EFFECT OF METHYL PARATHION ON THE PHYSIOLOGY OF TILAPIA MOSSAMBICA

BHAWNA SRIVASTAVA, REDDY P.B

Abstract: Parathion is an organophosphate insecticide and extensively used to control harmful insects of agriculture. The present study is aimed to study the toxicological impacts of parathion (Methyl Parathion) on oxygen consumption. The toxicity tests were conducted by static renewal bioassay method on the juveniles of teleost fish Tilapia mossambica (Peters). The fish of 15±1g was exposed to three different sub lethal concentrations of methyl parathion for 24,48,72,96 hours. The probit analysis showed that the LC50 (lethal concentration) for 24, 48, 72 and 96 h were 4.5, 2.8, 1.8 and 1.02 mg/L. One third (330 µg/1) one tenth (100 µg/1) and one fifth (200 µg /1) of the LC50 values were selected for sub lethal studies. Hepatic metabolic parameter like protein and glycogen were also determined as oxygen consumption is directly reflects their content.
Results show an initial increase in the oxygen consumption in low sub lethal concentrations (1/5th and 1/10th exposure) but a sharp decrease in oxygen consumption was observed in all sub lethal concentrations. Results also clearly reveal that protein, glucose and glycogen content of liver were significantly reduced in all experimental groups treated with sub lethal concentrations of parathion. The reduction in glycogen content of liver in all the experimental fishes might be due to the utilization of carbohydrates for energy production as a result of toxicant induced hypoxia which has also been reflected in oxygen consumption. Fish under sub lethal concentration were found to be under stress and duration of the exposure is found to be an important factor for inducing the toxic effects. Hence, respiratory dysfunction and hepatic metabolic markers like glucose and glycogen can be used as an index of Parathion toxicity.

Keywords: Hepatic markers, LC50 concentration, oxygen consumption, parathion

Introduction: Pesticides represent the major chemical pollutants of aquatic ecosystem. Studies proved that four different mechanisms have a role in metabolysis of methyl parathion [1]. Fishes are particularly very sensitive to the water pollution. Hence, various contaminants like pesticides and other xenobiotics may significantly influence some physiological and biochemical processes when they enter into the organs of fishes [2], [3], [4].More over the insecticides mainly crash on liver and affects the carbohydrate metabolism by declining the glycogen content [5].For that reason, an immense deal of previous research has described physiological mechanisms of toxicity in animals exposed to pollutants. On the contrary, effects of pollutants on fish behavior are not regularly studied. Since behavior links physiological function with ecological processes, behavioral markers of toxicity appear ideal for evaluating the effects of aquatic pollutants on fish populations. Toxicant exposure often completely reduces the performance of behaviors that are necessary for fitness and survival in natural ecosystems, frequently after exposures of lesser magnitude than those causing significant mortality. Unfortunately, the behavioral toxicity of many contaminants is still unknown, necessitate their future study. On the other hand, little toxicological research has required integrating the behavioral effects of toxicants with physiological processes. Oxygen uptake is widely used in physiology as a biological indicator that integrates the overall metabolic activity of an animal in response to specific environmental factors [6] because it reflects energy expenditure and, ultimately, the food requirements. The changes in the oxygen consumption of fish as an index of toxicity to various pesticides have been studied by several investigators [7], [8], [9], [10], [11].
Parathion is an organophosphate compound and is a potent insecticide.EPA has classified parathion as a Group C, possible human carcinogen [12]. Most of methyl parathion is discharged with urinary and some of it is discharged with stools [13].The methyl parathion which is entered into the body is completely and rapidly absorbed by gastrointestinal system. It was observed that the plasma cholinesterase enzyme (ChE) activity in the fish was inhibited in the rate of 90% right after 4 h following the application [14]. The entry of pesticides and other xenobiotics can lead to damage of liver function by damaging the sensitive vitamins and minerals, which are essential for the detoxification pathways. So, the liver must try to cope with every toxic chemical to maintain the homeostasis of the body [15]. Liver is the first organ where methyl parathion is metabolized and detoxified [16]. Therefore, the present study is aimed to investigate the effects of parathion on oxygen consumption and carbohydrate metabolism in a locally available fish, *Tilapia mossambica.*

Materials and methods: Study area: The study was conducted in the Chambal River at Nagda (22°24'35" N 75°3'38" E) in Ujjain district of Madhya Pradesh (Western India, Fig.1).

