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Research paper

Comparison of fatty acids profile and antioxidant enzyme level of cladoceran *Moina brachiata* (jurine, 1820) from freshwater bodies of Chennai

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Abstract

Omega-3 family (ω-3) of polyunsaturated fatty acids (PUFA) was considered as an important biochemical for the physiological function of all trophic level animals. In this study, we demonstrated the effect of algal diet on fatty acids composition (FA), antioxidant enzymes and DNA damage of *Moina brachiata* from Adyar River and Kolavoi Lake. 8 different fatty acids were identified in *M. brachiata* through GC-MS analysis and we noticed two PUFA (Eicosapentaenoic acid, EPA 20:5 (ω-3); Linoleic acid 18:2 (ω-6)). The dietary fatty acid accumulation and bioconversion capacity of *M. brachiata* have differed in two lakes fed with algal diet. The high amount of ω-3 PUFA was observed in *M. brachiata* fed with *Scenedesmus* sp. in Kolavoi Lake (35.84%) followed by Adyar River (33.78%). PUFA content was significantly declined in wild *M. brachiata* of Adyar River (17.44%) followed by Kolavoi lake (25.78%). On the other hand, high level of Malondialdehyde (MDA) and decreasing level of key antioxidant enzymes likes Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GSH) and DNA damage were observed in wild *M. brachiata* of Adyar River. Hence, the algal diet could enhance the level of antioxidant enzyme activity by decreasing the level of MDA and it does not show DNA damage on *M. brachiata*. Overall, the results obtained in this study explored that *Scenedesmus* sp., has the ability to enhance the PUFA content, antioxidant enzyme activity and prevent the DNA damage in *M. brachiata* which was declined in the wild animal due to the environmental stress conditions.

Keywords: *Antioxidant Enzyme; DNA Damage; Moina Brachiata; PUFA; Scenedesmus Sp.*

1. Introduction

Zooplankton plays a key role in the transfer of PUFA to organisms such as fish [1]. The accumulation of PUFA an invertebrate prey then influences fish production significantly [2]. The PUFAs EPA and DHA are hardly synthesized by most crustaceans and in general, can be regarded as an essential in the diet [3]. The accumulation of PUFA in aquatic invertebrates depends on an ordinal of environmental factors, such as water temperature [2] food type [4] and pollution [5]. In addition to such factors, phylogeny is a key determinant of the FA composition of animals [6]. For instance, two of the most significant large taxa of planktonic freshwater invertebrates, Cladocera and Copepoda, vary significantly in EPA and DHA percentages [7]. Fatty acids (FA) are one of the vital compounds transferred between plants to an animal in the aquatic food chain [8]. In addition, the significant compounds like highly unsaturated fatty acids (HUFA) particularly Eicosapentaenoic acid (20:5n-3,EPA) and Docosahexaenoic acid (22:6n-3,DHA) play a vital role in the health and other functions of all animals (Plankton, invertebrates, Fish and human) at any type of trophic levels [9]. On the other hand, lipid components are very sensitive to environmental changes and stress conditions. Thus the water pollution, Eutrophication and another environmental factor can affect and diverse the overall production of HUFA in the aquatic ecosystem [9].

The similarity between FA composition of Daphnia and their diets were determined by (Brett et al. 2006). Tough correlations among seston FA profiles in polar lipids and those in Daphnia have been shown in the field study [4].Moreover, Moreover, few authors have been reported a mismatch between FA composition of zooplankton and its food in natural water bodies [10], [1].

Antioxidant enzymes are important defensive mechanisms against ROS and like many other biochemical systems, their responses and effectiveness may vary across contaminants and species [11]. In *Daphnia* species, recent studies were reported on antioxidant enzyme responses, including CAT, SOD, GST and oxidative tissue damage (lipid peroxidation) to UV radiation to be dissimilar in oxygen concentration [12] but information on antioxidant protective system against pro-oxidant contaminants is absent.

In environmental monitoring, biomarkers grant an integrated detection system to identify environmental stressors [13]. Stress response biomarkers such as DNA damage reflect the physiological as well as the genetic susceptibility of organisms to environmental pollutants [14].DNA DNA is the carrier of hereditary information in successive generations, and any modification to its structures can cause a serious modulation in genetic traits [15], [16]. One technique for estimating DNA damage in aquatic organisms is the use of single-cell gel electrophoresis or comet assay [17].

