Impact of kerosene on morphological, behavioural changes, Oxygen consumption and rate of oxygen consumption) in the freshwater fish, *Channa marulius*

Mazher Sultana*1, Sivaraman D2 and Balamurugan K3

1Department of Advance Zoology and Biotechnology, Presidency College, Chennai – 05.
2Research Scholar, Bharathiar University, Coimbatore.
3Research Scholar, Presidency College, Chennai – 05.

Abstract
The present study deals with the effect of kerosene on the morphological, behavioural and physiological changes (oxygen consumption and rate of oxygen consumption) in the freshwater fish, *Channa marulius*. The experimental animals were collected from Bharath Fish Farm, Thiruvallor district, Tamilnadu. The fish were oxygen packed in polythene bags and brought to laboratory to acclimatize them with laboratory conditions. Water soluble fraction of kerosene water was used for LC50 determination. Each tank was filled with 20 litres of water with required concentrations of water soluble fraction of kerosene (100 to 800 ppm). Ten healthy fish were left in each tank for experimental purposes, after range finding assay. Percentage mortality at the end of 96hr was recorded. For the toxicological studies, the animals were subjected to different sub lethal concentrations of toxicants, viz., the water soluble fraction of kerosene at 75, 150, 300 ppm and a control (0 ppm), at 24, 48 and 96 hrs after regular intervals. The morphological and behavioural changes were observed and recorded. The physiological changes like, total oxygen consumption and the rate of oxygen consumption per unit body weight was studied by modified Winkler’s method (Durairaj, 1987). The results indicate that the rate of oxygen consumption was found to decrease in both acute and chronic exposure when compared with control. The results were plotted and discussed in details.

INTRODUCTION
One of the major public health concerns in recent years has been Environmental Pollution and its effects on the living organism. Population explosion, urbanization, industrialization and human apathy have all contributed towards increasing quantities of pollutants leading to an “ecological disaster”.

Pollution of water has emerged as one of the most significant environmental problem of recent times. Not only there is an increasing concern for rapidly deteriorating supply of water but the quantity of utilisable water also fast diminishing. The wide array of pollutants discharged into the aquatic environment may have physico-chemical, biological, toxic and pathogenic effects [1].

The Industrial growth and consequent pollution let into the freshwater system are a challenge for the fragile freshwater ecosystems. The ability of water bodies to clean themselves has been affected by the sheer quantity of waste generated by ever increasing population [2]. Freshwater ecosystems provide the most convenient and cheapest waste disposable systems, over exploitation and misuse of these unique systems result in environmental degradation, depletion and pollution causing health hazards. Aquatic systems are mainly polluted through domestic (sewage and nutrients), agricultural, aqua cultural and industrial wastes, endangering the existence of aquatic living resource on which millions of people depends for their existence. The protection of environment is necessary for the
existence of living beings as water is the elixir of life [3].

Among thousands of substance of anthropogenic origin, several classes of industrial contaminants have gained interest due to their persistence, toxicity, high levels, etc. These includes PAH, PCBs, dioxins, dioxin-like compounds, phenolic compounds benzoarfurans, plasticisers, detergents, metabolites of all these and many others etc., [4]. Water pollution has become a global problem. An increasing number of organic trace pollutants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dibenzo-p-dioxins, and organochlorine pesticides were produced by the development of chemical industries, resulting in the environment becoming burdened with foreign organic chemicals. Many of these contaminants ultimately entered the aquatic environment, either by direct discharge, hydrologic processes or by atmospheric deposition [5].

When the pollutants enter water bodies they can have direct or indirect impacts on the biota of aquatic systems. They often interfere with the normal functioning of an organism and its ability to live in harmony with the environment [6]. The changes they cause in behavior, growth, and reproduction of an organism will eventually result in undesirable effects at higher biological organization levels. The pollutants present in the water affect not only aquatic organism, but also public health as a result of bioaccumulation in food chain. The diversity of aquatic organism becomes limited with the extent of pollution. Therefore, there is a great need to assess the impacts of pollution in the aquatic environment.

