“Selected Papers on Physiology”

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<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Paper</th>
<th>Name of author</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>EFFECT OF CADMIUM CHLORIDE ON HISTOPATHOLOGICAL CHANGES IN KIDNEY TESTIS AND OVARY OF THE FRESHWATER FISH OPHIOCEPHALUS STRIATUS (CHANNA)</td>
<td>M. V. Lokhande and U. E. Bais</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>AND OVARY OF THE FRESHWATER FISH OPHIOCEPHALUS STRIATUS (NNA)</td>
<td>A. R. Jagtap and R. P. Mali</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>STUDIES ON THE EFFECT OF GARLIC AND CURRY LEAVES ON THE TOTAL PROTEIN CONTENT OF DIFFERENT TISSUES OF CHANNA PUNCTATUS</td>
<td>A. B. Harkal, R. S. Sonwane, V. Jadhav and R. P. Mali</td>
<td>26</td>
</tr>
<tr>
<td>4</td>
<td>RECOVERY IN LACTATE AND MALATE DEHYDROGENASE FROM MERCURY EXPOSED FRESHWATER FISH CHANNA PUNCTATUS</td>
<td>A. Shaikh, M. Rajyasree and R. P. Mali</td>
<td>32</td>
</tr>
<tr>
<td>5</td>
<td>ANTIBIOTICS INDUCED RESPIRATORY ACTIVITIES ON FRESH WATER FISH CHANNA PUNCTATUS FROM RIVER GODAVARI, DIST. NANDED.</td>
<td>S. D. Kothole, R. P. Mali and S. K. Afsar</td>
<td>41</td>
</tr>
<tr>
<td>6</td>
<td>NUTRITION FOR FISH HEALTH</td>
<td>S. O. Bondhare and R. P. Mali</td>
<td>45</td>
</tr>
<tr>
<td>7</td>
<td>DETERMINATION OF BLOOD UREA (SERUM)</td>
<td>M. V. Lokhande and G. D. Gore</td>
<td>58</td>
</tr>
<tr>
<td>Page</td>
<td>Title</td>
<td>Authors</td>
<td>Number</td>
</tr>
<tr>
<td>------</td>
<td>----------------------------------------------------------------------</td>
<td>----------------------------------------</td>
<td>--------</td>
</tr>
<tr>
<td>8</td>
<td>ANALYSIS OF HAEMOGLOBIN AND BLOOD CELL COUNT IN FRESH WATER FISH-LABEO ROHITA</td>
<td>N. R. Jaiswal and M. S. Kadam</td>
<td>65</td>
</tr>
<tr>
<td>9</td>
<td>EFFECT OF SYNTHETIC PESTICIDES ON AREAL RESPIRATION OF PERIPLANETAMERICA</td>
<td>J. T. Gawali, P. S. Managoli and P. B. Deshmukh</td>
<td>68</td>
</tr>
<tr>
<td>10</td>
<td>EFFECT OF FERTILIZERS UREA &amp; DAP ON RATE OF OXYGEN CONSUMPTION OF THE CRAB, BARYTELPHUSACUNICULARIS</td>
<td>M. Maqdoom and M. H. Mujewar</td>
<td>72</td>
</tr>
<tr>
<td>11</td>
<td>EFFECT OF DETERGENT SURF ON OXYGN CONSUMPTION BY BARYTELPHUSACUNICULARIS (WEST WOOD)</td>
<td>M.H. Mujewar and A. B. Harkal</td>
<td>78</td>
</tr>
<tr>
<td>12</td>
<td>SEASONAL METABOLIC VARIATION OF GLYCOGEN CONTENT IN FRESHWATER BIVALVE LAMELLIDENS CORRIANUS (LEA, 1834)</td>
<td>S.K.Padewar, R.P.Mali and L. M. Mudkhede</td>
<td>80</td>
</tr>
<tr>
<td>13</td>
<td>METALLIC IMPACT ON</td>
<td>R. P. Mali, U.</td>
<td>85</td>
</tr>
<tr>
<td>No.</td>
<td>Title</td>
<td>Author(s)</td>
<td>Page</td>
</tr>
<tr>
<td>-----</td>
<td>-----------------------------------------------------------------------</td>
<td>-----------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>14</td>
<td>BIOCHEMICAL ALTERATION ON PROTEIN AND AMINO ACID CONTENT OF MARINE CRUSTACEAN, SCYLLA SERRATA FROM WEST COAST OF INDIA.</td>
<td>M. Jayabhaye and N. G. Nagrale</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>SIMULATION OF GLYCOLYSIS USING MLP</td>
<td>Mohseena Thaseen</td>
<td>90</td>
</tr>
<tr>
<td>16</td>
<td>EFFECT OF MSG ON HUMAN HEALTH</td>
<td>S. A. Quadri, T. T. Shaikh and J.D. Shaikh</td>
<td>98</td>
</tr>
<tr>
<td>17</td>
<td>STUDY OF BODY MASS INDEX (BMI) STATUS OF SOME STUDENTS FROM NANDED (MAHARASHTRA)</td>
<td>M. K. Malviya</td>
<td>103</td>
</tr>
<tr>
<td>18</td>
<td>A COMPUTATIONAL-EXPERIMENTAL APPROACH FOR COMPUTATIONAL MODELING OF MITOCHONDRIAL METABOLISM</td>
<td>Mohseena Thaseen and M.M.V.Baig</td>
<td>109</td>
</tr>
<tr>
<td>19</td>
<td>PRODUCTION OF CELLULOLYTIC ENZYMES BY IMMOBILIZED CELLS.</td>
<td>S.S.Ingle and M.M.V.Baig</td>
<td>116</td>
</tr>
<tr>
<td>20</td>
<td>DETERMINATION OF SODIUM CONTENT FROM MANGIFERA INDICA PLANT FROM NANDED CITY</td>
<td>S.U. Sabry and A. B. Bhosle</td>
<td>127</td>
</tr>
<tr>
<td>21</td>
<td>ANALYSIS OF HEAVY METALS CONCENTRATION IN WATER AND SEDIMENT FROM BORI RIVER, NALDURG MAHARASHTRA.</td>
<td>A.D. Babare, R.R. Jadhav, R. M. Gochde and M.G. Babare</td>
<td>132</td>
</tr>
</tbody>
</table>
Effect Of Cadmium Chloride On Histopathological Changes In Kidney Testis And Ovary Of The Freshwater Fish Ophiocephalus Striatus (Channa)

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ABSTRACT:

Histopathological changes were observed in the kidney, testis and ovary of Ophiocephalus striatus exposed to the cadmium chloride. The fishes were exposed to median lethal concentration of 0.63 mg L\(^{-1}\) at 96 h. The histopathological change due to the toxicity of cadmium chloride in experimental kidney of fish in experimental (LC\(_{50}\)) at 96 hrs nephrotoxic lesions includes hydropic degeneration in uriniferous tabule, hyperchromatic nuclei & degeneration of glomerulus tubule observed in kidney. In experimental testis of fish (LC\(_{50}\)) at 96 hrs. necrosis of lobular (tubular) boundary, hemorrhage, & not visible of spermatogonium in the cells is observed in the testis. In experimental ovary of fish decreased frequency of ooyte maturation possibly due to the increased serum urea level in present work of hematological studies so it is clear that cadmium can indirectly affects gonadial organs of fishes. Also similar kinds of dysplasia result is observed in the ovary of experimental (LC\(_{50}\)) at 96 hrs fish.

Key Words: Cadmium chloride, kidney, testis and Ophiocephalus stratus.
INTRODUCTION:
Histopathology is promising field for research in aquatic toxicology as it provides the real picture of the toxic of xenobiotics in vital functions of a living organism.
[1] The extent of histopathological damages induced in the Tet fish and the amount of cell damages in relation to concentration of toxicants are utilized in assessing the toxicity of pollutants.
[2] Couch (1975) stated that gill, liver, intestine and kidney of fishes are best suited organs for histopathological studies.
Cadmium is naturally released to the environment from volcanic sources (some of 60% of total natural emission) and along with rain water it reaches up to the water bodies.
[3] Small amount of cadmium minerals are also associated with lead minerals. Cadmium occurs in earth crust along with zinc, lead-zinc compounds, lead zinc-copper ores. It is usually found as cadmium sulphide. The average concentration in earth curst is about 0.2 ppm cadmium compounds such as cadmium oxides, carbonates, sulphide and hydroxide are insoluble in water but cadmium flourides, bromide, chlorides, iodide, nitrate and sulphate are particularly water soluble. Cadmium is used for electroplating of metals.
[4] The histopathological studies on fish revealed that various toxicants produce pathological changes kidney, testis and ovary also seen toxic-teratological abnormalities. The objective of the present study is to highlight various histopathological changes in kidney, testis and ovary of Ophiocephalus striatus exposed to cadmium chloride for 96 h.

MATERIALS AND METHODS
Healthy test fish Ophiocephalus striatus weights 100 g and total length 20 to 25 + cm were selected and exposed to sublethal concentration (LC$_{50}$ 0.63 mg L$^{-1}$ at 96 h) of cadmium chloride. All the fishes were dissected out on the 4th day (96 h) at median lethal (LC$_{50}$) concentration of cadmium chloride along
with control. Gill, liver, stomach and intestine of experimental (LC₅₀) and control at 96 h fishes were separated and immediately transferred into 10% buffered formaldehyde solution. At the end of experiment fish was taken out, sacrificed and the tissue of kidney, testis and ovary excised out. The tissues were fixed in Bouin’s fluid and then they were processed by the usual procedure and embedded in paraffin wax. The tissues then processed for clearing by using xylene and transferred to molten paraffin (58-60°C). Prepared blocks for tissue holding section was done at 3 micron on rotatory microscope-after staining. Haematoxylin and eosin staining given nuclei in blue colour stain and rest tissues parts were identifying by pink colour staining.

**Mounting:**

Each individual stained tissue slide was mounted with paraffin. Place one drop of paraffin on cover slip and glass slide were reverse in position kept for drying for one day. These mounted slides of individual slide were used for microscopic examination.

**Microscopic examination:**

All the tissues microscopic view taken at high-resolution power with the help of Panasonic 7 megapixel digital camera. All the slides were observed under low and high resolution for their histological findings.

**Histological Study Of Kidney:**

The functions of excretion & osmoregulation are closely related and are performed by gills and kidneys in fishes. Kidney plays the most important part in the excretion of nitrogenous wastes & in maintaining the water-salt balance (homeostasis). Kidney perform vital functions by excreting excretory products, also it plays role in maintenance of internal environment. In addition of this excretory function kidneys perform many other functions like homeostasis maintenance of water balance, maintenance of electrolyte balance, maintenance of acid-base
balance, hemopoietic function endocrine function, and regulation of blood pressure & regulation of blood calcium level.

The kidney of fresh water fishes may also serve as an important excretory organ for minor nitrogenous compounds such as creatine creatinine & uric acid. So that histological assessment of kidney is very important diagnostic tool for assessment of health status of fishes.

**Histological Study Of Testes:**

Fishes reproduce by several methods are generally bisexual reproductive organs of male fish consist of a pair of tests which are elongated and flattened structure situated in either side ventral to the kidney in the posterior region of abdominal cavity. The tests remain attached to the body wall & the air bladder by means of mesorchia. Reproductive system is unique because it ensures the continuation of the species. Continuation of the generation depends only on the reproductive system.

Along with reproductive function of tests it performs gametogenic function as well as endocrine function in the fishes. So that assessment of histology of tests is a good diagnostic tool for checking the normal reproductive capacity of the organisms.

**Histological Study Of Ovary:**

Ovaries are the female reproductive organ which is paired elongated sac-like structure lying in the abdominal cavity ventral to the kidney. They are attached to the body wall by means of the mesovarium.

Both male and female fish gonads undergo marked cyclic morphology & histological changes before reaching full maturity & becoming ripe. The expulsion of gametes from the body in to the surrounding water is called spawning resulting in fertilization.

The histological assessment of ovary in fishes is very important diagnostic toll for checking reproductive capacity as well as the health status of female fishes. From the histological
observations we are concluded the reproductive capacity of the female fish & as well as the future fish production.

RESULTS:

In control fish normal renal corpuscle with glomerulus, normal structure of uriniferous tabule & proximal tubule is observed in kidney (fig:-1) where as in experimental (LC$_{50}$) at 96 hrs nephrotoxic lesions includes hydropic degeneration in uriniferou stabule hyperchromatic nuclei, & degeneration of glomerulus tubule observed in kidney. (fig:-2). In control fish at 96 hrs normal lobular(tubular) structure with spermatiogonium is observed in the testes (fig-3) where as in experimental (LC$_{50}$) at 96 hrs. necrosis of lobular (tubular) boundary, hemorrhage, & not visible of spermatogonium in the cells is observed (fig-4) in the tests.

In control fish at 96 hrs young oocytes mature oocytes & normal structure of wall of ovary is observed in the overy (fig-5) where as in experimental (LC50) at 96 hrs. fish karyolysis of ova, decreased frequency of oocyte maturation & dysplasia is observed (fig-6) in the overy.

DISCUSSION:

Gupta Ashok Kumar and Kumar Ashwini (2006) reported that degeneration and necrosis of glomerulus intestinal tissue & epithelium lining of renal tubule in the kidney of *Cirrhinus mrigala* fingerlings sub lethal exposed to mercury. Also concluded that degeneration in glomerulus poss0ibly leads to the reduced efficiency of kidney function & subsequently increased excretory products in the body. In present study degeneration of glomerulus possibly leads to the decreases the functional ability of kidney, for this reason excretaory nitrogenous products possibly increases in the body of experimental (LC$_{50}$) at 96 hrs fish. In chronic nephritis as well as nephrotoxic lesions of kidney may rises creatinine & urea level in the body Kidney failuare (renal dysfunction) is the
principle reason for increment in nitorgeous waste products in the body. Abdus salam bhuiyan & Badrunesa reported that vacuolation degeneration in kidney tubale, necrosis & hemorrhage in the kidney of spotted murrel *Channa punctatus* sub-lethal exposed to sumithion. Dubale and Shah mentioned the marked degeneration in the kidney of *Channa punctatus* exposed to cadmium concentration of 0.01, 0.03 and 0.05 ppm for period of 1-51 days.


Dhanpakiam and Premlata (1994)[14] observed hypertrophy of renal cells necrosis and degeneration of renal components in case of *Cyprius carpio* fingerlings on the effect of LC 50 (90 hrs) concentration of malathion & sevine, the kidney damage was severe with malation treatment than sevine. The teleostean kidney is one of the first organ to be affected by contaminant in the water.

Most common alteration found in the kidney of fishes exposed to water contamination are tubule degeration (cloudy swelling & hyaline droplets) and changes in corpuscle, such as dilation f capillaries in the glomerulus and reduction of bowman’s space (Takashima and Hibiya 1995).

Eisler and Gardner (1973) [9] reported that nephrotoxic lesions, including degenerative changes in tubular epithelium (cytoplasmic
vacuolation hydropic degeneration hyperchronatic nuclei) dilation of tubular lamina, tubular necrosis in the kidney of esturine teleostean fish exposed to the mixture of cadmium, copper and zinc salts. Above similar kinds of results is mentioned in case of kidney are Baker (1969), Bhatnagar and Shrivastava (1975), Trump et al., (1975) [15,16].

Nath and Kumar (1990)[17] investigated the histological impact of sub-lethal concentration of nickel on the gonads of both sexes of the fresh water tropical fish *Colisa fasciatus* following 96 hr exposure to 64 mg/L of nickel sulphate. Histological sections revealed degeneration of the germ cells in the testicular lobules, reduced spermatogenic activity, rupture of testicular lobule (tubule). They also concluded that reduced spermatogenesis is possibly causes the dysfunction of sex hormones & possibly leads to the severe problems in reproductive capacity of the fishes. Above similar kinds of results observed in experimental (LC$_{50}$) at 96 hrs in present study necrosis of lobular boundary & reduction in spermatogonium possibly leads to the severe problem in the reproductive capacity. It is very well documented that the role of hormones in the spermatogenesis. Follicle stimulating hormone is responsible for the initiation of spermatogenesis it binds with sertoli cells and spermatogonia and induces the proliferation of spermatogonia Estrogen formed from testosterone in setrtoli cells & necessary for the spermatogenesis.

In present work reduction in the spermatogonium & proliferation possibly due to the hyposecretion of testosterone & estrogen due to the cadmium toxicity. Ruby et al., (1979)[18] observed the reduced state of sperm development by damaging spermatogonia in juvenile male *Rainbow trout* exposed to the concentration of 0.01 & 0.03 mg/L of hydrogen cyanide at 12.5°C temperature also concluded that damaging the spermatogonia is possibly inhibition of proliferation. Shukla and Pandey (1984)[19] observed the rupture of testicular lobules
in the tropical perch exposed to 2.0 and 14.0 mg/lit. concentration of arsenic oxide for 15 and 30 days. Pundir and Saxena (1990) [20] reported damage of testicular tissue by hemorrhage & necrosis of Silver barb exposed to 26 mg/lit. of cadmium acetate for 96 hrs. period. Above similar kinds of result is observed in experimental (LC$_{50}$) at 96 hrs testes of fish in the present work. Sangalang and Halloran (1972) reported the general atrophy hypospermia (Lower mean index of spermatogenic development) necrosis of tubular boundry cells with hemorrhage congestion necrosis of primary germ cells, atrophy of seminiferous tubule & fibrosis is observed the testes of Brook trout exposed to cadmium. Walsh and Ribein (1975) [20] mentioned the atrophy hypospermia necrosis of tubular boundry hemorrhage congestion necrosis of primary germ cells atrophy of siminiperous tubules in the testes of fishes due to the pesticide poisoning. Tafanelli and summerfelt (1975) stated that exhaustion atrophy general atrophy hypospermia hemorrhage, vasodilatation congetion necrosis of primary germ cells with atrophy of somniferous tubule fibrosis in the tests of Gold fish exposed to cadmium.

From above discussion & present investigation it is clear that cadmium causes very adverse effects on the tests of fish & possibly responsible for the hypospermia and reproduction inability in the fishes.

Lesniak and Ruby (1982) reported the decreased maturation of oocyte i.e. reduced vitellogenesis in the ovary of Rainbow trout exposed to 0.01mg/lit. and 0.02 mg/lit. hydrogen cyanide for 20 days at $10^0C$. Also concluded that hydrogen cyanide probably inhibit the synthesis of female hormone due to which cellular proliferation & tissue growth is inhibited. Tafanelli and summerfelt (1975) observed hyperplasia of germinal epithelium, involution some ova, decreased frequency of oocyte maturation cytopasimc clumping fragmentation & Karyolysis of ova in the ovary of Gold fish exposed to cadmium.
Sivarajah *et al.*, (1978) reported the hyperplasia of germinal epithelium involution of ova decreased frequency of oocyte maturation Karyolysis of ova in the ovary of *Salmo gairdneri* exposed to Archlor. Abdus and Badrunesa (2001) stated that fragmented ova with abnormal shape arrangement were observed in spotted murrel, *Channa punctatus* exposed sub lethal to insecticides sumition also reported the complete breakage & dissolution of ovarias lamellae.

Jha and Jha (1994) [22] reported the impact of 30 days exposure to sub-lethal concentration of urea (416ppm) & ammonium sulphate (448ppm) on the ovary of *Heteropneustus fossilis* urea induced initial stimulation of vitellogenesis followed by subsequent arrest of ovarian growth also urea ammonium sulphate produce severe adverse effects as evident from large number of early non-vitellogeoic oocytes and traces of degenerated oocytes.

In present work decreased frequency of oocyte maturation possibly due to the increased serum urea level in present work of hematological studies so it is clear that cadmium can indirectly affects gonadal organs of fishes. Also similar kinds of dysplasia result is observed in the ovary of experimental (LC₅₀) at 96 hrs fish.

Eller (1971) [23] reported the hyperplasia of germinal epithelium involution of some ova decreased frequency of oocyte maturation cytoplasmic clumping fragmentation & karyolysis of ova in the ovary of *Cutthroat trout* exposed chronically to the insecticide endrin.

In present investigation the karyolysis of ova decreased frequency of maturation of oocytes & desplasia is observed in experimental (LC₅₀) at 96 hrs, so it is evident that cadmium effects those sex hormones which are responsible for the maturation of ova in the ovaries of female fish.

14 | Selected Papers on Physiology
Spehar (1976) found in *Jordanella floride* that reproduction was more sensitive towards cadmium toxicity than was embryo or larval survival while cadmium at concentration higher than 0.757 mg/lit inhibited spawning in the blue gill *Lepomis macrochirus* (Eaton J.E. 1974) [24] with spawning at lower exposure concentration being erratic.

Brown et.al., (1994) [25] found that continuous exposure of rainbow trout adults to cadmium concentration of 5.5 µg/lit didn’t affect their growth, but eggs obtained from *Rainbow trout* exposed to 1.8 and 3.4 µg/lit failed to develop to the fry stage. They also noted that oogenesis appeared to be delayed in *Brown trout* exposed to 9.3 and 29.1 µg/lit cadmium.

**CONCLUSION**

In experimental (LC$_{50}$) at 96 hrs of fish degeneration in uriniferous tubule, hyperchromatic nuclei and degeneration of glomerulus tubule is observed (fig:-2). Degeneration of glomerulus and uriniferous tubule possibly caused the decreased functional ability of kidney due to these reason excretory nitrogenous products (serum urea and serum creatinine) level is significantly increases in experimental fish in present investigation of haematological studies.

