ENZYMATIC ACTIVITY INDUCED BY PHOSALONE (35%) EC IN DIFFERENT TISSUES OF FRESHWATER FISH - *CTENOPHARYNGODON IDELLA* (GRASS CARP)

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ABSTRACT

Enzymes play significant role in food utilization and metabolism. The proteolytic enzymes participate in the breakdown of protein molecules into amino acids and these amino acids are in turn oxidized to give energy for body function. Biochemical analysis of biochemical components, Fish is important to estimate the physiological health of fish along with estimation of water pollution, discussed about the methods and materials to analysis the biochemical components of fish. fish *Ctenopharyngodon idella* of size 6±7 cm and 6±8 g weight were brought from a local fish farm kuchipudi, Guntur district of Andhra Pradesh, India and acclimatized at 28± 2^0 C in the laboratory for 15 days. Such acclimatized fish were exposed to sub lethal ($1/10^{th}$ 96 hr LC_{50}) and lethal (96 hr LC_{50}) concentrations of Phosalone for 24 hr lethal and sub lethal, 4 day 8 days. The vital tissues like muscle, brain, liver, gill and kidney of the fish were taken for the estimation of lactate dehydrogenase (LDH) activity, Estimation of Succinate dehydrogenase (SDH) activity, Malate dehydrogenase (MDH) activity, along with control exposures. Decreased in Enzymes values were observed in tissues of *Ctenopharyngodon Idella* exposure to Phosalone (35%) EC.

Keywords: *Ctenopharyngodon idella*, Phosalone, Enzymes, lactate dehydrogenase (LDH), Succinate dehydrogenase (SDH), Malate dehydrogenase (MDH).

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1.INTRODUCTION:

Enzymes play significant role in food utilization and metabolism. The proteolytic enzymes participate in the breakdown of protein molecules into amino acids and these amino acides are in turn oxidized to give energy for body function (Saravanam *et al.*, 2000). Enzymes are exceedingly efficient and very specific in terms of nature of reaction catalysed and the substrate utilized.The synthesis and final concentration of enzymes is under genetic control and is greatly influenced by very small molecules of substrate. The pesticide stress was known to induce significant change in protein metabolism; it is likely that the amino transferases were also considerably affected. Increased activities of AAT and ALAT in different tissues of fish either increased operation of transamination on increased synthesis of amino acids from other sources like glucose of fatty acids during exposed period of Phosalone (35%EC).

Lactate dehydrogenase (LDH) is hydrogen transferring enzyme that catalyzes the oxidation of L-lactate to pyruvate with the median of NAD+ as hydrogen acceptor. LDH, an indicator of anaerobic metabolism, was expected to exhibit increased activity at lower oxygen levels (Shwetha and Hosetti, 20011). It catalyzes the lost step in anaerobic glycolysis; the reversible oxidation of lactate to pyruvate, located in the cytoplasm of all cells in the body; it is consequently used as a quantitative marker enzyme for intact cells providing information cellular glycolytic pathway (Tugiyono and Gagnon, 2002; Ozmen *et al.*, 2007). Consequently, LDH play a major role in fish energy levels may be required in a short period of time (Cohen *etal.*, 2001; Monterio *et al.*, 2007; heavy metals and organochlorines (Ozman *et al.*, 2007); Pulp and paper mill effluents (Orrego *et al.*, 2011).

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 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 (Huang and Millar, 2013). The mitochondrial enzyme in the oxidative catabolism of sugars and such is used effectively as a marker of mitochondrial activity. It was concentrated in the chloride cells within the gills and has used as an indicator of osmoregulatory activity (Sonia Mukherjee *et al.*, 2007). The activity of SDH was decreased in different tissues like gill, brain, and muscle of freshwater fish *L.rohita* treated with copper or organochlorines (DDT and BHC) of organophosphates Diclorvos and Monochrotophos (Radhakrishnaiah *et al.*, 1992; Rajamannar *et al.*, 2000).

MDH is an enzyme that reversibly catalyzes the oxidation of Malate to oxalocelate using the reduction of NAD+ to NADH. This reaction is part of many metabolic pathways including the citric acid cycle. Malate dehydrogenase is also involved in gluconeogenesis, the synthesis of glucose from smaller molecules. Pyruvate in the mitochondria is acted upon by pyruvate carboxylase to form oxaloacetate, a citric acid cycle intermediate. In order to get the oxaloacetate out of the mitochondria, malate dehydrogenase reduces it to malate, and it then traverses the inner mitochondrial membrane. Malete dehydroginase (MDH) converts Malate to oxaloacetate but also pay a significant role in CO_2 fixation and in gluconeogenesis (Lehinnger, 2008).

