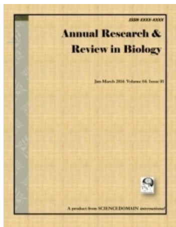

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Synergism of Heavy Metals on the Respiration of *Oreochromis mossambicus*

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Authors' contributions

Authors PSN and AKK designed the study. Author PSN carried out the study and derived the results results. Authors AKK and PSN analysed and discussed the results and author PSN presented the work under the supervision of AKK. Both authors have accepted to publish the manuscript.

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ABSTRACT

This study highlights the impact of heavy metals from electroplating industrial waste water on the respiration of the fish - *Oreochromis mossambicus*. Bioassays were conducted separately with chromium, nickel and mixed electroplating effluent on the fish. Results revealed quantum decrease in oxygen consumption levels in fish exposed to the highest concentrations of electroplating industrial effluent when compared to those exposed to chromium or nickel. The Two way ANOVA analysis exhibit significant influence of heavy metals in electroplating effluent on the respiration of fish. Synergism of heavy metals in electroplating effluent is projected in this work.

Keywords: Electroplating industrial effluent; bioassay; Oreochromis mossambicus; respiratory physiology; chromium; nickel; gill.

1. INTRODUCTION

Heavy metals as major constituents of industrial effluents are not usually remediated in the aquatic system by normal processes. Thus metals influx into aquatic organisms is inevitable

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and this entry tends to alter the physiology of organisms for instances transport across the plasma membrane, mitochondrial dysfunction and lysosome instability [1]. Mercury poisoning occupied the headlines through a well-known tragedy “Minamata Disease” caused in Japan due to mercury contaminated sea food [2]. The mixture of metals in industrial effluent may cause physiological disturbances to freshwater fish [3], such as chromosomal aberrations [4], testicular changes [5] and other biological effects [6].

The continuous input of metals into the aquatic environment directly through waste disposal or indirectly via settling of waterborne particles necessitates a continued assessment of their effects on the ecosystem [7]. This type of toxicity data is needed to evaluate the quality of the aquatic environments. The evaluation of impact of metals on the aquatic system involves not only the measurement of substances in the ecosystem but also the mechanism of toxicity and the factors which determine the availability of toxicants to the organism’s concerned [8].

Fish health in a water body is a useful index of water quality. No river or freshwater body can be considered to be of satisfactory quality unless fish can live and thrive in it. Fish actively avoid effluents that contain toxic chemicals or water low in dissolved oxygen, extreme pH and temperature or undesirable water quality characteristics [9].

Respiration is one of the essential parameters on which depend many of the vital functions like growth, reproduction and metabolism of fish [10]. Respiration rate has been identified as an indicator of sub- lethal stress in organisms exposed to stress and as a response of organisms to toxicants [2]. Oxygen budget of aquatic environment plays an important role in organic pollution and reduction in dissolved oxygen has a marked effect on many physiological, biochemical and behavioral processes in fish.

Disturbances in oxygen consumption of freshwater fish exposed to industrial effluents and heavy metals have been reported [9]. High loads of organics from industrial discharges therefore may cause chemical and biological effects, as well as anaerobiosis in water column. The nature and extent of pollution depend on biological oxygen demand and oxygen consumption in aquatic bodies [3]. Industrial effluents deplete dissolved oxygen and increase COD, causing heavy mortality of organisms by interfering with their respiratory physiology [7]. Similar findings in fish exposed to chromium [11], mercury, cadmium and chromium [12] and distillery effluent [13] exist.

Electroplating industry is a well-known cottage industry in Madurai .The wastewater discharged from this industry gets mixed with the Vaigai river water sources. Aqua farmers using this water to culture tilapia at a larger scale to cater to the need of the public. An understanding about the impact of this wastewater from electroplating effluent and its major toxic constituents, chromium and nickel on the respiratory physiology of tilapia (*Oreochromis mossambicus*) is being attempted to highlight the synergism of heavy metals in the industrial effluent.