Petroleum and its processing products are responsible for most of the recent surface and underground water pollution. Over 1970 to 1990 these materials were responsible for the majority of water pollution accidents that happened worldwide. These accidents were not caused by problems in sewage water treatment plants: most of them were due to negligent storage and handling, transport accidents, defects in equipment and the like - hence, all can be ascribed, either directly or indirectly, to the human factor. However, petroleum and products of its processing also penetrate into the aquatic medium with waste waters. Petrochemical industry is responsible for most of the pollution. Other important sources of pollution include engineering and metallurgical works and the car and truck repair and service shops. Most of these sources have been polluting waters for a long time [7].

It is expected that the water solubility of kerosene alkanes with chain lengths of greater than 11 carbons will be too low to give rise to acute aquatic toxicity [8]. The water solubility of the alkylbenzenes and two-ringed aromatics is expected to be high enough to contribute to aquatic toxicity. As mentioned above, spillages of kerosene into water can kill fish directly through toxicity, or indirectly via anaerobic conditions or through fouling of water. There are some reports of impacts on aquatic biota following such spillages with quite long recovery times [9].

Some of the ecotoxicity data for kerosene is as follows. The chronic study conducted by [10, 11] reported that there was no-observed effect concentration (NOEC) for the growth rate of American flagfish (Jordanella floridae) in 1.0 mg/L and juvenile flagfish was to be 1.5 mg/L. Furthermore, a chronic lowest observed effect concentration (LOEC) value (1.4 mg/L) was observed for rainbow trout (Oncorhynchus mykiss) growth. Acute toxicity (LC50) and effect of some petroleum hydrocarbons (Toluene, Quinoline, Pyridine and Naphthalene) on the metabolic index (oxygen consumption rate) of an estuarine fish Etroplus suratensis is reported. The LC50 values were lowest for naphthalene, suggesting that this hydrocarbon is most toxic. The oxygen consumption reduced significantly after 6 hours in all the cases. The reduction in oxygen consumption was maximum in naphthalene, reaffirming its high toxic nature. Initially the oxygen consumption rate was high in the petroleum contaminated tanks than in the control. However, it slowly decreased afterwards with slowing down in fish movement [12].

Studies of the accidental and intentional release of gasoline and fuel oils to the aquatic environment indicate that aquatic organisms are able to bioaccumulate some Total Petroleum Hydrocarbons (TPH) fractions, particularly PAHs [13] and [14]. Further studies are needed to determine the biomagnification potential of the TPH fractions, particularly PAHs, in the food chain within aquatic and terrestrial ecosystems.

Effects of pollutants are usually expressed first at the molecular/biochemical level. Changes at these levels can induce structural and functional change at a higher level, such as hormonal regulation, immune system, and metabolism in an organism. These changes may finally impair the growth,
reproduction and survival ability of the organism. A variety of changes observable or measurable at molecular, biochemical, cellular, or physiological level in individuals have been studied as biomarkers for investigating the present or past exposure of the individual to pollutants [15].

In spite of considerable literature available on the water qualities and management from abroad and to some extent from India, little is the information available regarding the Pollution - status of the Indian aquatic habitats, especially the fresh water habitats. Hence the present study was undertaken to fins put the impact of sub lethal concentration of kerosene on morphological, behavioural and physiological changes (total oxygen consumption and the rate of oxygen consumption) in _Channa marulius_ in the laboratory conditions.

**MATERIALS AND METHODS**

The fish without any structural, behavioral and clinical symptoms were chosen for experiments, after careful observation. Fish were divided into groups of ten each and exposed to different concentrations of toxicants, viz., 100 ppm, 200 ppm, 300 ppm, 400 ppm, 500 ppm, 600 ppm, 700 ppm and 800 ppm (water soluble fraction of kerosene) to determine 96hr LC50 values for the test toxicants. The test was conducted with 10 fishes each for 96hrs and observations were made continuously during the period of experimentation. The experimental groups were maintained in separate tanks and were categorized as follows: Suitable controls were maintained without water soluble fraction of kerosene and analyses were done on Zero day to 5th day of experimentation. Water soluble fraction of kerosene water was used for LC50 determination. Each tank was filled with 20 litres of water with required concentrations of water soluble fraction of kerosene (100 to 800 ppm). Ten healthy fish were left in each tank for experimental purposes, after range finding assay. Percentage mortality at the end of 96hr was recorded. Mortality was recorded for each day and cumulative percent mortality was arrived at the end of 96hrs for the different groups. Toxicity of the toxicants was assessed based on the mortality of experimental fish in the concerned exposed groups.