2. MATERIALS AND METHODS:

Biochemical analysis of biochemical components, Fish is important to estimate the physiological health of fish along with estimation of water pollution, discussed about the methods and materials to analysis the biochemical components of fish.

Fish *Ctenopharyngodon idella* of size 6±7 cm and 6±8 g weight were brought from a local fish farm kuchipudi, Guntur district of Andhra Pradesh, India and acclimatized at 28± 2^0 C in the laboratory for 15 days. Such acclimatized fish were exposed to sub lethal($1/10^{th}$ 96 hr LC_{50}) and lethal (96 hr LC_{50}) concentrations of Phosalone for 24 hr lethal and sub lethal, 4 day 8 days. The vital tissues like muscle, brain, liver, gill and kidney of the fish were taken for the estimation of lactate dehydragenase(LDH)activity,Estimation of Succinate dehydrogenase (SDH) activity, Malate dehydrogenase (MDH) activityalong with control exposures.

Estimation of Lactate Dehydrogenase (LDH) activity:

The lactate Dehydogenase activity (LDH) was estimated by the method of srikanthan and Krishna murthy (1955) with slight modifications. 2 percent homogenates of the tissue were prepared in 0.25 M ice-cold sucrose solution and centrifuged at 1000 rpm for 15 mins. The supernatant served as the enzyme sourse. 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-nitrophenyl)-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 mins, and by adding 5 ml of acetic acid stopped the reaction. Zero time controls were maintained by adding 5 ml of acitic 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.

Estimation of Succinate dehydrogenase (SDH) activity:

Succinate dehydrogenase (SDH) activity was estimated by the method of Nachlas *et al*.,(1960).4% homogenate(W/V) of the tissues were prepared in cold 0.25M sucrose solution and centrifuge at 1000rpm for 15 min. 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), 0.5ml of sodium succinate, 0.2ml 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 37ºC for 10min and reaction was stopped by adding 5ml of acetic acid. Zero time controls were maintained by adding 5ml of acetic prior to addition of homogenate. The intensity by OD colour developed was read at 495nm against a reagent blank in a spectrophometer (ELICO Model SL207). The activity was exposed as µ mole of formazan formed mg protein^{-1}h^{-1}.

Estimation of Malate dehydrogenase (MDH) activity:

Malate dehydrogenase (MDH) activity was estimated by the method of Nachlas *et a*l., (1960). 2% homogenate(W/V) of the tissues were prepared in cold 0.25M sucrose solution and centrifuge at 1000rpm for 15 min. the supernatant was used as the enzyme source to 0.6 ml of supernatant 0.5ml of phosphate buffer(pH-7.2), 0.5ml of Malate,0.5 ml of INT and 0.2ml of NAD^+ was added. The reaction mixture was incubated at 37ºC for 30min. 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 of colored developed was read at 495nm against a reagent blank in a (ELICO Model SL207) spectrophometer. The activity was expressed as µ mole of formazan formed mg protein^{-1}h^{-1}.

Statistical analysis:

Student's t-test and one way analysis of variance (ANOVA) of SPSS (20.0 version), SPSS Chicago, USA, was employed to calculated the significance of the differences between control and experimental means. P values of 0.05 or greater than were considered statistically significant (Fisher, 1950). Significance level was based on $P<0.05$. Results were expressed as ±S.D of five individual**.**

3.RESULT AND DISCUSSION:

Biochemical biomarkers can provide information about the detoxification process of pesticides in living organisms. When it can content with an organisms, the toxic agent or substance can be transformed by enzymes, which act to make the xenobiotics substance or toxic compounds a less toxic compound and facilitate its excretion (Santhosh and Martinez, 2012).

Lactate dehydrogenase (LDH):

The calculated value of lactate dehydrogenase (LDH) activity and the percent change over control along with standard deviation are given in the Table 1And Figure1 The activity levels of dehydrogenase in *Ctenopharyngodon idella* exposed to phosalone were expressed as micro moles of formazan/mg /protein/hr.