2. MATERIALS AND METHODS

2.1 Collection and Maintenance of Fingerlings

Healthy fingerlings of *Oreochromis mossambicus* collected from a local fish farm were transported to the laboratory in closed polythene bags filled with oxygen. During transportation, care was taken to reduce any hyperactivity and physical injuries to fish. Immediately after reaching the laboratory, disinfected dip treatment with 0.1% $KMnO_4$ was given as a precaution. Fish was acclimatized to lab condition of aquatic vegetation, for a period of one month. Only non- chlorinated ground water was used. During this period, the fish were fed with pellet diet having 38% crude protein Hardy [14].

2.2 Fish Feed

The feed for the test animals was constituted with peanut oil-cake, rice bran, fish meal, dry fish and tapioca flour in the ratio 3:2:2:2:1. The biochemical composition of the feed was tested using standard methods and presented in Table 1. The energy value of the formulated feed was estimated using an Oxygen bomb calorimeter.

Table 1. Proximate Composition (Mean ± SD) of the feed

Ingredient	Moisture (%)	Protein (%)	Fat (%)	Carbohydrate (%)	Fiber (%)	Fiber (%)	Calories (KJ. g-1)
Peanut Oilcake	10.77	30.42	9.80	25.40	-	14.16	9.8
Rice Bran	10.15	09.62	6.20	38.60	7.15	12.20	7.5
Fish Meal	2.00	26.40	18.30	32.30	8.50	13.10	16.8
Dry Fish	1.80	30.12	05.10	-	-	-	2.2
Tapioca flour	00.60	01.80	01.60	45.80	-	0.75	3.4

Total = 39.7 KJ.g-1

2.3 Collection and Characterization of Effluent

Effluent from the Electroplating industry was collected from the main points of discharge at Avaniyapuram, Madurai. The collected effluent was then transported immediately to the laboratory and the physical and chemical characteristics of raw electroplating effluent (Table 2) were estimated using Standard Methods APHA [15].

Table 2. Physico-chemical characteristics of Electroplating effluent

Parameter	Electroplating Effluent
Colour	yellowish green
Odour	unpleasant
pH	6.5
Alkalinity	140 mg.L^{-1}
Total solids	2,850 mg. $^{L -1}$
Suspended solids	990 mg. L^{-1}
COD	3400 mg. L^{-1}
Chromium	4.0mg. L -1
Cyanide	0.75mg. L^{-1}
Copper	5.7 mg. L^{-1}
Zinc	0.6mg $L.^{-1}$
Cadmium	0.4mg. L^{-1}
Nickel	5.0 mg. L^{-1}
Dissolved oxygen	4.2 mg. L^{-1}

2.4 Dilution Procedure

The undiluted electroplating industry effluent was taken as 100% solution. From this, selected effluent concentrations for the experiments were obtained by diluting it with clean non- chlorinated ground water. The physical and chemical characteristics of the ground water used for dilution were also analyzed (Table 3).

Table 3. Physico – Chemical characteristics of ground water used for dilution

Parameter	Value
Temperature	28.0^0C
pH	7.4
Dissolved oxygen	6.1
Organic nitrogen	0.3
Total hardness	2.4
Calcium	3.1
Chloride	4.2
Magnesium	2.4
Sodium	3.8

Except pH all value are expressed in mg.1^{-1}

2.5 Determination of Sub-lethal concentrations of Electroplating Industrial Effluent

In acute bioassay studies, the determination of LC_{50} is of prime importance as only then sub-lethal concentration in the experimental treatment of the test organisms could be chosen. Bioassays were conducted following the standard procedure [16]. Survival studies on the fish of 5.0±0.5g were carried out as described below. The test individuals were starved one day prior to the survival test.

Groups of 10 well-acclimated fish were introduced into cylindrical plastic aquaria containing 10 liters of test medium at 28±1ºC. Fish mortality was recorded at every 24hrs interval over a 96h period. Dead fish were removed immediately from the medium. The test medium was renewed daily with a freshly prepared one to give a constant effect of effluent on fish. During renewal, 80% of the test effluent medium was siphoned out and the same volume replaced with a fresh effluent medium with the least disturbance to fish. During the period of survival test, all fish were starved and kept in a sufficient oxygen loaded medium. Similarly, the LC_{50} values were determined for the fish exposed to chromium and nickel separately using Probit Analysis Finney [17].

