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Impact of Profenofos and Carbosulfan on Dehydrogenase activity of Freshwater fish, *Labeo rohita* (Hamilton)

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ABSTRACT

Experiments were intended to study effects of profenofos and carbosulfan on the activities of dehydrogenase of freshwater fish, *Labeo rohita*. Healthy freshwater fish, *Labeo rohita* (Hamilton) were collected from the Nandivelugu fishfarm, India. Profenofos and carbosulfan pesticides were purchased from local pesticide store in Guntur, India, based on the preliminary lethal toxicity tests (96 hr LC50 i.e. 100 µg l^{-1}; 1.2 mg l^{-1} and 1/10th 96 hr LC50 i.e.10 µg l^{-1}; 0.12mg l^{-1} and) were selected as lethal and sublethal concentrations. After the acclimation, fish were exposed to lethal and sublethal concentrations in groups of 10 fish in 15 L of the test water in test chambers for 1 and 8 days. Fluctuations in succinate dehydrogenase (SDH), lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) were observed in different tissues of the experimental animal. The activity of LDH was highly elevated following profenofos and carbosulfan exposure indicating increased anaerobic respiration to meet the energy demands where aerobic oxidation is lowered. Rapid depletion of SDH activity in all tissues of fish, *L. rohita* treated with sublethal and lethal concentrations of profenofos and carbosulfan. When compared to their respective controls. The general reduction in SDH activity due to pesticidal stress was associated with the inhibition of mitochondrial respiratory mechanism of dearrangement on ultra-structure, architectural integrity and permeability of mitochondria. Decreased MDH activity levels due to the inhibition exerted by oxaloacetate; maybe decrease in the activity of TCA cycle dehydrogenase is consistent with the disintegration of mitochondria of CO_2 formation from acetate. Both profenofos and carbosulfan intoxication exerted a profound influence on the dehydrogenase enzymes of fish *L. rohita* but comparatively profenofos treated fish tissues showed more deterioration when compared to carbosulfan treated fish.

Key words: Concentrations, Mitochondria, TCA cycle, Ultra-structure, Intoxication, Deterioration.

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1. INTRODUCTION

Pesticides were found to adversely affect a number of biological functions, thus causing harm to the non-target organisms. Organophosphate and cabamate compounds are known for this persistence in the environment and accumulation in the tissues for long periods for controlling the loss of produce due to pest attack and as a consequence of the demand for producing more food, there has been an increasing use of pesticides in developed countries (1-3). Many pesticides have been reported to produce a number of biochemical changes in fish both at lethal and sublethal levels. Changes in ion concentrations, organic constituents, enzyme activity and endocrinal activity as chemo regulators in fish have been attributed to pesticides (4, 5). It may also provide an early warning signal in stressed organism. The source of these parameters is the indicators responding to environmental effects and can also serve as markers for the xenobiotic exposure (6). The toxic effects of pesticides on non-target organisms in the environment can be studied by detecting changes in the organisms at the physiological, biochemical or molecular levels, providing early warning tools in quality of environmental monitoring (7). The analysis of biochemical parameters in fish can contribute the health status of animal and also environmental, habitat conditions, pesticides have been shown to cause alterations in the

activities of many enzymes concerning to cellular metabolisms (8). Lactate dehydrogenase (LDH) is hydrogen transferring enzyme that catalyzes the oxidation of L-lactate to pyruvate with the mediation of NAD^+ as hydrogen acceptor. LDH, an indicator of anaerobic metabolism, was expected to exhibit increased activity at lower oxygen levels (9). Alterations of normal LDH activity have been already reported in different fish by different authors exposed to environmental pollutants (10). Succinate dehydrogenase (SDH) is one of the important enzymes in the Krebs's cycle. It plays an important role in mitochondria, which are structures inside cells that convert the energy from food into a form that cells can use. Within mitochondria, the SDH enzyme links two important cellular pathways in energy conversion: the citric acid cycle and oxidative phosphorylation. This catalyzes the oxidation of succinate to fumarate (11). Malate dehydrogenase (MDH) is an NAD dependent enzyme which converts malate to oxaloacetate and reversible oxidation of fumarate to malate. It exists in two isozymic forms (a) mitochondrial (b) cytosolic. This enzyme not only converts malate to oxaloacetate but also play a significant role in CO_2 fixation and in gluconeogenesis (12). Any alterations in mitochondrial structure it inhibits the activity of MDH. Malate dehydrogenase (MDH) converts malate to oxaloacetate but also play a significant role in CO_2 fixation and in gluconeogenesis (12). Hence, the present study is undertaken to comprehend the LDH, SDH, and MDH alterations induced by profenofos and carbosulfan in different tissues of the fish, *Labeo rohita.*

2. MATERIALS AND METHODS

2.1. Animal selected

Labeo rohita is an edible freshwater fish occurring abundantly in the freshwater bodies, rivers, lakes and ponds in India (13). Large population of fish consumers prefers this fish, because of rich source of animal protein, tasty flesh and fewer bones. Besides its wide availability and commercial importance, this fish is known for its adaptability to laboratory conditions and suitability to toxicity studies (14, 15). Hence, this fish was selected as the experimental animal for this investigation.

2.2. Procurement and maintenance of fish

Healthy freshwater fish, *Labeo rohita* (Hamilton) size [6±7 cm total length (TL) and 6.5±7.5 g body weight] were collected from the Nandivelugu fish farm, Guntur district of Andhra Pradesh, India; fish were immediately transported in large plastic tanks with required aeration and brought to the laboratory. Then the fish acclimatized to the laboratory conditions in large cement (200L) tanks with sufficient dechlorinated ground water for 15 days at room temperature 28±2°C. During the acclimation period and subsequent periods of pesticides exposure, fish were held under a photoperiod of 12 hr light: 12 hr dark. The fish were fed with fish meal, rice bran and commercial fish pellets once in two days, at the same time water was renewed every day rich in oxygen (aeration) and feeding was stopped one day prior to the experimentation. Then the fish were separated into the batch of having the length of the fish 6±7cm and body size 6.5 ±7.5g were maintained in static water without any flow (16). All the precautions were followed (17). As the level of toxicity is reported to vary with the interference of various extrinsic and intrinsic factors like temperature, salinity, pH, hardness of water, exposure period, density of the animals, size and sex etc., precautions were taken throughout this investigation to control all these factors as far as possible. As a part of it, water from the same source has been used for maintenance of the fish. The size of the animals selected was also maintained strictly throughout the investigation.

2.3. Water quality

Since it is an established fact that chemistry of water influences the toxicity of chemical (18), care was taken to maintain uniformity of water quality used. The hydrographic conditions of water used for acclimatization and to conduct the experiments.