The LDH level of muscle, brain, liver, gill and kidney of control fish were almost stable. The control values of LDH in different tissues of the fish *Ctenopharyngodon idella* were in the order of: Liver >Muscle > Kidney > Gill > Brain

Under sublethal and lethal exposure to phosalone for 24hr, the activity levels of LDH were found to decrease in all the tissues of the fish *Ctenopharyngodon idella* the percent change in the activity levels of LDH, in the test fish were in the order of:

Phosalone sub lethal 24hr: Liver >Muscle > Kidney > Gill > Brain

Phosalone lethal 24hr: Liver >Muscle > Kidney > Gill > Brain

Under exposures to sublethal concentrations of phosalone for 4th and 8th days. The percent depletion of LDH level in the test tissues of *Ctenopharyngodon idella* was in the order of

Phosalone sublethal 4 days: Liver >Muscle > Kidney > Gill > Brain

Phosalone sublethal 8 days: Liver >Muscle > Kidney > Gill > Brain

Under lethal exposure of for 24hr, maximum percentage of depletion was in muscle (-28.08) and minimum percentage was observed in brain (-23.80). Under phosalone sublethal exposure 24hr,

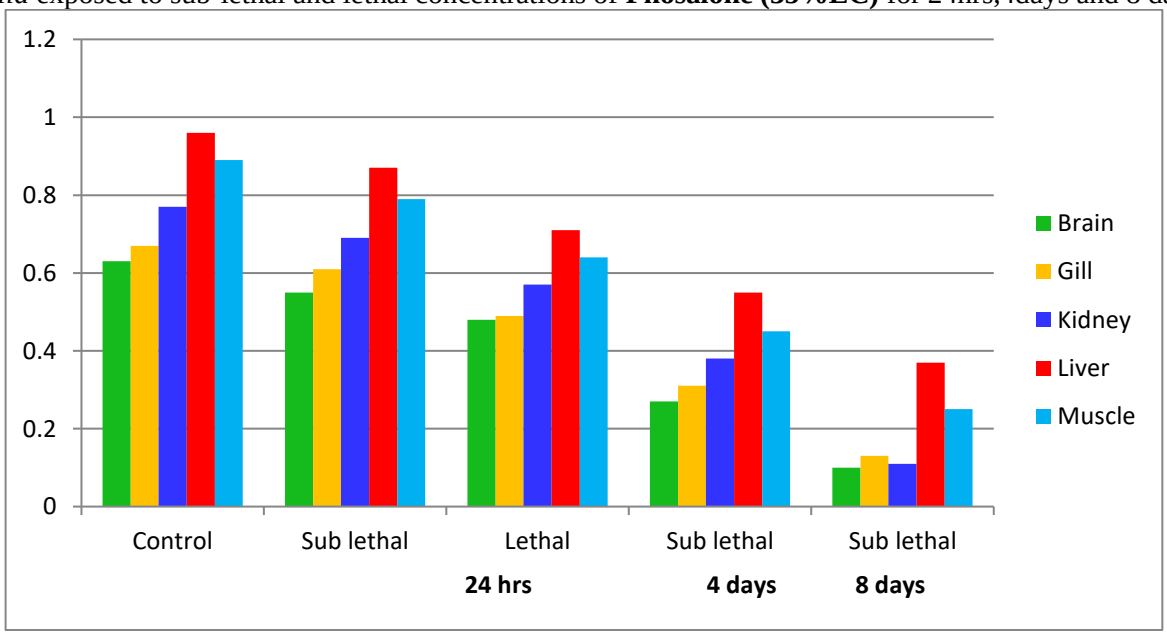
maximum percentage of depletion was observed in brain (-12.69) and minimum percentage was observed in gill (-8.95). Under phosalone sublethal exposure for 4 and 8 days, maximum percentage of depletion was (-57.14) in brain and (-42.70) in liver, minimum depletion was (-85.71) in kidney and (-61.45) in liver.

Table.1: Changes in the specific activity levels of Lactate dehydrogenase(LDH) (µ moles of formazan /mg protein/hr) and percent change over control in different tissues of the freshwater fish, *Ctenopharyngodon idella* exposed to sub-lethal and lethal concentrations of **Phosalone (35%EC)** for 24hrs,4days and 8 days:

Tissues	Control	24hrs Sub lethal	% change	24hr Lethal	% change	4days Sub lethal	% change	8days Sub lethal	% Change
Brain	0.63± 0.02	0.55± 0.03	-12.69	0.48± 0.03	-23.80	0.27± 0.03	-57.14	0.10± 0.03	-84.12
Gill	0.67± 0.03	0.61± 0.03	-8.95	0.49± 0.03	-26.86	0.31± 0.03	-53.73	0.13± 0.03	-80.59
Kidney	0.77± 0.05	0.69± 0.03	-10.38	0.57± 0.05	-25.97	0.38± 0.02	-50.64	0.11± 0.02	-85.71
Liver	0.96± 0.02	0.87± 0.04	-9.37	0.71± 0.03	-26.04	0.55± 0.05	-42.70	0.37± 0.04	-61.45
Muscle	0.89± 0.04	0.79± 0.03	-11.23	0.64± 0.02	-28.08	0.45± 0.04	-49.43	0.25± 0.03	-71.91

