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CONTENTS

Fluctuation of Serum TSH, FSH, Vitellogenin Level and Associated Ovarian ............................................ 109–116
Toxicity in Clarias batrachus (Linn.) Exposed to Pesticide
Prakriti Verma, Rajnikant Kumar and Nishat Arfin

Effects of Sodium-Heparin and Dipotassium EDTA on the Haematological Parameters and .......................... 117–122
Blood Cell Morphology of Freshwater Fish Schizothorax labiatus (McClelland, 1842)
Imtiaz Ahmed and Amir Maqbool

Histological Alterations in Gill, Liver and Kidney of Rainbow Trout following Fungal Infection............. 123–128
Debajit Sarma, Vimal Kohli, Sarika Singh Kushwaha, Jyoti Pandey, S. K. Mallik, Neetu Shahi,
Partha Das, S. K. Srivastava, Vineeta Joshi and M. S. Akhtar

Gonadal Development Stages of Wild Male Golden Mahseer, Tor putitora from Nainital ................. 129–134
Region of Uttarakhand, India
N. Shahi, J. Pandey, S. K. Mallik, D. Sarma and P. Das

Determination of Catalase Activity and Estimation of Zn, Mn and Fe in Moss Samples ..................... 135–144
Collected from Heavy Traffic Areas
Pramod Kumar Tandon and Manjul Misra

Isolation and Characterization of Microorganisms from Edible Bivalves as Potential ......................... 145–154
Agents for Bioremediation
Ekta Jaiswal, Shweta Sharma, Vitthal Mohite and Aparna Deshmukh

High Level of Estrogen in Male Oral Cancer Patients and Consumption of Smokeless Tobacco .......... 155–159
A. Nath, Priyanka, J. K. Singh, Shalini Singh, Preety Jain, Mohita Sardhana, Akhilesh Kumar,
Satish Kumar and Manish Singh

Study on Growth and Survival of Giant Freshwater Prawn, Macrobrachium .................. 161–165
rosenbergii, in Tarai Agroclimatic Regime of Uttarakhnad
Shashank Singh and R. S. Muley

Effect of Short Term Temperature on Physiological Body Indices of Two Estuarine ................ 167–174
Venerid Clams Katelysiaopima and Meretrixmeretrix (Mollusca: Bivalvia)
V. M. Lagade and D. V. Muley

Stress Responses of Biomolecules and Dehydrogenase Activity in the Tissues of Labeo .............. 175–183
rohita against Pollutant Load in the Lakes of Bangalore
B. Zutshi, N. Noor and G. Sreekala

Remediation of Heavy Metals through Aquatic Macrophytes from Water ................................. 185–192
Bodies of Bundelkhand Region of Uttar Pradesh
Gunjan Sharma, Jamshed Zaidi and Amit Pal

Impact of Textile and Fertilizer Industry Effluents on Cytology of Root Meristem ..................... 193–196
Cells of Hordeum vulgare L. Plant
Pramod Kumar Tandon, Induja Tripathi and Kumkum Mishra
Fluctuation of Serum TSH, FSH, Vitellogenin Level and Associated Ovarian Toxicity in *Clarias batrachus* (Linn.) Exposed to Pesticide

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**Abstract:** A comprehensive study of serum Thyroid Stimulating Hormone (TSH), Follicle Stimulating Hormone (FSH) and Vitellogenin level of control and Rogor (1µl/L and 2.5µl/L) treated *Clarias batrachus* were done by ELISA. The present study revealed a wave of hormone imbalance and significant variations in the concentration of serum TSH, FSH and Vitellogenin level due to Rogor toxicity. The mean value of Thyroid Stimulating Hormone (TSH) level varied between 0.2 ± 0.089µIU to 2.8 ± 0.141µIU after Rogor exposure as compared to normal 2.255 ± 0.187µIU value. Whereas, the mean value of Follicle Stimulating Hormone (FSH) fluctuate between 54.167 ± 3.017µIU to 181.883 ± 0.479µIU in comparison to normal value 144.5 ± 0.187 µIU. The mean value of vitellogenin ranges between 0.001 ± 0.001IU to 2.86144.5 ± 2.94IU in comparison to normal (2.24 ± 3.31 IU) value. Light and Electron microscopy studies of ovarian tissue of Rogor treated fish have shown various treatment related abnormalities. Under Light microscopy ovarian tissue of normal *C. batrachus* showed different stages of development in goocytes in the ovigerous lamellae extended from well developed germinal epithelium along with a few interstitial nutritive cells with distinct nucleus and nucleolus. However, Rogor exposure for 5 days, showed ruptured ovigerous lamellae, fusion of a number of young and maturing oocyte, shrinkage of nucleus and peripheral vacuolization which increased after 10 days and 15 Days exposure in both doses of Rogor (1µl/L and 2.5µl/L). Under Scanning electron microphotograph, three dimensional structures of ovarian cells also revealed grooves, pappilliations and protuberances on the surface of the ova which increased with dose and duration of exposure.

**Keywords:** *Clarias batrachus*, Dimethoate, FSH, TSH, Vitellogenin, Ovary.

**Introduction**

Pesticide poisoning in fish is considered to be very serious as fish form major food resources for mankind affecting the consumer’s health and may also adversely affect the yield of fish (Dubois, 1971). Toxicity tests with embryos and larvae are valuable for assessing potential impacts on growth, reproduction, and survival of organisms in polluted environments and are important tools for good environmental monitoring (Zagatto, 1999). Fishes occupying the highest tropic level of the water body, suffers the most due to the pesticides. Out of the different systems, reproductive system is one of the most important organ which gets affected to a great extent. Attempts have been made to study the deleterious effect of pesticides on ovary of fish (Dutta et al., 1994; Ramchandra, 2000; Verma and Nath 2003; Verma et al., 2004), thyroid gonad relationship (Kobayashi et al., 1998), follicle stimulating hormone (Okomassoun et al., 2002) and serum vitellogenin level (Montiverdian et al., 2000; Nath and Maitra, 2001; Verma and Rani, 2013; Okumara et al., 2004). However, the scanning electron microscopic studies related to the histopathological effects of Rogor at sub cellular level in the ovary of fish and its correlation with Thyroid-gonad hormone level have not been dealt in much detail. The present investigation has been done with the aim to study the effect of Rogor on serum TSH, vitellogenin and FSH level in correlation with the histopathological
and ultra structural changes in the ovary of air breathing fish *Clarias batrachus* (Linn.). The various hormonal and histopathological parameters were assessed during spawning season.

**Materials and Methods**
Live and healthy female species of *Clarias batrachus* (Linn.) average weight 60 ± 10 gm and 4.5” ± 2” lengths were procured from various wetland of North Bihar. The fishes were brought to the laboratory, disinfected with 0.1% KMNO₄ solution and kept for acclimatization in the standard laboratory condition in Plexiglas aquaria and plastic pools. To maintain normal water temperature, cooler and exhaust were used around the aquarium. The aerated tap water was changed daily. After 48 hours, fishes were fed with pellets of wheat flour and egg @ 5 % of their body weight. After, two weeks of properly acclimatization fishes were categorized into two groups. Group I – comprises normal and Group II – comprises for Rogor treated test fishes which were further subdivided into sub groups (6 fishes in each) according to the doses of pesticide. In the present investigation, commercially brand “Rogor (EC 30%)” has been purchased from the local supplier. The LC₅₀ of Rogor fish was performed by the technique described in the standard methods (APHA, 2005), the 96h. LC₅₀ of Rogor for *Clarias batrachus* was calculated as 4µl. The two doses considered in the experimental protocol were 1µl/L and 2.5µl/L, accordingly stock solution of Rogor was prepared using distilled water. Fishes were then treated with 1.0µl/L (lower) and 2.5µl/L (upper sub lethal) for 15 days. The control group was maintained in the tap water only. The water and pesticide solution were changed regularly.

**Sample Collection**
On the termination of exposure period of 5, 10 and 15 days blood sample of both control and experimental group were collected in a heparinized glass culture tube eppendorf by sterile syringe from caudal vein. The serum was separated by centrifuging blood at 5000 rev./min for 10 min at 4°C and stored at −20°C for further hormone assessment. Fishes were anaesthetized with MS222 and the ovarian tissues were carefully removed and fixed for light and electron microscopy in the neutral formalin and 2.5% glutaraldehyde in 0.1M Phosphate buffer (pH 7.4) at 4°C. The tissues were processed for Light Microscopy in laboratory as per routine method, finally stained with Haematoxyline and Eosin and viewed under Trinocoular Microscope, Labomed CXRIII. For Scanning Electron Microscopy ovarian tissues were washed in 0.1M phosphate buffer, dehydrated in graded series of alcohol and then in a mixer of absolute alcohol and amyl acetate (1:3, 1:1 and 3:1) and lastly in the pure amyl acetate. These dehydrated tissues were further dehydrated to critical point drawing in a critical point drier and Gold sputtered by gold coating machine and then viewed under Scanning Electron Microscope at SIF-EM Facility Unit Department of Anatomy New Delhi. Details of histopathological observations based on Light Microscopy (LM) as well as Electron Microscopy were depicted in Figures 4-7

**Hormone Assessment**
The hormone assessment of TSH FSH and Vitellogenin were done on Merck ‘mini mios’ ELISA reader, as per standard protocol of WHO (2002). All the hormone assessment was done for control and treated fish *C. batrachus*. TSH assay was done by Micro plate immune enzymometric Assay. The kit was procured from Omega Technique Chemical Incorporation. TSH is a solid phase sandwich ELISA method. The samples and Biotin labelled anti-TSH-HRP conjugate are added to the wells coated with streptavidin TSH in the serum binds to anti-TSH and form a sandwich with streptavidin coated wells. Unbound proteins are washed off by wash buffer. Upon the addition of the substrate, the intensity of colour is proportional to the concentration of TSH in the samples. The absorbance was measured at 450 nm against blank. FSH was done through Micro well FSH EIA.
techniques. The kit was procured from Syntron Bioresearch Incorporation. The Micro well FSH EIA is a solid-phase enzyme immunoassay based on the “Sandwich” principle. The FSH present in the test sample reacts simultaneously with one antibody immobilized on the micro well surface and with another antibody conjugated to horse radish peroxide enzyme. So an Ab-Ag-Ab-Enzyme complex was formed on the micro well surface. Then the unbound conjugate was removed by washing and the colour was changed upon exposure to the enzyme. The intensity of the colour is proportional to the FSH in the sample. The absorbances were measured at 450 nm against blank. The comp vitellogenin ELISA kit were purchased from Bioscience Laboratory, Norway. The lyophilized coup vitellogenin standard was calibrated against purified cat fish vitellogenin. And vitellogenin concentration (mg/mg = absorbance at 286 nm/0.66, range = 0.24 ng/ml.) were measured. For each blood serum TSH and FSH and Vitellogenin assay, six observations were taken. The arithmetic mean was calculated and subjected to statistical analysis. The evaluation of each parameter was performed by its mean ± SD. The obtained data were subjected to paired test. Values at P < 0.05, P < 0.01 and P < 0.001 were considered to be significant. All statistical analysis were done using Sigma Plot 8.0 version. The results obtained have been included in tabular form, pictorial statistical representation and graphical plates of the present work. A parallel comparison was undertaken between the serum TSH, FSH and vitellogenin level of control fish with the Rogor treated group, likewise, LM and SEM photomicrograph of ovarian tissue of Rogor treated fish were compared with control fish.

Results and Discussion

On perusal of the Fig. 1 Fluctuation of Thyroid Stimulating Hormone (TSH) level increased (2.8 ± 0.141µIU) after 5 days exposure of Rogor (1µl/L) whereas on 10th day it abruptly decreased (0.2 ± 0.089µIU) and then increased (2.8 ± 0.141µIU) after 15 days as compared to normal (2.255 ± 0.187µIU) level. Comparatively, by giving the high dose of 2.5µl/L TSH level increased abruptly (3.45 ± 0.104µIU) after 5 days and then after 10 days it decreased (0.583 ± 0.147µIU) but decreased less than the former dose. After 15 days it increased sharply (2.033 ± 0.186µIU) but did not reach to normal level. Fig. 2 represents follicle stimulating hormone (FSH) which was slightly decreased (132.3 ± 0.554µIU) after 5 days of treatment of Rogor in both dose (1µl/L and 2.5µl/L) in comparison to normal level. Whereas after 10 day it slightly increased (137.383 ± 0.147µIU) by the dose of 1µl/L and abruptly shoots up (54.167 ± 3.017µIU) at the dose of 2.5µl/L. After 15 days exposure of 1µl/L Rogor the FSH level sharply increased (181.883 ± 0.479µIU) but it abruptly decreased (137.133 ± 0.484µIU) after giving 2.5µl/L Rogor. Figure 3 represents, serum Vitellogenin level which slightly decreased from (2.20 ± 0.554IU) to (1.10 ± 0.2) after five days of Rogor treatment in both dose (1µl/L; 2.5µl/L) respectively as compared to normal (2.24 ± 3.31). However, on tenth day it sharply increased (2.82 ± 0.62) and (2.86 ± 2.94) respectively but after 15 days it fell down abruptly up to minimum level, which was difficult to estimate. The abnormal fluctuation of serum TSH, FSH and Vitellogenin level can be further correlated with the histopathological observations. Histopathological observations of ovarian tissue from control fish showed different stages of the oocytes in the ovigerous lamellae which
extended from well developed germinal epithelium along with a few interstitial nutritive cells. Nucleus and Nucleolus are distinct (Fig. 4.1.). However, 1µl/L Rogor treated for 5 Days, ovarian tissue revealed ruptured ovigerous lamellae, fusion of a number of young and maturing oocyte, shrinkage of nucleus and peripheral vacuolization (Fig. 4.2). After 10 day ovarian tissue showed extensive degeneration of the ovigerous lamellae and increased fusion of oocytes, The plasma membrane and theca cell get ruptured and shrinkage of nuclear material were increased. (Fig. 4.3). After 15 day almost all oocyte showed fusion, massive vacuolation, shrinkage of nuclear material and atretic follicle (Fig. 4.4). 2.5µl/L Rogor exposure for 5 days oocyte show ruptured germinal epithelium and ovigerous lamella. Most of the oocyte of stage 1, 2 and 3 undergoes atresia. Previtellogenic and vitellogenic oocyte showed shrinkage of

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**Fig. 2** Fluctuation of serum FSH level in control and Rogor treated *Clarias batrachus* (Linn.), (n = 6).

**Fig. 3** Fluctuation of serum Cortisol level in control and Rogor treated *Clarias batrachus* (Linn.), (n = 6).

**Fig. 4** Photomicrograph of transverse section of Ovary of *C. batrachus* stained with haematoxyline and eosin.

1. Control fish ovary showing different stages of the oocytes in the ovigerous lamellae (OL) extended well developed germinal epithelium (GE) nucleus (N) are distinct. Note few interstitial nutritive cells (INC) along GE. X 200
2. Section of ovary treated with 1µl/L Rogor for 5 days, showing ruptured ovigerous lamellae, a no. of young and maturing oocyte fusion, shrinkage of nucleus and peripheral vacuolization and increased atretic oocyte. (→). X 200
3. After 10 days showing extensive OL and increased fusion of different stages of oocyte, Note increased atresia of oocyte, increased shrinkage of nuclear material. X 200
4. After 15 days of exposure showing extensive degeneration almost all oocyte showing fusion, extensive shrinkage of nuclear material and atretic follicle. X 400
nuclear material leaving peripheral vacuolation (Fig. 5.1). After 10 Day the most striking feature was that all stages of oocyte undergoes atresia. The worst affected oocyte was stage S_1 to S_4 (Fig. 5.2), after 15 days oocyte showed complete acellularity along with absence of nuclear material (Fig. 5.3 and 5.4).

Scanning Electron Microphotograph of the ovarian tissue of control *Clarias batrachus* showed three dimensional surface morphology of ova, smooth, rounded surface with surface epithelium attached to it. Interdigitate blebs (microvilli) were also seen on the surface along with some electron dense macro granules scattered over the oocyte surface (Fig. 6.1). 1 µL Rogor, 5 days treated C. *batrachus* ovarian tissue showed a number of depression, striations and wavy appearance of the ova with scattered macro granules (Fig. 6.2). After 10 days showing prominent tetra and hexagonal veins, protuberances and sign of rupturing of ova (Fig. 6.3) which increases after 15 days (Fig. 6.4). However, 2.5 µL Rogor 5 days treated fish ovarian tissue showed grooves and depression (Fig.7.1). After 10 days follicular epithelium showed loosened and ruptured surface epithelium (Fig. 7.2). Superficial and deep furrow increased after 15 days with prominent raised portion showing acellularity (Fig. 7.3).

The present investigation in lights the Rogor exposure causes sharp fluctuation of various hormones and several structural deformities and abnormalities in the ovary of *Clarias batrachus*, at subcellular level. Pesticides have been found to enhance the incidence of follicular atresia depending upon the period of exposure under laboratory conditions. Rogor causes extensive damage to developing oocytes only after fifth day of the exposure followed by tenth and fifteenth day. The result has convincingly favoured by Mani and Saxena (1985) who studied the effect of sub lethal concentration of fenitrothion and carbofuran on the oocyte of *Channapunctatus* causing more atretic oocyte. In the present investigation cytoplasm clumping was observed which is in consistency with Saxena and Garg (1978). The dissolution of the ooplasmic contents and hence, vacuolation was observed in the cytoplasm of the oocytes of all the growing six stages (Singh and Sahai,1986) which probably interferes in the movement of yolk precursors thereby, inhibiting vitellogenesis. Literatures is available supporting suchim balances in serum hormones and ultrastructural changes due to pesticides on fish ovary (Deka *et al.*, 2012;Verma *et al*, 2004). These include clumping of cytoplasm, vacuolations and shrinkage in nuclear material in vitellogenic oocytes. Tripathy and Verma
Fig. 6 Scanning Electron Microphotograph of the ovary of *Clarias batrachus*.

1. Scanning Electron Microphotograph of the ovary of control *Clarias batrachus* showing oocyte with normal, smooth, rounded surface (→) with surface epithelium attached to it (↑). Note- the interdigitate blebs (B).
2. Oocyte of 1 µL/L Rogor, 5 days treated *C. batrachus* showing a number of depression (V), striations and wavy appearance (S) of the oocyte with scattered macrogranules (MG).
3. After 10 days ova showing prominent tetra and hexagonal veins (V), protuberances (↑) and sign of rupturing of ova (→).
4. After 15 days showing a number of depressions (D), striations and wavy appearance (S) of the oocyte surface with scattered macrogranules (MG), ruptured ova with oozing the cytoplasmic inclusions out of the ova (→).

(1993) and others have also reported that histomorphology of the teleostean ovary vis-a-vis maturation of oocytes is adversely affected by different biocides as observed presently in *Clarias batrachus* after Rogor exposure. Guraya (1979) considered destruction of yolk nucleus as one of the possible causative factors for oocyte atresia as the former is reportedly involved in active RNA-Protein synthesis prior to vitellogenesis. Similar result has also been observed that thickening of ovarian wall, basement membrane and atretic follicle increases with dose and duration of both 1µL/L and 2.5µl/l Rogor exposure. The treated fish reveals interdigitate furrow and polygonal striation on the surface of the oocyte which becomes more apparent with prolonged exposure in both doses under scanning electron microscope. Probably, disorganisation of this network contributes for the follicular deformity. Alterations in the cell surface reflects hormonal imbalance or other substances that interact with the cell (Flickinger, 1979). Teleost thyroid is associated with many physiological and environmental changes. It has been found to be involved in affecting growth reproduction, osmoregulation and energy metabolism (Turner, 1966; Matty, 1985). Increased serum TSH level and simultaneously decrease in

Fig. 7 Rogor treated fish ovary

1. 2.5 µL of Rogor 5 days treated fish ovary showing grooves (G) and more depression and follicular epithelium (FE) with shriveled surface. Magnified portion deep groove (G) on the oocyte surface. Bulbous protrusion also seen adhering the oocyte surface (↑).
2. Portion of the ovary of rogor treated *C. batrachus* (10 Days). Follicular epithelium (FE) loosened and ruptured. Superficial (r) and deep furrow (DF) observed.
3. Protuberances (p) prominent. Inter oocyte with a prominent raised portion, the protuberance (p). Oocytes with more deeper and wider furrow (DF).
the serum FSH level after fifth day treatment of Rogor coincides with the report of Hulburt (1977), who showed that the thyroid hormone act synergistically with gonadotropin. Similarly, high level of serum TSH and diminished level of serum vitellogenin after fifteen days of Rogor exposure is in corroboration with histological findings. Thus sub lethal dose of Rogor may be presumed to effect the oocyte directly disrupting normal function of the yolk nucleus by binding to mitochondrial protein and thereby, interfering energy production in the young oocyte of the present fish C. batrachus. Since the secretion of TSH is negative feedback (Turner, 1966) so its concentration sharply decreases after ten day Rogor treatment in both doses whereas serum FSH level slightly shoots up after ten day in the present findings. It can be correlated with the findings of Singh and Singh (1980) who expressed that pesticide cause low level of serum and blood gonadotropin in fish due to accumulation or direct toxic action on the ovary. It can be correlated with the increase in the follicular atresia after fifteen day of Rogor exposure due to decreased level of serum FSH level. Parallel to this finding Christians and Williams (2002) also suggested that FSH may play a role in egg size number trade off in oviparous vertebrates. Increase in serum vitellognin level and further decrease in fifteen day of Rogor exposure due to decreased level of serum FSH level. The present communications are in conclusion with detailed discussion that roger produces profound impact on the growth and maturation of oocytes in C. batrachus. Specially massive degeneration and atrophy of stage I–V oocytes. Thyroid has been reported to take active participation in growth processes in fish. Fish pituitary contains variable amount of TSH and FSH which causes involvement in growth, reproduction and other metabolic activities. The combined histopathological and hormonal imbalance will affect the process of oogenesis in fish. Specially vitellogenesis of the mature oocytes. The oocyte degeneration reflects a variety of pathological condition andultiately causes death. The increased Serum TSH and decreased FSH and vitellogenin level also reveals the extreme pathological condition which also serves as a good indicator of fish reproductive health.

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APHA (2005) Standard method for the examination of water and wastewater (21st Ed) A joint publication of the American Public Health Association (APHA), the American Water Works Association (AWWA), and the water environment Federation (WEF), 1368.


Introduction

In recent time haematological and immunological study of fish has assumed greater significance due to the increasing emphasis on fish culture and greater awareness of the pollution of natural fresh water resources (Blaxhhall, 1972). Haematological and biochemical parameters are used as health indicators to detect the structural and functional status of fish under stress condition (Pimpao et al., 2007; Suveltha et al., 2010). The haematological characteristics have also been used as diagnostic tool to evaluating physiological changes including fish health (Satheeshkumar et al., 2010). Many automatic clinical tools have been developed for mammalian haematological analysis, but due to the unavailability of these automatic tools for fish, manual methods are employed.

The interpretation of blood parameters is quite difficult, since variations in the blood are caused by internal and external factors including anticoagulants used, method of analysis, the storage temperature, and the time lapse between sample analyses. Fish blood contains a large number of thrombocytes due to which it clots quickly, and samples almost always require anticoagulant treatment. Anticoagulants are additives that inhibit the clotting of blood and thereby ensure that the concentration of the substance to be measured is changed as little as possible before the analytical process (Guder, 2001). Blood cells of various animals show different reactions to various anticoagulants (Witeska and Wargocka, 2011). In fish haematology the most commonly used anticoagulants are different salts of heparin and EDTA (Walencik and Witeska, 2007). EDTA acts by
binding calcium ions, which are essential in the coagulation cascade and for cell-to-cell interaction. Due to this property, the EDTA salt is used as a blood anticoagulant because it chelates calcium ions which promote blood clotting (Jain, 1993). Heparin binds to and accelerates the activity of antithrombin III, which inhibits the action of thrombin and other proteases necessary for coagulation (Harr et al., 2005).

Many workers have used EDTA salts as most appropriate for fish blood analyses (Blaxhall and Daisley, 1973; Sala-Rabanal et al., 2003), while others recommend heparin as the preferred anticoagulant (Walencik and Witeska, 2007; Ishikawa et al., 2010; Clark et al., 2011). Haematology provides useful information as long as the addition of an anticoagulant causes no alteration. The aim of the present study was to study the effects of sodium-heparin and di-potassium-EDTA on the haematological parameters and blood cell morphology of *Schizothorax labiatus* so as to suggest the better anticoagulant for routine fish blood analysis.

**Materials and Methods**

**Experimental Fish**

Adult specimens of *Schizothorax labiatus* (McClelland, 1842) weighing 250 ± 18.5 g and average length 25 ± 2.1 cm were obtained from a local fish farm. The fish were transported in polythene bags filled with water and oxygen and brought to wet laboratory at the Department of Zoology, University of Kashmir. To rule out any possible microbial infection, the fish were given a prophylactic dip in KMnO4 (5 mg L⁻¹) and stocked in indoor circular fish tank (water volume = 600 L) at 16.6 ± 1.4°C, D.O 6.6 mgL⁻¹ and pH 7.1-7.5 with 12:12 h photoperiod.

**Blood Sampling and Anticoagulant Treatment**

During blood sampling, fishes were netted carefully imparting minimum stress and placed in a fish trough. Using a chilled needle fitted to a 3 ml syringe, blood was drawn from the caudal vein. Blood smears were made on the spot from anticoagulant free blood, which were used as control to be compared against the blood smears prepared from anticoagulant treated blood. Blood sample was equally transferred to sterilized blood collection tubes, containing the respective anticoagulant i.e., Na-Heparin (20 I.U/ml) and K₂EDTA (1.0mg/ml) which were prepared by dissolving the dry salts of Na-Heparin and K₂EDTA (Loba Chemie, India) in phosphate buffer saline (PBS, pH 6.8). Blood vials were kept in an ice-bath until further analysis. All the haematological parameters were analyzed within 2 hours after sample collection.