Fish: Healthy and active juvenile fish *Tilapia mossambica* ranging from 7-9 cm in length and

weighing 10-15g were selected from the stock and used for experimental purpose. They were held in large water baths of 160L capacity and acclimatized for 14 days to laboratory conditions. Fingerlings were fed manually with commercial feeds twice every day (morning and afternoon). At first, the fingerlings were fed at a rate of 5% of the total body weight (TBW) of the fish and two weeks later 10% of TBW which was continued to the end to the experiment. Physico-chemical characters of water were examined by following the APHA. Fish were provided with commercial food pellets twice (9 and 16 h) at 12% initial body weight.(Manufacturer: Aakash Fish meal and oil, Porbandar, Gujarat, Composition: crude protein 60-65%, crude fat 10%, moisture 10%, Sand and silica 1%,Ash,20-30%,Histamine,500). The experiment was performed in glass aquaria with 100 liters of water capacity. Prior to the start of the experiment, it was assured that all aquariums were properly washed with distilled water to remove any sort of impurities and dust particles. Acclimated fish were not fed 24-hr prior to the start of the experiment and throughout acute toxicity tests.

Experimental Design: **Physico-chemical Parameters**: Water quality parameters like pH, temperature, and oxygen content of the solutions were measured on daily basis. The physical-chemical parameters of the experimental water were found to be within the limits of BIS standards. Temperature 24°C (1±°C), conductivity 25S/cm2, pH 7.5, and dissolved oxygen were found as 8 mg/L. The test organisms were not fed during the test period.

Determination of Lethal Concentration (LC) 50 value: The preparation of standard stock solution (1000 ppm) of methyl parathion and the determination of LC^{50} (the concentration at which 50% Methyl Parathion 50% EC or Foliodon 50 EC (Chemet Chemicals Pvt. Ltd. Gujarat) obtained from local market was used for the experiment. Methyl parathion is slightly soluble in water (55-60 mg/L @ 25^{C}, EPA [16].). Therefore ethanol was used as a medium to obtain proper circulation in the test solution. Successive dilutions of the stock solution were prepared using previously aerated, copper free and stored tap water. Stock solution of the parathion was prepared by dissolving methyl parathion (5mg) in ethanol (50ml) and the desired concentrations of test media in tap water were prepared by adding appropriate volumes of this stock solution into test aquarium. The water was continuously aerated. Acute toxicity study was carried out by following the standard guidelines [5].) to determine the lethal (LC^{50}) level of methyl parathion using static renewal method. Mortality of fish was recorded daily by handpicking and counting. Dead fishes were removed immediately from the test medium to avoid deterioration. Three set of replicates were performed for each concentration. A group of 10 healthy fishes were exposed to diverse concentrations of Methyl parathion to calculate the medium lethal concentration LC^{50} value using probit analysis method [17]. A semi-static system was used to expose the fish (average weight of 8.18 g) to the test chemical. 20-L glass aquaria were filled with copper free water. The behavioural pattern of fish was monitored regularly under above treatment conditions.

At first, a sample finding test was conducted to determine the range to be followed in the definitive test. In this test, the animals were exposed to a range of concentration in logarithmic scale such as 1, 2, 3, 4, 5, 6, 7 and 8 g/L. Static non-renewable bioassay was conducted in triplicate for each concentration of methyl parathion with four animals in each tub. No water exchange was done and the fishes were not fed during the period of experiment. Percentage mortality was recorded at 12, 24, 48, 72 and 96 h interval. Control group were subjected to ethanol at the maximum acetone volume used in the dilution of the dosing concentrations. The range of LC50 for tilapia (mean wt. 8.18 g) under given conditions was determined to lie between 500µg and 2mg/L. Hence for the definitive test, concentrations such as 300 µg, 600 µg 900 µg,1200 µg,1500 µg,1800 µg and 2100 µg /L methyl parathion concentration were selected. Experiment was conducted in triplicate for each concentration with 10 fishes in each tank. Fresh stock solutions were used for each exposure. The medium in which the animals were kept was changed for every 24 hours with freshwater in order to avoid the accumulation of excretory materials of animals and probable biodegradation products of pesticides. Different concentrations were used for each concentration 10 fishes were exposed in 20 liters of diluted solutions. After 96 hours the mortality of fish in each concentration was noted. The average mortality in each concentration was taken to determine the LC^{50} by graphical plots of percent mortality and probit mortality against log concentration. Dead fish were removed from each tank immediately. The data obtained from the experiment was processed by probit analysis using a Microsoft excel computer program.