2.4. Physico-chemical analysis of water

Turbidity–8 Silica units, Electrical conductivity at 28°C - 816 micro ohms/cm,Alkalinity-1, Phenolphthalein-Nil,Methylorange-472, Total hardness (as $CaCO_3$) - 232, Non carbonate hardness (as $CaCO_3$)-Nil, Calcium hardness (As N) -Nil, Sulphate (as SO_4) -Trace, Chloride (as Cl)- 40, Fluoride (as Fl)- 1.8, Iron (as Fe) -Nil , Dissolved oxygen-8-10 ppm, Temperature- 28±2°C. All the precautions laid by committee on toxicity tests to aquatic organisms were followed (17).

2.5. Preparation of stock solution

The stock solutions of both toxicants were prepared in 100% pure acetone; concentrations of profenofos and carbosulfan were taken in µg l^{-1} and mg l^{-1}. Control groups were maintained for each experiment and they were added with the quantity of acetone equal to the highest concentration used in the test.

2.6. Studies on Lethal toxicity

Experiments were carried out to assess the lethal responses of profenofos and carbosulfan by the experimental animals. The acute toxicity (96 hr LC_{50}) of both test toxicants for the freshwater fish, *L. rohita* was determined in the laboratory using the static renewal method according to (19). The containers of the test media are of 15 liters capacity; wherein for each test five containers were used and in each container 10 fish were introduced. The fish were exposed to different concentrations of profenofos and carbosulfan with five replicates for each concentration. Where maximum 10 fish were used per each concentration of the test toxicants, 10 fish were also maintained in separate container along with experimental groups, served as control. Water was renewed every day of test medium for every 24 hr with respective concentrations of the

carbosulfan and profenofos without oxygen (aeration). The data on the mortality rate of the fish was recorded and the dead fish were removed. The toxicity tests were conducted to choose the mortality range from 10% to 90% for 96 hr in static renewal systems. Finney Probit analysis (20), as recorded was followed to calculate the median lethal concentration (LC_{50}) values and its 95% confidence limits (21).

2.7. Selection of sub lethal and lethal concentrations

Toxicants may exist in the aquatic system at concentrations too low to cause rapid death directly; but they may impair the functioning of organisms. Though pesticide may not be present in lethal concentrations, accidental spillages may result in toxic concentrations. Hence, in the present investigation, 96 hr LC_{50} and $1/10^{th}$ of 96 hr LC_{50} values were selected as lethal and sub lethal concentrations for both toxicants to study the behavioral responses and physiological alterations in experimental animal.

2.8. Estimation of Lactate Dehydrogenase (LDH) (EC 1.1.1.27)

The lactate dehydrogenase activity (LDH) was estimated by the method of with slight modifications (22). Two percent homogenates of the tissue were prepared in 0.25 M ice-cold sucrose solution and centrifuged at 1000 rpm for 15 minutes. The supernatant served as the enzyme source. The reaction mixture of 2.0 ml contained 0.5 ml of lithium lactate, 0.5 ml of phosphate buffer, 0.2 ml of INT [(2-p-idophenol-3-(P-nitro phenyl)-5-(phenyl tetrazolium chloride)] and 0.2 ml of NAD and 0.6 ml of supernatant. The reaction mixture was incubated at 37°C for 30 minutes and by adding 5 ml of acetic acid stopped the reaction. Zero time controls were maintained by adding 5 ml of acetic acid prior to the addition of homogenate. The formazan formed was extracted overnight in 5 ml of cold toluene. The intensity of colour developed was read at 495 nm against a reagent blank in a spectrophotometer. The activity was expressed as µ moles of formazan formed/mg protein/hr.

2.9. Estimation of Succinate dehydrogenase (SDH) (EC.1.3.99.1) activity

Succinate dehydrogenase (SDH) activity was estimated by the method of (23). 4% Homogenate (W/V) of the tissues were prepared in cold 0.25M sucrose solution and centrifuge at 1000rpm for 15min. The supernatant act as enzyme source, the reaction mixture of 2ml contained the following 0.6ml of supernatant, 0.5ml of phosphate buffer (pH-7.2), 0.5 ml of sodium succinate, 0.2 ml of 2-(*p*-iodophenyl)-3(*p*-nitrophenyl)-5-phenyl tetrazolium chloride (INT) and 0.2ml of distilled water were added. The reaction mixture was incubated at 37°C for 30 min and reaction was stopped by adding 5 ml of acetic acid. Zero time controls were maintained by adding 5ml of acetic acid prior to addition of homogenate. The formazan formed was extracted overnight in 5ml of cold Toluene. The intensity by of color developed was read at 495 nm against a reagent blank in a spectrophotometer (ELICO Model SL207). The activity was expressed as µ moles of formazan formed mg $protein^{-1}h^{-1}$.

2.10. Estimation of Malate dehydrogenase (MDH) (EC 1.1.1.37) activity

Malate dehydrogenase (MDH) activity was estimated by the methods of (23). 2% homogenate of different tissues was prepared in ice cold 0.25M sucrose solution and centrifuged at 1000 rpm for 15 min. The supernatant was used as the enzyme source to 0.6 ml of supernatant 0.5 ml of phosphate buffer (pH-7.2), 0.5 ml of malate, 0.5 ml of 1NT and 0.2 ml of NAD^+ was added. The reaction mixture was incubated at 37°C for 30 min. Zero time controls were maintained by adding 5 ml of acetic acid prior to addition of homogenate. The formazan formed was extracted overnight in 5 ml of cold toluene. The intensity of colored developed was read at 495 nm against a reagent blank in a (ELICO Model SL207) spectrophotometer. The activity was expressed as µ moles of formazan formed mg $protein^{-1}h^{-1}$.

2.11. Statistical analysis

The statistical analysis of data was done using analysis of variance (ANOVA) followed by Duncan multiple range test as post hoc as test to calculate the significant difference (24). The analysis was made between the means of the control and profenofos and cabosulfan treated fishes. The significance of results was ascertained at $p < 0.05$. All the data are represented as means± standard deviation of the means.