**Total Erythrocyte Count (RBC) and Total Leucocyte Count (WBC)**

RBC and WBC counts were done simultaneously in a neubauer haemocytometer with Natt Herrick’s (1952) diluent (1:200). The blood cell counting was done under a microscope.

**Haemoglobin (Hb)**

Drabkin’s cyanomethemoglobin method (1946) was used to estimate the haemoglobin content of the blood. 20 µl of blood was mixed with 5 ml of Drabkin’s reagent (Loba Chemie, India), left to stand for 15 minutes and then absorbance measurement was done at 540 nm. Absorbance of test sample was plotted against that of haemoglobin standard (Ranbaxy India) to get the haemoglobin concentration. Haemoglobin test samples were centrifuged, prior to reading the absorbance, to remove dispersed nuclear material.

**Haematocrit (Hct)**

Blood samples (50µl) were taken in micro haematocrit capillaries and centrifuged in a micro centrifuge (REMI RM-12C BL, India) spun in at 12,000 rpm for 5 min to obtain haematocrit value which were reported as percentage. Blood indices including mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from...
RBC, Hct and Hb according to the following formulae: MCV = (PCV x 10)/ RBC, MCH = (Hb x 10)/ RBC and MCHC = (Hb x 100)/ Hct (Dacie and Lewis, 1991).

**Blood Cell Morphology**

Using grease free glass slides, two blood smears were prepared for each blood sample. After air drying, the smears were fixed in 100% methanol for 5-8 min and stained with May-Grunwald-Giemsa stain. Using a compound microscope (Magnus MLX-Tr, India) blood smears were examined at 1000x magnification. Digital microphotographs were obtained with a digital DSLR camera (Cannon EOS 70D) attached to microscope and blood cell morphometric analysis was done with ‘ImageJ’ image analysis software (National Institute of Health) calibrated to a stage micrometer.

**Statistical Analysis**

All statistical analyses were carried out with SPSS 11.5 (SPSS Inc., Chicago, IL, USA). Haematological parameters were summarized as mean ± standard deviation (SD) and differences between Na-heparin and K₂EDTA anticoagulated blood samples were statistically analyzed using the Student’s t-test. Results were considered significant at p<0.05.

**Results and Discussion**

**Haematological Parameters**

Table 1 shows the result of the blood samples treated with Na-Heparin (20 IU/ml of blood) and K₂EDTA (1.0 mg/ml of blood). Haematocrit values in all K₂EDTA treated samples were significantly higher (p < 0.05) and much more variable compared to those of heparin collected samples. The total RBC counts between blood samples obtained with K₂EDTA and those obtained with Na-Heparin showed significant (p < 0.05) differences. Mean corpuscular volume (MCV) was increased in K₂EDTA sample, while a significant decrease (p < 0.05) was found in mean corpuscular haemoglobin concentration (MCHC). Haemoglobin concentration, total WBC counts and leucocyte morphology did not differ significantly (p > 0.05) among the samples treated with Na-heparin and K₂EDTA.

**Cell Morphometric Analysis**

Characterization and identification of blood cells was done by light microscopy. Blood smear analysis revealed considerable erythrocyte swelling leading to significant enlargement in all the K₂EDTA treated samples (Fig. 1d) as compared to the untreated blood samples (Fig. 1a and 1b), while in heparinized samples, most of the erythrocytes were normal and intact (Fig. 1c). In Na₂EDTA treated samples, bare nuclei released by haemolysed erythrocytes were also observed (Fig. 1d). Leukocytes in K₂EDTA and Na-Heparin anticoagulated blood showed no morphological changes.

An elevation in haematocrit values and a decrease in haemoglobin concentration and RBC counts were observed in K₂EDTA treated samples. K₂EDTA also induced RBC swelling and erythrocyte haemolysis, while no significant change of haematological parameters was observed in Na-heparin treated samples. Similar observations were reported by Walencik and Witeska (2007) and Ishikawa et al. (2010). EDTA salt can cause acidification and an increase in pCO₂ (Smit et al., 1977), due to which haematocrit levels might have elevated. This phenomenon was observed by Korcock et al. (1988), Walencik and Witeska (2007) and Witeska and Wargocka (2011). Hattingh (1975) compared the effects of heparin and EDTA on haematocrit of five species of fish and found that EDTA had a tendency to increase haematocrit in fish, and in some species induced haemolysis, while heparin produced very little change in erythrocyte volume and haematocrit values. On incubation of blood for 20 min with 10 mg/mL of EDTA, a distortion of erythrocyte shape followed by lysis was observed in Blennius pholus (Mainwaring and Rowley, 1985). EDTA sequesters Ca²⁺ ions is responsible for the activation of Na⁺ and K⁺ ions in the cell membrane which allows the free
Table 1 Hematological parameters of Schizothorax labiatus (n=15) treated with anticoagulant sodium-heparin (20 I.U/ml of blood) and di-potassium-EDTA (1.0mg/ml of blood)*

<table>
<thead>
<tr>
<th>Anticoagulant used</th>
<th>Hct (%)</th>
<th>Hb (gdl⁻¹)</th>
<th>RBC (x10⁹/mm)</th>
<th>WBC (x10⁹/mm)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC (gdl⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na-Heparin</td>
<td>34.32 ± 2.01a</td>
<td>8.19 ± 0.43a</td>
<td>1.42 ± 0.07a</td>
<td>12.18 ± 3.20a</td>
<td>241.1 ± 8.96a</td>
<td>57.59 ± 1.16a</td>
<td>23.91 ± 1.31a</td>
</tr>
<tr>
<td>K₂EDTA</td>
<td>39.20 ± 1.95b</td>
<td>6.90 ± 0.70a</td>
<td>1.22 ± 0.06b</td>
<td>11.45 ± 2.71a</td>
<td>321.4 ± 8.38b</td>
<td>56.45 ± 2.77a</td>
<td>17.57 ± 1.01b</td>
</tr>
</tbody>
</table>

Hct, Haematocrit; Hb, Haemoglobin concentration; RBC, Red blood cell count; WBC, White blood cell count; MCH, Mean corpuscular haemoglobin; MCHC, Mean corpuscular haemoglobin concentration;

Values are mean ± SD. Means with different superscripts are significantly different (p<0.05)

A decrease in RBC count in Na₂EDTA treated samples was observed in the present study, which could be due to the haemolytic action of EDTA. EDTA treatment causing erythrocyte haemolysis have been reported in fish (Walencki and Witeska, 2007; Lataretu, 2013) and other vertebrates (Muro et al., 1998; Antwi-Baffour et al., 2013) leading to low RBC counts and decreased haemoglobin concentration. Witeska and Wargocka (2011) observed gradual destruction of erythrocytes in Na₂EDTA samples, where Na₂EDTA caused cell swelling, followed by disintegration of the outer membranes of erythrocytes, which resulted in release of the nucleus. Erythrocytes become more susceptible to lysis as EDTA increases their osmotic fragility. Increased osmotic fragility in the blood samples treated with EDTA was observed by Walencik and Witeska (2007) in Cyprinus carpio and Mafuvaadze and Erlwanger (2007) in ostriches, which eventually lead to a decrease in the RBC count. A gradual destruction of erythrocytes in Na₂EDTA samples was observed by Witeska and Wargocka (2011), where Na₂EDTA caused cell swelling, followed by disintegration of the outer membranes of erythrocytes, which resulted in release of the nucleus. Haemolytic action of EDTA can be attributed to the adverse effect of Na₂EDTA on erythrocyte membrane structure, permeability, and stability, which is probably related to decalcination induced by the chelating action of the anticoagulant (Blaxhall, 1972; Hattingh, 1975).

Fig. 1. Light micrographs of May-Grunwald-Giemsa stained blood film of Schizothorax labiatus. A and B: Control, showing normal and intact erythrocytes (R); C: Na-heparin treated, showing normal and intact erythrocytes (R) and leucocytes (L); D: K₂EDTA treated, showing erythrocyte swelling (S) and bare erythrocyte nuclei (N).

entry of water into the cell, promoting-swelling and its consequent lysis (Jain, 1993). Heparin is known to cause little alteration in corpuscular size and is considered a more suitable anticoagulant, because its varying concentration has little effect on the PCV values (Dubin et al., 1976). In the present study, Na-Heparin caused no significant alteration in RBC morphology, which also corroborate the observation made by Walencik and Witeska (2007), Ishikawa et al. (2010) and Witeska and Wargocka (2011) in common carp.
In conclusion Na-heparin is found as the preferable anticoagulant for blood sampling in Schizothorax labiatus as it imparts minimum changes to haematological parameters, while K2EDTA cannot be applied as an anticoagulant for haematological analysis, since it significantly affects the haematological parameters and alters the shape and size of blood cells, affecting red blood cell parameter readings.

Acknowledgements
The authors are grateful to the Head, Department of Zoology, University of Kashmir, Hazratbal, Srinagar, India, for providing the necessary laboratory facilities and also gratefully acknowledge the generous funding from the Department of Science and Technology (DST), Govt of India, New Delhi in the form of DST-FAST Track Young Scientist Project.

References
Histological Alterations in Gill, Liver and Kidney of Rainbow Trout following Fungal Infection


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Abstract: Histomorphological changes were observed to assess the effect of fungus on gill, liver and kidney of Rainbow trout (Oncorhynchus mykiss). The most frequent histological changes detected in the gills included hypertrophy, hyperplasia and fusion of secondary lamellae. Other lesions found were vacuolization, blood congestion, and increased melano-macrophage (MM) in liver. Shrinkage of glomerulus, increased Bowman’s space, increased MM and hematopoietic tissue were observed in the kidney.

Keywords: Histomorphology, Rainbow trout, Fungal infection.

Introduction

The rainbow trout (Oncorhynchus mykiss) is a commercially important coldwater fish which is generally cultured in Indian upland region. The colonization of aquatic fungi in teleost fish is a severe problem affecting both wild and cultured fish population. However, the fungal infection is more abundant in the captive environment which adversely affects fish industry. An external fungal infection cause lesions, subsequently become enlarge and may lead death (Hoffman, 1963). Stress, physical injury, malnutrition and poor water quality increase the susceptibility of fungal infections (Roth, 1972; Piper et al., 1983). Gill, kidney and liver are responsible for vital functions such as respiration, excretion, erythropoiesis, regulating blood pressure and the accumulation and biotransformation of xenobiotics in the fish (Hole, 1992; Gernhofer et al., 2001; Aguis and Robert, 2003). The alterations found in these organs are easier to identify than functional ones (Fanta et al., 2003), and serveas warning signs of damage to animal health (Hinton and Laurens, 1990). Hence, the study of tissue deformities in respect to fungal infection which is commonly encountered in cold water aquaculture needs to be studied in order to attain maximum yield. Keeping this in mind, the present study was carried out in order to understand and describe the degree of histological alterations in gill, kidney and liver following fungal infection.

Materials and Methods

Six adult live specimens (fungal infected as well as healthy rainbow trout) were collected from Experiment Field Centre of Directorate of Cold Water Fisheries Research (ICAR), Chamapawat (80° 07’ N, 29° 30’ N, and 1620 msl), Uttarakhand, India. After, being taken to the laboratory, fishes were dissected and the samples of gill, liver, kidney were fixed in Bouin’s fixative for 24 hours. After fixation, the samples were washed in water, dehydrated in graded ethanol solutions, cleared in xylene and embedded in paraffin. Paraffin blocks were sectioned 5–6 µm thick on a microtome (Microm HM 340E). The resulted sections were dewaxed and stained for histological purposes with haematoxylin and eosin (H and E) (Delafield, 1885). Histological sections were examined
through light microscope (Magnus MLX-DX) equipped with digital camera (Olympus E-420) at the magnification of 100x to 1000x.

**Results and Discussion**

**Gills**

The histological analysis of healthy fish gill indicated the primary gill lamella (PL) contains a centrally placed rod like axis (CA) with a row of secondary gill lamellae (SL) on both side of it. The secondary lamellae attached at their base with the primary lamellae free at their distal ends and covered by highly vascularized thin epithelial cells (EC). Inter lamellar region (ILR) is present between the two adjacent secondary lamella (Fig. 1a and 1b). However, histological observations in fungal infected rainbow trout clearly indicated the cytoarchitectural distortion of gills with marked, hypertrophy and hyperplasia of pillar and epithelial cells resulting in the fusion of secondary lamellae. Epithelium of secondary lamellae showed cloudy swelling. Inter lamellar region was reduced or completely absent in some places between the secondary lamella. Extensive disruption of primary lamella was also observed. More severe lesions were observed in the gill with hemorrhage due to rupture of lamellar epithelium (Fig. 1c and 1d).

The gills of fish are multi functional organs as they participate in respiration, osmoregulation, acid-base balance, excretion. They Remain in close contact with the external environment, which also makes them extremely sensitive to changes in the quality of the water. They are considered the primary target of the contaminants (Poleksic and Mitrovic, 1994; Mazon et al., 2002; Fernandes and Mazon, 2003; Au, 2004). According to the observations in the present study, fungi cause major histomorphological alterations in the gill of *O. mykiss* like hyperplasia, hypertrophy, completely fusion of secondary lamellae are examples of defense mechanisms. These may result in the increase of distance between the external environment and blood which act as a barrier. Similar observation were also seen by other workers (Mallatt, 1985; Hinton and Lauren, 1990; Poleksic and Mitrovic, 1994; Fernandes and Mazon, 2003). On the other hand, fusion of secondary lamella decreased inter lamellar distance, results in reduction in the diffusion conductance of the gills to respiratory gases (Greco et al., 1995; Perry et al., 1996), leading to impaired oxygen uptake by gills. The results of the present study also depict the same.

**Liver**

In general, the surface of liver is enclosed by serous membrane and connective tissue extends inward into parenchyma. The histological analysis of healthy liver tissue of rainbow trout indicates that the hepatocytes are rounded polygonal cells with spherical nucleus, arranged in distinct tubules. Tubules contain 5 to 7 hepatocytes arranged radially around with their apices surrounding a biliary lumen.

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**Fig. 1.** Photomicrographs of the gill of *O. mykiss* (a, b) normal gill showing the primary lamella (PL), secondary lamella (SL), central axis (CA), inter lamellar region (ILR), water channel (*), pillar cell (P), epithelial cell (E), erythrocytes (short arrow); (c, d) fungal infected gill showing hyperplasia in secondary lamellae (*), completely fusion of secondary lamella (FSL), hypertrophy (arrowhead), rupture of epithelium (black arrow), cloudy swelling (white arrow). H and E 400X (a,c), 1000X (b,d).
The basal aspects of hepatocytes faced the perisinusoidal space. Sinusoids appeared in the interstitial between hepatic tubules in our present experiment (Fig. 2a).

However, it was observed in the present study that fungal infection causes discrete pathological changes in the liver tissue of fish. These changes include blood congestion, increased accumulation of melano-macrophage and vacuole formation. Vacuolization observed are zones of total cell degeneration (Fig. 2b and 2c). Similar changes were also observed in the liver after exposure of various contaminants (Masud et al., 2001, 2003, Butchiram., 2009). Melano-macrophage aggregation at the site of blood congestion as these develop in association with chronic inflammatory lesions (Aguiss and Robert, 2003). The result of our study also revealed the same.

Kidney

The histological analysis of kidney of healthy-rainbow trout consists of the functional unit called nephrone which contains glomerulus, Bowman’s capsule, proximal, distal and collecting tubules. The hematopoietic tissue present between the tubules. Occasionally, Melano-macrophage centers located in hematopoietic tissue (Fig. 3a and 3b).

However, histological analysis of infected tissue indicated degeneration of glomerular tuft. Bowmen’s space of glomeruli became increased (Fig. 3c). Similar alterations in the kidney have also been reported in the fishes exposed to various compound such as zinc, lead, copper (Al-Zahaby et al., 1998), trichlorfon (Marcelo et al., 2002), nitrate (Iqbal et al., 2004), dimethoate (Singh, 2012), ethylenediaminetetraacetic acid (Ghorashi et al., 2013). It is known that maleno-macrophage contained macrophages and a variety of pigments including melanins, lipofuscin, ceroid and hemosiderin (Couillard et al., 1999). The accumulation of melanomacrophage was more apparent in fungal infected kidney tissue as observed in our study (Fig. 3c). Density of melanomacrophage

![Fig. 2. Photomicrographs of the liver of O. mykiss (a) normal liver showing the hepatocytes (H), sinusoids (SN); (b,c) fungal infected liver showing vacuole formation (V), melano-macrophage (MM), blood congestion (BC). H and E 1000X (a), 400X (b), 100X (c).](image)

![Fig. 3. Photomicrographs of the kidney of O. mykiss (a, b) normal kidney showing the proximal tubule (PT), distal tubule (DT), hematopoietic tissue (H), bowmen’s capsule (BC), glomerulus (G); (c) fungal infected kidney showing aggregation of melanomacrophage (*), increased Bowmen’s space (arrow), increased hematopoietic tissue (arrow head). H and E 400X (a,c), 1000x (c).](image)
increased in the condition of environmental stress, in the presence of cachectic disease and also develops in association with chronic inflammatory lesions elsewhere in the body (Vijayan and Leatherland, 1988; Agius and Roberts, 2003). Handy and Penrice (1993) also observed melano-macrophage in the kidney of trout \( (Salmo trutta) \) and Tilapia \( (Oreochromis mossambicus) \) exposed to mercuric chloride. Hematopoietic tissue formed a supporting matrix for the nephrons (Hickman and Trump, 1969). An extreme enhancement was also evident in hematopoietic tissue and it spread over large area, which may be an effort to provide additional support to degenerating nephrons.

The result of the present study revealed that histology may be an effective tool in describing the histomorphological alterations in the selective tissue of rainbow trout having fungal infection. As a result, the current effort will certainly help to understand the severity of fungal infection in the production system and thereby to take successful strategies to maximize the yield in the cold water aquaculture of rainbow trout.

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**References**


Histological alterations in gill, liver and kidney


**Gonadal Development Stages of Wild Male Golden Mahseer, Tor putitora** from Nainital Region of Uttarakhand, India


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**Abstract:** Golden mahseer, Tor putitora is an endangered fish of mid-Himalayan regions of India. It has good potential as aquaculture species for hill aquaculture, due to its high market demand. But the major constrains on introduction of this fish for aquaculture is its inability to breed in captivity and slow growth rate. Therefore, studies were carried out on its breeding biology to captive breed this fish for sustainable seed production. However, detailed study on reproductive pattern of adult golden mahseer is very limited. Therefore, in the present study, we have developed a macroscopic grading system for golden mahseer testes, which shows the testes development stages during the breeding season, which lasts from May–September in Bhimtal and Sattal lakes. The golden mahseer were sampled from February – September 2014, from Bhimtal and Sattal lakes. Altogether, 48 samples were collected during the sampling period. Based on macroscopic observation, the maturation stage of the testes was divided into five developmental stages: Immature (I), early spermatogenic (II), late spermatogenic (III), matured (IV) and spent (V).

**Keywords:** Tor putitora, Reproduction, Maturity, Testes.

**Introduction**

Golden mahseer, Tor putitora is an important food and game fish of mid-Himalayan regions of northern India. This fish has high demand as food, due to its good taste and nutritional content. Moreover, it is an excellent game fish, because of its fierce fighting nature (Jha and Rayamajhi, 2010). To fulfill the growing demand of fish eaters, indiscriminate fishing has been taken place over the last few decades, which has declined the natural population of T. putitora in its habitat. Moreover, construction of hydro-electric dams and habitat destruction has further reduced its population, and thus this species is now, listed under red data book as an endangered fish species (Khan and Sinha, 2000).

To revive the population of this indigenous fish, several attempts were undertaken (Das and Joshi, 1994). Ranching in natural water bodies and captive breeding of this fish has been initiated by central and state government departments. However, like many other fishes, T. putitora does not attain complete gonadal maturity in captivity, the seed production still depends on matured brooders collected from wild (Masuda and Bastola, 1984). Collection of brooders from wild is not a sustainable means of seed production, therefore, now attempts to breed this fish in captivity by using hormones have been given consideration (Shrestha et al., 1990). But, to captive breed this fish, it is important to know about its reproductive cycle and maturity stages.

Studies of gonad morphology at anatomical levels have long been done by fish biologists to identify reproductive cycles and to determine size-at-maturity. In fisheries, the determination of the reproductive state of a fish population in a specific area and time plays a very significant role, because these parameters are closely related to stock productivity and are the basis of many regulatory measures (Hunter and Maciewicz, 2003). Until recently, the reproduction of T. putitora had been

*Email: shahineetu@rediffmail.com*
studied sporadically on the basis of breeding biology with limited sample size (Sunder and Joshi, 1977). Macroscopic maturity scales of fish ovaries and testes are among the most frequently used indexes in assessments of fish reproductive condition (Hunter and Macewicz, 2003). This method is fast and inexpensive and can detect major reproductive events such as spawning season.

Generally, the majority of the macroscopic studies related to fish reproduction have been carried out for female, with sporadic studies being done on male reproduction (Parenti and Grier, 2004; Lowerre-Barbieri et al., 2011). But the males may have different reproductive parameters from females such as a different period of spawning seasons and a different size and age at maturity (Lowerre-Barbieri et al., 2011). Therefore, the knowledge of the reproductive biology of male fish is very significant for adopting any breeding and management measures.

In the present study, the detailed description of *T. putitora* testes developed at macroscopic (anatomical) level was carried out during the breeding season. The seasonal variability of gonad characteristics was used to build an anatomical scale.

**Materials and Methods**

**Sampling of Golden Mahseer**

From February to September 2014, 48 male *T. putitora* samples (Fig. 1) were collected from Bhimtal and Sattal lakes of Nainital district of Kumaun region of Uttarakhand. All the fishes were taken from local fisherman. The average total length and weight of the fishes were 32.78 ± 9.87 cm and 380 ± 68.90 g, respectively. The collected fishes were brought to the wet laboratory and after measuring morphometric parameters, sacrificed for gonad analysis. In the study, fishes above the age of 3.5 years were included for analysis and the age of the fishes were determined by counting the number of rings on their scale of dorsal region. All the fish were anaesthetized by clove oil (50 µL/L of water) before sacrifice. The gonads were collected for analysis by macroscopic characteristics.

**Macroscopic Analysis**

The macroscopic analysis and classification of the testes was carried out based on color, fullness, thickness, extrusion of milt, presence of blood spots and shape and length of gonad (Table 1).

**Results and Discussion**

The male fish had slender body and milky white milt oozes freely in matured golden mahseer. The testes of the *T. putitora* were divided into five gonadal developmental stages based on macroscopic observations (Fig. 2A–2E).

Immature (I) Testes: asymmetrical thin, elongated, paired with smooth edges and pale white in color (Fig. 2A).

Early spermatogenic (II) Testes: elongated, slightly thicker, off white and translucent (Fig. 2B) Blood spot were visible on surface.

Late spermatogenic (III) Testes: asymmetrical, pinkish white, opaque, lobular, enlarged (Fig. 2C) and larger in volume. Milt could not be striped at this stage.

Spermiation (IV) Testes: enlarged, massive, milky white, soft and anterior part is filled with white color milt (Fig. 2D). Viscous fluid present in the anterior part. blood vessels spread on the surface. Testes occupy almost entire length
Gonadal development stages of *Tor putitora*

Table 1 Major characteristics used in the macroscopic analysis of *Tor putitora* testes

<table>
<thead>
<tr>
<th>Feature</th>
<th>Classification levels for testes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color of the gonad</td>
<td>Grey, Brown, Red, Orange, White</td>
</tr>
<tr>
<td>Fullness</td>
<td>Empty looking, half full and full</td>
</tr>
<tr>
<td>Thickness</td>
<td>Thick, medium thick and thin</td>
</tr>
<tr>
<td>Pressure on abdomen</td>
<td>Semen released on gentle pressure to abdomen, Semen not released on pressure</td>
</tr>
<tr>
<td>Blood spots on gonad</td>
<td>Conspicuous, inconspicuous</td>
</tr>
<tr>
<td>Shape of the gonad</td>
<td>Triangular, rectangular, circular or too thin to characterize</td>
</tr>
<tr>
<td>Length of the gonad</td>
<td>Cover the entire length of the body cavity, half the length of the body cavity, less than half the length of the body cavity</td>
</tr>
</tbody>
</table>

**Fig. 2** Macroscopic appearance of *Tor putitora* testes collected from Bhimtal and Sattal lakes of Nainital district. A- anatomical class I; B- anatomical class II; C- anatomical class III; D- anatomical class IV; E- anatomical class V.

of the body cavity. Milt could be stripped from more than 70% of the males in this stage.

Post-spawning (V) Testes: in regression, flabby, blood red and deflated sacs (Fig. 2E). Milt could not be stripped from any of the male.

In the month of February to March, majority of the male fish were Immature (Fig. 3), where as in the June and July the number of fishes oozing milt increased drastically.

Due to indiscriminate capture of brooders and juveniles and habitat destruction, this fish is assessed as endangered and is in need of immediate conservation efforts to save it from becoming extinct. Golden mahseer productivity is reported to decline in the Tehri Dam in the Garhwal Himalaya, India. The stress on the population is not only because of its overexploitation, but also due to the rise in anthropogenic activities, especially the growing number of hydroelectric dams and irrigation projects which have fragmented and deteriorated its habitat (Jha and Rayamajhi, 2010). The species has the potential for being cultured in aquaculture ponds and ranched in rivers/artificial channels of Trans-Himalayan region. Strategies for preservation of existing stock by habitat conservation, development of seed production technology for restocking and culture have been undertaken to promote the population.

Several attempts were made to breed this fish in captivity. However, the rate of success...
was very low, and the collected brooders were mainly from nature. The main hindrance in development of a strategy for captive matura-
tion is that, its gonadal developmental stages were not studied in detail during the breeding season. There are few studies on its breed-
ing biology from nature. So to develop a successful strategy for the captive maturation of this species, it is very essential to know about its gonadal development stages in nature.