Values are the mean of five observations ;(±) indicates the standard deviation:
Values are significantly at $P< 0.05$

Fig.1: Changes in the specific activity levels of Lactate dehydrogenase(LDH) (µ moles of formazan /mg protein/hr) and percent change over control in different tissues of the freshwater fish, *Ctenopharyngodon idella* exposed to sub-lethal and lethal concentrations of **Phosalone (35%EC)** for 24hrs,4days and 8 days:



LDH is an important glycolytic enzyme which is present in the cells of almost all body tissues and changes in the enzyme activity may provide direct and indirect evidence of the cellular damage and can indicate the toxic mechanism. LDH is a terminal enzyme of anaerobic glycolysis, therefore, being of crucial importance to the muscular physiology, particularly in conditions of chemical stress, when high levels of energy may be required in a short period of time, Coppo (2002), Baghi, (1995).The effect of toxicant on enzymatic activity is one of the most important biochemical paratmeters, which is affected under stress (Das & Mukherjee 2003). The significant changes in enzymes activity of LDH indicate damage to any or all organs producing this enzyme such as liver or kidney injuries, (Young1999; De Coen; 2001).
LDH mediates inter-converson of lactate to pyruvate depending on the availability of co-enzyme NAD Amacher (2002). In the present

study, it was observed that the activity of LDH was decreased following sublethal and lethal exposures of endosulfan and fenvalerate in all the tissues of *Labeorohita* for 24 hrs and 15 days. The decrease in lactate dehydrogenase activity with a consequent increase in the levels of lactic acid suggests the predominance of anaerobic segment, glycolysis Amacher (2002).When an organ is diseased due to the effect of a toxicant, enzymatic activity appears to be increased or inhibited due to the active site being either denatured or distorted. Since some enzymes catalyze some steps in the metabolism of carbohydrate and protein, they are present in most tissues.

The increase or decrease in their level may be sufficient to provide information of diagnostic values (Begum2009). Ncibi (2008) was observedDecreased lactate deydrognase (LDH) activity in, *Channa punctatus* after exposure to monocroophos Significant decrease in LDH activity levels were observed in the tissues of *Channa Punctatus* exposed to Euphorbia royeleana latex Sambasiva Rao(1999), Decrease in LDH activities was observed after exposure to endosulfan and fenvalerate on fresh water fish *Clarias Batrachus*, which indicates decrease in aerobic and anaerobic capacity of fish, Shweta (2007). The degree of toxicity produced by the poisonous substance is dose independent upon environmental conditions such as tempatrature, pH, oxygen content and presence of residue molecules (Capkin *et al.*, 2006; Singh and Mishra, 2009; Gulfer *et al.*, 2009).

It is well known that protein, carbohydrates and lipid play a major role as energy precursors in fish under stress conditions. Enzymes play significant role in food utilization and metabolism. The proteolytic enzymes participate in the breakdown of protein molecules into amino acids and these amino acids and these amino acids are in turn oxidized to give energy for body function (Saravanan *etal.*, 2000). The significant decline of LDH activity in *L.rohita* further suggest the decrease in the glycolytic process due to the lower metabolic rate as a result of cyanide exposure (Bhattacharya*et al.*, 2009). Pesticides can induce noticeable changes in the activities of different enzymes in the freshwater animals like fish Tripathi (1990) Suneetha (2012).

LDH is considered to be the most important enzyme of the glycolytic pathway in animals, including fishes as it is the key enzyme located at the vital point between glycolysis and TCA cycle. As the LDH is a terminal enzyme of anaerobic glycolysis, it has crucial importance to the muscle physiology, particularly in conditions of chemical stress, when high levels of energy required in a short period of time. Baghi (1995), Coppo, (2002). It is likely that any fluctuation in the cellular environment alters the activity of LDH and the changes in its activity indicate the damage to any or all organs producing this enzyme such as liver and Kidney, De Coen and Jansen (2001).The changes in the activity of LDH provide a direct and indirect evidence of the toxic mechanism of the pesticides. Stress is an energy demanding process and the animal mobilizes energy substrates to cope with stress metabolically, Vijayan (1997).