**EXPERIMENTAL ANALYSES**

Healthy and normal _Channa marulius_ were exposed to the water soluble fraction of kerosene at sub lethal concentration of 75, 150, 300 ppm and a control (0 ppm) with reference to research work of Gabriel et al., [16] because their work was carried out in African cat fish treated with kerosene. Each experiment was set up in triplicate. The exposure lasted for 96 hrs. Various parameters were taken for the study of the effect of water soluble fraction of kerosene acting on the fish. The morphological and behavioural changes were observed and the rate of oxygen consumption was determined by the Winkler’s method after the introduction of fish to different concentration of toxicant at 96 hrs respectively. Rate of oxygen consumption was studied in the control and experimental groups by following the modified Winkler’s method [17]. The dissolved oxygen content of the water (APHA, 1998) [18] in the respiratory chamber was estimated, before the fish was released. After 96 hour, water from the respiratory chamber was carefully siphoned out avoiding air bubbles, and the dissolved oxygen content was estimated. The fish were weighed accurately to the nearest milligram after the fish were carefully wiped on a blotting paper. The physico-chemical characteristics of animal environment were determined. From this data, the unit oxygen consumption was calculated for each fish and expressed as ml/gm/hr body weight of fish. The data obtained was subjected to statistical analysis at probability level.

**RESULTS AND DISCUSSION**

The physico-chemical characteristics of water used during experiment were recorded as, temperature 29.3 to 30.6 °C; pH 7.50 to 7.72; oxygen content 5.85 to 6.70mg/l

Morphological changes observed in the fish treated with water soluble fractions of kerosene were due to the variation in the salinity. The remarkable variation was appearance of black spots over the body surface, the general color become dull and less shiny. Liberation of large amount of mucous and excreta from the kerosene treated fish were observed as compared with the control. The treated fish revealed variety of behavioral changes like, drifting up and down frequently with widely opened mouth, erratic movement of the operculum and resting flat at the bottom of the aquaria.

When compared with control values after 24, 48, 72 and 96 hrs of exposure in 75ppm of kerosene treatment the total oxygen consumption (2.220±0.45, 2.025±0.15, 1.975±0.80, 1.962±1.05ml/l) (table 1 and fig 1) and the rate of
oxygen consumption (0.3025, 0.2945, 0.2383, 0.2777/ml/gm/hr) decreased significantly (P<0.05) (table 2 and fig 2) and the percent change was found to be 1.94%, 30.38%, 38.38 and 32.81% respectively. Similarly for the exposure in 150 ppm (2.150, 2.002, 1.502, 1.005 ml/l and 0.3513, 0.2565, 0.3190, 0.2847/ml/gm/hr) and 300 ppm (2.003, 1.713, 1.322, 0.876 ml/hr and 0.3206, 0.3182, 0.2865, 0.3108 ml/gm/hr) of kerosene treatment the total oxygen consumption and the rate of oxygen consumption also decreased significantly (P<0.001) and the percent change was found to be 5.035%, 30.83%, 53.14 and 65.58% (150 ppm) and 11.53%, 40.81%, 58.75% and 70.00% (300 ppm) respectively.

The results showed decreased rate of oxygen consumption in the fish Channa marulius that may be due to direct effect of kerosene. The control showed maximum respiratory metabolism (table 1 Fig 1). The present study was found to be in consistent with the work of Vella on Monodonta atriculata due to the effect of mercury, which revealed that oxygen consumption decreased significantly with each progressive rise in mercury concentration.

Modern agriculture and industrial activities though result in higher food production and economy, also affects the environment. The toxicants and other undesirable chemicals ultimately reach the water bodies along with the rain water and destroy the quality of the water media causing imbalance to the environment leading to slow but steady deterioration of ecosystem, particularly the aquatic ones resulting in unabated mortality of aquatic fauna. The pollution of aquatic environment by toxicants adversely affects the survival of aquatic organisms including the commercially important fish species which form the dominant group of aquatic system [19] and [20].

Since aquatic environment is the ultimate sink for all pollutants, aquatic toxicity testing has become an integral part of the process of environmental hazard and evaluation of toxic chemicals. Generally, the potential impact of pollutants is more on the aquatic organism than in terrestrial environment. The International organizations have recommended the understanding of several bioassays to assess the ecotoxic risks to non target organisms and their environment like Environmental Protection Agency (EPA).