2.6 Respiration

The effect of sub- lethal concentrations (3, 6, 9% 96h LC_{50} value) of the chosen media viz., chromium, nickel and electroplating effluent on the oxygen consumption of tilapia (3.5+0.6gm) was studied.

Fish were exposed to the selected sub – lethal concentrations of chromium, nickel and electroplating effluent. Separate control was maintained for each medium for comparison. At each concentration, three sets of fish (5 fish in each set) were exposed in glass aquaria

(37x30x14 cm), containing 10 liters of the medium for 6 weeks. During the exposure period, fish were fed *ad-libitum* with prepared feed pellets daily.

The food remained was collected, using a siphon, immediately after feeding. In all the experiments, the medium was changed every day. The oxygen consumption rate of the fish exposed to the selected concentrations and control were estimated following the modified Winkler's iodometric method with the incorporation of sodium azide with alkaline iodide in order to eliminate any interference of oxidizing and reducing materials that may be present in the sample.

A simple flow through system as described by Beamish et al. [18] was adopted to estimate oxygen consumption in the present study. Non-chlorinated ground water was filled in a reservoir tank after filtering through a sintered glass column with glass wool. An aspirator bottle having a thin PVC tube served as a dosing apparatus (Mariotte bottle). Stock solution of the sample was prepared and placed in a Marriott bottle and allowed to flow into the reservoir tank. The required strength of the sample was maintained in the reservoir chamber by adjusting the flow of the effluent and water. A small magnetic stirrer was kept inside to ensure thorough mixing of the medium within the reservoir and also to keep the dissolved oxygen content of the water at saturation level.

From the reservoir, the test solution flowed to the constant surface chamber, the flow was regulated by a stopcock. The constant surface chamber was necessary to ensure a constant surface level and to regulate the rate of outflow throughout the experiment. Wide mouth glass jars with stoppers served as experimental and control chambers. Black paper with a small observation window was pasted on the outer surface of the experimental chamber to avoid disturbance to the fish. The water from the constant surface chamber was then allowed to pass simultaneously into experimental and control jars. This method was adopted for testing dissolved oxygen in the different concentrations of chromium, nickel and electroplating and in the control separately. Control medium was kept separately for each toxic medium.

The rates of oxygen consumption were estimated for the fish exposed to the different concentrations in the three toxic media after every week up to the sixth week of exposure. The fish were starved for four hours prior to the estimation of oxygen consumption in order to avoid the effects of any specific dynamic action.

During the estimation of oxygen consumption, individual fish was introduced into a respiratory chamber of exactly two liters capacity. The lid of the chamber was tightly closed down in such a way that there was no air space between the lid and the upper surface of the medium in the chamber. This arrangement was to avoid dissolution of atmospheric oxygen into the water in the course of the experiment. The fish was allowed to remain and respiring in the medium contained in the respiratory chamber for two hours. At the end of the experimental period, the dissolved oxygen content of the medium in the respiratory chamber was determined by following Winkler's iodometric method, as modified azide incorporation with alkaline iodide Welch [19].

The difference between the oxygen content of the medium without fish and that with the fish gave the amount of oxygen respired by the fish for two hours. The oxygen consumption rate of the fish was calculated by dividing the amount of oxygen consumed by the live weight of the fish. This was then converted to oxygen consumption rate per hour. The same procedure was repeated for the respiratory rates of fish exposed to chromium and nickel.

2.7 Statistical Analysis

ANOVA and Tukey's tests were conducted to understand the significant difference between and among the variables using SPSS 10 software package.

3. RESULTS AND DISCUSSIONS

3.1 Survival of Tilapia

The physicochemical characteristics of the test medium are presented in Table 2. The survival of tilapia exposed to different concentrations of the toxicant was studied.