Macroscopic study of the gonadal development stages provide information about the extension of the gonad in relation to the body cavity as well as the width of the gonad at the widest point. The maturity stages can be distinguished on size, shape, color and structure of the gonad. In male fish, there is a difference in maturity stages of testes, which occur due to sperm formation. However, color is a more prominent trait in females than males, as the variation in color between testes in different maturity stages is more subtle than for ovaries. The most significant macroscopic characteristics of testes in males are the volume of the testes compared to the body cavity as well as the appearance of the sperm duct and milt. The male reproductive cycle in general exhibit a smoother transition between stages than the female cycle.

In this study, we described the first detailed study of the male reproductive characteristics of wild Tor putitora from lakes of Kumaun region of Uttarakhand. Historically, macroscopic classifications of gonad development had been the major method for reproductive studies of fish, mainly in male reproduction (Gil et al., 2013). In case of golden mahseer, majority of the study is focused on its life cycle, breeding biology, length weight relationship, embryonic development and nutritional requirement (Hora and Mukerjee, 1936; Hora, 1939). Mahseer breeds several times during a year (Hora, 1939). T. putitora is known to breed in Himalayan Rivers during August to September (Hora and Mukerjee, 1936). Though the gonadal development stages were studied for tor mahseer, the same was not studied in detail for Himalayan mahseer. The gonadal development stages of the T. putitora were found to be near similar to tor mahseer. Majority of the male fish above the length of 30 cm, captured in the month of June and July were oozing milt. These fishes were more than 3 years old. It indicates that male get mature in monsoon month in this region. It may be due to the lowering in temperature and influx of freshwater, which stimulates their gonadal development. In this study we categorized the reproductive maturation of testes into 5 development stages based on macroscopic observation. This study was performed during the breeding season in Nainital region of Uttarakhand, India.

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References


**Introduction**

Atmosphere’s role in maintenance of the biosphere is in a healthy state. The load of pollutants discharge in the air, chemically dilute, and finally are brought back to earth’s surface to be eliminated. The air consists of complex mixture of a number of gases, water vapour and many fine particulate material like Nitrogen (78%), Oxygen (20.95%), Argon (0.93%), Carbon dioxide (0.03%), Neon (0.0015%), Helium (0.00052%), Methane (0.00015%), Krypton (0.001%), Hydrogen (0.00005%), Nitrous oxide (0.00005%), Xenon (0.000009%), and Ozone (0.00007%). There are more than 7,50,000 manmade chemicals present in our environment and to those 1000-2000 new ones are added every year. Massive production of such chemicals directly or indirectly releases thousands of tones of a variety of air pollutants into the atmosphere. Some of the air pollutants released into the atmosphere by man are CO, CO₂, SO₂, some other gases, vapours, odour and dusts of toxic metals like Lead, Arsenic, Asbestos, Nickel, Mercury, Vanadium, Zinc, various hydrocarbons, fluorides etc. Indeed quite a large number of heavy metals are essential to plant and animal (including human life). Few of these include iron, manganese, copper, nickel, zinc, cobalt, chromium and molybdenum. The toxicity of zinc to plants and fresh water algae ranges between 0.004 to 6.0 ppm. Zinc has been established as an essential trace element required for the functioning of several enzymes (Schroeder 1974, Underwood 1977). Concentration beyond limits can be highly toxic causing vomiting, dehydration, stomach pain, nausea, lethargy, dizziness and muscle in coordination (Sandstead 1975).

These investigations in relation to different air pollutants with bryophytes prove their utility as bioindicator in air pollution. Due to some special characters such as habitat diversity, structural simplicity, tot potency and rapid rate of multiplication, bryophytes can be taken for pollution monitoring studies. With this view, study has been carried out on two selected Bryophytes (Mosses) Octoblepharum albodum and Physcomitrium pyriforme, in Heavy Traffic areas of

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**Determination of Catalase Activity and Estimation of Zn, Mn and Fe in Moss Samples Collected from Heavy Traffic Areas**

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**Abstract:** Moss Physcomitrium pyriforme and Octoblepharum albodum samples were collected from some gardens and Monument areas and some Heavy Traffic areas. Samples were collected from soils and moist brick walls and analysed for determination of elements Zn, Mn and Fe. Samples collected from Garden and Monument areas treated as Control. Samples collected from Heavy Traffic areas showed higher level of elements in comparison to the samples collected from Garden and Monument sites. Higher content of Zinc, Manganese and Iron was found in the Heavy Traffic areas as compared to the Garden and monument areas. A parameter, catalase Activity has also been studied by using particular method to determine the effect of pollution on the metabolism of species.

**Keywords:** Heavy Metals, Pollution, Heavy Traffic areas, Moss, Biomonitoring.
Lucknow city (Uttar Pradesh), India to observe the effect of environment on these taxa with special reference to element Zn, Mn and Fe accumulation. Folkeson (1979) measured Zn concentration in *Pleurozium schreberi, Dicranum polysetum, Hylocomium splendens, Hypnum cupressiforme* from 57 sites in coniferous wood land at 1.6 - 7.0 km from a brass foundry in Sweden and found that Zn shows least variation in concentration between species. Zoltals (1988) sampled peat land within 250 km radius flinflon Monitoba near the smelter. He found the Zn concentration in the surface which is decreasing exponentially away from the source.

This paper also deals with the use of mosses as bio-indicator to collect the data of environmental pollution at some heavy traffic areas. Vehicular activity is the main cause of contamination of road side soil. It also depends on traffic density in the area, break oil quality and oil combustion techniques used in vehicles. Many other activities like fabricating works, collection and incineration of garbage, battery refilling, plating, using fertilizers in garden etc. are the main sources of environmental pollution in different heavy traffic areas of the city.

**Materials and Methods**

Lucknow, the capital city of Uttar Pradesh is situated on the northern Gangetic plain of India, between 26.50° North and 80.50° East at an elevation of 123 meters above sea level. Lucknow city is surrounded by its rural towns and villages. The total area covered by Lucknow is around 3204 square kilometers.

The Gomti River, the chief geographical feature, meanders through the city, dividing it into the Trans-Gomti and Cis-Gomti regions. Lucknow city is located in the seismic zone III. The climate is hot and humid in summers but winters are cold and chilly. Summers start by the end of March and remains till June. Monsoon starts immediately after the summers. Maximum and minimum temperature goes up to 310 C to 180 C respectively. Annual mean rainfall is 972 mm. October is the time when winter starts. This season is favorable for mosses.

Sites have been divided according to the traffic crowded roads and further divided into two groups according to the availability of moss species. Twelve different traffic crowded roads have been surveyed according to the availability of moss *Octoblepharum albifum* and these sites have been put in to group-A (Table 1) viz Ayurved Hospital, Haiderganj Crossing (site no. 1), DAV College (site no.2), Exone College, Campbell Road (site no. 3), Polytechnic Campus, Kanpur Road (site no. 4), Hindustan Aeronautical Limited (site no. 5), Yojana Bhawan (site no. 6), St. Francis College (site no. 7), Kapoorthala Crossing (site no. 8), I.T. College Crossing (site no. 9), Khurram Nagar Crossing (site no. 10), Krishi Bhawan (site no. 11) and Mill Road, Aishbagh (site no. 12). On the other hand 12 more sites have also been surveyed according to availability of moss *Physcomitrium pyriforme* and put in to group-B (Table 2) viz Medical College (site no. 13), R.B. Inter College (site no. 14), Literacy House, Kanpur Road (site no. 15), Thana Ghazipur, Faizabad Road (site no. 16), Charbagh Railway Station (site no. 17), AMC Road Sadar (site no. 18), Baradari, Kaiserbagh (site no. 19), Gandhi Museum (site no. 20), Doordarshan Kendra (site no. 21), Begum Hazrat Mahal Park Road (site no. 22), Daliganj, Railway Crossing (site no.23), Public Laundry, Nadan Mahal Road (site no.24). In order to collect moss samples of *Octoblepharum albifum* from pollution free zone gardens and monument places have also been surveyed (Table 3). Moss samples of group-A have been collected from Residency Ruins (site no. 1) and Dr. Bhim Rao Ambedkar Park (site no. 2), and moss samples of groups-B *Physcomitrium pyriforme* (Table 4) samples have been collected from Dilkusha Garden (site no. 3) and Kukrail Reserve Forest Picnic Spot (site no. 4) and all these four samples have been treated as control.
Determination of Catalase Activity and Estimation of Zn, Mn and Fe

For the estimation of elements Zn, Mn, Fe and catalase activity in moss material, the samples collected from different sites, were washed twice with de-ionized water and thereafter were shaken for 15 minutes to remove adhered soil particles from moss material. For the estimation of Zn, Mn and Fe the moss samples were oven dried for 48 hrs at 85°C. The known weight of dried plant material was well digested in a mixture (10: 1) of concentrated HNO₃ and HClO₃ (AOAC 1990). Residues were diluted in 25 ml distilled water and were filtered through Whatmen Filter Paper No. 11. The concentration of heavy metals in the solution was analysed by using Perkin-Elmer 280 Atomic Absorption Spectrophotometer. Analysis of the samples was carried out in triplicates. Concentrations of Elements were calculated by the formula.

\[
\frac{XV}{W}
\]

Where: \(X\) = reading in ppm on AAS  
\(V\) = final volume (ml)  
\(W\) = dry weight of moss in g

Catalase activity was analysed in 3.0 ml reaction mixture containing potassium phosphate buffer.

### Table 1 Showing Catalase activity (\(\mu\) moles H₂O₂ split / 100 mg fr.Wt and Zn, Mn, Fe concentration \(\mu\)gg⁻¹ in moss Octoblepharum albidum collected from Area-B Heavy traffic sites.

<table>
<thead>
<tr>
<th>Site No.</th>
<th>Area-A Heavy traffic sites</th>
<th>Zn Mean + SD (conc. in µgg⁻¹)</th>
<th>Mn Mean + SD (conc. in µgg⁻¹)</th>
<th>Fe Mean + SD (conc. in µgg⁻¹)</th>
<th>Catalase activity ((\mu) moles H₂O₂ split / 100 mg fr. wt Mean + SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ayurved Hospital, Haiderganj Crossing</td>
<td>195.60 + 18.26</td>
<td>168.00 + 6.70</td>
<td>1900.00 + 216.00</td>
<td>6.50 + 1.00</td>
</tr>
<tr>
<td>2</td>
<td>DAV College</td>
<td>200.00 + 8.16</td>
<td>130.00 + 14.14</td>
<td>2000.00 + 163.00</td>
<td>8.00 + 0.50</td>
</tr>
<tr>
<td>3</td>
<td>Exone College, Campbell Road</td>
<td>679.00 + 1.41</td>
<td>468.00 + 21.30</td>
<td>3006.00 + 429.00</td>
<td>10.00 + 0.50</td>
</tr>
<tr>
<td>4</td>
<td>Polytechnic campus, Kanpur Road</td>
<td>466.00 + 28.50</td>
<td>151.00 + 0.94</td>
<td>6600.00 + 0.00</td>
<td>8.75 + 0.75</td>
</tr>
<tr>
<td>5</td>
<td>Hindustan Aeronotical Limited</td>
<td>680.00 + 2.16</td>
<td>202.00 + 1.88</td>
<td>1020.00 + 172.00</td>
<td>6.75 + 0.25</td>
</tr>
<tr>
<td>6</td>
<td>Yojana Bhawan</td>
<td>147.00 + 0.81</td>
<td>232.00 + 22.60</td>
<td>3000.00 + 163.00</td>
<td>9.00 + 0.50</td>
</tr>
<tr>
<td>7</td>
<td>St. Francis College</td>
<td>321.00 + 4.64</td>
<td>207.00 + 2.05</td>
<td>2133.00 + 124.00</td>
<td>11.00 + 0.50</td>
</tr>
<tr>
<td>8</td>
<td>Kapoorthala Crossing</td>
<td>526.00 + 0.47</td>
<td>166.60 + 4.64</td>
<td>1666.00 + 94.20</td>
<td>7.75 + 0.25</td>
</tr>
<tr>
<td>9</td>
<td>I.T. College Crossing</td>
<td>490.00 + 8.16</td>
<td>203.00 + 38.60</td>
<td>1666.00 + 94.20</td>
<td>8.25 + 0.25</td>
</tr>
<tr>
<td>10</td>
<td>Khurram Nagar Crossing</td>
<td>195.00 + 18.35</td>
<td>269.00 + 20.67</td>
<td>1233.00 + 124.00</td>
<td>7.50 + 0.50</td>
</tr>
<tr>
<td>11</td>
<td>Krishi Bhawan</td>
<td>425.00 + 2.44</td>
<td>240.00 + 28.76</td>
<td>2826.00 + 115.80</td>
<td>8.00 + 1.00</td>
</tr>
<tr>
<td>12</td>
<td>Mill Road, Aishbagh</td>
<td>369.00 + 2.05</td>
<td>177.00 + 19.20</td>
<td>1733.00 + 94.20</td>
<td>7.75 + 0.25</td>
</tr>
</tbody>
</table>

All the values are the mean of three replicates + standard deviation.
Table 2 Showing Catalase activity (µ moles H$_2$O$_2$ split / 100 mg fr. Wt and Zn, Mn, Fe concentration µgg$^{-1}$ in moss Physcomitriumpyriforme collected from Area-B Heavy traffic sites.

<table>
<thead>
<tr>
<th>Site No</th>
<th>Area-B Heavy traffic sites</th>
<th>Zn Mean + SD (conc. in µgg$^{-1}$)</th>
<th>Mn Mean + SD (conc. in µgg$^{-1}$)</th>
<th>Fe Mean + SD (conc. in µgg$^{-1}$)</th>
<th>Catalase activity (µ moles H$_2$O$_2$ split / 100 mg fr. wt Mean + SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>Medical College</td>
<td>422.00 + 1.42</td>
<td>137.00 + 1.40</td>
<td>2200.00 + 0.00</td>
<td>6.00 + 1.00</td>
</tr>
<tr>
<td>14</td>
<td>R.B. Inter College</td>
<td>316.00 + 1.41</td>
<td>332.00 + 6.18</td>
<td>3466.00 + 94.20</td>
<td>7.25 + 0.25</td>
</tr>
<tr>
<td>15</td>
<td>Litracy House, Kanpur Road</td>
<td>665.00 + 4.02</td>
<td>155.00 + 0.81</td>
<td>3816.00 + 23.57</td>
<td>9.00 + 0.50</td>
</tr>
<tr>
<td>16</td>
<td>Thana Ghazipur, Faizabad Road</td>
<td>845.00 + 0.47</td>
<td>152.00 + 5.24</td>
<td>1376.00 + 11.77</td>
<td>6.50 + 1.00</td>
</tr>
<tr>
<td>17</td>
<td>Charbagh Railway Station</td>
<td>1456.00 + 40.20</td>
<td>221.00 + 6.23</td>
<td>1380.00 + 424.00</td>
<td>6.00 + 0.50</td>
</tr>
<tr>
<td>18</td>
<td>AMC Road Sadar</td>
<td>233.00 + 8.49</td>
<td>156.00 + 6.18</td>
<td>1753.00 + 37.71</td>
<td>6.25 + 0.03</td>
</tr>
<tr>
<td>19</td>
<td>Baradari, Kaiserbagh</td>
<td>145.00 + 0.81</td>
<td>388.00 + 6.23</td>
<td>3000.00 + 0.00</td>
<td>7.50 + 1.00</td>
</tr>
<tr>
<td>20</td>
<td>Gandhi Museum</td>
<td>245.00 + 4.08</td>
<td>428.00 + 1.63</td>
<td>2478.00 + 1.80</td>
<td>9.25 + 1.92</td>
</tr>
<tr>
<td>21</td>
<td>Doordarshan Kendra</td>
<td>422.00 + 1.41</td>
<td>383.00 + 6.84</td>
<td>2200.00 + 0.00</td>
<td>8.00 + 0.50</td>
</tr>
<tr>
<td>22</td>
<td>Begum HazratMahal Park Road</td>
<td>208.00 + 6.20</td>
<td>454.00 + 10.40</td>
<td>2706.00 + 9.42</td>
<td>8.50 + 0.50</td>
</tr>
<tr>
<td>23</td>
<td>Daliganj, Railway Crossing</td>
<td>315.00 + 1.69</td>
<td>336.00 + 1.24</td>
<td>2388.00 + 6.59</td>
<td>9.50 + 0.50</td>
</tr>
<tr>
<td>24</td>
<td>Public Laundry, NadanMahal Road</td>
<td>284.00 + 1.63</td>
<td>163.00 + 2.35</td>
<td>2013.00 + 18.80</td>
<td>7.50 + 0.50</td>
</tr>
</tbody>
</table>

All the values are the mean of three replicates + standard deviation.

Table 3. Showing Catalase activity (µ moles H$_2$O$_2$ split / 100 mg fr. Wt and Zn, Mn, Fe concentration µgg$^{-1}$ in moss Octoblepharumalbidum collected from Area A – Garden and Monumental.

<table>
<thead>
<tr>
<th>Site No</th>
<th>Area A – Garden and Monumental</th>
<th>Zn Mean + SD (conc. in µgg$^{-1}$)</th>
<th>Mn Mean + SD (conc. in µgg$^{-1}$)</th>
<th>Fe Mean + SD (conc. in µgg$^{-1}$)</th>
<th>Catalase activity (µ moles H$_2$O$_2$ split / 100 mg fr. wt Mean + SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>Residency Ruins(oa)</td>
<td>736.00 + 18.80</td>
<td>375.00 + 16.90</td>
<td>11506.00 + 21.90</td>
<td>10.00 + 1.00</td>
</tr>
<tr>
<td>26</td>
<td>Dr. BhimRao Ambedkar Parkoa(oa)</td>
<td>727.00 + 18.90</td>
<td>340.00 + 14.71</td>
<td>5733.00 + 124.72</td>
<td>10.50 + 1.25</td>
</tr>
</tbody>
</table>

All the values are the mean of three replicates + standard deviation.
pH 7.0, 11.0 mM \( \text{H}_2\text{O}_2 \) and moss homogenate following the kinetics at 250°C. Activity was determined Spectrophotometrically by monitoring the decomposition of \( \text{H}_2\text{O}_2 \) at 240 nm by measuring the time required for a decrease of absorbance from 0.45 to 0.40 (Aebi 1983).

The estimation of elements content and catalase activity data were subjected to variance analysis in order to discriminate real average effects from them, which may be due to chance error. The interpretation of the results was done on the basis of analysis of variance (ANOVA) test. The critical difference (CD) at 5 percent P level of probability were worked out to compare the treatments for their significance. Standard error of mean (SEm±) were computed in each case. The standard statistical methods were followed for statistical analysis of the data.

**Results and Discussion**

**Zinc Content**

**Area A - Garden and Monumental Sites**

At Residency Ruins (site no. 25) and at Dr. Bhim Rao Ambedkar Park (site no. 26) moss samples of *Octoblepharum albideum* showed Zn content 736.00 µg g\(^{-1}\) and 727.00 µg g\(^{-1}\) respectively. On the other hand at Dilkusha Garden (site no.27) and at Kukrail Reserve Forest Picnic Spot (site no.28) moss samples of *Phy-scomitrium pyriforme* showed Zn content 476.00 µg g\(^{-1}\) and 1560.00 µg g\(^{-1}\) respectively. Carballeira et al (2002) determined the level of Zn in the terrestrial mosses *Scleropodium purum* and *Hypnumcupressiforme* collected from 75 sampling sites in Galicia (N.W. Spain).

**Area B– Heavy Traffic Sites**

In order to evaluate the element contamination in all Heavy Traffic sites two moss species have been taken. Highest Zn contamination was observed in moss *Physcomitriumpyriforme* collected from Charbagh Railway Station (site no. 17) 1456.00 µg g\(^{-1}\). Higher concentrations were also found in samples of *Octoblepharum albideum* collected from Hindustan Aeronotical Limited (site no. 5) and Exone College, Campbell Road (site no. 3)680.00 µg g\(^{-1}\) and 679.00 µg g\(^{-1}\) and in the moss *Physcomitriumpyriforme* collected from Thana Ghazipur, Faizabad Road (site no.16) and Literacy House, Kanpur Road (site no. 15) 845.00 µg g\(^{-1}\) and 665.00 µg g\(^{-1}\) respectively.

Moderate value have been found in moss samples *Octoblepharum albideum* collected from Polytechnic campus, Kanpur Road (site no. 4), I.T. College Crossing (site no. 9), Krishi Bhawan (site no. 11) 466.00 µg g\(^{-1}\), 490.00 µg g\(^{-1}\), 425.00 µg g\(^{-1}\) respectively and followed by the samples of *Physcomitriumpyriforme* collected from Medical College (site no. 2) 422.00 µg g\(^{-1}\) and same in samples from Doordarshan Kendra (site no. 21). Moss *Octoblepharum albideum* procured from DAV College (site no.2), St. Francis College (site no.7) and Mill Road, Aishbagh (site no. 12) showed the range between 200.00 µg g\(^{-1}\)-369.00 µg g\(^{-1}\). On other hand in moss *Physcomitriumpyriforme*

**Table 4.** Showing Catalase activity (µ moles \( \text{H}_2\text{O}_2 \) split / 100 mg fr. wt) and Zn, Mn, Fe concentration µg g\(^{-1}\) in moss *Physcomitriumpyriforme* collected from Area A – Garden and Monumental.

<table>
<thead>
<tr>
<th>Site No.</th>
<th>Area -A Garden and Monumental</th>
<th>Zn Mean + SD (conc. in µg g(^{-1}))</th>
<th>Mn Mean +SD (conc. in µg g(^{-1}))</th>
<th>Fe Mean + SD (conc. in µg g(^{-1}))</th>
<th>Catalase activity (µ moles ( \text{H}_2\text{O}_2 )split / 100 mg fr. wt Mean + SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>Dilkusha Garden</td>
<td>476.00 + 4.32</td>
<td>276.00 + 13.69</td>
<td>6666.00 + 189.50</td>
<td>11.25 + 0.25</td>
</tr>
<tr>
<td>28</td>
<td>Kukrail Reserve Forest Picnic Spot</td>
<td>1560.00 + 16.77</td>
<td>284.00 + 0.81</td>
<td>8020.00 + 55.62</td>
<td>10.50 + 1.00</td>
</tr>
</tbody>
</table>

All the values are the mean of three replicates + standard deviation.
collected from R.B. Inter College (site no. 14), Gandhi Museum (site no. 20) Begum Hazrat Mahal Park Road (site no. 22), Daliganj, Railway Crossing (site no.23), and Public Laundry, Nadan Mahal Road (site no. 24), showed the accumulation of Zn between the range 245.00 µgg⁻¹-316.00 µgg⁻¹. Furthermore the lowest concentration in moss *Octoblepharum albium* at Ayurved Hospital, Haiderganj Crossing (site no. 1), and at Khurram Nagar Crossing (site no.10) 195.00 µgg⁻¹ have been found. Berg et al (1995) studied the data from a survey on atmospheric deposition of Zn in 495 moss samples of *Hylocomium splendens* collected in 1990 and analyses that Zn contributes the highest values in the dominant factors. Carballéira et al (2002) observed the level of Zn in the terrestrial mosses Scleropodium purum and *Hypnum cupressiforme* collected from 75 sampling sites in Galicia (N.W. Spain) were determined. It was found that the dominant lethology in sampling area had no influence on the estimated background levels.

**Manganese Content**

**Area A - Garden and Monumental Sites**

At Residency Ruins (site no. 25) and at Dr. Bhim Rao Ambedkar Park (site no. 26) moss samples of *Octoblepharum albium* showed the Zn content 375.00 µgg⁻¹ and 340.00 µgg⁻¹, respectively. At Dilkusha Garden (site no. 27) and at Kukrail Reserve Forest Picnic Spot (site no. 28) moss samples of *Physcomitrium pyriforme* the Zn content ranged between 276.00 µgg⁻¹ to 284.00 µgg⁻¹. Komai (1981) found the Mn concentration in surface soil of parks in the residential and less industrialized Kishiwada City of Japan ranged between 540-188 ppm.

**Area B – Heavy Traffic Sites**

The highest Mn accumulation has been found in the moss *Octoblepharum albium* collected from the site Exone College, Campbell Road (site no. 3) 468 µgg⁻¹ Followed by in the moss *Physcomitrium pyriforme* collected from the doordarshankendra (site no. 21) and Daliganj railway crossing (site no. 23) 428.00 µgg⁻¹ and 454.00 µgg⁻¹ respectively. The samples of moss *Octoblepharum albium* collected from Hindustan Aeronotical Limited (site no. 5), Yojna Bhavan (site no. 6), Mill Road, Aishbagh (site no. 12) showed the Mn accumulation range 202-269 µgg⁻¹ and in samples of *Physcomitrium pyriforme* collected from Charbagh Railway Station (site no. 17), Baradari Kaiserbagh (site no. 19), Begum Hazrat Mahal Park Road (site no. 22), Daliganj, Railway Crossing (site no. 23), ranged between 221-388 µgg⁻¹. Lowest accumulation have been found in *Physcomitrium pyriforme* at Medical College (site no. 13), 137 µgg⁻¹, Litracy House, Kanpur Road (site no. 15) 155 µgg⁻¹, Thana Ghazipur, Faizabad Road (site no. 16), 152 µgg⁻¹, AMC Road Sadar (site no. 18), 156 µgg⁻¹ and at Public Laundry, Nadan Mahal Road (site no. 24), 163 µgg⁻¹. Lowest range of Mn accumulation was found to be in moss *Octoblepharum albium* at Ayurved Hospital, Haiderganj Crossing (site no. 1), and at Khurram Nagar Crossing (site no.10) 195.00 µgg⁻¹, DAV College (site no. 2) 130 µgg⁻¹, Polytechnic campus, Kanpur Road (site no. 4) 151 µgg⁻¹ and KAPOORTHALA CROSSING (site no. 8) 166 µgg⁻¹.

