

USE OF AQUATIC ORGANISMS FOR WATER TREATMENT

*Thesis submitted to
University of Calicut*

*In partial fulfillment of
Doctor of Philosophy in Zoology*

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2003

CERTIFICATE

This is to certify that the thesis entitled " **Use of Aquatic Organisms for Water Treatment**" submitted by Ms. K. J. Shiny, for the degree of Doctor in Philosophy in Zoology of the University of Calicut, is a bonafide record of the research work done by her during the period from September 1999 to February 2003 in this institution under my guidance. This work has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar title or recognition.

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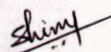

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DECLARATION

This is to declare that the thesis entitled "**Use of Aquatic Organisms for Water Treatment**" submitted by me for the degree of Doctor of Philosophy in Zoology of the University of Calicut, has not formed the basis for the award of any degree or diploma.

Calicut

27 February 2003


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ABBREVIATIONS

EC	Electrical Conductivity
DO	Dissolved Oxygen
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
NO ₃ -N	Nitrate nitrogen
PO ₄ -P	Phosphate phosphorous
SO ₄	Sulphate
Ca	Calcium
Mg	Magnesium
TSS	Total Suspended Solids
MPN	Most Probable Number
ND	Not Detectable
μ mhos/cm	Micro mhos per centimeter
ml	Millilitre
l	Litre
mg/l	Milligram per litre
g/l	Gram per litre
°C	Degree Celsius
μ	Micron
mm	Millimeter
cm	Centimeter
%	Percentage

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INTRODUCTION

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1. 0 INTRODUCTION

1. 0 INTRODUCTION

Water is one of the most important commodities which man has exploited than any other resource for the sustenance of life. Though evaporation and precipitation continuously purify water, pollution of water has emerged as one of the most significant environmental problems of recent times. The unique properties of water, which make it a universal solvent and a renewable resource, also make it a substance with greater tendency to get polluted. Water can be regarded as polluted when it changes its quality or composition either naturally or as a result of human activities, becoming less suitable for drinking, domestic, agricultural, industrial, recreational and any other uses for which it would have been otherwise suitable.

The overall aquatic environment is deteriorating both qualitatively and quantitatively owing to rapid urbanisation, industrialisation and growing population. Billions of litres of wastewater are generated everyday from various sources such as domestic sewage, industrial wastes, agricultural discharges containing detergents, toxic metals, thermal pollutants and radioactive materials in water. With the current emphasis on environmental health and water pollution issues, there is an increasing awareness of the need to dispose off these wastewaters safely. In the absence of suitable technology and facilities for treatment, they are inevitably discharged into fresh and marine waters. According to Trivedy (1998), about 7006.74 million litres of wastewater is generated per day in class I cities in India, out of which 59 percentage is collected and gets any kind of treatment while in class II cities about 1226.32 million litres of wastewater is

generated per day and only 15.54 percentage of which is collected and 5.44 percentage gets any kind of treatment. Most of the smaller towns and cities do not have any treatment facilities. In Kerala, about 51.5 percent of the population is completely without any sanitary provision (Sivadasan and Mohanan, 2000). Due to the lack of proper sewerage and sanitation facilities and very often, the lack of sense of social responsibility, large quantities of wastewater of domestic and industrial origin are discharged directly into roadside drains, gullies, canals, and rivers or into the soil. The effects of these practices are clearly observable - surface waters in and nearby such areas are seriously polluted and cause repulsive stench. Groundwater sources are contaminated chemically and bacteriologically and are not utilisable for domestic purposes. Groundwater pollution is particularly serious as eighty percent of the domestic needs are met from this source.

Nature, by virtue of its self-purification capacity can assimilate pollutants to a large extent. In using an aquatic system as a receiving water body for wastewater dilution, its ability to assimilate the waste materials and restore its own quality is relied upon. The capacity of any water body to accomplish this is dependent on a number of factors including the volume, strength of the waste, hydrography of the receiving body, flow and amount of waste dispersion, climate, temperature and efficiency of the reoxygenation process (Schmitz, 1996). However the global increase in wastewater produced annually warrants treatment measures before discharge.

Wastewater treatment although has great concern in public health, ecological, aesthetic and other points of view has a low priority, particularly in the developing countries where there are many competing demands on the limited

amounts of funds available for development. The high cost of construction and operation of sewage/effluent treatment plants is always a deterrent factor. Thus, within these limits, wastewater technology must be selected such that it has a bearing on local conditions such as climate, availability of land, equipment, power, skilled personnel and facilities for operation and maintenance of installations. Inappropriate transfer of technology from developed to developing countries can be avoided if the ability of simpler methods to obtain reliable and high quality treatment is recognised.

1. 1 BACKGROUND OF THE STUDY

Wastewater generated from domestic sources is the principal source of water pollution in the country. Increasing production and disposal of wastewater have accelerated eutrophication of surface waters and contamination of ground water sources. The characteristics of wastewater discharge vary depending on the population, land use and per capita water consumption. These wastewaters must be treated prior to disposal so that they do not degrade the quality of receiving water bodies. Conventional treatment systems include a combination of physical, chemical and biological processes to remove solids, organic matter, nutrients, heavy metals and pathogens from wastewater. These treatment processes are therefore not very attractive or economically justifiable especially in cash-strapped third world countries. The total cost of such plants are generally greater than those of comparable biological systems primarily due to cost of automatic control systems, sludge and chemical handling systems and their maintenance. The addition of chemicals to remove phosphorous and heavy metals as well as disinfection of water by chlorination has several after effects. The formation of

carcinogenic trihalomethanes on chlorination poses health risks to man. The above factors have revived an interest in the use of eco-friendly alternatives for waste treatment.

Heavy metals are among the most toxic contaminants in the environment and are non-biodegradable. The world consumption of metals has shown an upward trend during the last century. Although the heavy metal content in urban drainage systems generally does not reach the proportions found in industrial effluents, the problems caused by their presence, particularly in areas with dense population are of public concern. Metal containing effluents have unfavourable effects on wastewater treatment such as the inhibition of nitrification and interference in biological oxidation, thus lowering the rate of biodegradation. In addition, present technologies for metal removal from water, especially in low concentrations are highly expensive and cannot be afforded by third world countries.

The emergence of biological systems for wastewater treatment represents an alternative, ecologically safe approach for the removal of pollutants. Biological treatment systems are living systems, which make use of aquatic flora and fauna to either directly consume the waste materials or breaking them down and then removing it from solutions. Nature, by its delicately functioning systems, offers the best example of biotreatment.

In any water body, autotrophs take up nutrients and make themselves available for organisms higher in the food chain. The capacity of ecosystems that are dominated by aquatic filter feeders and plants to assimilate inputs of nutrients and organic matter has resulted in the use of such systems to wastewater

treatment. Aquatic treatment systems utilise several species of plants, invertebrate zooplankton as well as fishes in monoculture and polyculture operations. Aquatic macrophytes are capable of very high rates of growth associated with high levels of nutrient uptake especially of nitrogen, phosphorous and also metals. This process can be enhanced with a regulated harvesting regime and encouraging new growth. The concept of using fishes to treat polluted waters is very recent, although treated sewage had been used to fertilize fishponds and increase production.

The application of filter feeding invertebrates and fishes to consume organic matter in wastewater results in water quality improvement along with production of biomass, which can have alternative versatile uses.

A review of the work done during the past four decades on the utilisation of aquatic weeds for wastewater renovation or pollution abatement reveals that, very little work has been done in India. Although there is a vague idea about the bioremediation ability of some aquatic flora and fauna, there has been very little studies carried out on the potential of individual plant and animal species. Screening of metal tolerant macrophytes is highly essential for low cost and efficient treatment of toxic wastewaters. The selection of appropriate species for treatment process is necessary for effective field application. This study therefore has been attempted to evaluate the potential of some aquatic flora and fauna as receptors and bioreactors or processors of waste. Investigations were mainly focussed on the efficiency of test species to remove the pollutants from water. These studies on natural systems to combat water pollution may be of vital interest to sanitary engineers and wastewater treatment managers.

1. 2 WASTEWATER CHARACTERISTICS

Wastewater is the flow of used water from a community. The characteristics of wastewater discharges vary from location to location depending upon the population and industrial sector served, land uses and degree of separation between storm water and sanitary wastes. Domestic wastewater comprises spent water from kitchen, lavatory and bathroom as well as any other wastes that people may accidentally or intentionally pour down the drain. Daily per capita water use, quality of water supply and habits of the people also affect wastewater strength.

Characterisation of wastewater is essential for an effective and economic waste management programme. It helps in the choice of treatment methods, deciding the extent of treatment, assessing the beneficial uses of wastes and utilising the waste purification capacity of natural bodies in a planned and controlled manner.

Physically, wastewater is usually characterised by a grey colour, musty odour, a solids content of about 0.1 percent and 99 percent water content. With the passage of time sewage becomes stale, dark in colour with a pronounced smell due to microbial activity. The solids can be suspended as well as dissolved. Dissolved solids can be precipitated by chemical and biological process. Suspended solids can lead to the development of sludge deposits and anaerobic conditions when discharged into the receiving waters. Temperature of sewage is useful in indicating the solubility of oxygen, which affects the rate of biological activity. Extremely low temperature adversely affects the efficiency of sedimentation. Usually sewage has higher temperature than that of the water

supply. The hydrogen ion concentration of sewage is a valuable parameter in the operation of biological units. The pH of fresh domestic sewage is slightly more than the water supply to the community. However, onset of septic conditions may lower the pH while industrial wastes produce extreme fluctuations.

Chemically, wastewater is composed of organic and inorganic compounds, as well as various gases. Organic components consist of carbohydrates, proteins, fats and greases, surfactants, oils, pesticides and phenols. Inorganic components consist of heavy metals, nitrogen, phosphorous, sulphur, chlorides, alkalinity and toxic compounds. In domestic wastewater, the organic and inorganic portion is approximately 50 percent respectively. Gases commonly dissolved in wastewater are hydrogen sulphide, methane, ammonia, oxygen, carbon dioxide and nitrogen.

Biologically, wastewater contains various microorganisms, but the ones of concern are those classified as protista including bacteria, fungi, protozoa and algae. These are important in terms of wastewater treatment. Wastewater also contains many pathogenic organisms that generally originate from humans infected with disease or are carriers.

1.3 HISTORY OF WASTEWATER TREATMENT

Man's progress is interlinked with the history of water pollution. With the growth of civilisations, large settlements and towns began to appear giving rise to the problem of waste disposal. Several ancient cities developed elaborate sewer systems. Mohenjodaro and Harappa had drains for collection of wastewater. Wastewater disposal on land became a common practice almost throughout the world upto the seventeenth century. In the eighteenth century, as cities and towns began to grow, their condition worsened as garbage and wastes accumulated on

the streets. Though the development of sewers in early nineteenth century cleared the streets, it increased the load on the rivers as wastewaters were directly discharged into them. This initiated its conversion (treatment) by natural chemical and biological processes into substances that could be cycled in the environment, thereby returning the resources to these ecosystems. Pollution problems only occurred when wastes that were generated from resources collected over a large area were returned into a small area of the environment, so that it was ecologically overloaded (Mitchell, *et al.* 1990).

At the beginning of the twentieth century, it was realised that direct discharge of wastewater into streams caused health problems and must be treated to check pollution. The new engineering developments in handling and treating sewage remove organic matter, convert nitrogen, phosphorous and other chemicals to soluble forms. The reticulation of large amounts of waste to a single treatment work and the use of water as medium for disposal causes the carriage of immense quantities of biologically available nutrients into aquatic ecosystems, which, as a result often become eutrophicated.

1.4 CONVENTIONAL WASTEWATER TREATMENT PROCESS

Conventional wastewater treatment consists of a combination of physical, chemical and biological operations to remove solids, organic matter and nutrients from wastewater. The general terms used to describe different degrees of treatment, in order of increasing treatment levels are preliminary, primary, secondary and tertiary or advanced wastewater treatment. Disinfection to remove pathogens sometimes follows the last treatment step.

Preliminary Treatment

The objective of preliminary treatment is removal of coarse solids and other large materials often found in raw wastewaters, to enhance the operation and maintenance of subsequent treatment units. Preliminary treatment typically include coarse screening, grit removal and in some case comminution of large objects.

Primary Treatment

Primary treatment involves removal of settleable organic and inorganic solids by sedimentation, and removal of materials that float (scum) by skimming. Approximately 25-50 percent of the incoming BOD, 50-70 percent of the total suspended solids and 65 percent of the oil and grease are removed during primary treatment. Some organic nitrogen, organic phosphorous, and heavy metals associated with solids are also removed during primary sedimentation but colloidal and dissolved constituents is not affected.

Primary sedimentation tanks or clarifiers may be round or rectangular basins, 3-5 meter deep, with hydraulic retention time between 2-3 hours. Settled solids (primary sludge) are normally removed from the bottom of tanks by sludge rakes to central well from where it is pumped to sludge processing units in small sewage plants. Sludge is processed in a variety of ways including aerobic digestion, storage in sludge lagoons, direct application to sludge drying beds and land application.

Secondary Treatment

Secondary treatment is the further treatment of the effluent from primary treatment to remove residual organic and suspended solids. In most cases, it

follows primary treatment and involves the removal of biodegradable dissolved and colloidal organic matters using aerobic biological treatment processes by aerobic microorganisms (principally bacteria). These microorganisms metabolise the organic matter in the wastewater, thereby producing more microorganisms and inorganic end products (CO_2 , NH_3 and H_2O). Several aerobic biological processes are used for secondary treatment differing primarily in the manner in which oxygen is supplied and rate at which organisms metabolise the organic matter.

High rate biological processes are characterised by relatively small reactor volumes and high concentrations of microorganisms compared with low rate processes. High-rate processes include activated sludge processes, trickling filters or biofilters, oxidation ditches, and rotating biological contractors. Microorganisms must be separated from the treated wastewater by sedimentation to produce clarified secondary effluent. Secondary clarifiers (sedimentation tanks) remove the secondary sludge, which are contained with primary sludge for processing.

Tertiary Treatment

Tertiary or advanced wastewater treatment is employed when specific wastewater constituents that cannot be removed by secondary treatment must be removed. Individual treatment processes are necessary to remove nitrogen, phosphorus, additional suspended solids, refractory organics, heavy metals and dissolved solids.

Disinfections

The effluent from the treated wastewater is usually disinfected with chlorine before being discharged into receiving waters. The chlorine dosage depends upon the strength of the wastewater and other factors, but dosages of 5-15 milligrams per litre are common. Ozone and ultraviolet irradiation can also be used for disinfection, but these methods are not in common use.

1. 5 METAL POLLUTION

Humans have been introducing metals into their environment since they first gained knowledge of its many useful properties. Metals are found throughout the earth in rocks, soils and sediments primarily trapped in some stable form. Through natural processes such as weathering and erosion, small amounts are removed and enter in water. This is essential because many biochemical processes require a given amount of many of these metals, usually in trace quantities. However, with increase in standard of living and technological advances, the uses of metals increased and inevitably metal pollution also increased. The main sources of metal pollution in the environment are – industrial processing of ores and metals, use of metals and metal components in various industries, sewage, leaching of metals from garbage and solid waste dumps (Sarah, *et al.* 1994).

The metals are classified according to Nair (1984) as:

1. Very toxic – effects seen at concentration below 1 mg/l– *e.g.* cations of silver, beryllium, copper, mercury, tin, cobalt , nickel and lead.

2. Moderately toxic – effects appear at concentration between 1 and 100 mg/l.

e.g. anions of arsenic, manganese, molybdenum, selenium, vanadium and cations of aluminium, barium, cadmium, chromium, iron, manganese and zinc.

3. Scarcely toxic effects rarely appear *e.g.* strontium, titanium.

The priority list of pollutants compiled by the Environmental Protection Agency of United States contains the eight more widespread heavy metals – arsenic, cadmium, chromium, lead, mercury, nickel and zinc (Moore and Krishnamoorthy, 1984).

Metals are required for normal growth and reproduction of living cells. Sodium, potassium, calcium and magnesium are required by cells in macro quantities, whereas several other metals are required by cells in trace levels. Role of trace metals is well recognised. Some are an integral part of metalloenzymes and others take part in electron transfer. Thus, they are closely associated with energy and metabolic activities of the cell. However, metals in higher concentrations are considered as pollutants due to their toxicological effect. Man being at the top of the food chain is at a great risk of suffering from health hazards associated with toxic metals due to bioaccumulation. There is a growing concern worldwide regarding the metal pollution in aquatic environments and stringent measures are being adopted for its mitigation.

1. 6 CONVENTIONAL METAL TREATMENT PROCESS

Major objectives for removal of metals from aqueous solutions are toxicity removal that entails an environmental aspect and recovery of valuable metals, which involves a technological aspect. Various methods are available for heavy

metal waste management. The commonly used methods include precipitation, ion exchange, adsorption, reverse osmosis, electro dialysis and evaporation. Apart from these methods others like foam floatation, liquid membrane techniques, solvent extraction and crystallisation can also be used for heavy metal removal.

Metals can be precipitated either as hydroxides, carbonates or sulphides by adding the appropriate chemical to the aqueous metal solutions. Co-precipitation as well as adsorption onto flocculating agents, added to aid in settling the precipitates, appear to play a significant role in reducing the aqueous concentrations of the metal ions. Main limitations of this method are poor filterability of gelatinous sludge formed, production of large volumes of solid sludge requiring safe disposal and difficulty in achieving the permissible metal concentrations in the effluent especially in the presence of metal complexing agents in the wastewater.

Ion exchange methods are employed for recovery of precious metals from effluents having very low metal concentrations. The most essential component of this process is an ion exchange resin, which can exchange H^+ or Na^+ ions in lieu of the metal ions to be removed from the effluent. The effluent and resin are generally contacted in a packed bed reactor. The transfer of the toxic metal from liquid phase to solid phase is achieved within the bed, which gets exhausted in time and must be regenerated with acid or brine solution and reused for next cycle of metal removal. The major drawback of ion exchange process is its prohibitive cost.

Evaporation is a concentration process whereby water is evaporated from a solution, increasing the concentration of solute in the remaining solution. It was

reported by Stenson (1978) that evaporation was used for separation of chromium acid from wastewater. Reverse osmosis is a pressure driven membrane separation process using membranes such as cellulose acetate and polyamide. The main advantage of reverse osmosis is its low energy requirement. This is mainly used in plating industry for the treatment of rinse waters (Stenson, 1978).

Electrodialysis is a membrane process where separation is accomplished by the selective transport of ions through ion exchange membranes under the influence of an electrical potential applied across the membrane. It is a suitable technique for recovery of ionised species such as metal salts, cyanides or chromate from metal finishing wastewaters.

Adsorption, which involves interaction of heavy metals with the sorbent surface, is one of the preferred methods for heavy metal removal. Main advantages of the process are sludge free operation, recovery of sorbed metal and reuse of sorbent, which makes the process economical.

1. 7 NATURAL WASTEWATER TREATMENT SYSTEMS

As energy and construction costs have increased, new methods of wastewater treatment with potentially lower energy and capital costs have become increasingly attractive. Relatively simple wastewater technologies can be designed to provide low cost sanitation and environmental protection while providing additional benefits from the reuse of water. Effluents from conventional treatment facilities not only contain some unstable organic matter but also nutrients, which represent potential energy. It is therefore illogical to utilise external energy sources to dissipate energy stored within the wastewater. A natural treatment system makes use of aquatic animals and plants to improve

the water quality and reclaim nutrients. In addition, persistent toxic environmental contaminants such as pesticides, aromatic hydrocarbons and metals can be removed from wastewater through biological treatment process. Natural wastewater treatment systems include algal mass culture, macrophyte treatment systems (including constructed wetlands) and aquaculture. Natural systems may be utilised to treat agricultural, domestic and industrial wastewater. Climate, soil conditions, space, waste characteristics and other factors relate to its applicability.

1. 7. 1 Algal Mass Culture

Algae are a diverse group of microorganisms that can perform photosynthesis. Of these, unicellular algae (*e.g.* green algae, blue green algae) are of interest in wastewater treatment and recycling process. They are tolerant to changes in environmental conditions. The biological reactions occurring in algal ponds reduce the organic content and nutrients of wastewater by bacterial decomposition and convert them into algal biomass. The algae utilise solar energy and nutrients (or by-products) from bacterial oxidation, perform photosynthesis and synthesise new algal biomass. The excess algae and bacteria need to be regularly removed from the system for efficient performance.

Algal cells have high protein value and harvesting for animal and human consumption could be a financial incentive for wastewater treatment. Almost all the organic wastes such as municipal wastewater, agricultural and human wastes can be treated by algal systems. The main attraction of algal mass cultures is their great versatility for integration into multi-use systems.

Sewage and some industrial wastewaters can be used as culture medium for algae, without the addition of external nutrients, resulting in wastewater

treatment. Algae that are commonly used include *Spirulina*, *Chlorella*, *Scenedesmus* and *Micractinum*. Algae utilise ammonia as the principal source of nitrogen to build their proteinaceous cell material. About 80 percent of nitrogen in the waste is recovered in the algal cells. Phosphorous is not expected to become a limiting factor because of increased use of detergents in home and industry. Other nutrients are present in sufficient quantities in wastewater. The only constraint in algal technology is harvesting the small sized algae and it can be overcome by biological grazing using fish.

1. 7. 2 Macrophyte Treatment Systems

Aquatic plants require varying concentration of nutrients for optimum growth and they can accumulate more of nutrients than their need for growth. This luxury consumption allows plants to maintain a near optimum growth for a period of time after the nutrient becomes limiting. It also results in plants having a more significant effect on improving water quality in organically enriched waters by removing nutrients (especially nitrogen and phosphorous), heavy metals, phenols and refractory organics. Aquatic macrophytes include floating, submerged and emergent species, which could be used in monoculture and polyculture operations. The nutrient assimilation capacity is directly related to growth rate, standing crop and tissue composition (Reddy and De Busk, 1987). In such macrophyte systems apart from any physical removal process that might occur (especially sedimentation) the aquatic plants serve as living substrates for microbial activity, which removes BOD, nitrogen and achieves reduction in phosphorous and heavy metal levels (Wood, 1990). The basic function of macrophytes is therefore, to assimilate, concentrate and store contaminants on a

short term basis. Subsequent harvest of plant biomass results in permanent removal of stored contaminants from the treatment system (Reddy and De Busk, 1987). The common macrophytes used in wastewater treatment are given in Table 1.