Changes in the activity of the enzymes like LDH are sensitive to environmental pollutants like pesticides Devi (2003). Venkateswara Rao (2006) have reported an increase in the LDH activity in different organs of the exposed animals. The elevated lactate dehydrogenase (LDH) activity is a marker for tissue damage in fish (Ramesh *et al.*, 1993), hypoxic conditions (Das *et al.*, 2004), and muscular harm (Balint *et al.*, 1997) and serves as a good diagnostic tool in toxicology.LDH level which indicates the energy demands are met by anaerobic respiration through increase in LDH activity. Moreover, several investigators have reported that the oxygen consumption and the activities of liver respiratory enzymes were decreased considerably with an elevation of LDH activities in stressed animals.They suggested that the stressed animals were meeting energy requirement through anaerobic oxidation (Das and Mukerjee, 2000).

Succinate Dehydrogenage (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 reflection of SDH activity in all tissues of fish *Ctenopharyngodon idella* treated with lethal and sublethal concentrations of phosalone. When compared with controls. The calculated values of Succinate dehydrogenase activity and the present change over control along with standard deviation are given in Table 2 and Figure2.

The calculated value of SDH and standard deviation along with percent change over the controls is tissue specific viz., brain, liver, muscle, gill and kidney of fish *Ctenopharyngodon idella* exposed to lethal and sublethal concentrations of phosalone for 24hr, 4 and 8 days. In the tissue of 24hrs control fish, SDH activity was in the order of: Liver>Muscle>Kidney>Gill>Brain

The control fish, SDH activity was maximum in liver followed by muscle, kidney, gill and minimum in brain. The higher activity of SDH in

liver and muscle suggests higher distribution of mitochondria in the tissues, since succinate dehydrogenase (SDH) is mitochondrialy localized (Harper, 2008).

Phosalone sub lethal 24hr: Muscle > Liver > Kidney > Gill > Brain

Phosalone lethal 24hr: Muscle > Liver > Kidney > Gill > Brain

Under sublethal exposure to phosalone for 24hr the activity of SDH was found to decreased in all the tissues of test fish and, maximum decrease was observed in liver (-56.77) and minimum decrease was observed (-9.23) in brain. Under lethal exposure to phosalone for 24hr, the activity was found to decrease in all the tissues of test fish, maximum decrease was observed in Liver (-62.5) and minimum decrease was in kidney (-22.98).

Phosalone sublethal 4days: Muscle > Liver > Kidney > Gill > Brain

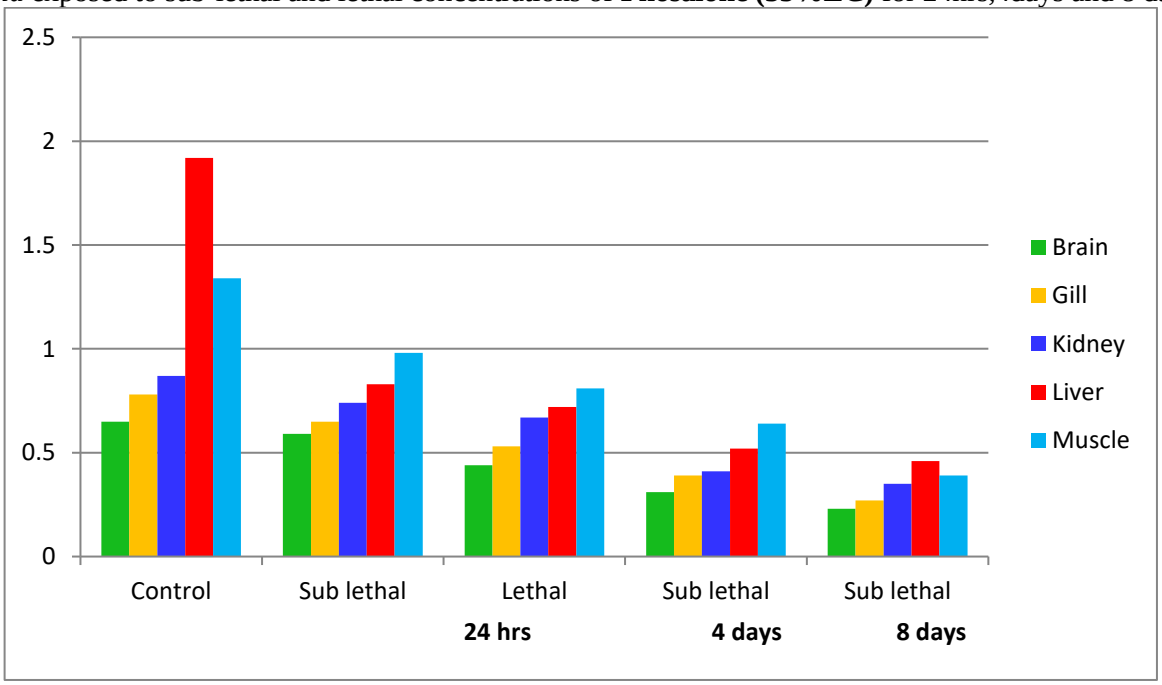
Phosalone sublethal 8 days: Liver > Muscle > Kidney > Gill > Brian

