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Effect of sublethal Concentration of phenyl mercuric Acetate on respiratory metabolism of Tilapia Mossambica, peters and Its Recovery

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ABSTRACT

The effect of a sublethal concentration of phenyl mercuric acetate (0.06 mg/L) on the whole body oxygen uptake, ventilation rate & tissue slice respiration of laboratory acclimatized freshwater fish, Tilapia Mossambica, peters was studied with the increase in exposure period the ventilation rate showed a significant declining trend. The whole animal oxygen uptake decreased significantly with the increase in exposure period. A maximum of 83.5% inhibition was observed on 28 d of exposure. The tissue slice respiration showed a declining trend in vivo with the increase in exposure period. Brain slice respiration declined significantly with the increase in exposure period. Exposed liver slices and muscle slices also behaved identifically. However, the effects were more pronounced at early periods of exposure in case of liver & muscle. Gill slice respiration showed significant variation and was much more pronounced then the other tissues. It showed highest inhibition indicating highest damage caused to the tissue by PMA in exposed fish, when compared to liver, muscle and brain of exposed fish. In in vivo studies, with the increase in PMA concentration the oxygen uptake reduced drastically and maximum reduction was observed at 0.5 mg/L. of PMA. When the exposed fishes were transferred to PMA free medium, partial recovery was observed in ventilation rate, whole animal uptake rate, liver slice respiration and gill slice respiration. No recovery was marked in brain slice respiration. In addition, further depletion was marked in recovery period. A positive significant recovery was marked in case of muscle tissues of the exposed fishes, in recovery medium.

INTRODUCTION

Oxygen consumption has been chosen as a parameter to assess the extent of stress, since it is a valuable indicator of energy expanded to meet the demands of an environmental alteration. Much work has been done pertaining to the effects of heavy metals on respiratory physiology. According to Mac Innes and Thurbeng (1973) silver depressed oxygen consumption in a mollusk, Nassarius absoletus. But in contrast, Therberg et al. (1974) reported that silver caused an increase in oxygen consumption in Crasstrea Virginica. O'Cornor and Fromm (1975) reported depletion in oxygen consumption of the gill tissues by methyl mercury in vitro in rainbow trout (Salmo qairdneri).

Vernberg and Vernberg (1972) reported that mercuric chloride had a damaging effect on the oxygen consumption of fiddler crabs. Uca Puygilator. Fox et al. (1975) made a comparative study on the effect of organic and inorganic mercury on brain slice respiration and metabolism in guinea pigs. Very little reference is available on the effect of organic mercury on the respiratory metabolism of fresh water fishes. Methyl mercury in fish is caused by bacterial methylation of inorganic, either in the environment or in bacteria associated with fish gills or gut. (Boening.D.W.2000). present investigation describes the effects of a sublethal concentration of phenyl mercuric acetate on the wholebody organ uptake, ventilation rate and tissue slice respiration of a fresh water fish Tilapia mossambica.

MATERIAL AND METHODS:

Exposure:

The test fish, Tilapia mossambica were maintained in laboratory aquaria and fed slices of boiled eggs throughout the experimental exposure and recovery period.

Tilapia fish were exposed in 100 L. glass aquaria at a concentration of 0.06 mg l-1 of phenyl mercuric acetate for 28 days and at a 7 days interval and the whole animal oxygen uptake was determined. The test solutions were changed constantly. Both control and exposed media were aerated for 24 hours per day and kept under normal photoperiodic cycles and at a room temperature of 28± 2oC.s

RESPIRATION:

Whole body oxygen uptake:

The whole animal oxygen uptake rates of the test and control fish were measured using 5 wide mouth 2 L. flasks. Each flask

containing the test solution and a test fish was hermetically sealed. A reference flask was kept without fish to check any change of oxygen concentration during the experiments, due to presence of micro-organisms. After 30 minutes, the dissolved oxygen of all experimental flasks were determined according to the modified Winkler's method (Ashby, 1973). The same procedure was repeated for the control fish. The reduction of the dissolved oxygen concentration equals the amount of dissolved oxygen consumed by the fish in 30 minutes. The oxygen uptake was expressed as mg of 02 g-1 h-1. The ventilation rates of Tilapia fish were counted by the number of opercular movements per minute.