The mortality values of fish exposed to various concentrations of the three media, for different time periods viz., 24, 48, 72, 96 h, observed as LC_{50} values .The LC_{50} value of chromium, which killed 50% of the test individuals in 24 h exposure, was 685 µg.l $^{-1}$ and it decreased to 434 µg.l $^{-1}$ when the exposure duration increased to 96h. Similar results were obtained by Palanichamy et al. [7] in *Mystus cavasius* and Venkataramreddy et al. [20] in freshwater fish. Similarly, the LC_{50}. Value of nickel in 24 h exposure was 1,491µg. l $^{-1}$ and it decreased to 983 µg.l^{-1} in 96 h exposure. Similar value was observed in the works of Pane et al. [21] in *Oncorhynchus mykiss* and Kedar N and Nishith [22] in freshwater perch. The LC_{50} value for the fish at 24 h exposure in Electroplating effluent was 13% and it decreased to 4 % at 96 h in the same medium. This indicated that as the duration of exposure in the test medium increased, the median lethal concentration decreased. A Two Way ANOVA test indicated significant differences in LC_{50} values of fish exposed to the different test media and over different times of exposure (F = 3.92; P<0.05) and this was further confirmed by Tukey's test.

Interestingly, the factor responsible for the mortality of fish during the bioassay was stress imposed by the test medium only, as there was no mortality in control groups. The 96hr LC_{50} of Electroplating effluent for *O. mossambicus* was found to be 4%, which contained 120 µg.l^{-1} Cr and 160 µg.l^{-1} Ni. Many workers have conducted a lot of work to study the LC_{50} value of different industrial effluents for freshwater fish. The 96hr LC_{50} value of 20% distillery effluent for *Cyprinus carpio* Ramakrishnan et al. [23], 2.5% tannery effluent for *Mystus keletius* (AntonyRaj et al. 24, Varadarajan and Subramanian 25), 3.11% Sugar mill effluent for *O.mossambicus* Haniffa and Jassentha [13] and 6.09, 8.35 and 8.28% paper mill effluent for *Anabas testudineus*, *Channa punctatus* and *Clarias batrachus* respectively Nanda et al. [4] have been reported. This clearly indicates that *O.mossambicus* is sensitive to several industrial effluents, and based on our results also sensitive to electroplating effluent.

The result also indicated that LC_{50} value of 4% in 96h for Electroplating effluent equivalent to 120 µg.l $^{-1}$ of chromium and 160 µg.l $^{-1}$ of nickel, was lesser than the LC_{50} values for individual metals viz., 434 µg.l $^{-1}$ chromium and 983 µg.l $^{-1}$ nickel. This showed that the combination of chromium and nickel and other toxic components present in the Electroplating effluent (EPE) resulted in greater toxic effect than the individual metals. Thus, the study revealed that tilapia was more sensitive to the Electroplating effluent than the individual metals and the order of toxicity can be written as: EPE>Cr>Ni.

3.2 Oxygen Consumption of Fish

The rates of oxygen consumption of the fish tilapia exposed to sub- lethal concentrations of toxic media are presented in Fig. 1.

Initially, the rates of oxygen consumption increased in fish exposed to the metals chromium, nickel and the electroplating effluent. Thus in the first and second week of exposures, the oxygen consumption of 701 and 704 mg. Kg $^{-1}$.d^{-1} in controls increased to 792 and 779 mg.Kg^{-1}.d^{-1} respectively in the highest sub-lethal concentration of 9% chromium (Fig. 1a).