Ethical guidelines from the Indian National Science Academy (INSA on Animal Care) were followed. Animals were kept in well aerated aquaria in a quiet and well-ventilated room, crowding was avoided, sufficient amount of nutritious food was provided, fishes were hold softly and only when necessary. The walls of the stock container were carefully cleaned and excreta were drain off on each day to avoid the accumulation of ammonia in the medium. Fish were

conditioned in stock container for 15 days before make use of them for the experiments. The test solution was replaced after every 24hrs.

Estimation of whole body oxygen consumption: The whole animal oxygen consumption was calculated for lethal and sub lethal concentrations in addition to control by following the method of Welsh and Smith(1953)[18] with slight modifications. The LC50 value of methyl parathion to Tilapia was found to be 1.09mg/L. Five sub lethal concentrations 55µg(1/5th),109 µg(1/10),164 µg(1/15th),236 µg(1/20th) and 345 µg(1/30th) of LC 50 were selected to study the oxygen consumption rate for 48 hr in fixed system with 12 hr interval. They were marked as A, B, C, D and E. Ten fish each were accommodated in 20L of test solution. The surface water of the control and test chamber was covered with a thin film of liquid paraffin, to prevent diffusion of atmospheric air into test medium. The amount of dissolved oxygen in water for every 12 hr was estimated by Winkler method [19]. The difference in dissolved oxygen content between initial and final water samples represents the amount of oxygen consumed by the fish. At the end of exposure each fish was weighed and placed in its respective test chamber and the experiment was run for a period of one hour. The same process was repeated for other fishes of the test chambers. The test water containing pesticide was renewed every 24 hours during the study period. Controls were also run simultaneously in tap water to obtain information on the normal rates of oxygen consumption of the fish. Water was renewed every day and 12-12 h of photoperiod was maintained daily during acclimatization and experiment periods. The fish were fed regularly with oil cake and rice bran. But feeding was stopped during acclimatization three days prior to exposure to the pesticide. Respiratory measurements were made using a closed chamber method and the dissolved oxygen was estimated by the Winkler method [20].The amount of oxygen consumed by the fish was expressed in ml/hr/1gm of tissue. Statistical significance of the differences in oxygen consumption between control and exposed fish at different methyl parathion concentrations were made by T-test at $p<0.01$ and dose-response dependency was tested by the Pearson correlation co-efficient (r) using excel software for Windows.

Effects of Methyl parathion on swimming behavior: In the current study opercular beat frequency (OBF), tail beat frequency (TBF), air in gulping, and surfacing phenomenon with other abnormal behavior were recorded at the initial (0 - 6), 24th, 48th, 72nd and 96th hrs in all concentration of control and experimental fish. Every change in the behavior and physiology of fish indicates the worsening of water quality, as fish are the biological markers of water quality.

Biochemical studies: Total protein estimation: The total Protein content of was estimated according to modified standard method of Lowery et al (1951) [21]. An amount of 5% homogenate of liver was isolated and precipitated with 5% trichloro acetic acid (TCA) and centrifuged at 3000 rpm for 15 minutes. The precipitate was dissolved in 1 ml of 1 N NaOH solution and 0.2 ml of extract taken into test tube and mixed with 5 ml of alkaline copper solution was added. To this 0.5% ml of 50 % folin phenol reagent was added. In the time following 30 minutes, the optical density was measured at 540 nm against a blank. The standard graph was plotted by using Lowry method with bovine serum albumin. The values were expressed as mg/g wet weight of the tissue.