In experimental (LC$_{50}$) at 96 hrs. testes of fish necrosis of tubular boundry, hemorrhage and absence of spermatogonium is observed (fig:-4). Hemorrhagic condition possibly reason for the reduced RBC count and reduced blood hemoglobin in the experimental fish of present investigation of haematological studies. Depletion of spermatogonium in the cells possibly leads to reduction in mature sperm and subsequently severe problem in the reproduction of fish. Also reproduction problem severely influenced the future production of fish.

In experimental (LC50) at 96 hrs. ovary of fish karyolysis of ova, decreased frequency of oocyte maturation and dysplasia is observed (fig:-6). All these conditions of above mentioned possibly create severe reproduction problems in
future of female fish. Decreased frequency of oocyte maturation possibly leads to unavailability of mature ova in future and subsequently affects on the future fish production. From above overall summery and conclusion it is clear that very trace quantity of cadmium caused the severe biochemical, haematological and histological changes in fresh water fish *Ophiocephalus (channa) striatus* and severely affects the normal metabolism of fish.

**ACKNOWLEDGMENT**

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Influence Of Sea Water On Respiratory Metabolism Of Fresh Water Fish, *CHANNA PUNCTATUS*, Godavari River, Nanded (M.S.)

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ABSTRACT
The present project work showed the study of effect of artificial sea on exposure to artificial sea water on the fish, *Channa* water on the respiratory metabolism of fresh water fish, *Channa punctatus*. The respiratory activity was studied by the estimation of total oxygen consumption and rate of oxygen consumption. The animals were exposed up to 96 hours of period of exposure. The obtained results were compared with the animals of control set. The respiratory activity was found to be declined *punctatus*. The obtained data was statistically analyzed and plotted in the form of graphs.

Key Words: Sea Water, Total Oxygen Consumption, Rate of Oxygen Consumption, *Channa punctatus*.

INTRODUCTION
Respiration is an essential physiological activity in all living organisms as oxygen is necessary to provide energy for life processes and for carrying out all other metabolic activities.

The pollution of water threatens the living systems and aquatic environment. The primary source of pollution is waste waters containing toxic substances in the form of pesticide residue, heavy metal salts, oils etc. as reported by Akberali *et al*. (1981). Modern civilization with rapidly growing industrial units, increase in the population, has lead to an accelerate
degradation of the fresh water resources. The pollutants are likely to affect the biological systems in different ways according to the ir chemical properties. The sum of physiological changes created particular pollutants is likely to be characteristics of these pollutants. Thus by observing the effects of polluted water and a set of physiological parameters. It might be possible to establish specific responses of that pollutant. From this it is easy to identify a pollutant on the basis of its physiological effect pattern.

Fishes are adapted for aquatic respiration, during which they take water in, through the mouth and passed through gill chambers covered by the operculum. The flow of water is continuous for almost the whole of the respiratory cycle. In its passage, the water gives up oxygen to the blood and takes away the carbon dioxide through diffusion. The process of oxygen is transported in the circulating fluid by hemoglobin present in the blood corpuscles. Oxygen uptake is widely used in physiology as a biological indicator that integrates the overall metabolic activity of an animal in response to specific environmental factors because it reflects energy expenditure and ultimately the food requirements. The metabolic rate of fish is usually measured by their rate of oxygen uptake from water.

The objectives of the present study are to evaluate the effect of artificial sea water on oxygen consumption on freshwater fish i.e. *Channa punctatus*. Respiration is an endless oxidative process in a living animal resulting in consumption of O₂ and production of CO₂. Therefore the calculations of oxygen consumed especially with reference to energy utilization by freshwater fishes can be expected throw lighten the physiological mechanism in animals.

**MATERIAL AND METHODS**

The freshwater fishes i.e. *Channa punctatus of* medium size were collected from Godavari River, Nanded. The fishes were acclimatized in the laboratory for 2-3 days prior to experiment. The fishes were feed with pieces of bivalve and earthworm. Feeding was stopped one day before the commencement of experiment. The fishes were divided into two sets I and II. Set II fishes were maintained as a control. The set I
were exposed to artificial sea water. The fishes between 40-50 gms were selected for present experiment. The oxygen consumed by fishes in each set was examined by keeping the fish in respiratory chamber. The weights of animals were noted at each time. The sets were continued for 24 hrs, 48 hrs, 72 hrs & 96 hrs.

**Estimation and measurement of oxygen consumption**

Oxygen consumption of the fishes in each set was measured by the method of Winkler as described by Welsch & Smith (1953). The fishes were weighed and placed in Winkler’s chamber and care was taken to make it air tight and free from leakage of water. The fish was allowed to stabilize in the chamber for few minutes. After few minutes water was collected into narrow bottle and dissolved oxygen was estimated by Iodometry method. After one hour the next sample was estimated in the same way. The difference between initial and final sample will give the actual oxygen consumed by the animal and expressed as oxygen consumption ml/hr/gm body weight of fish.

**RESULTS**

The rate of respiration of freshwater fish, *Channa punctatus* has been found altered when exposed to artificial sea water. The presence of different elements and heavy metals discharged into water resources cause hazardous effect on aquatic life. The uptake of oxygen in the present experiment was measured till the end of experiment i.e. up to 96 hrs periods of exposure. The results were compared with the values obtained for the total oxygen consumption and rate of oxygen consumption throughout the experiment.

The obtained values were compared with control group of animals in each set. The total oxygen consumption and rate of respiration by the control group of animals is 0.0156 ml/lit and 0.0117 ml/lit/hr respectively. The total oxygen consumed by the fresh water fish for 24 hr (0.0016), 48 hr (0.0028), 72 hr (0.015), and 96 hr (0.012) upon exposure to artificial sea water. The rate of oxygen consumption was found to be at 24 hr (0.0035), 48 hr (0.0058), 72 hr (0.0031) and 96 hr (0.0026).
From the above result it reveals that the total oxygen consumption and rate of oxygen consumption was found to be decreased suddenly at 24 hrs periods of exposure and later increased up to 72 hrs exposures and finally deceased. The same trend was observed in the rate of oxygen consumption. The values obtained in tabular form are as follows-

**Observation Table: Effect of artificial sea water on respiratory metabolism of fresh water fish, *Channa punctatus***

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Exposure Period (hours)</th>
<th>Total Oxygen Consumption (ml/lit)</th>
<th>Rate of Oxygen Consumption (ml/hr/lit/gm wet wt. of fish)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control</td>
<td>0.0156 ± 2.24 ml/lit</td>
<td>0.0117 ± 2.38 ml/lit/hr</td>
</tr>
<tr>
<td>2.</td>
<td>24 hours</td>
<td>0.0016 ± 1.78 ml/lit</td>
<td>0.0035 ± 2.44 ml/lit/hr</td>
</tr>
<tr>
<td>3.</td>
<td>48 hours</td>
<td>0.0028 ± 2.27 ml/lit</td>
<td>0.0058 ± 1.28 ml/lit/hr</td>
</tr>
<tr>
<td>4.</td>
<td>72 hours</td>
<td>0.0150 ± 1.44 ml/lit</td>
<td>0.0031 ± 2.16 ml/lit/hr</td>
</tr>
<tr>
<td>5.</td>
<td>96 hours</td>
<td>0.0129 ± 2.70 ml/lit</td>
<td>0.0026 ± 1.83 ml/lit/hr</td>
</tr>
</tbody>
</table>

(Each value is a mean of five observations ± Standard Deviation)

**DISCUSSION**

The change in rate of oxygen consumption is a good index of the metabolic capacity of an organism to face environment stresses. It is evident from the result of present study that the metallic pollutants exert influence by affecting the rate of oxygen consumption. The interpretation of metal induced changes in a respiration is complicated and varies from metal to metal and from species to species and From one experimental condition to other. The alteration in the normal respiratory metabolism is due to its intimate contact with polluted water which decrease the oxygen diffusing capacity of the gills. Metal
effects can be described as alterations of the biochemistry of the different sub cellular organelles.

Water resources are said to be polluted, when because of man’s activity in adding or causing the addition of matter to the water or altering the temperature, the physical, chemical or biological characteristics of the water are changed such an extent that its utility for any reasonable purpose or its environmental value is demonstrably depreciated. The higher concentration of toxicants brings about the adverse effects of an freshwater fishes which causes gill damage, skin of fishes, lack of availability of natural food to fishes, depletion of dissolved oxygen, reduction in maturation of oocytes, necrosis of seminiferous tubules and hypertrophy of cell etc.

Dissolved oxygen decrease due to mixing of sewage into river contains salts, minerals, heavy metals etc. From the above result it reveals that the total oxygen consumption and rate of oxygen consumption was found to be decreased suddenly at 24 hrs periods of exposure and later increased up to 72 hrs exposures and finally deceased. The pollutants induced changes in a respiration is complicated vary from metal to metal & from species to species & from one experimental condition to other.

Thus, from this it was clear that the artificial sea water affects the respiratory metabolism in fish life directly or indirectly. The artificial sea water salts, metals, minerals, pollutants etc. in water is highly vulnerable to pollution. The extent of damage depends on the quality and quantity of the pollutants and the species of fish. The present investigation shows the decreased rate of oxygen consumption when exposed to artificial sea water which is due to depletion of dissolved oxygen content of water and increase in BOD. The decrement may be due to the respiratory distress as a consequence of the impairment of oxidative metabolism. The pollutants also cause the damage of mucus membrane of the gill which directly affects the rate of respiration in freshwater fishes.
REFERENCES


Studies on The Effect of Garlic And Curry Leaves on The Total Protein Content of Different Tissues of CHANNA PUNCTATUS

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⁴Dept. of Zoology, Yeshwant Mahavidyalaya, Nanded.

ABSTRACT:

The present study deals with the effect of garlic and curry leaves on the fresh water fish channa punctatus was assessed the 96 hrs. LC50 of garlic and curry leaves were 15 mg/lit and 25 mg/lit. Respectively. The fresh water exposed to the experimental concentration of garlic and curry leaves for 96 hours. After the control 96 hrs. Exposures the total protein content were estimated in the muscle, kidney and heart. The result showed that the decrease in the total protein content in all experimental organs as compared to curry leaves.

Key word: - Total protein content, Garlic, Curry Leaves, Channa Punctatus, aqueous extract.

INTRODUCTION:

Proteins play a vital role in the biological functions and are, hence aptly called the building blocks for cellular components. In fish, proteins are the primary energy source and are involved in regulating physiological and metabolic processes in the body through hormones, enzymes etc., They play a vital role as energy precursors in fishes exposed to stress conditions (Ramalingam, 1980 Jones Nelson and Sunil Kumar, 1996; Anitha Kumari and Sree Ram Kumar, 1996;).
Proteins are of the most important and complete group of biological material comprising of nitrogenous constituents of the body and performing different function. Proteins are involved in several major physiological events. Therefore the assessment of protein content can be considered as diagnostic tool to determine the physiological phases of organisms as well as their health status.

Protein is an important constituent of all the cells and tissues which play vital role in the physiology of living organism. The fishes have little carbohydrate. Hence protein is used to meet the increased energy demand. Proteins are mainly involved in the architecture of the cell, which is the chief source of nitrogenous metabolism. Protein as one of the main sources of energy (Umminger, 1977) and it plays an important role in the maintenance of blood glucose (Jrueger et al 1968). It is the most fundamental and abundant biochemical constituent present in the animal body and the estimation of protein is considered to be important (Ravichandran et al 1994). Mule and Lomte (1995) have reported that the protein content of an animal is an important organic constituent, which plays a major role in cellular metabolism. The animal tissue protein level have a significant value which may show changes as per the biochemical condition of an organism. The tissue protein levels are determined by their rate of synthesis and degradation, and both these processes are well regulated (Segal, 1976) while alteration in degradative and synthetic rates have been reviewed (Sanchee, E. 1967).

**MATERIAL METHOD:**

The fishes, *Channa punctatus* were used for experimentation and collected from Godavari river district Nanded. They were maintained in glass aquaria and acclimated for ten days. Only healthy fishes ranging between 80 ± 2 Gms in weight and 20 ± 2 cm in length were selected for present work. They were subjected to sublethal concentration of garlic extract (15mg/lit) and curry leaves extract (25mg/lit).
Preparation of aqueous garlic extract:

The cloves of garlic (*Allium sativum*) and curry leaves (*Morraya koingii*) were collected from local market of Nanded city. Plant material was dried and grind to prepare the aqueous extract. Protein estimation was studied after exposure at 96 hours. Estimation of total protein was done by using Lowry method (Lowry et al; 1951) and values of proteins were expressed in terms of mg protein/gm wet weight of tissue.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Tissue</th>
<th>Control Value Garlic 96hrs mg/gm wt.wet</th>
<th>(LC50) Value Garlic 96hrs mg/gm wt.wet</th>
<th>(LC50) Value of Curry Leaves 96hr at mg/gm wt.wet of tissue</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Muscle</td>
<td>146.50 + 3.62</td>
<td>141.50 + 3.39</td>
<td>143.67 + 3.33</td>
</tr>
<tr>
<td>2</td>
<td>Kidney</td>
<td>88.00 + 3.74</td>
<td>75.50 + 2.74</td>
<td>84.33 + 3.67</td>
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<tr>
<td>3</td>
<td>Heart</td>
<td>80.33 + 3.98</td>
<td>68.67 + 3.78</td>
<td>72.83 + 5.31</td>
</tr>
</tbody>
</table>

Table: 1 Levels of Protein content in different tissues of *Channa punctatus* exposed to median lethal (LC$_{50}$) at 96 hour concentration of Garlic extract and curry leaves extract. Values are mean± SD of six samples *P<0.005, **P<0.001, ***P>0.01 significant when student ‘t’ test was applied between control and experiment groups.

**RESULT:**

The total protein content in muscle, kidney and heart of *Channa punctatus* exposed to LC$_{50}$ at 96 hour i.e. median lethal concentration of garlic and curry leaves has been given in Table(1). The Total protein content in muscle, kidney and heart of controlled *Channa punctatus* were 146.50, 88 and 80.33 mg/gm wet wt. of tissue respectively. While in Garlic extract exposed fish, the amount of total protein in tissues like muscle, kidney and heart were 141.50, 75.50 and 68.67 mg/gm wet wt of tissue respectively.
The curry leaves extract exposed fish showed the content of total proteins of various tissues such as muscle, kidney and heart were 143.67, 84.33 and 72.83 mg/gm wet wt. of tissues respectively.

Their was more decrease in total protein content of garlic aqueous extract exposed *Channa punctatus*. As compared to curry leaves exposed fish.

**DISCUSSION:**

Present investigation focused on the total protein content in different organs like muscle, kidney and heart of *Channa punctatus*. The total protein content in heart, muscle and kidney were gradual decreased in aqueous extract of garlic and aqueous extract of curry leaves exposed *Channa punctatus*.

[1] suggest that tobacco leaf dust caused a stress-induced effect on protein synthesis which must have led to the depletion in the serum protein. The decrease in serum total protein level suggested high protein hydrolytic activity due to the elevation of protease activity [2].

[2] reported decrease in serum total protein in snake head fish (*Channa punctatus*) exposed to sub lethal concentrations of lattices of *Euphorbia royleana*. [3] reported inhibition in the total serum protein of an air breathing fish *Heteropneustes fossilis* after exposure to different pesticides (DDT, YBHC and Malathion).

[4] reported depletion of protein due to proteolysis after exposing *Oreochromis mossambicus* to nominal concentrations of phenol. [5] pointed out that the decreased protein content might also be attributed to the destruction or necrosis of the cells and consequent impairment in protein synthesis machinery. The quantity of protein is dependent on the rate of protein synthesis, or on rate of its degradation. This may be affected by incorporating the impaired amino acids into polypeptide chains (Ram et. al., 2003). The depletion of protein fraction in liver and kidney tissues may have been due to their degradation and possible utilization for metabolic purpose which may indicate liver and kidney dysfunction (Fafioye et. al., 2005). The depletion of tissue protein-pesticides stress influences the
conversion of tissue protein into soluble fraction reacting in the blood for utilization. The significance reduction in liver and kidney total protein may be due to increase energy demand during stress/or altered enzymatic activities (Lettet et al., 1976).

Fayez A. B et al., (2011) reported that the Impact of methanol extract of *Adenium obesum* plant on some biochemical and biological parameters of *Bulinus truncatus* snails and he found the protein content in haemolymph and tissues of treated snails was greatly reduced than in untreated control snails. Sharma D. K. and Ansari B. A.(2011) studied effect of deltamethrin and a neem based pesticide achook on some biochemical parameters in tissues liver, ovary and muscle of zebra fish *Danio rerio* (Cyprinidae) the changes in total protein, total free amino acid content and in the liver, ovary and muscle of Zebrafish after exposure to 96 h LC5, LC10 and LC20 of deltamethrin and neem based pesticide Achook. It was found that the protein content was reduced to 45, 68, 65% (after deltamethrin exposure) and 54, 81, 85% (after achook exposure) as compared to the controls (100%) after 16 days in the liver, ovary and muscle, respectively at LC20 exposure.

Sarawoot P. et al., (2011) reported that the protective efficiency of *Thunbergia laurifolia* leaf extract against lead nitrate-induced toxicity in *Oreochromis niloticus*. He observed the gradual depletion in total protein content similar findings by (Evans et al., 2004)

Even through the protein contents were increased in various concentrations under the influence of different plant extracts.

Samson E. Abalaka et al.,(2011) reported that the evaluation of biochemical changes in *Clarias gariepinus* adults exposed to aqueous and ethanolic extracts of *Parkia biglobosa* pods. However, despite an increase in plasma total proteins concentrations with increasing extracts concentrations in fish exposed to aqueous extracts of *Parkia biglobosa*.

Velisek J. et al., (2009) reported that the Effects of acute exposure to bifenthrin on some haematological, biochemical and histopathological parameters of rainbow trout (*Oncorhynchus mykiss*). They found the total protein content is increased due to
bifenthrin is a synthetic analogues of natural pyrethrins produced by the ornamental plant *Chrysanthemum cinerariaefolium*.

**REFERENCES:**


Recovery in Lactate and Malate Dehydrogenase from Mercury Exposed Freshwater Fish CHANNA PUNCTATUS.

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ABSTRACT
Lactate and Malate dehydrogenase is important amongst the several molecules available in the cells and carbohydrates plays an important role in the cellular process. In the present investigation, fish, Channa punctatus treated with an equitoxic dose of 11 ppm of Mercuric nitrate and Mercuric acetate were scarified on 1, 4, 8, 12 and 15 days for recovery patterns in liver, muscle, kidney, gill and brain. Mercury toxicated fishes recovered after 15 days which depends on the physical condition of the fish.

Key words: Carbohydrate, Mercury, Channa.

INTRODUCTION
Pollution of the aquatic environment by heavy metals is a subject of great concern. These substances are generally discharged into the environment as a result of industrial processes and pose a problem because they are toxic and tend to accumulate in organisms. Minimata disease caused by the consumption of mercury contaminated shellfish and finfish taken from Minimata Bay in Japan, and Itai-Itai disease, caused by the consumption of food contaminated by cadmium in Japan (Ui 1972) have increased the awareness of toxic effects of the heavy metals on human beings and that has Prompted to take steps for their control. The modern industries are making use of various heavy metals such as iron, copper, nickel, platinum and Mercuric. Chemical pollution threatens the living systems and aquatic environment. Some of these metals are biologically
essential, but others like cadmium, Mercuric and mercury are highly hazardous to aquatic biota and normally occur in low concentrations. It is known that common forms of Mercuric poisoning results from mining, processing and commercial dissemination of Mercuric.

The natural emissions of mercury, mainly result from the degassing of the Earth’s crust and evaporation from water bodies, are two to four times larger than those from anthropogenic sources. The heavy metals are not only hazardous to aquatic animals but also alternatively to mankind; as human beings use most of the freshwater animals as a source of food material. The disposal of industrial and agricultural waste directly or indirectly into aquatic system burdens the ecosystem.

**MATERIALS AND METHODS**

*Channa punctatus* selected as test species is a representative of ray finned fishes in South India. They are well known for their air breathing ability, and can survive out of water in moist air for six days. It is selected as the test animal because of its euryhaline and eurythermal nature, and unique position in food chain. They are quite sturdy and ideally suited for experimentation in laboratory for longer periods.

Biochemical assays were done in different tissues from both experimental and control fishes. Fish, approximately of same size and weight was grouped into 6 batches. 2 batch of fish served as controls, 2 exposed to Mercuric nitrate and the remaining two exposed to Mercuric acetate for a period of 15 days. After a period of 15 days of exposure, a fish from each batch were transferred to Mercury-free water and scarified at the same intervals to observe the recovery. The values of different parameters were expressed as mean with standard error. Significance of the values obtained was tested using student ‘t’ test. Lactate and Malate dehydrogenase was estimated by modified method of Nachalas et.al, (1960).

**RESULTS AND DISCUSSION**

The results of the present investigation report the changes in the metabolite levels and enzyme profiles concerned with the carbohydrate metabolism in the tissues of fish during
exposure and recovery periods after Mercuric nitrate and Mercuric acetate intoxication. Analyses of the data performed at different exposure periods in the tissues such as liver, muscle, kidney, gill and brain – are as follows:

1) Lactate Dehydrogenase :

The activity of lactate dehydrogenase was increased in the early periods of exposure. However the activity was inhibited in the later periods of exposure in all the tissues.