3. RESULTS AND DISCUSSION

3.1. Lactate Dehydrogenase activity (LDH)

The calculated values of Lactate Dehydrogenase (LDH) activity and the percent change over control along with standard deviation are given in the Table 1, Table 2, Table 3, Table 4, Table 5 and Table 6 and Figure 1, Figure 2, Figure 3, Figure 4, Figure 5 and Figure 6. The activity levels of lactate dehydrogenase in *L. rohita* exposed to profenofos and carbosulfan were expressed as micro moles of formazan/mg protein/hr. The LDH levels of muscle, brain, liver, gill and kidney of control fish were almost stable. The control values of LDH in different tissues of the fish *L. rohita* were in the order of: Liver >Muscle >Kidney >Gill >Brain. Under lethal and sublethal exposures to profenofos for 24 hr, the activity levels of LDH were found to increase in all the tissues of the fish *L. rohita.* The percent changes in the activity levels of LDH, in the test fish were in the order of: Profenofos lethal 24 hr: Brain >Liver >Gill >Kidney>Muscle. Profenofos sub lethal 24 hr: Brain >Liver >Kidney > Gill> Muscle. Under lethal and sub lethal exposure to carbosulfan further changes in the activity levels of LDH were noticed. The increased activity levels of LDH for 24 hr in the test fish were in the order of: Carbosulfan lethal 24 hr:

Liver>Kidney>Gill >Brain >Muscle; Carbosulfan sub lethal 24 hr: Gill >Brain>Kidney >Liver >Muscle. On exposure to sub lethal concentrations of profenofos and carbosulfan for 8 days. The percent depletion of LDH levels in the test tissues of *L. rohita was* in the order of: Profenofos sublethal 8 days: Muscle>Kidney>Brain>Gill>Liver Carbosulfan sublethal 8 days: Kidney >Muscle>Brain>Liver>Gill. The LDH activity levels increased significantly in all exposures during the 8 days exposure period. Under lethal exposure of profenofos, maximum percentage of depletion was (-27.27%) in brain and minimum percentage was (-18.30%) in muscle. Under profenofos sublethal exposure for 24 hr, maximum percentage of depletion was (-14.54%) in brain and minimum percentage was (-9.85%) in muscle. Under lethal exposure of carbosulfan for 24 hr, maximum percentage of depletion was (-20.0) in liver and minimum percentage was (-12.67%) in muscle. Under carbosulfan sublethal exposure 24 hr, maximum percentage of depletion was (-9.37%) in gill and minimum percentage was (-5.63%) in muscle. Under profenofos and carbosulfan sublethal exposure for 8 days, maximum percentage of depletion was (19.04%) in muscle and (10.16%) in kidney, minimum percentage of depletion was (13.58%) in liver and (5.26%) in gill.

Table 1. Change in the specific activity levels of Lactate dehydrogenase (LDH) (µ moles of formazan/mg protein/hr) and percent change over the control in different tissues of the freshwater fish, *L. rohita* exposed to sublethal and lethal concentrations of profenofos and carbosulfan for 24 hr

		Profenofos				Carbosulfan			
Organs	Control	Sub lethal	Percent change	Lethal	Percent change	Sub lethal	Percent change	Lethal	Percent change
Liver	0.95± 0.01^{b}	0.82± 0.01^{a}	-13.68	0.70± 0.01^{a}	-26.31	0.88± 0.001^{c}	-7.36	0.76± 0.01^{a}	-20.01
Brain	0.55± 0.01^{d}	0.47± 0.01^{a}	-14.54	0.40± 0.01^{a}	-27.27	0.50± 0.03^{d}	-9.09	0.46± 0.01^{d}	-16.36
Muscle	0.71± 0.01^{d}	0.64± 0.01^{c}	-9.85	0.58± 0.01^{d}	-18.30	0.67± 0.01^{a}	-5.63	0.62± 0.03^{e}	-12.67
Gill	0.64± 0.01^{c}	0.55± 0.01^{d}	-14.00	0.49± 0.01^{a}	-23.43	0.58± 0.03^{e}	-9.37	0.53± 0.04^{c}	-17.18
Kidney	0.68± 0.005^{a}	0.58± 0.01^{b}	-14.71	0.51± 0.01^{c}	-25.00	0.62± 0.02^{a}	-8.82	0.56± 0.01^{a}	-17.64

Means are SD ± (n= 6) for a tissue in a row followed by the same letter are significantly different ($p < 0.05$) from each other according to Duncan's Multiple Range (DMR) Test.

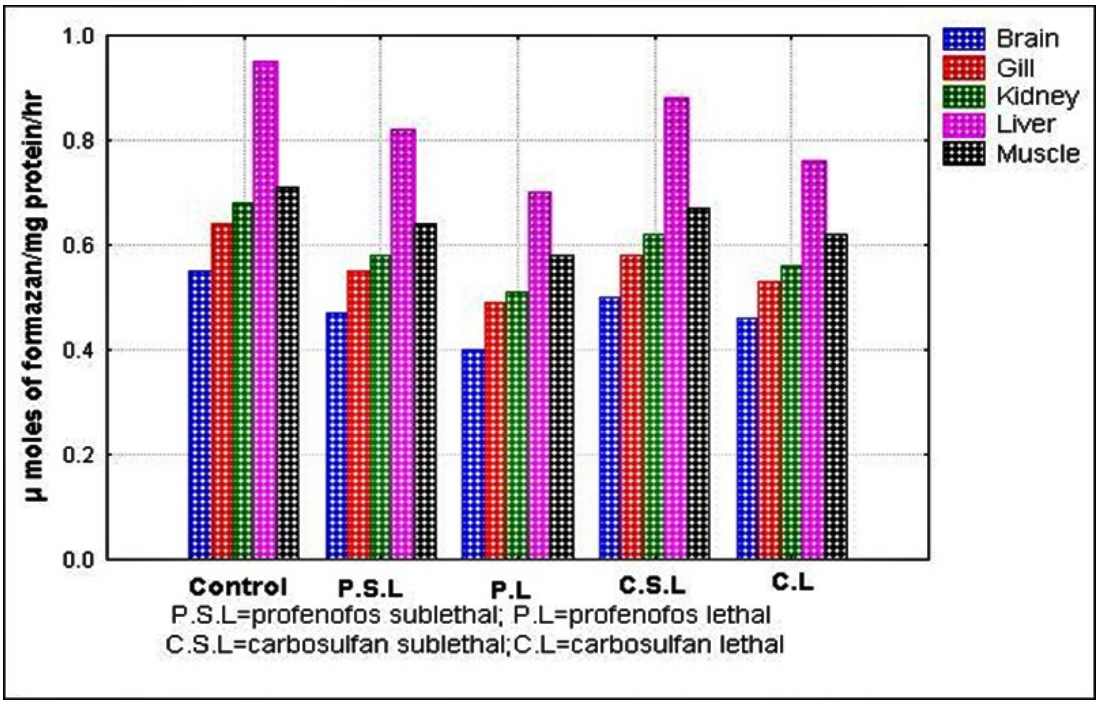

Figure 1. Change in the specific activity levels of Lactate dehydrogenase (LDH) (µ moles of formazan/mg protein/hr) and percent change over the control in different tissues of the freshwater fish, *L. rohita* exposed to sublethal and lethal concentrations of profenofos and carbosulfan for 24hr

Table 2. Change in the specific activity levels of Lactate dehydrogenase (LDH) (µ moles of formazan/mg protein/hr) and percent change over the control in different tissues of the freshwater fish, *L. rohita* exposed to sublethal concentrations of profenofos and carbosulfan for 8 days

	Profenofos			Carbosulfan	
Organs	Control	Sublethal	Percent change	Sublethal	Percent change
Liver	0.81± 0.03[a]	0.70± 0.04[d]	13.58	0.75± 0.01[c]	7.40
Brain	0.47± 0.001[d]	0.39± 0.01[a]	17.02	0.43± 0.03[a]	8.51
Muscle	0.63± 0.01[c]	0.51± 0.05[b]	19.04	0.57± 0.01[d]	9.52
Gill	0.57± 0.007[a]	0.49± 0.03[a]	14.03	0.54± 0.03[d]	5.26
Kidney	0.59± 0.005[b]	0.48± 0.01[a]	18.64	0.53± 0.02[b]	10.16

Means are SD ± (n= 6) for a tissue in a row followed by the same letter are significantly different ($p < 0.05$) from each other according to Duncan's Multiple Range (DMR) Test.