Tissue slice respiration:

Tissue and medium:

Both exposed and control fish were sacrificed. The brain, muscle, liver and gills were removed, washed in cold distilled water and kept in ice cold 0.25 M sucrose solution. Tissue slices were prepared approximately 0.35 mm thick using a razor blade and a recessed guide (Mc Ilwain, 1951) and floated into Kreb's ringer phosphate medium pre-gassed with oxygen. One to two slices were picked up on a bent wire, drained of excess fluid and the surface was soaked with the help of a Whatman filter paper and weighed in a single pan balance (MLW, Veb Fienwagetechinik, GDR) and transferred immediately to the medium. The weighed slices were subsequently transferred to the incubation medium in the Warburg flask.

The main compartment of the Warburg flask contained 3 Ml. incubation medium of the following composition: NaCl, 124 mM; KCl, 4.8 mM; Cacl2, 2.6 mM; MgSo4, 1.2mM; Na2HPO4, 16.0 mM; pH 7.4; and D-glucose, 10 mM. The pH of the medium was preadjusted to pH 7.4 with NaoH and the medium was pregassed with oxygen. The centre well of the flask had a filter paper wick and contained 0.2 ml of 0.3 N-NaOH to trap the evolved Co2.

Both control and experimental tissue were subjected to the same effects of swelling, ionic shifts and other changes occurring during preparation of slices., The slices under control condition were incubated in medium free of mercury compound for in vivo studies.

Incubation and Respiration:

Incubation took place in a Warburg bath, Temperature was maintained at 37oC. The reading of oxygen uptake and the preparation of tissue extract involved at 15 minutes equilibration of the tissue in a Warburg flask containing the incubation medium as mentioned above. Manometric reading of oxygen uptake and preparation of tissue extracts were carried out by the methods reported by Patel et al. (1973) and Fox et al. (1975).

ANALYSIS AND CALCULATION:

All calculations were made on the basis of wet weight of slices. Under our experimental conditions, dry weight of the tissue were mentioned as follows:

Brain tissue - 10.83% of the wet weight. Liver tissue - 12.93% of the wet weight. Muscle tissue - 15.62% of the wet weight. Gill tissue - 15.19% of the wet weight.

RESULTS:

VENTILATION RATE:

The ventilation rate in fish showed a significant declining trend with the increase in exposure period from 152 strokes per minute to 113 strokes per minute after 7 days of exposure to phenyl mercuric acetate. The exposed fish showed no significant variation upto 14 days of exposure. However, a significant decline in ventilation rate proceeded upto 28th day of exposure. The value depleted to 65 strokes per minute (Fig. 1 and Table-1). The data showed a partial recovery when the exposed fish was allowed to recover in PMA free medium for the same period of exposure. The result indicates, probable damage to the respiratory metabolic system linked to opercular movement of exposed fish. The control fish did not show any significant variation in ventilation rate throughout the experimental period.

WHOLE ANIMAL OXYGEN UPTAKE:

Initially the whole animal oxygen uptake was 0.843 mg of O2 g-1 h-1, but when the fish was exposed to PMA the whole animal oxygen uptake decreased linearly and significantly with the increase in exposure period (Fig. 2 & Table-2). After 7 d of exposure, the whole animal oxygen uptake depleted to 0.542 Mg. of O2 g-1 h-1 and a significant depletion was observed on 28 d exposure (0.142 mg of O2 g-1 h-1). The exposed fish were transferred to toxicant (PMA) free medium alter periodic exposure period (7, 14, 21 and 28 days).

The 7 d exposed fishes showed significant recovery when transferred to PMA free medium. After 7 days recovery, the fish could not recover to its pre-exposure level.

The 2nd batch of 14 days exposed fished were transferred to PMA free medium to estimate the period recovery. The exposed fishes could not recover to its pre-exposure level even after 14 days of recovery.

The third batch of 21 days exposed fishes were transferred to PMA free medium to find out the extent of damage and extent of recovery. The exposed fishes could not recover to its pre-exposure level. The extent of damage was deserved to be greater than 7th and 14th day exposed fishes. With the increase in recovery period the exposed fish did not show any significant recovery.

The final batch of 28th day exposed fishes were transferred to PMA free medium to determine the extent of stress and recovery. The damage was the highest in this batch of exposed fish when compared to other recovery sets. The exposed fishes could not recover even after 28 days of recovery. A higher rate of fish kill was marked in this set.