On 1st day of exposure maximum activity was noticed in liver (+8.05% Mercuric nitrate P < 0.05; +8.47% Mercuric acetate P < 0.01), kidney (+7.33% for Mercuric nitrate, +8.62% Mercuric acetate, P < 0.05) followed by gill (6.45% Mercuric nitrate P < 0.05 + 8.06% for Mercuric acetate P < 0.001) muscle (+5.67% Mercuric nitrate, P < 0.01, + 7.01% Mercuric acetate, P < 0.05). Insignificant elevation in activity was recorded in brain (+4.39% for Mercuric nitrate and +5.49% for Mercuric acetate).

On the 4th day of exposure all the tissues recorded an increase in LDH activity except kidney wherein significant inhibition was noticed ( -10.16% for Mercuric nitrate and - 10.38% for Mercuric acetate P < 0.05). Maximum enhancement was found in liver(+14.69% for Mercuric nitrate and + 17.00% for Mercuric acetate; P < 0.001) followed by gill (+11.75% for Mercuric nitrate, P < 0.01: +14.29% for Mercuric acetate; P < 0.001), muscle (+11.38% for Mercuric nitrate + 13.69 % Mercuric acetate;P < 0.01) and brain (+9.62% for Mercuric nitrate P < 0.01; +11.54% for Mercuric acetate P < 0.05).

On 8th day of exposure inhibition of LDH activity was recorded in all the tissues. Inhibition of enzyme activity was noticed till the end of exposure period (i.e. upto 15th day). Maximum inhibition was noticed in kidney (-17.65% Mercuric nitrate P < 0.05, -18.30% Mercuric acetate P < 0.01 followed by muscle (-10.06% Mercuric nitrate, -11.49% Mercuric acetate P < 0.05 gill (-8.36 % Mercuric nitrate, -9.97% Mercuric acetate, P < 0.05) and brain (-9.09% Mercuric nitrate; P < 0.01; -8.36% Mercuric acetate, P < 0.05).

On 12th day of exposure similar response was found in all the tissues. However the magnitude of depletion was more. The values were significant at P < 0.001; P < 0.01 and P < 0.05.
The percent inhibition ranged between -14.04% to -24.69% for Mercuric nitrate and -15.44% to -25.51% for Mercuric acetate.

On 15th day of exposure the maximum inhibition was observed in kidney (-28.19% Mercuric nitrate, -30.43% Mercuric acetate; P < 0.001) followed by liver (-28.55% Mercuric nitrate, -29.83% Mercuric acetate; P < 0.001) gill (-23.44% for Mercuric nitrate; P <0.05; -25.6%, Mercuric acetate P<0.001 and a brain (-22.41% for Mercuric nitrate and -23.79% Mercuric acetate; P < 0.001).

After transferring fish to mercuric free water the inhibitory response was continued with less magnitude of response in comparison to exposure period. The inhibition was gradually reduced during the subsequent exposure periods. The rates of reduction the inhibition was tissue-specific. On 15th day of recovery period the inhibition was narrowed down and the difference between the control and experimental values were statistically insignificant in liver (-2.12% Mercuric nitrate, -2.69% Mercuric acetate) muscle (-2.37% Mercuric nitrate, -3.11% Mercuric acetate) kidney (-1.44% Mercuric nitrate, -1.03% Mercuric acetate). Brain tissue recovered on 8th day. The gill of fish exposed to Mercuric nitrate recovered on on 8th day, but gills from Mercuric acetate exposed fish regained the normal levels on 12th day.

**Malate dehydrogenase:**

During 1st and 4th day of exposure periods malate dehydrogenase activity was increased. Later on a steady fall in activity was noticed. On 1st day maximum activity was elicited in muscle tissue(+7.94% for Mercuric nitrate and +12.69% for Mercuric acetate; P < 0.01) followed by gill (6.25% for Mercuric nitrate and +10.79% for Mercuric acetate; P < 0.05), kidney (9.73 for Mercuric nitrate and +10.62% for Mercuric acetate; P < 0.05), liver (8.49% for Mercuric nitrate and +9.81% P < 0.01) and brain (+5.56% for Mercuric nitrate and +6.48% for Mercuric acetate; P < 0.05).

On 4th day maximum enhancement was noticed in muscle (+19.23% Mercuric nitrate P < 0.01, +22.30% Mercuric acetate P < 0.001) followed by liver (+17.55% Mercuric nitrate,
+19.22% Mercuric acetate; P < 0.05), kidney (+17.39% Mercuric nitrate, +18.26% Mercuric acetate P < 0.05), brain (+10.00% Mercuric nitrate, +9.15% acetate, P < 0.001). The gill tissue recorded a drop in MDH activity (-7.88% Mercuric nitrate, -7.27% Mercuric acetate, P < 0.05).

In the 8th day of exposure inhibition in MDH activity was noticed in all the tissues. Maximum inhibition was seen in kidney (-16.36% Mercuric nitrate, -18.18% Mercuric acetate, P < 0.05) followed by liver (-15.10% Mercuric nitrate, 18.49% Mercuric acetate, P < 0.001), gill (-15.63% for Mercuric nitrate, -16.25% Mercuric acetate, P < 0.01), Muscle (-12.69% for Mercuric nitrate P < 0.05; -14.93% Mercuric acetate; P < 0.001) and brain (-4.71% Mercuric nitrate P < 0.01 -5.49% acetate P < 0.001).

On the 12th day of exposure depletion in MDH activity was noticed. The percent depletion ranged between -10.04% to -26.05% for Mercuric nitrate and -12.91% to -25.20% for Mercuric acetate. Kidney exhibited maximum inhibition (-26.05% for Mercuric nitrate, P < 0.01 -23.53% for Mercuric acetate P < 0.05) and minimum inhibition was noticed in brain (-10.04% for Mercuric nitrate; -12.91% for Mercuric nitrate; -12.91% for Mercuric acetate; P < 0.001). The percent inhibition in MDH activity was statistically significant at P < 0.001, P < 0.01, P < 0.05.

On the 15th day of exposure maximum inhibition in MDH activity was noticed in all the tissues. Out of all selected tissues kidney exhibited maximum drop in activity (-33.88% Mercuric nitrate P < 0.05; -35.54% Mercuric acetate P < 0.001) followed by liver (-32.39% Mercuric nitrate P < 0.01 -34.08% Mercuric acetate P < 0.001), muscle (-29.29% Mercuric nitrate P < 0.01; -31.43% Mercuric acetate P < 0.001), gill (-27.22% Mercuric nitrate, -29.44% Mercuric acetate; P < 0.05) and brain (-16.26% Mercuric nitrate, -16.87% Mercuric acetate; P < 0.001).

During recover periods inhibitory response was continued in all the tissues. However the inhibition was gradually reduced over remaining recovery periods. Liver, muscle, kidney reached near normal levels of MDH activity on 15th day, whereas brain and gill reached the control levels on 8th day 12th days respectively, by exhibiting statistically
insignificant variation over control levels. On 15\textsuperscript{th} day of recovery the liver exhibited variation of -2.11\% for Mercuric nitrate and -1.58\% for Mercuric acetate where as muscle exhibited -1.56\% and -3.13\% variation for Mercuric nitrate and Mercuric acetate. Kidney reported to show -4.59\% and -5.50\% variation over control for these two salts of Mercuric. The variation recorded in gill on 15\textsuperscript{th} day of recovery was +1.20\% and -0.60\% for Mercuric nitrate and Mercuric acetate. Brain exhibited minimum variation over control out of all tissues.

Discussion:
The present investigation is aimed to understand the alterations in various metabolites and enzymes of carbohydrate and energy metabolism during exposure and recovery periods after mercuric nitrate and mercuric acetate intoxication. Two heavy metal salts of Mercury i.e. mercuric nitrate and Mercuric acetate were selected in order to understand the relative toxicities of these salts. The alterations observed in various metabolites and enzymes appear to be tissue-specific and time-dependent. The differential responses of tissues during exposure to mercuric salts can be attributed to absorption, distribution and elimination kinetics of mercuric nitrate and mercuric acetate and also on the characteristics of tissues like vascularity, perfusion and residual blood volume (Villarreal and Villegas, 1987).

Lactate dehydrogenase an enzyme which catalyses the interconversion of lactate and pyruvate exhibited a tissue-specific and time-dependent responses. The responses appeared more in the organic form of Mercuric in comparison to inorganic. In the initial stages of toxicosis i.e., upto 4\textsuperscript{th} day of exposure the LDH activity was found enhanced in the liver, muscle, gill and brain. However, in kidney the enhancement was recorded only on the first day of exposure. The observed increase in LDH activity in all the tissues is in agreement with the earlier studies in fishes [1, 6-8]. The activity patterns of LDH in liver, muscle, gill and brain from 8\textsuperscript{th} day onwards and in kidney from 4\textsuperscript{th} day are simply reflected in the lactate levels of the tissues. The activity patterns of LDH during the early stages of toxicosis do not commensurate with the accumulation of lactate, and this seems to be an interesting deviation from
observations recorded from the various animal models during metal toxicosis. The enhancement in LDH activity suggests the possibility of conversion of pyruvate to lactate. In evidence to this the accumulation of lactate and depletion of pyruvate content was recorded during the early stages of Mercuric toxicosis in the present study.

Though the levels of pyruvic acid, lactic acid content depends on the activity patterns of LDH, a clear cut relationship in the responses could not be seen between these profiles, suggest the involvement of other factors for the observed responses in lactate, pyruvate content of the tissues.

MDH activities in the tissues, which were found inhibited during the later stages of exposures period in the study exhibited a gradual reduction in the inhibitory patterns during recovery period. Recovery was found to be tissue-specific and time-dependent. Brain was found to be recover the SDH & MDH activities on the 8\textsuperscript{th} day of recovery period while liver and kidney on the 15\textsuperscript{th} day of recovery period. The tissue-specific and time-dependent variations could be attributed to the variations in the eliminatory patterns of mercury toxicant, or to the incomplete mitochondrial organization which was suspected to undergo disorganization during exposure period (Diamond & Kench, 1974). The recovery in dehydrogenases could also attribute to the enhanced aerobic metabolism. In evidence to this a raise in the O\textsubscript{2} consumption was observed in a fresh water field crab exposed to Heavy metal during recovery process.

In conclusion the above observation during exposure to mercuric nitrate and mercuric acetate indicate a significant alteration in enzymes and metabolites related to carbohydrate metabolism. The alterations were more marked in mercuric acetate treated fish. However, the fish is able to recover the altered metabolites and enzymes after transferring them to clean water. The recovery studies are of considerable importance because post-exposure recovery undoubtedly promotes their chances of survival and offers a solution to the aquatic fauna from contaminated area. Recovery studies also suggest that when the pollution is detected in the correct time and when appropriate measures are taken the aquatic fauna may be protected.
Figure – 1: Lactate dehydrogenase activity in the tissues of *Channa punctatus* after Mercuric nitrate and Mercuric acetate intoxication

Figure - 2: Malate dehydrogenase activity in the tissues of *Channa punctatus* after Mercuric nitrate after Mercuric nitrate and Mercuric acetate intoxication.

REFERENCES


Antibiotics Induced Respiratory Activities On Fresh Water Fish

**CHANNA PUNCTATUS** From River Godavari, Dist. Nanded.

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² Vivekvardhini Day college, Jambagh, Hyderabad.

**ABSTRACT**

Today the medicine branch is the main support for cure all those diseases may caused due to toxic impacts, bacterial infections, viral infections to human being. The medical experts recommends various types of “Antibiotics” as a life line for chronic & acute diseases. All these antibiotics are not an exact sort of treatment because it might show many side effects on human as well as various animal bodies. To overcome such type of problems, the present investigation is based on the impact of antibiotics on some physiological aspects of fresh water fish – *Channa punctatus* from River Godavari, Dist. Nanded.

**INTRODUCTION**

Any change in the aquatic medium affects respiratory potentials of the fishes. The respiratory potentials of an animal are the important physiological parameters to assess the antibiotic stress because it is a variable indicator of energy expenditure in particular. The early symptoms of acute poisoning by pesticides and other substances are the alteration or failure of respiratory metabolism. A number of investigations on oxygen consumption of fishes have been reported. The adverse effect of several chemical contents in the fish mainly attributed to CNS, further are known to influence other physiological processes including respiration.
MATERIAL AND METHOD

In the present work, freshwater fish *Channa punctatus* is collected from Godavari river Nanded and brought to laboratory for experimentation purpose. Respiration is a vital life process as it is the sign of life & index of all biochemical activities & total oxygen consumption & rate of oxygen consumption per unit body weight was studied by modified Winkler’s method. The dissolved oxygen was estimated according to Winkler’s method by using specialized respiratory chamber. It was a black colored glass bottle facilitated by inlet, outlet & control openings. After taking all precautions about respiratory chamber & initial sample was collected. The animal was allowed to stay in the chamber for 1 hr.& at the end final sample was collected. By this method oxygen consumption in initial & final sample can be determined & the difference is taken as amount of oxygen consumed by animal during the concern time.

RESULTS AND DISCUSSION

The results of present work found to be highly sensitive. The fresh water fish *Channa punctatus* showed variations in rate of oxygen consumption & total oxygen consumption when treated with Cyprofloxacine up to 144 hrs. In the present investigation it was showed that gradual decreasing trend in total oxygen consumption & rate of oxygen consumption up to 144 hr. as compared to control. The result were tabulated in table no.1

The results calculated indicate that the total oxygen consumption of *Channa punctatus* decreased gradually when treated with increasing conc. i.e. 500, 1000, 1500, 2000, 2500, 3000 mg. The control set showed maximum respiratory metabolism. But when the animals were treated with Azithromycine they showed marked decline in the total oxygen consumption & rate of oxygen consumption.

It has been reported that oxygen consumption represents the physiological state of metabolic activity & may be an indicator of metabolic stress. The oxygen consumption is a very sensitive physiological process and the change in respiratory activity has been used as an indicator of stress in animal exposed...
to toxicants. Many workers have observed the decreasing trend in oxygen consumption when fish are exposed to pollutants. They have suggested reasons for the reduction of oxygen consumption as (i) the coagulation of mucus in the gills, which interfere with respiratory metabolism, (ii) due to abnormality in gill and other tissues and (iii) due to injury caused to the RBC, reduction in RBC count and haemoglobin content. The results of this study confirm the earlier report on oxygen consumption by fish in pesticide mixed water.

<table>
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<tr>
<th>Sr. No.</th>
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<th>Rate of Consumption</th>
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<td>0.0572±0.023</td>
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<td>24 hrs</td>
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<td>0.0480±0.031</td>
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<td>3</td>
<td>48 hrs</td>
<td>1.890±0.547</td>
<td>0.04021±0.054</td>
</tr>
<tr>
<td>4</td>
<td>72 hrs</td>
<td>1.276±0.144</td>
<td>0.02835±0.033</td>
</tr>
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<td>5</td>
<td>96 hrs</td>
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<td>0.02979±0.026</td>
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<tr>
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<td>7</td>
<td>144 hrs</td>
<td>0.730±√-0.004</td>
<td>0.0152±0.034</td>
</tr>
</tbody>
</table>
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NUTRITION FOR FISH HEALTH

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ABSTRACT:

Following a request from the Commission to address the risks and benefits as regards fish/seafood consumption related to relevant beneficial substances (e.g. nutrients such as n-3 long-chain polyunsaturated fatty acids) and the contaminant methylmercury, the Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a Scientific Opinion on health benefits of seafood consumption in relation to health risks associated with exposure to methylmercury. In the present Opinion, the NDA Panel has reviewed the role of seafood in European diets and evaluated the beneficial effects of seafood consumption in relation to health outcomes and population subgroups that have been identified by the FAO/WHO Joint Expert Consultation on the Risks and Benefits of Fish Consumption and/or the EFSA Panel on Contaminants in the context of a risk assessment related to the presence of mercury and methylmercury in food as relevant for the assessment. These included the effects of seafood consumption during pregnancy on functional outcomes of children’s neurodevelopment and the effects of seafood consumption on cardiovascular disease risk in adults. The Panel concluded that consumption of about 1-2 servings of seafood per week and up to 3-4 servings per week during pregnancy has been associated with better functional outcomes of neurodevelopment in children compared to no consumption of seafood. Such amounts have also been associated with a lower risk of coronary heart disease mortality in adults and are compatible with current intakes and recommendations in most of the European countries considered. These associations refer to seafood per se and include beneficial and adverse effects of nutrients and non-nutrients (i.e. including
contaminants such as methylmercury) contained in seafood. No additional benefits on neurodevelopmental outcomes and no benefit on coronary heart disease mortality risk might be expected at higher intakes.

**KEY WORDS:** Fishshellfish, benefit, neurodevelopment, coronary heart disease.

**INTRODUCTION**

Consumption of fish, our primary source of long-chain omega-3 polyunsaturated fatty acids EPA and DHA, is associated with numerous health benefits including improved infant cognitive and visual development, reduced risk of cardiovascular disease, reduced risk of non-alcoholic fatty liver, and reduced inflammation and positive clinical outcomes in inflammatory disease [1]. Increasing fish consumption is an easy way to improve the health of Hawaii’s residents. Work Dr. Watters has done with Hawaii State Department of Health indicates the median intake of fish in Hawaii is 5.3 ounces per week, which is below the recommended intake level of 8 oz per week by the American Heart Association. Healthy Seafood Hawaii is a project funded by the National Oceanic Atmospheric Administration to take seafood nutrition, culinary education and culinary education information into the homes of consumers. To achieve this goal Dr. Watters has been studying the topic of locally available fish from the following perspectives.

**Nutritional Enhancement of Long-Chain Omega-3s in Tilapia:**

Tilapia can be grown with feeds low in EPA and DHA, but this results in low EPA and DHA in the fish for consumption. In this study we changed the EPA and DHA of tilapia feed to increase the level of these beneficial fatty acids in the flesh of the fish[2].

**Fatty acid composition of aquaculture products in Hawaii**

With the expansion of aquaculture production and the introduction of numerous new species, nutritional data for many local aquaculture products is lacking. We have obtained and
have been analyzing over 25 different local aquaculture products for their fatty acid, mercury, and selenium contents.

In culturing fish in captivity, nothing is more important than sound nutrition and adequate feeding. If there is no utilizable feed intake by the fish, there can be no growth and death eventually results. Under-nourished or malnourished animals cannot maintain health and growth, regardless of the quality of the environment. Therefore, before any attempt at fish culture it would be wise to ask a fundamental question, “What and how should I feed my fish?” Faulty nutrition impairs fish productivity and affects their health; a fact the fish culturist should always keep in mind. Clinical disease often ensues when nutritional needs are not met. The borderline between reduced growth and diminished health and overt disease is difficult to define. Diets may hasten recovery from infection or slow the progress of an idiopathic disease or overcome environmental stress. However, diets may also cause nutrient imbalances, deficiency diseases, nutritional toxicoses, or may introduce infective agents. As a consequence, nutritionally-balanced and quality-controlled diets are of critical concern in fish production. As shown in Fig. 1, many steps of research, quality control, and biological evaluations must be exercised by various individuals and groups to develop and produce nutritionally-balanced diets for fish.

**ENERGY:**

Energy is not a nutrient. It is rather an end-product of absorbed macro-organic nutrients when they are oxidized and metabolized. All organic compounds in fish feed release heat upon combustion, and thus are potential sources of energy.

For salmonid fishes, most dietary carbohydrates, such as raw starch from plant feedstuffs, are not utilized as energy sources. Simple sugars can be utilized by salmonids but their use as energy sources in feed formulations is of no practical significance. Lipids and proteins, therefore, provide the primary dietary sources of energy Physiologically, lipids and proteins form an important part of a structure of a fish, but the need for energy can preclude their incorporation into tissues and may involve their catabolism as a source of energy, Thus, utilization
of the nutrients of each diet depends both upon the level of intake and upon the make-up of the diet. The over-riding importance of food as an energy source means that the major factor regulating the food intake of the animal is its energy value in relation to the animal’s energy needs. As a consequence, the concentration of the essential nutrients which must be provided in the diet to adequately meet the animal’s requirements is directly related to the energy value of the diet. Therefore, the biologically utilizable (metabolizable) energy content of a diet must be defined in relation to the other needs before one can estimate the effect of a diet upon the growth and well-being of the fish.

In practice, fish culturists must estimate the biologically available (digestible) energy content of a diet before they can determine the weight of feed that should be fed each day. A low energy diet which usually contains a high level of carbohydrate (starch and fiber) results in poor weight gain and feed efficiency in salmonids. Furthermore, the increased intake of a poorly utilizable feed results in increased excretion of feces which will pollute the aquatic system. Diets for salmonids should contain at least 14 MJ (3.35 Mcal) total digestible energy per kg feed; of this, at least 4 MJ or 1 Mcal/kg energy must be of lipid origin (Cho 1981).