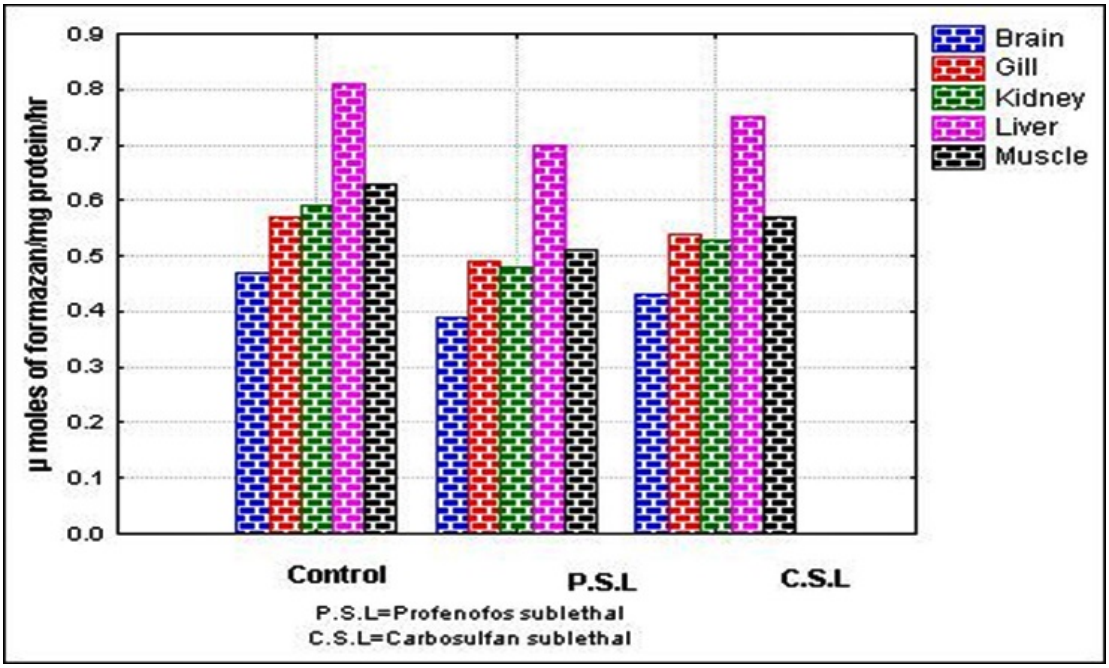

Figure 2. Change in the specific activity levels of Lactate dehydrogenase (LDH) (µ moles of formazan/mg protein/hr) and percent change over the control in different tissues of the freshwater fish, *L. rohita* exposed to sublethal concentrations of profenofos and carbosulfan for 8 days

In the present study, it is observed that the activity of LDH was highly elevated following profenofos and carbosulfan exposure indicating increased anaerobic respiration to meet the energy demands where aerobic oxidation is lowered. Further disruption of respiratory epithelium might have caused tissue hypoxia resulting in a decrease in oxidative metabolism which may be responsible for increase in LDH activity in toxicant stress (25). Lactate dehydrogenase (LDH) coverts the lactate to pyruvate and has very important role in carbohydrate metabolism. LDH involved in carbohydrate metabolism, any change in protein and carbohydrate metabolism may cause change in LDH activity (26). Elevated LDH activity in profenofos and carbosulfan treated fish *Labeo rohita* suggests that aerobic catabolism of glycogen and glucose has shifted towards the formation of lactate, which may have adverse effects on the organism (27). LDH activity depends on its five isoenzymes and the activity changes under pathological conditions (28). Metabolic enzymes such as citrate synthase and lactate dehydrogenase (LDH) are part of the respiratory enzymatic system (29), which can be affected by from the detoxification enzyme systems under stress conditions in fish (30). Normal activity of LDH patterns was found to be altered in situations of chemical stress (31). Elevation of LDH activity mosquito fish, *Gambusia holbrooki* after acute exposure to clofibric, reported that LDH activity in fish *Channa punctatus* significantly increased in skeletal muscle (2.2) fold followed by liver (1.8) fold, gill (1.6) fold and brain (1.4) in response to treatment with alphamethrin for 14 days, due to an increase in anaerobic respiratory activity and production of more lactate for completion of metabolic process (32, 33). Increasing concentration of both toxicant concentrations, there was a progressive increase LDH activity in gill, liver, kidney and brain of the fish *Labeo rohita*, may have due to stress induced increase in the rate of glycolysis. As the rate of glycolysis increases, the pyruvate is not routed to Krebs's cycle, rather catalyses to lactate; thereby shifting

the respiratory metabolism from aerobiosis to anaerobiosis. The increase of LDH activity during conditions favoring anaerobic respiration to meet the energy demands lowers the aerobic respiration (34). The earlier reports on *Tilapia mossambica* also support the present study (35, 36). Increased LDH activity in the liver and muscle of fish *Cyprinus carpio* exposed to drug carbamazepine (CBZ), due to disruption of respiratory epithelium might have caused tissue hypoxia resulting in a decrease in oxidative metabolism that may be responsible for in LDH activity in toxicant stress (27). In the present study, it was observed that the LDH activity in the fish *L. rohita* under exposure to lethal and sublethal concentrations of profenofos and carbosulfan was elevated, indicating that the anaerobic respirations arrived and aerobic respiration inhibited so as to meet the increased metabolic stress and to overcome the toxic stress. LDH is associated with cellular metabolic action, particularly in conditions of chemical exposure and stress when high levels of energy may be required in a short period of time.