TISSUE SLICE RESPIRATION:

Oxygen consumption and CO2 production was considered as a parameter for measuring the respiration rate of tissue slices of brain, liver, muscle and gill. With the help of Warburg's apparatus the CO2 evolution was measured, calculated and interpreted as oxygen uptake by the tissue slices. In vivo studies of brain, liver, muscle and gill showed a significant variation in oxygen uptake or CO2 production when compared to control values. With the increase in exposure period, the tissue slice respiration showed a declining trend in vivo. Brain slices showed little variation up to 14 days of exposure. The data decreased from 722.4 to 685.2 after 14 days of exposure. With the increase in exposure period the data declined maximum upto 408.6 after 28 days of exposure. When the exposed fishes were transferred to PMA free medium, no recovery altogether was marked. In addition, a higher degree of inhibition was marked. The oxygen uptake decreased from 408.6 to

365.6 and 371.3 µl of O2 g-1 h-1 after 14 and 28 days of recovery. (Fig.3 & Table-3). In contrast, exposed liver slices showed significant depletion in oxygen uptake during the early exposure period. A significant drastic decline in the data was marked when compared to control values. The data decreases from 816.4 to 285.6 µl of O2 g-1 h-1 after 28 days of exposure. When the exposed fish was transferred to PMA free medium liver slices showed further decrease upto 251.3 µl of O2 g-1 h-1 after 14 days of recovery. However, a partial recovery was observed after 14 days of recovery and the data showed on increase upto 272.6 µl of O2 g-1 h-1 after 28 days of recovery. Exposed muscle slices behaved identically with that of exposed liver slices. A significant depletion from 542.65 to 232.65 µl of O2 g-1 h-1 was observed after 28 days of exposure. When the exposed fishes were transferred to PMA free medium, muscle slices showed a positive significant recovery from the beginning. The exposed system could recover significantly from 232.65 to 341.62 µl of O2 g-1 h-1 after 28 days of recovery. Though the recovery was significant, however, the system could not reach to its pre- exposure level. A striking significant decrease was observed in case of gill when compared to control fish gill. The value decreased from 860.75 to 281.45 µl of O2 g-1 h-1 after 28 days of exposure. When the exposed fishes were transferred to PMA free medium, an insignificant recovery was marked even after 28 days of recovery. Gill showed the highest inhibition indicating highest damage caused to the tissue by PMA in exposed fish, when compared to liver, muscle and brain of exposed fish. Exposed brain was the least affected during the exposure period, however, the same declining trend continued during recovery period. Highest recovery was recorded only in exposed muscle slices in vivo after 28 days of recovery.

DISCUSSION

The toxicity of mercury to aquatic animals becomes apparent in a shorter period. Gibilin and Massaro (1973) and Olson et al. (1973) reported that the rapid absorption of inorganic mercury through the gills, skin and gastrointestinal tract. Mercurials have long been

recognized as agents which interact with and poison proteins in general and enzymes in particular. With the increase in concentration more enzymes were inhibited (Webb, 1966). In vivo studies are of urgent necessity, so as to demonstrate mercury toxicity to biological systems. In in vivo studies the residual mercury concentration in in vivo studies no major depression on the oxygen consumption was noticed. At high concentrations the inhibition of metabolism is almost complete.

The effect of inorganic mercury on the whole animal oxygen uptake and respiratory metabolism is as expected. At high concentration fungicide inhibition of metabolism was almost complete. Oxygen consumption decreased with the increase in exposure period. The ventilation rates of the exposed Tilapia fish decreased significantly when compared to control fish. With the increase in exposure period the whole animal oxygen uptake of Tilapia decreased significantly (Fig.2). Fox et al. (1975) reported that inorganic mercury has no effect on the oxygen consumption by the brain tissue slices up to 0.5 mM concentration of mercuric nitrate in contrast to organic mercury. Organo-mercurials inhibit the CO2 production at lower concentrations even (Fox et al. 1975) in brain slices of guinea pig.

In contrast, the observation of this study reveals no such increase at lower concentration of the toxicant. However, a decline in active metabolism which related to respiratory metabolism was marked with the increase in exposure period and pesticide concentration.

Heavy metals are known to effect the cytochrome systems when they attack directly the very first step of the electron transport system and the respiratory chain, as a result of which respiration is inhibited when the attack is on the subsequent steps the respiration rate is reduced and finally leads to death of the animal (Blair et al. 1975).