Table.2 Changes in the specific activity levels of Succinate dehydrogenase(SDH) (µ moles of formazan /mg protein/hr) and percent change over control in different tissues of the freshwater fish, Ctenopharyngodon idella exposed to sub-lethal and lethal concentrations of Phosalone (35%EC) for 24hrs,4days and 8 days:

Tissues	Control	24hrs Sub lethal	% change	24hrs Lethal	% change	4days Sub Lethal	% change	8days Sub lethal	% Change
Brain	0.65± 0.29	0.59± 0.03	-9.23	0.44± 0.03	-32.30	0.31± 0.03	-52.30	0.23± 0.03	-64.61
Gill	0.78± 0.04	0.65± 0.03	-16.66	0.53± 0.03	-32.05	0.39± 0.04	-50.01	0.27± 0.05	-65.38
Kidney	0.87± 0.14	0.74± 0.03	-14.94	0.67± 0.03	-22.98	0.41± 0.04	-52.87	0.35± 0.04	-59.77
Liver	1.92± 0.28	0.83± 0.02	-56.77	0.72± 0.03	-62.5	0.52± 0.03	-72.9	0.46± 0.03	-76.04
Muscle	1.34± 0.20	0.98± 0.07	-26.86	0.81± 0.04	-39.55	0.64± 0.03	-52.23	0.39± 0.03	-70.89

Values are the mean of five observations ;(±) indicates the standard deviation:
Values are significantly at $P< 0.05$

Fig.2. Changes in the specific activity levels of Succinate dehydrogenase(SDH) (µ moles of formazan /mg protein/hr) and percent change over control in different tissues of the freshwater fish, *Ctenopharyngodon idella* exposed to sub-lethal and lethal concentrations of **Phosalone (35%EC)** for 24hrs,4days and 8 days:



Under sub lethal exposure to phosalone for 4 days the activity of SDH was found to increase in all the tissues of test fish and, maximum decrease was observed in liver (-72.9) and minimum decrease was observed in gill (-50.01). Under sublethal exposure to Phosalone for 8days the activity of SDH was found to decrease in all the tissues of test fish, maximum depletion was observed in liver(-76.04) and minimum depletion was observed in kidney (-59.77).

SDH is a vital enzyme of citric acid cycle the reversible oxidation of succinate to fumarate. In the present investigation it can be visualized that there is a rapid depletion of SDH activity in all tissues of fish *Ctenopharyngodon idella* treated with lethal and sublethal doses of phosalone 35% EC when compared to their respective controls. In control fish, SDH activity was more in liver followd by muscle, gill tissues and minimum in kidney. The higher activity of SDH in liver and muscle suggests higher distribution of mitochondria in these tissues, since SDH is mitochondrially localized Lehninger (2008).

The general decrease in SDH activity during pesticides stress was associated with the inhibition of mitochondrial respiratory mechanism or dearrangement in ultra structure, architectural integrity and permeability of mitochondria, Tripathi,(2004).This prevents the transfer of electrons to molecular oxygen, resulting in the inhibition of SDH activity and shifting the aerobic metabolism to anaerobiosis, Shailendra(2010). The toxic effects of Dibutylphthalate (DBP) on the SDH levels of gill, liver and muscle partially agrees with that of Sastry *et al.,* (2002) who have reported quinolphos induced elevation in the activies of SDH in the intestine and inhibition of the same in other tissues (liver, kidney, gill, skeletal muscle, ovary and testis). On contrary, Samuel and Sastry (1981) have observed significant decrease in the activities of glucose-6-phosphate, hexokinase, LDH, SDH and MDH in *channa punctatus* on long term exposure to an organophosphate pesticide.
A similar decrement in the SDH activity was observed by (Abdel-Salam Mohamed Ibraik Ohaida *et al.*, 2010) in fish exposed to malthion and fenitrothion pesticides. The result of the present study are in agreement with those of (Ksheerasagar *et al.,* 2011; Suneetha, 2012) on *Labeo rohita.* The inhibition of NAD dependant, SDH activity indicated a decreased pass of intermediates into the citric acid cycle. This might be responshible for suppression of oxidative phased of tissue metabolism under pesticidal impact showing a shift from aerobic metabolism to anaerobic metabolism under pesticidal impact showing a shift from aerobic metabolism to anaerobic metabolism under the pesticidal stress (Abdel-Salam Mohamed Ibraik Ohaida *et al.,* 2010). The inhibition in LDH and SDH activities were observed in fish *Colisa fasciatus* due to toxicity of ethanolic extract of *Nerium indicum mill latex,* Tripathi (2004).

