MATERIALS & METHODS

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Fishes have been most popular test organisms because they are presumed to be the best understood organisms of the aquatic environment. These organisms are useful as food resources to man and have important role in the aquatic food chain. The test organisms are selected on the basis of their adequate availability, economic importance of the species and their interactions with heavy metals.

The important part of the fish fauna in wetlands are catfishes. In India there are about 158 species of inland catfishes from 50 genera and 13 families. The Indian families include Bagridae, Amblycipitidae Akysidae, Ariidae, Clariidae, Chacidae, Siluridae, Schilbeidae, Pangasiidae, Sisoridae, Heteropneustidae, Olyridae and Plotosidae (Talwar and Jhingran, 1991). Many of them have high economical and nutritive value. The highest diversity of catfish in India is in Northeastern Hills, Gangetic River System and the Estern Ghats (Thomas, *et. al.,* 2002). The catfishes generally, dwell in diverse habitats such as upland streams, large river channels and seasonal floodplain lagoons with broad feeding niches (Winemiller and Winemiller, 1996).

The problem in the field of industrial toxicity is to determine exact impact of a particular pollutant from number of factors existing in the environment on a given organism. It is difficult to assess the cumulative effect of pollutants in natural environment, as the fishes have different habitats, ecological niche, and degree of sensitivity to pollutants and range of adaptation to the changing environment. Effects of pollutants on fish are studied by various methods like, histological, pathological, clinical, physiological, bio-chemical, anatomical, biophysical, enzymological etc. Determining toxicity in aquatic environment and quick diagnosis of fish poisoning is important in studying indicator organism for early warning system.

The present study deals with the impact of industrial effluents on fish, therefore an attempt has made to study the impact of industrial effluents on the bio accumulation of heavy metals and biochemical composition in estuarine catfish *Mystus gulio* (Ham.)

Mystus gulio (Ham.) is a delicious catfish of the brackish water system. In West Bengal, it is locally known as "Nona-tengra", in konkan as "Shingati". It is a commercially important catfish species in coastal waters. It is a large bagrid catfish. It is

large (45 cm), silvery, schooling catfish. The catfish belonging to the order Siluriformes, found in the estuaries throughout India. It is highly preferred by the common people of West Bengal, as well as of Orissa, Andhra Pradesh, Tamil Nadu, Kerala, Karnataka, Maharastra and Gujarat. It breeds in sea waters adjacent to estuarine zone, and larvae/juveniles are available in the month of March – April / May. Due to increased anthropogenic activities and various ecological changes, the abundance of this fish is declining gradually.

The taxonomic classification of *Mystus gulio* (Ham.) is as follows.