Glycogen estimation: The glycogen was estimated by the standard method of Kemp et al [22]. (1954). A 2% homogenate of liver tissue was prepared in 80% methanol and centrifuged at 3000 rpm for 10 minutes. The tissue residue was suspended in 5 ml of trichloro acetic acid (TCA), boiled for 15 minutes at 1000C, and then cooled in running water. The solution was made up to 5 ml with TCA to compensate the evaporation and then centrifuged. From this, 2 ml of supernatant was taken into the test tube and 6 ml of concentrated H_2SO_4 was added and the mixture was boiled for 10 minutes. The mixture was cooled and the optical density was measured at 520 nm. The standard graph was plotted with D-glucose by using the above mentioned method. The glucose was converted to glycogen by the multiplication factor of 0.98 [23]. (Hawks, 1951) and is expressed as mg of glycogen/g wet weight of the tissue.

Statistical analysis: The data was subjected to T test, using Microsoft Excel-2007 and the significance difference was set up at $p < 0.01$. These values were expressed as Mean ± SD for all parameters in the experiment.

Results and Discussion: The water quality parameters of the experiments were determined and are presented in Table 1. Results clearly show that the water quality indicators are found to be within the limits of BIS/WHO standards. The elevated level of dissolved oxygen levels specify improved water quality. The slight increase in temperature could be endorsed to the season at sampling.

Table.1. Physico-chemical analysis of water used for experiment

Parameter	Value
Temperature (^{0}C)	25.7 ± 0.53
pH	7.6 ± 0.36

Turbidity (NTU)	21.3 ± 0.55
Conductivity (µScm-1)	401 ± 0.04
TSS (mg/l)	109 ± 1.53
Total alkalinity (mg/l)	64 ± 1.96
Chlorides (mg/)	212 ±1.08
COD (mg/l)	24.2 ± 1.53
BOD (mg/l)	34.6 ± 1.00
Sulphide (mg/l)	24.3 ± 1.53

The table.1 present ranges, mean, standard deviation and difference of the parameters under study.

Effects of methyl parathion on Behavior: Nervous system mainly controls the behavioral pattern in all animals. Furthermore the internal and certain physiological processes can also modify neuron function and these can result in behavior phenotypes. [24]. Behaviors are the actions and reactions taken to internal and external signals that are planned to place organisms in a safe position with respect to fitness. Circumstances and conditions that cause unexpected or impaired behaviors therefore have clear survival implications [25].

In the present investigation, the control fish exhibited normal and natural behavior i.e. they were active with their well synchronized movements. They were even aware by the smallest disturbance, but the fish exposed with parathion, exhibited irregular, erratic and darting swimming movements and loss of equilibrium which may due to inhibition of an enzyme acetylcholine esterase (AChE) activity leading to accumulation of acetylcholine in the end bulbs of neuron at synapses ending up with hyper stimulation [26]. They gradually became sluggish, hyper excited, restless and secreted excess mucus all over the body. Mucus secretion in fish forms a barrier between body and contaminated environment thus perhaps diminishes the contact of toxicant so as to minimize its irritating effect, or to eliminate it through epidermal mucus. Similar observations were made by Rao *et al.* (2003) [27] and Parma de Croux *et al.* (2002) [28]. It is also observed that opercular movements of gill raised at first in all exposure periods but decreased further progressively in lethal exposure compared to sub lethal exposure periods. The increased gill opercular movements observed initially may possibly compensate the increased physiological activities under stressful conditions [29]. Gulping of air by fish at surface may be a protective behavior which helps to keep the animal away from contact of toxic medium. Another reason might be more demand of higher oxygen level during the exposure period [30]. However, it was noticed that convulsive behavior was reduced just after 30 minutes and the swimming speed was also decreased with increasing duration and concentration of endosulfan and finally fish became lethargic.

Morphological symptoms: The occurrence of morphological abnormalities such as shedding of scales, discoloration, injury of skin, cracks and necrosis of fins, eye deformities, scoliosis (caudal bleeding), damaged skull, lower lip extension and abundant amount of mucus secretion all over the body were shown. The percentage of these abnormalities was higher at higher concentration (218µg-436 µg/l) and treatment duration (72 and 96 hrs.) It may be due to the inhibition of muscular AChE activity resulting in blockage of neuro transmission. At elevated level of pesticide concentration, scale reduction start, mild skin lesion observed from dorsal to lateral side of the body of fish, plentiful mucous, thickening of gills increases with the growing of concentration of methyl parathion. The fishes lost their original coloration and become almost pale yellow in colour.