3.2. Succinate Dehydrogenase (SDH) activity

Succinate dehydrogenase (SDH) is a vital enzyme of citric acid cycle, catalyses the reversible oxidation of succinate to fumarate. In this present investigation, it can be visualized that there is a rapid depletion of SDH activity in all tissues of fish *L. rohita* treated with sublethal and lethal concentrations of profenofos and carbosulfan. When compared to their respective controls. The calculated values of Succinate dehydrogenase activity and the percent change over control along with standard deviation are given in Table 3 and Figure 3. The calculated values of SDH and standard deviation along with percent change over the controls is tissue specific *viz*., brain, liver, muscle, gill and kidney of fish *L. rohita* exposed to lethal and sublethal concentrations of profenofos and carbosulfan for 24hr and 8 days. In the tissues of control fish, activity of SDH was in the order of: Liver>Muscle>Kidney>Gill >Brain. In control fish, SDH activity was maximum in liver followed by muscle, gill and minimum in kidney. The higher activity of SDH in liver and muscle suggests higher distribution of mitochondria in the tissues, since succinate dehydrogenase (SDH) is mitochondrial localized (37). Under lethal exposure to profenofos for 24 hr, the activity was found to decrease in all the tissues of test fish, maximum decrease was observed in liver (-38.51%) and minimum decrease was in kidney (-14.73%). In the subsequent periods of exposure also the activity was found to decrease with increase in period of exposure. The activity of SDH at 24 hr of lethal exposure to profenofos in the order of: Profenofos lethal 24hr: Liver>Brain>Muscle>Gill>Kidney. Under sublethal exposure to profenofos for 24 hr the activity of SDH was found to decrease in all the tissues of test fish and, maximum decrease was observed in liver (-32.59%) and minimum decrease was observed (-7.36%) in kidney. The activity of SDH at 24hr of sublethal exposure to profenofos in the order of: profenofos sublethal 24hr: Liver>Muscle>Brain>Gill>Kidney. Under sublethal exposure to profenofos for 8days the activity of SDH was found to decrease in all the tissues of test fish and maximum decrease was observed in gill (21.42%) and minimum decrease was observed (14.60%) in muscle. Profenofos sublethal 8 days: Gill>Brain>Liver>Muscle>Kidney. Under lethal exposure to carbosulfan for 24 hr, the activity of SDH was found to decrease in all the tissues but the intensity of decrease was greater when compared to sublethal concentrations. Maximum decrease in activity was found in the liver (-31.85%) and minimum decrease was observed (-14.73%) in kidney. The percent decrease in activity of SDH at 24hr of lethal exposure to carbosulfan was in the order of: Carbosulfan lethal 24 hr: Liver>Brain>Muscle>Gill>Kidney. Under sublethal exposure to carbosulfan for 24 hr and 8 days, the activity of SDH was found to decrease in all the tissues of test fish and maximum decrease was observed in liver (-24.44%) and (12.5%) in gill, minimum decrease was observed (-4.21%) and (6.09%) in kidney. Carbosulfan sublethal 24 hr: Liver>Brain>Muscle>Gill>Kidney; Carbosulfan sublethal 8 days: Gill>Brain>Liver>Muscle>Kidney. Similar decrement in the SDH activity was also observed by various workers in different species of fish exposed to different pesticides (38, 39), reported decrease in activities of LDH and SDH on fish *Colisa fasciatus* and *Labeo rohita* after exposure to cypermethrinreported that decreased SDH activity in fish *Tilapia mossambica* and *Clarias gariepinus* exposed to different type of chemicals, due to depletion in the oxidative metabolism at the level of mitochondria leading to depression of TCA cycle (40, 41). The general decrease in SDH activity during pesticide stress was associated with the inhibition of mitochondrial respiratory mechanism of dearrangement on ultra-structure, architectural integrity and permeability of mitochondria (9, 42). This prevents the transfer of electron to molecular oxygen, resulting in the inhibition of SDH activity and shifting to aerobic metabolism to anaerobiosis (43). The inhibition in LDH and SDH activities were observed in fish *Colisa fasciatus* due to toxicity of ethanolic extract of *Nerium indicum* mill latex (44).

Table 3. Change in the specific activity levels of Succinate dehydrogenase (SDH) (µ moles of formazan/mg protein^{-1}/hr^{-1}) and percent change over the control in different tissues of the freshwater fish, *L. rohita* exposed to sublethal and lethal concentrations of profenofos and carbosulfan for 24hr

	Profenofos					Carbosulfan			
Organs	Control	Sub lethal	Percent change	Lethal	Percent change	Sub lethal	Percent change	Lethal	Percent change
Liver	1.35 ±0.01^{b}	0.91 ± 0.01^{a}	-32.59	0.83 ± 0.04^{c}	-38.51	1.02 ± 0.03^{b}	-24.44	0.92 ± 0.03^{d}	-31.85
Brain	0.55 ± 0.02^{d}	0.49 ± 0.04^{a}	-10.95	0.42 ± 0.02^{b}	-23.60	0.51 ± 0.02^{d}	-7.27	0.45 ± 0.02^{b}	-18.18
Muscle	1.18 ± 0.04^{a}	1.01 ± 0.04^{c}	-14.41	0.92 ± 0.04^{d}	-22.05	1.08 ± 0.04^{a}	- 8.47	0.98 ± 0.03^{a}	-16.94
Gill	0.62 ± 0.001^{a}	0.56 ±0.04^{b}	-9.67	0.50 ± 0.03^{b}	-19.35	0.59 ± 0.03^{b}	- 4.83	0.53 ± 0.02^{a}	-14.51
Kidney	0.95 ± 0.03^{c}	0.88 ±0.03^{a}	-7.36	0.81 ± 0.04^{a}	-14.73	0.91 ± 0.004^{d}	-4.21	0.86 ± 0.03^{d}	-9.47

Means are SD ± (n= 5) for a tissue in a row followed by the same letter are significantly different ($p < 0.05$) from each other according to Duncan's Multiple Range (DMR) Test.