Floating Macrophytes

Floating plants are of two types - floating unattached and floating attached which are anchored in the soil. The roots of floating plants are good habitat for the bacteria responsible for waste stabilisation. The leaves and stems are above the water receiving sunlight directly. Floating plants utilise atmospheric oxygen and carbon dioxide. Remainder of their sustenance is obtained from nutrients in the waters in which they grow.

Several genera of floating macrophytes have been used for wastewater treatment including *Eichhornia*, *Salvinia*, *Lemna*, *Pistia* and other plants. Floating macrophyte ponds can serve in stripping effluents of nutrients and at the same time produce easily harvestable biomass. Leaf canopy shades out algae and prevents its growth. They are also known to absorb heavy metals and refractory organics from effluents.

Submerged Macrophytes

Aquatic macrophytes that grow below the water surface are called submerged macrophytes. Often they form a dense wall of vegetation from the bottom of the water surface. Submerged species can grow only where there is sufficient light and may be adversely affected by factors such as turbidity and excessive populations of planktonic algae, which decrease the penetration of light into water. Submerged species that are able to survive in highly enriched waters

include *Potamogeton*, *Ceratophyllum*, *Hydrilla*, *Elodea*, *Myriophyllum* and *Najas*. In warm waters *Hydrilla verticillata* dominates all other submerged plant species. Submerged plants remove soluble nutrients and provide substratum for attachment of algae, diatoms, bacteria and stalked ciliates. Silt and detritus accumulate on the stem and leaves of the plant. An important consideration in the design of a submerged macrophyte system is shallow water depth (< 2 meters), as the euphotic zone should extend to the bottom.

Emergent Macrophytes

These are rooted plants that extend above the water surface and can survive in conditions where rapid or extensive fluctuations in water level occur, generally in wetlands. In recent years, natural and artificial wetlands as well as marshes have been used to treat raw sewage and partially treated effluents. Natural wetlands are unmanaged, whereas artificial systems are designed to maximise performance by providing the optimum conditions for emergent macrophyte growth.

Wastewater biological oxygen demand and nitrogen are removed by bacterial activity. Aerobic treatment takes place in the rhizosphere, and anaerobic treatment taking place in the surrounding soil. Suspended solids are aerobically composted in the above ground layer of vegetation formed from dead leaves and stems. Nutrients and heavy metals are removed by plant uptake.

The emergent macrophytes used for wastewater treatment include *Typha*, *Juncus*, *Scirpus*, *Phragmites* and *Eleocharis*. The growth rate and pollutant assimilative capacity of emergent macrophytes such as *Phragmites* and *Scirpus* are limited by the culture system, wastewater loading rate, plant density, climate

and management factors. Emergent macrophytes have more supportive tissue than floating macrophytes, and therefore greater potential for storing nutrients over a longer period. Consequently, frequent harvesting is not necessary to achieve maximum nutrient removal although harvesting above ground biomass once a year would improve overall nutrient removal efficiency.

Table 1. List of Common Plants Used in Waste Water Treatment

No	Scientific name	Common name	Family
Floating Plants			
1	<i>Eichhornia crassipes</i>	Water hyacinth	Pontederiaceae
2	<i>Salvinia molesta</i>	Water fern	Salviniaceae
3	<i>Pistia stratiotes</i>	Water lettuce	Araceae
4	<i>Lemna sp.</i>	Duckweed	Lemnaceae
5	<i>Spirodela sp.</i>	Greater duckweed	Lemnaceae
6	<i>Azolla sp.</i>	Water velvet	Azollaceae
Submerged Plants			
7	<i>Elodea sp.</i>	Elodea	Hydrocharitaceae
8	<i>Hydrilla verticillata</i>	Water thyme	Hydrocharitaceae
9	<i>Ceratophyllum demersum</i>	Coontail	Ceratophyllaceae
10	<i>Potamogeton sp.</i>	Pondweed	Potamogetonaceae
11	<i>Myriophyllum sp.</i>	Water milfoil	Haloragaceae
12	<i>Ludwigia sp.</i>	Water primrose	Onagraceae
Emergent Plants			
13	<i>Scirpus lacustris</i>	Bulrush	Cyperaceae
14	<i>Phragmites communis</i>	Common reed	Poaceae
15	<i>Alternanthera philoxeroides</i>	Alligator weed	Amaranthaceae
16	<i>Typha sp.</i>	Cattail	Typhaceae

1. 7. 3 Aquaculture

Aquaculture is one of the oldest techniques used for wastewater treatment. The natural processes of an aquatic food chain break down organic material. Depending on the climatic conditions, systems can contain smaller plant and animal organisms such as microscopic bacteria, zooplankton, phytoplankton and

larger species including fishes. Systems range from indoor and outdoor containerised mesocosms to large wastewater fed aquaculture ponds.

The principle of all models of aquaculture for treatment of domestic sewage has been primarily dilution, oxidation, reduction of BOD, COD and suspended solids along with nutrient recovery by employing the biotic components in an aquatic ecosystem. Several food chains operate in these systems, rendering the influent nutrient deficient and less harmful to the environments to which they are discharged.

There are a large number of fish species that could possibly be employed to improve wastewater quality. Herbivorous and omnivorous species are most suited for this purpose. Polyculture of several species, each with a particular niche is desirable. High fecundity, rapid growth and ability to survive under harsh environmental conditions are positive attributes for fishes selected for wastewater treatment. The fauna used for wastewater treatment include:

1. Protozoa

Protozoa are microscopic animals, which multiply by simple fission and feed upon bacteria and organic detritus. Although protozoa are abundant in wastewaters, their contribution to wastewater treatment was considered negligible until recently. Protozoa have a significant role due to the continual cropping of bacterial population, which dissipates energy from water. Removal of excess bacteria and suspended organic debris serves to clarify water. Some of the genera of protozoa that have been isolated from stabilisation ponds include *Paramecium*, *Colpoda*, *Euplotes*, *Arcella*, *Oxytricha*, *Vorticella*, *Amoeba* and *Didinium*.

2. Arthropoda

(i) Insects - Midge fly larva

The larvae of midge fly (blood worm) can survive in waters having low dissolved oxygen levels. The larvae feed on the entrapped food particles and some species consume benthic organic detritus. They are important in removing algae and promoting mixing at the mud-water interface. Bloodworm culture is particularly attractive as it is the preferred fish feed.

(ii) Crustaceans

Copepods, cladocerans and anostracans contribute to energy dissipation through biological recycling by their consumption of algae, protozoa, bacteria and other suspended organic matter. Cladocerans include *Daphnia*, *Moina*, *Chydorus*, and *Bosmina*. Copepods are *Cyclops*, *Mesocyclops* and *Diaptomus*. The thoracic legs help in filter feeding. *Daphnia* are able to survive in waters with very low levels of dissolved oxygen. Haemoglobin content of the blood increases and animals become reddish in colour.

Brine shrimps are crustaceans (anostracans) whose controlled culture has been evaluated as a means of removing algae and other organic suspended matter from saline wastewaters (McShan *et al.* 1974). They feed continuously and food consists of both organic and inorganic particles. *Artemia salina* species, which has several strains, holds promise for clarification of wastewaters of cities and industries situated near the sea.

3. Molluscs

Molluscs (clams, mussels, oysters and scallops) live in waters having insufficient food to support mass culture of filter feeding crustaceans. *Corbicula*

are small clams native to south and south east Asia, where they are harvested and used for human and livestock food. Clams are tolerant to highly enriched waters if some oxygen is present and sufficient water flow occurs to continually disperse toxic catabolic products. They are capable of rapidly removing algae, phosphorous, colloidal material and fecal coliforms from water. Utilisation of clams for biological clarification of wastewater will be feasible if large stable populations are available.

4. Fishes

There are a number of fish species that could possibly be employed to improve wastewater quality. Feeding habits of fish, niche, tolerance to adverse conditions and suitability to local conditions are some of the factors to be considered in the choice of fish for wastewater treatment. Herbivorous and omnivorous species are most suited and polyculture practices, which combine fish with different feeding habits in proportions that effectively utilise the existing nutrients in water, yield good results. The fishes that appear to have the greatest potential for use in wastewater treatment systems are the Cyprinids (carps-minnows), Cichlids (tilapia) and Poecillids (mollies). The common fishes with potential for use in wastewater treatment systems are listed in Table 2.

There are several instances where stocking of fishes in ponds has led to an improvement in water quality parameters by reduction in BOD and removal of algae (Wrigley *et al.* 1988). An added advantage is the economic value of fishes. The main attractions of such systems are a high fish production potential and low maintenance costs. Minor disadvantages of such systems include aversion by the

public for fish grown in sewage, primarily because of health reasons. However sewage fed fisheries of West Bengal is an excellent example of such systems.

Table 2. List of Common Fishes used in Wastewater Treatment

No.	Common name	Scientific name	Feeding habit
1	Silver carp	<i>Hypophthalmichthys molitrix</i>	Phytoplankton
2	Bighead carp	<i>Aristichthys nobilis</i>	Phytoplankton, zooplankton, suspended solids
3	Black carp	<i>Mylopharyngodon piceus</i>	Snails, crustaceans, mussels
4	Grass carp	<i>Ctenopharyngodon idella</i>	Vegetation, variable
5	Common carp	<i>Cyprinus carpio</i>	Phytoplankton, zooplankton, insect larvae
6	Tilapia	<i>Tilapia sp.</i>	Plants, plankton detritus, invertebrates
7	Catfish	<i>Ictalurus sp.</i>	Crustaceans, algae , insect larvae
8	Fathead minnows	<i>Pimephales promelas</i>	Phytoplankton, zooplankton
9	Golden shiner minnows	<i>Notemigonas crysoleucas</i>	Insect larvae, zooplankton, algae
10	Mosquito fish	<i>Gambusia affinis</i>	Crustaceans, detritus, insect larvae
11	Catla	<i>Catla catla</i>	Zooplankton and detritus
12	Rohu	<i>Labeo rohita</i>	Decayed vegetation, epiphytes
13	Mrigal	<i>Cirrhinus mrigala</i>	Decayed vegetation, benthic animals, epiphytic plankton
14	Green sailfin mollies	<i>Poecilia latipinna</i>	Attached algae

1. 8 COMPARISON BETWEEN CONVENTIONAL AND NATURAL TREATMENT SYSTEMS

Conventional wastewater treatment relies on large scale plants. It is the preferred form of wastewater treatment in developed countries to a large extent because it is a well known technology in which civil engineers throughout the world are trained. It also minimises the area required for treatment per capita, which is an important consideration in urban areas, where space is at a premium. Conventional treatment also shortens the period that the wastewater effluent remains in retention ponds and so can treat more effluent over a period of time. However it has some disadvantages.

- ◆ High capital and maintenance costs for plants and supporting infrastructure.
- ◆ Retrofit or replacement of piping is labour and resource intensive
- ◆ Not easily adaptable to new technologies or to varying scales
- ◆ No alternative in the event of a disaster
- ◆ Does not promote water conservation
- ◆ Intensive use of nutrients and reuse of water is not optimised
- ◆ Water quality benefits are inconclusive

Ecological alternatives to wastewater treatment are newer technologies often unknown and resisted by the engineering and public works committees. Their land requirements are greater than conventional plants and therefore can be feasible in sub-urban and rural areas. These systems are effective options in developing countries, which would like to keep mechanisation to minimum and use innovative methods appropriate to local conditions. Like conventional centralised treatment facilities, natural biological treatment systems can help

reduce the level of biological oxygen demand and disease causing organisms in water bodies.

In spite of land constraints, where feasible, ecological alternatives provide the following advantages:

- ◆ Reclamation of nutrients
- ◆ May be built on any scale
- ◆ Flexible and adaptable to a variety of sites
- ◆ Are more transparent
- ◆ Provide useable open space for recreation
- ◆ Low capital and maintenance costs
- ◆ Little or no chemical use
- ◆ Decentralised locations
- ◆ Educational value

1. 9 ADDED USES OF BIOTIC SYSTEMS IN TREATMENT

APPLICATIONS

Human needs are indefinite and so is the search for new resources to meet these needs. Aquatic plants are categorised as pests and efforts are made to eradicate it. Simultaneously, alternative uses of flora and fauna for several purposes including wastewater treatment are being worked upon. In the recent years, attention has been focussed on the utilisation of flora and fauna after use in such treatment systems. This is partly because economic returns from such systems will counter the costs of installation and labour.

The algae from wastewater treatment applications can be fed to fish, poultry and livestock owing to its high protein content. The fuel characteristics of

dry algae (average heat content 6 Kcal/g) are similar to those of medium grade bituminous coal and suitable for use as energy source (Benemann *et al.* 1980). Significant amount of lipids are present in algal cells, which can be used, for industrial purposes such as manufacturing surfactants, grease, textiles, food additives, cosmetics and pharmaceuticals. Microalgae may also serve as a source of carotenoids (Polprasert, 1996).

Macrophytes after use in wastewater treatment can be harvested and used directly as feed for livestock, fish and poultry. They can also be used to generate biogas. It also holds potential for use as organic fertiliser, namely as compost, mulch, ash, green manure or biogas slurry (Table 3). Weeds such as *Phragmites* and water hyacinth can be used as a source of fibre and pulp for making paper. Dried water hyacinth petioles are woven into baskets and purses (NAS, 1976).

There are many species of herbivorous fish that feed on aquatic weeds, thus converting them into valuable food. The Chinese grass carp, *Ctenopharyngodon idellus* and *Tilapia* feed voraciously on many aquatic weeds, including submerged weeds (Polprasert, 1996).

The plants used for metal removal from solutions however cannot be used as feed, due to the high concentration of metals in their tissues. They can be harvested and used for the generation of biogas. Metal extraction of the residual sludge can be used to recover valuable metals like silver, gold and cadmium.

The plants after wastewater treatment can be dried, ground and used as biosorbents instead of living systems in areas of high metal concentration, such as effluents of metal industries. Such metal removal systems are not subjected to metal toxicity limitations and there are no requirements for growth media and

nutrients. It is also possible to recover the sorbed materials by elution using sorption-desorption cycles. They are cost-effective when compared to ion exchange resins.

The aquatic fauna such as daphnia, midge larvae, and brine shrimp can be used as fish food or as additives in pet food. Clams and fishes can be used for human consumption after a period of depuration not less than two weeks in clean water to eliminate most of the pathogens. A major problem regarding the consumption of fish raised on wastes is its public acceptability. Public education to wash, remove the intestines, cook the fish and clams well before consumption, will be a safe measure. As far as taste and texture are concerned, observations by various workers indicate that fish grown in well treated domestic wastes are equal or even superior in taste and odour to non-wastewater cultivated fish (Allen and Hephher, 1976). Raising carnivorous fish for human consumption using the herbivorous fish from waste fed ponds, as feed is another option, either directly or after incorporation in fishmeal. The biological value of protein in fishmeal is 74 - 89 percent and hence suitable for feeding to pigs and poultry.

Table 3. Conversion of Vascular Aquatic Plants to Useful Products

Pollution Removal Applications	Harvested Plants - Processing Alternatives		Products
Removal of Heavy Metals from Chemical and Industrial Wastewaters	Anaerobic Fermentation → Methane Gas ↓ Residual Sludge → Metal Extraction Process → Silver, Gold, Cadmium, Mercury, Lead etc.		Methane Gas
	Biosorbent for Industrial Effluents		Metal Removal
Removal of Nitrates and Phosphates from Domestic Sewage	Anaerobic Fermentation → Methane Gas ↓ Residual Sludge → Dried using Methane Gas or Solar Energy → Agricultural Fertilizer (Bagged or Bulk)		Methane Gas
	Chopped and Dried Plant Material	Animal Feed Processing → Potable Food Processing →	Additive for Cattle, Swine and Poultry Feeds Protein Supplement Flour or Meal Cereal Ingredient
	Composted →		Yard and Garden Mulch (Bagged or Bulk)

REVIEW OF LITERATURE

Shiny K J “Use of aquatic organisms for water treatment” Thesis. Department of Life Science, University of Calicut, 2003

2. 0 REVIEW OF LITERATURE

2.0 REVIEW OF LITERATURE

It is a universally acknowledged fact that water becomes a critical resource as the growing population demands more and various anthropogenic activities reduce its quantity and spoil its quality. Water pollution is a growing concern of communities globally and emphasis is being given on low cost eco-friendly wastewater treatment (Lowe, 1990). Wastewater treatment can be effectively carried out through biological processes involving the activity of organisms such as bacteria, algae, plants and animals. The environmental technologies are aimed at recycling and recovery of nutrients or pollutants in contrast with the early approach of treatment technologies (Olguin and Hernandez, 1998)

The participation of an array of species in the water purification process, including higher animals and plants were established at an early date (Weston and Turner, 1917). Natural systems in the developed areas of the world are primarily viewed as an economical means of renovating wastewater, although Torrel (1978) proposed that food could be produced in enriched treated wastewaters. Duffer and Moyer (1978) reported that aquaculture technology could produce clean water cheaper than conventional waste treatment methods. The natural treatment methods include algal culture, macrophyte treatment systems and aquaculture.

Evans and Williams (1975) referred to the luxury metabolism of algae like *Chlorella*, *Scenedesmus* and *Chlamydomonas* that are able to take up nitrogen and phosphorous when present in the medium in high concentrations. This phenomenon has obvious advantages in terms of sewage purification. The algal induced elevation of pH (due to utilisation of carbon dioxide in photosynthesis and shift in carbonate-bicarbonate equilibrium and hydroxyl ion formation) has

been used to bring about effective purification of sewage waste in an experimental system (Hemens and Mason, 1968; Hemens and Stander, 1969). Algae have been considered as key oxygenators and also as a useful source of protein (Kosaric *et al.* 1974). Algae in association with bacteria have been shown to play a vital role in the degradation of organic matter in sewage (Abbot, 1950). Studies by Patil, (1991) showed significant enhancement in pH and dissolved oxygen, while reduction in biochemical oxygen demand, phosphate and ammonia nitrogen were observed. The use of microalgal cultures has been proposed for biological secondary or tertiary treatment of wastewater (Goldman, 1979; Shelef and Soeder, 1980; de la Noue and Ni Eidhin, 1988 and Oswald, 1989). The alga *Chara* is an excellent nutrient absorber, improving water clarity in a eutrophic lake from one metre to about three metres (Denike and Geiger, 1974).

Much of the attention focussed on aquatic plants has been directed towards their elimination from water bodies, since dense stands of vegetation can impede navigation and threaten the balance of biota in aquatic systems. Literature abounds with papers reporting the detrimental effects of aquatic weeds on aquatic ecosystems (Joy, 1978; Bhaskar, 1976; McVea and Boyd, 1975; Gopal and Sharma, 1981; Gupta, 1979; Majid, 1986; Reddy and Sacco, 1981). In the past years, there has been considerable emphasis on the use of aquatic plants in pollution control. Cornwell *et al.* (1977) and Sheffield (1967) were among the first scientists to demonstrate the nutrient removal potential of aquatic plants. In the early 1960's NASA began actively researching into the use of aquatic plant systems for treatment of wastewater. Studies by Boyd (1970) showed that aquatic plants possess a tremendous capacity of absorbing or translocating nutrients and

other substances from water, thus bringing down the pollution load (Tchobanoglous *et al.* 1979). Evolution has allowed different macrophytes to occupy various ecological niches, depending upon their ability to occupy water logged and oxygen stressed environments (Wood, 1990). Lagoons with floating plants such as water hyacinth have been investigated since the seventies (Boyd, 1970) and are currently used in at largescale for the treatment of municipal wastewater. The constructed wetlands with emergent plants such as reeds and bulrush have been studied and applied more recently (Olguin *et al.* 1998).

It was shown as early as 1970 that water hyacinth has the capacity to remove nitrogen and phosphorous from secondary sewage treatment effluent pond (Steward, 1970). Since then a large number of trials all over the world showing the tremendous capacity of water hyacinth for the removal of a variety of waste constituents such as nutrients, suspended particles, organic matter and heavy metals (Cornwell, *et al.* 1977; Dinges, 1976 a; Wolverson and Mc Donald, 1976. Boyd (1970) reported that upto 200 kg/ha nitrogen and 320 kg/ha of phosphorous could be removed by using water hyacinth. The ability of water hyacinth to treat industrial wastes was studied by John (1984); Mehrotra and Aowal (1982). Trivedy *et al.*(1983) and Trivedy and Pattanshetty (2002) worked with dairy wastes. Trivedy and Khomane (1985) studied the removal of nutrients from distillery waste, textile mill waste and metal work waste by water hyacinth.

The use of duckweeds (Lemnaceae) and other small aquatic plants in wastewater treatments has been tested because of various advantages such as high growth rates (Brix and Schierup, 1989; Korner and Vermaat, 1998; Lambers and Poorter, 1992), wider geographic range and ability to survive in cold climates. In

principle, high nutrient removal efficiencies can be achieved due to rapid growth rates (Shiomi and Kitoh, 1987). Controlled duckweed culture contributes to water quality improvement by the direct plant uptake of organic and mineral matter, suppression of algal growth and by creating a habitat suited for the growth of zooplankton (Ehrlich, 1966 and Dinges, 1973). Duckweeds such as *Lemna gibba* have been utilised to treat municipal wastewater in Israel and a very high BOD removal (97 percent) has been reported (Oron and Porath, 1987). Taking into consideration the fact that duckweed may recover a high percentage of nitrogen from wastewater and may accumulate a protein content as high as 40 percent, it has been utilised to recover nitrogen and phosphorous from anaerobic effluents from digested pig waste (Hernandez *et al.* 1997). Naskar *et al.* (1986) studied the yield of *Wolffia arrhiza* with different sewage concentrations and its efficacy as a carp feed.

Azolla, a cosmopolitan free floating fern, owing to its nitrogen fixing ability has been used for several decades as green manure in rice fields (Peters and Meeks, 1989). There is an increasing interest in its utilisation as a decontaminant plant in low-cost wastewater treatment systems because of its capability to remove nitrogen and phosphorous from the water (Shiomi and Kitoh, 1987; Schaarpenseel and Knuth, 1987) and efficient accumulation of heavy metals (Sela *et al.* 1989; Zhao and Duncan, 1999). Studies by Forni *et al.* (2001) advocate utilisation of the fern to remove nitrogenous compounds (mainly nitrate) from aquaculture wastewater.