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 fresh water fish *Ctenopharyngodon idella* treated to sublethal and lethal concentrations of phosalone for 24hr, 4days and 8 days, time period were in Table 3 and Figure3. In the tissues of control fish, activity of MDH was in the order of:
Control: Liver > Muscle > Kidney > Gill > Brain
24hrs sublethal days: Liver > Muscle > Kidney > Gill > Brain
24hrs lethal days: Liver > Muscle > Kidney > Gill > Brain

In control fish decreased activity of MDH was noticed in liver followed by muscle, kidney, gill and minimum in brain. Under lethal exposure to phosalone for 24hr was found to decrease in all the tissues of test fish, maximum decreased was in brain (-50.94) and minimum decreased in muscle (-20.22). Under sublethal exposure to Phosalone for 24hr was found to decrease in all the tissues of test fish, maximum decreased was in gill (-27.69) and minimum decreased in muscle (-8.98).
Sublethal 4 days: Liver > Muscle > Kidney > Gill > Brain
Sublethal 8 days: Liver > Muscle > Kidney > Gill > Brain

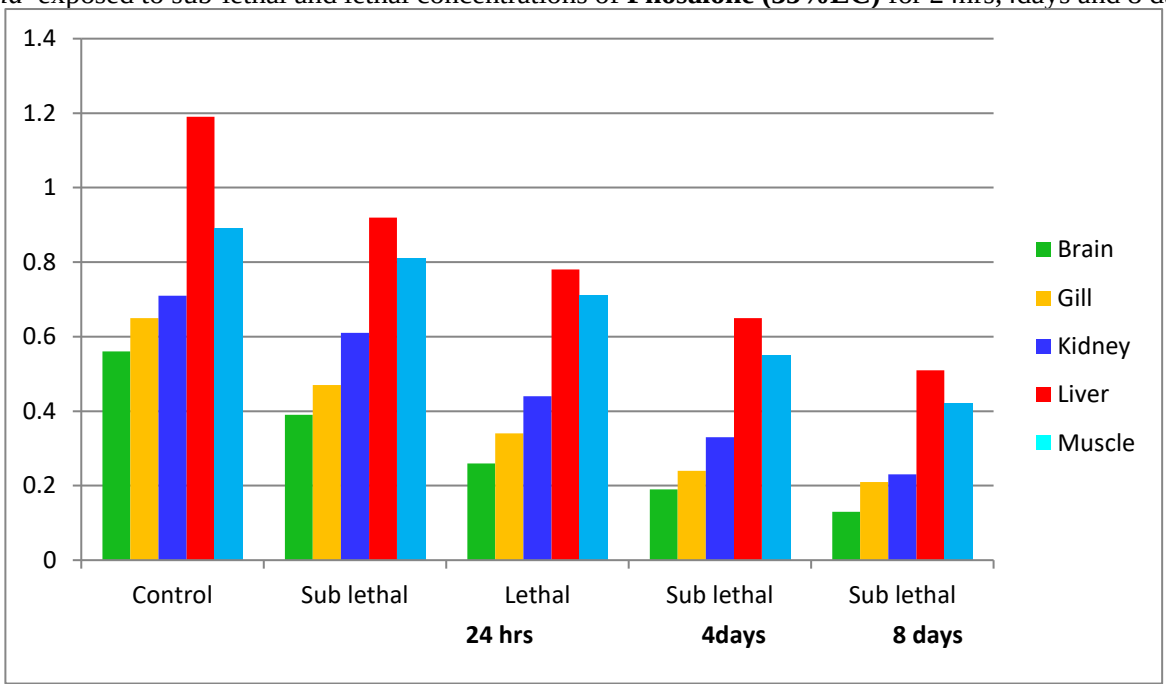
Under sublethal exposure to phosalone for 4 days, the activity of MDH was found to decreased in all the tissues of test fish and maximum decrease was in (-64.15) brain and minimum decrease in muscle (-38.20). Under sublethal exposure to phosalone for 8 days, the activity of MDH was found to decrease in all the tissues of test fish and maximum decrease was in brain (-75.47) and minimum decrease in muscle (-52.80).