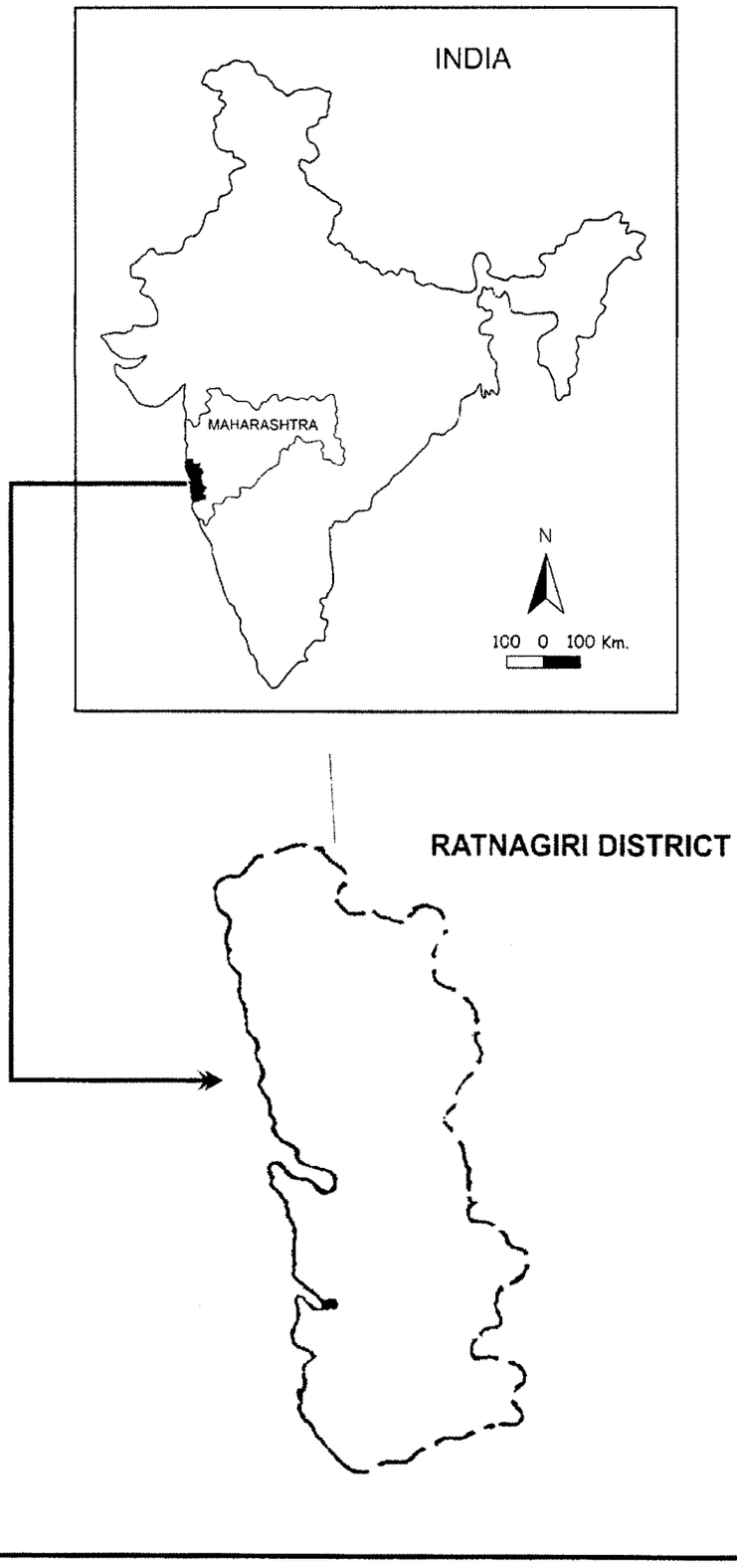
Classification:

Kingdom: Animalia.	Subkingdom: Bilateria.
Infrakingdom: Chordonia.	Branch: Deuterostomia.
Phylum: Chordata.	Subphylum: Vertebrata.
Infraphylum: Gnathostomata.	Class: Osteichthyes.
Subclass: Actinopterygii.	Infraclass: Actinopteri.
Superdivision: Neopterygii.	Division: Halecostomi.
Subdivision: Teleostei.	Infradivision: Elopocephala.
Cohort: Clupeocephala.	Subcohort: Otocephala.
Division: Ostariophysi.	Order: Siluriformes.
Family: Bagridae.	Genus: Mystus
Mystus gulio (Hamilton, 1822),	(Eschmeyer, W.N. 1998: 2460)

Acute toxicity tests (96 h) under static bioassay were carried out to assess the effect of heavy metals in industrial effluents on the fish species tested. The behaviour and mortality along with LC_0 and LC_{50} values of this fish was determined. The impact of acute as well as chronic exposure on the biochemical composition of various tissues of the fish was determined. Finally the accumulation of heavy metals in major tissues like liver, kidney, gill and muscle from test fish after acute exposure was estimated.