Thus, the aim of our present work is to study the comparisons of FA Profile, antioxidant enzyme level and DNA damage of cladoceran (*M. brachiata*) from Adyar River and Kolavoi Lake along with algal diet.

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2. Methods

Study Sites

2.1. Adyar river

Adyar River (80º16' E; 12º49' N) runs its way through the southern part of the Chennai city, Tamil Nadu, India and enters the sea near Adyar (Fig.1 A).

2.2. Kolavoi lake

Kolavoi Lake has placed in the Chengalpet 58 Km away from the Chennai city. It is one of the biggest lakes situated about 200m on the northeast of Chengalpet and close to Pulipakkam village, running parallel to the national highway (Fig. 1B). This lake receives water from 12 tanks, and the surplus flows into Palar, Neenjal and Madura rivers. It is one of the largest water bodies with 894 hectares and a maximum depth of 4. 5meters. At present, 17 villages are benefiting from this lake. It has various species of plants and animals. The total ability of the tank is 476.69 Mct, with one filling. Lake water has been used for agriculture, recreation and fishing behavior.

A) Adyar River



B) Kolavoi Lake



Fig. 1: Study areas.

2.3. Collection and maintenance of cladoceran

Cladoceran (*M. brachiata*) was collected using 60 µm mesh size plankton net and identified using the authenticated monograph [18]. Cladoceran had been maintained at 28°C to 31°C and under 12D: 12L photoperiod cycle for 30 days. The culture water was 0.45 µm Millipore filtered freshwater (cellulose filter paper), with 7 to 7.9 mg L-1 dissolve oxygen and a pH ranging from 7.90 to 8.25. Cladoceran was fed with an algal diet of *Scenedesmus* sp.

2.4. Fatty acid analysis

Lipids were extracted by chloroform–methanol (2: 1, v/v). After evaporation of the solvent, the total lipid extracts was methylated. Methyl ethers of fatty acids (FAMEs) were analyzed and identified using a Gas Chromato Graph–Mass Spectrometer (JEOL GCMATE II GC-MS, Agilent Technologies 6890N Network GC system for Gas Chromatography, SAIF, IIT- Madras, Chennai). The conditions are described in detail elsewhere [19].Peaks of FAMEs were identified by their retention time and mass spectra in comparison to those in the database (Agilent Technologies, IIT Madras) and to those of available authentic standards (Sigma, United States). The quantification of fatty acids was calculated according to the peak area of an internal standard.

2.5. Assay of lipid per oxidation and antioxidant enzyme activity

Lipid per oxidation as a measure of thiobarbituric acid reactive substances (TBARS) was measured by following the standard procedure (Carlos Barata et al. 2005). Malondialdehyde (MDA) forms as an intermediate product of the peroxidation of lipids and serves as an index of the intensity of oxidative stress. The intensity of the pink color formed during the reaction was read on a spectrophotometer at 532 nm. The activity of Superoxide Dismutase (SOD) in cladoceran homogenate was assayed according to the method of [20].The unit of enzyme activity is defined as the enzyme required for 50% inhibition of pyrogallol auto-oxidation. The results have been expressed as unit/min/mg protein. The activity of Catalase (CAT) in cladoceran homogenate was assayed following the standard protocol [21] using Hydrogen Peroxide (H_2O_2) as substrate. The activity of Catalase was measured on a spectrophotometer and has been expressed in µmole/min/mg protein.

2.6. DNA damage analysis

DNA damage on *M. brachiata* was analyzed by DNA Fragmentation. DNA fragmentation was measured directly on recovered DNA by loading samples of extracted DNA onto Agarose gel [22]. DNA was extracted with a mixture of phenol and chloroform, precipitated with ethanol, dried and dissolved in TE buffer. DNA samples were analyzed by electrophoresis on 1 % Agarose gel containing ethidium bromide (0.5 µg/mL) and then visualized under UV illumination and visualized using a gel documentation system (GELSTON 1312, Medicare, India).

2.7. Statistical analysis

All data are presented as means ± SD. All analyses were done using Prism statistical software (Graph Pad, San Diego, CA). A p-value < 0.05 were considered significant.