The gill surfaces of the freshwater teleports in intimate contact with the water are particularly susceptible to aquatic contaminants, and changes in respiratory metabolism (Schaumburg et al. 1967). Since the respiratory and circulatory systems are intimately connected, any significant change in one system would reflect in the other. The gills are the primary site for the active absorption and respiratory exchange of gases. Because of the insufficiency of low solubility, the gills in obtaining the required oxygen for survival, low solubility of oxygen and increased toxicity of water, large amount of water must be passed over the gills to meet the oxygen demands of the fish (O' Connor and Fromm, 1975). At the first introduction of the toxic chemical to the aquatic system, the ventilation rate increased. When the fish slowly acclimatized to the concentration of the toxicant and increased in the toxicant residual concentration in different organs of the fish like- gill, liver, brain, muscle, the physiological activity decreased with the crease in ventilation rate. With prolonged exposure to a particular concentration, the fish ultimately faces death due to high accumulation of the toxicant inside the body tissue and also due to the inhibition of all metabolic enzymes and finally attack on central nervous system. The interpretation of metal induced changes in respiration is complicated by the fact that such alterations differ from metal to metal, from speciesto species and from one experimental condition to another. Although much information is available on the uptake and accumulation of heavy metals, very few studies have been conducted in detail on the physiological effects of metals of freshwater fishes. Further work is necessary to study the effects of phenyl mercury on respiratory enzymes and mitochondrial respiration. Further studies using cell fractionations and tissue homogenation will help for a comprehensive assessment of mercury pollution.

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TABLE-1: Ventilation rate (Strokes/Minute) of *Tilapia Mossambica*, exposed to 0.06 $Mg.1^{-1}$ Phenyl mercuric acetate at different days of exposure and recovery. Date are the mean of 5 samples.

	Exposure in Days					Recovery in Days				
	0	7	14	21	28	0	7	14	21	28
Control	152	157	158	154	156	156	156	153	155	154
Exposed	152	113	112	82	65	65	72	82	91	96

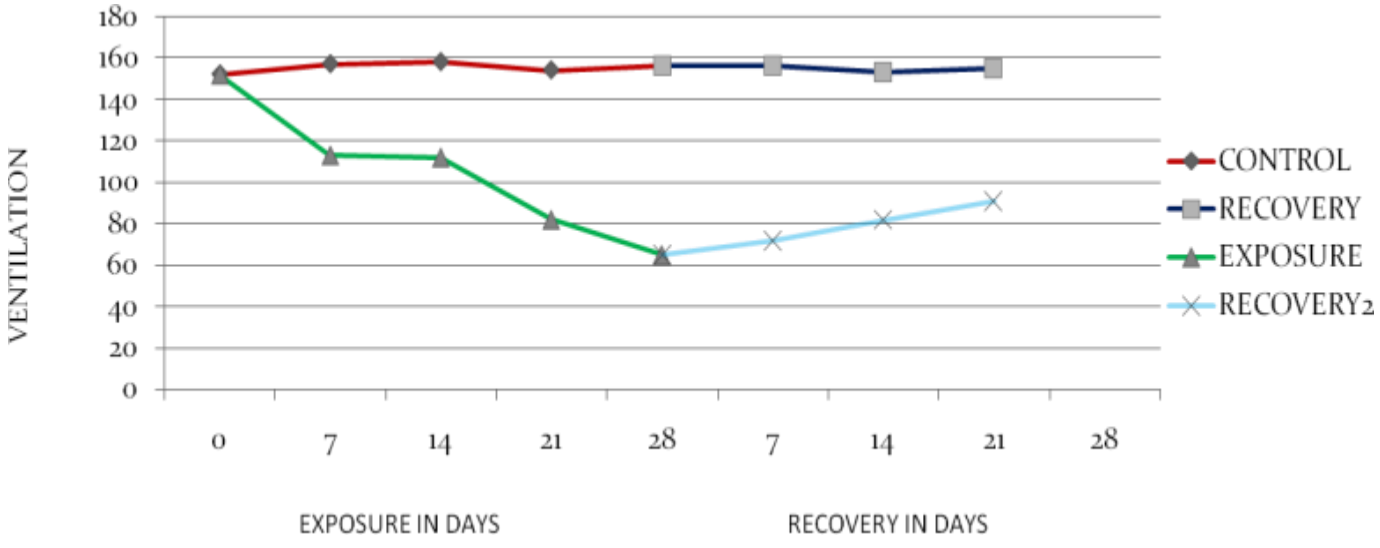

FIGURE-1 : Ventilation rate (Strokes/Minute) of Tilapia Mossambica, exposed to 0.06 Mg.1-1 Phenyl mercuric

acetate at different days of exposure and recovery. Date are the mean of 5 samples.