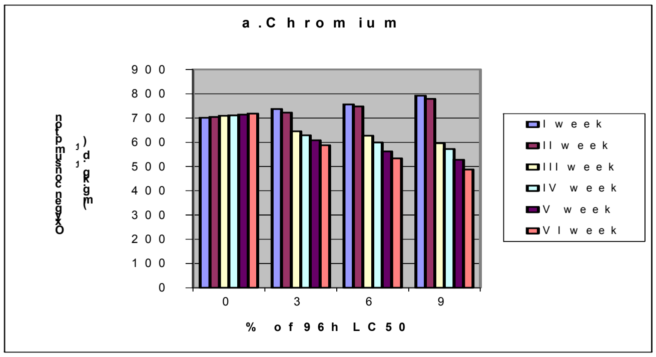

Fig. 1a. Effect of Chromium on the Oxygen consumption rate of fish (*O. mossambicus*)

The increases in percentage were 13 and 11 in the first and second weeks of exposures respectively that corroborates the findings of (3,21). However, from the third week onwards, the rates of oxygen consumption decreased. Thus in the third, fourth, fifth and sixth weeks of exposure, the rates of oxygen consumption were 709, 711, 714 and 718 mg.Kg $^{-1}$.d^{-1} in the controls which decreased to 596, 572, 528 and 488 mg.Kg $^{-1}$.d^{-1} respectively in the highest sub- lethal concentration of 9% chromium (Fig. 1a). The decreases were 16%, 20%, 26% and 32% respectively. Similar trends were observed in *Catla catla* [26] and in *Channa punctatus* [12].

A similar trend was identified in the rates of oxygen consumption of fish exposed to nickel. Thus in the first and second weeks of exposure, the oxygen consumption increased to 816 and 786 mg.Kg $^{-1}$.d^{-1} respectively in the highest sub lethal concentration of 9% nickel (Table 1b). The increases were 16% and 12% respectively. However, from the third week onwards, the oxygen consumption rates decreased. Thus in the third, fourth, fifth and sixth weeks of exposure, the rates of oxygen consumption decreased to 551, 505, 462 and 399 mg.Kg $^{-1}$.d^{-1} respectively in the highest sub-lethal concentration of 9% nickel (Table 1b). The decreases were 22%, 29%, 35% and 44% respectively. (Varadarajan and Subramanian [25] and Senthamilselvan et al. [27] have observed the similar findings in their works.

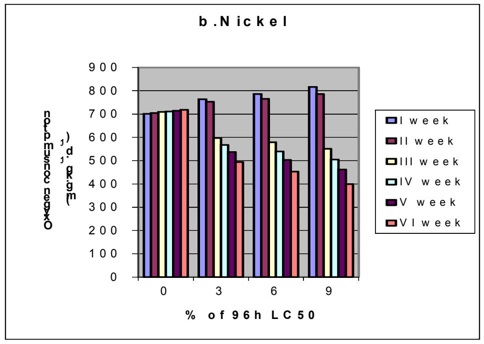

Fig. 1b. Effect of Nickel on the Oxygen consumption rate of fish (*O. mossambicus*)

The same trend in the rates of oxygen consumption was observed in fish exposed to the electroplating effluent. Thus in the first and second weeks of exposure, the oxygen consumption rates increased to 862 and 811 mg.Kg $^{-1}$.d^{-1} respectively in the highest sub-lethal concentration of 9% electroplating effluent (Fig. 1c). The increases were 23% and 15% in the first and second weeks of exposure respectively. However, from the third week onwards, the oxygen consumption decreased in fish exposed to electroplating effluent. Thus in the third, fourth, fifth and sixth weeks of exposure, the rates of oxygen consumption decreased to 479, 403, 316 and 306 mg.Kg $^{-1}$.d^{-} respectively in the highest sub - lethal concentration of 9% electroplating effluent (Fig. 1c). The decreases were amounted to 32%, 43%, 56% and 57% respectively.

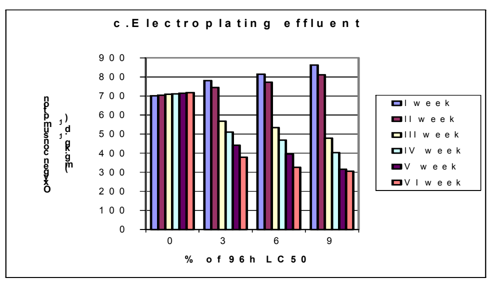

Fig. 1c. Effect of Electroplating effluent on the Oxygen consumption rate of fish (*O. mossambicus*)

The same trend in the rates of oxygen consumption of fish exposed to 3% and 6% sub-lethal concentrations in all the selected test media were observed (Fig. 1a-c).