3. Results

3.1. Fatty acid composition of *M. brachiata* fed with algal diet from both lakes

The fatty acid composition of *M. brachiata* fed with algal diet *Scenedesmus* sp. in Adyar River and Kolavoi Lake are presented in Table 1. Totally, 8 different fatty acids were identified. The major fatty acids of Cladoceran Tetradecaphonic acid (C14:0),Tridecanoic acid (C13:1) , Heptadecanoic acid (C17:0), Palmitic acid (C16:0) belonging to Saturated Fatty acids (SFA); Oleic acid(C18:1), Palmitoleic acid(C16:1) belonging to Monounsaturated Fatty acid (MUFA) and Eicosapentaenoic acid (C20:5) and Linoleic acid (C18:2) (PUFA) were observed (Table

1). The *Scenedesmus* sp., has a large amount of Eicosapentaenoic acid C20:5 (35.84%) and Linoleic acid C18:2 (12.92%) in Kolavoi lake than in Adyar River (33.78% & 13.05). Moreover, a sharp increase in FA concentration of (26.69%) was observed in cultured animal than in the wild animal. Cladoceran fed with *Scenedesmus* sp., contained C14:0;C13:1;C20:5;C17:0 ; C16:0; C18:1;C16:1 and C18:2 fatty acids (Table 1).

In general, saturated and unsaturated fatty acids are predominant in freshwater Cladoceran than in the marine Cladoceran species. The only exception to this finding was *M. brachiata* which showed a large amount of PUFA (35.84%) in Kolavoi Lake (Table 1). Furthermore, the SFA was mainly composed of mixtures of 13:1; 14:0; 16:0 and 17:0 and represented about 35.84% of the total lipid composition.

Table 1 Presence of different types and total fatty acids content in *M. brachiata* at Adyar and Kolavoi Lake (Wild and fed with *Scenedesmus* sp)

S. No	RT	Name of the Compound	Molecular formula	Adyar wild *M. brachiata* (%)	Adyar fed with *Scenedesmus* sp (%)	Kolavoi wild (%)	Kolavoi Fed with *(%) Scenedesmus* sp.
1	14.67	Tetradecanoic Acid (S) C14:0	$C_{15}H_{30}O_2$	3.09	2.42	2.87	2.07
2	14.95	Tridecanoic acid (S) C13:1	$C_{13}H_{26}O_2$	1.20	2.61	2.54	3.85
3	16.95	Eicosapentaenoic acid C20:5(PU)	$C_{20}H_{30}O_2$	17.44	33.78	25.78	35.84
4	17.22	Heptadecanoic Acid C17:0 (S)	$C_{17}H_{34}O_2$	10.47	13.38	12.58	13.77
5	18.23	Palmitic acid C16:0 (S)	$C_{16}H_{32}O_2$	6.34	9.50	8.94	11.76
6	19.0	Oleic acid C18:1 (MU)	$C_{18}H_{34}O_2$	39.40	20.67	27.34	15.94
7	19.7	Palmitoleic acid C16:1 (MU)	$C_{16}H_{30}O_2$	10.90	4.57	7.65	3.85
8	20.45	Linoleic acid C18:2 (PU)	$C_{18}H_{32}O_2$	11.16	13.05	12.27	12.92

In Adyar River the monounsaturated fatty acid (MUFA) content C18:1 (39.40%), and C16:1 (10.90) was found to be higher in wild *M. brachiata* than in cultured animal C18:1 (20.67%) and C16:1 (4.57%), whereas the polyunsaturated fatty acid Eicosapentaenoic acid recorded the highest value in Fed with *Scenedesmus* Sp., diet (35.84%) than in MUFA. The polyunsaturated fatty acids 20:5, 18:2, Linoleic acid and EPA were found high concentration than in a wild animal at Kolavoi Lake.