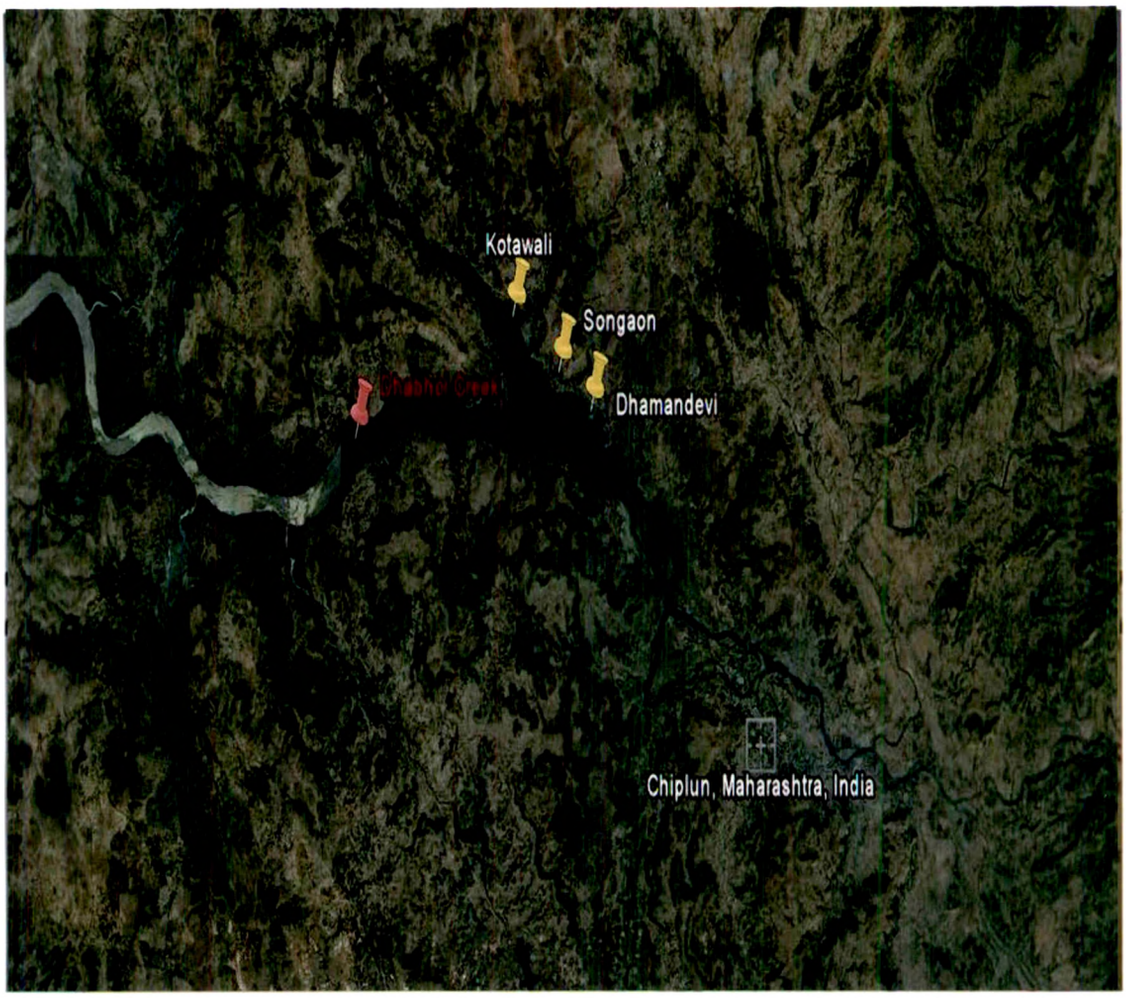
The present work encompasses effect of industrial effluents on fish species from Dabhol creek. The study extended over a period of 12 months, December, 2007 to November, 2008.

Fig. 1 INDEX MAP



Annexure I

Map showing locations of sample collection.



The investigation on physical, chemical and biological parameters of water were carried out during the above mentioned period. The experimental work was carried out partially in the field and partially in the laboratory.

Study area:

The study area the Dabhol creek (Annexure I) lies between lat. 17^0. 34' 54 . 30" N to 73^0. 10' 39.20" E, (Annexure - I) is a marginal marine biotope characterized by an estuarine delta and a shallow bay. These types of habitats are continuously influenced by changes in salinity due to fresh water discharge, high suspended load, high deposition of organic matter, temperature fluctuations characterizing shallow coastal ecosystems and high biological productivity.

The river Vashisti joins the Arabian Sea at Dabhol forming the Dabhol creek system. Western Ghats bounds the coastal segment in this region in the east and Arabian Sea in the west. A number of head lands and clefts intersect the coast, shoals and sand bars are present at the mouth of the Vashisti River and they show a seasonal shift (ANON, 1997).

The creek forms a major sink for anthropogenic pollutants released from various industries situated at Lote M. I. D. C. area (Raje, *et. al.,* 2005, Pria and parivartan, 1998).

Selection of Sampling Stations:

The three sampling stations chosen for the study are: Kotawali – The site where the industrial effluents are discharged, Songaon - a site 10 km away from the Kotawali and Dhamandevi - where the river Vashishti joins the creek (Plate No. I, II and III).