Information of the acute toxic effects of hexavalent chromium on survival and physiology of fishes is limited in Indian context [21-23] and its effects on the widely consumed Indian major carp, Labeo rohita which forms an important link in the aquatic food chain are not known. Knowledge of acute toxicity of a xenobiotic often can be very helpful in predicting and preventing acute damage to the aquatic life in receiving water as well as in regulating toxic waste discharges. In view of this, short- term acute toxicity test were performed on Rasbora daniconius over a period of 96hrs to determine the LC50 value so as to elucidate the acute effect of the chromium present in the tannery effluent on the survival, oxygen consumption and some physiological parameters of the fish by Mazher Sultana and Saraswathy [24].

The behaviour and condition of the fishes in both the control and test solution was noted every 24 hrs up to 96 hrs. The fishes showed a marked change in their behaviour when exposed to different concentration of the test solution. The behavioural manifestation of acute toxicity like copious secretion of the mucus, loss of scales, decolourization, surfacing and darting movement were observed in C. marulius exposed to sub lethal concentration of the kerosene at 24 to 96 hrs. After 72 hrs exposure, the fishes exhibited lethargy and erratic movements suggesting loss of equilibrium at high concentration. At the time of death transient hyper activity was also observed in Rasbora daniconius on exposure to tannery effluents by [24].

Heavy metals have long been recognized as serious pollutants of the aquatic environment they cause serious impairment in metabolic, physiological and structural system when present in high concentration [25]. Fish are largely being used for the assessment of the quality of aquatic environment and as such can serve as bioindicators of environmental pollution [26-28]. The aquatic organisms can metabolically control the intake of metal ions up to the threshold level of concentration but above the same the organism cannot control the excess metal and the excess metals are accumulated in the body of the organisms and may shoot up to a level higher than that of the ambient waters [29].

The toxicity of any toxicant is either acute or chronic. The chronic studies include both histochemistry and pathology. Mode of action of different chemicals varies leading to varied effects on various body tissues. Some toxicants exert their effect locally at the portal of entry, resulting in damage to external surface of the body. Thus,
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various toxicants and chemicals with their varied modes of action on different tissues bring about certain architectural changes ultimately culminating in either death of the organism or making the organism less liable for its survival. Respiration is an essential physiological activity in all living organisms as oxygen is necessary to provide energy for life processes and for carrying out all other metabolic activities. Respiration is a vital process through which the organisms obtain oxygen from external environment and utilize it for the energy generation during oxidative metabolism [30]. The activity of animal can be measured in terms of oxygen uptake. Aquatic animals have to pass large quantities of water over their respiratory surface and are subjected to relatively greater risk of exposure to the toxic substances [31]. A perusal of the available information reveals that heavy metal induced alterations in the respiratory function of fishes differ not only from metal to metal but also the sites of the action. The decrease in the oxygen consumption of *Labeo rohita* exposed to chromium indicates the onset of acute hypoxia under metallic stress was observed by Vutukuru [32] in his study. But in the present investigation the total oxygen consumption was enhanced and rate of oxygen consumption under the stressed situation of the metallic toxicity which also indicates the protective measure to ensure the adaptability of the fish for its survival.

The drop in the metabolic activity of *C. marulius* exposed to Kerosene can also be attributed to clogging of gills by mucus. Gills are vital respiratory and osmoregulatory organs and cellular damage induced by the metal might impair the respiratory function of the fish. These findings clearly suggest that the decreased respiratory activity can account for the drop in the metabolic activity of the fish is a significant change as observed in the present investigation.

The change in rate of oxygen consumption is a good index of the metabolic capacity of an organism to face environment stresses. It is evident from the result of present study that the kerosene exerts influence by affecting the rate of oxygen consumption [33]. The interpretation of metal induced changes in a respiration is complicated and varies from metal to metal and from species to species and from one experimental condition to other. The alteration in the normal respiratory metabolism is due to its intimate contact with polluted water which decreases the oxygen diffusing capacity of the gills [32, 25]. Hiltibran [34] noted that zinc can disrupt energy production by inhibition of oxygen uptake in the blue gill fish. Baby and Menon [35] have observed that mercury, cadmium and zinc act as a respiratory depressant rate of animals in toxic media including zinc sulphate is attributes to the asphyxiation which ultimately leads to the failure of respiratory metabolism which may be caused centrally in the brain through respiratory surface. Some water born metals can bind to gills of fresh water fish and disrupt the ionoregulatory and respiratory functions of the gills [36]. Mali and Ambrose [37] reported that the changes in rate of oxygen consumption of fresh water female crab, on exposure to ZnSO4 showed decrease in oxygen consumption and it is evident and in agreement with earlier obtained results of the workers in different aquatic animals which exhibit enhancement or decrease in oxygen consumption of exposed animals and also showed variable result in the same animal.