A Two way ANOVA test indicated significant decrease in the rates of oxygen consumption of the fish exposed over the periods in the test media ($p<0.05$). Similar statistically significant differences were found in the rates of oxygen consumption of fish exposed to the different sub-lethal concentrations of the test media ($p<0.05$). The statistics also showed significant interaction between sub- lethal concentrations and the periods of exposure ($p<0.05$ (Table 4).

Table 4. ANOVA. Oxygen consumption of fish exposed to toxicants

Toxicant	**$F_{(5, 48)}$ value Different periods of exposure**	**$F_{(3, 48)}$ value Different concentrations**	**$F_{(15, 48)}$ value Interaction between time and concentration**
Cr	609.16	340.18	120.18
Ni	724.28	453.14	253.86
EPE	876.28	632.29	540.17

Table value of $F_{(3, 48)}$) = 2.81; $F_{(5, 48)}$ = 2.42; $F_{(15, 48)}$ = 1.89; $p < 0.05$

Tukey's multicomparison test on the rates of oxygen consumption of fish indicated significant differences (q>3.387; *p<0.05*) between the control and each sub - lethal concentration of the test medium. The test also revealed statistical variations in the rates of oxygen consumption of fish exposed to sub - lethal concentrations of the test media (Table 4a).

Table 4a. ANOVA. Tukey's Multicomparison test between control and sub-lethal concentrations

SLC	**$F_{(3,48)}$ value**
3%	150.72
6%	192.15
9%	214.27

Table value of $F_{(3, 48)}$) = 2.81; p value = < 0.05

The reduction in oxygen consumption may be due to the malfunctioning of gills, which have direct contact with metals and effluents dissolved in the medium. Haniffa et al (28) have also observed damage caused to gill structure due to pollutants. Likewise injury to the gill structure and abnormal accumulation of mucus on the gills of Bleached Kraft mill effluent–treated fish, leading to reduced efficiency of oxygen exchange in the gills have been reported Pamila et al. [11]. Pane et al. [21] has shown that high concentrations of toxic chemicals in water medium caused coagulation of gill mucus of fish and thereby altered respiratory metabolism. Similar reports have been published by Haniffa and Jaseentha [13] in the toxicity of distillery effluent on *Sarotherodon mossambicus.*Thus accumulation of mucus on the gills, asphyxiation and inhibition of enzyme system at mitochondria level could result in the decrease of oxygen consumption Hopkins et al. [29].

Heath [10] also indicated that pollutants cause alterations in the structure of mitochondrial membrane, which prevent the intake of oxygen into mitochondria and hence, reduction in the rate of oxygen consumption. Similar observations were reported in fish exposed to industrial effluents Bashamohideen et al. [30]; in *Heteropneusteus fossilis*, *Mystus vittatus* and *C.striatus* following toxicants intoxication Venkatramreddy et al. [20]; and in *Lates calcarifer* exposed to chromium Senthamilselvan [27]. The drop in oxygen consumption of *O. mossambicus* treated with heavy metals, observed in this work, indicates the onset of hypoxia in fish under stress, which triggers metabolic pathways of fish. This can trigger some biochemical changes in different tissues of the fish body.

According to Larsson et al. [8], synergistic effects of metals in combination may be due to intrinsic affinity of the individual metal for critical sites, or the relative concentration and distribution of metals within the target organ sites and antagonism due to partial occupation of receptor sites with lesser toxic metals, which leads to the blocking of higher toxic metal.

The critical sites in most cases are SH-groups Haniffa et al., [28]. It could be concluded that synergistic effect of chromium and nickel in the electroplating effluent definitely influenced the respiratory physiology of *O. mossambicus.* This result highlights lower oxygen consumption rates in fish exposed to electroplating effluent medium compared to those of the other test media.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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