchers had found that catalase activity was decreased instead of increasing as it witnessed for other antioxidant enzymes. Hence, they have considered that these alterations were all time-dependent. According to the findings of these researchers, some secondary lamellae of the gill tissues showed tendency to fuse at 48 h of exposure, Whereas, histological examination exposed the clubbing and fusion of secondary lamellae, hyperplasia of the respiratory epithelium and a thickening of the basal membrane in the secondary lamellae at 72 h exposure. At 96 h, detachment of the epithelial layer triggered by strong edema at the primary lamellae and some areas in the gill tissues with comprehensive shrinkage of the secondary lamellar cells were also observed by them. Therefore, they have recommended those antioxidant enzymes as useful early-warning bioindicators of environmental pollution by malathion in the areas where it is proposed to be used in pest control activities. During the entire experimental period, they have not reported any mortality of the fishes.

In a study entitled "Malathion induced histological modifications in gills and kidney of *Carassius auratus gibelio*" carried out by Cristina *et al*., 2008, gills and kidney tissues were exposed to 0.05 mg/l malathion to see the histological changes for24 hours exposure period.These changes included vacuolization of cytoplasm, changes in cell and nuclear volumes, detached cubic epithelial cells lining and renal tubuli from the basal membrane. They had observed these damages continued in subsequent 48 and 72 hours exposure time to sublethal malathion. These included increased cytoplasm acidophily in epithelial cells and appearance of melanomacrophages aggregates following 48 hours' exposure. In addition to these changes the researchers had also noticed necrotic renal tubuli, pycnotic and hypertrophic nuclei inside the cells. Following 72 hours of exposure, they had reported contraction of glomeruli, enlargement of Bowman's space, penetrating lymphocytes into the renal epithelium and narrowed lumen of renal tubuli. Hence, they had assumed that the changes in the size and structure of the epithelial cells, the narrow lumen of the renal tubuli and nuclear changes indicated alteration in kidney physiology and functions. According to the finding of this research, malathion treated fishes showed histological changes like epithelial ruptures, secondary lamellae fusion and hyperplasia of branchial epithelium in the gills structure following 48 hours exposure. After 72 hours, vascular congestion was observed by the investigators. As stated by the researchers, lamellar fusion and secondary lamellae hyperplasia possibly be protective behaviour which might decrease gill vulnerability. The researchers assumed that structural changes like lamellar fusion and secondary lamellae hyperplasia would have induced suffocation in the fish resulting increase in the diffusion distance between the respiratory blood and xenobiotics.

In a study carried out by Magar and Shaikh, 2013 on effect of malathion on detoxifying organ likekidney and liver , the fresh water fish *Channa punctatus* exposed to sublethal concentration of commercial grade malathion (50% EC) for period of 7 days exhibited histopathological alterations in the experimental organs. Both of them had recorded highly degenerative renal and collecting tubules with severe necrosis and spaces in Bowman's capsule as well as shrinkage of glomeruli and renal tubules. According to the findings of this research work, liver tissues of *Channa punctatus* exposed to malathion for 4 days showed degeneration of cytoplasm and vacuolization of hepatocytes. Pugazhvendan *et al.,* 2009 investigated the Effect of Malathion Toxicity in the Freshwater fish *Ophiocephalus punctatus* and observed histopathological alterations on the fish. They have estimated the LC_{50} value of the malathion for the freshwater fish, *Ophiocephalus punctatus* which was found to be 16µl/L. The upper and lower 90% confidence limits reported by the researchers were found to be 20 µl/L and 12 µl/L respectively. According to the findings of the research, the protein content in the muscle of the control fishes displayed highest activity at 7th day (10.25 ± 0.4mg/g) whereas the lower value was witnessed at 0th day i.e. (8.5 ± 0.4mg/g) in the control group. On the other hand, fishes exposed to malathion showed very lowest value of muscle protein i.e., (6.25 ± 0.1mg/g) following 7th day of experiment. The histology of brain was observed to be normal and uniformly aligned by the neural cells of control fish. The fishes exposed to malathion exhibited disintegrated and severe damaged neural cells. The broke down of neural bundles were seen. Likewise, normal architecture was reported by the investigators in the gills of control fishes. The researchers had observed severe damage, noticeable edema and active secretion of mucous in the experimental fish gill. Epithelial cells of the secondary lamellae were observed to be destructed, few lamellae were curled which finally led to congestion and haemorrhage of gills. Gills lost it natural colour following 7 dyas of exposure of the pesticide.