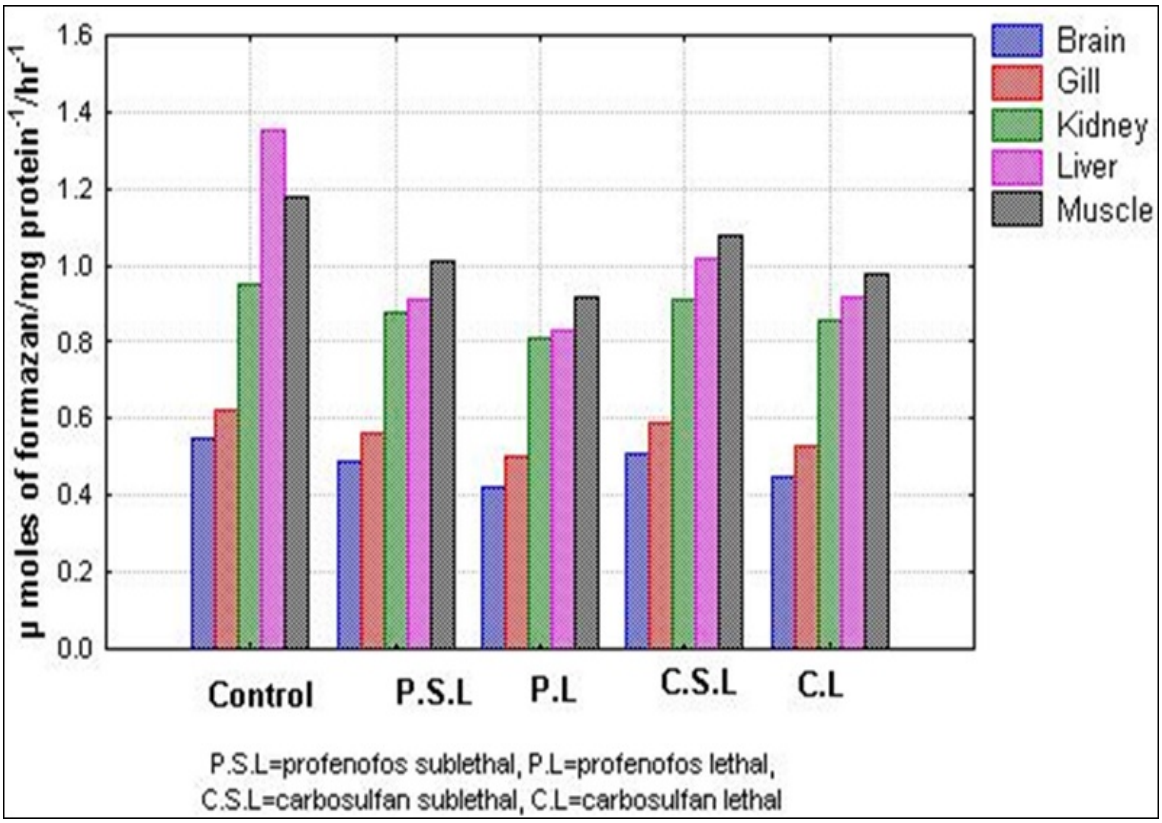

Figure 3. Change in the specific activity levels of Succinate dehydrogenase (SDH) (µ moles of formazan/mg protein^{-1}/hr^{-1}) and percent change over the control in different tissues of the freshwater fish, *L. rohita* exposed to sublethal and lethal concentrations of profenofos and carbosulfan for 24hr

Table 4. Change in the specific activity levels of Succinate dehydrogenase (SDH) (µ moles of formazan/mg protein^{-1}/hr^{-1}) and percent change over the control in different tissues of the freshwater fish, *L. rohita* exposed to sublethal concentrations of profenofos and carbosulfan for 8 days

	Profenofos			Carbosulfan	
Organs	Control	Sublethal	Percent Change	Sublethal	Percent Change
Liver	1.03±0.001^{a}	0.86± 0.01^{b}	16.5	0.95± 0.05^{a}	7.76
Brain	0.43± 0.01^{c}	0.35± 0.03^{d}	18.60	0.41± 0.01^{d}	4.65
Muscle	0.89± 0.007^{a}	0.76± 0.04^{d}	14.67	0.83± 0.04^{a}	6.74
Gill	0.56± 0.01^{b}	0.44± 0.04^{a}	21.42	0.49± 0.04^{b}	12.5
Kidney	0.82± 0.03^{c}	0.73± 0.006^{d}	10.97	0.77± 0.01^{c}	6.09

Means are SD ± (n= 5) for a tissue in a row followed by the same letter are significantly different ($p < 0.05$) from each other according to Duncan's Multiple Range (DMR) Test.

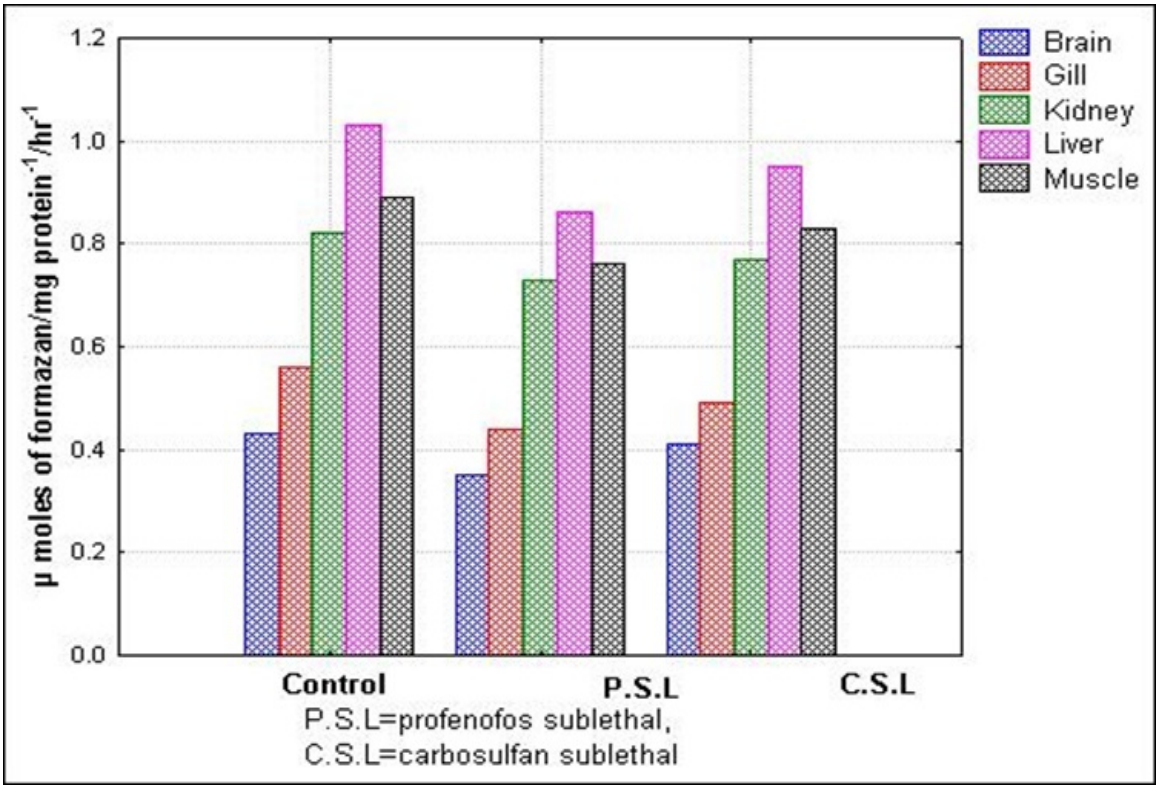

Figure 4. Change in the specific activity levels of Succinate dehydrogenase (SDH) (µ moles of formazan/mg protein^{-1}/hr^{-1}) and percent change over the control in different tissues of the freshwater fish, *L. rohita* exposed to sublethal concentrations of profenofos and carbosulfan for 8 days

A similar decrement in the SDH activity was observed by in fish exposed to malathion and fenitrothion pesticides (45). The results of the present study are in agreement with those of, on albino mice and *Labeo rohita* (38, 46). The inhibition of NAD dependent, LDH activity and SDH activity indicated a decreased pass of intermediates into the citric acid cycle. This might be responsible for suppression of oxidative phase of tissue metabolism under pesticidal impact showing a shift from aerobic metabolism to anaerobic metabolism under profenofos and carbosulfan stress.