Present study undertaken to study the effect of sublethal levels of kerosene on the total oxygen consumption in the fish *C. marulius* also revealed decrease in oxygen consumption of exposed animals to various concentration of the toxicants at 24, 48, 72 and 96 hrs. Similarly the rate of oxygen consumption in the fish *Rasbora doniconius* exposed to different concentration of tannery effluent decreased when compared to the control fish. Similar studies of Siddiqui [38] on *Clarius batrachus* revealed decreased trend in rate of oxygen consumption when exposed to sub-lethal concentration (1.5 ppm) of copper sulphate. The control set showed maximum respiratory metabolism.

It has been reported that the oxygen consumption represents the physiological state of metabolic activity and may be an indicator of metabolic stress; the pollutant may induce stress to exposed animals [24]. Many researchers have shown the harmful effect of heavy metal on histological structure of gills of fish [39-41]. The effect of ammonia on respiratory activity of air breathing fish *Clarius batrachus* showed that the acute and sub acute treatment on animal lead to severe disruption of oxidation reduction process and suppressed tissue respiration [42]. The studies of Tilak and Sathyavardhana, [43] showed the amount of oxygen consumption initially increased and then gradually

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decreased when the fish, Channa punctatus were treated with fenvalerate.
Siddiqui [38] suggested that the copper sulphate may induce alteration in gill structure, disintegration or rupture of respiratory epithelium and coagulation of mucus films over the gill surface of Clarias batrachus which could be the reason for gradual decrease in oxygen consumption. Mazher Sultana and Saraswathy [24] also reported decrease in the total oxygen consumption and the rate of oxygen consumption significantly in Rasbora doniconius due to the impact of sublethal concentration of tannery effluents in their studies. The above results corroborated with the present findings in Channa marulius due to the impact of sublethal concentration of kerosene.

Table 1. Effect of Sub-lethal concentration of kerosene on Total Oxygen Consumption in Channa marulius and Control

<table>
<thead>
<tr>
<th>S.No</th>
<th>Exposure period</th>
<th>Control fish</th>
<th>75ppm</th>
<th>150 ppm</th>
<th>300ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24</td>
<td>2.264±0.07</td>
<td>2.220±0.45</td>
<td>2.150±0.25</td>
<td>2.003±0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.94%</td>
<td>5.035%</td>
<td>11.53%</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>48</td>
<td>2.894±1.12</td>
<td>2.025±0.15</td>
<td>2.002±0.16</td>
<td>1.713±0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30.03%</td>
<td>30.83%</td>
<td>40.81%</td>
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</tr>
<tr>
<td>3</td>
<td>72</td>
<td>3.205±0.28</td>
<td>1.975±0.80</td>
<td>1.502±0.14</td>
<td>1.322±0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>38.38%</td>
<td>53.14%</td>
<td>58.75%</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>96</td>
<td>2.920±0.18</td>
<td>1.962±1.05</td>
<td>1.005±0.008</td>
<td>0.876±0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32.81%</td>
<td>65.58%</td>
<td>70.00%</td>
<td></td>
</tr>
</tbody>
</table>

The readings are mean values of total oxygen consumption of five readings ± SD

Table 2. Effect of Sub-lethal concentration of kerosene on Rate of Oxygen Consumption (per unit body weight) in Channa marulius and Control

<table>
<thead>
<tr>
<th>S.No</th>
<th>Exposure period</th>
<th>Control fish</th>
<th>75 ppm</th>
<th>150 ppm</th>
<th>300 ppm</th>
</tr>
</thead>
<tbody>
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</tr>
<tr>
<td>3</td>
<td>72</td>
<td>0.2645</td>
<td>0.2383</td>
<td>0.3190</td>
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</tr>
<tr>
<td>4</td>
<td>96</td>
<td>0.2932</td>
<td>0.2777</td>
<td>0.2847</td>
<td>0.3108</td>
</tr>
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</table>
REFERENCES

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