RESULT AND DISCUSSION:

TABLE 1

Capture fisheries and aquaculture production together with fish consumption in the top five ranked aquaculture producers

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>China</td>
<td>15.8</td>
<td>15.8</td>
<td>41</td>
<td>41</td>
<td>4.2</td>
<td>8.9</td>
<td>33.5</td>
<td>33.5</td>
</tr>
<tr>
<td>India</td>
<td>4.3</td>
<td>4.6</td>
<td>5</td>
<td>2</td>
<td>94</td>
<td>2.1</td>
<td>7.4</td>
<td>5.2</td>
</tr>
<tr>
<td>Vietnam</td>
<td>2.5</td>
<td>2.8</td>
<td>10</td>
<td>88</td>
<td>5.7</td>
<td>14.7</td>
<td>33.6</td>
<td>6.4</td>
</tr>
<tr>
<td>Indonesia</td>
<td>5.7</td>
<td>2.7</td>
<td>3</td>
<td>210</td>
<td>4.0</td>
<td>8.4</td>
<td>28.9</td>
<td>7.5</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>1.6</td>
<td>1.5</td>
<td>15</td>
<td>105</td>
<td>4.4</td>
<td>10.4</td>
<td>19.7</td>
<td>13.5</td>
</tr>
</tbody>
</table>
Fishery resources are an important source of both macro- and micro-nutrients for humans. Globally fish accounts for about 17 percent of animal protein intake. This share, however, exceeds 50 percent in many countries. Despite the low overall African per capita consumption noted above, in West African coastal countries the proportion of dietary protein that comes from fish is very high: 72 percent in Sierra Leone, 55 percent in Ghana and Gambia and 43 percent in Senegal. Also in Asia and some small island states the contribution is high: 70 percent in the Maldives, 60 percent in Cambodia, 57 percent in Bangladesh, 54 percent in Indonesia, 55 percent in Sri Lanka (FAO 2012). Official data on fish consumption in developing countries may also be underestimated as these data fail to capture fish bought in small rural markets, as well as fish caught for consumption by household members or produced in home farms. In addition, fish consumption is affected by location, seasonality, time and household socio-economic status.

The central role that fish plays in the diet in some developing countries is exemplified by using data from Bangladesh. Data from the Household Income and Expenditure Survey 2010 showed that fish is by far the most frequently consumed nutrient-rich food group, followed by leafy vegetables, fruit, eggs, milk and meat (Bangladesh Bureau of Statistics 2012). In the 14 days preceding the survey, 71% of households had consumed fish, whereas only 2% had consumed meat. A summary of results from several consumption studies – national, different locations and seasons, from 1962 to 1999, in rural Bangladesh showed fish intake ranging from 15 g/capita day to 96 g/capita/day. Poor households had smaller fish intakes than rich households, and a larger proportion of the total fish intake was made up of small fish species in poor households compared to rich households. A large proportion of small fish is consumed as dried fish. The diversity of fish species consumption in Bangladesh is high, especially in rural Bangladesh. Survey data from four rural locations in 1992 showed that a total of 75 different fish species were consumed at household level (Thilsted 2012). However, reduced intake of diverse fish species, as well as a decreased proportion of small fish in total fish intake, has been reported with time. At the same
time, with aquaculture expansion, intake of few, large farm species: carps, tilapia (Oreochromis niloticus) and pangasius (Pangasianodon hypophthalmus) have increased throughout Bangladesh (Thilsted 2013). An adequate animal protein supply can be obtained from other sources (e.g. meat from terrestrial animals) and there has been a tendency, as incomes rise, for the more affluent to increase their purchases from these sources. However, as they are generally more expensive than the cheapest fish the poor cannot make such life style choices and remain critically dependent on fish. Traditionally the major focus of nutritionists was on the macronutrients providing energy and protein. Increasingly today the role of micronutrients - vitamins and minerals – in the diet, particularly of the poor, is recognized as having a limiting effect on development and health. Micronutrient deficiencies affect hundreds of million people, particularly women and children in the developing world. More than 250 million children worldwide are at risk of vitamin A deficiency, 200 million people have goitre and 20 million are mentally retarded as a result of iodine deficiency, 2 billion people (over 30 percent of the world’s population) are iron deficient, and 800 000 child deaths per year are attributable to zinc deficiency. Rural diets in many countries are not particularly diverse, and thus, it is vital to have a good food sources that can provide all essential nutrients to their diets. Foods from the aquatic environment are a complete and unique source of both the macro-and micronutrients required in a healthy diet. The benefits, as well as the potential risks, of fish consumption are well documented in the report of an FAO/WHO Expert Consultation on the risks and benefits of fish consumption (FAO/WHO 2011) that concluded that the benefits far outweigh the risks, which were principally from mercury and dioxins. The experts found convincing evidence of beneficial health outcomes from fish consumption for: • reduction in the risk of death from coronary heart disease improved neurodevelopment in infants and young children when the mother consumes fish before and during pregnancy It is the essential long-chain omega-3 fatty acid docosahexaenoic acid (DHA) that is important for optimal brain and neurodevelopment in children and eicosapantaenoic acid (EPA) that improves
cardio-vascular health. Although many vegetable oils contain omega-3 fatty acids this is in the form of alpha-linolenic acid (ALA), which must be converted metabolically by chain length extension to EPA and DHA. However, the conversion from ALA into EPA and DHA is limited in humans, making it difficult to rely only on vegetable oil during the most critical periods of life. It has been demonstrated that the metabolic pathway in the human male is only 5 percent efficient, although the rate in females is higher, indicating a bigger requirement, (Burdge et al 2005). Omega-3 fatty acids in the form of DHA rather than ALA are therefore needed to secure an optimal brain and neural system development in neonates and infants. This is particularly important during pregnancy and the first two years of life (the 1000 day window). Fish consumption also provides health benefits to the adult population. There is strong evidence that fish, in particular oily fish, lowers the risk of coronary heart disease (CHD) mortality by up to 36 percent due to a combination of EPA and DHA (FAO/WHO 2011)[7]. In addition to the health benefits of these macro-nutrients fish is also an important provider of a range of micro-nutrients, not widely available from other sources in the diets of the poor. More and more attention is given to fisheries products as a source of micronutrients such as vitamins and minerals. This is in particularly true for small sized species consumed whole with heads and bones, which can be an excellent source of many essential minerals such as iodine, selenium, zinc, iron, calcium, phosphorus, potassium, vitamins A and D, and several B vitamins. There are significant variations between species and between different parts of the fish. Seafood is almost the only natural source of iodine, and iron and zinc are found in significant amounts, particularly in fish species eaten with bones, such as small indigenous fish species. Some of these small fish species, for example mola (Amblypharyngodonmola) have a very high content of vitamin A in the form of dehydroretinol and retinol. As most small fish are eaten whole, with bones, they are a rich source of highly bioavailable calcium (Roos et al., 2007). In addition, fish enhances the bioavailability of iron and zinc from the other foods in a meal (Aung-Than-Batu et al., 1976).
Data from a study in rural northern Bangladesh in 1997 showed that small fish intake met about 40% of the vitamin A and 32% of the calcium recommendations of an average household, in the peak fish production season (Roos et al., 2006). In Cambodia, a traditional daily meal of rice and sour soup made with the iron-rich small fish, trey changwaplieng (Esomuslongimanus) can meet 45% of the daily iron requirement of a woman. As the edible parts of large fish do not include the bones, viscera and organs, the micronutrient content is much lower than that of small fish (Table 3). For example, only 20 grams of trey changwaplieng from Cambodia contains the daily needs of iron and zinc for a child. Mola from Bangladesh, is reported to have a vitamin A level of > 2,500 µg RAE in 100 g of fish; based on a RDA of 500 µg for a child, 140 g of this fish will be enough to cover a child’s weekly needs of vitamin A. (Roos et al 2007) Table 3 gives the micronutrient composition of a number of small and large fish species from Bangladesh. Traditional wisdom among Bangladeshi women is that specific small fish species have health benefits including being: good for/protect eyes, full of vitamins, good for pregnancy and lactation, give strength and build up the blood. Wild and farmed fish are a healthier alternative to almost any other meats. Farmed fish have a more constant nutrient composition compared to their wild counterparts, whose environment, food and access to food varies during the year. The environment of farmed fish can be monitored and managed to secure an optimal product. By controlling the composition of aquaculture feeds and other inputs, fish with good health and healthy fish products with optimal nutritional composition can be produced.

**Consumer safety of fish:**

Recent years there has been a lot of publicity on the potential risks from fish consumption, related to the presence of pollutants or contaminants. Much of this has been sensationalist but there are real problems that need to be faced not only with chemical contaminants but also with fish-borne diseases caused by poor hygiene and lack of effective food control. In order to maximize the contribution of fish to human nutrition, not only the fish supply needs to be improved, but the consumer safety
aspects need to be considered. In general, fish is considered a safe food and guidelines for safe production, handling and processing are available in the FAO/WHO Codex Code of Practice for Fish and Fishery Products (CAC/RCP 52-2003). However, even in the developed world it is apparent that these guidelines are not followed. For instance in the United States, from 2001-2010 there were 657 outbreaks of illness involving 5603 cases associated with fish and fishery products. Poultry was associated with 458 outbreaks involving 11,338 cases, fresh produce with 696 outbreaks involving 25,222 cases and beef with 363 outbreaks involving 7528 cases (CSPI 2013). Most of the illnesses associated with fish were due to scombrototoxin (histamine and other biogenic amines caused by improper handling of certain species of fish) and ciguatoxin (toxin derived from certain microalgae found in many tropical areas of the world). In the EU, during 2011, 78.9 percent of the 71 outbreaks associated with fin fish were due to scombrototoxin and 4.2 percent were due to ciguatoxin. In the case of shellfish (crustaceans and molluscs), 40.5 percent of the 42 outbreaks in 2011 were due to calciviruses (noroviruses) and 16.7 percent were due to algal biotoxins (EFSA and ECDC 2013). In some parts of Asia, where there is a practice of consuming raw fish, human infestations caused by fish-borne trematodes (liver flukes) are commonly reported and there is some evidence that these are spreading to other countries through fish exports to migrant communities.

Nutritional disorders caused by vitamin deficiencies can impair the utilization of other nutrients, weaken the health of the fish, and lead to disease. It is well known that pantothenic acid deficiency results in nutritional or clubbed gill disease. However, this condition may not be as specific as reported because feeding a diet containing 10 mg pantothenic acid/kg feed (NRC recommends 40 mg/kg) for 5 mo did not produce either the described deficiency signs or growth depression at our laboratory.

Ascorbic acid (Vitamin C) is the most unstable vitamin required in fish diets (Hilton et al. 1977).
Therefore, the extent of destruction of ascorbic acid in a feed gives some indication of manufacturing methods and storage conditions.

In addition to losses associated with manufacturing and storage, there is some loss of vitamins due to leaching during the feeding process. However, at least for salmonids, the leaching of vitamins from properly manufactured dry pellets and granules is not a major problem. Most published data on leaching losses were obtained under very artificial conditions.

Nutritional deficiency signs usually develop gradually, and it is difficult to detect clear signs in the early stages. However, the culturist may obtain indirect clues of vitamin deficiency from such signs as poor appetite, reduced weight gain, and poor feed efficiency.

MINERALS

In fish, minerals perform important roles in osmoregulation, intermediary metabolism, and in formation of the skeleton and scales (La11 1981). Mineral requirements of fish are difficult to study because many minerals are required in only trace amounts and others are absorbed from water in significant quantities through the gills as well as from the diet. It is also very difficult to obtain mineral-free feed ingredients for experimental diets. Most practical diets for salmonids provide the major mineral requirements through fish meal which is also a major source of protein. However, diets which rely heavily on plant protein sources must be supplemented with carefully balanced mineral premixes. The minerals required in finfish diets include calcium, zinc, manganese, cobalt, selenium, and water stable with a minimum amount of fines. Proper feeding of a quality diet should be considered as a high priority in the daily routine on fish culture stations. Wasted feed depletes oxygen levels, causes gill damage, and supports fungal and bacterial growth, all of which can lead to disease problems. Because it is One must be cautious in applying these tables to modern diets which have higher nutrient densities and availabilities. The main factors influencing feed intake of fish are water temperature, the energy content of the diet, and expected growth.
Therefore, an estimation of feed intake needed must be based on these fundamental factors. If a group of fish is not feeding actively or growing as expected.

Fish as a Source of Omega-3 Fatty Acids
As mentioned, fish are unique among all foods in having an abundant supply of long-chain omega-3 fatty acids in their oil. Much recent research indicates that consumption of such fatty acids has a beneficial effect in lessening the risk of heart attack and, in some cases, perhaps other medical conditions (Lands and Bimbo, 1983; Lands, 1986, Stansby, 1985). Dozens of research papers have been published in the past several years on this subject (e.g., see Simopoulos et al., 1986). For individuals who want to increase their intake of long-chain omega-3 fatty acids, the best way at present is to consume species of fish having a moderately high fish oil content (about 5-10 percent). Such species have no more oil than occurs in even the leanest varieties of meat (which of course contains no omega-3 fatty acids). Such marine species of fish would include, for example, salmon (all species), herring, sardines, sablefish, mackerel, and many others. Other species of fish with lower fat content would furnish smaller, but still important, amounts of omega-3 fatty acids.

Possible interventions
Interventions to overcome the above constraints are wide ranging and will involve a number of actors including: international organizations, governments and civil society organizations, industry and academia. The theme of integration of efforts to reduce undernutrition is taken up in the State of Food and Agriculture (FAO 2013), which enjoins the entire food system – from inputs and production, through processing, storage, transport and retailing, to consumption to contribute much more to the eradication of malnutrition.

CONCLUSION:
In recent years, with dramatic rises and increased volatility in food prices, there is a risk that the diets of the poor will become even less diverse and more dependent on starchy
staples. There is therefore a renewed emphasis on the production, access, distribution and utilization of common, micronutrient-rich foods. Fish, especially nutrient-rich small fish, from the wild and from aquaculture, can play a vital role in improving human nutrition, but this will require changes to government policies, investment in infrastructure and encouragement of research. Means must be found to reduce post-harvest losses in fisheries, better utilize processing waste and to make use of the large quantities of small pelagic fish that are available for direct human consumption. International organizations such as FAO, bilateral agencies such as USAID, through Feed the Future and DFID, the CGIAR through the CGIAR Research Programs, governments, NGOs and the private sector have all initiated programmes and interventions that provide a platform for fish to contribute to human nutrition. These should be further strengthened and coordinated.

REFERENCES:


Determination of Blood Urea (Serum Urea) After Exposure of Lethal and Sub-Lethal Concentration 96 Hours LC$_{50}$ of Cadmium Chloride on The Freshwater Fish, OPHIOCEPHALUS STRIATUS (CHANNA)

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Abstract:
In the present investigation serum urea level increased significantly in experimental (LC$_{50}$) at 96 hrs fishes over control. Serum urea level is increases slightly but steadily in sub-lethal (10% of LC$_{50}$) during 24-96 hrs. An increased serum urea level is due to the, decreased capacity of kidney to eliminate urea i.e. kidney dysfunction like degeneration of glomerulus serum creatinine level is increased significantly in experimental (LC50) at 96 hrs and sub-lethal (10% of LC 50) during 24-96 hrs over the control. Increased serum urea level in experimental fishes possible reason for increment in serum creatinine level. Both urea and creatinine possibly increased due to the nephrotoxic lesions of kidney observed in histological study of present work. Increased urea and creatinine level is clearly indicates renal dysfunction of kidney.

Key Words: Blood Urea, Cadmium Chloride, Ophiocephalus striatus.

Introduction:
Determination of serum urea (or blood urea nitrogen, BUN) is the most widely used screening test for the evaluation of kidney function. The test is frequently requested along with the serum creatine or creatinine test since simultaneous determination of these two compounds appares to aid in the
differential diagnosis of pre-renal, renal & post renal hyperuraemia (increased serum concentration of urea). Pre-renal cause could be cardiac-related or due to increase protein catabolism, renal cause includes glomerulonphrititis chronic nephrititis, nephritic syndrome & other condition. Post-renal causes include obstruction of the urinary tract.

An increase serum urea concentration above normal is due to the one of the factors like decreased capacity of the body to eliminate urea, excessive urea formation (i.e. increased protein catabolism), concentration of blood (i.e. dehydration). Above these three mechanisms are often interlinked i.e. more than one may be operating at the same time. In many cases it is important to have some means of evaluating the ability of kidney to remove waste products from the blood. Several “kidney function” tests have been devised for this purpose & many of them utilized blood urea determination.

MATERIALS AND METHODS:
Specimen:
Serum specimen is used for determination of urea. Serum can be used directly which is more convenient but requires a high quality automatic pipette to dispense a small quality of specimen (50 µL). The alternative procedure requires 1:20 dilution of serum. Protein free filtrate (protein removed by 4% TCA) can also used. Since urea may be lost through bacterial action, the specimen should be analyzed within two hours after blood collection or should be preserved by refrigeration. The blood urea is Determine by Berthelot’s Method.

Principle:
The urea of serum is hydrolyzed by specific enzyme urease & is converted to ammonia & carbon dioxide. The reaction is buffered with EDTA which also serves to chelate any heavy metals ions that might otherwise inactivate urease. The ammonia is estimated by the Berthelot reaction in which it reacts with phenol & alkaline hypochlorite to form P-quinone chloroimine. The P-quinone imine reacts with another molecule of phenol to form indophenol, which in alkaline solution dissociates to yield a blue indophenol dye. The reaction is
catalyzed by sodium nitroprusside. The final dilution of serum sample is so great precipitation of proteins is not necessary.

**Reagents :**

1] **Buffered Urease :**

   Using good quality commercial urease with an activity of 800 to 1000 sumner units per gram. Dissolve 150 mg urease & 1.0 gm EDTA in about 80 ml double distilled water. Using a pH meter (systronics) adjust pH to 6.5. Make up to 100 ml. Store in plastic bottle in a refrigerator (stability = 1 month).

2] **Phenol colour reagent :-**

   Dissolve 25.0 gm analytical grade phenol in 480 ml distilled water in 50 ml volumetric flask. Dissolve separately in 50 ml distilled water 125 mg analytical grade sodium nitroprusside & add this to the phenol solution. Make 500 ml with distilled water. Store in a dark brown bottle in a refrigerator (stability = 2 month).

3] **Alkaline hypochorite reagent :-**

   Dissolve 12.5 gm analytical grade NaOH in 400 ml of distilled water in a 500 ml volumetric flask. Add about 20 ml of commercial bleach. Make volume to 500 ml with distilled water.

4] **Working Standard Solution: -**

   Dissolve 215 mg pure dry urea in distilled water in a 500 ml volumetric flask, make to volume & mix. Transfer to a bottle & add a few drops of chloroform as a preservative, shake well & store in the refrigerator. This standard contains 20 mg urea nitrogen.

**Procedure :**

1. In to each of three test tubes marked test, blank & standard, pipette 0.2 ml of buffered urease preparation. Add 20 ml of serum to the tube marked test & 20 ml of working standard solutions to the standard tube.
2. Incubate all three tubes for 15 minutes at 37°C.
3. Remove all three from water bath pipette 1 ml of phenol colour reagent in each of 3 tubes, mix by gentle lateral shaking, then add 1 ml of alkaline hypochlorite reagent & mix again. Add the standard reagents in the said order.
4. Return tubes to 37°C water bath for 15 minutes.
5. Remove the tubes from water bath & add 10 ml of distilled water to all the tubes. Mix by inversion, covering the mouth of the tubes with stopper not with the thumb.
6. Read optical density (absorbance) of the test & standard solutions at 630 nm in colorimeter using the blank solution set to zero absorbance. If 630 nm is not available use nearest one in available.
7. If the absorbance reading of test solution is above 0.8, dilute both test & blank solutions with more distilled water until the absorbance of the test solution falls within the range 0.2 to 0.8. Calculate the test value & multiply by the appropriate dilution factor.

RESULTS AND DISCUSSION:
In the present investigation the serum urea level is significantly increases in experimental (LC₅₀) fishes 11.91% over the control. In sub-lethal (10% of LC₅₀) concentration serum urea is slightly but steadily increases during 96 hrs. In sub-lethal exposure as the exposure period is increases serum urea level is also increases. (Table: 1, 2).

In present study urea is significantly increased over the control. Teleost fishes are primarily ammonotelic but their blood contains significant amount of urea. Renal disorder associated with damaged kidney; in present work of experimental kidney shows nephrotoxic lesion in histological studies possibly caused the inability to excreatory function. Kidney dysfunction is probable reason for the increased serum urea level in experimental fish. In present work kidney of experimental fish shows nephrotoxic lesions in histological studies possibly the reason for increased serum urea level[3]. Stated that glomerulonephrititis, there is a net diminution in the filtration of urea, less urea is eliminated & blood urea consequently rises, this is usually occur when severe kidney lesion is occur. Above
similar kinds of result is occur in experimental kidney of fish in histological studies in present work. The free amino acid which is involved in urea cycle responsible for the urea synthesis. In present work of Biochemical studies kidney & liver shows highest increment in free amino acids. This result support increased serum urea level in experimental fish.

Stated that kidney dysfunction is mainly responsible for the increased serum urea level in fresh water cat fish *Clarias lazera* stated the increased serum urea level in Chinese grass carp *Ctenopharygodon idella* exposed to sub-lethal doses of mercuric chloride. He also concluded that increased urea level is associated with dehydration, increased protein catabolism, hemorrhage in to soft tissue or body cavity, acute renal failure, chronic renal disease etc.

**CONCLUSION**

From above discussion it is clear that increased urea level is associated with increased proteolysis, increase free amino acids, liver dysfunction, kidney nephrotoxic lesions etc. Similar kinds of biochemical (increased free amino acids & decreased protein level) studies & histological (nephrotoxic lesions) results in experimental fish supports above conclusion about increased serum urea level in the present study.

**ACKNOWLEDGMENT:**

Authors are thankful to Principal Dr. G.N. Shinde, Indira Gandhi (Sr.) College, CIDCO, Nanded for providing laboratory facilities during the research work.