Determination of Lethal Concentration (LC) $_{50}$ of Methyl Parathion: LC50 is the most widely accepted tool for acute toxicity study and it is the concentration of a test chemical which results in 50% mortality of the experimental animals in a particular length of exposure (96 h). Results of probit analysis showed the median lethal concentration LC^{50} of estimated methyl parathion to *Tilapia mossaambica* for 96 h of exposure to be 1.09mg/L, the lower bound and upper bound 95% lethal confidence limits for methyl parathion indicated a wide range of values (0.916 to 1.329). The results confirmed that parathion was toxic to the fish *Tilapia mossaambica* and toxic effects are found to be both time and concentration dependent. The test result of the 96 h LC50 of *Tilapia mossaambica* treated with parathion in the present study was slightly higher than the 96 h LC value of 0.5949 ppm in *Channa punctatus* [31]. [32], [33], [34].

The LC50 values of Methyl parathion 50% EC for the fish Tilapia mossambica for 24, 48, 72 and 96 h in static exposure are 2.61 mg/L, 2.11mg/L, 1.80 mg/L and 1.0949 mg/L respectively. The 96 h LC50 value of methyl parathion found in the present study was comparatively less than the many values reported earlier, which could be due to the age and hardness of the test species, size of animal, and rearing system. Hii et al [35]. (2007) also found significant differences between 24 and 96 h LC^{50} value as they renewed 56% of the test solution each day. Low value of 96th hour LC^{50} (median lethal concentration) of 1.09µg/L indicates that methyl parathion is highly toxic to fish. In the present study slope functions of the toxicity curve also clearly indicate the high acute toxicity of parathion insecticide. Steep slope functions of the toxicity curves are due to rapid absorption of the insecticides and rapid onset of effects. Flat slope

functions indicate slow absorption, rapid excretion, detoxification or delayed toxicity of the toxicant [36]. (Caquet *et al.*, 2012). Our results are in agreement with Phipps and Holcombe (1985) [37] for catfish *Lctalurus punctatus*, Holcombe et al., (1982) [38]. For *Pimephales promelas*, Phipps and Holcombe (1985) [37] for *Pimephales promelas* and *Carrasius auratus* and Ferguson et al., [39] (1966) for *Gambusia affinis*.

Oxygen consumption: The amount of oxygen consumption by an animal reflects its total metabolic rate and consequently the energy yield. It is well known that pesticides can cause respiratory distress (or) even failure by affecting respiratory centers of the brain (or) tissue involved in breathing (O'Brien, [40]. 1967). It may be due to changes in the architecture of gill under parathion stress which altered the diffusing capacity of gill with consequent hypoxic/anoxic conditions. Comparative data on the whole animal oxygen consumption of control and experimental fish, calculated per gram body weight in lethal and sublethal concentrations of methyl parathion 50% EC formulation for *Tilapia mossambica* was given in table 2 and Fig.2.

Oxygen is necessary for many metabolic processes that are very important to aerobic life. Like all other organisms, fish are vulnerable to the effects of different biotic and abiotic factors on antioxidant defenses in fish [41]. (Reddy, 2012). Evaluation of whole body oxygen consumption by the fish is valuable for measurement of sub lethal effects of toxicants and is an indicator of physiological state of animal. The degree of damage depends on the toxic potentiality of a particular compound or pesticide accumulated in the tissue [42]. It is clearly evident from the studies (Table 2& Fig.2) that methyl parathion affected oxygen consumption of *T. mossambica* under different sub lethal concentrations. It was seen that experimental fish exhibited an increased tendency in oxygen consumption during the early hours of exposures i.e. 2 to 4 hours and later on a gradual drop off was observed during the successive study period. The initial increase in oxygen consumption in experimental fish may be due to the stress caused by the pesticide on the fish making it active to combat the stress, thus gaining an increased requirement of energy. The increase in activity might be to boost up oxidative metabolism for an increased supply of energy to combat the toxic stress [43]. This probably accounts for an elevation in oxygen consumption. The decrease in oxygen consumption rate in treated fish may due to lowering down of energy requirements which can be considered as adaptive and even strategic [44]. Besides, the treated fish might have overcome the pesticide toxicity by activating the process of detoxification. The depletion of the oxygen consumption in the present study may be due to the ineffectiveness of the gill due to rupture in the respiratory epithelium. From these results it can be assumed that, during sublethal exposure of the toxicant, the fish may be adapting to supplement the physiological adjustment for elimination of the chemical stress. Our findings are in accordance with findings of various authors [45]. In controls also, the rate of oxygen consumption was slowly decreased and this can be endorsed to the starved conditions and the reduced metabolic rates of the starved fish. The fish are in more stress during first hour and later they are showing signs of recovery. All the way through the experimental period the fish showed severe respiratory distress with rapid opercular movements leading to the higher amount of toxicant uptake. The fluctuated response in respiration may be endorsed to respiratory distress as a consequence of the destruction of oxidative metabolism as in *Tilapia mossambica* [46] due to cypermethrin toxicity.