Salvinia, another member of ferns, has not been subject to extensive research until recently. *Salvinia molesta* has been evaluated for upgrading

effluent quality at the Maroochydwore wastewater treatment facility in Queensland (Dinges, 1982). Harvey and Fox (1973) revealed that *Salvinia rotundifolia* was rejected as a candidate species for nutrient removal from wastewater because it produced large amounts of detrital material and was found to have low digestibility value. Experiments by Olguin *et al.* (1998) revealed that bioaccumulation factors for cadmium were in the range of 2595-2932 for copper, 2730-3211 for lead and around 783-784 for chromium. Nitrogen and phosphorous removal capacity of *Pistia stratiotes* (water lettuce) on tropical freshwater ponds has been studied by Tripathi *et al.* (1991).

Controlled culture of submerged vegetation for the improvement of quality of treated wastewater effluent is in an early experimental stage. Study utilising submerged aquatic vegetation for improvement of wastewater quality had been conducted at the Michigan State University (Bahr, *et al.* 1974, 1977). Culture basins contained *Elodea canadensis*, *Ceratophyllum demersum*, *Potamogeton foliosus* and the epiphytic algae, *Cladophora*. Bahr *et al.* (1977) reported that after the system had been in operation for two years, the incoming nitrate level of 15 mg/l was reduced to 0.01 mg/l and phosphorous was reduced from 4 mg/l to about 0.03 mg/l during passage through the four basins. Serafy *et al.* (1988) and Killgore *et al.* (1989) have mentioned that *Hydrilla* helps in suppressing summer algal blooms and provides protection to small fauna seeking food and shelter from predators, by creating structural complexity in the water column.

Studies on the utilisation of emergent plants grown in artificial environments for treatment of wastewater commenced in the early 1950's at the Max Planck Institute in Germany. Seidel *et al.* (1978) evaluated over 200 species

and selected two plants, *Scripus lacustris* (bulrush) and *Phragmites communis* (common reed) for artificial marsh systems. Spangler *et al.* (1976) performed experiments using bulrush in artificial marsh for wastewater treatment and found that BOD₅ reduction in the unit was 92 percent; COD was reduced by 44 percent and total phosphorous by 35 percent. In a study by Soto *et al.* (1999), presence of *Scripus lacustris* was responsible for 30 percent removal of total nitrogen, 20 percent removal of total phosphorous and a 99 percent pathogen removal. Investigations by Gersberg *et al.* (1986) showed that nitrogen removal efficiency of *Scripus validus* and *Phragmites communis* was greater than unvegetated beds. Similarly BOD removal was 5.3 and 22.2 mg/l for *Scripus* and *Phragmites* respectively, which is significantly lower than that for unvegetated beds (36.4mg/l).

Engineering analyses have shown that in some locations, the cost of secondary and advanced domestic wastewater treatment can be reduced by utilising aquatic macrophyte based systems rather than conventional treatment methods (Reddy and De Busk, 1987).

The presence of protozoa in biological used water treatment processes had been recognised, but only recently the significance of these organisms has begun to emerge. There is considerable amount of evidence in literature to show that protozoa in pure culture are able to flocculate particulate matter and bacteria. This can aid in both clarification of the effluent and the formation of sludge (Curds and Hawkes, 1975). Furthermore it is seen that when protozoa are not present in full-scale plants, the effluent obtained is turbid and of inferior quality (Curds and Cockburn, 1970). The reduction of the numbers of bacteria from sewage to

effluent following aerobic treatment was noted by Curds *et al.* (1968). The dominant types of protozoa found in these processes feed mainly on bacteria (Sandon, 1932) and the predatory activity could be responsible for bacterial removal. Protozoa are known to feed upon pathogenic bacteria, including those that cause diseases such as diphtheria, cholera, typhus and streptococcal infections as well as fecal bacteria such as *E. Coli* (Curds, 1975).

Preliminary lab scale studies conducted at the University of Texas Medical Branch at Galveston, Texas (Songer *et al.* 1974; McShan *et al.* 1974 and Trieff *et al.* 1976) demonstrated that controlled brine shrimp culture was effective in the removal of undesirable bacteria and other suspended matter from domestic wastewaters. Pilot scale studies on the controlled culture of *Artemia salina* for clarification of industrial wastewaters have been conducted at the Dow chemical Texas Division facility at Freeport, Texas. Reduction in BOD and suspended solids was reported. Brine shrimp have been used in laboratory and pilot scale experiments in a two-step process where the brine shrimp were expected to clarify the effluent from algal ponds. Removal of BOD and suspended solids averaged 89 percent in the pilot scale shrimp tanks that were aerated and heated during winter months (Milligan *et al.* 1980). The environmental and management requirements for this shrimp culture may not permit cost-effective full-scale systems. The organism exhibited no significant accumulation of industrial waste chemicals.

The fact that aquaculture technology can produce clean water cheaper than conventional waste treatment methods was studied by Duffer and Moyer (1978). Milligan *et al.* (1980) experimented on the sequential use of bacteria, algae and brine shrimp to treat industrial wastewaters at pilot plant scale. The combination

of complementary filter feeders to control excessive suspended solids and nutrients in stabilization ponds has been tried by Smith (1993) and observed 99 percent reduction of algal biomass.

Loedolff (1964) evaluated the role of cladocera in a stabilization pond system in South Africa and reported that a significant reduction in turbidity was observed at times when cladoceran populations were high. Kryutchkova (1968) studied that stabilisation of polluted waters occurs by the conversion of unstable organic matter into zooplankton bodies and through the mineralization of organic matter.

Mass *Daphnia* culture for the treatment of wastewater has been evaluated by Ehrlich (1966), Dinges (1972, 1973 and 1974) and Gram (1973). Three species, *Daphnia pulex*, *Daphnia magna* and *Daphnia similis* are best suited for controlled mass culture (Dinges, 1982). Scheithauer and Bick (1964) cultured *Daphnia magna* and *Daphnia pulex* in a duck pond and in laboratory microcosms. It was estimated that the filtering rate of *Daphnia* varied from 140-300 ml per mg dry weight of *Daphnia* per day.

Dinges (1973) noted that some stabilization ponds in Texas having high populations of *Daphnia* were macroscopically clear. A pilot scale pond having a capacity of 37.8 m³ was constructed at the wastewater treatment facility at Texas, to evaluate the feasibility of establishing and utilising controlled *Daphnia* culture for the removal of algae from stabilisation pond effluent (Dinges, 1982). Gram (1973) evaluated algal nutrient removal by *Daphnia pulex* at the Las Vegas wastewater treatment system at Calabashes, California. Heimbuch (1978),

Uhlmann (1971) and Gordon (1977) also performed studies on *Daphnia* under laboratory conditions.

Gellis and Clarke (1935) working on cladocera found that they needed particulate food and could possibly utilise colloidal particles and bacteria.

Kawasaki *et al.* (1982) reported the aquacultural approaches to recycling of dissolved nutrients in secondarily treated domestic wastewater using *Scenedesmus*, *Daphnia* and *Hypophthalmichthys*. Kaur and Dhawan (1992) discussed the role of biological wastes in carp culture for recycling wastes, reducing pollution and for producing protein at a low cost.

Donaszy (1974) stated that the efficiency of a fishery established for wastewater treatment might only be successful if fish production is a secondary objective. Hallock and Ziebell (1970) investigated the feasibility of establishing a sport fishery in activated sludge treatment plant effluent that has been passed through a sand and gravel filter. Trimberger (1972) reported upon the production of fathead minnows in stabilization ponds for use as forage for game fish. Coleman *et al.* (1974) evaluated the potential of fish production in wastewater stabilization ponds at a treatment facility in Oklahoma City.

Allen and Dennis (1974) cultured coho salmon (*Onchorhynchus kisutch*) and chinook salmon (*Onchorhynchus tshowytscha*) fry and fingerlings in a mixture of stabilization pond effluent and seawater at Arcata, California. Dinges (1976 c) placed golden shiner minnows, fat head minnows and gold fish in the fourth and final cell of an experimental integrated wastewater treatment system at the Williamson creek wastewater treatment facility in Austin, Texas.

Buck (1976) studied the Munich carp ponds where BOD₅ of the inflowing wastewater was about 60 mg/l and effluent BOD₅ ranged from 2-6 mg/l. Chambers (1978) evaluated the use of fish culture for algae removal in a 142 ha stabilisation pond serving the Exxon refinery complex at Texas. *Tilapia*, golden shiner minnows, fathead minnows and mullet were tested for TSS removal. Golden shiner minnows affected algal reduction over a period of one month. The extremely high reproductive rate of *Tilapia*, which is considered detrimental in the culture of these fishes for food, is an asset in wastewater treatment systems.

Henderson (1976) described the feeding mechanisms of silver and big head carps. The gill rakers of silver carp form a spongy mass that enables them to remove particles as small as four microns in diameter from water. Buck *et al.* (1978) and Maddox *et al.* (1978) have investigated the feasibility of utilizing these two carps and other fish species in treating swine wastes. Chinese grass carp has been demonstrated to consume a variety of aquatic vegetation (Schuster, 1952; Cross, 1969; Blackburn and Sutton, 1971; Mitzner, 1978). Bailey (1978) reported that this species has high potential for use in integrated wastewater treatment systems for the controlled cropping of submersed vegetation and duckweed.

Colt *et al.* (1979) suggest that the filter feeding sacramento blackfish is a potential species for use in wastewater treatment. Blackfish were grown for a brief period in an experimental high rate algal pond to remove nitrate from agricultural drainage waters (Brown, 1971).

Heavy metals enter wastewater from a variety of sources including naturally occurring geological weathering of rocks and sediments, anthropogenic sources such as runoff, landfill leachates, domestic wastewater, mining wastes and

various industrial wastewaters, particularly from electroplating, electronic and metal finishing industries (Sarah, *et al.* 1994). Metal containing effluents have unfavourable effects on wastewater treatment such as the inhibition of nitrification and interference in biological oxidation (Becker, 1983), lowering the rate of biodegradation to complete cessation of the process (Jain *et al.* 1992). Methods such as chemical precipitation and reverse osmosis for removal of metal ions from solutions result in incomplete removal, has high reagent or energy requirements and generate toxic sludges which are difficult to dewater (Kapoor and Viraraghavan, 1998).

Researchers have been looking for cheaper, more efficient methods to remediate metal contaminated waters and reduce the growing public health risk (Runnels *et al.* 1992). Bioremediation has emerged as a technology for accumulation of metals by use of living organisms (Tiemann *et al.* 2000). Biological systems have proven to be quite effective at removing metal ions from contaminated solutions in a low cost and environmentally friendly manner (Atlas, 1995). Utilisation of aquatic plants for removal or recovery of metals from wastewaters has been studied by several authors (Wolverton *et al.* 1976, O'Keeffe and Hardy, 1984, Muramoto and Oki, 1983, Panda, 1996). The efficiency of metal removal by different plants depends on pattern of uptake by individual plant and also their tolerance to each metal (Santra *et al.* 2001). Plants such as *Eichhornia crassipes*, *Hydrocotyle umbellata*, *Lemna minor*, *Scirpus lacustris*, *Phragmites karka*, *Bacopa monnieri* and *Azolla pinnata* are effective in removing metals from aquatic systems (Chandra *et al.* 1997). Studies by Farango and Parsons (1985) demonstrated that water hyacinth can remove platinum from a

trade effluent and the possibility of large-scale use has been discussed. Considerable work on metal contaminated waste effluents has been carried out for the removal of copper (Sutton *et al.* 1971), cadmium and nickel (Wolverton, 1975 a), silver, cobalt, strontium (Wolverton and Mc Donald, 1975 b) mercury and lead (Wolverton and Mc Donald, 1975 c), by water hyacinth. Zhao and Duncan (1999) studied the batch removal of chromium by *Azolla filiculoides*. Zayed (1998) observed that *Lemna minor* showed promise for the removal of cadmium, selenium and copper from contaminated wastewater since it accumulates high concentrations of these elements. *Lemna minor* has been reported to remove chromium and copper in laboratory conditions upto 1 mg/l (Abdel Wahaab *et al.* 1995).

The aquatic flora and fauna used for wastewater treatment can be harvested for a multitude of uses. The high productivity of aquatic plants makes economic and possible environmental gains from their utilisation impressive (Gopal and Sharma, 1979). These plants can be used as animal feed (Baldwin, *et al.* 1974), fish food (Sutton, 1974; Opuszynski, 1972), manufacture of paper (Morton, 1975), compost and soil conditioner, mulch in coconut and cocoa groves (Menon, 1971), biofertiliser (*Azolla*), biogas (Jogalekar and Sonar, 1986; Abbasi and Nipanay, 1983), as a source of protein for animal and human consumption (Sharma, 1971; Nayar and Madhusoodanan, 1979) and carotene (Ingole and Bhole, 2001). The medicinal value of some aquatic weeds have been also been reported. The potential of water hyacinth to cure goitre has been investigated (Anonymous, 1974). Its flowers have been used in skin diseases and as an antimicrobial agent (Agarwal, 1997). *Pistia* has been used as a cure for wounds,

skin diseases, coughs and asthma. *Lemna* has uses in hormone therapy. Rootstocks of *Lagenandra* are poisonous (Gupta, 1979), yet medicinal uses have been reported (Agarwal, 1997). *Myriophyllum* is also used in medicines. *Ceratophyllum* is used in scorpion stings and biliousness and its extract is antimicrobial (Agarwal, 1997).

The plants used for metal remediation needs effective disposal. They can be dried, ashed or composted. The plant residues may be recycled as a source of metal. Plants containing valuable metals like copper and nickel can be burnt and the metals recovered from the residue (Wolverton, *et al.* 1976). Plants that concentrate mundane metals such as lead could be dried and buried in protected vaults.

The harvested fauna also has several uses including fish feed, human and livestock food. Wastewater grown *Daphnia pulex*, on a dry weight basis was found to contain 65.3 percent protein, 18.6 percent fat and 16 percent ash (Dinges, 1973). Harvested *Daphnia* could either be frozen or fed directly to fish. It can also be employed as protein additive in pet and livestock foods. Brine shrimp (dry or frozen) and midge fly larvae have good potential as they are in demand as staple fish food. Clams are harvested and used for human consumption (Sinclair and Isom, 1963). Fishes from wastewater aquaculture systems can be used after a depuration period of several weeks for pathogen control. Buras *et al.* (1987) experimentally found the critical concentration of standard plate count bacteria to be 5×10^4 per ml. Bacterial concentrations higher than this were found to cause their appearance in the meat of fish reared in the ponds.

In general, natural systems are very effective in removing potentially harmful chemicals and biological agents, including viruses (Anonymous, 1979). Natural treatment systems utilizing wastewater input are considered as being valuable for recycling nutrients and organic to produce food, fuel and fertilizer in developing nations (Eusebio *et al.* 1976; Anonymous, 1977, Mc Garry, 1978 and US National Academy of Sciences, 1979).

Health implication related to various types of natural treatment systems have been addressed by several investigators (Bryan, 1974; Carpenter *et al.* 1974; Kerfoot and Jacobs, 1974; Kerfoot and Redmann, 1974; and Stanford and Tuburan, 1974).

Aside from technology and economics, resistance to implementation of natural wastewater treatment systems may be expected because some of the concepts are new (Eichbaum, 1976). Economic considerations of various natural treatment systems have been discussed by Henderson and Wert (1976) and Tchobanoglous, *et al.* (1979).

MATERIAL AND METHODS

Shiny K J “Use of aquatic organisms for water treatment” Thesis. Department of Life Science, University of Calicut, 2003

3. 0 MATERIAL AND METHODS

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3. 0 MATERIAL AND METHODS

Aquatic macrophytes with potential for wastewater treatment and aquatic fauna with filter feeding /omnivorous habits including protozoans, crustaceans and pisces were selected for treatment studies. Experimental studies were conducted in the laboratory to evaluate the pollutant abatement capacity of the selected flora and fauna. Experimental and control sets were maintained for a fixed period with different stocking rates of test species in selected concentrations of wastewater. Three replicates were maintained for each experiment. Physico-chemical and bacteriological parameters of the experimental water samples were carried out at pre-experimental and post-experimental stage. The relationship between biomass of test species and rate of removal of significant water quality parameters were studied and statistical interpretations were made.

3. 1 ANALYSIS OF PHYSICO-CHEMICAL AND BACTERIOLOGICAL PARAMETERS

The water samples from the experimental and control sets were subjected to physico-chemical and bacteriological analysis as per APHA (1995), Manivasakam (1996), Trivedy and Goel (1984) and Abbassi (1998).

Samples for physico-chemical parameters were collected in polythene bottles. The bottles were thoroughly cleaned, followed by repeated washing with distilled water. The containers were rinsed thrice with the sample before collection. For bacteriological examination, glass bottles, previously sterilised at 120°C under 15 lb pressure for 15 minutes in an autoclave were used.

The water samples for metal analysis were filtered through Whatman No. 1 filter paper, collected in acid washed bottles and preserved immediately by acidifying with concentrated HNO₃ to pH < 2.

The samples were analysed as quickly as possible and if immediate analysis was not done, they were stored under refrigeration. Different methods of preservation of samples, which vary in terms of physical conditions, nature and concentration of chemicals was followed as per Table 4. The instruments and analytical methods followed are given in Table 5.

Table 4. Methods of Preservation of Water Samples

No	Parameters	Preservation Methods	Maximum Storage Time
1	Temperature	-	Measured immediately
2	pH	-	2 hours
3	Electrical Conductivity (EC)	Refrigeration	28 days
4	Dissolved Oxygen (DO)	Titration can be delayed, after acidification for a maximum of 8 hours	Immediately titrated
5	Biological Oxygen Demand (BOD)	Refrigeration at 4 ⁰ C	6 hours
6	Chemical Oxygen Demand (COD)	Add H ₂ SO ₄ to pH < 2 and refrigerate	7 days
7	Hardness	Add HNO ₃ to pH < 2	6 months
8	Nitrate - Nitrogen (NO ₃ - N)	Refrigeration	As soon as possible
9	Phosphate – Phosphorus (PO ₄ - P)	Refrigeration	48 hours
10	Sulphate (SO ₄)	Refrigeration	28 days
11	Calcium (Ca)	Add HNO ₃ to pH < 2	28 days
12	Magnesium (Mg)	Add HNO ₃ to pH < 2	28 days
13	Metals	For dissolved metals, filter immediately, add HNO ₃ to pH < 2	6 months
14	Total coliforms, Fecal coliforms and <i>E. coli</i>	Refrigeration	Maximum 6 hours

Table 5 Instruments and Analytical Methods followed for Water Analysis

No	Parameters	Methods	Instrument and Model	Unit
1	Temperature	Thermometry	Mercury Thermometer	°C
2	pH	Electrometry	pH Meter (Systronics 335)	-
3	Electrical Conductivity (EC)	Electrometry	Conductivity Meter (Elico 180)	μ mhos/cm
4	Total suspended solids	Filtration	-	mg/l
5	Dissolved Oxygen (DO)	Winkler's Azide Modification	-	mg/l
6	Biological Oxygen Demand (BOD)	Winkler's Azide Modification	-	mg/l
7	Chemical Oxygen Demand	Dichromate reflux method (Titrimetry)	-	mg/l
8	Total Hardness (TH)	EDTA Titrimetry	Titration	mg/l
9	Nitrate –Nitrogen (NO ₃ - N)	Photometry	UV-Visible Spectrophotometer (Hitachi 220)	mg/l
10	Phosphate (PO ₄ - P)	Stannous Chloride Method	UV-Visible Spectrophotometer (Hitachi 220)	mg/l
11	Sulphate (SO ₄)	Turbidimetry	Digital Nepheloturbidity meter (Systronics 132)	mg/l
12	Calcium (Ca)	EDTA Titrimetry	-	
13	Magnesium (Mg)	EDTA Titrimetry (Mg by calculation)	-	mg/l
14	Metals	Atomic Absorption	Atomic Absorption Spectrophotometer (Elico 173)	mg/l
15	Total coliforms	Multiple Tube Fermentation Technique	-	MPN/100 ml
16	Fecal coliforms	Multiple Tube Fermentation Technique	-	-
17	<i>E. coli</i>	Indole Test	-	-

3. 1. 1 Physical and Chemical Parameters

Temperature

Water temperature is the amount of heat contained in water. One of the biologically important temperature effects is the decrease in oxygen solubility with increasing temperature. Other important processes, such as biological growth rates, rates of chemical and biological reactions and the solubility of particulate compounds are also affected by temperature.

Method

Thermometry

Procedure

Temperature readings were taken with mercury filled celsius thermometer of 0-50 °C with a scale marked for 0.1°C.

pH

pH is the concentration of hydrogen ions in water and measures the intensity of acidity or alkalinity. pH is an important water quality parameter of both natural and wastewater since the range suitable for chemical and biological reactions as well as life is quite narrow and critical. pH by itself is not a contaminant but is important as a characterisation parameter, since it may reveal contamination. Wastewater with an adverse concentration of hydrogen ions is difficult to treat by biological means and if the concentration is not altered before discharge it may alter the pH of receiving waters. High pH values hasten scale formation, reduce germicidal potential of chlorine and induce the formation of trihalomethanes which are toxic. pH below 6.5 starts corrosion in pipes, thereby releasing toxic metals such as zinc, lead, cadmium and copper.

Method

Potentiometry using digital pH Meter

Procedure

pH meter was standardised using standard buffer solution of pH 4 and 9. The pH value of the samples was then measured directly by immersing the electrode in the sample.

Electrical Conductivity

Conductivity is the measure of the capacity of a substance or solution to conduct electric current. It is the reciprocal of resistance. Conductivity is a good and rapid measure of the total dissolved solids. The presence of salts and contamination with wastewater increases the conductivity of water and is therefore indicative of pollution. Conductivity is highly dependent on temperature. It can be used to evaluate variations in dissolved mineral concentration of raw water/wastewater and consequently in controlling water treatment processes.

Method

Conductometry, using digital conductivity meter

Procedure

After proper washing of the cell with distilled water, the conductivity cell was dipped into the sample and reading was recorded.

Total Suspended Solids

Total suspended solids gives a measure of the amount of particulate material, both organic and inorganic, present in water. Suspended solids include both organic matter, such as various microorganisms, algae, plant material and

inorganic material including sand, silt and clay particles. Polluted waters are commonly turbid and improvement is marked by greater clarity.