**Table:** 1 Levels of serum urea in *Ophicoephalus (channa) striatus* exposed to median lethal (LC50) at 96 hrs. & sub-lethal (10% of LC50) at 24 - 96 hrs.
<table>
<thead>
<tr>
<th>Parameter (mg/dl)</th>
<th>Condition</th>
<th>Exposure Sub-lethal (10 % of LC50)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Median lethal (LC50) at 96 hrs.</td>
</tr>
<tr>
<td>Serum Urea</td>
<td>Control</td>
<td>5.54± 0.54</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>6.20± 0.41*</td>
</tr>
</tbody>
</table>

Values are mean ± SD of six replicates * P < 0.05, ** P < 0.01, *** P > 0.01, significant when student’s ‘t’ test was applied between control & experimental groups.

**Table: 2** Variation in the levels of serum urea content in *Ophiocephalus (channa) striatus* in terms of % increase (↑) over control exposed to median-lethal (LC50) at 96 hrs. & sub-lethal (10% of LC50) at 24 - 96 hrs. concentration of cadmium chloride (cdcl2)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median Lethal (LC50) at 96 hrs. % ( □ )</th>
<th>Exposure Sub-lethal (10 % of LC50)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hrs. % ( □ )</td>
<td>48 hrs. % ( □ )</td>
</tr>
<tr>
<td>Serum Urea</td>
<td>11.91</td>
<td>1.81</td>
</tr>
</tbody>
</table>

**REFERENCES**


Analysis of Haemoglobin and Blood Cell Count in Fresh Water Fish- 
*Labeo rohita*

N. R. Jaiswal, M. S. Kadam  
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**ABSTRACT**  
There are variations in blood cell count and percentage haemoglobin in different fish species, these variations are remarkable in planktophagous and carnivorous fishes found in fresh water reservoirs. In relation to change in habitat, seasonal changes, parasitic infection, effect of pollutants, relative physiological activities shows difference in haematological parameters. The blood sample of fish –*Labeo rohita* were examined to study the blood cell count and haemoglobin percentage from Godavari river at Nanded (Maharashtra).  

**KEY WORDS:** Haemoglobin , erythrocyte, leucocytes, *L. rohita* .

**INTRODUCTION**  
The haematological study in fishes is significant to understand the comparative physiology, in the study of phylogenic relationship and to know the effect of pollutants on fish life. The study is also important in fisheries management especially to manage the artificial feeding and ecological parameters for effective fish culture .The remarkable work on fish haematology was by [1,2]-worked on the haemotology of Channa sp. Later on other Scientist contributed in fish haematological studies are Marachi (1959), Priston (1960), Pradhan (1961), Srivastava (1968),Joshi(1990), Bhat and Singh (1981), Sharma and Joshi (1992). Recently it was observed that the haematological parameter greatly changes with seasonal change, Yeragi and Lendhe (2004).
MATERIALS AND METHODS:

The fish *Labeo rohita* were collected from Godavari river at Nanded (Maharashtra). The fishermen were requested to collect the blood samples from the selected fishes. The blood was collected from the aorta with the help of syringe and blood was transferred to a 5 ml capacity with stopper containing EDTA as anticoagulant. Blood sample were collected by using physical restrain from the live fishes at the site of fish catch of the river, without any anesthesia. Percentage of haemoglobin was determined by Cynohaemoglobin method outlined by Blanhall and Daisley (1973). Blood cells were counted using Neubeur’s hamecytometer using Hayem’s solution as diluting fluid.

RESULTS AND DISCUSSION

All the observations were from the healthy, non injured individuals. The selected haematological parameters were - Haemoglobin (Hb) for its percentage concentration from the blood, total erythrocytic count (TEC) for million/cu. mm. Leucocytes, amount of P.C.V, amount of M.C.V, amount of M.C.H, amount of M.C.H.C, amount of R.D.W- S.D. counting was in percentage concentration of the total R.B.C indices.

The haemoglobin percentage in *Labeo rohita* was 13.9 mgs/dl, the TEC was 1.77 million /cu mm. The percentage concentration of Leucocytes count is in given sample 2,60,000 per cu.mm, amount of P.C.V in given sample is 25.4%, amount of M.C.V in given sample is 143.5 femtolitres, the amount of M.C.H in given sample is 78.5 pico-grams, the amount of M.C.H.C in given sample is 54.7%, amount of R.D.W- S.D in given sample is 00.1 fl. Breazible et.al. (1981) found less Monocytes 0.4% in carnivorous fish as compared to 2.0% in omnivorous fish , S.P Chavan et.al.(2010).

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Effect of Synthetic pesticides on areal respiration of *PERIPLANETA AMERICANA*

J. T. Gawali, P S. Managoli and P. B. Deshmukh
Dept. Of Zoology, NES’Science College Nanded,(M.S)

**ABSTRACT:**
In the present study; the effect of pyrethroids containing mosquito mat and mosquito coil on the rate of respiration *Periplaneta americana* was investigated. In present study it was concluded that mosquito mat is more effective than mosquito coil.

**Keywords:** Pyrethroids, Chrysanthemum, synthetic pesticides.

**INTRODUCTION:**
Pesticides are the chemicals which are based on plant extracts such as pyrethroids which are commonly used for destruction of pest management. Insecticides are also toxic to many nontarget organisms. Pyrethroid is a pesticides which are synthetic analogous of pyrethrins. Pyrethroid is synthesized from flower of chrysantamum from Cinerariafolium. Pyrethroids are currently major pesticides used against pest. It acts almost as a contact poison. The pesticidal properties of pyrethroids are derived from ketoalcoholic esters of Chrysanthemum and pyrethroids acids. The Pyrethroids acids are strongly lipophillic and rapidly penetrate many insects and paralyse their nervous system. Pyrethroid act specially by disrupting potassium and sodium ion exchange process in insect nerve fibres and interrupts the normal transmission of nerve impulses. Synthetic pyrethroids such as Deltametrin which have of the most widely used pesticides owing to their action. Deltamethrin is consider powerful synthetic pyrethroids, its poisoning occurs through cuticular penetration or oral uptake.

One common Pyrethroid is allethrin which is one of the ingredients of mosquito coil. It is widely known as efficient mosquito repellent. The active ingredients found in mosquito
coat may include pyrethrum, a natural powdered material from a kind of Chrysanthemum plant.

**Material and Method:**

Selected healthy and active cockroaches Periplaneta Americana were (0.4-0.6 g.) weighed. 05 cockroaches were placed in the respiratory chamber which was clean and dry. Each chamber was sealed by a cork fitted with two glass capillary tubes; one of the tubes was bent in 90\(^\circ\) while another was shorter in length without a bend. At the base of straight tube a small bag containing NaOH was tagged. The bent tube was marked by using a strip of graph paper and a colour drop was introduced. After that keep the cockroaches in desicator chamber under mosquito coil and mosquito mat step by step put it in respiration chamber. The respiration by the cockroaches inside the chamber caused release of CO\(_2\). The CO\(_2\) released was absorbed by NaOH. The decrease in the air pressure causes the entry of atmospheric air through the straight tube. The entry of fresh air was indicated by the movement of the coloured drop in the bend tube. From this movement the rate of O\(_2\) consumed/gram wet wt/hr was calculated.

**RESULT:**

Effect of mosquito mat and mosquito coil on rate of respiration was calculated. Mosquito mat was observed to be more effective than mosquito coil.

**Table:** Effect of pesticide on rate of whole body respiration of cockroach *Periplaneta americana*.

<table>
<thead>
<tr>
<th>Pesticide repellent used</th>
<th>Control (cm)</th>
<th>Experimental (cm)</th>
<th>S.D. (control)</th>
<th>S.D. (Exp.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mosquito mat</td>
<td>2.76</td>
<td>1.55</td>
<td>0.282</td>
<td>0.89</td>
</tr>
<tr>
<td>Mosquito coil</td>
<td>5.11</td>
<td>1.61</td>
<td>0.682</td>
<td>0.392</td>
</tr>
</tbody>
</table>
DISCUSSION:
A mosquito coil is widely known as an efficient insect repellent. The major active ingredient of mosquito coil is pyrethrin accounting for about 0.3-0.4% of coil man when a mosquito coil is burned the insecticides evaporate with smoke. The remaining components of mosquito coil are organic filters binders dyes, and other additives capable of smouldering well the combustion of the remaining materials generated large amounts of sub micrometer particle can reach the lower respiratory tract & may coated with wide range compounds some of which are carcinogens such as polycyclic aromatic hydrocarbons. synthetic pyrethroids and pyrethrins affect both the peripheral and central nervous system of insects by acting on the voltage gated sodium channel proteins found in cell membrane by prolonging the opening of these channels, pyrethrins and Pyrethroid stimulate nerve cells to produce repetitive discharges causing paralysis and possible of insect death. Due to allethrin stress containing mosquito coil it causes depletion in the level of carbohydrate reserves in cockroach Periplaneta americana. Due to exposure of allethrin containing mosquito coil increase in protein level in cockroach Periplaneta americana was observed.

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Selected Papers on Physiology 171
Effect of Fertilizers Urea and DAP on Rate of Oxygen Consumption of The Crab, *BARYTELPHUSA CUNICULARIS*

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² Dept of zoology, Shri. Renukadevi Mahavidyalaya, Mahur

ABSTRACT:
The rate of oxygen consumption of the crab, *Barytelphusa cunicularis* increases from 24 to 72 hours & steep decrease was observed at 96 hours on Exposure to two fertilizers Urea and DAP, the lethal concentration of Urea 3.5 ppm & DAP 0.25 ppm respectively

**Key words**: Urea and DAP fertilizers, rate oxygen consumption of crab *Barytelphusa cunicularis*.

INTRODUCTION:
Environmental pollution especially water pollution, has been increasing by addition of pesticides, fertilizers, herbicides and chemicals which altering the toxicity of aquatic media. The rate of toxicity of water also changes due to rapid industrialization, civilization deforestation and toxicants find their ways in to the fresh water bodies and have produced unexpected consequences on aquatic fauna.

In modern agricultural operations, a variety of fertilizers such as ammonium chloride, Diammonium phosphate and urea are used for increase in products of agriculture. The excess amount of these chemicals produces unwanted residues, which pose a great threat to aquatic organisms, tees these chemicals when enter in the water body, accumulated in different organs of aquatic organisms and interfere with various biological processes like respiration.

Oxygen consumption is a measure of the metabolic state of the animals. Hence it is considered as vital parameters and
indicates the physiological and metabolic alterations of the animal. It is known that the respiratory rates alter under the influences of several biotic and abiotic factors.

Use of fertilizers and pesticides is of fend associate with pollution problems. Accumulation of nitrate in ground water causes increase percentage of nitrate in drinking water which pose serious problems for the consumers and public health in general. Other problems include contamination of soil by heavy metals cadmium through addition of phosphate Fertilizer and nutrient leading to eutrophication and deterioration of water quality.

Several others have reported that the principal effect of organic fertilizers in pond to increase the productivity than in turn result to increase in fish yield. Mertzger and Boyd (1980) showed that 5.6 Kg/ha of ammonium phosphate is suitable for pond with low or moderate exploitation. It is known fact that fertilizers are degraded by micro flora and fauna in water as reported by, Miller,(1975) and Bard, et al.,(1976); Hunt and Boyd,(1981) reported that the indiscriminate use of fertilizers can causes the lowering pH condition of fresh water system.

However the scanty information is available on oxygen consumption in fresh water crab, Barytelphusa cunicularis with respect to study of the effect of commonly used fertilizers i.e. Urea (3.5ppm) lethal concentration and DAP at (0.25ppm)lethal concentrateration on oxygen consumption.

MATERIALS AND METHOD:

The fresh water crab, Barytelphusa cunicularis was used for experiment to observe the effect of fertilizers Urea at (3.5 ppm) and DAP at (0.25 ppm) on oxygen consumption. During the period of experiment the temperature of the water is 27 C .

The water was changed every day ,only the male crab was used for experiments, an average weighing the crabs are used about (30 to 40 gms)and animals were not feed during the experimental period. After different time period such as 24, 48,72,and 96 hours. The oxygen consumption was studied by using the apparatus devised by [10]Saroja,(1959).

The amount of dissolve oxygen in the sample was determined by the slandered Winkler’s method as given by

Selected Papers on Physiology 173
Welsh and Smith,(1960). The oxygen consumed by animal at each successive time interval of 24,48,72 and 96 hours was calculated. The value are expressed as CC of O$_2$ /hr/gm wet wt.

**RESULT:**

The effect of fertilizers causes increase in the rate of oxygen consumption of fresh water crab, *Barytelphusa cunicularis* in lethal concentration of Urea at (3.5 ppm) and DAP at (0.25 ppm). Initially in both fertilizers the rate of oxygen consumption increases from 24 hours to 72 hours, and steep decrease at 96 hours in both fertilizers.

Due to stress and suffocation of fertilizers effect, but Urea shows more decrease in rate of oxygen consumption effect than DAP. It is clear that different fertilizers exert their effect differently to same animals, The result shown in Table 1.1

**Table 1.1**

<table>
<thead>
<tr>
<th>Time (Hours)</th>
<th>control</th>
<th>Concentration of Fertilize (Urea)</th>
<th>Concentration of fertilizers (DAP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24±</td>
<td>0.099±0.007</td>
<td>0.12±0.0005</td>
<td>0.13±0.001</td>
</tr>
<tr>
<td>48±</td>
<td>0.11±0.003</td>
<td>0.13±0.001</td>
<td>0.15±0.002</td>
</tr>
<tr>
<td>72±</td>
<td>0.12±0.0005</td>
<td>0.15±0.003</td>
<td>0.16±0.002</td>
</tr>
<tr>
<td>96±</td>
<td>0.098±0.002</td>
<td>0.11±0.005</td>
<td>0.14±0.002</td>
</tr>
</tbody>
</table>

**Discussion:**

The alterations in rate of O$_2$ consumption are valuable indicator of stress and are frequently used to evaluate changes in metabolism due to environmental alteration. *Barytelphusa cunicularis* exposed to lethal concentration of Urea and DAP
exhibit increase in the rate of oxygen consumption from 24 hours and maximum at 72 hours in both fertilizers and decrease at 96 hours, it is more in Urea than DAP. This indicate that the long term exposure of toxicants causes increase in osmotic work of animals at cellular level resulting in rate of oxygen consumption, this is due to formation of mucus film over the body surface and gills must be taking place which interferes the respiratory functions and other vital activity of gills.

According to Skidmore & Tovell, (1972); Randal & Shelton, (1968) observed the restlessness increased opercula movement in Ranibow troutr when exposed to the pesticide.

The gills are the main organ for osmoregulation surface and site for ammonia excretion, aquatic crab is phyllobranchiatic gill Regnault, 1987, featuring a single cell layered epithelium covered by anion selective cuticle. The gills of aquatic crabs are multifunctional organs in addition to their function in excretion of nitrogenous waste products, they are also responsible for gas exchange. Mertzger and Boyd (1980) studied ammonia and enzyme positively linked and involved in ammonia transport.

In present investigation result indicates, that rate of oxygen consumption of fresh water male crab, *Barytelphusa cunicularis* exposed to lethal concentration of fertilizers Urea at 3.5 PPM & DAP at 0.25 PPM showed significant alterations. The rate of oxygen consumption is found to be increased up to 72 hours & decline at 96 hours in both fertilizers Urea & DAP as compare to control. This may be due to suppression of metabolic activity of animals or due to disruption of the gills & coagulation of gills mucus and inhibition of enzyme system at mitochondrial level.

REFERENCES:


Effect of Detergent Surf on Oxygen Consumption by *Barytelphusa cunicularis* (West Wood)

M. H. Mujewar and A. B. Harkal
Department of Zoology
Shri Renukadevi Collage, Mahur, India

**ABSTRACT**

The rate of oxygen consumption of the crab *Barytelphusa cunicularis* decreased from 24 hours onwards when exposed to lethal concentration of Surf at a concentration of 0.4 ppm.

**Key words:** Detergent, oxygen consumption rate, *Barytelphusa cunicularis*

Detergents show poisonous effects on aquatic flora and fauna. They destroy external mucus membrane of the gills of aquatic organisms and decrease breathing ability. Phosphate present in the detergents can lead to the formation of algal blooms which release toxins and deplete oxygen in water. As oxygen consumption is a measure of metabolic state of animal, it is considered as an important parameter which indicates physiological and metabolic alterations in the animals. It is known that respiration alters under the influence of biotic and abiotic factors[1]. relationship between respiration with pollution has reviewed earlier by Roberts (1972)[2]

Normal respiratory area of gills may get altered, due to the contact with polluted water. Hence, current investigation was undertaken to determine oxygen consumption of tropical freshwater amphibious field crab *Barytelphusa cunicularis* exposed to the solution (0.4 ppm) of Surf.

Fresh water crab, *Barytelphusa cunicularis*, used during present study were collected from Banshelki dam, Lature District and were brought in to the laboratory. They were kept in aquaria
with freshwater., the temperature of which varied between 26-30°C. The animals were fed with frog leg muscles before exposing them to required concentration of Surf. Water of the aquarium was changed daily.

The experiment was conducted with crabs subjected to lethal concentration of detergent (0.4 ppm) The crabs of average weight 40-50 gm were used and the amount of dissolved oxygen was determined [3]

The data on oxygen consumption has been expressed in terms of ml/hr/gm. Wt. liter. As an average of five observations along with standard deviation (Table 1). The animals exposed to the detergent exhibited decreasing trends up to 96 hrs. as compared with unexposed (Control) animals. Hence it is concluded that the detergent was toxic.

**TABLE 1:** Oxygen Comsumption (ml/hr/gm.wt/lit) By *Barytelphusa Cunicularis* exposed to (0.4ppm) Surf

<table>
<thead>
<tr>
<th>Exposure time (hrs)</th>
<th>Control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>0.1600 ± 0.0054</td>
<td>0.160 ± 0.0100</td>
</tr>
<tr>
<td>48</td>
<td>0.1500 ± 0.0044</td>
<td>0.155 ± 0.0044</td>
</tr>
<tr>
<td>72</td>
<td>0.1400 ± 0.0070</td>
<td>0.148 ± 0.0057</td>
</tr>
<tr>
<td>96</td>
<td>0.1400 ± 0.0044</td>
<td>0.140 ± 0.0044</td>
</tr>
</tbody>
</table>

**REFERENCES :**
1. Prosser, L.L. (1973) in “Comparative animal physiology” (III Ed) W.B. Sauders Co; philadelphia U. S. A.
Seasonal Metabolic Variation of Glycogen Content in Freshwater Bivalve *Lamellidens corrianus* (Lea, 1834)

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ABSTRACT

Glycogen is a multibranched polysaccharide of glucose that serves as a form of energy storage in animals. Glycogen is important organic substances required by an organism in cellular process. In metabolic pathways and biochemical reactions glycogen have been known to act as the energy supplier, under extreme stress condition. In the present investigation glycogen is found maximum in gonads throughout summer season, increasing in rainy season and minimum in winter season followed by mantle, hepatopancreas, Adductor Muscle and gills. Keywords: Glycogen, Lamellidens corrianus, Seasonal.

INTRODUCTION

The chief carbohydrate in the tissues is glycogen, while glucose is an utilizable sugar found in the tissues and the body fluids. Glycogen is reversibly converted to glucose under the influence of hormonal mediated enzyme activities. The equilibrium in glycogenesis and glycogenolysis tends to maintain blood sugar at a steady state. The oxidation of glucose is mediated by catabolic pathways viz., glycolysis, Krebs cycle, electron transport system and hexose mono-phosphate shunt, which constitute the major segments of carbohydrate metabolism. Thus carbohydrate metabolism gained importance in the physiology of animals. According to Prosser, (1984) [1] the synthesis and degradation of glycogen will not occur simultaneously at any significant rate. The other probability for
occurrence of depletion in glycogen levels might be due to dephosphorylation of phosphorylase ‘a’ and specific protein phosphatases. Glycogen is a multibranched polysaccharide of glucose that serves as a form of energy storage in animals. The polysaccharide structure represents the main storage form of glucose in the body.

MATERIAL AND METHOD

Bivalves Lamellidens corrianus sample (75-80 in shell length) were obtained from fishermen’s catch. In the present study they were collected from Godavari River near distinct Nanded of Maharashtra in India. Immediately after bringing to laboratory, hard shells of these freshwater bivalves were brushed and washed with fresh and clean water to remove algal biomass, mid and other waste material. The cleaned animals were kept for 12 hrs in laboratory conditions under constant aeration. For glycogen analysis, animals were dissected and soft body tissues like mantle, hepatopancreas, gonad, Adductor muscle and gill were removed. 100 mg of each wet tissues were taken for biochemical analysis. Estimation of glycogen was done by Anthrone method using glucose as standard and values of glycogen were expressed in terms of mg glycogen/gm wet weight of tissue.

3. RESULTS AND DISCUSSION

Glycogen estimation observed during experimental work has been given in Fig 1 for Lamellidens corrianus.

Glycogen content in Lamellidens corrianus:

1) Gonad :- The seasonal change in the glycogen content in gonad of L.corrianus are shown in (Table and Figure, 5). The percentage of glycogen was found to be maximum in summer and varies from 10.0463±0.2352 to 11.1 121±0.2342 , increasing in rainy season and varies from 4.3302±0.5203 to 9.1012±0.3414 , whereas it is minimum in winter season and varies from 3.1317±0.3204 to 5.0400±0.2231 .
2) **Mantle** :- The seasonal change in the glycogen content in mantle of *L.corrianus* are shown in (Table and Figure, 5). The percentage of glycogen was found to be maximum in summer and varies from $10.1213\pm0.7415$ to $12.1220\pm0.1202$, increasing in rainy season and varies from $2.2210\pm0.5213$ to $6.6413\pm0.4201$, whereas it is minimum in winter season and varies from $6.6413\pm0.4201$ to $6.6413\pm0.4201$.