This outcome suggests that the altered rates of respiration of Tilapia may also serve as a quick biological monitor to evaluate the impacts of endosulfan on other biotic communities in the aquatic sources.

Fig.2.Effect of sub lethal concentration of methyl parathion on oxygen consumption of Tilapia mossambica (ml/mg wet wt/hr): Gills are the most important respiratory organs and all metabolic pathways depend upon the efficiency of the gills for their energy supply. Any damage to these vital organs causes a series of destructive events, which eventually lead to respiratory distress [47]. Prominent secretion of mucus layer over the gill lamellae has been observed during pesticide stress. Secretion of mucus over the gill restricts the diffusion of oxygen [46] which may finally decrease the oxygen uptake by the fish. The xenobiotics may cause structural anomalies of gill [48] or the membrane functions are disturbed by an altered permeability [49] oxygen uptake rate would even quickly reduced. Conversely, the metabolic rate (in relation to respiration) of fish could be increased under chemical stress. Kalavathy *et al.* (2001) [50] reported that the dimethoate is efficiently absorbed across the gill and diffuse into the blood stream resulting toxic to fish. The analysis of data from the present investigation evidenced that endosulfan is highly toxic and had intense impact on both behaviour and oxygen consumption in *Tilapia mossambica in* both lethal and sub lethal concentrations. Consequently it has led to the distorted fish respiratory physiology. Differences in the oxygen consumption rate in experimental fish treated with endosulfan are perhaps due to damaged oxidative metabolism and pesticide induced

respiratory stress. Hence, dysfunction of behavior and respiration can serve as an index of pesticide toxicity.

Biochemical analysis: Results are shown table 4 and Fig.4**.** The change in the biochemical parameters (increase or decrease) is dependent on the health status and metabolism of the individual. Most of the toxicants including pesticides act as metabolic depressor and influence the metabolism of biologically active molecules such as proteins, glycogen, carbohydrates and lipids [46] (David, M. et al, 2002). In the present study total protein content significantly and gradually decreased in experimental fish gradually along with increase in the pesticide concentration. The depletion in protein content in liver might be due to the damaged or low protein synthesis under the toxic stress condition as reported the earlier workers [51],[52],[53].Besides, special catabolic reactions like proteolysis, formation of lipoproteins to repair the cell and to fulfill energy requirement in cells are also responsible for reduction of protein content [51], [54].

Glycogen is a polysaccharide and major reserve food and first source of energy for many animals. It plays a significant role in the glucose cycle that can be quickly transferred to meet an unexpected need for glucose [55] (Sadava, E.D et al, 2011). In the present experiment, results clearly indicated that the glycogen content of liver was decreased with increased Methyl parathion concentration. The reduction of liver glycogen content in all the experimental fishes might be due to the utilization of carbohydrates for energy production as a result of toxicant induced hypoxia which has also been reflected in oxygen consumption. Similar reports were observed in common carp *Cyprinus carpio* exposed to sub lethal concentrations of endosulfan showing a decrease in levels of plasma glucose and slight variation in the serum protein [56].The depletion in glycogen content may also due to active glycogenolysis and glycolytic pathway to provide excess energy in stress condition [57]. Similar findings were also found in the glycogen content of the liver of *Colisa fasciatus* and *Sarotherodon mossambicus* exposure to various toxicants including Thiodan and Arsenate [58], [59], [60], [61], and [62]. In the present investigation the reduction in liver glycogen might be due to the hypoxic condition created by pesticides during the period of experimentation.