Method

Filtration

Principle

Total suspended solids are the retained material on a standard glass fibre filter paper after filtration of a well-mixed sample of water or wastewater.

Procedure

100 ml of a well-mixed sample was filtered through a weighed standard glass fibre filter paper under vacuum. The residue retained on the filter was dried to a constant weight at 103-105° C. The increase in the weight of the filter represents the total suspended solids.

Calculation

$$\text{Total suspended solids (mg/l)} = \frac{(A-B) \times 1000}{\text{Sample volume (ml)}}$$

Where A= Weight of filter + dried residue

B = Weight of filter

Dissolved Oxygen

Dissolved oxygen is one of the most important parameters in water quality assessment as its presence is essential to maintain the higher forms of life and the effects of waste discharge in a water body are largely determined by the oxygen balance of the system. Oxygen can be rapidly removed from the waters by discharge of the oxygen demanding wastes. The chemical and biochemical processes in a water body are largely dependent upon the presence of oxygen.

Low oxygen concentrations are generally associated with heavy contamination by organic matter.

Low oxygen in water can kill fish and other organisms present in water. Organisms have specific requirement for oxygen – game fish need at least 5 mg/l and coarse fish about 2 mg/l of dissolved oxygen.

Method

Winkler method with Azide modification

Principle

When manganous sulphate is added to the sample containing alkaline potassium iodide, manganous hydroxide is formed, which is oxidised by the dissolved oxygen of the sample to basic manganic oxide. On addition of sulphuric acid, the manganic oxide liberates iodine equivalent to that of dissolved oxygen originally present in the sample. The liberated iodine is titrated with a standard solution of sodium thiosulphate using starch as indicator.

The presence of certain oxidising and reducing materials may effectively interfere with the determination of oxygen by converting iodide ions to iodine or vice-versa. The azide modification removes the interference of such substances, especially nitrite, which is destroyed by sodium azide.

Reagents

Manganous sulphate

Alkali -iodide azide

Conc. sulphuric acid

Starch indicator

Standard sodium thiosulphate

Procedure

Sample was taken in a BOD bottle without bubbling or trapping of air. 2 ml of manganous sulphate followed by 2 ml of azide was added well below the surface through the walls. The brown precipitate obtained was allowed to settle and 2 ml of concentrated sulphuric acid was added and shaken well to dissolve the precipitate. The sample was then immediately titrated against sodium thiosulphate using starch as indicator. Disappearance of the initial dark blue colour indicates the endpoint.

$$\text{DO, mg/l} = \frac{(\text{ml} \times \text{Normality}) \text{ of Sodium thiosulphate}}{\text{Volume of sample taken}} \times 8 \times 1000$$

Biochemical Oxygen Demand

Biochemical Oxygen Demand refers to the quantity of oxygen required by bacteria and other microorganisms in the biochemical degradation and transformation of organic matter under aerobic conditions. BOD approximates the amount of oxidisable organic matter present in the solution and the BOD value can be used as a measure of waste strength. It is highly important to know the amount of organic matter present in the waste treatment system in process designing and loading calculations as well as the measure of treatment plant efficiency and operation. BOD is a test of great value in the analysis of sewage, industrial effluents and grossly polluted waters. It is also useful in stream pollution control management and in evaluating the self-purification capacities of streams.

Reagents

Phosphate buffer

Magnesium sulphate

Calcium chloride

Ferric chloride

Manganous sulphate

Alkali -iodide azide

Conc. sulphuric acid

Starch indicator

Standard sodium thiosulphate

Principle

The basic principle underlying the BOD determination is the measurement of the dissolved oxygen content of the sample before and after five days of incubation at 20 °C.

Procedure

Samples in duplicate were diluted with dilution water. One set of sample dilutions and dilution water was used to find out the initial DO content. The other set of sample dilutions and dilution water were kept in the BOD incubator at 20 °C in the dark for 5 days and BOD content was determined.

Calculation

$$\text{BOD, mg/l} = \frac{D_1 - D_2}{P}$$

D_1 = DO of diluted sample before incubation

D_2 = DO of diluted sample after incubation

P = Decimal fraction of sample used

Chemical Oxygen Demand

Chemical Oxygen Demand is a measure of the oxygen equivalent of that portion of the organic matter in a sample that is susceptible to oxidation by a strong chemical oxidant. COD test is widely employed as a means of measuring the pollution strength of domestic and industrial waters, as the result can be obtained within three hours, while a BOD₅ takes at least five days. COD determinations are of great importance where BOD values cannot be determined accurately due to the presence of toxins and other such unfavourable conditions for growth of microorganisms. COD values are taken as basis for calculation of the efficiency of the treatment plants and also figure in the standards for discharging industrial /domestic effluents in various kinds of waters.

Method

Dichromate reflux

Principle

A boiling mixture of chromic and sulphuric acid destroys most types of organic matter. A sample is refluxed with known amounts of potassium dichromate and sulphuric acid. The unreacted dichromate is back titrated with a standard solution of ferrous ammonium sulphate. The amount of oxidisable organic matter, measured as oxygen equivalent, is proportional to the potassium dichromate consumed.

Reagents

Standard potassium dichromate

Silver sulphate - sulphuric acid

Ferrous ammonium sulphate (0.1 N)

Mercuric sulphate

Ferroun indicator

Procedure

20 ml of the sample was taken in a 500 ml refluxing flask. 1 gm mercuric sulphate, antibumping granules and 30 ml of sulphuric acid were added. The flask was attached to the condensor and refluxed for 2 hours. The excess potassium dichromate was titrated with ferrous ammonium sulphate after cooling using ferroun indicator. A blank was also refluxed and titrated in the same manner.

Calculation

$$\text{COD, as mgO}_2/\text{l} = \frac{(A - B) \times N \times 1000 \times 8}{\text{Volume of sample (ml)}}$$

A= ml ferrous ammonium sulphate used for blank

B = ml ferrous ammonium sulphate used for sample

N = normality of ferrous ammonium sulphate

Nitrate - Nitrogen

Nitrate nitrogen is the most highly oxidised form of nitrogen found in wastewaters. The most important sources of nitrate are the biological oxidation of organic nitrogenous substances which come in sewage and industrial wastes or produced indigenously in waters. Runoff from agricultural fields is also high in nitrate. Nitrate is also the key nutrient after phosphate in accelerating eutrophication. Groundwaters have significant quantities of nitrates due to leaching with the percolating water, contamination by sewage and other wastes rich in nitrates. High amount of nitrates are generally indicative of pollution. In

waste treatment systems, high nitrate values denote the aerobic conditions and stability of the wastes.

Method

Cadmium reduction method

Principle

Nitrate is quantitatively reduced to nitrite while passing through a column containing amalgamated cadmium filings. The nitrite thus produced is determined by diazotising with sulphanilamide and coupling with N (1- naphthyl) ethylene diamine to form a highly coloured azo dye that is measured colorimetrically.

Reagents

Ammonium chloride solution

Amalgamated cadmium filings

Sulphanilamide reagent

N (1- naphthyl) ethylene diamine

Hydrochloric acid

Standard nitrate solution

Procedure

90 ml of sample was taken and 2 ml of concentrated ammonium chloride was added and passed through the cadmium column. The first 30 ml was discarded and remaining 50 ml of the reduced sample was collected. 1 ml of sulphanilamide was added followed by 1ml of N (1- naphthyl) ethylene diamine after two minutes. The colour developed was measured at 543 nm using spectrophotometer.

Phosphate-Phosphorus

Phosphate is a critical nutrient that leads to the process of eutrophication. The major sources of phosphate are domestic sewage, agricultural effluents with fertilisers and industrial wastewaters. Phosphate is a major element of domestic wastewater due to the utilisation of synthetic detergents. Higher concentration of phosphorous is therefore indicative of pollution. Phosphorous occurs in natural and wastewaters in the form of various phosphates.

Method

Stannous chloride method

Principle

Ammonium molybdate reacts with phosphate to form molybdophosphoric acid, which is reduced to blue coloured complex 'molybdenum blue' by the addition of stannous chloride.

Reagents

Ammonium molybdate

Stannous chloride

Standard phosphate solution

Procedure

50 ml of the sample was taken and 2 ml of ammonium molybdate solution and 5 drops of stannous chloride were added. The intensity of colour was measured using a spectrophotometer at 690 nm wavelength, after ten minutes, but before twelve minutes.

Hardness

Hardness is the capacity of water for reducing and destroying the lather of soap and increasing the boiling point of water. Hardness in water is due to the natural accumulation of salts from contact with soil and geologic formations or it may enter from pollution by industrial effluents. Calcium and magnesium are principal cations causing hardness. Total hardness indicates the concentration of calcium and magnesium ions only because these are present in higher concentrations over other cations.

Method

EDTA titrimetry

Principle

In alkaline condition (pH about 10), EDTA reacts with calcium and magnesium ions to form a soluble chelated complex. Calcium and magnesium ions develop wine red colour with Eriochrome Black T under alkaline conditions. When EDTA is added as a titrant, calcium and magnesium divalent ions get complexed resulting in a sharp change from wine red to blue, which indicates end point of the titration.

Reagents

Standard EDTA solution (0.01 M)

Ammonium chloride ammonium hydroxide buffer

Eriochrome Black T indicator

Standard Calcium solution

Procedure

50 ml of sample was taken and 2 ml of ammonia buffer and a pinch of Eriochrome Black T was added and titrated against 0.01 M EDTA until wine red colour changed to blue.

Calculation

$$\text{Total Hardness, (mg/l) as CaCO}_3 = \frac{V \times M \times \text{Equivalent weight of CaCO}_3}{\text{Volume (ml) of sample taken}} \times 1000$$

V = Volume of EDTA (ml); M = Molarity of EDTA

Calcium

Calcium is an important macronutrient in aquatic environment and is needed especially in large quantities by molluscs and vertebrates. The presence of calcium in water is mainly due to its passage through or over deposits of limestone, dolomite, gypsum and other gypsiferous materials. Disposal of sewage and industrial wastes are also important sources of calcium. Concentration of calcium is reduced at a higher pH due to its precipitation as CaCO_3 . The importance of calcium lies in its disadvantages in household and industrial uses. High concentrations are not desirable in washing, and bathing owing to its suppression of lather formation with soap. Scale formation in boilers takes place by high calcium along with magnesium.

Method

EDTA titrimetric method

Principle

The pH of the sample is made sufficiently high (12-13) to precipitate magnesium as hydroxide and calcium ions that remain are allowed to react with

EDTA in the presence of murexide indicator. Change from pink to purple colour indicates the endpoint of the reaction.

Procedure

50 ml of the sample was taken and 2 ml of sodium hydroxide and 0.1-0.2 g of indicator was added and titrated immediately with 0.01 M EDTA to proper end point.

Calculation

$$\text{CaH (mg/l) as CaCO}_3 = \frac{V \times M \times \text{Equivalent weight of CaCO}_3}{\text{Volume (ml) of sample taken}} \times 1000$$

$$\text{Ca} = \text{CaH} \times 0.4$$

Magnesium

The principal sources in the natural waters are various kinds of rocks. Sewage and industrial wastes are also important contributors of magnesium. Magnesium adds to the hardness of waters and with calcium poses the problem of scale formation in the boilers.

Method

Magnesium by calculation.

Magnesium may be calculated as the difference between total hardness and calcium hardness.

Calculation

$$\text{MgH mg/l} = \text{Total hardness} - \text{Calcium hardness}$$

$$\text{Mg as CaCO}_3 = \text{Magnesium hardness} \times 0.243$$

3. 1. 2 Analysis of Metals

Zinc

Zinc is one of the commonest elements in the earth's crust. It is a very essential micronutrient, needed for protein synthesis, normal cell differentiation and growth in both plants and animals. However it can be toxic in excess. A number of enzymes including alkaline phosphatase, aldolase alcohol dehydrogenase, carboxypeptidase and lactic dehydrogenase are zinc dependent.

Some zinc is released into the environment by natural processes, but most comes from anthropogenic activities such as mining, galvanising, paint, pigments, printing, fertilisers, brass alloy manufacturing, pharmaceuticals, insecticides, cosmetics, municipal waste etc. This discharge increases its concentration in appreciable amounts in the waters (Trivedy and Goel, 1984).

Manganese

Manganese occurs as an oxide in minerals (pyrolusite, manganite). Manganese is needed in trace amounts by living organisms. It activates many enzymatic reactions associated with the metabolism of organic acids, carbohydrates, nitrogen and phosphorous. The element is involved in the photosynthesis of plants. (Mukhopadhyay and Sharma, 1991). Manganese finds applications in ceramic, dry batteries, matches, glasses, steel alloys such as ferromanganese and copper manganese. The acetate, borate, oleate and carbonate salts find use as drying compounds in varnishes, oils and mordant in dyeing.

Iron

Iron is one of the most abundant elements, and all kinds of waters, including groundwater have appreciable quantities of iron. Iron exists in both

ferrous and ferric forms. In addition to the natural sources of iron, its use in fertilisers, textile mills, tanneries, iron and steel industries, corrosion of pipes and pumps can also increase its concentration in distribution systems. Iron is essential for the formation of an iron porphyrin complex which is required for chlorophyll synthesis (Basiouny *et al.*1977). The rate of photosynthesis is lowered in iron deficiency (Mohapatra and Rengarajan , 2000).

Iron is considered as a nuisance in excessive quantities. Iron in excess of 0.3 mg/l causes staining of clothes and utensils. Higher concentration of iron is also not suitable for processing of food, beverages, ice, dyeing, bleaching and other uses. Iron in higher concentrations may also cause vomiting. High concentration of iron in waters produces yellow flocculent mass of ferric hydroxide that is acidic and makes the water deficient in oxygen, causing fish kills.

Chromium

Chromium is primarily used in electroplating industry, as a component of alloy metal. Chrome tanning, wood preservative, explosives, ceramics, dyes, paints and paper industry. It is also used as a corrosion inhibitor, in fertilisers, rubber goods and petrochemicals. Municipal wastes contain considerable amounts of chromium (Mohapatra and Rengarajan, 2000).

Copper

Copper is an essential component of many enzymes. It is used with sulphate as a pesticide, algicide and fungicide, as a fertiliser, in motor vehicle, aircraft plating finishing and petroleum refining. Other uses are in coolant water

discharge, corrosion of pipelines, municipal drainage, pulp and paper board mills (Mohapatra and Rengarajan, 2000).

Procedure

Metals were analysed immediately or acidified as per Standard Methods (APHA, 1995). Metal concentration of the metals was determined using Atomic Absorption Spectrophotometer.

3. 1. 3 Bacteriological Parameters

Sample Collection

The grab samples were collected in presterilized bottles. The pre-sterilized sampling bottle was lowered in the water column. The bottle was opened under water, filled up to $\frac{3}{4}$ th of its capacity and closed under water. The samples were preserved immediately at 4⁰C in an icebox, transported to the laboratory and analyzed within 24 hours.

Principle

Multiple tube fermentation technique was used for the enumeration of microorganisms and biochemical tests were used to detect the various groups such as coliforms, fecal coliforms and *E.coli*.

Reagents

Buffered dilution water

Mac Conkey Broth

Brilliant Green Lactose Bile Broth (BGLB)

Peptone water

Kovac's (Indole) Reagent

Phosphate buffer

Procedure

I. Test for Total Coliforms

Presumptive Test

Mac Conkey Broth of single and double strength were prepared in tubes containing inverted Durham's tubes, sterilized in an autoclave at 15 lb pressure for 15 minutes without any air bubbles. If the water was highly contaminated, the sample was serially diluted 1000 times (up to 0.001 ml dilution) using phosphate buffer solution. 10 ml and above volume of sample was inoculated in double strength and 1 ml and its below was inoculated into single strength medium and incubated at 37°C for 48 hours. The colour change and gas formation indicates the positive presumptive test.

Confirmatory Test

The medium used for confirmed test was BGLB Broth. The required broth was distributed in tubes containing Durham's tubes and sterilized. One or two loop full of positive cultures from positive presumptive tube was inoculated into the BGLB tubes and incubated at 37 °C for 48 hours. The formation of gas in the Durham's tubes of BGLB tube at any time within 48 ± 2 hours indicated a positive confirmation.

II. Test for Fecal Coliforms

This procedure was adopted to differentiate coliforms of fecal origin from those of non-fecal origin. Fecal coliforms are those coliforms which can ferment lactose at 44.5°C within 24± 2 hours with the production of acid and gas in Brilliant Green Lactose Bile Broth cultures.

All the presumptive positive tubes of the coliform test were sub-cultured at the end of 24 – 48 hours into BGLB Broth and incubated at 44.5⁰C for 24 hours in an incubator. Gas formation within 24 hours indicated a positive reaction for fecal coliforms.

III. Rapid Test for *E.coli*

The positive tubes of BGLB Broth (fecal coliforms) were sub-cultured in tubes of peptone water. The required peptone water medium was prepared and distributed in test tubes and sterilized. The inoculated tubes were incubated at 44.5⁰C for 24+ 2 hours. After incubation, the test for indole production was conducted by adding few drops of Kovac's Reagent.

A pinkish red coloured ring indicated a positive test, while yellow colour indicated a negative reaction.

Based on the number of positive tubes at each dilution, the most probable number (MPN) *i.e.*, coliform bacteria per 100 ml of the sample were calculated referring to the standard probability table. In case of unlikely combinations, the following formula was used to calculate MPN/100ml.

$$\text{MPN/100ml} = \frac{\text{No. of positive tubes} \times 100}{\sqrt{\text{ml sample in negative tubes} \times \text{ml sample in all tubes}}}$$

3. 2 SELECTION OF TEST SPECIES

The aquatic fauna for experimental studies were selected based on the following criteria:

- ◆ Availability of species
- ◆ Resistance to handling and transport

- ◆ Ability to survive in wastewaters
- ◆ Species which can be cultured on a large scale
- ◆ Species with filter-feeding/omnivorous/ detritivorous habit.

Aquatic flora were selected based on the following criteria:

- ◆ Rapid growth rate
- ◆ Hardiness
- ◆ Ease of harvest
- ◆ Useful products on harvesting
- ◆ High mineral absorption ability
- ◆ Low in water content
- ◆ Non toxicity to animals

3. 3 COLLECTION OF TEST SPECIES

1. Aquatic Macrophytes

Aquatic plants were collected from local ponds and acclimatised in laboratory conditions. They were identified as per Gupta (1979).

2. Invertebrates

Paramecium and *Daphnia* were collected from local ponds and cultured in laboratory conditions. They were identified as per Davis (1955) and Kotpal (1972).

3. Fishes

Healthy fish fingerlings of test fishes ranging in size from 3.5-5 cm were collected from Ullalam Fish Farm/Department of state fisheries, Kerala. They were identified as per Day (1878).

3.4 CULTURE OF TEST SPECIES

1. *Paramecium* Culture

Paramecium was cultured by the hay infusion method for ciliates. First 1 litre of pond water or tap water was boiled and a handful of hay (Size one inch length and 8-10 numbers in 100 ml of water) was added to it. This was allowed to boil for an additional 10 minutes. The mixture was cooled and allowed to stand for 2 days before inoculating it with *Paramecium*.

2. *Daphnia* Culture

Daphnia were obtained by field collection from the nearby ponds. One of the simplest culture media used for rearing *Daphnia* is the manure-soil medium developed by Banta and modified by Anderson (1944), by supplementing it periodically with yeast.

The medium was made by mixing 5 g dried sheep manure, 25 g garden soil or sandy mud, and 1 litre pond or tap water. This mixture was allowed to stand for 2 days at room temperature and then strained through bolting cloth with mesh openings of approximately 0.15 mm. During straining, some of the finer soil particles also pass through the cloth. The filtrate was set aside for a week or more and the residue discarded. To make the final medium, one part filtrate was mixed with 6-8 parts pond or dechlorinated tap water. The original filtrate may be kept indefinitely before the final medium is made.

The final medium could be used for individual or mass culturing. For individual rearing, 100 ml medium was dispensed into 125 ml wide mouth glass vessels and inoculated with one *Daphnia* per bottle. A day after inoculation, 1ml of a suspension containing 1mg active dry yeast in water was added to each bottle on alternate days. This provided the optimum quantity of yeast for maximum reproduction. Water was added from time to time to replace that lost by evaporation and in removal of young ones. To retard evaporation, the vessels were covered with a perforated cover to permit diffusion of air.

3.5 ACCLIMATION OF TEST SPECIES

Test species for an experiment were obtained from a common source and at one time. Healthy neonates of *Daphnia* were selected. In the case of fish, uniformly sized fingerlings, with the largest individual not more than 50 percent longer than the shortest were selected. Care was taken to ensure that the organisms were not damaged during collection, transfer and transporting process.

The characteristics of water such as pH, temperature, dissolved oxygen and quantity of water required by the organisms was confirmed. The organisms were held in stocking tanks and observed for two days for any sign of distress or disease thus allowing them a period of acclimation to laboratory conditions (Plate 1). Dead and abnormal individuals were immediately removed. The organisms were fed with pellet feed during this period. The tanks were covered to prevent the organisms from jumping out.

The tanks and nets were cleaned thoroughly and water changed twice a week. Uneaten food that collected on the bottom was removed within 24 hours.



Plate 1 Acclimation of Fishes



Plate 2 *Paramecium caudatum* Ehrenberg showing cilia

While handling, care was taken to prevent mechanical injury to the organisms. The test organisms were ensured to be in healthy condition before the experiment.

Aquatic plants were also acclimated by keeping them for seven days in laboratory conditions. Signs of wilting or decay and chlorosis were noted and such plants were discarded. The presence of pests or disease was also observed.

3. 6 CHARACTERISTICS OF TEST SPECIES

3. 6. 1 Aquatic Fauna

1. *Paramecium caudatum* Ehrenberg

Phylum - Protozoa

Class - Ciliophora

Subclass - Ciliata

Order - Holotricha

Family - Parameciidae

Paramecium commonly called slipper animalcule, occurs in freshwater ponds and ditches. It is about 0.3 mm in length. The body is covered with cilia in longitudinal rows, which acts as a locomotory apparatus (Plate 2). *Paramecium* chiefly feed on bacteria, diatoms and other organic particles, which are swept down the peristomial groove into the cytostome by ciliary action.