3.3. Malate Dehydrogenase (MDH)

The calculated values of MDH and standard deviation along with percent change over the control is tissue specific: *viz.*, brain, liver, muscle, gill and kidney of freshwater fish *L. rohita* treated to sublethal and lethal concentrations of profenofos and carbosulfan for 24 hr and 8 days**.** In the tissues of control fish, activity of MDH was in the order of: Control 24 hr: Liver>Muscle>Kidney>Gill>Brain; Control 8 days: Liver>Muscle>Kidney>Gill>brain. In control fish decreased activity of MDH was noticed in liver followed by muscle, gill and kidney and minimum in brain. Under lethal exposure to profenofos and carbosulfan for 24 hr, the activity was found to decrease in all the tissues of test fish,

maximum decrease was in liver (-27.77) and minimum decrease in muscle (-20.87). In subsequent periods of exposure, the activity of MDH further decreases with increase in the period of exposure. The activity of MDH at 24 hr of lethal exposure to profenofos was in the order of: Profenofos lethal 24 hr: Liver>Kidney>Gill>Brain>Muscle. Under sublethal exposure to profenofos for 24 hr and 8days, the activity of MDH was found to decrease in all the tissues of test fish and Maximum decrease was in liver(-19.44) and (21.73) in kidney, minimum decrease was observed in brain (-12.0) and (10.09) in liver. Profenofos sublethal 24 hr: Liver>Brain>Gill>Muscle>Kidney; Profenofos sublethal 8 days: Kidney>Brain>Muscle>Liver>Gill. Under lethal exposure to carbosulfan for 24 hr activity of MDH was found to decrease in all the tissues but the intensity of increase was greater when compared to sub lethal concentrations, maximum decrease in activity was found in the liver (-16.66) and minimum (-8.79) in muscle, the decrease was observed in activity of MDH at 24 hr of lethal exposure to carbosulfan was in the order of: Carbosulfan lethal 24 hr: Liver>Brain>Kidney>Muscle>Gill. Under sublethal exposure to carbosulfan for 24 hr and 8days, the activity of MDH was found to decrease in all the tissues of toxicant treated fish and maximum decrease was observed in liver (-16.66) and (17.39) in kidney and minimum decrease was observed in (-7.04) and (5.49) in liver. Carbosulfan sublethal 24 hr: Liver>Kidney>Brain>Muscle>Gill. Carbosulfan sublethal 8 days: Kidney>Muscle>Brain>Gill>Liver.

Table 5. Change in the specific activity levels of Malate dehydrogenase (MDH) (μ moles of formazan/mg protein^{-1}/hr^{-1}) and percent change over the control in different tissues of the freshwater fish, *L. rohita* exposed to sublethal and lethal concentrations of profenofos and carbosulfan for 24hr

	Profenofos					Carbosulfan			
Organs	Control	Sub lethal	Percent Change	Lethal	Percent Change	Sub lethal	Percent Change	Lethal	Percent Change
Liver	1.08± 0.01^{b}	0.87± 0.04^{c}	-19.44	0.78 0±.02^{a}	-27.77	0.90 ±0.01^{c}	-16.66	0.86 ±0.02^{d}	-20.37
Brain	0.69± 0.003^{a}	0.58± 0.02^{a}	-15.94	0.54 ±0.04^{d}	-21.73	0.60 ±0.04^{d}	-13.04	0.57 ±0.01^{b}	-17.39
Muscle	0.91± 0.02^{d}	0.80± 0.03^{b}	-12.03	0.72 ±0.02^{c}	-20.87	0.83 ±0.02^{a}	-8.79	0.76 ±0.01^{a}	-16.48
Gill	0.71± 0.04^{a}	0.62 ± 0.02^{d}	-12.67	0.55 ±0.03^{b}	-22.53	0.66 ±0.02^{d}	-7.04	0.60 ±0.03^{c}	-15.49
Kidney	0.78± 0.002^{a}	0.69 ±0.003^{a}	-11.53	0.58 ±0.02^{a}	-25.64	0.71 ±0.04^{b}	-8.97	0.63 ±0.04^{a}	-19.21

Means are SD ± (n= 5) for a tissue in a row followed by the same letter are significantly different ($p < 0.05$) from each other according to Duncan's Multiple Range (DMR) Test.

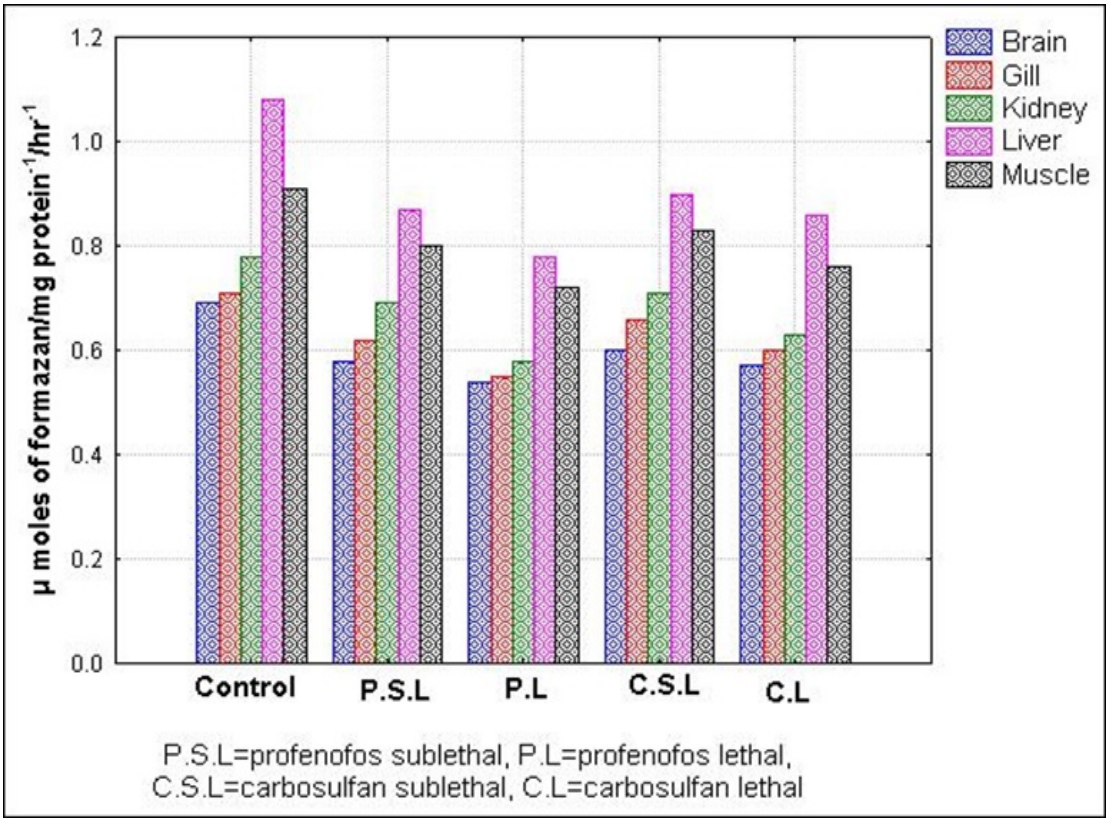

Figure 5. Change in the specific activity levels of Malate dehydrogenase (μ moles of formazan/mg protein^{-1}/hr^{-1}) and percent change over the control

in different tissues of the freshwater fish, *L. rohita* exposed to sublethal and lethal concentrations of profenofos and carbosulfan for 24 hr