Manganese (Mn) is an essential trace element for living being but toxic at higher concentrations. Pyrolusite (MnO₂) is the commonest source of manganese. It is used in ceramics, dry batteries, electrical coils, matches, glasses, dyes, welding rods, fertilizers and iron alloys. Gupta (1995) examined the content of Mn in *Plagiothecium denticulatum*, *Bryumargentenum* and *Sphagnum spp.* in Shillong (Meghalaya) north eastern India. Samples were collected from urban and suburban areas of Shillong while *Sphagnum sp.* was collected from suburban sites only and the study showed that *Sphagnum sp.* accumulated higher amount of Mn and reversed trend was discerned for Mn in *Plagiothecium denticulatum*.

**Iron Content**

**Area A - Garden and Monumental Sites**

At Residency Ruins (site no. 25) and at Dr. Bhim Rao Ambedkar Park (site no. 26) moss samples of *Octoblepharum albium* showed
the Fe content 11506.00 µgg⁻¹, and 5733.00 µgg⁻¹ respectively. At Dilkusha Garden (site no. 27) and at Kukrail Reserve Forest Picnic Spot (site no. 28) moss samples of Physcomitriumpyriforme the Fe content ranged between 6666.00 µgg⁻¹ and 8020.00 µgg⁻¹. Abass (1998) studied the bioaccumulation of Fe in mosses collected from polluted and unpolluted areas of southern parts of Nigeria.

Area B – Heavy Traffic Sites

The highest accumulation of Fe has been demarcated in the samples of Octoblepharumalbidum collected from the Polytechnic campus, Kanpur Road (site no. 4) 6600.00 µgg⁻¹ and followed by Exone College, Campbell Road (site no. 3) 3006 µgg⁻¹, moss Physcomitriumpyriforme showed the highest accumulation range between 3816-3000 µgg⁻¹ in the samples collected from Litracy House, Kanpur Road (site no. 15), R.B. Inter College (site no. 14), and Baradari, Kaiserbagh site no. 19). Rest of the samples showed the accumulation range between 1376-2706 µgg⁻¹ collected from different sites- Medical College (site no. 13), Thana Ghazipur, Faizabad Road (site no. 16), AMC Road Sadar (site no. 18), Gandhi Museum (site no. 20), Doordarshan Kendra (site no. 21), Begum Hazrat Mahal Park Road (site no. 22), Daliganj, Railway Crossing (site no. 23), Public Laundry and Nadan Mahal Road (site no. 24), Folkeson (1978) studied the Fe content in 5 moss species in 57 sites in coniferous woodland at 1.6-7.0 km from a brass foundry and larger amount of Fe accumulated in HypnumCupressiforme.

Catalase Activity

Area A - Garden and Monumental Sites

At Residency Ruins (site no. 25) and at Dr. Bhim Rao Ambedkar Park (site no. 26) moss samples of Octoblepharumalbidum showed the Catalase Activity 10.00-10.50 µ moles H₂O₂ split / 100 mg fr. wt. respectively. At Dilkusha Garden (site no. 27) and at Kukrail Reserve Forest Picnic Spot (site no. 28) moss samples of Physcomitriumpyriforme the Catalase Activity 11.25 and 10.50 µ moles H₂O₂split / 100 mg fr.Wt. Enzymatic activity in bryophytes have already been reported by various authors. Hebant and Suire (1974) analysed the activity of enzymes- acid phosphatase, cytochrome oxidase, b-fructosidase oxalic acid oxidase, peroxidase and succinate dehydrogenase in moss Dicranum.

Area B – Heavy traffic sites

Highest Catalase Activity was found in the moss samples of Physcomitriumpyriforme collected from Daliganj, Railway Crossing (site no. 23) 9.50µ moles H₂O₂ split / 100 mg fr. wt. followed by Gandhi Museum (site no. 20) 9.25µ moles H₂O₂ split / 100 mg fr. wt. followed by Litracy House, Kanpur Road (site no. 15) 9.00 µ moles H₂O₂ split / 100 mg fr. wt. At Doordarshan Kendra (site no. 21), Begum Hazrat Mahal Park Road (site no. 22) ranged between 8.00- 8.50 µ moles H₂O₂ split / 100 mg fr. wt. samples collected from Medical College (site no. 13) and R.B. Inter College (site no. 14), Thana Ghazipur, Faizabad Road (site no. 16) Charbagh Railway Station (site no. 17), AMC Road Sadar (site no. 18) Catalase Activity ranged between 6.00-7.50 µ moles H₂O₂ split / 100 mg fr. wt.

Samples of moss Octoblepharumalbidum collected from St. Francis College (site no. 7) showed the highest Catalase Activity 11.00 µ moles H₂O₂ split / 100 mg fr. wt. followed by Exone College, Campbell Road (site no. 3) 10.00 µ moles H₂O₂ split / 100 mg fr. wt. At DAV College (site no. 2), Polytechnic campus, Kanpur Road (site no.4), Yojana Bhawan (site no. 6), I.T. College Crossing (site no. 9)and Krishi Bhawan (site no. 11) ranged between 8.00-9.00 µ moles H₂O₂ split / 100 mg fr. wt. Samples collected from Ayurved Hospital, Haiderganj Crossing (site no. 1), Hindustan Aeronotical Limited (site no. 5), Kapoorthala Crossing (site no. 8), Khurrum Nagar Crossing (site no. 10) and Mill Road, Aishbagh (site no. 12) showed Catalase Activity 6.50, 6.75, 7.75,
7.50 and 7.75 µ moles H₂O₂ split / 100 mg fr. wt. respectively.

Catalase Activity at all these sites might have shown the variation due to pollution affect on habitat of mosses resulted into effect on growth of mosses. At all these sites effect of variable vehicular activities might be the cause of variation in the Catalase Activity. The catalase activity have been observed in Plagiochasma and Riccia. Udar and Chandra (1960) also examined the enzymatic activity in hepatics.

The data indicate that mosses Octoblepharumalbidum collected from the site Exone College, Campbell Road are highly contaminated with element Zn and Mn, Hindustan Aeronautical Limited, Kapoorthala Crossing with element Zn. Polytechnic campus, Kanpur Road and Yojana Bhawan are highly contaminated for the element Fe. In case of moss samples of Physcomitriumpyriforme collected from the sites Charbagh Railway Station, Thana Ghazipur, Faizabad Road and Doordarshan Kendra are also found to be contaminated for the element Zn. Samples collected from the site R.B. Inter College and Litarcy House, Kanpur Road are contaminated for the element Zn, Mn and Fe and Baradari, Kaiserbagh are for Mn and Zn.

At Garden and monuments sites Residency Ruins, Reserve Forest Picnic and Dilkusha Garden Fe has been found in high value in both the mosses. Kukrail Reserve Forest Picnic Spot is highly contaminated for Zn. Catalase activity have also been found high in samples collected from Garden and monuments sites.

The present study shows no similar pattern of elements accumulation in both moss samples in Group-A and Group-B at all sites. Different concentrations of elements observed at different sites indicates that the vehicular pollution is not only the cause of heavy metal accumulation in mosses but some other commercial activities being done in these areas also cause the contamination in moss sample. Data also indicates that Zn, Mn and Fe accumulation in both moss samples collected from number of Garden and Monument sites were found to be higher as compared to the elements accumulation found in Heavy Traffic sites. Moss samples taken from the garden soil also showed higher catalase activity and were found to be indirectly proportionate to these areas showed that any type of pollution can cause this decrease in metabolic activities of mosses.

Acknowledgements

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References


Determination of Catalase Activity and Estimation of Zn, Mn and Fe

Introduction

Water pollution is a major concern in today’s life. To a large extent, uncontrolled and unregulated industrial activities along with cultural practices are the primary reason for deterioration of fresh water and sea water quality. Not only the aquatic life and environment are under a serious threat, but also these pollutants become part of the food chain leading to serious health effects in the human population (Kaur, 2012; Sahasrabudhe and Pathade, 2011). In various parts of India, idol immersion of deities is practiced as part of culture and tradition. Idols made of Plaster of Paris are painted with different coloured dyes and at the end of festivities, the idols in large numbers are immersed in nearby lakes, rivers and seas (Watkar and Barbate, 2014). Plaster of Paris is not biodegradable while coloured dyes used are industrial grade chemicals. Dyes with dark metallic colours such as green, orange, blue, gold and silver are preferred to make the idols look attractive and sparkling (Kaur, 2012). Paints which are used may also contain toxic and carcinogenic metals like Cu, Zn, Pb, Hg, As, Cr, Fe, Co and Cd (Kaur, 2012). After the immersion of the idols, these foreign chemicals become part of marine environment. Dyes reduce oxygen level and increase acidity level of water, causing death of marine life and disturbing the ecological balance. The effect of these chemicals has been previously looked
by Reddy and Kumar (2001) and Dhote et al. (2001). They showed that non-biodegradable materials, synthetic paints and toxic chemicals used for colouring idols lead to increase in hardness of water and significantly alter the water quality, thus affecting the marine life.

Microorganisms can play an important role in degradation of toxic pollutants. Industrial dyes like Malachite green and Congo red are widely used in textile industry and agriculture, due to easy availability, cost effectiveness and greater efficiency (Cao, 2009). However, these dyes are a serious health hazard to aquatic flora and fauna (Hassanshahian and Mohamadian, 2011). Due to carcinogenic effect of Malachite green, developed countries like USA have banned its use. Previous studies have shown the ability of intestinal bacteria and *Staphylococcus aureus* to degrade Malachite green into colourless, less toxic compounds (Cao, 2009).

Bivalves are a class of marine and freshwater molluscs. They include clams, scallops, mussels and oysters. One of the subspecies, clams, is found in inter-tidal zone of seas and have long been part of diet of coastal population. Clams harbour microorganisms in their gills which are exposed to organic and metal pollutants. The aim of the present study is to isolate and characterize microorganisms present in gills of clams. Clam samples were collected from two different locations: 1-Coast of Mumbai, a large metropolitan area with a history of large textile industry since 1865 (The Cotton Mills, 1997) and where idol immersion during Ganesh Chaturthi festival is practiced in large numbers (Bansal, 2010). 2-Alibaug which is a beach resort about 96 km from Mumbai with a small population and comparatively less pollution. A number of studies have been done on the effect of metals and other pollutants in coastal water, sediment and different tissues of bivalves (Sunita, 1987; Tendulkar, 1996); however, not much attention has been paid on the presence of microorganisms in gills of bivalves and their bioremediation potential. The present study focused on microorganisms isolated from gills of bivalves which were studied by

**Materials and Methods**

**Sample Collection and Isolation of Bacteria**

Bivalve samples were collected from the coast of Mumbai and Alibaug. Gills of bivalves were cut into pieces and added to sterile saline suspension under aseptic condition. The growth medium used for the isolation, activation and further studies of microorganisms was modified halophilic agar (Dundas, 1977; Gibbons, 1969). The medium consisted of casein acid hydrolysate (10 g/l), yeast extract (10 g/l), protease peptone (5 g/l), trisodiumcitrate (3 g/l), potassium chloride (2 g/l), magnesium sulphate (25 g/l), sodium chloride (80 g/l) and agar (20 g/l). Cultures were incubated at 37 ºC for 24 hours.

**Characterization of Microbial Isolates**

Microorganisms were characterized for their Gram-nature, motility and colony characteristics. The isolates were tested for their salt tolerance on halophilic agar medium supplemented with sodium chloride between 8–25%. Biochemical tests were conducted including glucose fermentation, acid production from glycerol, tryptophan utilization, Methyl Red and Voges-Proskauer (MR-VP) test, citrate utilization and reduction of nitrate to nitrite. Further, enzyme tests including catalase, starch hydrolysis, casein hydrolysis, urease and arginine decarboxylase were carried out.

**Effect of Dyes on Strains**

Resistance of the isolated cultures to dyes was qualitatively analysed using gradient plate method. Three different dyes commonly used for colouring Ganesh idols Fluo orange, Fluo green and Fluo blue were sourced from Matrix Speciality, Mumbai. The dye tests were run in parallel with Malachite green. Stock solution
Isolation and characterization of microorganisms

147

of dyes were prepared at 1mg/ml and then serially diluted to 0.1, 0.01 and 0.001 mg/ml. Different growth media was prepared by mixing halophilic agar with 1 ml of the dye solution at different concentrations and plates were prepared with concentration gradient. Cultures were streaked from lower concentration side to higher concentration side and incubated at 37°C; bacterial growth was studied after 24 hours. Continuous growth of the culture on the streaked area across the plate indicated resistance of the isolate to the dye incorporated in the halophilic agar. Cultures with growth only at lower concentration side of the plate were categorized as sensitive, while cultures without growth at both sides were categorized as highly sensitive.

Effect of Dyes on Marine Microorganisms

Sea water sample was collected from Mumbai coast and marine micro-organisms were tested against the three dyes–Fluo orange, Fluo green and Fluo blue along with Malachite green using spread plate method with halophilic agar media. A control plate without dye was also run in parallel. Total number of microorganisms was counted on each plate using colony count method in terms of Colony Forming Unit (CFU).

Tolerance of Strains to Heavy Metal Compounds

The isolated cultures from bivalves were tested for heavy metal tolerance using agar cup well method. Zinc sulphate (ZnSO\textsubscript{4}.7H\textsubscript{2}O), cobalt chloride (CoCl\textsubscript{2}.6H\textsubscript{2}O), copper sulphate (CuSO\textsubscript{4}.5H\textsubscript{2}O) and lead nitrate (Pb(NO\textsubscript{3})\textsubscript{2}) solution were prepared at 10, 20, 40 and 80 mM; all salts were sourced from Loba Chemie, Mumbai. Plates were prepared by bulk seeding 1 ml of isolated culture in molten agar media, followed by adding 50 µl of metal salt solution of various concentrations in the agar cups. The plates were then placed in incubator at 37°C and bacterial growth and Zone of Inhibition (ZOI) were studied after 24 hours. Cultures which showed growth without any inhibition were classified as resistant, cultures which showed zone of inhibition were classified as sensitive and cultures which showed no growth were classified as highly sensitive.

Results and Discussion

Characterization of Isolates

Eight different microbial cultures (five from Mumbai and three from Alibaug) were identified from bivalves samples based on colony shape, size and morphology. The isolates from Mumbai samples were named as MS1, MS3, MS4, MS9, MS11 and isolates from Alibaug as AS1, AS2 and AS3. The isolates from Mumbai exhibited variable colony characteristics, Gram nature and morphology. MS4 and MS11 were found to be Gram-positive and cocci-shaped, MS9 was Gram-positive and rod-shaped and MS1 and MS3 were Gram-negative and rod-shaped. All the isolates from Alibaug were Gram-negative and rod-shaped. Except MS4, MS9 and MS11, all isolates were found to be motile. All the eight cultures showed growth at 8% NaCl concentration but no growth was observed at 15% and 25% NaCl concentration. Hence, the cultures are halo tolerant but not extremophiles. The results of biochemical and enzyme tests for the eight isolates are presented in Table 1.

Effect of Dyes on Isolates

Qualitative results of the dye tests are summarized in Table 2. Photographs of culture after dye tests are shown in Figure 1, 2, 3 and 4 for Fluo orange (0.1 mg/ml), Fluo green (0.1 mg/ml), Fluo blue (0.1 mg/ml) and Malachite green (0.01 mg/ml) respectively. Isolated cultures AS1, AS3, MS3, MS4, MS9 and MS11 were found to be resistant to Fluo orange, Fluo green and Fluo blue at 0.1 mg/ml, while AS2 and MS1 were found to be highly sensitive at 0.01 mg/ml. AS1, AS3, MS9 and MS11 were also found to be resistant to Malachite green at 0.01 mg/ml but were sensitive at 0.1 mg/ml. AS2 and MS1 were found to highly sensitive even at 0.001 mg/ml to Malachite green.
Table 1 Biochemical, enzymatic and other characteristics of the isolated strains.

<table>
<thead>
<tr>
<th>Biochemical Tests</th>
<th>AS1</th>
<th>AS2</th>
<th>AS3</th>
<th>MS1</th>
<th>MS3</th>
<th>MS4</th>
<th>MS9</th>
<th>MS11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphology</td>
<td>rod</td>
<td>rod</td>
<td>rod</td>
<td>rod</td>
<td>cocci</td>
<td>rod</td>
<td>cocci</td>
<td></td>
</tr>
<tr>
<td>Gram staining</td>
<td>–ve</td>
<td>–ve</td>
<td>–ve</td>
<td>–ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Motility</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Acid production from glucose</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Acid from glycerol</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Indole production from tryptophan</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Methyl Red (MR)</td>
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<td>+</td>
<td>+</td>
<td>–</td>
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<td>–</td>
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<tr>
<td>Voges-Proskauer (VP)</td>
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<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Citrate</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Reduction of nitrate to nitrite</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<td>Catalase</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>Starch hydrolysis</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Casein hydrolysis</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Urease</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Arginine Decarboxylase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

1 “–ve”: Gram-negative, “+ve”: Gram-positive
2 “–”: no growth, “+”: growth

Table 2 Sensitivity of the isolates to test dyes using gradient plate method.

<table>
<thead>
<tr>
<th>Metals</th>
<th>Conc. (mg/ml)</th>
<th>AS1</th>
<th>AS2</th>
<th>AS3</th>
<th>MS1</th>
<th>MS3</th>
<th>MS4</th>
<th>MS9</th>
<th>MS11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluo orange</td>
<td>0.001</td>
<td>R</td>
<td>HS</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>R</td>
<td>HS</td>
<td>R</td>
<td>HS</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>R</td>
<td>HS</td>
<td>R</td>
<td>HS</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Fluo green</td>
<td>0.001</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
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<td>R</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>R</td>
<td>HS</td>
<td>R</td>
<td>HS</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>R</td>
<td>HS</td>
<td>R</td>
<td>HS</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Fluo blue</td>
<td>0.001</td>
<td>R</td>
<td>HS</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>R</td>
<td>HS</td>
<td>R</td>
<td>HS</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>R</td>
<td>HS</td>
<td>R</td>
<td>HS</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Malachite green</td>
<td>0.001</td>
<td>R</td>
<td>HS</td>
<td>R</td>
<td>HS</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>R</td>
<td>HS</td>
<td>R</td>
<td>HS</td>
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<td>R</td>
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</tr>
<tr>
<td></td>
<td>0.1</td>
<td>S</td>
<td>HS</td>
<td>S</td>
<td>HS</td>
<td>HS</td>
<td>HS</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

1R: Resistant, S: Sensitive, HS: Highly Sensitive
Fig. 1 Resistance of microbial isolates to Fluo orange at 0.1 mg/ml. Growth was observed at both low and high concentration side for all isolates except AS2 and MS1.

Fig. 2. Resistance of microbial isolates to Fluo green at 0.1 mg/ml. Growth was observed at both low and high concentration side for all isolates except AS2 and MS1.

Fig. 3 Resistance of microbial isolates to Fluo blue at 0.1 mg/ml. Growth was observed at both low and high concentration side for all isolates except AS2 and MS1.

Fig. 4 Resistance of microbial isolates to Malachite green at 0.01 mg/ml. Growth was observed at both low and high concentration side for AS1, AS3, MS9 and MS11.
**Effect of Dyes on Marine Microorganisms**

Figure 5 presents the number of microorganisms grown on marine sample plates with and without dye. Highest number of colonies (296 CFU/ml) was seen in sample without any dye. The plates which had the test dyes incorporated showed significantly less number of colonies as compared to the control. 39 microbial colonies were observed on the plate with Fluo orange and 108 colonies on the plate with Fluo green. No growth was observed in plates containing Fluo blue and Malachite green. These results suggest that the dyes have a strong toxic effect on microorganisms found in sea water.

**Effect of Heavy Metals on Isolates**

Resistance of isolates from bivalves to heavy metal compounds is presented in Table 3. Among the eight isolates, MS11 showed the highest tolerance – up to 80 mM of ZnSO₄, CuSO₄, CoCl₂ and PbNO₃. For the other isolates the resistance was variable. MS4 was

**Table 3** Sensitivity of the isolates to heavy metal compounds using agar cup method.

<table>
<thead>
<tr>
<th>Metals</th>
<th>Conc. (mM)</th>
<th>AS1</th>
<th>AS2</th>
<th>AS3</th>
<th>MS1</th>
<th>MS3</th>
<th>MS4</th>
<th>MS9</th>
<th>MS11</th>
</tr>
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<tbody>
<tr>
<td>ZnSO₄</td>
<td>10</td>
<td>HS</td>
<td>HS</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>HS</td>
<td>HS</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>HS</td>
<td>HS</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>HS</td>
<td>HS</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>HS</td>
<td>HS</td>
<td>S (22 mm)²</td>
<td>S (18 mm)</td>
<td>S (19 mm)</td>
<td>HS</td>
<td>HS</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>HS</td>
<td>HS</td>
<td>S (21 mm)</td>
<td>S (20 mm)</td>
<td>HS</td>
<td>HS</td>
<td>R</td>
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<tr>
<td>CuSO₄</td>
<td>10</td>
<td>HS</td>
<td>HS</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>HS</td>
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<td></td>
<td>20</td>
<td>HS</td>
<td>HS</td>
<td>S (19 mm)</td>
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<td>40</td>
<td>HS</td>
<td>HS</td>
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<td>S (15 mm)</td>
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<tr>
<td></td>
<td>80</td>
<td>HS</td>
<td>HS</td>
<td>HS</td>
<td>HS</td>
<td>HS</td>
<td>HS</td>
<td>HS</td>
<td>S (18 mm)</td>
</tr>
<tr>
<td>CoCl₂</td>
<td>10</td>
<td>HS</td>
<td>HS</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>HS</td>
<td>HS</td>
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<td>HS</td>
<td>HS</td>
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<td></td>
<td>40</td>
<td>HS</td>
<td>HS</td>
<td>S (20 mm)</td>
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<td>HS</td>
<td>HS</td>
<td>S (25 mm)</td>
</tr>
<tr>
<td>PbNO₃</td>
<td>10</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>HS</td>
<td>R</td>
<td>HS</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>HS</td>
<td>R</td>
<td>HS</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>S (17 mm)</td>
<td>R</td>
<td>R</td>
<td>HS</td>
<td>HS</td>
<td>HS</td>
<td>R</td>
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</tr>
<tr>
<td></td>
<td>80</td>
<td>S (19 mm)</td>
<td>S (20 mm)</td>
<td>R</td>
<td>HS</td>
<td>HS</td>
<td>HS</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>

¹ R: Resistant, S: Sensitive, HS: Highly Sensitive

²Size of Zone of Inhibition
found to be the most sensitive – no growth was observed with ZnSO₄, CuSO₄, CoCl₂ and Pb(NO₃)₂ even at 10 mM concentration.

In the present paper, we studied Gram-nature and phenotypic character of bacteria isolated from gills of clams. The current results are comparable with the published work (Schweinemann and Felbeck, 1985; Baldi et al., 2013; Espinosa et al., 2013). Bivalves carry large number of Gram-negative and Gram-positive bacteria within their gills, siphons and hepatopancreas (Baldi et al., 2013). Dando et al. (1985) showed that Myrteaspiniferarclacl collected from Ypsesund, Norway carry large number of Gram-negative, sulphuroxidizing bacteria. Other strains of clam like Lucinid collected from inshore lagoon in Bermuda contain chemosynthetic bacteria in their gills (Schweinemanns and Felbeck, 1985).

Eight cultures were isolated in the current study – five from coast of Mumbai and three from coast of Alibaug. It is interesting to note that not only the cultures found in the two samples (Mumbai, Alibaug) were different but also their resistance to dyes and heavy metals were different. It indicates that the local marine environment is affecting the microflora. Compared to Alibaug, the marine environment in Mumbai is more polluted. We have obtained more resistant strains of microorganisms from Mumbai samples indicating survival and growth of microorganisms which have adapted to the local environment. Among the eight isolates, MS11 was found to be most resistant to the dyes (Fluo orange, Fluo green, Fluo blue, Malachite green) and metals (Zn, Cu, Co, Pb) used in this study. MS11 isolate was found in Mumbai sample and is Gram-positive and cocci-shaped. Detailed biochemical analysis (Table 4) identified MS11 as Staphylococcus arlettae. Schleifer et al. (1984) isolated Staphylococcus arlettae from skin of mammals and bird and found it to be Gram-positive, non-motile and cocci-shaped bacteria, which matches with the current results.

Microbial biodegradation is a natural and environment friendly process, compared to chemical processes, to convert toxic compounds into non-toxic end products (Saratale et al., 2011). Microorganisms use carbon and nitrogen as source of energy and lead to biodegradation of synthetic dyes. Klebsiella and Bacillus have been found to use glucose, starch, sucrose and yeast extract as carbon source and peptone as nitrogen source resulting in degradation of Turquoise blue and Malachite green dyes (Joshi et al., 2013; Ramezani et al., 2013). Cultures isolated in the current work use yeast extract and peptone from halophilic agar as nitrogen source and casein and beef extract as carbon source.