3) **Hepatopancreas** :-

The seasonal change in the glycogen content in hepatopancreas of *L.corrianus* are shown in (Table and Figure, 5). The percentage of glycogen was found to be maximum in summer and varies from $10.1000\pm0.2110$ to $11.0120\pm0.3220$, increasing in rainy season and varies from $4.1121\pm0.4120$ to $7.4041\pm0.4403$, whereas it is minimum in winter season and varies from $4.2021\pm0.3210$ to $6.2134\pm0.3010$.

**Adductar Muscle** :-

The seasonal change in the glycogen content in Add.muscle of *L.corrianus* are shown in (Table and Figure, 5). The percentage of glycogen was found to be maximum in summer and varies from $9.0220\pm0.3130$ to $10.4202\pm0.2230$, increasing in rainy season and varies from $7.1200\pm0.1254$ to $9.1010\pm0.1022$, whereas it is minimum in winter season and varies from $4.1211\pm0.4212$ to $5.270\pm0.3201$.

5) **Gills** :-

The seasonal change in the glycogen content in gill of *L.corrianus* are shown in (Table and Figure, 5). The percentage of glycogen was found to be maximum in summer and varies from $7.1220\pm0.2728$ to $10.2130\pm0.6$, increasing in rainy season and varies from $6.5210\pm0.5312$ to $9.2023\pm0.2112$, whereas it is minimum in winter season and varies from $4.2210\pm0.2120$ to $5.1202\pm0.4226$.

The present study revealed that, there is significant variation in the biochemical composition in different body tissues according to seasonal changes. Seasonal changes in glycogen content may
be of great importance in relation to energy metabolism necessary for growth and reproduction. Organic constituents like glycogen act as key substances for different metabolic activities. All the tissues show increasing order glycogen contents in rainy season, which is correlated with highest body activities of animal during this season. And due to increase inflow and turbidity of water and to cope up with new environmental change. It might be due to favourable environmental lots of food availability and the period of growth with the gonadal development. Similar conclusions were reported in M. edulis, in British water by Williams, 1969 and Nagabhushanam, 1978. Bivalves generally store carbohydrates in large amounts during their growing season and use them over the rest of the year; Glycogen found to be maximum in body tissues during winter season as compared with monsoon and summer season Glycogen is the primary energy store in bivalves. In the entire body organ it is observed that glycogen contents are significantly accumulated is found to be more during summer season. The relationship of the energy transfer between different tissues, their capacity of reserve amounts under food availability, and their positive relationship with the high temperature and gonadal maturation have been shown in different species of bivalve 111ork111sk such as scallops. The relative content of glycogen varies seasonally. These changes are principally related to the reproductive cycle and the season maximum shell growth. Similar characteristics have been observed in other bivalve Lyropecten (Nodipecten) nodosus.

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4. Biochemical composition and energy allocation in the tropical scallop Lyropecten (Nodipecten) nodosus during the
months leading up to and following the development gonads. Aquaculture. 199: 63-72.


Metallic Impact on Biochemical Alteration on Protein and Amino Acid Content of Marine Crustacean, *SCYLLA SERRATA* from West Coast of India. R. P. Mali, U. M. Jayabhaye and N. G. Nagrale

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**ABSTRACT:**

Today pollution of biosphere is threatening existence of human being on the earth along with live nature. Metal pollution of sea is visible and indirect than other types of marine pollution but it effects on marine ecosystem and man which disturbs the natural balance. The impacts of metals directly affect and alter the molecular and biochemical constituents of aquatic organisms. Hence in present investigation the crab *Scylla serrata* has been studied after exposure of heavy metals such as cadmium sulphate and mercuric sulphate for 24, 48, 72, 96 and 120 hours exposure. It was observed that the protein content was gradually decreased due to enhanced proteolysis while the amino acid content was gradually increased to meet the required demand for energy requisites in addition to carbohydrates and fats to make up and adjust for heavy toxicity activities.

**Keywords:** Heavy Metal Toxicity, *Scylla serrata*, Cadmium sulphate, Mercuric sulphate.

**INTRODUCTION:**

The severe alterations in natural aquatic ecosystem of the world are the burning problem of this century. The discharge
of all wastes including chemical, domestic and agricultural are the uncontrolled sources of water pollution.

The content of heavy metals in water is not only serious for aquatic fauna but also to human being. The effect of heavy metals on aquatic organisms is currently attracting wide spread attention, especially in the field of water pollution. Before the past four decades, there was a little concerned about heavy metals related environmental contamination. However, latter on due to heavy industrialization, it became evident that a metallic salt causes harmful and deleterious effects on biological systems. The study of heavy metal toxicity to marine ecosystem, showing vast alterations in various biological constituents such as proteins and amino acids to which help in understanding alterations to overcome the toxic effects and hence the changes induced by heavy metal toxicity. The marine crab, *Scylla serrata* has been selected as one of the important indicator organism from west coast of Indian Ocean as it is highly sensitive to any aquatic changes.

**MATERIALS AND METHODS:**

The marine crustaceans, *Scylla serrata* were collected from November 2013 to January 2014 through local fisherman of Mumbai coast Dist. Mumbai and transported to Nanded through Railway.

The crabs were brought to the laboratory and acclimated for seven (7) days. Then they were treated with various concentrations of cadmium sulphate and mercuric sulphate to study the LC 50 values. The protein contents were estimated and free amino acids were estimated.

**RESULTS:**

The total protein contents in different tissues of *Scylla serrata* exposed to cadmium sulphate and mercuric sulphate showed decreasing trend as well as the amino acid content were gradually increased as compared to control from 24 hours to 120 hours. The results are as tabulated.
DISCUSSION AND CONCLUSION:

In present investigation the protein level decreased gradually when crabs were exposed to heavy metals such as of cadmium sulphate and mercuric sulphate. This decrease is attributed to severe stress exerted by of cadmium sulphate and mercuric sulphate reported that the depletion of protein content was due to increased proteolysis. This depletion creating protein deficiency which ultimately affect the survival status of crab showed the total protein was decreased in crab *Barytelphusa guerini* after exposed to zinc sulphate and copper sulphate. Similar observations were made by in *Scylla serrata* was reported by Due to impact of Heavy metals, animal shows high sensitivity towards the amino acid concentrations. In present investigation free amino acid contents in different tissues like muscles, chelate legs and hepatopancrease of crab, *Scylla serrata* exposed to cadmium sulphate and mercuric sulphate showed gradually increased trend of amino acid concentration than the control set. The results are coinciding with various researchers were observed that free amino acids content showed significant increased when fish *Channa punctatus* exposed to metal polluted water. reported the remarkable changes in free amino acids contents of freshwater female crab *Barytelphusa guerini* exposed to cadmium sulphate. Reported The amino acids are essential building blocks of the animal cell. Essential amino acid composition increased free amino acid level after exposure to cadmium chloride in *Ophiocephalus striatus*. The increased level of amino acids may be due to proteolytic activity.

REFERENCES:

various tissues of *Barytelphusa guerini*. J. Advan. Zoo.. 12(2) pp 155-120


**Table-1:** Cadmium sulphate and Mercuric sulphate exposed tissues of *Scylla serrata* shows protein contents in mg/100 mgs wet weight of tissues.

<table>
<thead>
<tr>
<th>Exposure Period (Hours)</th>
<th>Exposure of tissues to Cadmium sulphate</th>
<th>Exposure of tissues to Mercuric sulphate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscles</td>
<td>Chelate Legs</td>
</tr>
<tr>
<td>Control</td>
<td>22.7 ± 0.19</td>
<td>20.87 ± 0.31</td>
</tr>
<tr>
<td>24</td>
<td>22.1 ± 0.42</td>
<td>18.47 ± 0.12</td>
</tr>
<tr>
<td>48</td>
<td>20.56 ± 0.34</td>
<td>17.67 ± 0.56</td>
</tr>
<tr>
<td>72</td>
<td>17.91 ± 0.37</td>
<td>16.10 ± 0.34</td>
</tr>
<tr>
<td>96</td>
<td>16.34 ± 0.18</td>
<td>14.96 ± 0.87</td>
</tr>
<tr>
<td>120</td>
<td>18.34 ± 0.63</td>
<td>13.37 ± 0.21</td>
</tr>
</tbody>
</table>

(Mean values of protein contents of six samples and ± SD)
Table-2: Cadmium sulphate and Mercuric sulphate exposed tissues of *Scylla serrata* shows amino acid contents in mg/100 mgs wet weight of tissues.

<table>
<thead>
<tr>
<th>Exposure Period (Hours)</th>
<th>Exposure of tissues to Cadmium sulphate</th>
<th>Exposure of tissues to Mercuric sulphate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscles</td>
<td>Chelate</td>
</tr>
<tr>
<td>Control</td>
<td>23.19 ± 0.9</td>
<td>23.37 ± 0.07</td>
</tr>
<tr>
<td>24</td>
<td>24.11 ± 0.32</td>
<td>23.02 ± 0.03</td>
</tr>
<tr>
<td>48</td>
<td>25.10 ± 0.22</td>
<td>24.42 ± 0.57</td>
</tr>
<tr>
<td>72</td>
<td>26.44 ± 0.06</td>
<td>25.21 ± 0.16</td>
</tr>
<tr>
<td>96</td>
<td>27.41 ± 0.34</td>
<td>26.88 ± 0.11</td>
</tr>
<tr>
<td>120</td>
<td>28.23 ± 0.34</td>
<td>27.94 ± 0.15</td>
</tr>
</tbody>
</table>

(Mean values of amino acid contents of six samples and ± SD)
Simulation Of Glycolysis Using MLP

Mohseeena Thaseen
Dept of Computer Science and Information Technology, SSBE’S Institution of Technology & Management, Nanded.

ABSTRACT:
Energy produce to do work is essential aspect for survival of life. This energy is generated through a metabolic reaction within the cells; one among them is glycolysis process which is included in the respiration system of the human being, plants and even in animals. However glycolysis is the first process of Cell respiration, its main aim is to breakdown glucose to form two pyruvates within cytoplasm, it produces 1ATP’s and 2 NADH utilizing only 2 ATP’s in the process.

System dynamics facilitate Non-Linear activities easily used by simulation, via the principle of divide and conquer in designing and learning the network of glycolysis. This network is referred as multi-layer perceptron (MLP). A Simulations exhibit the behavioral complexity of globally integrated independent functions through Activation function system.

Keywords : Glycolysis, Multi-layer perceptron (MLP), Activation Function.

INTRODUCTION:
Cell consists of regulatory networks of proteins that controls the arrangement of cell replication by growth and by isolating with respective to time constrain of a cell-life. A cell is enclosed with concentrated aqueous solution of molecules contained in membrane, called plasma membrane. The components and their interactions in a network results in an insightful, to overcome this problem the biologist has proposed that “cells are complex Systems” [1]
System changing with respective to time consequences in the system’s dynamic behavior. System Dynamic mainly deals with non-linear behavior [2] of a complex process, which diverse
into mechanistic models i.e. direct approach - Model[3]. Simulation (predict system), sometimes referred to as in silico (computer simulation). However the biological behavior simulation can be completed under specific conditions carried out by numerical software packages. Non-linear data doesn’t have to follow any specific model, since nonlinearity is taken by means of biochemical interactions [4], which are the zoological allotters for the rate and specifications of different chemical reactions undergoes inside cells.

In this paper cell respiration first process, i.e. glycolysis is simulated using Multi-layer Percetron (MLP) and its rate of reactants and products in a biochemical reactions is modeled through stoichiometric dynamics.

1.1 The System Structure for Glycolysis:
Glycolysis is the most primordial metabolic pathways in living cells; however it is universal across the all species of living organisms and is catalyzed by soluble enzymes recited in the cytosol of the cells. This process is regarded as a linear pathway; furthermore glycoltic pathway has two phases [5]. The energy investment and energy generation with or with presences of oxygen (Aerobic and Anaerobic respectively).

**Fig 1:** Biological view of glycolysis

*[Selected Papers on Physiology]*
Step 1: Glucose + ATP (hexokinase) → Glucose-6-P + ADP  
Step 2: Glucose-6-P (Isomerase) → Fructose-6-P  
Step 3: Fructose-6-P + ATP (Phosphofructokinase) → Fructose 1;6 Di-P  
Step 4: Fructose 1;6 Di-P (Aldolase) → 3: PGAL + DHAP  
Step 5: 3: PGAL + NAD + iP + H2O (Phosphoglycerate Dehydrogenase) → 1:3 Di-PGA + NADH2  
Step 6: 1:3 Di-PGA + ADP (Phosphoglycerate Kinase) → 3: PGA + ATP  
Step 7: 3: PGA (Phosphoglycerate mutase) → 2: PGA  
Step 8: 2: PGA (Enolase) → PEPA(3C) + H2O  
Step 9: PEPA(3C) + ADP → Pyruvic Acid(3C) + ATP  

The metabolic reaction requires total eleven variables and ten independent variables which results in the complete process.

[II] Motivation:  
The main purpose behind this modulation is the biological neural network (BNN) structure [6]. Metabolism is the entire chemical reactions that take place in an organism. Neural network try to emulate the working functionality of biological neural system, as many billions of neurons resembling as nodes a processing unit and their interconnections by means of individual synaptic weights and connecting one neuron to the next associated neuron to form the networks, and this kind of network is modeled as perceptron. And which is further more trained to differentiate between linear and non-linear the output of process [7].

Figure 2. Artificial Node - Threshold logical unit

The mathematically representation:

\[ x_1 + x_2 + \ldots + x_n \]  
Where X and Y the neuron’s receives inputs from excitatory \( x_1 + x_2 + \ldots + x_n \) with identical weights are effectively positive integers and Inhibitory inputs have \( x_{n+1} + x_{n+2} + \ldots + x_{n+m} \) for one way output with unrestricted is represented.
The synapses accumulate parameters are called as “weights” that can manipulate the data in the calculation

**[III] Activation Function:** It shows the output node of given input node similarly it defines the output layer of MLP when an input layer is given to any network. The most commonly used nonlinear functions of activation function are

1. The Sigmoid (logistic) 2. Tangent hyperbolic
An activation function is defined as the output of a neuron in terms of its input (i.e. induced local field)

There are three types of activation functions
1. Threshold function (This function is also termed the *Heaviside* function)

2. Piecewise linear: amplification inside linear

3. Sigmoid function: it is the combination of above both i.e. threshold function & linear function
   i. Logistic function whose domain is (0,1)

   ii. The hyperbolic tangent whose domain is (–1, –1)

   iii. Algebraic sigmoid function whose domain is
The interconnection is done in layers—the first layer will attain the input, hence it is called input layer, the next layer is intermediate or hidden layer where the processing takes place and the final is the output layer as name itself states it contains the output of the complete modeled, and this modeled is named as Multi-Layered perceptron (MLP).

**Figure 5.** Multi layered perceptron model.
Mathematical representation:

Where $x_j$ is the $j^{th}$ component of input vector of the neuron. It consists of an activation function $z = \psi(x, \ldots, x_n) : n \rightarrow$ and a possibly non-linear output or transfer function $\psi(x, \ldots, x_n) = \text{Activation function} z = \psi(x, \ldots, x_n) : n \rightarrow$ and a possibly non-linear output or transfer function $\psi(z): (x, \ldots, x_n) = \sum_{i}^{N} w_{i} x_{i}$

The output functions that are commonly applied in the neural network are linear, sigmoidal and tan-sigmoidal.

Since each neuron has its weight vector $w_{ij}$ which is representing the strength of the synaptic connection between the neuron $i$, if the synaptic weight is positive then it corresponds to an excitatory synapse and negative weight to an inhibitory.
synapse and a zero to synaptic weight having no connection between the neurons. An additional constant of weight vector called “bias” is used with input value of +1, or -1 to model MPL from linear [8] to an affine Transformation. However the learning algorithms estimate weights repetitively by minimizing a cost function so that if any error between the desired and neural network model output it can be neutralized.

This layered model is directed graph with each layer completely connected to the next associated layer. And hence each node is a processing unit with a non-linear [9] activation function, and which is trained using training algorithm to be modified in the linear form.

This model approach is used to solve in computer neuroscience and which get approximate solution for extremely complex problems.

**[IV] MULTI-LAYER PERCEPTRONS (MLPS):**

A multilayer perceptron (MLP) is best suitable for feed-forward artificial neural networks, that maps input data with appropriate outputs. As mentioned earlier MLP contains multi – layers with nodes arranged in a directed graph, with each layer connected to the next layer. MLP is an adaptation of linear perceptron [10].

As depicted from figure 5. The input layer of MLP usually contain 0(zero) layer, with many hidden layers (intermediated layer) and finally output layer. The hidden layers can be N layers, with N layers of weights. Two layer multi-Layer perceptron are represented in the following form.

Frequently, the input layer is layer 0, and intermediated hidden - N layer network of weights and N non-input layers of processing units. Thus a two layer Multi-Layer Perceptron takes the form:
From the above figure 6 the number of layers can be added, considering the activation function \( f(n)(x) \) for each different layers. where (n) is the n nodes of that particular layer.

[V] CONVERTING LINEAR TO NON-LINEAR:

The glycolytic pathway is ever-present and is being found in every cell of all living things. The glycolytic pathway process in both the presence (aerobic) or absence of oxygen (anaerobic such as fructose, galactose, and mannose are also metabolized ). And glucose act as a stimulate energy being oxidized, as it oxidized by glycolytic enzymes & coenzyme nicotinamide adenine dinucleotide (NAD+) converted to its oxidized from NAD+ to NADH, which is form in presences of oxygen.

In animals cells also the same process of glycolysis is taken place for the energy formation. Similarly the same process in neural network minimizes the interaction between the neurons through weights of the components, as specified the neuron interacting is being considered as N-Neurons/Nodes. Which provides the robustness and reliability in resultant neurons, these approaches is regard as automatic or knowledge-based to determine the structure of pathway process build in neural networks.

[VI] CONCLUSION:

In this paper more Infosys is given to understand the essential features for formulating linear reaction taken place into the process of glycolysis into non-linear formulation structure via Multi-layered perceptron (MLP). According to [10,
previous papers indicating its dynamic system process for metabolism, has indicated its stoichiometric matrix, in future may be training and learning concept could be elucidated which will be the continuation of this papers.

REFERENCES:

Effect of MSG On Human Health

S. A. Quadri, T.T. Shaikh and J.D. Shaikh
Maulana Azad College Aurangabad, India.

ABSTRACT:
Monosodium Glutamate (MSG) used as food all over the world. It is usually saturated fat containing glutamic acid or is a non essential amino acid, generally it is expressed as E number E 621 and HS code 29224220 (Harmonised system code) commonly used in Chinese food item but still most of the people don’t know its side effects on our health, children and pregnant woman are vulnerable to it. In general, if a food is processed you can assume it contains MSG (or one of its pseudo-ingredients). So if you stick to a whole, fresh foods diet, you can pretty much guarantee that you'll avoid this toxin. Making a decision to avoid MSG in your diet as much as possible is a wise choice for nearly everyone. It does take a bit more preparation and time in the kitchen to organize food at home, using fresh, locally grown ingredients. Present study has revealed some health hazards of MSG.

Key words: MSG, glutamic acid, E number, kitchen, Chinese food and Health.

INTRODUCTION
Monosodium Glutamate (MSG) commonly known’s as Ajinomoto occur naturally in seaweed, dairy products, meat, parmesan Cheese, fish and mushrooms. MSG is used in Chinese food broth, ripe tomato ketchup, soya sauce, and different chemical food industries as flavour enhancer [1-4]. MSG is cheap and readily available which enhance food taste. Food and Drug and Administration (FDA) has classified MSG as food ingredients but its use remains controversial.

Role of MSG in Food:
MSG is commonly used in many Chinese food items. It is used in pizza, hot dogs, burgers, noodles, all ready to cook and instant food etc. MSG is sold in supermarket, malls, foods stores. It is used as preservative and to enhance the taste of food. Therefore the children’s become mostly habitual of such food [5-8].

**List of ingredients that ALWAYS contain MSG:**

<table>
<thead>
<tr>
<th>Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autolyzed Yeast Glutamate</td>
</tr>
<tr>
<td>Calcium Caseinate</td>
</tr>
<tr>
<td>Glutamyl Acid</td>
</tr>
<tr>
<td>Gelatin</td>
</tr>
<tr>
<td>Hydrolyzed Protein</td>
</tr>
<tr>
<td>Monopotassium Glutamate</td>
</tr>
<tr>
<td>Monosodium Glutamate</td>
</tr>
<tr>
<td>Sodium Caseinate</td>
</tr>
<tr>
<td>Textured Protein</td>
</tr>
<tr>
<td>Yeast Extract</td>
</tr>
<tr>
<td>Yeast Nutriet</td>
</tr>
</tbody>
</table>

**These ingredients OFTEN contain MSG or create MSG during processing**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Seasonings</th>
<th>Natural Flavors and Flavorings</th>
<th>Natural Pork Flavoring</th>
<th>Natural Beef Flavoring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural Chicken Flavoring</td>
<td>Soy Sauce</td>
<td>Soy Protein Isolate</td>
<td>Soy Protein</td>
<td>Bouillon</td>
</tr>
<tr>
<td>Stock</td>
<td>Broth</td>
<td>Malt Extract</td>
<td>Malt Flavoring</td>
<td>BarleyMalt</td>
</tr>
<tr>
<td>Anything Enzyme Modified</td>
<td>Carrageenan</td>
<td>Maltodextrin</td>
<td>Pectin</td>
<td>Enzymes</td>
</tr>
</tbody>
</table>

**Choosing to be MSG-Free**

Making a decision to avoid MSG in your diet as much as possible is a wise choice for nearly everyone. Admittedly, it does take a bit more planning and time in the kitchen to prepare food at home, using fresh, locally grown ingredients. But knowing that your food is pure and free of toxic additives like MSG will make it well worth it. Plus, choosing whole foods will ultimately
give you better flavor and more health value than any MSG-laden processed food you could buy at your supermarket.