Pugazhvendan *et al,* 2009, in their studies, observed normal histological architecture of liver in control fish but the histopathology of experimental fish liver showed proliferation of ducted cells and appearance of small spaces between hepatic cells. The liver tissue was found to be degenerative and scattered with necrosis.They had observed normal texture of ovary in control fish. In experimental fish, inhibited oocyte development, broken germinal vesicles, disappearance of yolk granules and many disturbed oogonia were observed by the investigators. Magar and Dube, 2013 investigated effect of Sub Lethal Concentration of Malathion on Metabolic Profiles and Histological Studies in Heart Tissue of *Channa punctatus* and found normal structure of heart of *Channa punctatus*. In malathion treated fish, cardiac muscle were observed to be shown congestion and atrophy. The investigator observed

vacuolar degeneration of muscle fibrealong with the separation of Cardiac muscle fibres from each other. They have also noticed haemorrhage and haemolysis along with aggregation of inflammatory cells in the myocardial fibres of malathion treated fish.

Effect on Respiratory Responses

Magar and Shaikh, 2012, made an attempt to investigate the effect of malathion on oxygen consumption of fresh water fish *Channa punctatus* by exposing a sublethal dose of 0.8 ppm for a period of 24 hours, 48 hours, 72 hours and 96 hours. In this investigation total oxygen consumption in treated group of fishes was recorded to be 2.37, 1.71, 0.86 and 0.76 ml (C.C.) of O2/animal/hr during 24, 48, 72 and 96 hours respectively compared to 2.78 ml (C.C.) of O2/animal/hr recorded in control fishes. The calculated rate of oxygen consumption was found to be 0.067, 0.048, 0.024 and 0.021 ml (C.C.) of O2/gm/hr during 24, 48, 72 and 96 hours respectively which was reduced as compared with control (0.079 ml (C.C.) of O2/gm/ hr). The result indicated that the rate of oxygen consumption was reported to be decreased subsequently from 24 hours to 92 hours in the experimental fish compared to control.

respiratory distress as symptoms of pesticide toxicity and reported possible cause of the decreased oxygen consumption of the malathion treated fish as the absorbance of additional pesticide through the gills of the fish

Bioaccumulation of malathion

According to the Thurston Country review summary, malathion was considered to have moderate potential to bind to fish or animal fat and tissues and the octanol/water partition coefficient can indicate about this. A range of bioconcentration factors from 87 – 119 was reported from studies on freshwater fish, which indicated moderate accumulation of this pesticide in fishes. It was confirmed from the study on the fish accumulation that malathion was quickly eliminated from the fish when fishes were moved to clean water (depuration) (Thurston County Review Summary on malathion, 2012). The majority of the data available on the bioaccumulation of malathion suggested that malathion might be bioconcentrated but it is quickly metabolized or depurated from the tissue of aquatic organisms. Hence, the pesticide was not expected to be biomagnified in the food chain in such a way that it would create exposure threats to human (US EPA, 2000, Howard, 1991).

Table 1. Showing LC_{50} values of Malathion on fishes (compiled by Author)

Sl No	Test Species	Duration	LC50 value	Reference
1	*Clarias batrachus (Linn.)*	96 h	1 ppm	Venkataraman and Sandhya Rani, 2013
2	*Clarias gariepinus*	DO	8.22 mg/L	Zubair Ahmad, 2012.
3	*Tilapia mossambica (Peters).*	48 h	5.8 ppm	Kabeer *et al.*, 1980.
4	*Labeo rohita*	96 h	9.0 µl/L.	Vineetkumar Kallappa Patil, and Muniswamy David, 2009.
5	*Carassius auratus*	96 h	4.71 mg/L	Saeid Shahbazi Naserabad *et al*., 2015.
6	*Ctenopharyngodon idella Val.*	24 h 48h 72h 96h	3.728 mg/l, 2.838 mg/l 2.444 mg/l 2.138 mg/l	Salwa M. and Abou El Ella, 2008.
7	*Clarius batrachus*	48h 96h	0.31 ppm 0.25 ppm	Yogesh *et al.,* 2009.
8	*Ophiocephalus punctatus*	96h	16 µl/L	S.R. Pugazhvendan *et al.,* 2009.
9	*Gambusia affinis*	96h	0.7 ppb	
10	*Onchorhynchus mykiss*	96h	4.1 ppb	
11	*Lepomis macrochirus*	96h	30 ppb	
12	*O. clarki*	96h	174 ppb	USEPA, 2006.
13	*Perca flavescens*	96h	263 ppb	
14	*Tilapia mosambica*	96h	2000 ppb	
15	*Cyprinus carpio*	96h	6590 ppb	
16	*Oryzia latipes*	96h	40,000 ppb	
17	*Ameiurus melas*	96h	11.8 mg/L	Martinez and Leyhe, 2004.
18	*Hetereopneustes fossilis*	96h	15.3 mg/L	
19	*Juvenile C. mrigala*	96h	9.32 mg/L	Rauf, 2015.
20	*Coho Salmon*	96h	174 µl/L	
21	*Cutthroat salmon*	96h	239 µl/L	
22	*Rainbow trout*	96h	97 µl/L	Mayer and Ellersieck, 1986.
23	*Brown trout*	96h	101 µl/L	
24	*Lake trout*	96h	109 µl/L	