### Table 4 Detailed biochemical analysis for identification of MS11.

<table>
<thead>
<tr>
<th>Test</th>
<th>AMY</th>
<th>PIPLC</th>
<th>dXYL</th>
<th>ADH1</th>
<th>BGAL</th>
<th>AGLU</th>
<th>APPA</th>
<th>CDEX</th>
<th>AspA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Result</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>(+)</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Test</td>
<td>BGAR</td>
<td>AMAN</td>
<td>PHOS</td>
<td>LeuA</td>
<td>ProA</td>
<td>BGURr</td>
<td>AGAL</td>
<td>PyrA</td>
<td>BGUR</td>
</tr>
<tr>
<td>Result</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Test</td>
<td>AlaA</td>
<td>TyrA</td>
<td>dSOR</td>
<td>URE</td>
<td>POLYB</td>
<td>dGAL</td>
<td>dRIB</td>
<td>ILATk</td>
<td>LAC</td>
</tr>
<tr>
<td>Result</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Test</td>
<td>NAG</td>
<td>dMAL</td>
<td>BACI</td>
<td>NOVO</td>
<td>NC6.5</td>
<td>dMAN</td>
<td>dMNE</td>
<td>MBdG</td>
<td>PUL</td>
</tr>
<tr>
<td>Result</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Test</td>
<td>dRAF</td>
<td>O129R</td>
<td>SAL</td>
<td>SAC</td>
<td>dTRE</td>
<td>ADH2s</td>
<td>OPTO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Result</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

151
Elisangela et al. (2009) isolated *Staphylococcus arlettae* from activated sludge produced by textile industry in Brazil and showed that the isolate has the ability to decolourize and degrade azo dyes using a sequential microaerophilic/aerobic process. *Staphylococcus aureus* has been demonstrated to degrade Malachite green rapidly without the detection of leucomalachite green (Cao, 2009). With respect to heavy metals, Kumar et al. (2013) studied the resistance of *Staphylococcus sp.* and showed their potential to remove chromium and lead from solid waste. *Staphylococcus arlettae* isolated from arsenic contaminated site of West Bengal, India has the ability to remove arsenic from liquid media and to promote plant growth (Srivastava et al., 2013). In the present study, *Staphylococcus arlettae* was isolated from gills of bivalves collected from coast of Mumbai. Halotolerance and resistance to toxic chemicals suggest that the isolated bacteria *Staphylococcus arlettae* could be useful in bioremediation processes in marine environments.

**References**


Isolation and characterization of microorganisms


Introduction
Oral cancer is the most common form of cancer and cancer-related deaths among Indian males. India accounts for 86% of the world’s oral cancer cases, according to the study conducted in February 2011 by the National Institute of Public Health. Ninety percent of these cases are due to chewing tobacco, unlike in the west countries where smoking is the main reason. Furthermore, globally oral cancer accounts for 267,000 new cases and 128,000 deaths of which two-third cases are contributed by the developing countries (Parkin et al., 2002). Nearly, 8 million deaths annually are expected by 2030 due to tobacco consumption around the world (WHO, 2014). More than 50% oral cancer occur due to consumption of smokeless tobacco in India (Boffetta et al., 2008) and it has been shown to associate with cancers of the lip, oral cavity, pharynx, digestive, respiratory and intrathoracic organs (Pednekar et al., 2011). Estrogen and testosterone both is an important hormone for male and female. Estrogen has an important role in the development of breast cancer, ovarian cancer etc. There are very few studies available which describe the increased oral cancer incidence and level of estrogen in male oral cancer patients and use of smokeless tobacco consumption among the same type of cancer patients. Therefore, the present study has been aimed to find out the prevalence of oral cancer due to consumption of smokeless tobacco, sub-site distribution and level of estrogen in the oral cancer patients in Bihar. In the present study 126 patients suffering from oral cancer were selected randomly. The background data obtained were categorized into sex-wise as well as age-wise oral cancer incidence, carcinoma site, frequency of oral cancer and the type of tobacco addiction among the surveyed oral cancer patients. Estrogen level in male oral cancer patients was assessed by ELISA-kit method. Addition of khaini among all of the smoke and smokeless tobacco consuming persons in Bihar was found higher. Prevalence of oral cancer was four times higher in male patients than the female and the level of estrogen hormone in male oral cancer patients were found elevated. In conclusion, high oral cancer incidence has been observed among people of Bihar. Most popular form of smokeless tobacco is khaini mainly used by illiterate people. Elevated level of estrogen hormone in male oral cancer patients may be associated with the increased incidence of oral cancer among people of Bihar. It may further provide some evidence to understand the whole mechanism of oral cancer.

Keywords: Oral cancer, Estrogen, Smokeless tobacco, ELISA
to the DNA which led to DNA damage or cell division of the cells.

Use of smokeless tobacco in some part of the India is acceptable mainly in Eastern, Northern and North-eastern parts of the country. Only 20% of the tobacco consumed by weight is consumed as cigarettes, 40% consumed as bidi and the rest in smokeless form (WHO 1997). In India, at least 800,000 deaths every year are related to tobacco use, out of which 700,000 are related to smoking (Reddy and Gupta, 2004). The prevalence of use of tobacco was reported to be much higher in the North-eastern states compared to other parts of India (IIPS and ORC 2000). Bihar is situated in the eastern region of India between 83°-30’ to 88°-00’ longitude. Geographical area of Bihar is of 38,202 sq mi (98,940 km²) and it is a 3rd largest state by population. Due to cheap labour cost mainly, Bihar is the 6th largest tobacco producing state in India. 90% population of Bihar depends upon agriculture. The land is fertile and produces several types of agricultural products including tobacco. Tobacco plays a significant role in the development of state’s economy. The tobacco use is very prominent in Bihar. According to Global Adult Tobacco Survey (2009-10), 26% Indians aged 15 and above use smokeless tobacco products. In India, khaini, gutka, betel quid with tobacco, powdered tobacco snuff and other smokeless tobacco were used by 11.6%, 8.2%, 6.2%, 4.7% and 4.4% respectively (IIPS, 2009). There are very few studies available which correlate the high incidence of oral cancer with high level of estrogen in male oral cancer patients and use of smokeless tobacco consumption among the same type of cancer patients. Therefore, the present study has been designed to find out the prevalence of oral cancer due to consumption of smokeless tobacco, sub site distribution and level of estrogen in the oral cancer patients in Bihar.

Results and Discussion

Over the past several centuries, tobacco has been used in India. People use tobacco in two forms mainly i.e. smokeless or smoking tobacco. Smoked tobacco includes cigarette, bidi, cigars etc. and smokeless tobacco contains khaini, gutka, pan with tobacco, pan masala etc. People with lower economic status usually use these form of smokeless tobacco. It has been observed that most susceptible period of tobacco use is early childhood in India (WHO 2012). According to WHO, nearly 250 million adults consume smokeless tobacco in 11 countries of South-East Asia region which contributes 90% of total global smokeless tobacco users (WHO 2014). Use of smokeless tobacco has been associated with the increased risk of oral cancer. Khaini is the most common form of smokeless tobacco used among less educated or illiterate people in India. At the beginning of the study, we found that oral cancer is the most common in the man of middle aged group between 50–60 years. Almost similar finding has been seen in

Materials and Methods

This is a Hospital record data based study of the Oral cancer patients, visiting Mahavir Cancer Sansthan Hospital from different districts of Bihar. In this study 126 patients suffering from oral cancer were selected randomly. The data obtained were categorized into sex-wise as well as age-wise oral cancer incidence, carcinoma site, frequency of oral cancer and the type of tobacco addiction among the surveyed oral cancer patients.

Tobacco use was broadly classified into four categories; smokeless, smoker, mixed (smokeless tobacco users as well as smokers) and non users. Smokeless tobacco uses include Gutka (an industrially manufactured tobacco product, containing areca nut, tobacco and other ingredients), Khaini (tobacco-lime mixtures), Pan and Betel quid (containing fresh betel leaf, lime, catechu, areca nut and tobacco,) which are very common in Bihar and other parts of the India. Estimation of estrogen in serum was done in 18 male oral cancer patients by the ELISA kit method.
India as well as in different parts of the world (Murthy et al., 2011; Petit, 2003). Smokeless form of tobacco use was predominant, and majority of the oral precancers were detected in employees using the smokeless form in a recent study (Ulap et al., 2011).

In the present study, out of 126 oral cancer patients, 101 male and 25 female oral cancer patients were found. Figure 1 shows that prevalence of oral cancer in male patients. It was found nearly four times higher than in female patients. Figure 2 shows the co-relation between incidence of oral cancer and age-group of patients. Among the age groups, the highest oral cancer incidence was found among the group of 50–60 years of oral cancer patients.

Table 1 shows the site of carcinoma and frequency of oral cancer incidence in people of Bihar, India. Lower gingivo labial mucosa and tongue were the most prevalent site of oral cancer patients having the highest percentage of incidence 25.21 and 23.53 respectively.

In Figure 3, pie-chart indicates prevalence of khaini addiction (50%) among all smoke and smokeless tobacco consuming persons in Bihar, India. This result is consistent with some other literatures available in India. Furthermore, in addition to smoke and smokeless tobacco addiction, Pan and Gutka also contributed a significant role in the development of oral cancer.

**Table 1** Carcinoma site and frequency of incidence in Bihar, India.

<table>
<thead>
<tr>
<th>Site</th>
<th>Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower gingiva-labial mucosa (Lower alveolus)</td>
<td>25.21</td>
</tr>
<tr>
<td>Tongue</td>
<td>23.53</td>
</tr>
<tr>
<td>Buccal mucosa</td>
<td>15.12</td>
</tr>
<tr>
<td>Oesophagus</td>
<td>9.24</td>
</tr>
<tr>
<td>Tonsil</td>
<td>8.41</td>
</tr>
<tr>
<td>Lower lip</td>
<td>4.21</td>
</tr>
<tr>
<td>Upper labial mucosa (Upper alveolus)</td>
<td>3.36</td>
</tr>
<tr>
<td>Larynx</td>
<td>2.52</td>
</tr>
<tr>
<td>Pharynx</td>
<td>0.84</td>
</tr>
<tr>
<td>Others</td>
<td>4.20</td>
</tr>
</tbody>
</table>

Fig. 1 Sex-wise incidence of oral cancer in Bihar.

**Fig. 2** Age wise incidence of oral cancer in Bihar.

**Fig. 3** Tobacco addiction among the surveyed oral cancer patients in Bihar, India.
Figure 4 shows the elevated level of estrogen hormone in male and female oral cancer patients. Estimation of estrogen hormone was done in 18 male oral cancer patients. Maximum level was found as 81.5 pg/ml. Out of 18 male oral cancer patients, 14 patients have the estrogen concentration greater than 40 pg/ml and minimum concentration was detected as 15.5 pg/ml in the male oral cancer patients. Estrogen has been a suspected carcinogen for the development of cancer. Some studies confirmed the positive association of elevated estrogen level with breast, ovary and endometrium cancer.

Our hospital based data provide the evidence that khaini is most prevalent risk factor among the smokeless tobacco using oral cancer patients and it is also associated with the increased risk of oral cancer with the two sites Lower gingivo-labial mucosa and tongue mainly. Government of India has banned the use of tobacco in tooth powder and toothpaste in 1992 and the Supreme Court of India upheld the decision of Government in 1997. These products however, continue to be available openly in the market (Sinha et al., 2003). People are using these products therefore, it is the need of time to make people aware of the adverse effects of tobacco. Further, a very significant result has been found in the present study. Elevated estrogen levels in male oral cancer patients have been observed. The exact mechanism behind this is still unclear but it may be due to conversion of testosterone to the estrogen by the enzyme aromatase. It has been shown that level of testosterone hormone in male circulation is greater than the circulation of postmenopausal women. Furthermore, testosterone level in male is similar to the $K_m$ of aromatase, hence conversion of testosterone to estrogen has been occurred in extragonadal sites resulting the elevated level of estrogen in male (Simpson et al., 1999).

In conclusion, prevalence of oral cancer is very high in males in Bihar. Most popular form of smokeless tobacco is khaini which is used by illiterate people mainly. High oral cancer incidence has been observed among these people in Bihar. Elevated level of estrogen hormone in male oral cancer patients may be associated with the increased incidence of oral cancer among people of Bihar. It may further provide some evidence to understand the whole mechanism of oral cancer.

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Study on Growth and Survival of Giant Freshwater Prawn, *Macrobrachium rosenbergii*, in Tarai Agroclimatic Regime of Uttarakhand

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G. B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand), India – 263145

Abstract: Giant freshwater prawn, *Macrobrachium rosenbergii*, is a high value cultured aquatic species, commonly called as 'scampi' in trade circles. An experiment was conducted at Instructional Fish Farm of the College of Fisheries, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar in four rectangular earthen ponds each of the size of 0.033 ha in tarai agroclimatic regime of Uttarakhand. The stocking of PL 10 was done @ 60000/ha. The pre-stocking management of the ponds included dewatering, drying, liming, organic manuring and watering of ponds. The water quality parameters were monitored regularly. The larvae were fed with starter feed upto 60 days of rearing when the larvae attained average weight of around 4g. Thereafter, juveniles were fed with grower feed @ 5% body weight twice a day in two equal splitted doses. The sampling of the prawn was done at fortnightly intervals. The average weight of the prawn at the time of stocking was 0.04g while after 180 days of culture at the time of harvesting the prawn attained average weight of 25.37 ± 14.21g with 76.95% survival. The average absolute growth rate of 0.140 g/day and specific growth rate of 1.55% was achieved during the experimental period. The calculated gross production of 1183.159 kg prawn/ha/crop of 180 days was obtained. The result of the study inferred that the culture of giant freshwater prawn, *M. rosenbergii*, is feasible in tarai agroclimatic regime of Uttarakhand. The fish farmers of the area can earn their sustainable livelihood through scampi farming.

Keywords: *Macrobrachium rosenbergii*, Growth, Survival, Production.

Introduction

Freshwater prawn is a highly priced commodity among the freshwater aquaculture species and share a large amount of total aquaculture production. About 200 species of *Macrobrachium* are found all over the world, of which 30 species abound Indian waters. The most important culturable species of genus *Macrobrachium* is *rosenbergii*. This prawn is commonly called as scampi in trade circles. In nursery ponds, stocking density varies from 200 to 400/m². However, farmers generally adopt a moderate stocking density of 50 to 100/m² without any aeration and water circulation. After 30–40 days of culture in nursery ponds the juveniles are transferred to grow-out ponds with varying stocking density of 5-10/m². Most farmers, stock at a moderate stocking density of 6/m² under semi-intensive type of monoculture system. *Artemia* is most preferred food of prawn larvae. Formulated pelleted feeds are usually given in grow-out culture twice daily at the rate of 3–5% of the biomass in feeding trays. Recently, farming of *M. rosenbergii* had been concentrated in maritime states due to ready availability of stocking material i.e. post larvae. But, it has also got entry into inland areas where brackish or saline soil or water is available. Tarai region is a unique agro-climatic zone where underground water level is very high and climate is hot and humid. There is no record of giant freshwater prawn farming in this region. In India, culture of giant freshwater prawn at stocking densities of 30000–50000/
ha has shown production levels of 1.0–1.5 t/ha in a culture period of 6-8 months (Ayyappan, 2007). In the first feasibility study of rearing *M. rosenbergii* in South African inland ponds in 1981, promising yield of 1.2 t/ha was obtained in 172 days, with survival at 76% and a mean weight of nearly 29g (Taylor *et al.*, 1992). Culture of the giant freshwater prawn, *Macrobrachium rosenbergii*, has drawn the attention of aqua-culturists and fish farmers in many parts of the world because of its fast growth, adaptability to the poly-culture environment and artificial feed, greater disease resistance than its marine counterparts and high market demand. The popularity of this species has resulted in receiving the increasing attention of researchers in search for innovative culture technology (New, 2002). Various types of shelters/hideouts like PVC pipes, earthen pipes, aquatic weeds and used tyres have been used in prawn culture ponds. The present investigation is aimed to assess culture feasibility of *Macrobrachium rosenbergii* in tarai region of Uttarakhand, including study on survival and growth performance of scampi under semi-intensive monoculture in earthen ponds.

**Materials and Methods**

The experiment was carried out for a period of 180 days from 28th May to 25th November, 2011 at the Instructional Fish Farm of the College of Fisheries, G.B. Pant University of Agriculture and Technology, Pantnagar. The climatic condition of Pantnagar is humid, sub tropical and is characterized by hot dry summer and extremely cold conditions. The study was carried out in the four outdoor earthen rectangular ponds (P1, P2, P3 and P4) of size 23m x 14.35m x 1.2m. Supply of water was ensured through a tube well oxygenated water to maintain average water depth of 0.80 ± 0.10 m throughout the experimental period. The ponds were dewatered, dried and liming was done @ 500kg/ha followed by initial manuring @ 2500kg raw cattle dung /ha. The used tyres and broken PVC pipes were used as hide-outs as shelter for prawns. Healthy stock of *M. rosenbergii* was procured from CIFE-sub centre, Rohtak, Haryana. The PL 10 was packed in oxygen filled polythene bags having 1000 larvae. The sealed polythene bags were kept in carton boxes. Transportation was done through road. The seed was properly acclimatized and stocked at the rate of 60000/ha (2000/experimental pond of 330 m²). Supplementary feeding was done with starter feed (30% protein) and grower feed (26% protein) @ 5% body weight of the prawn twice a day in the two equal splitted doses during late morning and late afternoon. Prawns were sampled fortnightly for growth and health monitoring. The analysis of pond sediment viz. soil pH, soil organic carbon, total nitrogen, available phosphorus, available potassium and soil texture was done. The water quality parameters viz. water- temperature, pH, dissolved oxygen, free CO2 and total alkalinity in ponds were monitored regularly. The quantitative and qualitative analysis of plankton was also done. The average weight of prawn was measured at fortnightly intervals by using hide-outs or by netting out prawn in equal numbers from each pond and weight of each prawn was measured separately. Absolute growth rate (g/day) was evaluated by using the following formula given by Wood *et al.* (1983).

\[
\text{Absolute growth rate} = \frac{W_1 - W_0}{T}
\]

Where, \(W_1 = \text{Prawn weight at the end of study, g; } W_0 = \text{Prawn weight at start of study, g and } T = \text{Time interval in days}\)

On the basis of collecting average weight data, specific growth rate (SGR) was calculated by using following formula-

\[
\text{SGR} = \frac{\ln \log (\text{final weight}) - \ln \log (\text{initial weight})}{\text{Time (days)}} \times 100
\]

Survival rate of the prawn was also calculated by using following formula

\[
\text{Survival rate } (%) = \frac{\text{Nos. recovered}}{\text{Nos. stocked}} \times 100
\]
The gross production of *M. rosenbergii* from experimental ponds was estimated at the end of study in terms of kg/ha/crop of 180 days. Data collected from the experiment were subjected to one way analysis of variance (ANOVA) test using the statistical package (STPR 43).

**Results and Discussion**

Analysis of variance showed that the difference in water quality among experimental ponds was non-significant and within acceptable limits for culture of giant freshwater prawn. The plankton population consisted of Chlorophyceae, Bacillariophyceae, Cyanophyceae and Euglinophyceae from phytoplankton whereas Rotifers, Copepods and Cladocerans from zooplankton. The details of minimum, maximum and average weight of prawns at 15 days interval are included in Table 1. The similar result was obtained by Davassi (2011) at the end of 6 months culture where he found final average weight of 28.06g, 32.02g, 35.50g, 25.05g and 22.00g when the initial weight of the prawn was 1.00g, 1.03g, 0.90g, 0.94g and 0.97g respectively on different diets. In the culture period of 180 days, Sarkar *et al.* (1998) recorded final weight of 68 ± 4.9 g, 65 ± 4.0 g and 78 ± 3.9 g respectively in feed, cow dung and cow dung + feed management regimes in earthen ponds.

The details of absolute growth rate of giant freshwater prawn evaluated at every 15 days intervals are presented in Table 2. The value of specific growth rate is used to compare growth on daily basis. The SGR was estimated at 15 days intervals and presented in Table 3. Gupta *et al.*, (2011) observed the specific growth rate of scampi as 1.84 ± 0.46 to 2.24 ± 0.56 on different diet composition. Felix and Jayaseelan (2006) found the highest SGR of post larvae of *M. rosenbergii* on 40% protein feed (6.635 ± 0.095) and lowest on 25% protein feed (5.148 ± 0.072) when they fed the scampi with feeds containing different protein levels (15–45% protein feed). Davassi (2011) estimated SGR ranging from 1.73 to 2.04 on different diets.

In the present study the maximum survival was observed in pond P1 (Table 4). Average survival was observed as 76.95%. Davassi (2011) found very low survival rate i.e. 13.33-40.00% on different diets. In the study on *Macrobrachium*
Singh and Chauhan

Fig. 1 Survival rate of giant freshwater prawn in experimental ponds.

Table 2 Absolute growth rate (g/day) of Macrobrachium rosenbergii in different Experimental ponds.

<table>
<thead>
<tr>
<th>Days</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>Pooled</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 days</td>
<td>0.0180</td>
<td>0.0193</td>
<td>0.0206</td>
<td>0.0213</td>
<td>0.0200</td>
</tr>
<tr>
<td>30 days</td>
<td>0.0206</td>
<td>0.0233</td>
<td>0.0266</td>
<td>0.0266</td>
<td>0.0240</td>
</tr>
<tr>
<td>45 days</td>
<td>0.0233</td>
<td>0.0266</td>
<td>0.0253</td>
<td>0.0266</td>
<td>0.0253</td>
</tr>
<tr>
<td>60 days</td>
<td>0.1000</td>
<td>0.0973</td>
<td>0.1020</td>
<td>0.1220</td>
<td>0.1053</td>
</tr>
<tr>
<td>75 days</td>
<td>0.1140</td>
<td>0.1160</td>
<td>0.1106</td>
<td>0.1040</td>
<td>0.1113</td>
</tr>
<tr>
<td>90 days</td>
<td>0.2333</td>
<td>0.2346</td>
<td>0.2340</td>
<td>0.2233</td>
<td>0.2313</td>
</tr>
<tr>
<td>105 days</td>
<td>0.1580</td>
<td>0.3386</td>
<td>0.3546</td>
<td>0.3866</td>
<td>0.3093</td>
</tr>
<tr>
<td>120 days</td>
<td>0.1520</td>
<td>0.1900</td>
<td>0.2673</td>
<td>0.2820</td>
<td>0.2233</td>
</tr>
<tr>
<td>135 days</td>
<td>0.0833</td>
<td>0.1873</td>
<td>0.1360</td>
<td>0.2580</td>
<td>0.1660</td>
</tr>
<tr>
<td>150 days</td>
<td>0.0873</td>
<td>0.2146</td>
<td>0.2166</td>
<td>0.2220</td>
<td>0.1853</td>
</tr>
<tr>
<td>165 days</td>
<td>0.1020</td>
<td>0.0753</td>
<td>0.2240</td>
<td>0.2026</td>
<td>0.1506</td>
</tr>
<tr>
<td>180 days</td>
<td>0.2080</td>
<td>0.0740</td>
<td>0.1533</td>
<td>0.1546</td>
<td>0.1366</td>
</tr>
</tbody>
</table>

Table 3 Specific growth rate (% bw/d) of Macrobrachium rosenbergii in different Experimental ponds.

<table>
<thead>
<tr>
<th>Days</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>Pooled</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 days</td>
<td>5.93</td>
<td>6.11</td>
<td>6.28</td>
<td>6.36</td>
<td>6.20</td>
</tr>
<tr>
<td>30 days</td>
<td>2.01</td>
<td>2.09</td>
<td>2.21</td>
<td>2.13</td>
<td>2.09</td>
</tr>
<tr>
<td>45 days</td>
<td>1.30</td>
<td>1.34</td>
<td>1.19</td>
<td>1.24</td>
<td>1.26</td>
</tr>
<tr>
<td>60 days</td>
<td>2.71</td>
<td>2.48</td>
<td>2.48</td>
<td>2.76</td>
<td>2.61</td>
</tr>
<tr>
<td>75 days</td>
<td>1.52</td>
<td>1.51</td>
<td>1.40</td>
<td>1.22</td>
<td>1.41</td>
</tr>
<tr>
<td>90 days</td>
<td>1.76</td>
<td>1.74</td>
<td>1.72</td>
<td>1.60</td>
<td>1.70</td>
</tr>
<tr>
<td>105 days</td>
<td>0.78</td>
<td>1.45</td>
<td>1.50</td>
<td>1.60</td>
<td>1.35</td>
</tr>
<tr>
<td>120 days</td>
<td>0.59</td>
<td>0.58</td>
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<td>0.78</td>
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<tr>
<td>135 days</td>
<td>0.28</td>
<td>0.48</td>
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<td>0.57</td>
<td>0.42</td>
</tr>
<tr>
<td>150 days</td>
<td>0.27</td>
<td>0.46</td>
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<td>0.41</td>
<td>0.41</td>
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<tr>
<td>165 days</td>
<td>0.28</td>
<td>0.15</td>
<td>0.40</td>
<td>0.33</td>
<td>0.30</td>
</tr>
<tr>
<td>180 days</td>
<td>0.50</td>
<td>0.14</td>
<td>0.25</td>
<td>0.23</td>
<td>0.24</td>
</tr>
</tbody>
</table>

malcolmsonii juveniles fed with different feeds for 60 days, Soundarapanadian et al. (2002) observed survival ranging from 92.1 ± 0.6 to 95.6 ± 0.9%. Gupta et al. (2011) found survival of scampi varying from 63.80 ± 7.30 to 77.70 ± 10.20% that is more or less similar to the present experiment. Sarkar et al., (1998) found the survival of 80 ± 2.7 to 84 ± 3.0% under three different management regimes.

With 6156 prawns recovered (76.95% survival) each averaging weight of 25.37g, a gross production of 156.177 Kg was obtained from combined area of 1320 m² of four experimental ponds. This equals calculated gross production of 1183.159 kg prawn/ha/crop of 6 months. Fatema et al. (2011) recorded production of giant freshwater prawn ranging from 529.32 to 715.19 Kg/ha/crop of 120 days. Nagarathinam et al. (2000) obtained estimated production of M. rosenbergii in monoculture grow-out ponds as 984.34 and 1662.26 Kg/ha/crop of 180 days for two different stocking densities.

It is inferred from the results that culture of freshwater prawn, M. rosenbergii, is feasible in tarai agroclimatic region of Uttarakhand. Thus, the fish farmers of the area may adopt culture practice of scampi to earn their sustainable livelihood.
Study on growth and survival of giant freshwater prawn

Acknowledgement
The authors are thankful to Dean, College of Fisheries, G. B. Pant University of Agriculture and Technology, Pantnagar for providing facilities to carry out present work.