<table>
<thead>
<tr>
<th>Number</th>
<th>Name</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-620</td>
<td>Glutamic Acid</td>
<td>Might cause similar problems as MSG(621), young children should avoid it</td>
</tr>
<tr>
<td>E-621</td>
<td>Monopotassium glutamate (MSG)</td>
<td>Can be allergen, not permitted in foods for infants and young children</td>
</tr>
<tr>
<td>E-622</td>
<td>Monopotassium Glutamate</td>
<td>Can cause nausea, vomiting, diarrhea, abdominal cramps, Typical products are low sodium salt substitutes</td>
</tr>
<tr>
<td>E-623</td>
<td>Calcium glutamate</td>
<td>No known adverse effects</td>
</tr>
<tr>
<td>E-624</td>
<td>Monoammonium L-glutamate</td>
<td>No known adverse effects</td>
</tr>
<tr>
<td>E-625</td>
<td>Magnesium di-L-glutamate</td>
<td>No known adverse effects</td>
</tr>
<tr>
<td>E-627</td>
<td>Disodiumguanylate</td>
<td>Not permitted in foods for infants and young children</td>
</tr>
<tr>
<td>E-631</td>
<td>Dosodium inosinate</td>
<td>Not permitted in foods for infants and young children</td>
</tr>
<tr>
<td>E635</td>
<td>Sodium5'ribonucleotide</td>
<td>not permitted in Australia</td>
</tr>
<tr>
<td>E636</td>
<td>Maltol</td>
<td>no known adverse effects</td>
</tr>
<tr>
<td>E637</td>
<td>Ethyl maltol</td>
<td>no known adverse effects</td>
</tr>
</tbody>
</table>

According to the FDA, MSG Symptom Complex can involve symptoms such as:

- Numbness
- Burning sensation
- Tingling

100 | Selected Papers on Physiology
• Facial pressure or tightness
• Chest pain or difficulty breathing
• Headache
• Nausea
• Rapid heartbeat
• Drowsiness

Other effects of MSG:

1) MSG is slow killer when it consumed in large amount sever impairment to brain cells takes place.
2) MSG causes Allergies and side effects include chest pain, shortness of breath, Nausa, headaches, migration, hyperacidity, constipation heart palation and sweating.
3) MSG stimulates pancreases even the glucose level in normal in blood and feel hungry.
4) MSG cause respiratory problems including asthma.
5) MSG increase the gross weight of the heart and cause cardiac muscle by pertrophy.
6) Many food like broths, Soup and some kind of meat which is use MSG as a flavouring in danger (Fawzi Elshobaki)
7) MSG is generally safe in small amount but if it is in prolong and real harm (Warns Elshobaki).
8) MSG causes Obesity.
9) Depression.
10) Fatigue and disorientation (Russell Blaylock).

Some reports also indicates that, If body contains a high level of MSG, it gives way to free glutamates. This affects babies while in their mothers’ womb, as the placental barrier breaks down blocking the food supply to the baby. The placental barrier allows only good substances to enter. However, without the barrier, your baby is exposed to the good and bad, even allergens and germs [9-10].

CONCLUSION:

According to recent study of momjunction.com, Maggi noodles have dangerously high levels of lead and monosodium glutamate (MSG). A recent press report had stated that tests
performed by the food safety authorities in Uttar Pradesh had revealed that Maggi samples contained 17.2 parts per million (ppm) of lead, which is many times the permitted limits that range between 0.01 and 2.5 ppm. Both ingredients are extremely harmful, especially for children, which is the brand’s (Nestle) target market.

Finding revealed that continuous and increased use of MSG can significantly effects the health problems and person become addict chiefly children become addict to it.

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Study of Body Mass Index (BMI) Status of Some Students from Nanded (Maharashtra)

M. K. Malviya
Department of Zoology, Pratibha Niketan College, Vazirabad, Nanded, M.S., India.

ABSTRACT:
BMI is a statistical measure of body weight based on a person's weight and height. BMI has been used in Clinical practice by the WHO as the standard for recording obesity statistics since the early 1980s. We randomly selected 618 students, out of which 500 were from college and 118 students from school, both located at Nanded district in Maharashtra state. The anthropometric measurement of students were recorded in the year 2013-2014. Findings were tabulated and calculated for BMI, which represented graphically. We found maximum percentage of underweight students than normal BMI. Underweight BMI may be due to many different factors such as lack of awareness about healthy diet, economic background of family, number of family members, stress, tension, restlessness, study, etc affecting their diet and health.

Key words: Body Mass Index, students,

INTRODUCTION
BMI or Quetelet index is defined as the individual's body mass divided by the square of their height – with the value universally being given in units of kg/m² gives an indication of nutritional status. BMI or Quetelet index was invented between 1830 and 1850 by the Belgian polymath Adolphe Quetelet during his study on social physics. The healthy BMI range for adults is 18.5 to 24.9. However, children are constantly growing, which makes it difficult to have set values for BMI cut-offs. For adults who have stopped growing, an increase in BMI is usually
caused by an increase in body fat. But as children grow, their amount of body fat changes and so will their BMI. For example, BMI usually decreases during the preschool years and then increases into adulthood. For this reason, a BMI calculation for a child or adolescent is interpreted differently from an adult’s, and takes into account the age and sex of the child or adolescent. BMI can be calculated with reference to their age according to different age groups as it has different ranges for being overweight, underweight or normal weight.

**Research Methodology**

For our data collection we randomly selected 618 students from school and college of Nanded town. Out of which 118 students belong to Z.P. School, Dhanagarwadi, District Nanded, learning in 1st to 9th standard and 500 students from XI, XII and Undergraduate level of Pratibha Niketan College, Nanded (Maharashtra). The height and weight of total 249 male and 251 female from college students; 118 students from Z. P. school (61 males and 57 females) were measured. The data was collected by taking anthropometric measurements of students in the school and college time. The data was collected in the year 2013-2014. We recorded the data in tabular form, calculated student’s BMI by using formula and tally it with internationally recommended BMI chart (CDC BMI-for-age growth charts for girls and boys 0 to 20 years).

**Anthropometric Measurement**

We recorded the BMI readings in the department of Zoology, Pratibha Niketan College, Nanded (Maharashtra) and campus of Z.P. School, Dhanagarwadi, District Nanded whereby the student mobility occurs. Students are asked to remove their shoes, belongings like jacket, coat, hair ornaments, etc. Student’s height and weight measured successively until coincide readings.

**Height** was measured by using portable/wall mounted stadiometer with movable head piece.

The stadiometer was fixed from the 200 cm mark up against the wall from the floor. The students were asked to stand with his/her back to the height rule. The back of the head, back,
buttocks, calves and heels should be touching the upright, feet together. The head piece of the stadiometer was lowered so that the hair pressed flat.

Weight of students measured by using weighing machine in kg. The weighing machine was placed nearby to the stadiometer. The weighing machine should be placed on a hard-floor surface..

RESULT AND DISCUSSION:
We have recorded BMI readings of total 705 students including 404 male and 301 female. Out of which 118 students from Z.P. School, Dhanagarwadi, District Nanded and 587 students from Pratibha Niketan College, Nanded (Maharashtra).

BMI of Z.P. School students, Dhanagarwadi, District Nanded
118 Students from Z.P. School include 61 males and 57 females of 1st to 9th class having age between 5 to 14 years. We found maximum 70.33 % students have underweight BMI status and minimum 29.96 % showed normal BMI. Only 1.69 % female students were obese but not a single overweight have been observed.

BMI of students from Pratibha Niketan College, Nanded (Maharashtra).
We examined total 500 students including male and female, from Pratibha Niketan College, Nanded (Maharashtra) studying in XI, XII and UG class. All these students age ranges between 16 to 20 years. We measured BMI of total 150 students from XIth class, 185 from XIIth and 165 from UG class including both male and female. We observed 245 students having underweight BMI status, 238 students have normal BMI status, 14 students having overweight BMI status and 3 students having obese BMI status.

CONCLUSION
College student ranges between 16 to 20 years age showed maximum 49 % underweight BMI status than minimum 47.6 % normal. Overweight BMI status 2.8% and obese BMI
status 0.6% has been found. Where as school student ranges between 5 to 14 years age showed maximum 70.33 % underweight BMI status than minimum 27.96 % normal . Overweight BMI status 2.8% and obese BMI status 0.6% has been found. Most of the school students does not aware of their health so we found maximum BMI percentage of underweight. Their poor economic family background, more number of family members, careless about proper health, eating disorder or malnutrition, diseases, genetic disorder, and school mid day meal may be the possible reason. The present investigation may be monitor the effects of school-based physical activity, nutrition programs, medical care and policies, achieving health objectives, motivating parents and their children to make healthy and safe lifestyle changes. School-based BMI-surveillance data can be used to identify the percentages of students in the population who are obese, overweight, normal weight, and underweight; surveillance does not involve informing parents of their child's weight status.

BMI calculation formula:

Table 1: BMI status of students- Pratibha Niketan College, Nanded

<table>
<thead>
<tr>
<th>Sex/BMI</th>
<th>Class</th>
<th>Normal</th>
<th>Underweight</th>
<th>Overweight</th>
<th>Obese</th>
<th>Total</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Male</td>
<td>XI</td>
<td>15</td>
<td>41</td>
<td>2</td>
<td>00</td>
<td>58</td>
<td>249</td>
</tr>
<tr>
<td></td>
<td>XII</td>
<td>54</td>
<td>40</td>
<td>1</td>
<td>2</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td></td>
<td>UG</td>
<td>49</td>
<td>41</td>
<td>4</td>
<td>00</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>No. of</td>
<td>XI</td>
<td>40</td>
<td>49</td>
<td>3</td>
<td>00</td>
<td>92</td>
<td>251</td>
</tr>
<tr>
<td>Female</td>
<td>XII</td>
<td>45</td>
<td>41</td>
<td>1</td>
<td>1</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td></td>
<td>UG</td>
<td>29</td>
<td>39</td>
<td>3</td>
<td>00</td>
<td>71</td>
<td></td>
</tr>
</tbody>
</table>
Table 2: BMI status of students

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>BMI Status</th>
<th>No. of college students</th>
<th>No. of school students</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>238</td>
<td>33</td>
</tr>
<tr>
<td>2</td>
<td>Underweight</td>
<td>245</td>
<td>83</td>
</tr>
<tr>
<td>3</td>
<td>Overweight</td>
<td>14</td>
<td>00</td>
</tr>
<tr>
<td>4</td>
<td>Obese</td>
<td>03</td>
<td>02</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>500</td>
<td>118</td>
</tr>
</tbody>
</table>

Graph: showing BMI status and Number of students in school and college

X-Axis= Number of students; Y-Axis= BMI status

REFERENCES
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A Computational-Experimental approach for Computational Modeling of Mitochondrial Metabolism

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\textsuperscript{2}Dept of Botany and Biotechnology, Yeshwant Mahavidyalaya, VIP Road, Nanded.

ABSTRACT:

Computational models have represented to understand biological processes and phenomena in Systems Biology. This paper describes a simple model with some new ideas in the area with a particular application. A computational tool for mitochondrial systems biology has been developed as a simulation model. The general model consists of many enzymatic reactions and metabolites, representing the respiratory chain, the TCA cycle. It is based on previously published enzyme kinetics studies in the literature. The model can be easily extended and modified so that mitochondrial researchers can integrate the information / data in their own developed models to evaluate it in the system of the organism.

Mitochondrial energy transduction has been conventionally studied with the thermodynamic models developed earlier. More recently, kinetic or thermo-kinetic models have been proposed for the understanding of the control and regulation of mitochondrial energy metabolism and where it communicates with cytoplasm and other sub cellular organelles.

Keywords: Mitochondrial metabolism, TCA cycle, Computational model
INTRODUCTION

Many earlier dynamic kinetic models were built to predict time evolution of metabolic pathways energy metabolism in eukaryotic cells in Mitochondria were also studied by dynamic simulation of the respiratory chain model and the TCA cycle model.

The earlier studies investigated a group of pathways in mitochondria by reductionist approach whereas the present approach integrates several pathways like the glycolysis, Krebs cycle, oxidative phosphorylation, β-oxidation of the fatty acid and the metabolite transport system. This provides a holistic approach towards the dynamic performance of energy metabolism in mitochondrion. The modeling environment for the metabolic pathway in mitochondrion can be easily extended and modified. Therefore, it permits researchers working on mitochondrion for implementation of their own models to evaluate their hypotheses in an organism system.

The present approach comprises of modular building of models where the module represents the available data or information and the kinetic scheme available for that process.

This approach has taken into account the functional and regulatory interactions of reconstructed networks which forms the basis of analyzing the dynamics of complex physiological responses.

MATERIALS AND METHODS

2.1. Pathway Information

The essential biochemical information regarding the metabolic pathways and transport processes used in the modeling was obtained from biochemistry or physiology textbooks and some biochemical databases - KEGG, Kyoto Encyclopedia of Genes and Genomes (http://www.genome.jp/kegg/) and BRENDA, a comprehensive enzyme information database (http://www.brenda-enzymes.org).

2.2. Databases

Repository databases of models for model construction were used. Repositories of models available in EMBL-EBI
Selected Papers on Physiology | 111

European Bioinformatics Institute the IUPS Physiome Project repository (http://models.cellml.org) were used. Pathways Logic Models (http://mcs.une.edu.au/~iop/Bionet/index.html). The models available in these databases are kept as per the series of standards in language, annotations (e.g., SMBL, CellML) and are available for download to the public.

2.3. Kinetics

Experimental data to limit the modules’ kinetics were obtained from in vitro conditions.

2.4. Tools

Computational tools to work with the modules were used in the available software/platform like MATLAB and Mathematica.

[III] RESULTS 3.1. The Model

The mitochondrial model is a dynamic simulation model that contains many enzymatic reactions and metabolites of the respiratory chain - the TCA cycle (Figure 1). Every metabolite and enzymatic reaction is located in the various space like matrix, inner membrane or outer membrane of mitochondrion, inter-membrane space and cytoplasm of cell that surrounds the mitochondrion. The concentrations of all the enzymes and the metabolites in the cytoplasm are assumed to be constant. The evolution of time of all the enzymatic reaction and concentrations of the metabolite can be recorded. It allows the variation in concentration of metabolite to simulate their effect on the mitochondrial metabolism [4].

3.2. Pathways yield energy

A well-organized and prevalent process of cellular respiration utilizes oxygen as a reactant to complete the breakdown of a variety of organic molecules. Mitochondrion forms the most dynamic site for the biochemical and enzymatic reaction of cellular respiration in a eukaryotic cell. Food in the form of organic material is used as fuel for respiration. The end products are carbon dioxide and water.
The overall process is:
Organic compounds + O₂ → CO₂ + H₂O + energy (ATP + heat). Carbohydrates, fats, and proteins can all be used substrate for energy generation but the most preferred is considered to be glucose.

C₆H₁₂O₆ + 6O₂ → 6CO₂ + 6H₂O + Energy (ATP + heat)

The breakdown of glucose is exergonic with a ΔG of −686 kcal per mole of glucose.

A part of this energy is utilized to synthesized ATP for performing cellular activity.

**Figure 1:** The Kreb cycle

3.3. Construction

The major approaches for computational modeling considered in to either inductive approach or hypothesis driven science. The inductive approach comprises of large-scale modeling approaches. In this modeling generally it is considered that significant modeling features emerge from their simulations and analysis. The hypothesis driven approach try to seek the simplest model that can encompass the key features of a system.
steady with the level of available experimental data. The hypothesis-driven approach is easily recognizable among numerous works exploring the control of mitochondrial respiration using single integrated equation models. In the recent times, the majority of work follows an inductive approach that aims for the construction of relatively large-scale mechanistic models.

The model is based on information generated by many researchers over a longer time comprising of quantitative studies of the organelle. The overall enzymatic reactions are represented by rate equations and total kinetic parameters. The other parameters are computationally estimated to fulfill the Lineweaver–Burk plots of each enzyme using the special genetic algorithm implemented. The initial metabolite concentrations are taken from the literature (or set around their $K_m$ values of the enzymes. The matrix volume is in correspondence with the data of rat liver mitochondria. Simulations are performed by mathematical integration of the rate equations simulated in the cell reactor that make use of the fourth-order Runge–Kutta method.

**Figure 2:** A simple machine model at steady state

The present Computational model is model of systems inspired by the model of an information processing system. In its most common manifestation a model is a digital computer but need not be as restrictive as that in practice [9]. The key issues
involve in the present model is that system is always interacting with their environment (Figure 2) where the information processing models exhibits the communication and concurrency with the model. The models may be amenable to automated analysis as well as simulation. The most basic discrete model is the finite state machine with a set of internal states; a set of external inputs ± events; a set of system outputs ± actions [10, 11].

[IV] CONCLUSION

The mitochondrial model integrates the dynamics of the respiratory chain, the TCA cycle to enable metabolic pathway simulations in a whole mitochondrial scale. The rate of reaction and ability to increase/ decrease metabolite concentration while running the simulation can be done. The study the mitochondrial metabolism in special conditions can be customized in the model by substituting the kinetic parameters, the initial concentration of enzymes and metabolites or importing new reactions.

REFERENCES

Production of cellulolytic Enzymes by immobilized cells

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²Department of Botany and Biotechnology, Yeshwant Mahavidyalaya Nanded. (M.S.)

ABSTRACT

A number of opportunities awaits the application of immobilized cells in bioconversion studies. The increasing requirement for glucose as substrate for the production of fructose, ethanol etc. has led to development of bioconversion process. The bioconversion of higher carbohydrates, like starch, cellulose etc. polymers of glucose can yield abundance of glucose from waste or unutilized biomass. with the aim to efficiently manage and utilized the generated agricultural waste in the so called backward region of Maharashtra Marathwada, the problem of food and energy can be solved. Selecting the two mostly widely cultivated crops of this region generating large quantities of agricultural waste cotton and pigeon pea, work was undertaken to utilizes the wastes efficiently. Isolating the dominant and known cellulolytic fungi from the local soils and employing them in bioconversion studies was done. Aspergillus niger, Penicillium digitatum and Trichoderma lignorum were studied for production of cellulolytic enzymes in free as well as immobilized state. These enzymatic studies were carried out on CMC as well as medium based on Agro waste. Higher amount of enzymes were synthesized on agro waste medium by all the three fungi. pH and temperature affected synthesis of enzymes in both the state.

INTRODUCTION

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fructose, ethanol etc. has led to development of bioconversion process. The bioconversion of higher carbohydrates, like starch, cellulose etc. polymers of glucose can yield abundance of glucose from waste or unutilized biomass. with the aim to efficiently manage and utilized the generated agricultural waste in the so called backward region of Maharashtra Marathwada, the problem of food and energy can be solved. Selecting the two mostly widely cultivated crops of this region generating large quantities of agricultural waste cotton and pigeon pea, work was undertaken to utilizes the wastes efficiently.

Isolating the dominant and known cellulolytic fungi from the local soils and employing them in bioconversion studies was done. *Aspergillus niger*, *Penicillium digitatum* and *Trichoderma lignorum* were studied for production of cellulolytic enzymes in free as well as immobilized state. These enzymatic studies were carried out on CMC as well as medium based on Agro waste. Higher amount of enzymes were synthesized on agro waste medium by all the three fungi, pH and temperature affected synthesis of enzymes in both the state.

**Material and Method**

**Isolation of fungi from soil**

Dilution plate method was employed for isolation of fungi from soil. One gram of the soil was transferred to a conical flask containing 100ml sterile water and was shaken vigorously. lml of the sample was added to 9 ml sterile water similarly further dilutions were made. The most suitable dilution that gave measurable number of colonies per petriplate was found to be 1:1000. One ml of soil suspension from this last dilution was transferred aseptically to petriplates in triplicates and melted CMCN agar medium at 45°C was poured over it. The suspension was thoroughly mixed with the above media by rotating the petriplates and then allowed to solidify. Petriplates were incubated at room temperature for 5 days in inverted position. Daily observations were recorded for appearance of colonies and number of colonies developed. When a new colony appeared, it was transferred to PDA slants for further studies. The fast growing and dominant fungus from each soil sample was selected for further studies in the production of cellulases.
Media based on agricultural waste

When agricultural waste based media were used for production of cellulolytic enzymes, CMC in CMC Nitrate medium was replaced by wastes such as Cotton stalk and Pigeon pea stalk. The straws/stalk freshly collected from fields following harvest were ground to powder using 40 mesh pulverizer and sieving through specially designed sieves (120μ). The powder so obtained was used as carbon source. All these powders were treated with appropriate quantities (1:10v/v) 4% NaOH for 3 hours at 100˚c to delignify the material. The powders were used after freeing them from alkali by repeated washings.