3.2. Level of lipid per oxidation and another antioxidant enzyme of *M. brachiata* fed with algal diets

In the present study, the level of lipid peroxidation and other antioxidant enzymes (SOD, CAT, and GSH) were analyzed in *M. brachiata* from Adyar River and Kolavoi Lake. Whole-body lipid peroxides level was analyzed by the formation of MDA. MDA is formed by oxidation of polyunsaturated fatty acids in the presence of lipid peroxidation and reacts with two molecules of thiobarbituric acid to produce a pinkish red chromogen. The *M. brachiata* fed with algal diet *Scenedesmus* sp. supplementation was significantly decreased (25.13±1.77, 11.65 ± 1.55 Unit/mg-1) in Kolavoi Lake and Adyar River. The level of lipid peroxidation, which was increased in wild *M. brachiata* of Adyar River (38.7±1.5, 19.70 ± 1.50 Unit/mg-1) (Fig. 2). Fig.3 shows the SOD, CAT and GSH activities of *M. brachiata* fed with algal diets. A small but significantly decrease in the level of SOD was observed in *M. brachiata* fed with *Scenedesmus* sp, in Adyar River (5.97±0.252 Unit/mg-1) than in Kolavoi lake (9.747 ± 0.828 Unit/mg-1). Significant differences were observed for total GSH and CAT activities (GSH 68.4± 3.10, 74.0± 1.388 and 37.1± 1.414, 41.1± 1.564 Unit/mg-1 were respectively) being maximum in the Kolavoi Lake than in the Adyar River. The lower level of SOD, CAT, and GSH activities was observed in Adyar River because of the high level of lipid peroxidation production. In addition to that the level of SOD, CAT and GSH were enhanced in *M. brachiata* diet with *Scenedesmus* sp. Hence, the level of antioxidant enzyme level was significantly decreased in Adyar River (Fig. 3A, B & C).

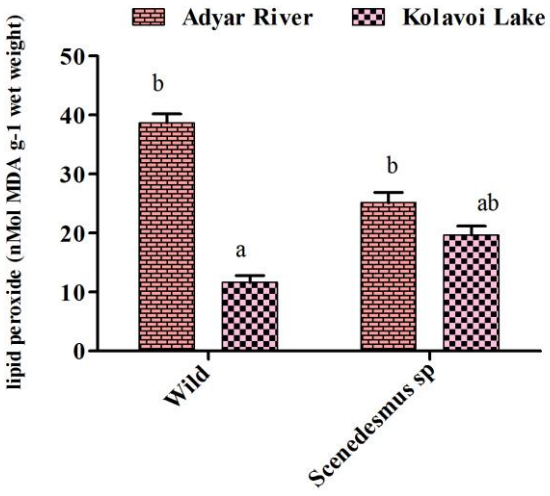


Fig. 2: The Level of Lipid Peroxide in Whole Body of Wild *M. brachiata* and Fed with *Scenedesmus* Sp., in Adyar River and Kolavoi Lake. Values Are Expressed As Mean ± S.D (Number of Pools N=3). Level of Significance: Mean Above the Bars of Each Parameters with Different Letters at Significantly Different At (P<0.5).