References
Effect of Short Term Temperature on Physiological Body Indices of Two Estuarine Venerid Clams *Katelysiaopima* and *Meretrixmeretrix* (Mollusca: Bivalvia)

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Abstract: In the present investigation, the physiological body indices like hepatopancreas index (HI), gonadal index (GI) and condition index (CI) of two estuarine clams (*Katelysiaopima* and *Meretrixmeretrix*) were estimated after exposing to three experimental temperatures (20, 25 and 35°C). In experimental design, both the clam species were exposed to selected temperatures for 192 hours (8 days) and during the exposures the body indices were determined at each 48 hours of interval. After exposing to experimental temperatures, in both the clams, all body indices like HI, GI and CI were considerably declined (p<0.001) after 192 hours of exposure. The highest decline in body indices has been noted particularly at high temperature (35°C) followed by low temperature like 20°C and 25°C. On the basis of obtained results, it confirms that, the gonadal index was closely related to hepatopancreas index and increment of condition index is directly associated with gonadal development. Relatively, *M. meretrix* species was more sensitive towards temperature change and it has been evident from the maximum decline in body indices than *K. opima*. Hence, the quantitative analysis of body indices among clams might be useful in assessing the probable effect of short-term change in water temperature on physiological process.

Keywords: Physiological body indices, Clams, Hepatopancreas, Temperature.

**Introduction**

Coastal zones like estuaries, creeks, backwaters and lagoons are ecologically as well as economically important and are among those areas which strongly affected by global climate change (Laning *et al.*, 2010). The environmental changes, affects the metabolic activities and biological performance of an organism, however the mechanism of action of environmental factors and their risks on marine ectothermal organisms that are not yet fully understood (Melzner *et al.*, 2009; Portner, 2010).

Among environmental factors, temperature plays the crucial role in all facets of organisms life (Gillooly *et al.*, 2001). To measure the environmental or temperature consequences, the behaviour and physiological alterations are considered as functional attributes to identify the stress. Generally, stress conditions cause energy limitation, which reduces the energy investment in to production hence, it will have the direct impact on the fitness of an organism (Laning *et al.*, 2010).

Physiological fitness of an organism under various environmental stressor particularly temperature can be ascertained by assessing the physiological body condition indices like hepatopancreas index, gonad index and condition index. According to the literature, hepatopancreas and gonad are the major organs of invertebrate, which stored the reserve nutrients and these reserves are mobilized according to seasonal cycle (Galap *et al.*, 1997; Lomovasky *et al.*, 2004) or reserved
nutrients of hepatopancreas are utilized for gonadal development (Sokolowicz et al., 2006). Condition index has been employed by various authors to determine the physiological activity like reproduction and growth (Li et al., 2009; Mladineo et al., 2007; Celik et al., 2012; Li et al., 2011). Literature reported that, the condition index among bivalve species fluctuates by change in water temperature, food availability and season (Flores-Vergara et al., 2004; Delgado et al., 2004; Ojea et al., 2004; Orban et al., 2006).

Earlier, few authors have reported the effect of temperature on the condition index and gonad maturity of bivalves (Newell et al., 1982; Mac Donald and Thompson, 1985; Martinez and Perez, 2003). In recent, Suja and Muthiah, (2009) studied the synergistic effect of starvation and temperature on hepatopancreas somatic index, gonad somatic index and condition index of Marcia opima from Tuticorin Bay. Matias et al., (2008) documented the effect of temperature on condition index of Ruditapes decussates.

This attempt has been ascertained to detect the effect of short-term exposure of temperature on hepatopancreas index, gonad index and condition index of two commercially important venerid clams (Katelysiaopima and Meretrixmeretrix) of Bhatye estuary, Ratnagiri coast. This study eventually evaluates the physiological fitness or the probable effects of both decline and elevated temperatures on sessile organism. Present study might be useful in understanding the nutritional value of commercially important clams at a different regime of temperature and this information may benefit from bivalve aquaculture as well as fishery point of view.

**Materials and Methods**

**Animal Collection and Maintenance**

In this study, experimental clams like Katelysiaopima (Gmelin, 1791) and Meretrix meretrix (Linnaeus, 1758) were collected from Bhatye estuary during low tide with the help of local fishers during April 2010. The collected clams brought to the laboratory, carefully cleaned to remove the adhered fine sediments. All clams were segregated according to their shell length, only mature/average size clams were selected for experimental purpose. The average shell length (38-42 mm) of K. opima and (45-50 mm) of M. meretrix has chosen for further experimentation. After segregation and selection, clams were acclimated in plastic container (3 × 2 ft) for 48 hours at room temperature (30 ± 1°C).

**Experimental Design**

Healthy clams were exposed to three experimental temperatures (20°C, 25°C and 35°C) along with a control group (30 ± 1°C) for 8 days i.e. 192 hours. The 20°C and 25°C experimental groups were considered as low temperatures, while 35°C group treated as a high temperature. The low temperatures (20°C and 25°C) were maintained by ice-cold water, while high temperature (35°C) was controlled by thermostat. In each group of experimental temperatures, thirty (30) clams were exposed. During experimental exposure, water from the container was changed after every six hours. Throughout the experimentation, 38 ppt sea water was used.

**Experimental Analysis**

During 192 hours of exposure, five individuals were removed after every 48 hours from experimental sets to assess physiological body indices like hepatopancreas index, gonad index and condition index. These individuals were sacrificed and flesh and shells were separated. The hepatopancreas and gonad were carefully separated from each other, and partially dried by blotting paper to remove the extra water. After blotting, tissues were weighed on monopan electronic digital balance. For drying, the flesh and shells were kept in oven at 60°C up to 72 hours to determine the constant dry meat and shell weight.
In experimental analysis, hepatopancreas index (HI) and Gonadal index (GI) was estimated as described by Giese (1959). The condition index (CI) was analyzed as suggested by Rainer and Mann (1992). Formulae for HI, GI and CI are as follows,

1. Hepatopancreas index (HI) = \frac{\text{Wet weight of hepatopancreas (g)}}{\text{Wet weight of meat (g)}} \times 100

2. Gonadal index (GI) = \frac{\text{Wet weight of gonad (g)}}{\text{Wet weight of meat (g)}} \times 100

3. Condition index (CI) = \frac{\text{Meat dry weight (g)}}{\text{Dry shell weight (g)}} \times 100

Statistical Analysis

All the results of HI, GI and CI were the mean of five separate analyses with ± SD. One-way ANOVA was used to test the significant difference between means of experimental exposure (20°C, 25°C and 35°C) with the means of control group (30 ± 1°C). All statistical difference was accepted at the 0.05 level of significance using the Graph Pad software version 5.04.

Results and Discussion

In the present investigation, physiological body indices like hepatopancreas index (HI), gonad index (GI) and condition index (CI) were studied in two clam species Katelysia opima and Meretrix meretrix under short-term exposure of temperatures (Table 1 and 2). In both the clam species, all body indices have been affected significantly after exposure to both increasing and decreasing temperatures.

Hepatopancreas Index (HI)

After 192 hours of temperature exposure, in K. opima the hepatopancreas index (HI) was reduced significantly (46%) at high temperature i.e. 35°C followed by low temperatures like 20°C (44%) and 25°C (33%), while there was (55%, 40% and 28%) reduction at 20°C, 25°C and 35°C in M. meretrix species respectively. During exposure of 192 hours, at all temperature ranges (20°C, 25°C and 35°C) the K. opima clam showed decline (p<0.01) in HI at 48 hours, while 96 hours onwards it was declined slowly up to 192 hours. In M. meretrix, at 20°C, HI was decreased (p>0.05) at 48 hours, while it was declined (p<0.05) at 96 hours. The significant (p<0.001) decrease in HI was noticed at 192 hours, but less decline was recorded at 144 hours. At 25°C, the HI was declined (p=0.05) at 48, 96 hours, 144 hours, while (p<0.001) decline at 192 hours respectively. At high temperature (35°C), the HI was successively decreased from 48 to 192 hours of exposure. At 48 hours (p<0.05) and 96 hours (p<0.01) decline was recorded, however, it was reduced (p<0.001) 192 hours.

In both the clams, maximum reduction of HI was recorded particularly at high temperature i.e. (35°C). Relatively, maximum decline in HI was observed in M. meretrix especially at high temperature however, from 20°C and 25°C ranges the maximum decline was noted in K. opima clam.

Among the environmental factors, temperature is one of the most vital and relevant abiotic factors, which controls all facets of organisms at biological and ecological levels (Heilmayer et al., 2004; Resgalla Jr. et al., 2007). Temperature regulates the extent of species distribution, physiological processes such as feeding, respiration, growth and reproduction (Davenport, 1979; Newell and Branch, 1980; Shumway, 1982). Therefore, in organism adaptation to varying environmental temperature is major challenge in evolutionary adaptation. The adaptation activity is totally relies on the large extent of the organisms ability to compensate metabolic rate under high influence of temperatures at both short-term and long-term exposures (Hochachka and Samero, 2002; Portner, 2002 a, b). According to literature, various body indices like hepatopancreas index, gonad index and condition index has been utilized to assess the physiological measure like reproductive output (Suja and Muthiah, 2009). According to Sokolowicz et al., (2006) the reserved materials and their fluctuation in the hepatopancreas tissue is subjected to gonadal development.
In this study, after exposure to various temperature ranges the hepatopancreas index (HI) in both the clam species was maximally reduced at high temperature; about 46% and 55% decrease was recorded in *K. opima* and *M. meretrix* respectively. The hepatopancreas functionally plays intermediary role in metabolism and it also acts as a store house for fat bodies (Smith *et al.*, 1975; Bhide *et al.*, 2006).

It is generally accepted that, the nutrients are principally stored in the hepatopancreas tissue or digestive gland. These nutrients are utilized for growth and development, even in reproductive development the hepatopancreas supplies nutrients to gonad for gonadal development. In the present study, after exposure to various temperature maximum reductions in HI was occurred at high temperature. The metabolic rate increases, consequently the scope for growth reduces at high temperature (Widdows, 1978). In context of increased metabolic rate, clam might be utilized available nutrients to sustain at undesired condition instead of to use for growth and development. Therefore, in order to survive at increased temperature regime, the depletion in HI was occurred.

Sastry, (1968) reported that, the hepatopancreas index of scallops *Aequipectenirradians* was depleted at high temperature. He suggested that, the available reserves in scallops have been utilized for maintenance at high temperature regime. Suja and Muthiah (2009) observed considerable difference in digestive index of unfed treatment however, in fed treatment there was no significant change was recorded in HI at two rearing temperatures (23°C and 28°C). Sastry (1968) suggested that, the digestive gland index was maximum during vegetative and rearing stages.

**Gonadal Index (GI)**

Gonad index (GI) also decreased significantly in both the clams when exposed to both low and high temperatures. In *K. opima*, after 192 hours exposure, maximum decline (40%) in GI was occurred at high temperature like 35°C than (27% and 24%) at 20 and 25°C. In *M. meretrix* clam also recorded similar trend of decline GI, the maximum decline (41%) in GI was noted at high temperature (35°C) followed by (36%) at 20°C and (31%) at 25°C respectively.

During 192 hours of exposure, in *K. opima*, the GI was declined significantly (p<0.01) in *K. opima* at 48 hours of exposure, while it was (p<0.05) declined in *M. meretrix* clam. However, 96 hour onwards the GI was declined successively up to 192 hours in both the clams species. Overall, after 192 hours exposure, the highest decline in GI was detected in clam *M. meretrix* than *K. opima*.

**Condition Index (CI)**

Condition index (CI) in both the clams has been declined considerably after exposing to both low and high temperatures. Condition index of *K. opima* was decreased significantly at all experimental temperatures after 192 hours of exposure. After 8th day of exposure, the maximum reduction in CI was recorded...
Effect of short term temperature on physiological body indices

At high temperature (35°C) than lower ranges (20°C and 25°C). At high temperature, CI was reduced to 32%, while 26% and 19% reduction was recorded at 20°C and 25°C temperature groups respectively. In this clam, at 20 and 25°C the CI was declined (p>0.05) during exposure i.e. from 96-144 hours however, at 48 and 192 hours CI was noticed with less (p<0.05) reduction. In case of high temperature (35°C), CI was slightly (p<0.05) reduced from 96 to 192 hours, but significant (p<0.01) reduction was noticed at 48 hours.

In *M. meretrix* clam maximum reduction in CI was noticed at high temperature than low temperatures. After 8th day of exposure, 39% reduction was recorded at 35°C, while at 20°C, 36% reduction and at 25°C, 17% reduction was recorded respectively. In all temperature ranges, CI was suddenly declined at 48 hours however, later i.e. from 96 to 192 hours, there was slow decline in CI. During 192 hours exposure of both high and low temperatures, the CI was considerably declined. At high temperature (35°C), the CI was declined slightly (p<0.05) at 48 hours, however significantly (p<0.01) at 96-120 hour and (p<0.001) declined at 144-192 hours respectively. At low temperature like (20°C), the CI was non-significantly (p>0.05) declined at 48 hours, while (p<0.01) at 96, 144 hours and (p<0.001) reduction was recorded at 192 hours respectively. However, at 25°C the CI was decreased (p>0.05) from 48-144 hour, while (p<0.05) reduction at 192 hours.

In recent past, several authors well documented the studies on physiological body indices in bivalves and they have reported a strict relationship between the condition index increment and the gonadal development (Hamida *et al*, 2004; Ojea *et al*, 2004; Mladineo *et al*, 2007). Similarly, Abraham (1996) and Suja and Muthiah
Table 1 Effect of temperature on HI, GI and CI of *Katlephas opima* clam.

<table>
<thead>
<tr>
<th>Exposure hours</th>
<th>Control (30±1°C)</th>
<th>20°C</th>
<th>25°C</th>
<th>35°C</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>HI</td>
<td>GI</td>
<td>CI</td>
<td>HI</td>
</tr>
<tr>
<td>48 h</td>
<td>3.36 ±0.44</td>
<td>3.54 ±0.48</td>
<td>2.03 ±0.18</td>
<td>3.23 ±0.11***</td>
</tr>
<tr>
<td>96 h</td>
<td>3.40 ±0.42</td>
<td>3.51 ±0.31</td>
<td>2.44 ±0.14</td>
<td>3.21 ±0.18***</td>
</tr>
<tr>
<td>144 h</td>
<td>3.34 ±1.42</td>
<td>3.48 ±1.01</td>
<td>2.15 ±0.41</td>
<td>2.11 ±0.42 NS</td>
</tr>
<tr>
<td>192 h</td>
<td>3.32 ±0.62</td>
<td>3.48 ±0.60</td>
<td>2.08 ±0.42</td>
<td>1.67 ±0.36**</td>
</tr>
</tbody>
</table>

HI= Hepatopancreas index, GI= Gonadal index, CI= Condition index. All results are the mean of five observations with ± SD.

Table 2 Effect of temperature on HI, GI and CI of *Meretrix meretrix* clam.

<table>
<thead>
<tr>
<th>Exposure hours</th>
<th>Control (30±1°C)</th>
<th>20°C</th>
<th>25°C</th>
<th>35°C</th>
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<tr>
<td></td>
<td>HI</td>
<td>GI</td>
<td>CI</td>
<td>HI</td>
</tr>
<tr>
<td>48 h</td>
<td>4.36 ±1.03</td>
<td>5.83 ±1.42</td>
<td>3.21 ±0.48</td>
<td>3.38 ±0.62 NS</td>
</tr>
<tr>
<td>96 h</td>
<td>4.21 ±0.88</td>
<td>5.73 ±1.40</td>
<td>3.22 ±0.47</td>
<td>3.24 ±0.44*</td>
</tr>
<tr>
<td>144 h</td>
<td>4.15 ±0.56</td>
<td>5.50 ±0.64</td>
<td>2.99 ±0.44</td>
<td>2.62 ±0.28**</td>
</tr>
<tr>
<td>192 h</td>
<td>4.04 ±0.82</td>
<td>5.33 ±1.44</td>
<td>2.90 ±0.23</td>
<td>2.42 ±0.26**</td>
</tr>
</tbody>
</table>

HI= Hepatopancreas index, GI= Gonadal index, CI= Condition index. All results are the mean of five observations with ± SD.

(p< 0.001) = ***, (p< 0.01) = **, (p< 0.05) = * and (p>0.05) = NS.

(2009) observed direct relationship between condition index and gonadosomatic index.

In the present study, both clam species showed considerable decrease in condition index (CI) at high temperature. It clearly indicates that, the CI of clams is closely associated with the gonad index. At stress condition, there was a limitation of both oxygen and food hence, the organism suppressed their oxygen consumption and ceasing all activity in order to conserve the energy and maintenance at undesired environment (Laing *et al.*, 1987). The probable reason for declining the gonadal and condition index might be the availability of food at different temperature regime. Because insufficient quantity of nutrients may suppressed the gonadal development and maturation (Suja and Muthiah, 2009) ultimately the physiological condition of clams may affected. Recently, Yap and Al-Barwani, (2012) recorded decline in condition index when mussel *Perna viridis* subjected to stress condition.

Finally we concluded that, the physiological measures like hepatopancreas index, gonadal index and condition index amongst both the clam species were significantly declined when exposed to three experimental temperatures (20, 25 and 35°C). At all experimental temperatures,
the physiological body indices were declined significantly however, highest decline in body indices has been noticed at high temperature (35°C) followed by 20°C and 25°C. Relatively, maximum decline in the indices was recorded in M. meretrix species than K. opima. Based on this observation, it assumed that, both the clam species are very sensitive to changing temperature particularly to high temperature (35°C) and comparatively M. meretrix species is more sensitive towards temperature change than K. opima.

Acknowledgement
The authors are very thankful to Retd. Prof. U. H. Mane, Director, Centre for Coastal and Marine Biodiversity, Bhatye, Ratnagiri, for his valuable suggestion and guidance throughout research work.

References


Introduction

Water is a limited resource which cannot be produced as and when required by the technological means. The quality of water depends on the concentration of its physico-chemical parameters such as pH, DO, TDS, alkalinity, BOD, COD, temperature (natural), etc. With recent development of industries and sudden population growth, pollutants are constantly being discharged into fresh water bodies (lakes) which change the properties of water and adversely affect the flora and fauna of that particular water ecosystem. The degree of toxicity produced by the pollutants is dose independent upon environmental conditions such as temperature, pH, oxygen content and presence of residue molecules (Singh and Mishra, 2009). Several species of fish are susceptible to deleterious effects when exposed to heavy metals, pesticides and other environmental stressors (Arehon and Plump, 1990). Among the various pollutants, heavy metals, in particular, are widespread contaminants released into aquatic systems from numerous factories and industries. Some metals are known to be toxic even at low concentrations, including arsenic, cadmium, mercury and lead (Lee et al., 2009). Others such as copper and cobalt, are known to be essential elements and play important roles in biological metabolism at very low concentrations (Lee et al., 2009). The presence of heavy metals in fishes from the Coastal waters of Kapar...
and Mersing, Malaysia water was reported by Bashir et al. (2013). The indestructible nature and long term toxic effects of heavy metals including lead (Pb), nickel (Ni), manganese (Mn), zinc (Zn), cadmium (Cd) and chromium (Cr) to man as a result of consumption of organisms obtained from polluted rivers has raised scientific and environmental concerns (Kumar et al., 2012). Heavy metals are among the most persistent pollutants in aquatic ecosystem because of their resistance to decomposition in natural conditions (Khan, 2011). The danger is that heavy metals even at low concentrations in fish and water have a particular significance in ecotoxicology and their toxic effects have been widely published for a number of water bodies (Ekeanyanwu et al., 2011).

In the present investigation, Labeo rohita, a freshwater fish was sampled from the two polluted lakes, Vengaiah lake and Yellamallapally (lakes A and lake B respectively) and from control fish farm, for analyses of biochemical parameters and enzymatic activity of its muscle and gill tissues. The fish reared in lake A which received sewage and domestic waste from surrounding habitation and lake B which is adjacent to Cipla pharmaceutical company, were analysed. Fish from Hebbal fish farm were taken as control. Biomolecules as proteins, carbohydrates and lipids play a major role as precursors in fish under stress conditions. It is known that pollutants produce metabolic changes by way of influencing enzyme system. Decrease of protein level was reported by Dhapte et al. (2006) in fish Nemacheilus botia exposed to endosulfan. Decrease in glycogen content was also reported by Sobha et al. (2007) in the tissues of freshwater fish, Catla catla exposed to the heavy metal toxicant cadmium chloride. Cholesterol is required to build and maintain membranes. The industrial effluents from tannery, electroplating and textile mills caused marked depletion in biochemical composition (glycogen, protein and lipid) in tissues such as gill, liver, muscle and kidney of the fish, Labeo rohita (Muley et al., 2007). Dehydrogenases are the redox enzymes. Succinic dehydrogenase (SDH) is a primary enzyme in the oxidative catabolism of sugars. Malate dehydrogenase (MDH) an enzyme in citric acid cycle that helps in the conversion of malate into oxaloacetate (using NAD+) as a catalyst and vice versa where as lactate dehydrogenase (LDH) converts pyruvate to lactate in absence or in short supply of oxygen. LDH is widely used as a biomarker of lesions in organ and tissue in toxicology. Ramakritinan et al. (2005) studied impact of distillery effluent on the activity of enzymes like LDH and SDH in the muscle, liver and brain tissues fresh water fish, Cyprinus carpio. The present study was aimed to compare and correlate the variations in biochemical constituents and enzymatic activity of the tissues of fish with the physico-chemical parameters of the selected water bodies to recognise changes in the metabolic pathways due to the presence of xenobiotic substances.

Materials and Methods

Sampling of water was done in acid washed (Nelson’s water sampler) bottles from control site (Hebbal Fish farm), experimental sites of Lake (A) and (B) located in Bangalore city. Standard methods were used for determination of the physico-chemical parameters e.g., temperature, pH, BOD, COD, DO, TDS, phosphates, sulphates, nitrates and alkalinity etc., (APHA et al., 2005). DO was fixed on site as per the standard method. Trace metals were analysed by using Atomic Absorption Spectrophotometer. The values of physico-chemical parameters were compared with the Indian standard values provided by BIS: 10500-1991 (Revised 2012). Test fish, Labeo rohita were sampled with the help of dragnet - brought to the lake bank alive from the three water bodies simultaneously along with water samples. Fish were anaesthetized using MS222 to retain the characteristics of biochemical constituents and enzymes of the tissues. The tissues such as muscle and gill were carefully excised out of the body of the fish at the site and transferred to buffer solution for analysis of biochemical
Stress responses of biomolecules and dehydrogenase activity

constituents and dehydrogenases’ activities. Proteins were estimated by Lowry’s method (Lowry et al., 1951), glycogen content by Anthrone reagent (Seifer et al., 1950) by standard glucose solutions and cholesterol was estimated by Zlatki’s, method (Zlatkis et al., 1953). Succinic, malic and lactic dehydrogenases were estimated by continuous Spectrophotometric rate determination method (Bergmeyer and Bernt, 1974). One way ANOVA was applied to statistically evaluate the results and to find significant differences (Tukey’s multiple comparison test) within the physico-chemical parameters of control fish farm, lake A and lake B, and correlation with the biochemical and dehydrogenases activity of tissues of fish.

Results and Discussion

Results: The physico-chemical characteristics of water samples from Hebbal fish farm, lake A and lake B were statistically analyzed and the data represented in Table- 1 showed that due to the presence of trace metals, low values of DO and pH and high values of all the remaining parameters, lake B (Yellamallappa chetty lake) was assessed to be industrially polluted when compared to the lake A (Vengaiah lake). Hebbal fish farm was taken as control site. All water parameters of control site were compared with lake A and lake B and inturn with the standard BIS: 10500-1991(Revised 2012). Physico-chemical parameters of lake A and B when compared to BIS standard revealed presence of high levels of temperature, total suspended solids, chemical oxygen demand, biological oxygen demand, conductivity, turbidity and alkalinity. Trace metals’ content such as, aluminium, cadmium, copper, iron, lead and mercury in the present study showed relatively high level in water samples of lake B when compared to BIS values.

The present study on biochemical profile and enzymatic activity in muscle and gill tissue of fish were analysed statistically as shown in Table 2 and 3. An appreciable reduction was recorded in the level of protein and glycogen content in the two tissues of fish from lake B (Protein - 13.10 ± 0.89 and 8.26 ± 0.42 and glycogen - 2.20 ± 0.18 and 1.63 ± 0.08 respectively) when compared to those of lake A (Protein - 17.03 ± 0.57 and 16.22 ± 0.45 and glycogen - 3.45 ± 0.14 and 2.03 ± 0.12 respectively) and control ones (Protein - 18.33 ± 0.82 and 17.83 ± 1.17 and glycogen - 4.08 ± 0.25, 2.24 ± 0.10 respectively). Cholesterol content in the muscle and gill tissue (3.2 ± 0.22 and 2.4 ± 0.14) of fish from lake B showed a significant increase when compared to those of lake A (2.2 ± 0.09 and 1.6 ± 0.09) and control ones (2.0 ± 0.09 and 1.4 ± 0.14).

With similar lines as biochemical profile the succinic dehydrogenase activity in the muscle and gill of control fish showed a higher level (43.33 ± 0.50 and 20.58 ± 0.55 respectively) when compared to those of lake A (41.52 ± 0.91 and 18.9 ± 0.4 respectively) and Lake B (33.38 ± 0.78 and 12.8 ± 0.72 respectively). Inspite of the differences in the physico-chemical parameters of two lakes and control site, the muscle tissue showed a higher SDH activity than gill tissue in general but a reducing trend was observed from the fishes sampled from control site to polluted lake A and B. A marked reduction was also recorded in malate dehydrogenase activity of muscle and gill tissue sampled from lake B (35.83 ± 2.14 and 16.32 ± 0.81) and also lake A when compared to those of control ones (47.83 ± 1.17 and 22.75 ± 1.17) respectively. But the activity of lactic dehydrogenase of the muscle tissue of lake B showed a significant increase of 383.67 ± 4.84 when compared to control fish and of those observed in the tissues from lake A (326.33 ± 4.41). An increase of LDH activity found in fish from lake A and B was significant in the muscle tissues when compared to those in the gill tissue. Thus, out of the three dehydrogenases, the activity of succinic and malic dehydrogenase was lower but of lactic dehydrogenase was higher in the muscle and gill tissue of the fish sampled from lake B. An insignificant variation in biochemical levels and dehydrogenase activity in both the tissues from Lake A were observed when compared to those of control ones.
### Table 1: Physico-Chemical parameters of fish farm (Control), Vengaiah lake (A) and Yelemallappa Chetty lake (B).