2. *Daphnia magna* Straus

Phylum - Arthropoda

Class - Crustacea

Subclass - Brachiopoda

Order - Cladocera

Family - Daphnidae

Daphnia (water flea) are filter feeding crustaceans which get their name from their jerky movements through water. They are found in ponds and lakes throughout the temperate and tropical areas of the world. Their size ranges from 0.2-6 mm. The body is laterally compressed and is almost entirely enclosed in a large bivalved carapace (Plate 3). The thoracic setose legs help in filtering suspended matter from water to the mouth. *Daphnia* are an important link in food chains in water bodies.

3. *Cyprinus carpio communis* Linn.

Phylum - Chordata

Class - Pisces

Subclass - Actinopterygii

Order - Cypriniformes

Family - Cyprinidae

The common carp is the native of temperate regions of Asia and is widely distributed all over the world. It is omnivorous, fast growing and a non-predator species. It is a hardy fish that can tolerate wide ranges in pH, temperature and salinity. The carp has a small mouth for bottom feeding (Plate 4). Food is sucked from vegetation or strained and sorted from mouthfuls of inedible sediment by the fish's gill rakers and muscular soft palate. Grown fish eat decaying organic matter, zooplankton, insect larvae, worms, molluscs and also submerged plants.

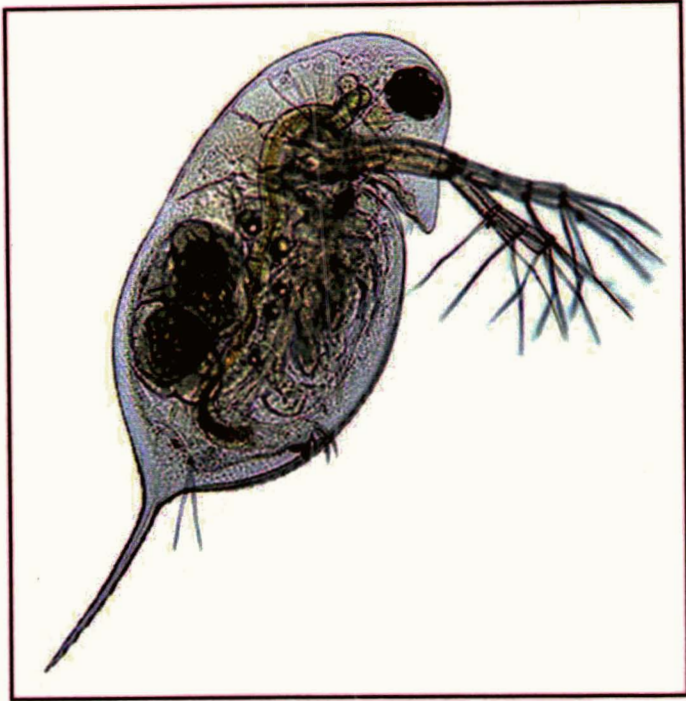


Plate 3 *Daphnia magna* Straus



Plate 4 *Cyprinus carpio* L.

4. *Catla catla* Hamilton

Phylum - Chordata

Class - Pisces

Subclass - Actinopterygii

Order - Cypriniformes

Family - Cyprinidae

One of the three Indian major carps, catla is a surface feeder (Plate 5). It filters the plankton available in surface waters. Its mouth is upturned and gill rakers are specially adapted for filtering specific food organisms from water.

5. *Labeo rohita* Hamilton

Phylum - Chordata

Class - Pisces

Subclass - Actinopterygii

Order - Cypriniformes

Family - Cyprinidae

Rohu is one of the major carps. It is a bottom and column feeder. Major part of the diet is decaying vegetation, phytoplankton and zooplankton. The lower lip of the mouth is fringed. The gill rakers form broad sieve-like structures across the gill slits for filtering and retention of food in the buccopharynx (Plate 6).

6. *Cirrhinus mrigala* Hamilton

Phylum - Chordata

Class - Pisces

Subclass - Actinopterygii

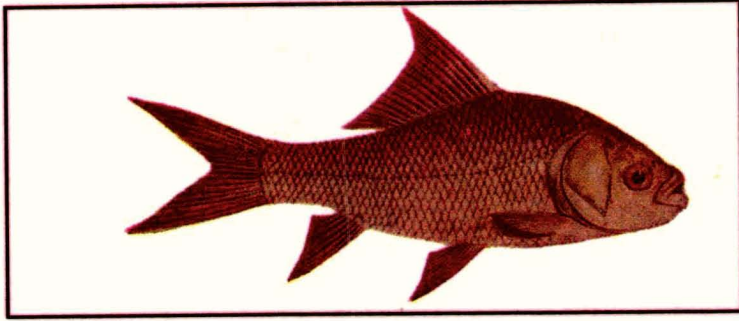


Plate 5 *Catla catla* Hamilton

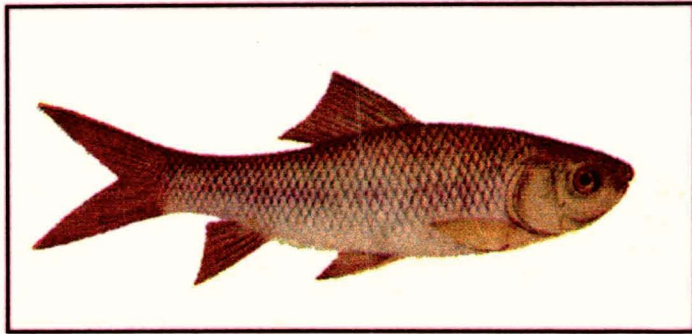


Plate 6 *Labeo rohita* Hamilton

Order - Cypriniformes

Family - Cyprinidae

One of the Indian major carps, mrigal is a bottom feeder. Diet consists mainly of semi-decayed organic matter. It feeds on plant and animal material in the bottom. Mouth is rounded to suit its feeding habit (Plate 7).

7. *Tilapia mossambica* Peters

Phylum - Chordata

Class - Pisces

Subclass - Actinopterygii

Order - Perciformes

Family - Cichlidae

Tilapia are fast growing, hardy, omnivorous fish. It shows parental care. Fry feeds on diatoms and other unicellular planktonic and epiphytic algae. Adults are omnivorous, voracious feeders, feeding on vegetation, worms, insects and detritus (Plate 8).

8. *Lebistes reticulatus* Peters

Phylum- Chordata

Class -Pisces

Subclass-Actinopterygii

Order-Cyprinodontiformes

Family-Poeciliidae

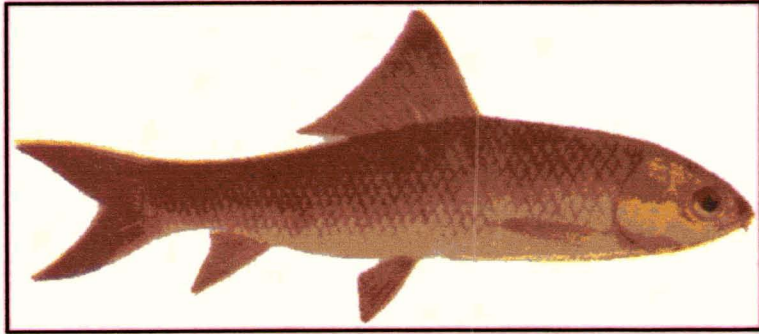


Plate 7 *Cirrhinus mrigala* Hamilton

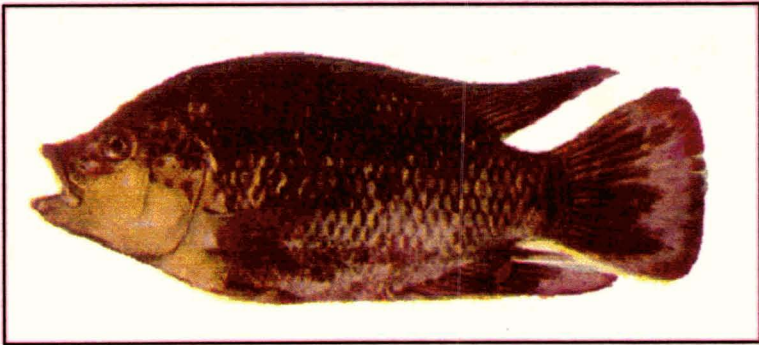


Plate 8 *Tilapia mossambica* Peters

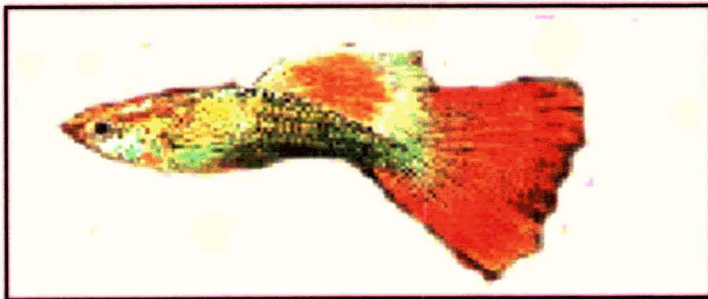


Plate 9 *Lebistes reticulatus* Peters

Guppies are small fishes, attaining a maximum size of 5 cm. They are voracious omnivores. The mouth is upturned to feed on the surface waters. Guppies are live bearers (Plate 9).

2. 6. 2 Aquatic Flora

1. *Azolla rubra* R. Br. (Water Velvet)

Class -Filicopsida

Order- Salviniiales

Family –Azollaceae

Azolla is a floating water fern, triangular in shape, with slender, horizontal rhizome. It has two lobes, upper lobe pubescent with unicellular papillate hairs and cavities which houses a population of symbiotic cyanobacterium, *Anabaena azollae*. *Azolla* float on the water surface individually or in mats (Plate 10). Plant diameter ranges from 1-2.5 cm for small species such as *A. pinnata*, to 15 or more for *A. nilotica*.

2. *Salvinia molesta* Mitchell (Water Fern)

Class - Filicopsida

Order- Salviniiales

Family –Salviniaceae

Salvinia molesta is a free-floating fern with cylindrical, spongy, horizontal rhizome covered with brownish hairs and conspicuous nodes and internodes. Each node bears two unequal, subopposite, folded, aerial leaves with characteristic egg-beater-like hairs on the upper surface. Lateral to the small leaf there is a branch and a submerged organ with a short cylindrical stalk and a terminal tuft of root-

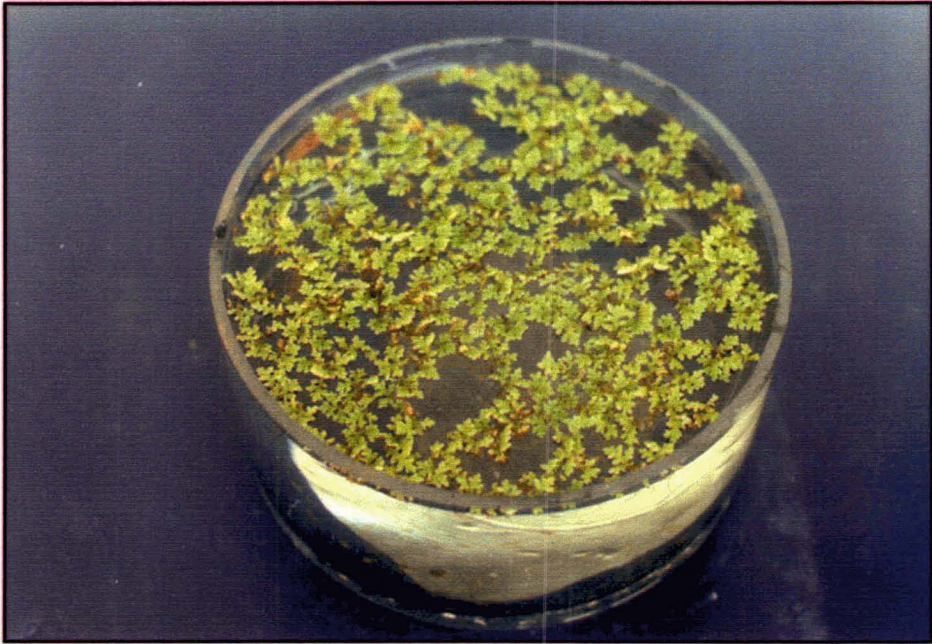


Plate 10 *Azolla rubra* R. Br.

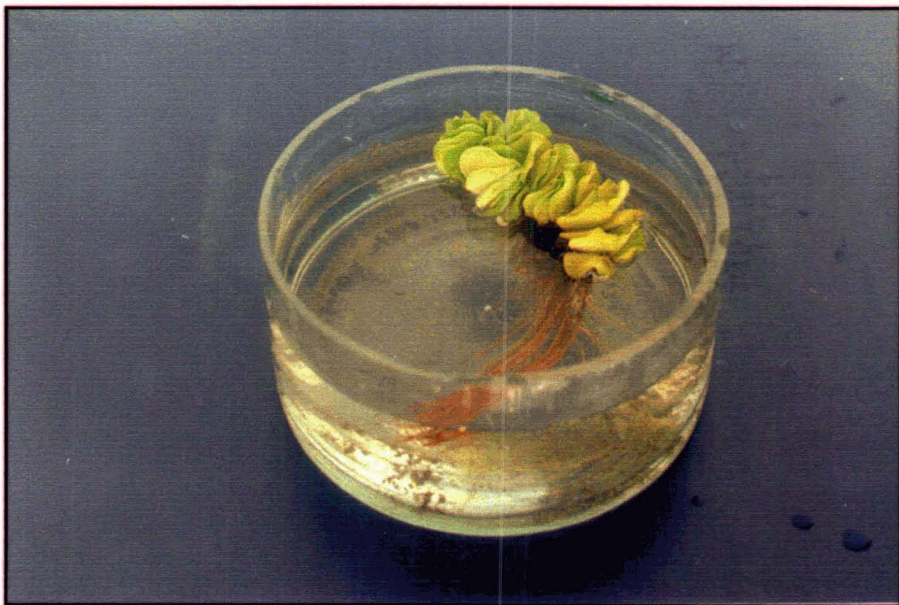


Plate 11 *Salvinia molesta* Mitchell

like appendages (Plate 11). Dominant features of *Salvinia* are its tremendous growth and reproductive rates. Individuals have a size doubling time of 2-4 days (Gaudet, 1973; Mitchell, 1979).

3. *Lemna perpusilla* Torrey (Duckweed)

Class -Monocotyledonae

Order - Arales

Family – Lemnaceae

Lemna are small floating aquatic plants found worldwide, seen growing in thick blanket like mats on still nutrient rich fresh and brackish waters. The plant consists of a flat, ovoid frond and a short root. There is no leaf, stem or specialised structures (Plate 12).

They can double their mass in less than two days under ideal conditions of nutrient availability and temperature. Duckweeds grow well in raw wastewaters.

4. *Spirodela polyrrhiza* (L.) Schleid (Giant Duckweed)

Class -Monocotyledonae

Order - Arales

Family – Lemnaceae

Spirodela are the largest of duckweeds. Fronds are disc shaped, often purplish on the underside with hanging 4-10 long roots each (Plate 13). Turions supplement frond division in the regeneration of the plant.

5. *Wolffia globosa* (Roxb.) Hartog & Plas. (Water Meal)

Class -Monocotyledonae

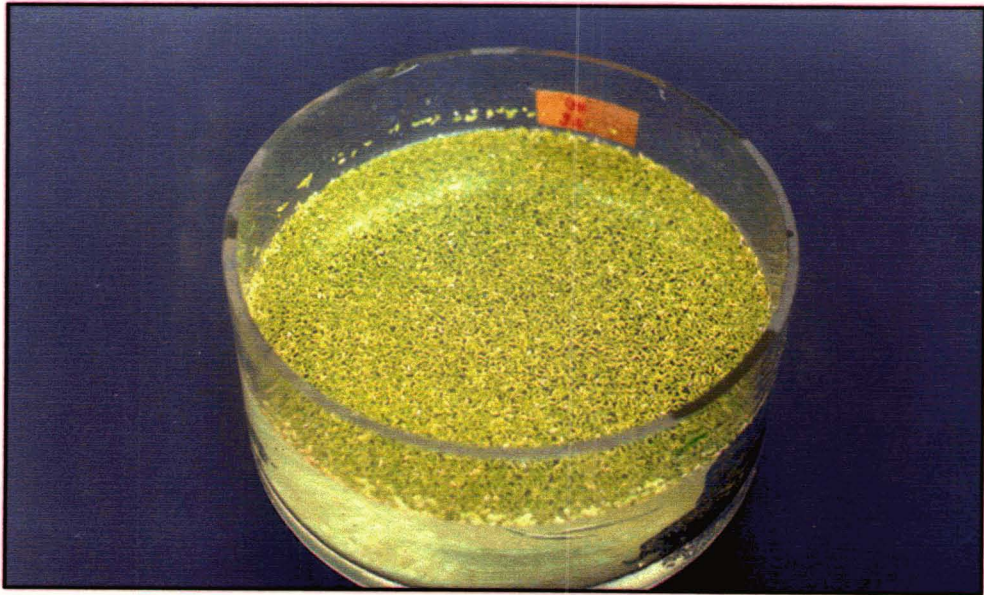


Plate 12 *Lemna perpusilla* Torrey

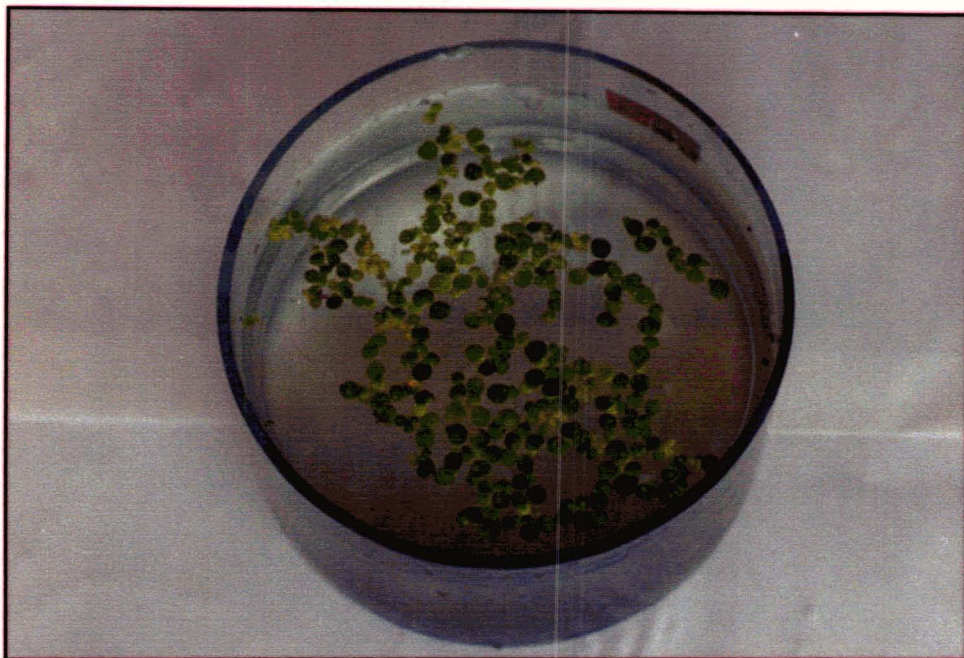


Plate 13 *Spirodela polyrrhiza* (L.) Schleid

Order - Arales

Family – Lemnaceae

Wolffia are one of the smallest flowering plants of the world. They are 1 to 1.5 mm, disc shaped, subglobose like sand grains, rootless, floating proliferous, bearing flowers on the upper surface (Plate 14). The plants form a green carpet on the water surface. It is commonly found in still or sluggish waters often mixed with other duckweeds but sometimes forms a pure stand.

6. *Pistia stratiotes* L. (Water Lettuce)

Class – Monocotyledonae

Order – Arales

Family – Araceae

Free floating perennial plant, with the plant body comprising of a shell like rosette of tongue shaped wide leaves, clustered on the top of a small tuber (Plate 15). Plants extend to form mats by offsets and buds. Buoyancy is provided by air chambers in their leaves and other green parts. Leaves are light green, with parallel ridges, covered in short hairs leaf margins wavy, top margins scalloped. Roots are feathery and numerous, hanging submersed beneath floating leaves.

7. *Hydrilla verticillata* (L. f.) Royle (Water Thyme)

Class – Monocot

Order – Hydrocharitales

Family – Hydrocharitaceae

It is the most dominant submerged weed in India, infesting ponds, lakes, canals and at some places even rivers. Besides shallow waters, it infests deep

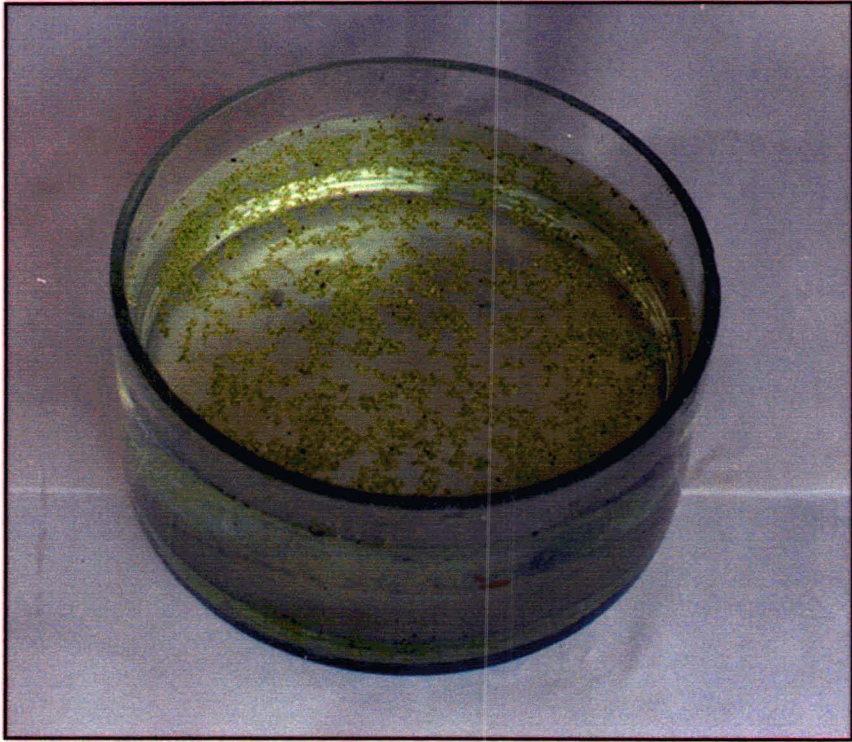


Plate 14 *Wolffia globosa* (Roxb.) Hartog and Plas.