Conclusions: We found that the sub lethal exposure of the parathion, an organophosphorous pesticide proved to be highly toxic to *Tilapia mossambicus* and which affected the rate of oxygen consumption, depletion in total proteins and glycogen levels liver.

The experimental data of the present investigation reveals that oxygen consumption decreases with the time of exposure to the parathion. This initial increase is perhaps a reaction to the sudden inception of toxicity that led to acceleration of oxidative metabolism. On the other hand, prolonged exposure to toxicants had altered gill morphology, which in turn decreased the oxygen consumption resulting in asphyxia. Further it can be also assumed that probably fish absorbed a greater amount of pesticide through the gills which are in direct contact with the toxic test medium as the teleost fish *Macrognathus aculeatum* [62]. It is further concluded that the appraisal of the biochemical parameters is significant to know the wellbeing status of fish under restricted and wild habitat. However, it is very difficult to predict the changes in fish of natural water bodies on the basis of haematological indices due to age, seasonal, and sexual fluctuations. Therefore a detailed investigation of the variety of pathological changes in blood indices is required in order to find general regulations of transformation in the blood system of fish caused by various pollutants as well as pesticides. Future studies should include a more detailed analysis of the effects of chronic parathion exposure on these biomarkers to further assess the impact of the pesticide on mammalian models.

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Table.1. Determination of Lethal Concentration (LC) $_{50}$ of Endosulfan for Tilapia mossambica

Group	Number of Species	Doseµg/L	24h	48h	72h	96h	% Mortality
1	10	Control	–	–	–	–	–
2	10	200	–	–	–	–	–
3	10	400	–	–	–	1	10
4	10	600	–	–	1	2	20
5	10	800	–	1	1	3	30
6	10	1000	–	1	1	4	40
7	10	1200	1	2	2	6	60
8	10	1400	2	3	4	7	70
9	10	1600	4	5	6	7	70
10	10	1800	5	2	7	9	100

Table 2. Oxygen consumption (ml of oxygen consumed/gm/hr wet wt. of fish/) of the fish, *Tialpia mossambica following* exposure to different sub lethal concentrations of Methyl parathion

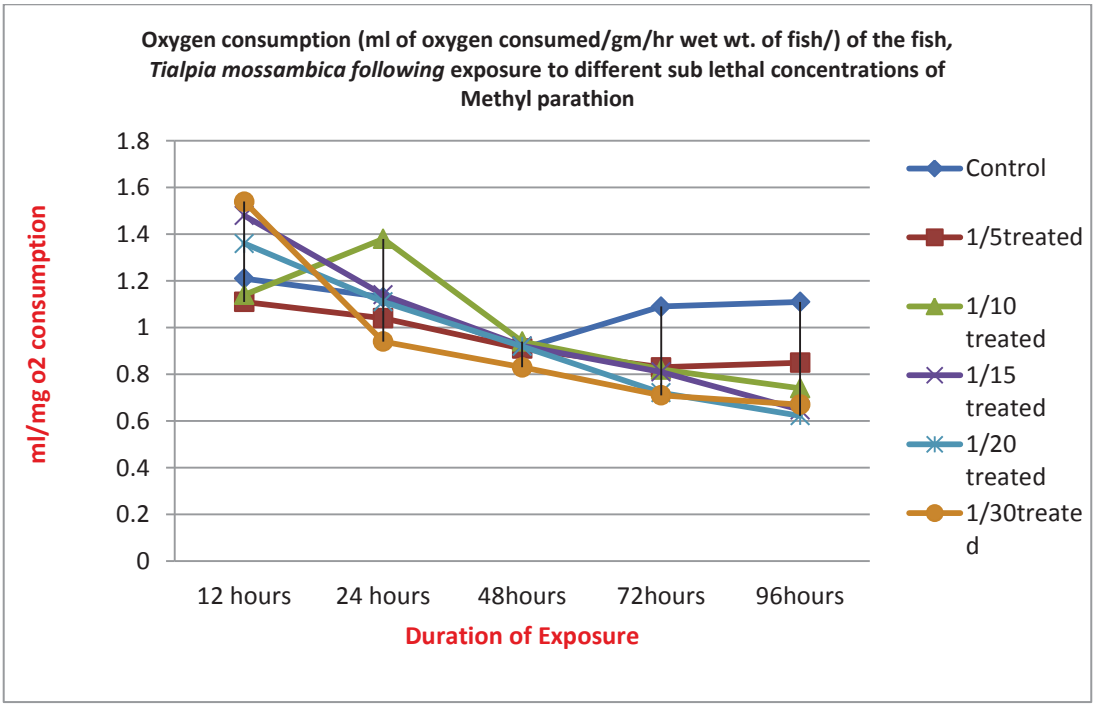
Duration	control	1/5 treated	1/10treated	1/15 treated	1/20 treated	1/30treated
12hr	1.21	1.11	1.144	1.48	1.36	1.54
24hr	1.13	1.04	1.38	1.14	1.11	0.94
48hr	0.91	0.91	0.94	0.922	0.92	0.83
72hr	1.09	0.83	0.82	0.81	0.721	0.71
96hr	1.11	0.85	0.74	0.648	0.622	0.67