Same result was reported by Fahmy, 2012. Kabeer *et al.,* 1980, in their study Effect of malathion exposure on some physical parameters of whole body and on tissue cations of teleost, *Tilapia mossambica* (Peters), the rate of oxygen consumption was reported to be increased considerably during the first 24 h of exposure, but it was decreased subsequently and yet this was statistically not considered significant. According to the researchers the initial increase in the rate of oxygen consumption suggested the acceleration of oxidative metabolism which declined after 24 h when compared to normal fish. Decline in oxygen consumption was in accordance with the reduced levels of oxidative enzymes after 48 h of malathion exposure. Kabeer *et al.,* 1980 cited Ferguson and Goodyear, 1967 and Ferguson *et al.,* 1966 and stated

Effects on chromosome

Journal of pesticide reform, 2003 cited description of the National Institute for OccupationalSafety and Health (NIOSH) who analysed 29 laboratory studies published between 1978 and 1995 and based on the genetic damage caused by malathion and they hadstated malathion as a mutagen. These studies included tests on bacteria, fruit flies, mice, hamsters, fish, and human cell cultures. Moradi *et al*., 2012 conducted a study for the evaluation of DNA damage in *Cyprinus carpio* (L. 1758) exposed to malathion using Single Cell Gel Electrophoresis and found that the fish specimens exposed to diverged concentrations of malathion revealed

Table 2. Showing LC_{50} values and ecotoxicity of Malathion on fishes

Species	Scientific name	% a.i.	LC_{50} (96h) (ppb)	Toxicity category
Bluegill sunfish	*Lepomis macrochirus*	95	30	very highly toxic
Redear sunfish	*Lepomis microlophus*	95	62	very highly toxic
Rainbow trout	*Oncorhynchus mykiss*	95	4.1	very highly toxic
			32	very highly toxic
			30	very highly toxic
Yellow perch	*Perca flavescens*	95	263	highly toxic
Largemouth bass	*Micropterus salmoides*	95	250	highly toxic
Carp	*Cyprinus carpio carpio*	95	6590	moderately toxic
Fathead minnow	*Pimephales promelas*	95	8650	moderately toxic
Channel catfish	*Ictalurus punctatus*	95	7620	moderately toxic
Salmon	*Oncorhynchus kisutch*	95	170	highly toxic
Cutthroat trout	*Oncorhynchus clarki*	95	174	highly toxic
Brown trout	*Salmo trutta*	95	101	highly toxic
Lake trout	*Salvelinus namaycush*	95	76	very highly toxic
Black bullhead catfish	*Ameiurus melas*	95	11700	slightly toxic
Green sunfish	*Lepomis cyanellus*	95	146	highly toxic
Walleye	*Stizostedion vitreus*	95	64	very highly toxic
Tilapia	*Tilapia mossambica*	95	2000	moderately toxic
Goldfish	*Carassius auratus*	95	10700	moderately toxic

Source: EFED chapter and Pesticide Ecotoxicity Database.

significantly greater DNA damage in their blood cells than the control group. Fishes were exposed to different non-lethal concentrations (0.5, 1.5 and 3 mgL-1) of the malathion for 96 hour. The amount of DNA damage in the cells was estimated by the researchers from visual classification of cells into five type "comets" according to the tail length (Cavas, 2011; Lee and Steinert, 2003); 0: undamage, 1: low damage, 2: moderate damage, 3: high damage and 4: complete damage. Genetic Damage Index (GDI) was calculated by them using the following formula.

GDI = (n1+2n2+3n3+4n4) / (Σ / 100), where, n1: Minimum damage, n4: Maximum damage, Σ: Total number of the cells.

The DNA damage reported by the researchers was observed to be concentration dependent ($P<0.05$). The fish exposed to the lower concentration of malathion (0.5 mgL-1) shown relatively an insignificant GDI compared to control. Similar results were reported by Kumar *et al.,* 2010 on *Channa punctatus*. Kushwaha *et al.,* 2000 on *C. punctatus* following exposure to malathion.

Lethal concentration (lc_{50}) and ecotoxicity of malathion on fishes

Lethal Concentration (LC_{50}) and ecotoxicity of Malathion on different species of fishes are presented in the Table 1 and Table 2.

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