The details of preparation of the medium are specified along with the experiments. All above media were sterilized in autoclave at 15-lbs/sq. inch pressure for 20 minutes.

Identification

After isolation from dilution plates pure cultures of individual fungi were obtained, each fungus was grown separately on CMCN medium and its growth and sporulation were recorded.

Comparing morphology of hyphae, conidiophores and conidia with standard manuals generic and specific names were determined. Sets of cultures were sent to mycological division, department of plant pathology IARI India and their identifications were confirmed.

Immobilization in Polyurethane foams

For the natural attachment method using of polyurethane foam cubes as the carrier for fungal immobilization. Polyurethane foam had macropores larger than hundreds of micron. mm of cubes of polyurethane foam were cut with sharp blade from the bulk. Cubes such obtained were transferred to flask containing the GN medium. The flask along with the cubes were autoclaved at 15lbs for 20 min. spore suspension was used to inoculate. The foam cubes and incubated at 27±2˚C. The cubes after regular time interval were observed for the growth of mycelium in pores these cubes were then thoroughly washed.
with sterilized distilled water and transferred to enzyme production medium.

**Enzyme assays**

The fungi which were isolated in the course of this study were assayed for FPase, CMCase and \( \beta \)-glucosidase.

Assay for Cellulases was done following method of Berghem and Petterson (1973) with slight modifications.

**For FPase enzyme:** 100 mg of Filter paper dust was suspended in 1 ml of 0.01 M Sodium acetate buffer of pH 4.8 was incubated with 2 ml of crude enzyme solution.

**For CMCase enzyme:** 2ml of crude enzyme solution was mixed with 4 ml of CMC and 1ml of the 0.01 M Sodium acetate buffer of pH 4.8 and were incubated at 27\( ^\circ \)C. Aliquots were drawn from the mixture at regular time interval and the release of glucose due to the enzyme activity was assayed by 3 5 Dinitrosalicylic acid method (Miller, 1959) using D glucose as standard.

**For glucosidase:** Activity of \( \beta \) glycosidase was assayed by the method of Eberhart *et al* (1963) using p-nitro \( \beta \) glucoside as substrate. The reaction mixture consist of 50 mg of p-nitro \( \beta \) glucoside in 2 ml of 0.01 M acetate buffer at 4.8 pH and 1 ml crude enzyme preparation incubated at 27\( ^\circ \)C. At regular time intervals aliquot were drawn and added with 0.1 N NaOH and the release of p-nitro phenol was from substrate was estimated for absorbance at 420 nm in a spectrophotometer. Soluble reducing sugars (equivalent to glucose) were released from filter paper was estimated and the Cellulase activity was expressed in enzyme units. Enzyme activity is expressed in Units (U). 1 enzyme unit is equivalent to amount of enzyme required to release 1 \( \mu \) mole of D glucose (for FPase and CMCase) or P nitro phenol (For \( \beta \)-glucosidase) per minute from respective substrate.
**Dinitrosalicylic acid reagent:** 1 ml of DNSA reagent was added to 1 ml of culture filtrate and was kept in boiling water bath for 5 min. Before cooling 0.3 ml of 40% K-Na tartarate solution was added to the reaction mixture. The sodium bisulphite was added in the DNSA reagent just before use and percentage transmittance was recorded using 575 nm wavelength filter with colorimeter (Erma). Glucose was used as the reference standard (Miller, 1959).

A known aliquot of the digested sample was pipetted out into a 50ml volumetric flask and 10 ml of HNO₃– vanadomolybdate regent was added. The volume was made up to 50ml using distilled water, the contents were mixed thoroughly by shaking and left to stand for 30 min. The intensity of the colour developed was read at 420nm using a Elico spectrophotometer and the values for unknown were obtained from a standard curve prepared by different concentrations of P using KH₂PO₄.

**Immobilization of fungi in Polyurethane foam matrix**
Experiments were conducted to study the efficacy of Polyurethane foam matrix to immobilized cells of various fungi included in the study. So, six different concentration of cells suspension were used to study immobilization in three different number of cube of Polyurethane foam. The cells immobilized in the cubes were subjected to shaker in the flask and the filtrate was examined for the presence of cells/conidia as described. *Aspergillus niger* conidia were immobilized in Polyurethane foam. The colonies develop after plating the filtrate indicates that, with increase in the number of cubes. The number of cells in the filtrate were also more. The number of cubes affected the binding of cell were the maximum cells were 30 from 10 cubes with 22 from 20 cubes and 10 from 40 cubes with 1X10⁷ cells/ml. In *Penicillium digitatum* the maximum cells were 40 from 10 cubes with 32 from 20 cubes and 18 from 40 cubes with 1X10⁷ cells/ml. and in *Trichoderma lignorum* the maximum cells were 40 from 10 cubes with 30 from 20 cubes and 18 from 40 cubes with 1X10⁷ cells/ml.
Production of cellulases by *Aspergillus niger* immobilized in polyurethane foam

Experiments were conducted to study the production of cellulases by *Aspergillus niger* immobilized in polyurethane foam. The medium was incubated with free and immobilized cells. The filtrate after regular interval of time was assessed for the production of enzymes.

The enzymes produced by free and immobilized cells of *Aspergillus niger* in polyurethane foam varied. The enzymes produced by immobilized cells were higher than free cells in CMC medium. The maximum enzymes were produced on 9th day where immobilized cells, enzymes produced 0.18 U/ml Fpase, 0.50 U/ml CMCase and 0.20 U/ml β-glucosidase where free cells produced 0.17 U/ml Fpase, 0.46 U/ml CMCase and 0.14 U/ml β-glucosidase (T.No. 1). by *Penicillium digitatum* the maximum enzymes were produced on 9th day where immobilized cells, enzymes produced 0.25 U/ml Fpase, 0.44 U/ml CMCase and 0.19 U/ml β-glucosidase where free cells produced 0.19 U/ml Fpase, 0.40 U/ml CMCase and 0.13 U/ml β-glucosidase (T.No. 2). and by *Trichoderma lignorum* the maximum enzymes were produced on 8th day where immobilized cells, enzymes produced 0.12 U/ml Fpase, 0.29 U/ml CMCase and 0.22 U/ml β-glucosidase where free cells produced 0.11 U/ml Fpase, 0.23 U/ml CMCase and 0.14 U/ml β-glucosidase (T.No. 3).

**Table 1:** Production of cellulases by *A. niger* in CMC nitrate medium by free and immobilized cell

<table>
<thead>
<tr>
<th>Age of culture filtrate (Days)</th>
<th>Cellulase activity (U / ml)</th>
<th>Free cell</th>
<th>Immobilized cell</th>
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Table 2: Production of cellulases by *P. digitatum* on CMC nitrate medium free and immobilized cell

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<th>Age of culture filtrate (Days)</th>
<th>Cellulase activity (U / ml)</th>
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<th>Immobilized cell</th>
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Table 3: Production of cellulases by *T. lignorum* on CMC nitrate medium free and immobilized cell

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<th>Age of culture filtrate (Days)</th>
<th>Cellulase activity (U / ml)</th>
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<th>Immobilized cell</th>
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Similarly the enzymes produced by immobilized *A. niger* cells were higher than free cells in cotton agro waste medium. The maximum enzymes were produced on 9th day where immobilized cells were produced 0.20 U/ml Fpase, 0.36 U/ml CMCase and 0.22 U/ml β-glucosidase, where free cell produced 0.17 U/ml Fpase, 0.34 U/ml CMCase and 0.19 U/ml β-glucosidase (T.No.4). By *P. digitatum* the maximum enzymes were produced on 9th day where immobilized cells were produced 0.22 U/ml Fpase, 0.25 U/ml CMCase and 0.18 U/ml β-glucosidase, where free cell produced 0.20 U/ml Fpase, 0.23 U/ml CMCase and 0.13 U/ml β-glucosidase (T.No.5). And by *T. lignorum* the maximum enzymes were produced on 9th day where immobilized cells were produced 0.19 U/ml Fpase, 0.35 U/ml CMCase and 0.27 U/ml β-glucosidase, where free cell produced 0.14 U/ml Fpase, 0.30 U/ml CMCase and 0.14 U/ml β-glucosidase (T.No.6).

**No. 4 Production of cellulases by A. niger in cotton agro waste medium by free and immobilized cell.**

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T.No.5 Production of cellulases by *Penicillium digitatum* on cotton agro waste by medium free and immobilized cell

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T.No.6 Production of cellulases by *Trichoderma lignorum* on Cotton agro waste medium by free and Immobilized cells

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<td>0.15</td>
<td>0.27</td>
<td>0.16</td>
</tr>
<tr>
<td>09</td>
<td>0.14</td>
<td>0.30</td>
<td>0.14</td>
</tr>
<tr>
<td>10</td>
<td>0.15</td>
<td>0.32</td>
<td>0.11</td>
</tr>
</tbody>
</table>
RESULT AND DISCUSSION

In all the samples from cultivated soils, members of Deuteromycetes were dominant both in quality and quantity. *Aspergillus* gave the highest counts although quantitative difference was crop specific. In all five *Aspergillus* were isolated and their colony forming units ranged between 2000 to 5000 per gram of soil. Soybean field soil favoured the maximum number of fungi. Twenty-three species belonging to eleven genera were recorded. In a detailed study of soil fungi from Marathwada (Chapalgaonker, 1972) it was observed that the Mucorales and Deuteromycetes dominated soil mycoflora. In all seventyone fungi were isolated on Waksman's medium. In this study thirty four fungi have been isolated and Deuteromycetes were dominant. *Aspergillus niger*, dominant in soils of the world, again exhibits a wide substrate adaptability. Its capacity to degrade major soil polysaccharides—pectin, cellulose, starch and lipids is responsible for its dominance (Domsch, 1970).

When conidia of *Aspergillus niger* *Penicillium digitatum* and *Trichoderma lignorum* where entrapped in other matrix like Ca-alginate and polyacrylamide gel the leaching of cell after 4 hours of stabilization was very negligible. It was found that the adsorption of fungal conidia on PU foam the efficiency varied hence PU foam offered best option for implication purpose.

In the cellulose production studies by *Aspergillus niger*, *Penicillium digitatum* and *Trichoderma lignorum* there was a little difference in the enzyme production in CMC as well as agro waste based medium by free cell but marked and enhanced production was observed with immobilized cells. So immobilized cells inspite of different substrate and different organism proved to be superior in all cases studied (Joseph 1986). The variation in CMC and agro waste based medium may be due to presence of other component like hemicellulose. where as CMC is pure. The effect of pH and temperature on cellulose production was recorded. The synthesis was favoured by pH 6-6.5 in free and immobilized state. The maximum enzyme were released at 40-45° C IN either states. The immobilization of cells was pH and temperature insensitive (Mane, 2001).
REFERENCES
Determination of Sodium Content from *MANGIFERA INDICA* Plant from Nanded City

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**ABSTRACT**

The plants of Nanded region of Maharashtra are potential source of nutritionally and phytochemically important compounds. The animals and human beings in this region are fully dependent on these plants for food, fodder, fibre and fuel. The plant species growing in this region besides their medicinal importance may contain sufficient amount of nutrients to be considered as livestock feed. The present investigation deals with evaluation of Sodium contents of Leaves of Mango (*Mangifera indica*) plant. The Leaves of the selected plant species taken for present investigation were collected from six region of Nanded City. The samples were dried powdered and then used for estimation of mineral i.e. Sodium. The Average Sodium content in Mango Plant Leaves is ranges from 185.98 to 251.9 ppm during the study period.

**KEY WORDS:** Sodium, plant, leaves, nutrient, Nanded, etc.

**INTRODUCTION**

The remarkable progress that has been made in the science of Medical Elementology during the past few decades has not only opened avenues for research on human health related aspects but also aroused the interest of the pharmaceutical industries containing elements reported to be essential for human health. The varieties of formulations are available and used worldwide.

It has been reported that out of 110 known elements, 81 elements present in living organism, which were then biologically classified. Plant body is composed primarily of carbohydrate
proteins, amino acids, nucleotides, lipids and porphyrins. The plant parts used by desert dwellers have not been analyzed fully from nutritive value point of view. Life cannot be sustained without adequate nourishment. Man needs adequate food for growth, development and to lead an active and healthy life. Minerals are also a type of nutritive contents.

Macronutrients are required in large quantities (more than 100 mg/ltr of water) to the plant and usually participate in body construction (C, H, O, N, S, P, K, Mg, Ca, Fe, Na). Micronutrients are required in smaller quantities (100 mg/ltr of water) and usually participate in various metabolic activities. These mineral constitute major part of the animal diet. Some of these are important in various metabolic activities also. These nutritive contents are found in all green plants. The primary productivity of the green autotrophic plants is the main base for the present existence of entire biosphere. It determines the carrying capacity of earth for human beings. Great importance is being laid on the rate of energy storage in diverse ecosystem by green plants. Primary productivity is the gain in the weight of organic matter generated by photosynthesis in a given period of time. Net production is that part of gross photosynthetic production, which is accumulated in plant after metabolic activities and hence becomes available for utilization as food.

Study Area

The district Nanded is located on Deccan plateau region of southern India. The main trend of the hills is from NW to SE in parallel ranges with offshoots generally running in perpendicular direction. The Satamala ranges enter the districts after the Penganga valley just west of Mahur. To the south of the Satamala ranges, the Nirmala hill ranges run parallel to them and to the east of the Peneganga they are linked to the former by offshoot hill which are aligned more or less to the course of the river which in turns forms the district boundary. The study area is situated within Godavari valley region. The landform is developed by moderately dissected plateau deposition of soil on the flood plains of the Godavari River. The sampling locations are near the Godavari River in Nanded City.
Material and Methods

The present investigation deals with evaluation of Sodium contents of Leaves of Mango (*Mangifera indica*) plant. The Leaves of the selected plant species taken for present investigation were collected from Chaitanya Nagar, Taroda Naka, Sneh Nagar, Hingoli Naka, Pawadewadi Naka and Baba Nagar region of Nanded City of Nanded district. All Plant leaves were collected in polythene bags. The samples were dried powdered and then used for estimation of mineral i.e. Sodium.

RESULTS AND DISCUSSION

The present work was therefore an attempt to evaluate the Sodium (Na) content of selected Mango (*Mangifera indica*) plant in six location of Nanded City i.e. Chaitanya Nagar, Taroda Naka, Sneh Nagar, Hingoli Naka, Pawadewadi Naka and Baba Nagar during April, 2014 to March, 2015 has been determined.

From the figure 1, the Average Sodium content in April, 2014 to March, 2015 of Chaitanya Nagar (185.98 ppm) location is less and Baba Nagar is higher (251.9 ppm) as compare to the other locations from Nanded City. In April, 2014 the Sodium content is zero, while in May, June and July 2014 is slightly less as compare to the other month’s sodium content. In the months of May 2014, June 2014, July 2014 and August, 2014 the sodium content is lesser as compare to other months sodium content in all months in all locations.
DISCUSSION

The average Sodium and Potassium content of Mango plants and six sampling location in the month of April, 2014 to March, 2015 are shown in the Table 1.

Table 1: Average Sodium (Na) Content from Selected Plant Materials of Nanded City

<table>
<thead>
<tr>
<th>Location</th>
<th>Mango</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chaitanya Nagar</td>
<td>185.98</td>
</tr>
<tr>
<td>Taroda Naka</td>
<td>188.98</td>
</tr>
<tr>
<td>Sneh Nagar</td>
<td>197.87</td>
</tr>
<tr>
<td>Hingoli Naka</td>
<td>213</td>
</tr>
<tr>
<td>Pawadewadi Naka</td>
<td>220.03</td>
</tr>
<tr>
<td>Baba Nagar</td>
<td>251.9</td>
</tr>
<tr>
<td>Minimum</td>
<td>185.98</td>
</tr>
<tr>
<td>Maximum</td>
<td>251.9</td>
</tr>
<tr>
<td>Average</td>
<td>209.63</td>
</tr>
</tbody>
</table>

In the Mango Plant Leaves the Average Sodium content in April, 2014 to March, 2015 of ranges from 185.98 to 251.9 ppm (Table 1). The Average Sodium content in April, 2014 to March, 2015 of Chaitanya Nagar Location is less and Baba Nagar is higher as compare to the other locations from Nanded City. The average Sodium content value of the plant is 209.63 ppm.

CONCLUSION

In the Mango Plant Leaves the Average Sodium content in April, 2014 to March, 2015 of ranges from 185.98 to 251.9 ppm. The Average Sodium content in April, 2014 to March, 2015 of Chaitanya Nagar Location is less and Baba Nagar is higher as compare to the other locations from Nanded City.

REFERENCES

Analysis of Heavy Metals Concentration in Water and Sediment from Bori River, Naldurg Maharashtra.

A.D. Babare¹, R.R. Jadhav², R. M. Gochde¹ and M.G. Babare¹,

ABSTRACT

The present communication deals with the study of analysis of heavy metal concentration in water and sediment from Bori River Naldurg, Maharashtra. The work was carried out during the year 2014 (January to December).

The results of the present work shows that, the heavy metal content from the non-polluted zone of Bori River (Naldurg) water was found below detectable level. Samples from polluted zone showed variations in their heavy metal content in River water and sediment.

The higher concentration of heavy metals is due to the local domestic and agricultural wastes which caused severe damage to the aquatic flora and fauna of Bori River.

Key-words – Bori River-Heavy metals- Water sediment.

INTRODUCTION

Underground water plays an important role in the overall water balance of the environment. As a reservoir, it has an enormous capacity to store water in rainy periods which can be utilized in dry periods. Ground water is primary source of fresh water in several towns and rural areas. It is widely used as a source of water for drinking, irrigation and other purposes. The industrial wastes have the greatest potential for polluting the
water. Affected ground water quality, which is not useful for drinking purpose. Ground water pollution causes irreparable damage to soil, plants and animals including Humanbeings. Polluted ground water is the cause for the spread of epidemics and chronic diseases in man.

Nanded is the one of the most important district of Maharashtra, because Sikh's tenth Guru Guru Gobind Singhji's tomb is located at Nanded. Nanded is also declared as Holy City. In the year 2008 in Nanded there is Guru-ta-Gaddi procession. For this procession sikh bandhus came in crores. They should live in Nanded as well as beside Nanded. As well as regularly local persons beside Nanded city suffer from health hazards like flurosis due to excess quantity of fluorine in drinking water. So defloridity of drinking water from this area is necessary. Because of the poor economic conditions and illiteracy, people of this area are not at all conscious about the pollution of water and its control. The water contains excess fluoride more than 3 mg/lit. Therefore in this area dental flurosis is of frequent occurrence in elderly persons. They also show bent bones. Keeping the above facts in mind the present investigation was undertaken to study the fluoride contents in water. The quality of drinking water is analysed by analyzing about five parameters.

MATERIALS AND METHODS:

Seven water samples were collected in two liters polythene cans each in the year 2003-2004. In Jalswaraj Project of Dept of Water Supply and Sanitation Govt. of Maharashtra already detected excess fluoride from these Bore-wells. We have selected seven sampling stations from which maximum use of water was taken by the villagers for drinking purposes, because there is no alternate source of drinking water supply system available in these villages.

Seven Bore-wells were chosen from Asarjan, Asarjan Camp, New Hasapur, Old Hasapur, Brahmanwada, Trikutwadi & Punegaon. The water samples for analysis were collected during the period of June 2012 to Oct. 2012.

The Polythene cans brought to the laboratory and analyzed. Fluoride is determined by fluoride meter, TDS by
Water Analyser kit and Iron, Nitrate, Chloride by the standard methods of APHA.

**RESULTS:**

The appearance of all water samples were pale yellow on visual observation. They exhibit no odor no taste. The observations of physico-chemical parameters are summerised in Table No.1. The average values of different parameters at different locations are different but the general trend observed about higher levels of fluoride at all locations as well as nitrates and T.D.S. are excess at some sampling station.

In present investigation the average fluoride values are at Trikutwadi 8.3 mg./lit, at Punegaon 13.5 mg./lit, at Brahmanwada 9.19 at Old Hasapur 4.5 at New Hasapur 5.5 mg./lit, Asarjan Camp 19.3 mg/lit, and at Asarjan – 10.2 mg/lit, other parameters are well within permissible limit.

**DISCUSSION:**

The increasing industrialization, urbanization, and developmental activities to cope up with the population explosion have brought inevitable water crisis. Water, the universal solvent, serves as an unavoidable medium for the livelihood of the human beings, plants and animals. The available fresh water is hardly 0.3 to 0.5 % of the total water on the earth.

The factors namely T.D.S.is excess quantity responsible for wide spread gastric disorders in this area including frequent outbreak of water borne disease. In the present investigation the fluoride in bore-well is excess at all sampling stations. Fluoride in the range of 0.5 to 1.5 mg./lit, is beneficial to human being, but consumption of fluoride in higher quantity i.e. more than 1.5 mg/lit for long time through drinking water load of fluoride, which is a chronic disease characterized by mottling of teeth and softening of bones, ossification of tendons and ligaments. Also Iron is found to be higher in some samples.

In present investigation nitrates are also higher at some sampling stations. Vegetation growth in nitrate rich soil may cause Eutrophication. Vegetation growth causes toxic effect in cattles.
Thus the quality of drinking water beside Nanded city in these seven villages is not safe for drinking purpose.

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