Water sampling:

The water samples were collected from the three sampling stations for physicochemical analysis from December, 2007 to November, 2008.Water samples were collected in clean and wide mouth plastic containers in the morning hours between 8.00 to 10.00 a.m. For each station separate containers were used. Then samples were brought to the laboratory for further analysis. The readings were recorded for all experiments through out the period of investigation and the data is statistically analyzed for comparison of water quality parameters and seasonal changes by using graphics.

The measurement of temperature, pH, were carried out right in the field. Other parameters such as Dissolved oxygen, Free Carbon-dioxide, Salinity, Nitrates and Phosphates were analyzed in the laboratory. The standard methods of APHA (1981) were used for physico-chemical analysis of water. These are listed below:

Temperature:

Surface water temperature was recorded at the sites using mercury thermometer, the range of thermometer was from 0 - 100 ^{0}c.

pH:

pH is equal to negative log $_{10}$ of hydrogen ion concentration.

$$pH = - \log_{10} [H^{+}]$$

pH measures the concentration of hydrogen ions in water. The pH of sample was measured in the field using pocket- sized electronic model HANNA pH meter. (pH ep^{R})

Salinity:

The water samples were collected from the selected spots and salinity was recorded.

Dissolved oxygen:

Dissolved oxygen in water samples was determined with the help of Winkler's Tritrometric method. Starch was used as an indicator. The result were expressed in mg/lit.

Free Carbon- dioxide:

Free carbon-dioxide was determined by titrating the samples using strong alkali (such as carbonate free NaOH) to pH 8.3. At this pH all free carbon-dioxide is converted into bi-carbonates. Phenolphthalein is used as an indicator. The results are expressed in mg / lit.

Nitrate (N):

Nitrate ions react with brucine in strong sulphuric acid solution to form yellow colour, which was measured calorimetrically at 410 nm. The reaction is highly dependent upon the heat generated during the test. Erma Model colorimeter was used for measurement of optical density (O.D.) of sample. A calibration curve was prepared by plotting the absorbance against the concentration. O.D. of the sample was compared with standard curve to calculate nitrate content (mg / lit) in the sample.

Plate No. I.

Spot A

Arrow indicating release of effluents in Dabhol Creek.

Stressful condition of mangrove and other vegetation due to spilt effluents.

[illegible] Slide1.JPG 002 HD
115 047 -1 N+1-2--- 2099.9/ 100.0

Plate No. II.

Spot A

Leakage of effluent carrying pipe line of Lote MIDC.

Collection of sample during low tide

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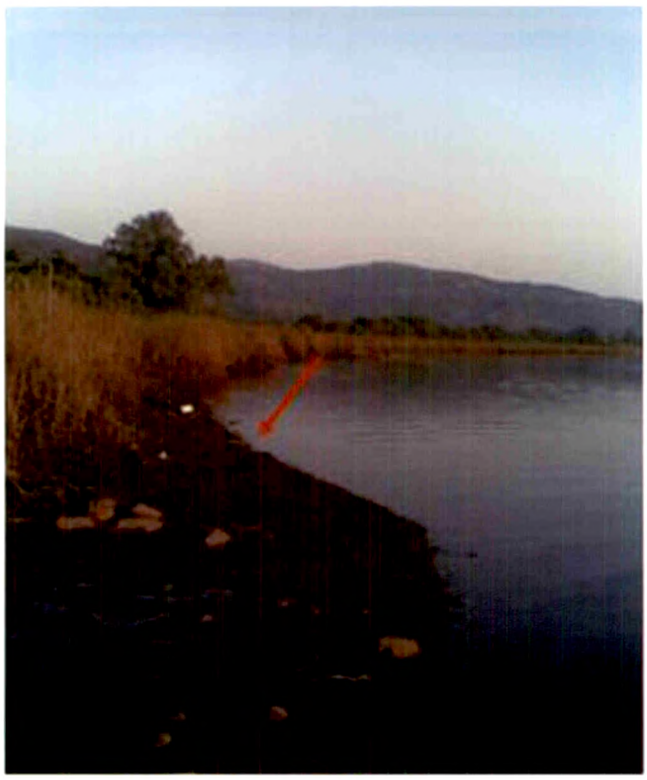
111 042-1 N+1-2--- 2099.9/ 100.0

Plate No. III.