Plate 15 *Pistia stratiotes* L .

waters upto 12 metres. It has branching tender stems, linear, lanceolate leaves and fine filiform adventitious roots. The shoots ramify fast forming dense masses (Plate 16). It has the ability to photosynthesise under light conditions lower than that required by most other submerged plants.

8. *Ceratophyllum demersum* L. (Coontail)

Class – Dicotyledonae

Order - Ranales

Family - Ceratophyllaceae

Ceratophyllum demersum is a perennial, submerged plant. The leaves are dichotomously cleft into filiform minutely toothed lobes, located in whorls at nodes (Plate 17). Propagation occurs by plant fragments and overwintering by terminal turions. It is seen growing in polluted waters also.

9. *Lagenandra toxicaria* Dalz.

Class- Monocotyledonae

Order- Arales

Family – Araceae

Lagenandra is commonly seen along the banks of streams, rivers and ponds. It is an aquatic herb with thick creeping rootstock and large petioled, elliptical-oblong leaves (Plate 18).



Plate 16 *Hydrilla verticillata* (L.f.) Royle

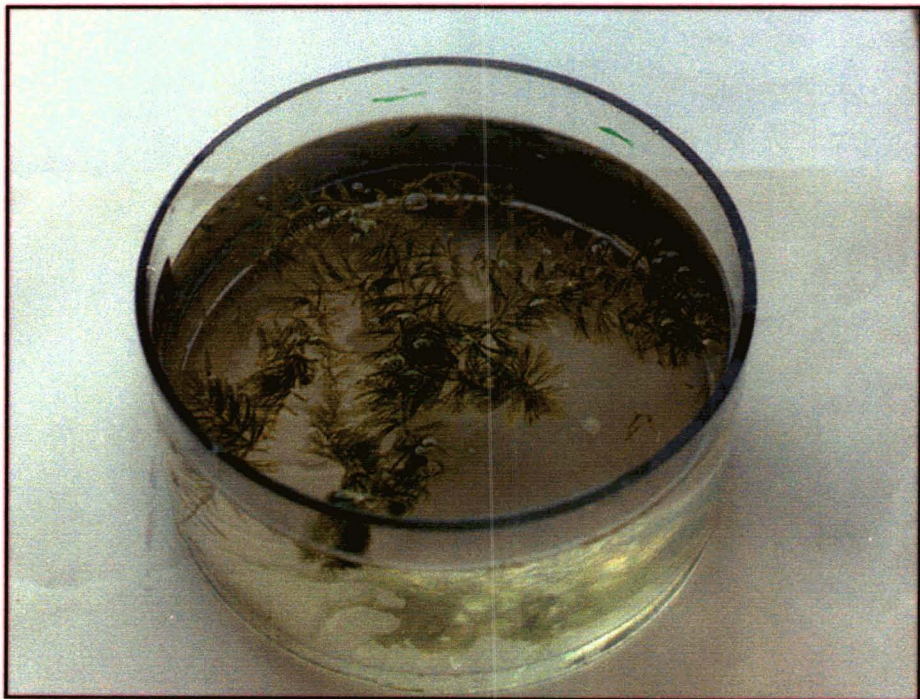


Plate 17 *Ceratophyllum demersum* L.



Plate 18 *Lagenandra toxicaria* Dalz.

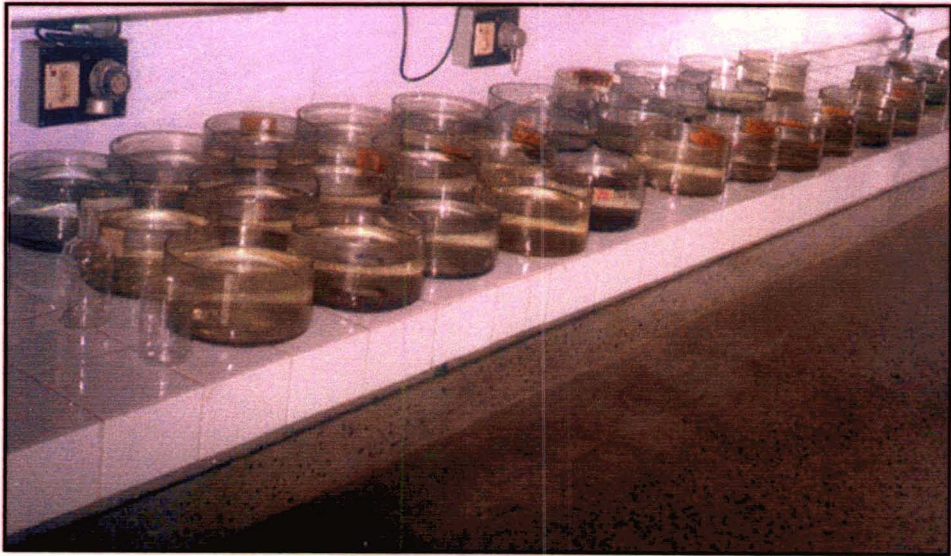


Plate 19 Experimental Setup

3.7 EXPERIMENTAL DESIGN

3.7.1 Wastewater for Experiments

Wastewater from Kozhikode corporation was used for the experiments. The corporation with a population of 4,19,831 has no integrated sewerage system. Wastewater is indiscriminately discharged without any treatment into the sea, Canoly canal and wetlands/ waterlogged areas. The drains receive domestic wastes, hospital and hotel wastes as well as storm runoff.

Wastewater was collected from the drains of the city from four different locations at the same time (grab sample) and then mixed to give an integrated sample of wastewater. This gives the mean characteristics of the sampled wastewater. The integrated sample collected for conducting various experiments was then analyzed for various physico chemical and bacteriological parameters as per APHA (1995). Table 6 gives the range of values for wastewater samples collected during the study.

Table 6 Range of Values for Wastewater

No	Parameter	Range of values
1	Temperature	29-30
2	pH	7- 8
3	Electrical conductivity	619-3150
4	Total hardness	200-250
5	Calcium	50-70
6	Magnesium	15-25
7	Nitrate nitrogen	0.5-2.45
8	Phosphate phosphorous	1.8-2.2
9	Sulphate	12-18
10	Total Suspended Solids	220-300
11	Dissolved Oxygen	0-1.2
12	Biochemical Oxygen Demand	180-300
13	Chemical Oxygen Demand	370-480
14	Total Coliforms -MPN/100 ml	50 x10 ⁸

3. 7. 2 Experimental Set up

Aliquots of the integrated wastewater sample were introduced into dechlorinated tap water to prepare experimental wastewater for treatment with aquatic flora and fauna. 5, 10, 15 and 20 ml of wastewater per litre of water was used to prepare experimental wastewater for the invertebrates, *Paramecium* and *Daphnia*. 10, 20, 30, 40 and 50 ml of wastewater per litre of water was used for fishes. 50, 100, 150, 200 and 250 ml wastewater/l water was used for macrophytes. The experiments were carried out in 2.5 litre capacity troughs filled with 2 litre of experimental wastewater. Controls were maintained for each concentration of experimental wastewater to determine whether the treatment effects due to presence of the test species was significant (Plate 19). The physicochemical and bacteriological characteristics of experimental and control sets were analysed at pre experimental and post experimental stage.

Aquatic Fauna

The selected species of aquatic fauna included *Paramecium caudatum* Ehrenberg, *Daphnia magna* Straus among invertebrates. Fishes selected for the experiments were *Cyprinus carpio* L, *Tilapia mossambica* Peters, *Catla catla* Hamilton, *Cirrhinus mrigala* Hamilton, *Labeo rohita* Hamilton, and *Lebistis reticulates* Peters. Two litres of experimental wastewater was taken in glass troughs and the test species were introduced into them after ascertaining their number/weight. *Paramecium* was enumerated using haemocytometer and *Daphnia* by direct counting. The wet weight of fishes was taken. The test organisms were retained in the experimental set up for a period of 7 days.

Paramecium was introduced at 20 numbers/ml and *Daphnia* was introduced at 3 different numbers - 10, 20 and 30/100 ml of experimental wastewater. Fishes were experimented with two varying biomass 3g/l and 4g/l in each wastewater experimental set. After the treatment period, the organisms were removed and water from the troughs analysed for various water quality parameters.

Aquatic Flora

The aquatic flora selected as test species for experiments in this study include the two ferns *Azolla rubra* R.Br. and *Salvinia molesta* Mitchell; the three duckweeds - *Lemna perpusilla* Torrey, *Spirodela polyrrhiza* (L.) Schleid and *Wolffia globosa* (Roxb.) Hartog and Plas. as well as *Pistia stratiotes* L., *Hydrilla verticillata* (L.f.) Royle, *Ceratophyllum demersum* L. and *Lagenandra toxicaria* Dalz. Experimental water was prepared using 50, 100, 150, 200 and 250 ml wastewater/l of dechlorinated tap water. Physicochemical and bacteriological characteristics of the experimental wastewater were analysed at pre and post experimental stage. Two litres of experimental wastewater was taken in 2.5 l capacity troughs. The plants were blotted and weighed before introducing them into the troughs. 2 gm/l of *Azolla*, *Lemna* and *Spirodela* were taken while 5 gm/l of *Pistia*, *Salvinia*, *Ceratophyllum* and *Hydrilla* were used for the experiments. The detention period for all the plants was 7 days, after which the plants were removed and water analysed for various physicochemical and bacteriological parameters. Test procedures for experiments with aquatic flora and fauna are summarized in Table 7.

Table 7 Summary of Test Procedures for Aquatic Fauna and Flora

No	Test Organism	Biomass or Number	Treatment Period	Sewage Dilutions
Aquatic Fauna				
<u>Invertebrates</u>				
1	<i>Paramecium caudatum</i> Ehrenberg	20/ml	7	5-20 ml/l
2	<i>Daphnia magna</i> Straus	10,20 &30/ml	7	5-20 ml/l
<u>Vertebrates</u>				
3	<i>Cirrhinus mrigala</i> Hamilton	3,4 g/l	7	10-50 ml/l
4	<i>Labeo rohita</i> Hamilton	3,4 g/l	7	10-50 ml/l
5	<i>Catla catla</i> Hamilton	3,4 g/l	7	10-50 ml/l
6	<i>Cyprinus carpio</i> L.	3,4 g/l	7	10-50 ml/l
7	<i>Tilapia mossambica</i> Peters	3,4 g/l	7	10-50 ml/l
8	<i>Lebistes reticulatus</i> Peters	3,4 g/l	7	10-50 ml/l
Aquatic Flora				
9	<i>Azolla rubra</i> R. Br.	2 g/l	7	50-250 ml/l
10	<i>Salvinia molesta</i> Mitchell	5 g/l	7	50-250 ml/l
11	<i>Lemna perpusilla</i> Torrey	2 g/l	7	50-250 ml/l
12	<i>Spirodela polyrrhiza</i> (L.) Schleid	2 g/l	7	50-250 ml/l
13	<i>Wolffia globosa</i> (Roxb.) Hartog & Plas.	2 g/l	7	50-250 ml/l
14	<i>Pistia stratiotes</i> L.	5 g/l	7	50-250 ml/l
15	<i>Hydrilla verticillata</i> (L. f.) Royle	5 g/l	7	50-250 ml/l
16	<i>Ceratophyllum demersum</i> L.	5 g/l	7	50-250 ml/l
17	<i>Lagenandra toxicaria</i> Dalz.	60 g/l	7	50-250 ml/l

3. 8 STATISTICAL ANALYSIS

The data generated from the experiments were subjected to statistical analysis with SPSS software. The mean and 95 % confidence intervals were estimated in respect of various water quality parameters.

The data were classified in the following hierarchy: (i) dilution, (ii) before and after experiments and (iii) with or without test species. Analysis of variance (ANOVA) was performed in relation to the hierarchy of data (Snedecor and Cochran, 1989). Besides main effects, two-way interactions also were included in the analysis. However, three way interactions was not included as preliminary analysis indicated that it did not lead to meaningful interpretations.

The functional relations of different water quality parameters were assessed by fitting appropriate polynomial regression equations with dilution as independent variable.

3. 9 PHYTOREMEDIATION OF METALS

3. 9. 1 Experiments with Macrophyte Species

Studies were conducted to determine the relative ability of a few species of macrophytes to remove metals from spiked solutions. Stock solutions for different metals were prepared by dissolving required amount of potassium dichromate, manganous sulphate, ferrous ammonium sulphate, copper and zinc metals. From these stock solutions desired concentrations (0.25-10 mg/l) of metal solutions were prepared, using dechlorinated tap water after ascertaining that the metals under study were below detectable limits.

All the plants were collected, cultured and acclimatised as for experiments with wastewater. Healthy plants were selected, washed thoroughly, blotted, weighed and placed in individual metal solutions of copper, chromium, manganese, zinc and iron. The metal solutions of 0.25-10 mg/l were taken in glass containers since adsorption of the metals per unit area on glass container was found to be minimum in earlier studies (Ingole and Bhole, 2000). Macrophytes such as *Pistia*, *Hydrilla*, *Ceratophyllum* and *Salvinia* were weighed (wet weight after blotting) and introduced at 5 grams per litre while smaller plants such as *Azolla*, *Lemna* and *Spirodela* were introduced at 2 grams per litre. The treatment period was seven days and analysed for metals using Atomic Absorption Spectrophotometer. Table 8 represents the details of metal uptake studies with macrophytes.

Table 8 Metal Removal Studies with Aquatic Macrophytes

No	Scientific Name	Weight (g)	Period of Treatment	Metal (mg/l)
1	<i>Azolla rubra</i> R.Br.	2	7	Copper , manganese, iron, chromium, zinc
2	<i>Salvinia molesta</i> Mitchell	5	7	Copper , manganese, iron, chromium, zinc
3	<i>Lemna perpusilla</i> Torrey	2	7	Copper, manganese, iron, chromium, zinc
4	<i>Spirodela polyrrhiza</i> Schleid	2	7	Manganese, iron, chromium, zinc
5	<i>Wolffia globosa</i> (Roxb.) Hartog and Plas.	2	7	Manganese, iron, zinc
6	<i>Pistia stratiotes</i> L.	5	7	Copper, manganese, iron, chromium, zinc
7	<i>Hydrilla verticillata</i> (L.f.) Royle	5	7	Copper, manganese, iron, chromium, zinc
8	<i>Ceratophyllum demersum</i> L.	5	7	Copper, manganese, iron, chromium, zinc

3. 9. 2 Experiments with Dried Plants

The plants used for removal of nutrients and improving water quality of wastewater experiments showed prolific growth. The effective utilization of plants used in such treatment systems include their use as manure, mulching and compost.

In this study, alternate uses were explored. Column studies were carried out with dried samples of *Azolla*, *Hydrilla*, *Salvinia* and *Lemna* harvested from the experimental sets with wastewater to test its adsorption/retention capacity for removal of metals. The plant material was dried at 60⁰ C, ground and sieved. A column of 2 cm diameter and holding capacity of 60 ml was filled with 0.5 g dried plant. The flow rate was adjusted to 5 ml/min. Zinc, manganese and copper solution of 20 mg/l was prepared from stock solution. Metal solution in the column was maintained at 50 ml level. The eluted samples were collected at intervals of 15 minutes and analysed using Atomic Absorption Spectrometer.

RESULTS AND DISCUSSION

Shiny K J “Use of aquatic organisms for water treatment” Thesis. Department of Life Science, University of Calicut, 2003

4. 0 RESULTS AND DISCUSSION

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4. 1 TREATMENT STUDIES WITH AQUATIC FAUNA

4.0 RESULTS AND DISCUSSION

4.1 TREATMENT STUDIES USING AQUATIC FAUNA

4.1.1 General

An array of aquatic animals such as invertebrates, molluscs and fishes are increasingly being used in biological wastewater treatment systems. In the present study, experiments were conducted using selected invertebrates and vertebrates to determine their ability to remediate experimental wastewaters. The invertebrates experimented include *Paramecium caudatum* Ehrenberg, and *Daphnia magna* Straus. The vertebrates include six fish species - *Tilapia mossambica* Peters, *Cyprinus carpio* L., *Catla catla* Hamilton, *Labeo rohita* Hamilton, *Cirrhinus mrigala* Hamilton and *Lebistes reticulatus* Peters.

The role of protozoans in wastewater treatment has been recognised only in the recent years, although their presence was known since long. *Paramecium* is a common protozoan, whose filter feeding habit is a major contribution to wastewater treatment.

Daphnia are zooplanktonic cladocerans, which aid in biorecycling by consumption of algae, protozoa, bacteria and suspended organic matter. Stabilisation pond effluent with *Daphnia* at high population level were found to be macroscopically clear and contained few coliform bacteria (Dinges, 1976 b). The filtering efficiency of setae of thoracic appendages is mainly responsible for this (Plate 20).

The main features of growing fish in nutrient rich waters are high fish production potential and low maintenance costs. Improvement of the water quality has not been conclusively determined. Some authors suggest that it may help to

improve the efficiency of water treatment systems (Schroeder, 1975) but according to others (Anderson *et al.* 1978) fish may increase the process of eutrophication, leading to a reduction in water quality.

The materials present in wastewater can either be consumed directly by the fish, or be utilised by bacteria and protozoans, which in turn form food for fish. Direct consumption of materials could be possible at the fairly diluted water, where concentration of wastes does not interfere with fish life.

Certain fishes and some fishes at its juvenile stage are adapted to remove even small phytoplankton (less than 20 μm). Mucous secretion from the gill surface and other specialised structures appears to entrap food particles and the gill rakers prevent the resultant slime from escaping in the outward current (Colman and Edwards, 1985).

The anatomy of gills aid in filtering water effectively, more apparently in filter feeding fishes. The gills bear gill lamellae and gill rakers, of which the latter project into the pharyngeal cavity and are arranged in 2-3 rows (Plate 21). In carnivorous species, gill rakers are long, hard and teeth like forming rasping organs. The individual filaments of gill rakers are situated very close in filter feeders and herbivores like *Labeo rohita*, *Cirrhinus mrigala* and *Tilapia* and can remove small sized particles from water (Khanna, 1993). The filtering efficiency increases considerably from carnivorous to herbivorous fishes.

Experiments were conducted to evaluate the ability of selected aquatic flora and fauna for improvement of water quality of polluted waters. The relationship between biomass/number of test species and removal of pollutants was also studied. Experimental sets with various test organisms were maintained

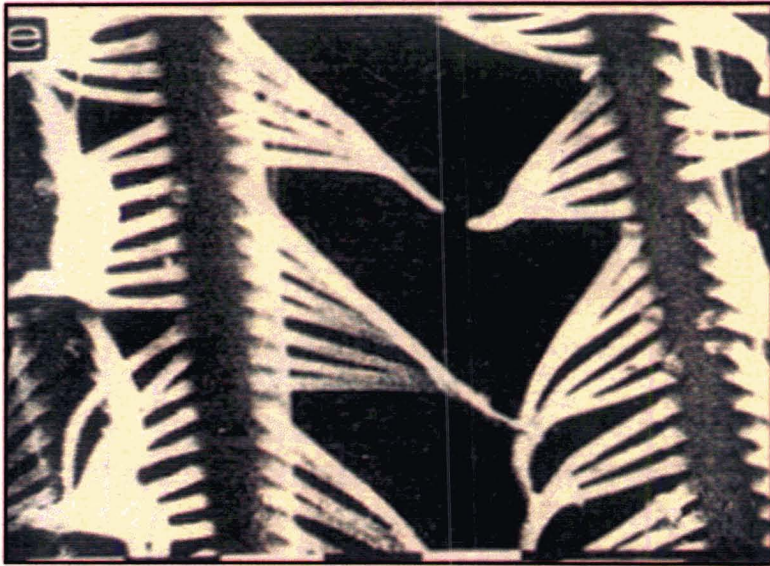


Plate 20 Interstitial spacing on setae of limb of *Daphnia*

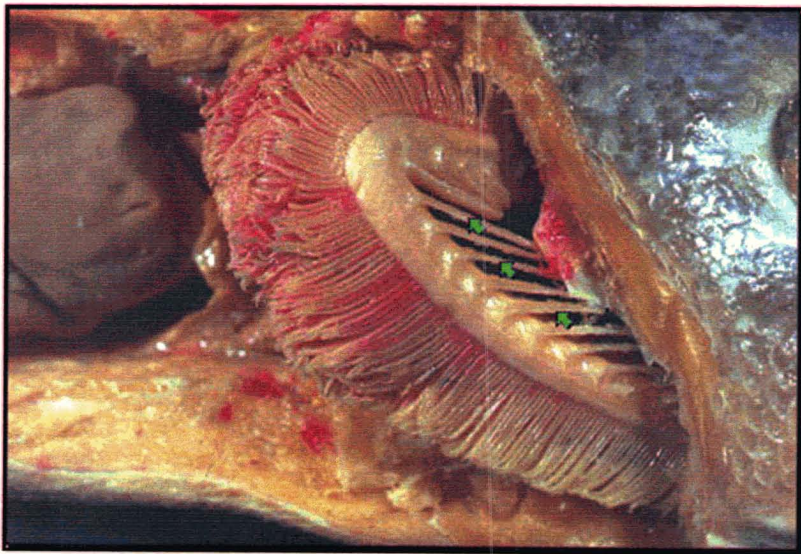


Plate 21 Gill Rakers of a Fish

for a fixed period with different stocking rates in various concentrations of wastewater. Analysis of the physicochemical and bacteriological parameters were carried out at pre experimental stage and post experimental stage. The parameters studied include temperature, pH, electrical conductivity, total suspended solids, nitrate nitrogen, sulphate, phosphate phosphorous, dissolved oxygen, biological oxygen demand, chemical oxygen demand, hardness, calcium, magnesium, total coliforms, fecal coliforms and *E.coli*.

4. 1. 2 Invertebrates

1. *Paramecium caudatum* Ehrenberg

In the experiments conducted using *Paramecium* as test organism (20 numbers/ ml) for a treatment period of seven days, considerable extent of removal of pollutants was observed. The physicochemical and bacteriological characteristics of test waters are represented in Table 9. Among the parameters studied, considerable reduction was observed in BOD, COD, TSS and coliforms.

BOD removal was maximum (24.07 percent) in the experimental set with least wastewater (5 ml/l) when compared to its control (3.88 percent). With increase in the wastewater concentration (10, 15 and 20 ml/l), the reduction in BOD showed a decreasing trend at 6.45, 8.82 and 6.94 percent. The maximum reduction in BOD in control sets was only 3.8 percent. Fig.1 represents the percentage reduction in BOD in *Paramecium* treated experimental sets for seven days.