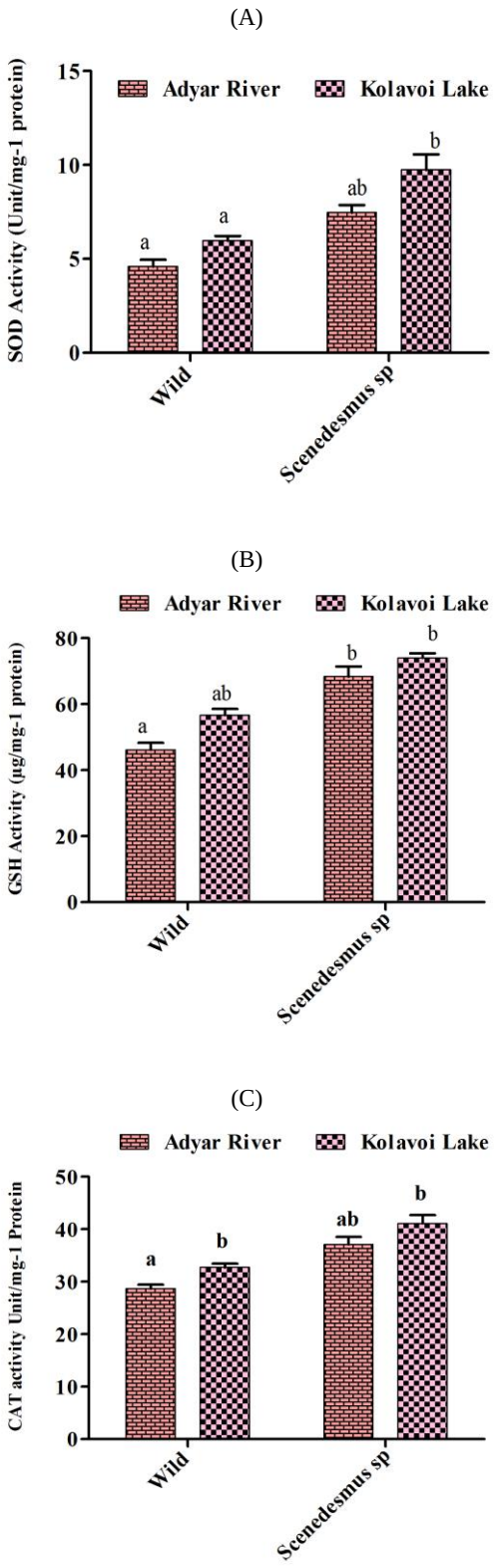


Fig. 3: The Level of Antioxidant Enzyme Activity Changes in Wild *M. brachiata* and Fed with *Scenedesmus* Sp., In Adyar And Kolavoi Lake (A) Superoxide Dismutase (SOD) Activity (B) Reduced Glutathione (GSH) Activity (C) Catalase (CAT) Activity. Values are Expressed as Mean ± S.D (Number of Pools N=3). Level of Significance: Mean Above the Bars of Each Parameters with Different Letters at Significantly Different at (P<0.5).

3.3. Level of DNA damage in *M. brachiata* fed with algal diet

The results shown in Figure 4 indicated that wild *M. brachiata* collected from Adyar River showed fragmented DNA due to severe DNA damage when compared to Kolavoi Lake. Hence the algal diet with *M. brachiata* does not show any DNA damage in Adyar River and Kolavoi Lake.

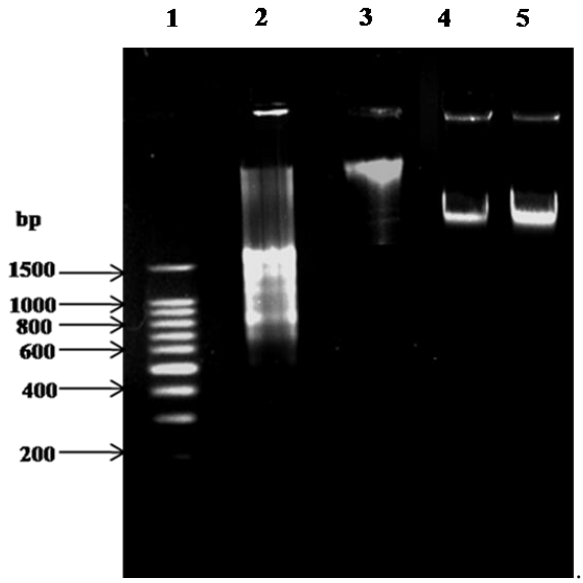


Fig. 4: DNA Damage (DNA Fragmentation) of *M. brachiata* (1) Ladder (2) Wild Individual of Adyar River (3) Fed with *Scenedesmus* Sp M. Brachiata From Adyar River (4). Wild Individual of Kolavoi Lake (5) Fed with Scenedesmus Sp. From Kolavoi Lake

4. Discussion

In the past few decades, the biochemical composition of zooplankton from different trophic levels has received much attention from the aquatic ecologists, since it contain Omega-3 family of the long-chain highly unsaturated fatty acids, such as Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) and its well-known for essential physiological importance for all animals, including humans [23].

We found significant differences in Fatty acid percent level of *M.brachiata* from Adyar River and Kolavoi Lake. It appeared that, the dietary supplementation plays a vital role in the alteration of a polyunsaturated profile of Cladoceran. Obviously, cladocerans in all environments had comparatively invariant taxon- specific Fatty acid composition. Our data on the moderately low variability of FA composition in Cladoceran are in good agreement with the findings of [24]. Likewise, Masclaux et al. [24] observed that differences among the polyunsaturated fatty acids six compositions of different Cladocerans species of Daphniidae supplemented with different kinds of diet as *Cryptomonas* sp., and *Scenedesmus obliques*. There are a lot of comparisons of the FA composition of Cladocerans [9].