TABLE-2: Effect of Phenyl mercuric acetate on the whole body oxygen up take (Mg of 0_2 g^{-1} h^{-1}) of *Tilapia Mossambica*, after different days of exposure and its recovery. Data are mean of 5 samples.

	Exposure in Days					Recovery in Days				
	0	7	14	21	28	0	7	14	21	28
Control	0.843	0.853	0.846	0.851	0.848	0.848	0.844	0.853	0.864	0.851
Exposed	0.843	0.542	0.357	0.160	0.142	0.142	0.164	0.159	0.243	0.292

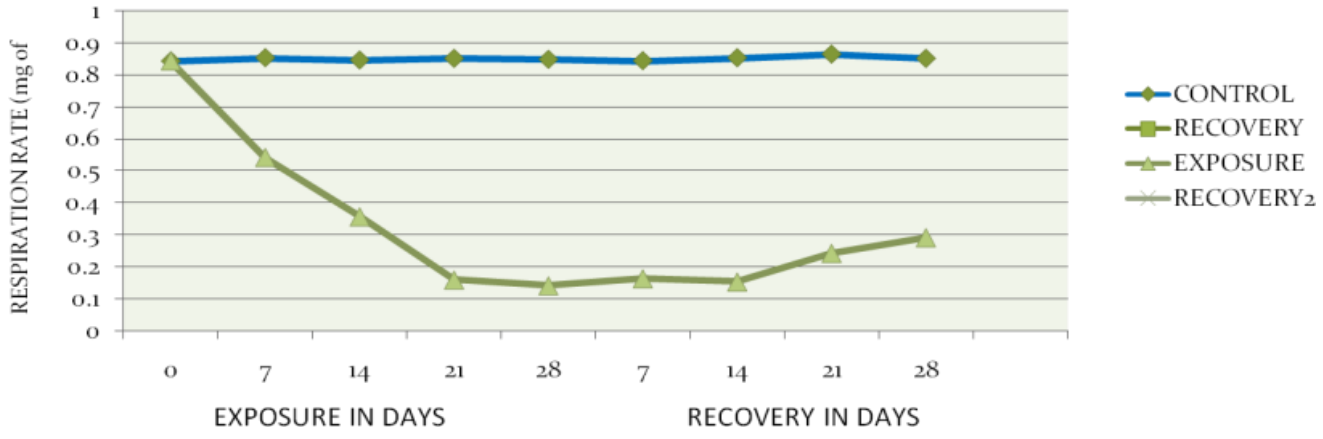

FIGURE:2 Effect of Phenyl mercuric acetate on the whole body oxygen up take (Mg of 02 g-1 h-1) of Tilapia Mossambica, after different days of exposure and its recovery. Data are mean of 5 samples.

TABLE-3: Change of oxygen uptake(μ of 02 g-1 h-1) by brain, liver, muscle tissue slices and gill of Tilapia exposed to 0.06 Mg. L-1 phenyl mercuric acetate in vivo for different days of exposure and its recovery. Date are the mean of 5 samples.

TABLE-3: Change of oxygen uptake(μ of 0_2 g^{-1} h^{-1}) by brain, liver, muscle tissue slices and gill of Tilapia

Tissues studied		Exposure in Days					Recovery in Days				
		0	7	14	21	28	0	7	14	21	28
Brain	Control	722.4	718.6	719.5	721.4	722.2	722.2	718.2	722.5	721.6	732.5
	Exposed	722.4	711.4	685.2	532.3	408.6	408.6	382.5	365.6	385.6	371.3
Liver	Control		812.6	808.4	806.5	8146	814.6	811.5	809.6	810.9	808.3
	Exposed		731.1			258.6	258.6	282.6	251.3	261.4	272.6
Muscle	Control	542.65	522.31		536.37		51.53	539.82			
	Exposed	542.65	436.91	364.44	296.56	232.65	232.65	238.31	271.43	315.15	341.62
	Control	860.75	844.33	854.62	858.66	849.24	849.24	854.31	855.65	849.65	852.38
	Exposed	860.75	641.39	511.40	462.39	281.45	281.45	282.44	288.42	291.45	302.45

FIGURE-3: Change of oxygen uptake (μ of 0_2 g^{-1} h^{-1}) by brain, liver, muscle tissue slices and gill of Tilapia exposed to 0.06 Mg. L^{-1} phenyl mercuric acetate in vivo for different days of exposure and its recovery. Date are the mean of 5 samples

exposed to 0.06 Mg. L^{-1} phenyl mercuric acetate in vivo for different days of exposure and its recovery. Date are the mean of 5 samples.