COD showed reduction of 34, 36.8, 33 and 30.3 percent in 5-20 ml/l experimental sets. This was comparatively greater than controls for the same

Table 9 Experimental Sets Treated with *Paramecium caudatum* Ehrenberg

Period of Treatment -7 days
Number -20/ml

E- Experiment
C- Control

Parameters	5ml Wastewater/l			10 ml Wastewater/l			15 ml Wastewater/l			20 ml Wastewater/l		
	Before	After		Before	After		Before	After		Before	After	
		E	C		E	C		E	C		E	C
Temp	27.30	26.20	26.50	27.00	26.50	26.20	27.40	26.50	26.30	27.50	26.70	26.50
pH	6.35	5.40	6.12	7.65	6.62	6.69	8.20	6.45	7.93	8.91	7.20	8.30
EC	63.00	55.00	59.50	68.20	64.2	67.80	71.50	65.20	70.50	73.90	69.40	72.60
TSS	45.00	5.00	40.00	59.00	11.80	52.00	67.00	17.42	61.00	78.00	27.30	71.00
DO	6.23	6.18	6.20	6.15	6.11	6.13	5.92	5.85	5.89	5.67	5.60	5.65
BOD	5.40	4.10	5.19	6.20	5.80	6.20	6.80	6.20	6.80	7.20	6.70	7.12
COD	14.21	9.38	13.50	11.69	7.39	10.20	12.59	8.44	11.63	14.40	10.04	13.41
NO ₃ -N	0.90	0.40	0.87	1.50	1.20	1.47	1.90	1.60	1.83	2.30	2.00	2.30
PO ₄ -P	0.004	ND	0.003	0.007	ND	0.005	0.010	0.006	0.009	0.030	0.016	0.026
SO ₄	0.43	0.32	0.39	0.49	0.39	0.41	0.51	0.46	0.50	0.94	0.83	0.92
TH	8.00	6.00	8.00	16.00	12.00	14.00	22.00	18.00	20.00	28.00	21.00	26.00
Ca	1.60	0.70	1.30	4.10	2.40	3.20	5.60	3.30	4.00	9.80	4.90	8.50
Mg	0.98	0.72	0.86	1.48	1.40	1.45	1.95	1.91	1.92	2.00	1.95	1.97
T.C	2400	1100	2400	2400	920	2400	2400	920	2400	2400	1100	2400
F.C	+ ve	-ve	+ ve	+ ve	-ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
E.Coli	+ ve	-ve	+ ve	+ ve	-ve	+ ve	+ ve	-ve	+ ve	+ ve	-ve	+ ve

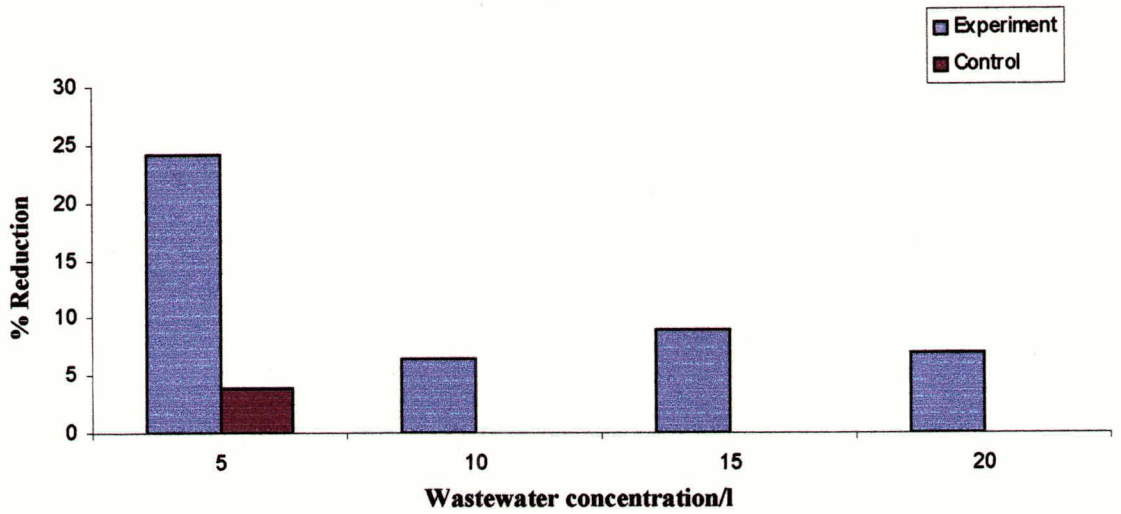


Fig.1 Percentage Reduction of BOD in Experimental Sets Treated with *Paramecium caudatum* Ehrenberg 20 Nos./ml

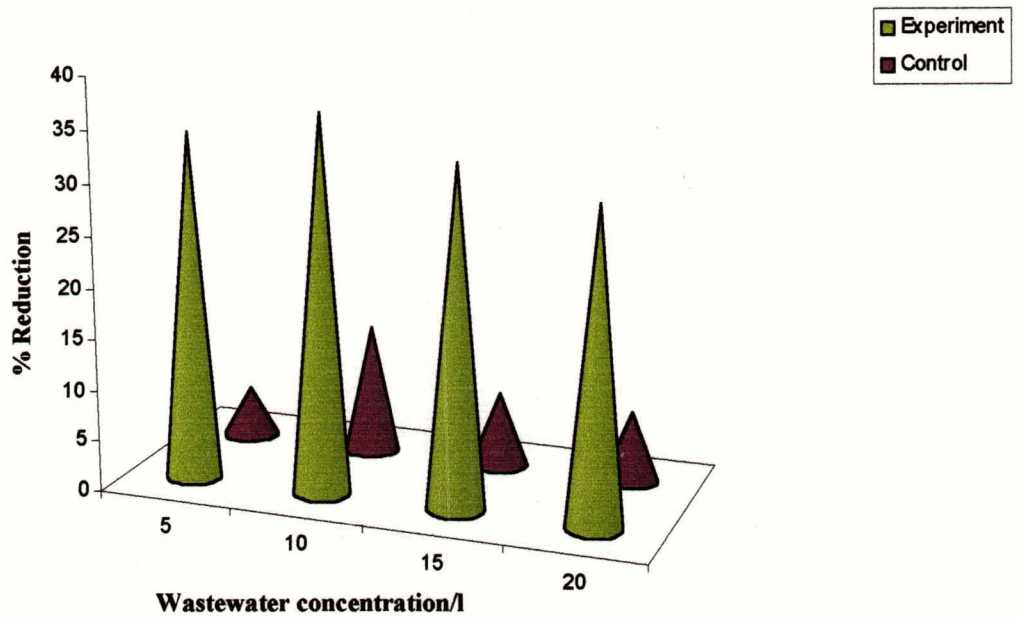


Fig. 2 Percentage Reduction of COD in Experimental Sets Treated with *Paramecium caudatum* Ehrenberg 20 Nos./ml

experiment - 4.9, 12.74, 7.62 and 6.87 percent. Fig. 2 represents the reduction in COD.

Reduction in total suspended solids as high as 88 percent was observed in 5 ml/l wastewater experimental sets. This was significant since its control had only 11.1 percent reduction. Higher wastewater concentrations showed 80, 74 and 65 percent reduction in TSS. The controls for 10-20 ml wastewater showed maximum of 11.86 percent reduction. Fig.3 represents the reduction in TSS obtained using *Paramecium*.

The maximum reduction in total coliforms in the experiments was 61.66 percent in 10 and 15 ml wastewater experimental sets, while 5 and 20 ml sets had 54.16 percent reduction. The control for these experiments had no reduction in total coliforms. Fig.4 represents the reduction in coliforms obtained using *Paramecium*. Faecal coliforms and *E.coli* were absent in the experimental sets treated with *Paramecium* in 5 and 10 ml/l wastewater.

Electrical conductivity, pH and dissolved oxygen showed a slight decrease in all the concentrations of experimental wastewaters. The nutrients, nitrate and phosphate showed decrease when compared to controls. Fifty five percent reduction in nitrate was observed in 5 ml /l wastewater experimental sets, while its control had only 3.3 percent reduction. Higher wastewater concentrations gave reductions of 13.3, 15.78 and 1.3 percent when compared to their respective controls which had 2, 3.68 percent and nil reduction.

Phosphate was below detectable level in experiments with 5 ml wastewater/l. In higher wastewater experimental sets (15 and 20 ml wastewater/l),

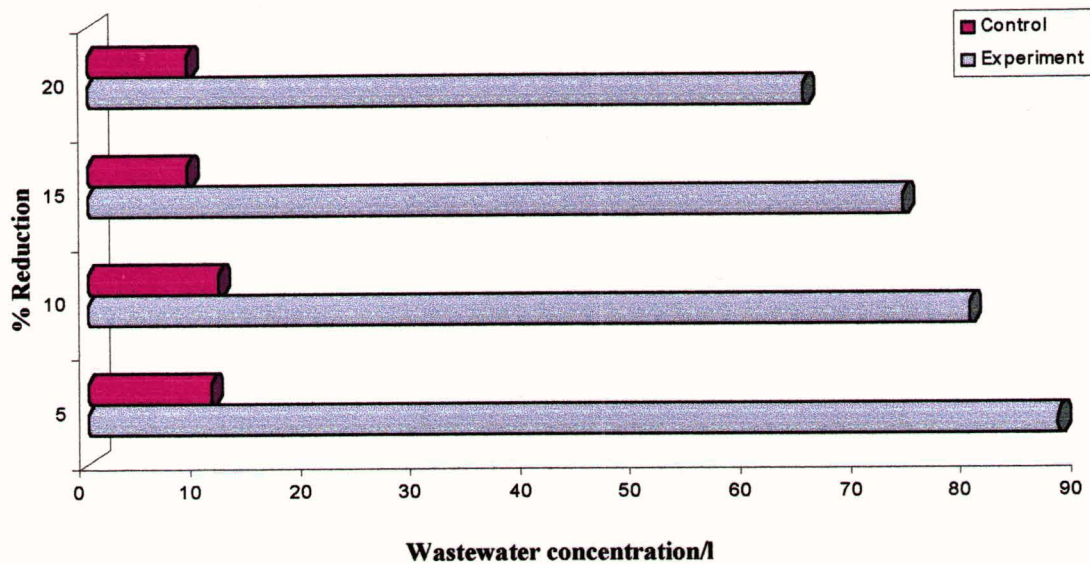


Fig. 3 Percentage Reduction of TSS in Experimental Sets Treated with *Paramecium caudatum* Ehrenberg 20 Nos./ml

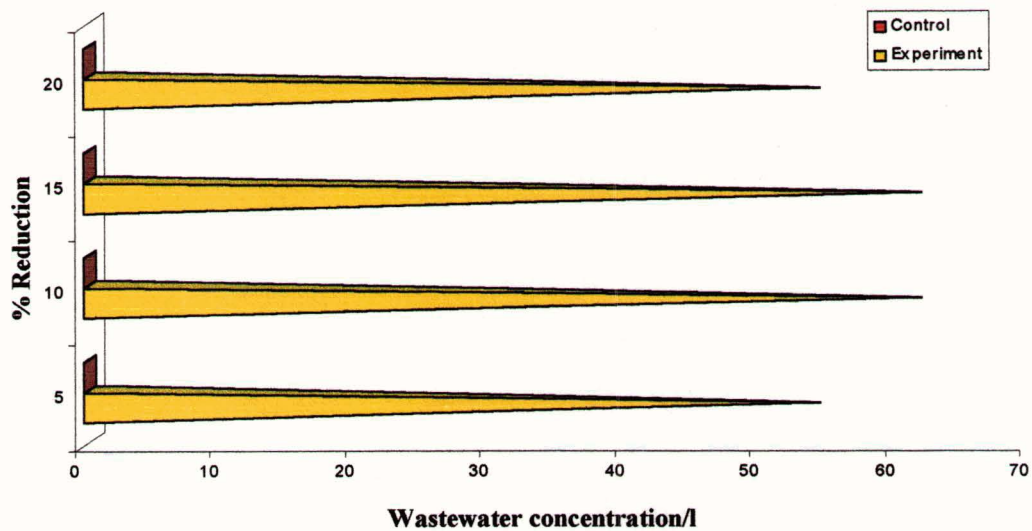


Fig. 4 Percentage Reduction of Coliforms in Experimental Sets Treated with *Paramecium caudatum* Ehrenberg 20 Nos./ml

40 and 47.3 percent reduction was observed compared to 10 and 13.3 percent in the controls.

Sulphate showed very slight reduction of 25.58 percent with 5 ml experimental wastewater. Percentage reduction in sulphate decreased (22.4, 9.8 and 11.7 percent) in higher wastewater concentrations of 10, 15 and 20 ml/l.

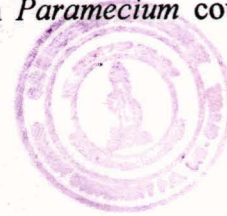
Hardness of experimental wastewater decreased by 25 percent in 5, 10 and 20 ml wastewater and 18 percent in 15 ml wastewater experimental set. Calcium and magnesium also reduced considerably corresponding with decrease in hardness. Hardness in control sets showed a maximum reduction of 18.18 percent only.

The role of *Paramecium* in reduction of various water quality parameters such as BOD, COD, TSS and coliforms could be attributed to its feeding habit. *Paramecium* is a heterotrophic filter feeding protozoan. It feeds chiefly on bacteria, organic particles and detritus, which are swept down the peristomial groove to the cytostome by the action of cilia.

Curds (1975) found that in the case of *Paramecium caudatum*, two mechanisms were responsible for clarification of effluent and sludge formation (Hawkes, 1960). The ciliate was able to secrete a soluble polysaccharide (a polymer of glucose and arabinose) into the medium, which changed the surface charge of the suspended colloidal particles present. Secondly, particles ingested during cyclosis were glued together by a mucin. The soluble organic compounds and colloidal materials present in wastewater are used by the protozoan for its nutrition. The reductions in TSS and other parameters could be attributed to this.

The results of the present study are in agreement with the observations of Curds *et al.* (1968).

Protozoa are known to feed on bacteria, including pathogenic strains causing diphtheria, cholera, typhus and streptococcal infections as well as faecal bacteria such as *E. coli* (Curds, 1975). Relatively lower total coliform counts and absence of *E. coli* in the experimental sets treated with *Paramecium* could be attributed to this.



2. *Daphnia magna* Straus

Experiments were conducted using *Daphnia magna* at three varying numbers 10, 20 and 30/100 ml of experimental wastewater for a treatment period of seven days. The water quality characteristics at pre and post experimental stages are presented in Tables 10 to 12. The results indicate that *Daphnia* was capable of excellent removal of coliform bacteria (98 percent) and suspended solids (94 percent). Reduction in BOD and COD were also appreciable. Maximum reduction was observed in the above mentioned parameters in the treatments with 30 numbers of *Daphnia*/ 100 ml when compared to 20 and 10 numbers/100 ml.

98.25 percent reduction in total coliforms count was recorded when 30 *Daphnia*/100 ml experimental wastewater was introduced in 5 ml/l wastewater experimental set. Control for this had only 37.18 percent removal. In 10, 15 and 20 ml wastewater concentrations, 86.64, 43.67 and 66.38 percent reduction in total coliforms was observed, which was significantly greater than their respective controls 16.66, 15.21 and 14.66 percent (Fig. 5).

Table 10 Experimental Sets Treated with *Daphnia magna* Straus

Period of Treatment-7 days
Number-10/100ml

E- Experiment
C- Control

Parameters	5 ml Wastewater/l			10 ml Wastewater/l			15 ml Wastewater/l			20 ml Wastewater/l		
	Before	After		Before	After		Before	After		Before	After	
		E	C		E	C		E	C		E	C
Temp	29.30	29.00	29.10	29.50	29.30	29.40	29.60	28.90	29.10	29.60	29.40	29.50
pH	7.35	7.42	7.23	7.23	7.52	7.72	7.30	7.05	6.92	7.42	7.15	7.02
EC	85.30	82.10	85.10	87.60	85.20	86.90	98.50	96.60	98.00	107.10	105.30	106.00
TSS	54.00	4.86	52.00	63.00	8.19	60.00	72.00	14.40	69.00	83.00	23.24	79.00
DO	6.25	6.21	6.10	6.13	6.10	6.12	5.94	5.89	5.92	5.25	5.13	5.21
BOD	6.07	4.60	5.30	6.14	4.76	5.20	6.36	4.87	5.24	6.50	5.24	5.34
COD	30.35	16.09	29.40	23.61	14.17	22.60	22.71	14.31	20.30	22.41	14.80	20.40
NO ₃ -N	1.20	0.60	1.10	1.83	1.30	1.80	2.30	1.80	2.29	2.80	2.40	2.77
PO ₄ -P	0.007	0.006	0.007	0.02	0.017	0.019	0.050	0.045	0.043	0.080	0.074	0.076
SO ₄	0.62	0.45	0.60	0.87	0.67	0.85	0.91	0.73	0.89	1.20	1.08	1.17
TH	16.00	12.00	16.00	20.00	18.00	20.00	28.00	26.00	26.00	32.00	30.00	30.00
Ca	4.20	2.70	4.00	6.50	4.70	6.00	9.80	7.60	9.50	12.00	9.30	11.70
Mg	2.50	2.27	2.40	2.90	2.40	2.70	3.94	3.44	3.90	4.86	4.81	4.85
T.C	900	409	565	4300	2176	3583	2400	3605	5426	12000	7500	10240
F.C	+ ve	-ve	+ ve	+ ve	-ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
E.Coli	+ ve	-ve	+ ve	+ ve	-ve	+ ve	+ ve	-ve	+ ve	+ ve	+ ve	+ ve

Table 11 Experimental Sets Treated with *Daphnia magna* Straus

Period of Treatment -7 days
Number - 20/100ml

E- Experiment
C- Control

Parameters	5 ml Wastewater/l			10 ml Wastewater/l			15 ml Wastewater/l			20 ml Wastewater/l		
	Before	After		Before	After		Before	After		Before	After	
		E	C		E	C		E	C		E	C
Temp	29.30	29.00	29.10	29.50	29.30	29.40	29.60	28.90	29.10	29.60	29.40	29.50
pH	7.35	7.40	7.23	7.25	7.53	7.72	7.30	7.06	6.92	7.42	7.20	7.02
EC	85.30	81.50	85.10	87.60	83.90	86.90	98.50	95.20	98.00	107.10	104.70	106.00
TSS	54.00	4.32	52.00	63.00	6.93	60.00	72.00	11.52	69.00	83.00	19.09	79.00
DO	6.25	6.18	6.20	6.13	6.09	6.12	5.94	5.76	5.92	5.25	5.08	5.21
BOD	6.28	4.23	5.49	6.59	4.59	5.58	6.67	4.70	5.49	6.90	5.26	5.67
COD	20.93	10.68	19.87	19.38	11.25	19.15	19.05	11.63	18.72	13.80	8.56	12.60
NO ₃ -N	1.20	0.37	1.10	1.83	1.20	1.80	2.30	1.72	2.29	2.80	2.32	2.77
PO ₄ -P	0.007	0.006	0.007	0.02	0.019	0.019	0.050	0.048	0.043	0.080	0.078	0.076
SO ₄	0.62	0.44	0.60	0.87	0.66	0.85	0.91	0.73	0.89	1.20	1.06	1.17
TH	16.00	10.00	16.00	20.00	16.00	20.00	28.00	24.00	26.00	32.00	28.00	30.00
Ca	4.20	2.40	4.00	6.50	4.60	6.00	9.80	7.50	9.50	12.00	9.20	11.70
Mg	2.50	2.25	2.40	2.90	2.50	2.70	3.94	3.85	3.90	4.86	4.77	4.85
T.C	3900	1500	2450	4300	2048	3583	4600	2591	3900	7500	3764	6400
F.C	+ ve	-ve	+ ve	+ ve	-ve	+ ve	+ ve	- ve	+ ve	+ ve	+ ve	+ ve
E.Coli	+ ve	-ve	+ ve	+ ve	-ve	+ ve	+ ve	-ve	+ ve	+ ve	-ve	+ ve

Table 12 Experimental Sets Treated with *Daphnia magna* Straus

Period of Treatment -7 days
Number -30/100 ml

E- Experiment
C- Control

Parameters	5 ml Wastewater/l			10 ml Wastewater/l			15 ml Wastewater/l			20 ml Wastewater/l		
	Before	After		Before	After		Before	After		Before	After	
		E	C		E	C		E	C		E	C
Temp	28.30	28.70	28.70	29.50	29.40	29.60	29.60	29.30	29.50	29.60	29.40	29.50
pH	7.35	7.41	7.23	7.25	7.51	7.72	7.30	7.06	6.92	7.42	7.17	7.02
EC	85.30	81.20	85.10	87.60	83.20	86.90	98.50	95.02	98.00	107.10	104.30	106.00
TSS	54.00	3.24	52.00	63.00	6.30	60.00	72.00	7.92	69.00	83.00	16.60	79.00
DO	6.25	6.17	6.20	6.13	6.01	0.85	0.91	0.71	0.89	1.20	1.03	1.17
BOD	6.18	2.81	5.40	6.33	3.08	6.12	5.94	5.53	5.92	5.25	4.95	5.21
COD	20.60	9.75	19.80	15.07	7.58	5.28	6.54	3.26	5.54	6.95	4.11	5.93
NO ₃ -N	1.20	0.33	1.10	1.83	1.11	1.80	2.30	1.69	2.29	2.80	2.27	2.77
PO ₄ -P	0.007	0.006	0.007	0.02	0.02	0.019	0.05	0.048	0.043	0.080	0.079	0.076
SO ₄	0.62	0.19	0.60	0.87	0.65	0.85	0.91	0.71	0.89	1.20	1.03	1.17
TH	16.00	8.00	16.00	20.00	14.00	6.00	9.80	7.10	9.50	12.00	8.90	11.7
Ca	4.20	2.00	4.00	6.50	4.20	2.70	3.94	3.78	3.90	4.86	4.73	4.85
Mg	2.50	1.88	2.40	2.90	2.52	1.80	2.30	1.69	2.29	2.80	2.27	2.77
T.C	1100	19	691	2300	353	1916	6400	3605	5426	7500	2521	6400
F.C	+ ve	-ve	+ ve	+ ve	-ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
E.Coli	+ ve	-ve	+ ve	+ ve	-ve	+ ve	+ ve	-ve	+ ve	+ ve	-ve	+ ve