The FA composition establishes for full body *M.brachiata* was also characterized by a high concentration of PUFAs including Eicosapentaenoic acid C20:5 (35.84%) and Linoleic acid C18:2 (12.92%) and low concentration of MUFAs i.e., Oleic acid C18:1(15.94%), Palmitoleic acid C16:1 (3.85%) in Kolavoi lake. The earlier results are in concord with results found by [25]. Lau et al. [6] also described the Adult *Daphnia magna* showed the lower level of MUFA, but rather high level of PUFA. Although, the Cladoceran *M. brachiata* diet with *Scenedesmus* sp., showed increasing amount of EPA and decreasing amount of AlA and LIN at Kolavoi Lake. Fascinatingly we noticed the small amount of Arachidonic acid (Ann) in *M. brachiata* diet with *Scenedesmus* sp. at both lakes. It may be owing to the cause that, the green micro-

algae *Scenedesmus* sp., contains high amount of Alpha-linoleic acid (ALA) and Linoleic acid (LIN) [26]. The Cladoceran *M. brachiata* have the capacity to synthesis ARA and EPA through the bioconvert process of dietary precursors like LIN and ALA respectively present in the *Scenedesmus* sp. In this difference, the previous information on different species of *Daphnia* does not show ARA as well as an exhibited very low amount of EPA in wild-type [27]. In addition, its diet with microalgae *Cryptomonas* sp showed the little amount of Docosahexaenoic acid (DHA) and diet with *Scenedesmus* sp., showed the little amount of ARA and high amount of EPA [2].

FA composition diverse with Adyar River and Kolavoi Lake in wild animal with Adyar River having less PUFA substance i.e., Eicosapentaenoic acid C20:5 (17.44%) and Linoleic acid C18:2 (11.16%) and elevated amount of monounsaturated fatty acids like Oleic acid C18:1(39.40%), Palmitoleic acid C16:1(10.90%) than algal diet. On the other hand, it is important to point out that *Daphnia* fatty acid composition like another freshwater crustacean was characterized by having lower levels of polyunsaturated fatty acids when compared with marine species, which may result in a minor risk for lipid peroxidation [28].

Naturally, all cladocerans are able to bioconvert the dietary FA into EPA. It indicates that the Cladocerans are able to synthesizing EPA, a significant PUFA necessary for Cladocerans using a cheap food source like phytoplankton [29].

Lipid peroxidation is a chain reaction initiated by free radical through the oxidation of polyunsaturated fatty acid. A number of studies have reported that exposure to stress increased the production of free radical generation and cause oxidative damage to the biological membrane through increased levels of lipid peroxidation, protein carbonyl contents followed by decreased antioxidant defense system [30], [31]. In the current study, the level of an antioxidant enzyme was considerably decreased in Adyar Lake because of a high level of lipid peroxidation production when compared to Kolavoi Lake.

M. brachiata was found to possess key antioxidant enzyme activities like CAT, SOD, and GSH parallel to another Daphnia sp [32]. In our study, Adyar River has the declining level of GSH, CAT and SOD then the Kolavoi Lake. Distinction of antioxidant enzyme activities between species is difficult due to a scarcity of existing data on invertebrate's species and the use of different units and methods.

Ramanibai & Shanthi [33] reported that Adyar river sediment sample exhibited highest accumulation of heavy metals Pb, Zn, Co, Ni, Cr, and Cu. According to Silambarasan et al. (2012) Adyar River has the high level of heavy metals such as Zinc (Zn), Cadmium (Cd), Copper (Cu), Iron (Fe) and Lead (Pb) in the sediment and water sample. In this relation Aboul-Ela et al. [34] reported that iron, and coppers were the main influencing factor that caused the clear rise of both DNA damage and lipid peroxidation level and decrease the enzymatic antioxidant activity in fish individuals of *M. cephalic* collected from Abu-Qir Bay and Sidi-Barrani, Egypt. Likewise, in the Present study results showed M. brachiata has severed DNA damage and greater level of lipid peroxidation due to the oxidative stress caused by high level of heavy metals accumulation in the Adyar River. The elevated amount of toxic heavy metals in the aquatic ecosystem can induce oxidative stress at the subcellular level to the aquatic organism and be leading to oxidative damage to DNA and proteins as well as alteration on lipid peroxidation and other antioxidant enzymes responses [34].

In the present study, *M. brachiata* collected from Adyar River and Kolavoi Lake. The results showed the low amount of fatty acids contents, high level of lipid peroxidation activity and decreasing level of antioxidant enzyme activity like GSH, SOD, and CAT and also it showed severe DNA damage in Adyar River when compared to Kolavoi Lake. According to the available literature and the collected data, the present study concluded that over-accumulation of heavy metals in the Adyar River leads to oxidative stress to the aquatic organism like phytoplankton, zooplankton, and fishes and it ultimately affected the human population. Thus, the necessity of continuous biomonitoring of this river is higher required to reduce the pollutant contents and ease the risk to an aquatic organism.

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