Spot B



Spot C

Arrow indicate sample collection sites

Phosphate (P):

Phosphate in water reacts with ammonium molybdate to form molybdophosphonic acid, which gets reduced to complex of blue colour in the presence of stannous chloride ($Sncl_2$). The absorption of light by this blue colour was measured at 690 nm with the help of Erma model colorimeter. The concentration of phosphate (mg / lit) was calculated by comparing optical density of each sample with calibration curve.

Heavy Metal Analysis:

Heavy metal analysis from water samples:

Water samples were collected from three sampling stations namely Kotawali, Songaon and Dhamandevi during December, 2007 to November, 2008. In the laboratory they were treated with 1:1 Perchloric acid and nitric acid and refrigerated to 4^0C temperature to prevent change in volume due to evaporation. Then heavy metal content was analyzed by Atomic Absorption Spectrophotometer (Perkin Elmer Model No. 3030 USA) and values are expressed in ppm.

Heavy metal analysis from sediments:

Sediment samples were collected in clean polythene bags from selected sampling stations. Then the samples were brought to the laboratory for further analysis.

In the laboratory, the sediments were air dried, pulverized with grinder, sieved through 80 mesh sieve and stored in clean polythene bags. The samples were analyzed for detection of heavy metals. The required sediment samples were digested in 50 ml perchloric acid and 50 ml concentrated nitric acid in 1:1 ratio for 48 hours. The digested samples were flittered and the filtrate were subjected for detection of heavy metals by using Atomic Absorption Spectrophotometer (Perkin Elmer Model No. 3030 USA) and values were expressed in ppm.

Collection of fishes:

Irrespective of sex, healthy specimens of *Mystus gulio* (Ham.) having a body weight of 58 ± 2 g and length of 18 ± 3 cm were collected from Dabhol creek, Maharashtra, India. Collected fishes were acclimated to the laboratory conditions for 30

days in large plastic aquaria containing estuarine water. During acclimation they were fed with pelleted fish food and minced boiled chicken eggs. Water was renewed after every 24 h with routine cleaning of the aquaria, leaving no faecal matter and unconsumed food. Feeding was, however, stopped 24 h before sacrifice. After the expiry of 30 days of exposure, the specimens were photographed (Plate No. IV). Then 5 fish each from the respectively marked experimental, as well as control aquaria, were sacrificed. For estimating the biochemical content, the gills, kidneys, liver and muscle were pooled from the experimental fishes as well as control fishes separately and the tissues were used for analysis. All the dissection instruments and glassware were acid washed and rinsed with de ionized water. Metal concentrations in samples were measured using a Perkin Elmer Atomic Absorption Spectrophotometer- No. 3030 and expressed in ppm. Obtained data were subjected to standard statistical processing based on random sampling of three different samples of experimental, as well as control groups, of each tissue at each sampling period.

Bioaccumulation of heavy metals from fish organs:

The fish species *Mystus gulio* (Ham) exposed to industrial effluent were carefully dissected to segregate gills, muscles, liver and kidney to determine the concentration of heavy metals in them. Each tissue was dried in oven at 60 ^{0}C for 72 hours; dried tissues were pulverized in mortar, kept in polythene bags and stored in the refrigerator. The powdered 100 mg sample was digested with 10ml nitric acid and perchloric acid mixture (1:1) till clean solution was obtained. The digested samples were cooled at room temperature and filtered through Wattman Filter Paper. The filtrate was then diluted with concentrated HCL (5ml), are again diluted with glass distilled water (35ml). Test solutions were then analyzed for different trace metals using Atomic Absorption Spectrophotometer (Perkin Elmer Model No. 3030 USA) and finally the concentration quantified is expressed in ppm (Lithnor, 1975).

Biochemical Analysis:

The fish species *Mystus gulio* (Ham) exposed to industrial effluents were carefully dissected to segregate gills, muscles, liver and kidney. These tissues then

blotted, weighed and used for biochemical estimations. Glycogen was estimated by De Zwaan and Zandee, (1972), Protein by Folin Phenol method (Lowry, *et. al.,* 1951) and total lipids by Barnes and Black Stock, (1973). The experiments were repeated three times and mean values were expressed as mg / 100 mg wet weight tissue. Three replicates were subjected for statistical analysis for comparison of mean to find out significant differences.

The values obtained for all the biochemical analysis were expressed as mean ± SE. Statistical differences for biochemical values were determined using analysis of variances (ANOVA).