Table 6: Effect of sub lethal concentration of Methyl parathion on total proteins and total glycogen content in liver of *Tilapia mossambica*

Duration(Hr)	Control**(X ± SD)**	Treated with Sub-Lethal concentrations**(X ± SD)**		
		1/10	1/20	1/30

	Protein (mg/g)	Glycogen mg/g)	Protein(mg/g)	Glycogen mg/g)	Protein (mg/g)	Glycogen mg/g)	Protein (mg/g)	Glycogen mg/g)
24	151.11	39.18	131.11	38.18	130.2	41.18	141.11	48.08
48	149.57	40.21	130.17	33.14	129.11	31.10	126.14	27.11
72	159.2	40.31	119.12	28.11	115.01	24.2	114.12	20.11
96	148.44	41.21	118.14	22.03	98.14	18.41	89.53	17.81

Results are mean (X ± SD) of 5 observations indicates the standard deviation values and are significant at P < 0.05



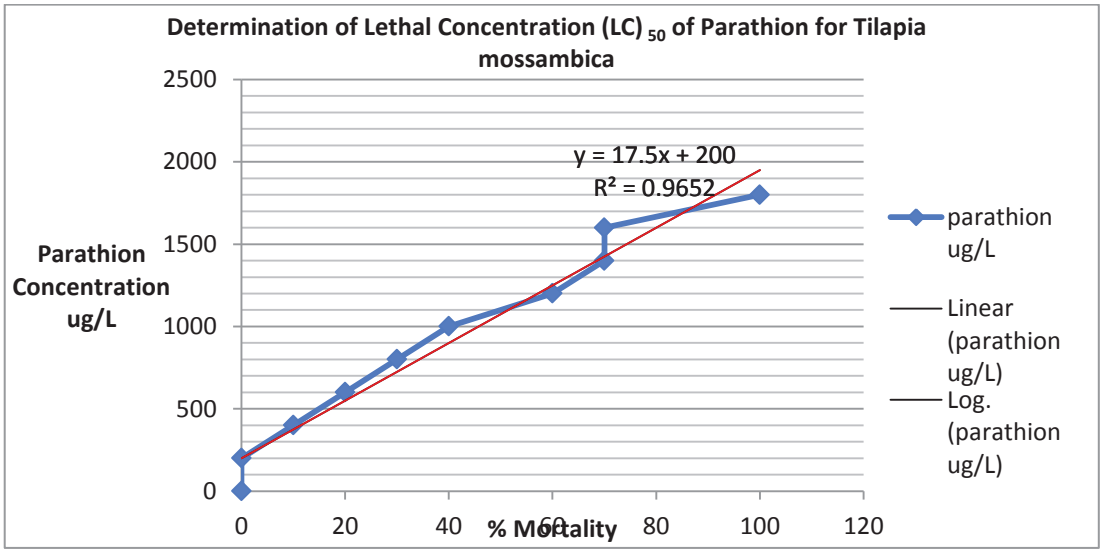


Fig.1. Determination of Lethal Concentration (LC) $_{50}$ of Parathion for Tilapia mossambica

Bhawna Srivastava,DAV, College, Kanpur, Uttar Pradesh
Reddy, P.B,Govt.PG College, Ratlam, Madhya Pradesh