<table>
<thead>
<tr>
<th>Sl. no</th>
<th>Parameters</th>
<th>Standards BIS: 10500-1991 (Revised 2012)</th>
<th>Control (farm)</th>
<th>Lake A</th>
<th>Lake B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Temperature (°C)</td>
<td>22-28</td>
<td>26 ± 0.63</td>
<td>26 ± 0.63</td>
<td>28 ± 1.26</td>
</tr>
<tr>
<td>2</td>
<td>pH Value</td>
<td>06.50 - 08.50</td>
<td>7.87 ± 0.08</td>
<td>7.65 ± 0.16</td>
<td>6.85 ± 0.74</td>
</tr>
<tr>
<td>3</td>
<td>Color (Pt-Co scale)</td>
<td>5 - 25</td>
<td>3.1 ± 0.63</td>
<td>4.3 ± 0.63</td>
<td>6.1 ± 0.49</td>
</tr>
<tr>
<td>4</td>
<td>Odor</td>
<td>UOB</td>
<td>UOB</td>
<td>UOB</td>
<td>Fishy</td>
</tr>
<tr>
<td>5</td>
<td>Turbidity, NTU</td>
<td>05 - 20</td>
<td>7.8 ± 0.15</td>
<td>21 ± 0.89</td>
<td>34.2 ± 2.14^a</td>
</tr>
<tr>
<td>6</td>
<td>Conductivity mmho /cm</td>
<td>300</td>
<td>483 ± 9.54</td>
<td>837 ± 42.80^a</td>
<td>1207 ± 35.15^a</td>
</tr>
<tr>
<td>7</td>
<td>Total Alkalinity as CaCO₃, mg/l</td>
<td>200 - 600</td>
<td>202 ± 1.38</td>
<td>290 ± 1.60^a</td>
<td>544 ± 11.07^a</td>
</tr>
<tr>
<td>8</td>
<td>Total Dissolved solids, mg/l</td>
<td>500 - 2000</td>
<td>420 ± 7.69</td>
<td>750 ± 1.41^a</td>
<td>985 ± 2.93^a</td>
</tr>
<tr>
<td>9</td>
<td>Total Suspended solids, mg/l</td>
<td>100</td>
<td>92 ± 0.82</td>
<td>150 ± 0.82^a</td>
<td>260 ± 4.73^a</td>
</tr>
<tr>
<td>10</td>
<td>D.O, mg/l</td>
<td>4.0 - 6.0</td>
<td>3.7 ± 0.05</td>
<td>3.7 ± 0.05</td>
<td>1.2 ± 0.08</td>
</tr>
<tr>
<td>11</td>
<td>B.O.D, mg/l</td>
<td>2 - 6</td>
<td>6 ± 0.76</td>
<td>24 ± 1.21</td>
<td>113 ± 1.33^a</td>
</tr>
<tr>
<td>12</td>
<td>C.O.D, mg/l</td>
<td>200</td>
<td>76 ± 1.72</td>
<td>126 ± 2.25^a</td>
<td>374.7 ± 2.88^a</td>
</tr>
<tr>
<td>13</td>
<td>Total Phosphorus, mg/l</td>
<td>-</td>
<td>0.35 ± 0.01</td>
<td>1.02 ± 0.01</td>
<td>2.42 ± 0.01</td>
</tr>
<tr>
<td>14</td>
<td>Nitrates as NO₃, mg/l</td>
<td>45 - 100</td>
<td>2.13 ± 0.28</td>
<td>2.25 ± 0.20</td>
<td>3.87 ± 0.10</td>
</tr>
<tr>
<td>15</td>
<td>Sulphates as SO₄, mg/l</td>
<td>200 - 400</td>
<td>62 ± 0.52</td>
<td>103 ± 1.21^a</td>
<td>210 ± 0.52^a</td>
</tr>
<tr>
<td>16</td>
<td>Aluminium as mg/l</td>
<td>0.03- 0.2</td>
<td>0</td>
<td>0.067 ± 0.002</td>
<td>3.7 ± 0.089</td>
</tr>
<tr>
<td>17</td>
<td>Arsenic as mg/l</td>
<td>0.05</td>
<td>0</td>
<td>0</td>
<td>0.003 ± 0.001</td>
</tr>
<tr>
<td>18</td>
<td>Cadmium as mg/l</td>
<td>0.01</td>
<td>0.001</td>
<td>0.04 ± 0.01</td>
<td>0.124</td>
</tr>
<tr>
<td>19</td>
<td>Copper as mg/l</td>
<td>0.05 – 1.5</td>
<td>0.013</td>
<td>0.03</td>
<td>0.32 ± 0.01</td>
</tr>
<tr>
<td>20</td>
<td>Iron as mg/l</td>
<td>0.3 - 1</td>
<td>0.04 ± 0.008</td>
<td>0.13 ± 0.022</td>
<td>3.68 ± 0.004</td>
</tr>
<tr>
<td>21</td>
<td>Lead mg/l</td>
<td>0.05</td>
<td>0.004 ± 0.001</td>
<td>0.04 ± 0.012</td>
<td>0.37 ± 0.020</td>
</tr>
<tr>
<td>22</td>
<td>Zinc as mg/l</td>
<td>5 - 15</td>
<td>0.54 ± 0.02</td>
<td>1.88 ± 0.01</td>
<td>2.68 ± 0.01</td>
</tr>
<tr>
<td>23</td>
<td>Mercury, mg/l</td>
<td>0.001</td>
<td>0</td>
<td>0</td>
<td>0.028</td>
</tr>
</tbody>
</table>

UOB: Unobjectionable, BDL: Below Detectable Limits

The superscripts a and b indicate statistical mean differences at p < 0.001 and 0.01 respectively.

**Discussion:** Physico-chemical and toxicological bioassay is the basic tool for evaluation of a complex industrial waste (Webner *et al.*, 1989). Conductivity, alkalinity, total dissolved solids, total suspended solids, nitrates, sulphates, biological oxygen demand and chemical oxygen demand of lake B showed significant variation in its level when compared to those of control site resulting in its algal growth, eutrophic and anaerobic condition. Murdoch *et al.* (2001) also reported that phosphate and nitrate are responsible nutrients for increase in algae growth and other toxic blooms which leads to eutrophication of lakes causing decrease in dissolved oxygen and a simultaneous increase in BOD. High levels of TDS, TSS, total phosphorus etc and trace
Stress responses of biomolecules and dehydrogenase activity

Table 2: Levels of protein, glycogen and cholesterol in the different tissues of L. rohita from fish farm (Control), Vengaiah lake (A) and Yelemallapa Chetty lake (B).

<table>
<thead>
<tr>
<th>Biochemical constituents</th>
<th>Tissue</th>
<th>Control</th>
<th>Lake A</th>
<th>Lake B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>Muscle</td>
<td>18.33 ± 0.82</td>
<td>17.03 ± 0.57</td>
<td>13.10 ± 0.89</td>
</tr>
<tr>
<td></td>
<td>Gill</td>
<td>17.83 ± 1.17</td>
<td>16.22 ± 0.45</td>
<td>8.26 ± 0.42</td>
</tr>
<tr>
<td>Glycogen</td>
<td>Muscle</td>
<td>4.08 ± 0.25</td>
<td>3.45 ± 0.14</td>
<td>2.20 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>Gill</td>
<td>2.24 ± 0.10</td>
<td>2.03 ± 0.12</td>
<td>1.63 ± 0.08</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Muscle</td>
<td>2.0 ± 0.09</td>
<td>2.2 ± 0.09</td>
<td>3.2 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>Gill</td>
<td>1.4 ± 0.14</td>
<td>1.6 ± 0.09</td>
<td>2.4 ± 0.14</td>
</tr>
</tbody>
</table>

Values expressed mg/g wet weight of tissues. Values are expressed as Mean ± S.D; sample size (n) = 6
Statistically significant mean difference at p < 0.0001.

Table 3: Succinic-, Malic- and Lactic dehydrogenase activity in muscle and gill tissues of L. rohita from fish farm (Control), Vengaiah lake (A) and Yelemallapa Chetty lake (B).

<table>
<thead>
<tr>
<th>Dehydrogenases' activity</th>
<th>Tissue</th>
<th>Control</th>
<th>Lake A</th>
<th>Lake B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Succinic dehydrogenase (SDH)</td>
<td>Muscle</td>
<td>43.33 ± 0.50</td>
<td>41.52 ± 0.91</td>
<td>33.38 ± 0.78</td>
</tr>
<tr>
<td></td>
<td>Gill</td>
<td>20.58 ± 0.55</td>
<td>18.9 ± 0.4</td>
<td>12.8 ± 0.72</td>
</tr>
<tr>
<td>Malic dehydrogenase (MDH)</td>
<td>Muscle</td>
<td>47.83 ± 1.17</td>
<td>43.83 ± 1.47</td>
<td>35.83 ± 2.14</td>
</tr>
<tr>
<td></td>
<td>Gill</td>
<td>22.75 ± 1.17</td>
<td>20.53 ± 0.86</td>
<td>16.32 ± 0.81</td>
</tr>
<tr>
<td>Lactic dehydrogenase (LDH)</td>
<td>Muscle</td>
<td>300.83 ± 4.45</td>
<td>326.33 ± 4.41</td>
<td>383.67 ± 4.84</td>
</tr>
<tr>
<td></td>
<td>Gill</td>
<td>124.67 ± 4.50</td>
<td>152.33 ± 4.18</td>
<td>212.67 ± 5.16</td>
</tr>
</tbody>
</table>

Values expressed as U/ml enzyme. Values are expressed as Mean ± S.D; sample size (n) = 6
Statistically significant mean difference at p < 0.0001.

Metals like cadmium, lead and mercury more than the permissible BIS standard values caused degradation of water quality of lake B. Similar results were recorded by Adeyemo (2003) in his work on consequences of pollution and degradation on Nigerian aquatic environment on fisheries resources and Murdoch et al. (2001) during his work on watershed inventory and stream monitoring methods. Heavy metals are natural trace components of the aquatic environment, but their levels have increased due to contamination of lake water with domestic, industrial and agricultural wastes causing greatest threat to the health of aquatic ecosystem (Joshi et al., 2002). This problem of environmental pollution and its deleterious effects on aquatic biota, including fish is receiving focus during the last few decades (Jagadeesan et al., 2001). Almost all the trace metals that contaminate the system get readily absorbed by plants and then animals reported to be relatively toxic at levels slightly above than those required for maintaining normal metabolic activities of the body (Chakraborty et al., 2004).

Fishes are sensitive to contamination and the pollutants may disrupt some physiological and biochemical processes when they enter the organs of fishes (Tulasi et al., 1992) as also observed in the present study on Labeo rohita from lake B. Fish at a higher level of food chain
accumulate a significant amount of pollutants and this accumulation depends on the intake and elimination from the body (Karadede et al., 2004). Exposure of aquatic organisms to metal pollution is a major concern today. The present study showed alteration in the physiological condition and metabolic activities of test fish, *Labeo rohita* sampled from lake B when compared to those observed in lake A and control site. This can be attributed to the exposure of fish to the significant variation in levels of physico-chemical parameters including trace metals. Since metals act as mutagenic/genotoxic compounds, interfering with xenobiotic metabolic pathways affecting glycolysis, the Krebs cycle, oxidative phosphorylation, protein, amino acid metabolism as well as carbohydrate and lipid metabolism as reported by Drastichova et al. (2005) in his cytochemical studies of carp neutrophil after acute exposure to cadmium.

Fish are an important source of protein to man; muscles and gills are the vital organs which have direct contact with the medium through which pollutants enter into their body. In the present study, since high levels of nutrient and trace metals were recorded from water of lake B and subsequently variation in the levels of biomolecules and dehydrogenase activity in the muscle and gill tissue of test fish from the same lake was observed. Therefore, enzymes, could be used (as biomarkers) to identify possible environmental contaminations before the health of aquatic organisms is seriously affected (Barnhoorn, 1996) and to develop water quality indices (Mekkawy et al., 2009). Biochemical approaches can be used to provide an early warning of potentially damaging changes in stressed fish. Reduction in protein and glycogen content in both the tissues of fish in the present study proves that protein has undergone hydrolysis and oxidation whereas glycogen has been utilised rapidly to meet the increasing demand for energy for the survival of fish which is stressed due to the degraded water quality of lake B. De Smet and Blust (2001) also supported this observation by stating that exposure to cadmium caused an increase in the role of proteins for the energy production to combat stress and also due to excessive proteolysis to overcome the metabolic stress, as deposited protein in the cytoplasm may be used to replace the loss of proteins during physiological stress (Patil et al., 2011).

According to Cicik and Engin (2005), cadmium stress caused alteration in the glycogen content through glycolysis or hexose monophosphate pathway in muscle and liver tissue of *Cyprinus carpio* and by Paraquat in African Catfish (Kori-Siakpere et al., 2007). These results are in agreement with the present work on muscle and gill tissue of *Labeo rohita* from lake B which was contaminated with significantly high level of trace metals (Al, Cd, Fe, Pb, Zn and traces of Hg). Depletion in glycogen content may be due to rapid glycogenolysis and inhibition of glycogenesis through activation of glycogen phosphorylase and depression of transferase (Jha and Jha, 1995). The decline in glycogen might be partly due to its utilization in the formation of glycoprotein and glycolipids, which are the essential constituents of various cells and other membranes (Vutukuru, 2005).

Stimulation of cholesterol observed in both the tissues of fish may be due to their exposure to pollutants, lipophilic in nature present in lake B. Meenakumari et al. (2010) reported that cholesterologenesis increases on the interference of xenobiotics with feedback mechanism. In similar lines with the present study, Muley et al. (2007) reported that an increase in cholesterol level can be attributed to the inhibition of steroidogenesis. Under stress conditions, liver undergoes metabolic changes which disturb the excretory mechanism leading to an increase in cholesterol content (Goksyrr et al., 1994).

The exposure of common carp to heavy metals significantly elevated the concentrations of red blood cells, blood glucose and total cholesterol level (Vinodhini and Narayanan, 2009). A decreasing trend in all the biochemical constituents (total proteins, carbohydrates and lipids) in the tissues of *Channa orientalis* was
reported by Hymavathi and Rao (2001) from the habitat polluted by slaughterhouse wastes when compared to the fishes of unpolluted habitat of Mudasarlova stream of Visakhapatnam. The changes exhibited by the biochemical constituents in muscle and gill tissues in the present study due to degradation in the water quality of the lakes can be attributed to proteolysis, glycogenolysis with activation of glycogen phosphorylase and non utilisation of cholesterol to overcome metabolic stress for their survival in non conducive environment of lake B. Degraded condition of the water quality of lake B required surplus oxygen intake by the fish causing an increase in respiratory activity. This resulted in excess utilisation of glycogen and protein in gill when compared to muscle tissue.

Activity of dehydrogenases can be considered as marker of mitochondrial abundance indicating state of fish health and its physiological condition. Inhibition of mitochondrial oxidation of succinate may lead to drop in energy production resulting in suppression of SDH activity which indicates impairment of oxidative metabolism. In similar lines MDH is also dependent on the TCA products and inhibition in its activity is a result of reduction in oxidative metabolism. In the present study, the pollutants in lake B induced a marked decrease in SDH and MDH activity in fishes causing disturbances in oxidative process in the muscle and gill tissue of fish of the lake. These observations tally with reports by Almeida et al. (2002) in muscle of Oreochromis mossambicus exposed to cadmium and by Rajamannar and Manohar (2000) in different tissues (gill, liver, muscle and brain) of adult or fingerling of L. rohita exposed to lethal or sublethal concentrations of lead and copper or organochlorine (DDT and BHC) and organophosphorous (Dichlorvos and Monocrotophos) compounds resulting in a gradual decrease in SDH activity. The significant elevation of LDH in the present study in both muscle and gill tissue regardless of the water bodies suggested that pyruvate; the end product of glycolysis is not routed to Kreb’s cycle but to the lactic acid cycle. This is in agreement with the reports of cadmium stress on the metabolic response of Nile Tilapia by Almeida et al. (2001). Decline in SDH and MDH activity and concurrent increase in LDH activity in the tissues of fish from lake B due to the degradation of water quality indicated that fishes were unable to bear the pollutant stress which resulted in fall of oxidative metabolism in TCA cycle and which in turn resulted in shift towards anaerobiosis at organ level. Similar results were reported by Ramkrithinan et al. (2005). The statistical correlation between water parameters and dehydrogenases activity as well as biomolecule constituents revealed that fish is surviving in hypoxic condition with high levels of BOD and COD and variation in the nutrient level and other parameters in lake B. The changes in enzymes may be induced secondarily as a result of damaged cells or these effects may be due to accumulation of trace metals and fluctuation in metabolism of biomolecules in muscle and gill tissues.

The above results indicate that the respiratory activity of fish under stress was affected and it tried to bear the toxic effects by undertaking an anaerobic metabolism due to adverse environmental conditions. Thus, under anaerobic (stressful) conditions the elevated activity of LDH and reduced activity of SDH and MDH reflects the metabolic capacity of tissues after long term exposure to pollutants in water bodies. The effects of these enzymes probably are only a part of general metabolic response to stressful conditions due to significant variation in physico chemical parameters of lakes and may not be specific for trace metals. Fish flesh provides an excellent source of nutrition for human diet and it has relatively high digestibility, biological and growth promoting value but the nutritive value of the fish in question has reduced due to its exposure to various pollutants and is assumed to be unhealthy for human consumption. Therefore, management, conservation and periodic monitoring of these lakes are suggested for the survival of its flora and fauna.
Acknowledgement
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References


Stress responses of biomolecules and dehydrogenase activity


Introduction

Metals occur naturally in the earth’s crust and are neither created nor destroyed by anthropogenic or biological process. However, their redistribution by the minerals and metals industry (mining and smelting), power generation, fossil fuel combustion, and many other industrial processes may be of concern since an increase in metal concentrations in our environment could pose a threat to human and ecosystem health (Nriagu, 1991). As a consequence of the industrial revolution there is an enormous and increasing demand for heavy metals that leads to high anthropogenic emission of heavy metals in the biosphere (Vangronsveld and Cunningham, 1998). Nonradioactive As, Cd, Cu, Hg, Pb and Zn and radioactive Sr, Cs, and U are the most important metallic pollutants (Raskin et al., 1997). These metals become an environmental concern when their concentrations begin to affect human health and the environment. A common characteristic of heavy metals regardless of whether they are biologically essential or not, they may already exert toxic effects at low concentrations (Kabata-Pendas and Pendias, 2001). Unlike organic molecules, toxic metals cannot be degraded but only be remediated. It requires consequently the intervention of mankind. In societies like our India with developing economics, the optimum development, efficient utilization and effective management of their water resources should be the dominant strategy for economic growth. But in recent years unscientific management and use of this resources for various purpose almost invariably has created undesirable problems in its wake, water logging and salinity in the case of agriculture use and environment pollution of various limits as a result of mining, industries and municipal use (Rai and Pal, 2001; Kumar et al, 2008; Kumar and Pal, 2011).

Phytoremediation, an emerging cleanup

Remediation of Heavy Metals through Aquatic Macrophytes from Water Bodies of Bundelkhand Region of Uttar Pradesh

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Abstract: The Bundelkhand region – approximately an area of 70,000 square kilometers with 21 million people, comprising 13 districts of Madhya Pradesh (MP) and Uttar Pradesh (UP) – is facing its worst ever drought spell in living memory. There are numbers of century old historical lakes in this region which are getting polluted day by day due to the growth of the small scale industrial corridor, nutrient loading and rapid anthropogenic activities. The increasing levels of metals in the aquatic ecosystem, their entry into food chain and the overall health effects are of major concern to researchers in the field of ecology. There are out of twelve existing plants in different water bodies, four aquatic macrophytes namely Eichorniacrassipes, Pistiastratiotes, Lemma minor and Vallisneriaspiralis were selected for phytoremediation study on the basis of their abundance in selected study areas. Concentration of six trace metals have been estimated in above mentioned macrophytes i.e., Copper (Cu), Chromium (Cr), Iron (Fe), Manganese (Mn), Lead (Pb), and Zinc (Zn). From a phytoremediation perspective, E. crassipes and Pistiastratiotes are promising plant species for remediation of polluted water bodies of Bundelkhand region.

Keywords: Bundelkhand region, Eichhornia, Pistia, Lemma, Vallisneria, Heavy metals.
technology for contaminated groundwater and wastewater that is both low-tech and low-cost, is defined as the engineered use of green plants (including grasses, forbs, and woody species) to remove, contain, or render harmless such environmental contaminants as heavy metals, trace elements, organic compounds and radioactive compounds in any aquatic systems (Kumar and Pal, 2011; Sarma, 2011; Singh et al., 2012). Macrophytes are considered as important component of the aquatic ecosystem not only as food source for aquatic invertebrates, but also act as an efficient accumulator of heavy metals (Devlin, 1967; Chung and Jeng, 1974; Rahman and Hasegawa, 2011; Ndimele and Jimoh, 2011; Pant et al., 2011). They are unchangeable biological filters and play an important role in the maintenance of aquatic ecosystem. Now a day, for cleaning the water resources, various techniques such as flocculation, sedimentation, carbon-absorption exchange etc. are available. However, the chemical and energy cost associated with these advanced techniques has been a serious constraint in adapting them in a developing country like India. In this context, a phytoremediation system which utilizes naturally occurring aquatic plants in waste water to absorb toxic metals could provide an inexpensive mean to indicate and remove toxic metals from polluted water bodies.

The present study has been conducted with following objectives -

I. Analysis of physico-chemical properties of selected water bodies.

II. Observation of metal concentration in selected areas.

III. Macrophytic observations in different study areas

IV. Metal concentration of selected macrophytes in field condition.

Materials and Methods

Study Area: The Bundelkhand region lies between approximately 23°10’ and 26°27’ North latitude and 78°4’ and 81°34’ East longitudes, and comprise four districts of Chitrakut division, three districts of Jhansi division, five district of Sagar divisions and one district of Gwalior division. There are enormous historical water bodies in this region. Three aquatic bodies namely Laxmi Taal, Antiya Taal and Baruwa Sagar have been selected for present study which is shown in the map (Figure 1) and periodic water samples have been collected from these lakes of Jhansi district of Bundelkhand region of Uttar Pradesh, India which is located at 25°12’ – 25°16’ N and 78°18’ – 79°23’E.

Laxhmi Taal (Lake): It is situated a few meters from the gate of historical Jhansi city in east. The area of lake is 0.162 km². It is polluted mainly anthropogenic activities as the sewage system of Jhansi directly input their organic load into this lake.

Antiya Taal (Lake): The lake is shallow with an area of 0.03 km² and is surrounded by residential house all side. The lake receive enormous quantity of organic matter from the nearest residential houses which causes them heavy polluted and which has accelerated the process of eutrophication.

Barua Sagar (Lake): Barua Sagar is a historical place located about 25 km from Jhansi in Uttar Pradesh, India. It is situated on the bank of the Betwa River; the place is named after the Barua Sagar Taal, a large lake created about 260 years ago when Raja Udit Singh of Orchha built the embankment. Area is 4.64 km²; altitude is 210 mtrs above MSL.

Preservation and analysis of water samples were based on standard methods shown in Table 1 and by the method of American Public Health Association (APHA, 2005).

Analysis of Heavy Metals in Water: The water samples were taken in evaporating dishes and acidified to methyl orange with conc. HNO₃. Further 5 ml conc. HNO₃ was added and evaporated to 10 ml. Then it was transferred to a 125 ml conical flask. 6 ml of conc. HNO₃ and 2 mL HClO₄ (70%) were added. After that heated gently, till white dense fumes of HClO₄
Remediation of heavy metals through aquatic macrophytes

Fig. 1 Location of the selected water bodies for study.

appear. The digested samples were cooled at room temperature, filtered through Whatman No. 42 and finally the volume was made up to 100 ml with double distilled water. The solution was used for the determination of heavy metals. The metals were analyzed by Atomic Absorption Spectrophotometer (Perkin Elmer).

Plant Sampling: After the identification of tolerant and sensitive aquatic macrophytes from study areas, four existing aquatic macrophytes were selected on the basis of their abundance as well as passive biomonitor for estimating the toxicity status induced by heavy metals.

The plant species *Eichhornia crassipes* (Mart.) Solms, *Pistiastratiotes* L., *Lemna minor* L., *Vallisneria spiralis* L. have been selected for the study of heavy metal content. Healthy aquatic plants were collected by hand, washed with lake water then after distilled water to remove periphyton and sediment particles. The collected plant species were preserved in plastic bags, labeled carefully and brought to the laboratory.

Analysis of Heavy Metals in Aquatic Plant Species: Heavy metals were analyzed in harvested plants which were thoroughly washed with distilled water, and dried in an oven at 80°C for 48 hr. Dried plant tissue (1 gm) were digested in HNO₃ (70%) and HClO₄ (70%) (3:1 v/v). Heavy metals in aquatic plants species was estimated by using Perkin – Elmer atomic absorption spectrophotometer.

Results and Discussion

Present investigation following water bodies e.g. Laxhmi Lake, Antiya Lake, and Baruwa Sagar respectively have been selected in Jhansi district of Bundelkhand region. Observations and finding of present work have been tabulated and are as follows:

Physico-chemical Properties in Different Water Bodies: The data of physico-chemical properties of respective study areas are presented in the Table 2. On the basis of eighteen physico-chemical parameters it may
Sharma et al.