Table 6. Change in the specific activity levels of Malate dehydrogenase (MDH) (µ moles of formazan/mg protein^{-1}/hr^{-1}) and percent change over the control in different tissues of the freshwater fish, *L. rohita* exposed to sublethal concentrations of profenofos and carbosulfan for 8 days

	Profenofos			Carbosulfan	
Organs	control	Sublethal	Percent Change	Sublethal	Percent Change
Liver	0.92±0.01^{b}	0.81±0.04^{a}	10.91	0.86±0.01^{a}	5.49
Brain	0.61±0.05^{d}	0.52±0.02^{a}	14.71	0.56±0.04^{d}	8.19
Muscle	0.82±0.01^{a}	0.71±0.03^{c}	13.44	0.73±0.02^{c}	10.9
Gill	0.66±0.006^{a}	0.59±0.02^{d}	10.60	0.62±0.002^{a}	6.01
Kidney	0.69±0.04^{c}	0.54±0.003^{b}	21.73	0.57±0.01^{a}	17.39

Means are SD ± (n= 5) for a tissue in a row followed by the same letter are significantly different ($p < 0.05$) from each other according to Duncan's Multiple Range (DMR) Test.

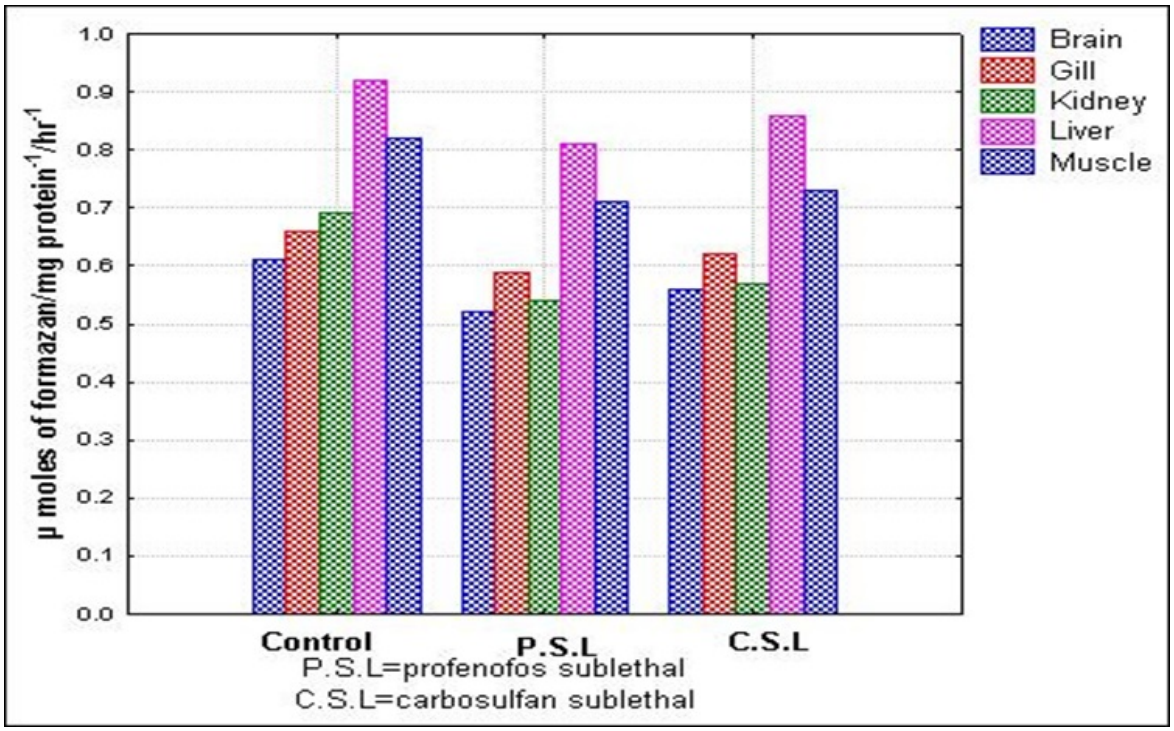

Figure 6. Change in the specific activity levels of Malate dehydrogenase (MDH) (µ moles of formazan/mg protein^{-1}/hr^{-1}) and percent change over the control in different tissues of the freshwater fish, *L. rohita* exposed to sublethal concentrations of profenofos and carbosulfan for 8 days

Decreased MDH activity levels due to the inhibition exerted by oxaloacetate; because of decrease in the activity of TCA cycle dehydrogenase is consistent with the disintegration of mitochondria of CO_2 formation from acetate. The decrement of MDH activity suggests that there is a shift in the respiratory metabolism towards anaerobiosis, depletion in MDH levels in different tissues of rat exposed to pesticide cypermethrin stress Reported that, the activity of SDH and MDH levels decreased in the tissues of freshwater mussel *Lamellidens marginalis* exposed to sublethal concentration of copper sulphate, decreased MDH activity due to inhibition of oxidative metabolism in mussel by copper stress (47-49). Decreased MDH levels in the tissues of *Clarius batrachus* and *Matrinaxa bryconcephalus* on exposure to endosufan and folidol (50, 51). In the present study indicate that both pesticides profenofos and carbosulfan caused alterations in the MDH activity of fish *L. rohita* but comparatively profenofos treated fish showed more decrement when compared to carbosulfan treated fish. MDH indicates that both pesticides significantly inhibits aerobic, as well as anaerobic metabolism in exposed animals.

4. CONCLUSION

Based on the experimental data, our study finds profenofos and carboslfan to be highly toxic to freshwater fish *Labeo rohita*, at ecologically pertinent concentration. The effect was comprehensible to be both time and concentration dependent and indicating that the anaerobic respirations arrived and aerobic respiration inhibited so as to meet the increased metabolic stress and to overcome the toxic stress. The alteration in the LDH, SDH and MDH activities can be taken as good biomarker or indicator of the profenofos and carbosulfan induced stress.

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AUTHORS CONTRIBUTION

This work was carried out in collaboration among all authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this paper.

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