Table.3: Changes in the specific activity levels of Malate dehydrogenase(MDH) (µ moles of formazan /mg protein/hr) and percent change over control in different tissues of the freshwater fish, *Ctenopharyngodon idella* exposed to sub-lethal and lethal concentrations of **Phosalone (35%EC)** for 24hrs,4days and 8 days:

Tissues	Control	24hrs Sub lethal	% Change	24hrs Lethal	% change	4days Sub Lethal	% change	8days Sub lethal	% Change
Brain	0.53± 0.03	0.39± 0.03	-26.41	0.26± 0.04	-50.94	0.19± 0.03	-64.15	0.13± 0.03	-75.47
Gill	0.65± 0.04	0.47± 0.03	-27.69	0.34± 0.03	-47.69	0.24± 0.03	-63.07	0.21± 0.05	-67.69
Kidney	0.71± 0.04	0.61± 0.06	-14.08	0.44± 0.04	-38.02	0.33± 0.04	-53.52	0.23± 0.03	-67.60
Liver	1.19± 0.34	0.92± 0.05	-22.68	0.78± 0.04	-34.45	0.65± 0.03	-45.37	0.51± 0.03	-57.14
Muscle	0.89± 0.04	0.81± 0.03	-8.98	0.71± 0.06	-20.22	0.55± 0.03	-38.20	0.42± 0.04	-52.80

Values are the mean of five observations ;(±) indicates the standard deviation:
Values are significantly at $P< 0.05$

Fig.3: Changes in the specific activity levels of Malate dehydrogenase(MDH) (µ moles of formazan /mg protein/hr) and percent change over control in different tissues of the freshwater fish, *Ctenopharyngodon idella* exposed to sub-lethal and lethal concentrations of **Phosalone (35%EC)** for 24hrs,4days and 8 days:



Malate dehydrogenase 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 plays a significant role in CO_2 fixation and in gluconeogenesis Lehninger (2008).

Malate+ NAD+ Oxaloacetate+$NADH^+$+H^+

Fish responds to toxicants by altering their enzyme activities and the inhibition of these enzyme activities has been used to indicate the tissue damage Webb and Rose (2005). Biochemical indicators, know as biomarkers, can serve as early warning signs of envirnonmental pollution or stress indication to soil organisms, and can be divided in three classes: exposure biomarkers, effect biomarkers, and susceptibility biomarkers Schlenk, (1999). Biomarkers of exposure are related with cellular or molecular responses indicating an interaction between an organism and a xenobiotic agent, Roberts (2004). The effectiveness of biomarkers has been demonstrated in several studies on the toxicity of pesticides to fish Ramesh and Srinivasan (2009).

In the present study decreased MDH activity levels due to the inhibition exerted by oxaloacetate,

because of decrease in the activity of TCA cycle dehydrogenases is consistent with the disintegration of mitochondria and CO2 formation from acetate. Decreased MDH levels in the tissues of *Clarius batrachusand, Matrinaxa byconcephalus* was exposure to endosufan and folidol (Rajinikant and Shukla, 1997; Lucia *et al.,* 2004). Alterations in MDH activity were observed in fish *Labeo rohita* exposed to endosulfan and fenvalerate (Suneetha, 2012).

Decreased in MDH values were observed in tissues of *Clarias batrachus* exposure to endosulfan Tiwari (2009). A reduction in MDH activity was observed in matrinxa, *Brycon cephalus* after exposure to Folidol 600, Archanakumta(1998). . Decrease in the malate dehydragenase levels were observed in tissues of *Clarias batrachus* on exposure to edosulfan (Rajikanth Mishra and Shukla, 1997). A reduction in MDH activity was observed in *Brycon cephalus* after exposure to Folidol 600(Lucia *et al., 2004).*

4.CONCLUSION:

Decreased Lactate dehydrogenase (LDH) activity observed in lethal and sublethal concentrations in different exposure periods. Lactate dehydrogenase converts that lactate to pyruvate and has very important role in carbohydrate metabolism. Alterations in the specific activity of Succinate dehydrogenase (SDH) in different tissues fish *Ctenopharyngodon idella* after sublethal and lethal exposure of Phosalone. SDH activity was significantly inhibited at 24 hr sublethal and lethal exposure. The changes in the SDH activity over control were observed. SDH is a FAD dependent enzyme and facilitates the activity levels decrease. Under Phosalone induced stress, the activity level of malate dehydrogenase (MDH) was found to be decreased in all the tissues. The decrease in MDH activity over control were observed, MDH catalyses the oxidation of malate to oxaloacetate, but also plays an important role in CO_2fixation and gluconeogenesis. Fluctuations in MDH (Malate dehydrogenase) activity denote alterations in the oxidative metabolism of substrate malate to oxaloacetate.

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