**Table 1** Methodologies and instruments used for analytical work.

<table>
<thead>
<tr>
<th>1</th>
<th>pH</th>
<th>Electronic</th>
<th>Microprocessor based Portable Soil &amp; Water Analysis kit / Jyoti</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Electrical Conductivity (EC)</td>
<td>Electronic</td>
<td>Microprocessor based Portable Soil &amp; Water Analysis kit / Jyoti</td>
</tr>
<tr>
<td>3</td>
<td>Temperature</td>
<td>Electronic</td>
<td>Microprocessor based Portable Soil &amp; Water Analysis kit / Jyoti</td>
</tr>
<tr>
<td>4</td>
<td>Total Hardness</td>
<td>Titration method</td>
<td>---------------</td>
</tr>
<tr>
<td>5</td>
<td>Alkalinity</td>
<td>Titration method</td>
<td>---------------</td>
</tr>
<tr>
<td>6</td>
<td>Total Dissolved Solid (TDS)</td>
<td>Electronic</td>
<td>Microprocessor based Portable Soil &amp; Water Analysis kit / Jyoti</td>
</tr>
<tr>
<td>7</td>
<td>Dissolved Oxygen (DO)</td>
<td>Electronic</td>
<td>Microprocessor based Portable Soil &amp; Water Analysis kit / Jyoti</td>
</tr>
<tr>
<td>8</td>
<td>Biochemical Oxygen Demand (BOD)</td>
<td>Winkler's iodometric method</td>
<td>BOD Incubator</td>
</tr>
<tr>
<td>9</td>
<td>Chemical Oxygen Demand (COD)</td>
<td>Reflux digestion method</td>
<td>COD Incubator</td>
</tr>
<tr>
<td>10</td>
<td>Nitrate</td>
<td>Spectrophotometric method</td>
<td>UV Visible Spectrophotometer / Elico, SL 159</td>
</tr>
<tr>
<td>11</td>
<td>Phosphate</td>
<td>Spectrophotometric method</td>
<td>UV Visible Spectrophotometer / Elico, SL 160</td>
</tr>
<tr>
<td>12</td>
<td>Sulphate</td>
<td>Spectrophotometric method</td>
<td>UV Visible Spectrophotometer / Elico, SL 161</td>
</tr>
<tr>
<td>13</td>
<td>Sodium</td>
<td>Flame photometric method</td>
<td>Flame Photometer / Systronic 130</td>
</tr>
<tr>
<td>14</td>
<td>Potassium</td>
<td>Flame photometric method</td>
<td>Flame Photometer / Systronic 131</td>
</tr>
<tr>
<td>15</td>
<td>Calcium</td>
<td>Titration method</td>
<td>---------------</td>
</tr>
<tr>
<td>16</td>
<td>Magnesium</td>
<td>Titration method</td>
<td>---------------</td>
</tr>
<tr>
<td>17</td>
<td>% Na</td>
<td>Formulated</td>
<td>---------------</td>
</tr>
<tr>
<td>18</td>
<td>Sodium Absorption Ratio (SAR)</td>
<td>Formulated</td>
<td>---------------</td>
</tr>
<tr>
<td>19</td>
<td>Chlorophyll and Carotenoid estimation</td>
<td>Centrifugation &amp; Spectrophotometric method</td>
<td>Remi R-4C DX &amp; UV Visible Spectrophotometer / Elico, SL 161</td>
</tr>
<tr>
<td>20</td>
<td>Heavy Metals</td>
<td>AAS method</td>
<td>Atomic Absorption Spectrophotometer / Perkin Elmer,</td>
</tr>
</tbody>
</table>

be concluded that Laxmi Taal is more contaminated and followed by Antiya Taal and Baruwa Sagar respectively. This is because discharges of domestic and small scale industries of Jhansi city are come to the Laxmi Taal and Antiya Taal directly through the inlets. Baruwa Sagar is less polluted due to the far away of populated areas and only agricultural discharges are coming in to this lake through runoff from in and around adjacent agricultural lands and could be use for irrigation only after minor treatment (Mau-rya et al., 2012).

**Metal Concentration in Different Water Bodies:** The data of heavy metal concentration in water of respective study areas are presented in the Table 3. Among nine metals, except Al and Cd all are found that the beyond the limit of Indian standard. Presence of Pb in water may be recognized more harmful for any organisms. The average concentration of lead was significantly higher (1.526 mg/l) in Laxmi Lake and followed by Antiya Taal. The high level of Pb in water of lake could be attributed to the small scale industrial and agricultural discharge and
Table 2 Physico-chemical properties in water of selected areas.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Parameters</th>
<th>Laxmi Taal</th>
<th>Antiya Taal</th>
<th>Baruwa Sagar</th>
</tr>
</thead>
<tbody>
<tr>
<td>01.</td>
<td>Temp</td>
<td>24.29 ± 0.045</td>
<td>24.18 ± 0.078</td>
<td>16.95 ± 0.016</td>
</tr>
<tr>
<td>02.</td>
<td>pH</td>
<td>7.46 ± 0.020</td>
<td>7.44 ± 0.025</td>
<td>7.525 ± 0.008</td>
</tr>
<tr>
<td>03.</td>
<td>EC</td>
<td>931 ± 0.523</td>
<td>629 ± 0.762</td>
<td>481.7 ± 1.23</td>
</tr>
<tr>
<td>04.</td>
<td>Al</td>
<td>240 ± 0.843</td>
<td>235 ± 0.432</td>
<td>60.975 ± 0.67</td>
</tr>
<tr>
<td>05.</td>
<td>TH</td>
<td>197 ± 0.432</td>
<td>242 ± 0.467</td>
<td>111.475 ± 0.92</td>
</tr>
<tr>
<td>06.</td>
<td>NO₃</td>
<td>1.58 ± 0.010</td>
<td>1.49 ± 0.03</td>
<td>1.975 ± 0.03</td>
</tr>
<tr>
<td>07.</td>
<td>DO</td>
<td>3.92 ± 0.02</td>
<td>5.85 ± 0.021</td>
<td>7.025 ± 0.001</td>
</tr>
<tr>
<td>08.</td>
<td>Mg</td>
<td>19.3 ± 0.03</td>
<td>22.24 ± 0.08</td>
<td>26.9 ± 1.12</td>
</tr>
<tr>
<td>09.</td>
<td>TDS</td>
<td>625 ± 0.657</td>
<td>437 ± 0.467</td>
<td>229 ± 2.32</td>
</tr>
<tr>
<td>10.</td>
<td>PO₄</td>
<td>0.54 ± 0.02</td>
<td>0.75 ± 0.02</td>
<td>0.717 ± 0.015</td>
</tr>
<tr>
<td>11.</td>
<td>SO₄</td>
<td>18.0 ± 0.02</td>
<td>21.7 ± 0.02</td>
<td>16.4 ± 1.73</td>
</tr>
<tr>
<td>12.</td>
<td>BOD</td>
<td>2.57 ± 0.035</td>
<td>4.42 ± 0.037</td>
<td>3.925 ± 0.08</td>
</tr>
<tr>
<td>13.</td>
<td>COD</td>
<td>185 ± 0.656</td>
<td>143 ± 0.873</td>
<td>35.25 ± 1.31</td>
</tr>
<tr>
<td>14.</td>
<td>Na</td>
<td>263 ± 0.63</td>
<td>246 ± 1.34</td>
<td>258.5 ± 1.43</td>
</tr>
<tr>
<td>15.</td>
<td>K</td>
<td>44.3 ± 0.04</td>
<td>38.7 ± 0.05</td>
<td>39.25 ± 1.14</td>
</tr>
<tr>
<td>16.</td>
<td>Ca</td>
<td>42.3 ± 0.10</td>
<td>56.0 ± 0.06</td>
<td>48.575 ± 0.08</td>
</tr>
<tr>
<td>17.</td>
<td>Na%</td>
<td>69.7 ± 0.02</td>
<td>67.73 ± 0.65</td>
<td>69.245 ± 0.92</td>
</tr>
<tr>
<td>18.</td>
<td>SAR</td>
<td>47.50 ± 0.168</td>
<td>39.30 ± 0.459</td>
<td>42.395 ± 0.06</td>
</tr>
</tbody>
</table>

Values are Mean ± SE (n = 3); Units: - Concentration in mg/l, except pH; Temperature (ºC); EC (µS/cm); SAR (meq/l)

dust which holds a huge amount of lead from the combustion of petrol in automobile cars. Because of Batteries, radiators for cars and trucks, and some colors of ink also contain lead which also had been available nearby this lake and flow through drainage in these lakes. Similar observation was found by Hoo (2004) in Labu river of Malaysia; Yadav and Kumar (2011) in Kosi river; Zaidi et al. (2011) in Betwa and Pahuj rivers of India.

Macrophytic Observations in Different Study Areas: The availability of macrophytic composition in selected aquatic bodies and their variation of abundance in respective study areas are depicted in the Table 4 and summarized as follows -

Aquatic macrophytic diversity and its role in understanding the fresh water ecosystem dynamics have tremendous significance. In present investigations an initiative have been taken to identify the tolerant and sensitive aquatic macrophytes grown in different selected study areas during the year 2013 to 2014 respectively. There are total twelve species of aquatic macrophytes have been found in selected study areas such as *Eichhornia crassipes* (Mart.) Solms, *Hydriilla verticillata* (L.F.) Royle, *Ipomoea aquatic* (Forssk.), *Vallisneria spiralis* (L.), *Najas graminea* (Del.), *Pistiastratiotes* (L.), *Typhadomingensis* (Pers.), *Nymphaea lotus* (L.), *Potamogeton crispus* (L.), *Chara spp.* (L.), *Cyperus spp.*(L.), and *Lemna spp.* (L.).

All the twelve above mentioned species have been found in Laxhmi Lake and except *Najas Species* remaining eleven species were also found in Antiya taal and Baruwa Sagar although they showed variation in abundance according to the seasonal variations. *Eichhornia crassipes* was found abundantly in almost every season throughout the experimental period of the year. *Hydriilla verticillata*, *Vallisneria spiralis*, *Chara spp.* and *Lemna Spp.* was found in winter and pre-monsoon seasons.
Table 3 Average Metals concentrations in water of selected areas.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Metals</th>
<th>Laxmi Taal</th>
<th>Antiya Taal</th>
<th>Baruwa Sagar</th>
<th>Indian Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>01.</td>
<td>Al</td>
<td>0.0075 ± 0.0003</td>
<td>0.006 ± 0.001</td>
<td>0.018 ± 0.001</td>
<td>NA</td>
</tr>
<tr>
<td>02.</td>
<td>Cd</td>
<td>0.0015 ± 0.0003</td>
<td>0.002 ± 0.0005</td>
<td>0.002 ± 0.0001</td>
<td>.01</td>
</tr>
<tr>
<td>03.</td>
<td>Cr</td>
<td>0.338 ± 0.007</td>
<td>0.161 ± 0.013</td>
<td>0.064 ± 0.002</td>
<td>.05</td>
</tr>
<tr>
<td>04.</td>
<td>Cu</td>
<td>0.070 ± 0.008</td>
<td>0.015 ± 0.005</td>
<td>0.016 ± 0.001</td>
<td>.05</td>
</tr>
<tr>
<td>05.</td>
<td>Fe</td>
<td>1.497 ± 0.011</td>
<td>1.304 ± 0.005</td>
<td>0.672 ± 0.012</td>
<td>.3</td>
</tr>
<tr>
<td>06.</td>
<td>Pb</td>
<td>1.526 ± 0.024</td>
<td>1.187 ± 0.001</td>
<td>1.174 ± 0.002</td>
<td>.05</td>
</tr>
<tr>
<td>07.</td>
<td>Mn</td>
<td>1.649 ± 0.023</td>
<td>1.846 ± 0.021</td>
<td>2.695 ± 0.003</td>
<td>.05</td>
</tr>
<tr>
<td>08.</td>
<td>Ni</td>
<td>1.429 ± 0.017</td>
<td>NA</td>
<td>NA</td>
<td>.02 *</td>
</tr>
<tr>
<td>09.</td>
<td>Zn</td>
<td>0.020 ± 0.006</td>
<td>0.007 ± 0.001</td>
<td>0.076 ± 0.004</td>
<td>5</td>
</tr>
</tbody>
</table>

Values are Mean ± SE (n = 3); Unit: - concentration in mg/l, NA = not available *WHO Standard

Table 4 Identification of tolerant and sensitive aquatic macrophytes grown in the study areas.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Species name</th>
<th>Laxmi Taal</th>
<th>Antiya Taal</th>
<th>Baruwa Sagar</th>
</tr>
</thead>
<tbody>
<tr>
<td>01.</td>
<td><em>Eichhorniacrassipes</em>(Mart.) Solms</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>02.</td>
<td><em>Hydrillavericillata</em>(L.F.) Royle</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>03.</td>
<td><em>Ipomoea aquatic</em> (Forssk.),</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>04.</td>
<td><em>Vallisneriaspiralis</em> (L.)</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>05.</td>
<td><em>Najasgraminea</em> (Del.)</td>
<td>+</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>06.</td>
<td><em>Pistiastratiotes</em>(L.)</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>07.</td>
<td><em>Typhadomingensis</em> (Pers.)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>08.</td>
<td><em>Nymphaea lotus</em> (L.)</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>09.</td>
<td><em>Potamogetoncrispus</em> (L.)</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>10.</td>
<td><em>Chara spp.</em> (L.)</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>11.</td>
<td><em>Cyperus spp.</em> (L.)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>12.</td>
<td><em>Lemna spp.</em> (L.)</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

+++ = High, ++ = Medium, + = Low; NA = Not Available

Metal Concentration in Plant Species (macrophytes) in Field Condition: The data of heavy metal concentrations in selected plant species in the field condition are presented in the Table 5. Metals accumulation by selected plant species have been compared with the average concentrations of metals in the water bodies and depicted in Figure 2. There are four aquatic macrophytes namely *Eichonia crassipes*, *Pistia stratiotes*, *Lemna minor* and *Vallisneria spiralis* were selected for phytoremediation study on the basis of their abundance in selected study areas. Concentration of six trace metals have been estimated in above mentioned macrophytes i.e., Copper (Cu), Chromium (Cr), Iron (Fe), Manganese (Mn), Lead (Pb), and Zinc (Zn) for their bioaccumulation abilities.

In recent years, it has been reported that some plants species; known as hyperaccumulators, which are usually present in heavy metal-contaminated areas, have the ability to accumulate unusually high concentration of heavy metals without dramatically being
physiologically impacted (Hu et al., 2010). The level of toxic metals (Pb, Cr, Hg etc) can be reduced from contaminated water by a number of aquatic plants taken up by the roots system and transported to the stems and leaves without showing toxicity syndrome has confirmed by many studies (Rai et al., 1995; Cardwell et al., 2002; Abidal and Harikrishna 2010; Sarma, 2011). Maximum accumulation of Cu, Fe, Cr, Pb and Zn was recorded in *Eichhornia crassipes* where as Mn was observed maximum in *Pistia stratiotes*. Therefore as per Table 5 and Figure 2, among four mentioned species *Eichhornia crassipes* may be consider one of the promising species followed by *Pistia stratiotes*, *Vallisneria spiralis* and *Lemna Minor* respectively for phytoremediation of heavy metals from aquatic bodies of Bundelkhand region.

The lakes and reservoirs, all over the study area without exception, are in varying degrees of environmental degradation. The degradation is due to encroachments eutrophication (from domestic/municipal and small scale industrial discharges). There has been a quantum jump in population during the last century without corresponding expansion of civic facilities resulting in lakes and reservoirs, especially the urban ones, becoming sinks for contaminants. Finally it may be concluded that, accelerated eutrophication is a widespread and significant threat to aquatic water bodies of Bundelkhand region. Among the most threatened Lakes are those located in or near urban settlements (Laxhmi and Antiya Lake) which can result in a rapidly increasing nutrient load in Lake because of uncontrolled point and non-point effluent as well as domestic discharges. Thus, although lake ecosystems constitute an essential resource for many ecosystem services and human activities, urban lakes often exhibit serious degradation that interferes with these services and benefits. Present work suggests that, phytoremediation has become an effective and affordable technological solution used to extract or remove inactive metals and metal pollutants from contaminated water bodies. This technology is environmental friendly and potentially cost-effective. Hence, native species of macrophytes like *Eichhornia crassipes*, *Pistia stratiotes*, *Vallisneria spiralis*and *Lemna spp.* were efficient and cost effective for accumulation of heavy metals from contaminated water bodies of this region and they may be recommended as phytoremediator species.

![Fig. 2 Metal accumulation in various plants in response to metal content in water.](image)

Table 5 Average metals concentration in selected aquatic macrophytes grown in the study areas.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Metals</th>
<th><em>Eichhornia crassipes</em> (Mart.) Solms</th>
<th><em>Lemna minor</em> (L.)</th>
<th><em>Pistia stratiotes</em> (L.)</th>
<th><em>Vallisneria spiralis</em> (L.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01.</td>
<td>Cu</td>
<td>0.283 ± 0.002</td>
<td>ND</td>
<td>0.073 ± 0.002</td>
<td>0.052 ± 0.003</td>
</tr>
<tr>
<td>02.</td>
<td>Fe</td>
<td>3.528 ± 0.01</td>
<td>2.758 ± 0.05</td>
<td>2.946 ± 0.05</td>
<td>3.083 ± 0.04</td>
</tr>
<tr>
<td>03.</td>
<td>Cr</td>
<td>1.285 ± 0.04</td>
<td>ND</td>
<td>ND</td>
<td>0.025 ± 0.001</td>
</tr>
<tr>
<td>04.</td>
<td>Mn</td>
<td>0.875 ± 0.006</td>
<td>0.001 ± 0.0001</td>
<td>2.512 ± 0.013</td>
<td>2.409 ± 0.07</td>
</tr>
<tr>
<td>05.</td>
<td>Pb</td>
<td>0.691 ± 0.02</td>
<td>0.190 ± 0.003</td>
<td>0.454 ± 0.006</td>
<td>0.273 ± 0.006</td>
</tr>
<tr>
<td>06.</td>
<td>Zn</td>
<td>3.223 ± 0.007</td>
<td>0.026 ± 0.001</td>
<td>0.302 ± 0.01</td>
<td>0.264 ± 0.002</td>
</tr>
</tbody>
</table>

All values are mg/L (ppm)
Acknowledgement
Authors are thankful to University Grant Commission, New Delhi for financial support to carry out this research work.

References


Impact of Textile and Fertilizer Industry Effluents on Cytology of Root Meristem Cells of *Hordeum vulgare* L. Plant

Pramod Kumar Tandon*, Induja Tripathi and Kumkum Mishra

Department of Botany, University of Lucknow, Lucknow (U.P.), India – 226007

Abstract: Cytotoxic effects of effluents of both sugar and textile industries were studied in the root meristem cells of *Hordeum vulgare* plants. A comparative investigation on the somatic cells have been made on the basis of cytological observations such as mitotic index and other chromosomal abnormalities. Result of the present study indicated that both the industrial effluents have inhibitory effect on mitotic index and induced several chromosomal abnormalities.

Keywords: Cytotoxic effect, Industry effluents, Root meristem, *Hordeum vulgare*, Somatic cells, Mitotic index, Chromosomal abnormalities.

Introduction

Almost all industries generate hazardous wastes. They usually contain complex mixture of chemical and substantially contaminate ground and surface water reservoirs which are used for drinking purpose (Mumtaz, 1995; Dewhurst *et al.*, 2002; Chandra *et al.*, 2005) and might become hazardous to human health by inducing genetic alterations. Plant systems can detect a wide range of genetic damages including gene mutation and chromosome aberration (Maluzynska and Juchimiuk, 2005). Plant roots are extremely useful in biological testing. Therefore, the observation of root tip constitutes a rapid and sensitive method for environmental monitoring. Thus *Hordeum* root tips have been used for this study.

Materials and Methods

Seeds of *Hordeum vulgare* were used as test material which were soaked in tap water for 24 hrs and allowed to germinate when their root length became 1 – 1.5 cm, were transferred to petridishes containing effluents of both textile and fertilizer industry, respectively of different concentration (25, 50, 75 and 100%). After 24 hrs, roots were washed and fixed in fresh and chilled Carnoy’s fluid containing 1:3 acetic acid alcohol and put in refrigerator (4°C). These root tips were stored in 90% alcohol and were used for preparing squashes. Mitotic squash preparation was made by the method of Darlington and La Cour (1976). Data were analysed by analysis of variance (ANOVA) and compared for level of significance by Duncan’s Multiple Range Test.

Results and Discussion

Various concentrations of textile fertilizer industry effluent (25, 50, 75 and 100% each) were used to demonstrate the effect on mitotic index in *H. vulgare* at 24 hr was 6.183 in control whereas after treatment it was 4.148, 3.565, 2.051 and 1.393 at different concentrations of effluent (Table 1), indicating that frequency of chromosomal and mitotic aberration were increased with increase in concentration of effluent. The percentage of chromosomal aberration at 24 hr. It was 3.75, 7.32, 11.54 and 15.00 at different concentrations of textile industry effluent found in control. In the same effluent the percentage of mitotic aberrant cells at 24 hr was 6.870 in control and 5.480, 9.244, 19.700 and 28.570 at different concentration of effluent (Table 1).
While mitotic index in control was 6.183, 5.604, 4.492, 3.856 and 2.321 at various concentration of fertilizer industry effluent showed mitotic index was significantly decreased with increased fertilizer effluent concentration (Table 2). Textile industry effluents also showed chromosomal and mitotic abnormalities such as C-metaphase, bridges laggard, stickiness, unsynchronized and multipolar arrangement of chromosome were observed. However, the frequency of chromosomal and mitotic aberration were increased significantly after treatment with different concentrations of fertilizer industry effluent (Table 2). The percentage of chromosomal aberration was found to be nil in control while 1.25, 1.85, 2.78 and 4.55 at 25, 50, 75 and 100% concentrations of effluent, respectively. The percentage of mitotic aberrant cells at 24 hrs was 0.870 in control and 2.577, 5.263, 14.286, 19.444 at different fertilizer concentrations of effluent. Chromosomal aberrations like break and fragment were observed whereas aberrations such as C-metaphase, bridges, stickiness, unsynchronized, multipolar arrangement of chromosomes were observed (Table 2)

**Table 1** Mitotic index and percentage of different abnormalities in root meristem cells of *Hordeum vulgare* L. treated with different concentrations of textile industry effluent for 24 hour.

<table>
<thead>
<tr>
<th>Effluent concentration (%)</th>
<th>Number of cells scored</th>
<th>Number of dividing cells</th>
<th>Mitotic index</th>
<th>Number of metaphase scored</th>
<th>Chromosomal aberration</th>
<th>Mitotic aberration</th>
<th>Total mitotic aberration</th>
<th>% aberrant cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3720</td>
<td>230</td>
<td>6.183 (±0.002)</td>
<td>-</td>
<td>- - - - - -</td>
<td>1 - 1 - - - 2</td>
<td>0.870</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>3520</td>
<td>146</td>
<td>4.148 (±0.007)</td>
<td>80 2 1 3</td>
<td>1 1 1 1 1 1</td>
<td>8</td>
<td>5.480**</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>3338</td>
<td>119</td>
<td>3.565 (±0.003)</td>
<td>82 4 2 6</td>
<td>5 3 - - 2 1 1 11</td>
<td>9.244***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>3218</td>
<td>66</td>
<td>2.051 (±0.004)</td>
<td>26 2 1 3</td>
<td>3 3 2 1 2 2 13</td>
<td>19.70***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>3016</td>
<td>42</td>
<td>1.393 (±0.002)</td>
<td>20 2 1 3</td>
<td>2 4 2 - 1 3 12</td>
<td>28.57***</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± S.E. (n = 3), One way ANOVA of mitotic index (F = 229460.52*)

* = Significant at p < 0.001

** = Significant from the control p < 0.01 (x^2 test)

*** = Significant from the control p < 0.001 (x^2 test)
Table 2 Mitotic index and percentage of different abnormalities in root meristem cells of *Hordeum vulgare* L. treated with different concentrations of fertilizer industry effluent for 24 hour.

<table>
<thead>
<tr>
<th>Effluent concentration (%)</th>
<th>Number of cells scored</th>
<th>Number of dividing cells</th>
<th>Mitotic index (%)</th>
<th>Number of metaphase scored</th>
<th>Chromosomal aberration</th>
<th>Mitotic aberration</th>
<th>Values</th>
<th>% aberrant cells</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>3720</td>
<td>230</td>
<td>6.183 (±0.002)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1 –</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>3462</td>
<td>5.604 (±0.005)</td>
<td>80</td>
<td>1 – 1</td>
<td>1.25</td>
<td>3 –</td>
<td>5.77</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>3384</td>
<td>4.492 (±0.004)</td>
<td>54</td>
<td>1 – 1</td>
<td>1.85</td>
<td>5 –</td>
<td>8.26</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>3268</td>
<td>3.856 (±0.013)</td>
<td>36</td>
<td>1 – 1</td>
<td>2.78</td>
<td>7 2</td>
<td>14.28</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>3102</td>
<td>2.321 (±0.003)</td>
<td>22</td>
<td>1 – 1</td>
<td>4.55**</td>
<td>8 1</td>
<td>19.44</td>
</tr>
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Values are mean ± S.E. (n = 3), One way ANOVA of mitotic index (F = 50587.99*)

*= Significant at p < 0.001

**= Significant from the control p < 0.05 (x^2 test)

***= Significant from the control p < 0.001 (x^2 test)

Results of the present study indicate that effluent of textile industry can induce greater genotoxicity that effects fertilizer industry effluent. Mitotic index decreased progressively with increased effluent concentrations. Similar findings, remove the were already given by Baskar *et al.* (2002), El-Shahaby *et al.* (2003), Chandra *et al.* (2004), Shobha (2004), Sreekumar *et al.* (2009), Bakare *et al.* (2009).

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**References**


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