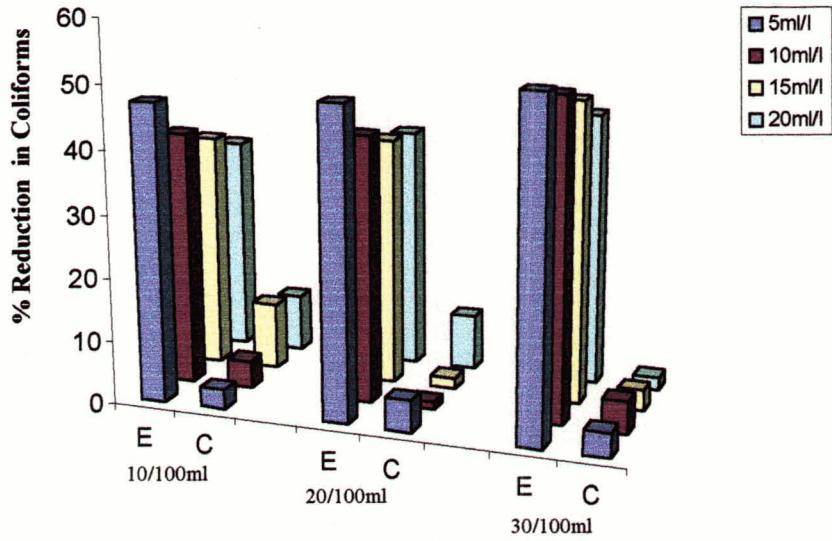


Fig. 5 Percentage Reduction of Coliforms in Experimental Sets Treated with *Daphnia magna* Straus

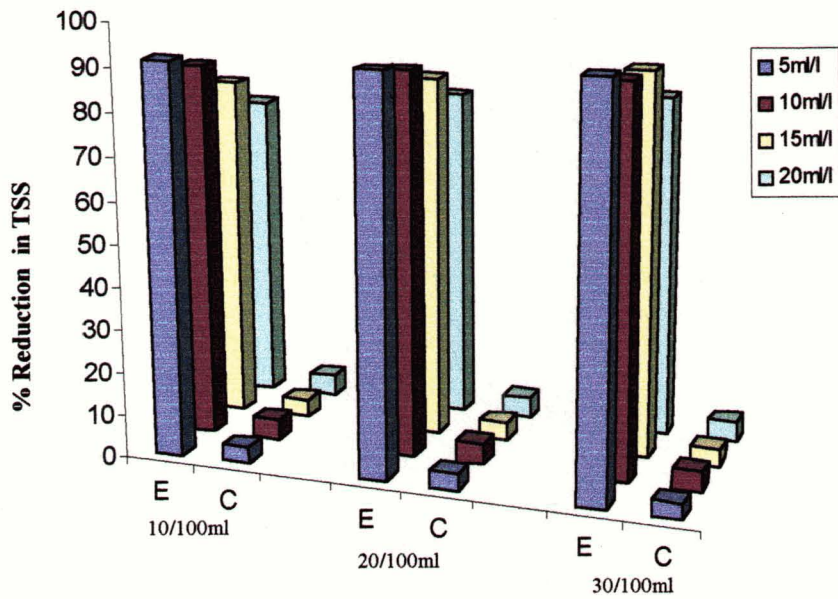


Fig. 6 Percentage Reduction of TSS in Experimental Sets Treated with *Daphnia magna* Straus

Daphnia at 20 numbers/100 ml showed 61.63, 43.67 and 49.81 percent reduction in total coliforms, in the four wastewater experimental sets. When *Daphnia* at 10 numbers/100 ml were introduced in similar experimental wastewater, 54.52, 49.39, 43.67 and 37.54 percent reduction in total coliforms was observed.

Total suspended solids in the experiments with 5 ml wastewater and *Daphnia* at 30 numbers/100 ml showed attenuation of 94 percent, while its control had only 3.7 percent reduction, implying that this could be due to the presence of the organism. In higher wastewater concentrations, 90, 89 and 80 percent reduction in TSS was recorded (Fig. 6). Controls had 4.76, 4.16 and 4.81 percent removal by natural methods. *Daphnia* at 20 numbers/ 100 ml could reduce TSS by 92, 89, 84 and 77 percent in the four wastewater concentrations experimented. 91, 87, 80 and 72 percent reduction was observed when 10 *Daphnia*/100 ml were used for the experiments.

Daphnia at 30 numbers/100 ml were capable of reducing BOD of the four wastewater experimental sets (5-20 ml wastewater/l) by 54.53, 51.33, 50.13 and 40.86 percent, while corresponding controls had reductions of 12.63, 15.38, 17.61 and 17.89 percent. When 20 numbers/100 ml were used, the BOD reduction was 32.62, 30.46, 29.53 and 23.78 percent in the four experimental sets. 24.26, 22.46, 23.42 and 17.61 percent removal in BOD removal was observed when *Daphnia* were introduced at 10 numbers/100 ml. Thus a decreasing trend in BOD removal was noticed when the number of organisms per ml decreased. Fig.7 represents the percentage removal in BOD.

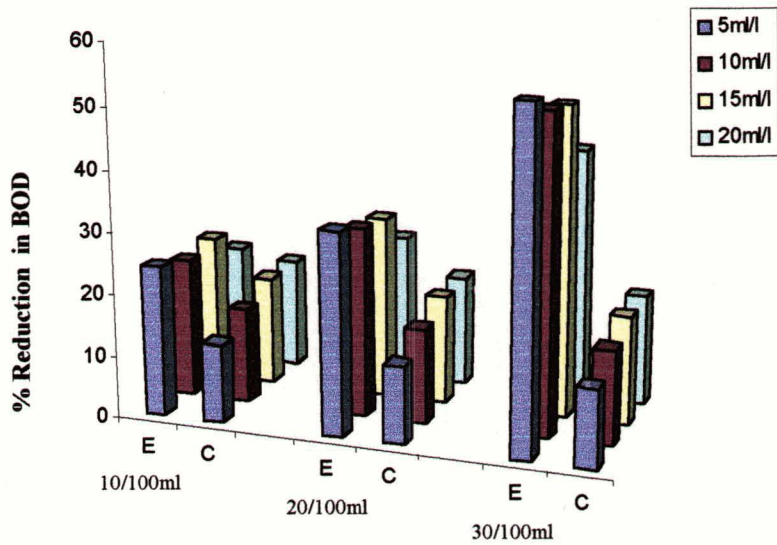


Fig. 7 Percentage Reduction of BOD in Experimental Sets Treated with *Daphnia magna* Straus

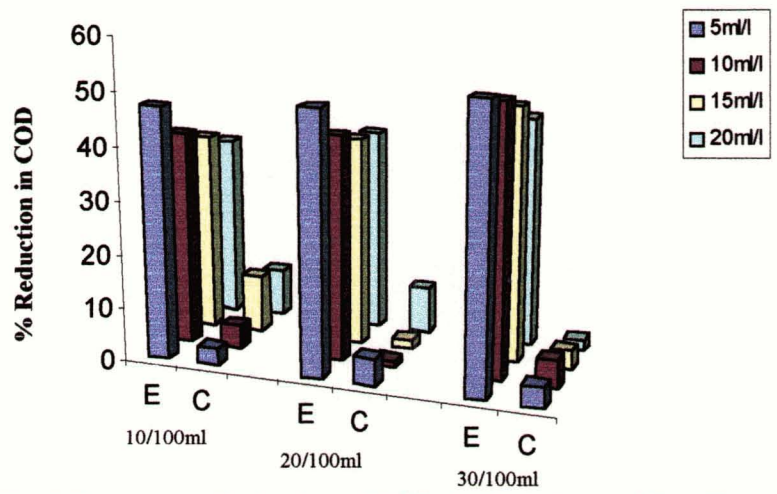


Fig. 8 Percentage Reduction of COD in Experimental Sets Treated with *Daphnia magna* Straus

Removal efficiency of COD by *Daphnia* was a maximum of 52.7 percent when 30 numbers/100ml were used in experimental set with 5ml wastewater/l, its control showed a removal of only 3.88 percent. Higher wastewater concentration showed 50.3, 47.5, and 43.1 percent reduction in COD while the controls had 5, 10, 3.40 and 1.97 percent removal. When 20 numbers /100ml of *Daphnia* were experimented, 49, 42, 39 and 38 percent reduction in COD was observed. Lower reduction were noticed when 10 *Daphnia*/100 ml were used for treatment (47, 40, 37 and 34 percent). Fig. 8 represents the percentage of removal of COD.

The animal creates a current of water by means of rhythmic movements of its appendages, which flows into the chamber formed by the valves of the carapace into the barbs of filtering setae of the third and fourth pair of appendages (Lavens and Sorgeloos, 1996). The distance between the barbs of setae retains food particles as small as 0.5 μm (Loedolff, 1964). The efficiency of the filter allows the uptake of algae, bacteria, detritus and colloidal particles, small particles of food are collected and concentrated in the food groove and then ingested.

Daphnia can regulate their feeding rate and this is in proportion to the concentration of food available (Rigler, 1961 a). When excess food is available, the collection and ingestion of food is regulated and excess food is rejected from the food groove in the form of a bolus. The rate of rejection increases with increasing concentration of food (McMahon and Rigler, 1963). In wastewater treatment systems, this may be a very important process in removing suspended organic and inorganic matter, since excess food is rejected as boluses, which settles, contributing to rapid flocculation. When the food concentration falls below a certain threshold, even detritus and benthic food can be an important food

source. In this case the water current produced by the animals swimming on the bottom whirls up the material, which is eventually ingested (Lavens and Sorgeloos, 1996).

The filtering rate of *Daphnia* is estimated to vary from about 140-130 l/mg dry weight of *Daphnia* per day (Richman, 1958). Lampert and Taylor (1985) observed that *Daphnia* species can handle 5-30 ml per day, which implies that if present in reasonably high numbers may process substantial proportions of their habitat everyday.

pH in the experimental sets treated with *Daphnia* showed a slight increase with respect to controls. Electrical conductivity reduced slightly and this could be attributed to the reduction in suspended solids. Phosphate showed very negligible reduction in all the experimental wastewaters. The cladocerans remove phosphorous principally by feeding on phytoplankton and bacteria, some of it is returned in excretions (Rigler, 1961 b). While there is evidence that *Daphnia* reabsorb a portion of their soluble phosphate excretions through epizootic bacteria, *Daphnia* excrete phosphate to the media four times faster than the uptake rate (Rigler, 1961b). The phosphate excretion rate is highly variable and dependent upon body size, temperature and food level (Peter and Rigler, 1973).

Nitrate in the experimental sets showed a maximum decrease of 72 percent. A maximum of 30 percent reduction in sulphate was observed in 30 numbers/100 ml *Daphnia* treated wastewater sets. In other concentrations, a minimum of 10 percent reduction was recorded.

Hardness removal was a maximum of 50 percent in the treatment with 30 *Daphnia* in 5ml/l wastewater concentration. Other concentrations showed 30, 21.4

and 25 percent reduction in hardness. Control for this experimental set had maximum of 7.14 percent reduction. Samples treated with 20 *Daphnia* had 37.5, 20, 20 and 12.5 percent reduction in hardness. In samples treated with 10 *Daphnia*/100ml, 25, 10, 7.14 and 6.25 percent reduction in hardness was recorded.

Decrease in calcium concentration upto 52.3 percent was recorded in experimental set with least wastewater and maximum organisms per ml (5 ml wastewater and 30 *Daphnia*/100ml), while its control had only 4.76 percent reduction. Higher concentrations of wastewater gave 33.84, 27.55 and 25.83 percent removal when compared to controls, which had negligible reduction of 7.69 percent. When 20 *Daphnia*/100 ml was used in the four wastewater dilutions, reductions of 42.85, 29.23, 23.46 and 23.3 percent was obtained. *Daphnia* make use of calcium in the formation of their chitinous carapace and this could be the reason for the decrease in calcium concentration in the experimental sets.

Magnesium showed a maximum reduction of 24.8 percent in the experimental sets treated with 30 *Daphnia*/ 100 ml (5 ml wastewater/l), while its control set had only 4 percent reduction in magnesium. Other dilutions recorded 13.1, 5.9 and 2.67 percent reductions.

4. 1. 3 Vertebrates

1. *Tilapia mossambica* Peters

Experiments were conducted using *Tilapia* at two varying biomass 3 and 4 g/l. They were introduced in experimental sets with 10, 20, 30, 40 and 50 ml wastewater/ litre for a detention period of seven days. The physico-chemical and

bacteriological characteristics of the water quality parameters studied are presented in Table 13 and 14.

Among the parameters, appreciable removal was recorded in TSS and coliforms followed by BOD and COD when compared to controls. Higher reductions were observed when 4g/l of *Tilapia* were used for the experiments.

Removal efficiencies in TSS up to 91.3 percent were recorded in experimental sets (10 ml wastewater /l) with *Tilapia* at 4g/l (Fig. 9). 90.2 percent reduction was obtained in the same wastewater concentration when 3g/l of fish was used, while control had only 1.77 percent reduction in TSS. The reduction in TSS in this study was higher than that obtained by da Silva *et al.* (2000).

In higher wastewater concentrations, 89.5, 85.87, 83.5 and 75.2 percent reduction in TSS was recorded with 3g/l and slightly better removal was obtained with 4g/l fish for these experiments. 90.6, 87.5, 84.2 and 77.3 percent reduction for 20, 30, 40, and 50 ml in water/ litre was obtained. Control for both biomass had only 2.51, 4.74, 3.57 and 3.38 percent reduction. Studies on selective grazing of fish juveniles on microparticles using maize starch by Matena and Simek (1997) revealed that *Tilapia* was able to consume grains > 4 μ m while silver carp consumed grains > 8 -10 μ m. The reduction in TSS may presumably be attributed to this reason.

Coliform counts were substantially reduced during the treatment period in 10 ml wastewater/ litre experimental set. 82.08 percent reduction in 4g/l and 80.83 percent reduction in 3g/l *Tilapia* treated experimental set was obtained, which is considerably greater than the reduction in control set (12.5 percent).

Table 13 Experimental Sets Treated with *Tilapia mossambica* Peters

Period of Treatment -7 days
Weight - 3 g/l

E- Experiment
C- Control

Parameters	10 ml Wastewater/l			20 ml Wastewater/l			30 ml Wastewater/l			40ml Wastewater/l			50 ml Wastewater/l		
	Before	After		Before	After		Before	After		Before	After		Before	After	
		E	C		E	C		E	C		E	C			
Temp	30.20	29.60	30.00	30.10	29.80	30.50	30.50	31.80	29.90	30.40	29.40	30.20	29.80	29.60	30.20
pH	7.36	7.39	7.40	7.43	7.50	7.40	7.46	7.53	7.42	7.53	7.62	7.47	7.61	7.69	7.57
EC	112.30	118.40	109.10	119.60	126.70	121.60	133.20	137.50	144.50	147.50	156.30	150.20	159.40	165.20	161.20
TSS	118.30	11.59	116.20	123.40	12.96	120.30	139.20	19.90	132.60	145.60	24.02	140.40	162.30	40.25	156.80
DO	6.00	6.70	6.20	5.23	5.81	5.42	5.00	5.72	5.13	4.86	5.02	4.92	4.52	4.80	4.73
BOD	12.60	5.52	10.30	14.30	6.71	13.40	18.70	10.27	17.60	21.90	13.27	19.30	25.80	16.20	22.20
COD	26.25	11.73	24.70	34.05	16.48	32.60	40.65	20.60	38.40	54.75	34.93	51.90	66.15	46.23	63.10
NO ₃ -N	0.96	1.21	0.98	1.23	1.59	1.36	1.64	2.08	1.73	2.06	2.64	2.12	2.52	3.18	2.73
PO ₄ -P	0.08	0.10	0.07	0.10	0.13	0.11	0.12	0.15	0.14	0.15	0.18	0.16	0.18	0.21	0.19
SO ₄	0.80	0.10	0.90	2.20	2.60	2.20	3.60	3.90	3.70	4.80	5.00	4.90	5.20	5.60	5.30
TH	48.00	52.00	56.00	52.00	60.00	66.00	68.00	76.00	74.00	80.00	96.00	88.00	100.00	108.00	104.00
Ca	11.20	6.40	13.06	6.40	17.60	8.12	17.60	19.66	19.30	20.80	27.20	25.60	27.20	43.20	28.20
Mg	4.86	8.70	5.66	8.70	3.88	11.00	5.83	6.51	5.62	6.80	7.77	5.83	8.74	9.72	9.06
T.C	24x10 ⁴	46 x10 ³	21 x10 ⁴	39 x10 ⁴	10 x10 ⁴	23 x10 ⁴	46 x10 ⁴	14 x10 ⁴	43 x10 ⁴	75 x10 ⁴	28 x10 ⁴	64 x10 ⁴	90 x10 ⁴	46 x10 ⁴	75 x10 ⁴
F.C	+ ve	-ve	+ ve	+ ve	-ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
E.Coli	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve

Table 14 Experimental Sets Treated with *Tilapia mossambica* Peters

Period of Treatment -7 days
Weight - 4 g/l

E- Experiment
C- Control

Parameters	10 ml Wastewater/l			20 ml Wastewater/l			30 ml Wastewater/l			40ml Wastewater/l			50 ml Wastewater/l		
	Before	After		Before	After		Before	After		Before	After		Before	After	
		E	C		E	C		E	C		E	C			
Temp	30.20	30.30	30.10	30.10	30.00	30.10	30.50	30.40	30.40	30.40	30.40	30.60	29.80	29.70	29.80
pH	7.36	7.40	7.35	7.43	7.45	7.41	7.46	7.48	7.42	7.53	7.59	7.47	7.61	7.71	7.57
EC	112.30	119.30	109.10	119.60	128.30	121.60	133.20	139.60	144.50	147.50	158.20	150.20	159.40	167.30	161.20
TSS	118.30	10.29	116.20	123.40	11.60	120.30	139.20	17.40	132.60	145.60	23.00	140.40	162.30	36.84	156.80
DO	6.00	6.50	6.20	5.23	5.73	5.42	5.00	5.52	5.13	4.86	4.93	4.92	4.52	4.75	4.73
BOD	12.60	5.15	10.30	14.30	6.41	13.40	18.70	9.67	17.60	21.90	12.68	19.30	25.80	15.32	22.20
COD	26.25	11.23	24.70	34.05	16.27	32.60	40.65	19.75	38.40	54.75	32.19	51.90	66.15	44.51	63.10
NO ₃ - N	0.96	1.30	0.98	1.23	1.72	1.36	1.64	2.16	1.73	2.06	2.93	2.12	2.52	3.46	2.73
PO ₄ -P	0.08	0.11	0.07	0.10	0.14	0.12	0.12	0.17	0.14	0.15	0.18	0.16	0.18	0.23	0.19
SO ₄	0.80	0.12	0.90	2.20	2.70	2.20	3.60	4.00	3.70	4.80	5.20	4.90	5.20	5.90	5.30
TH	48.00	52.00	54.00	52.00	60.00	64.00	68.00	76.00	72.00	80.00	96.00	88.00	100.00	108.00	102.00
Ca	11.20	6.40	12.60	6.40	17.60	19.20	17.60	19.66	18.63	20.80	27.20	25.60	27.20	43.20	33.60
Mg	4.86	8.70	5.46	8.70	3.88	3.89	5.83	6.51	6.17	6.80	7.77	5.83	8.74	9.72	4.37
T.C	24x10 ⁴	43 x10 ³	21 x10 ⁴	39 x10 ⁴	97 x10 ³	23 x10 ⁴	46 x10 ⁴	13x10 ⁴	43 x10 ⁴	75 x10 ⁴	24 x10 ⁴	64 x10 ⁴	90 x10 ⁴	39 x10 ⁴	75 x10 ⁴
F.C	+ ve	-ve	+ ve	+ ve	-ve	+ ve	+ ve	+ve	+ ve	+ ve	+ve	+ ve	+ ve	+ve	+ ve
E.Coli	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve

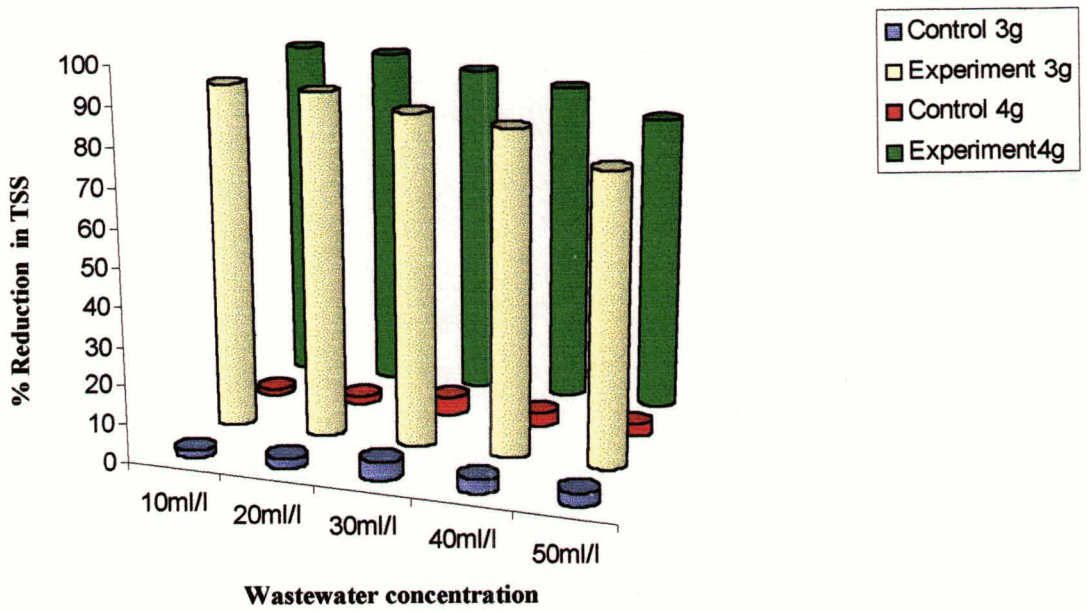


Fig. 9 Percentage Reduction of TSS in Experimental Sets Treated with *Tilapia mossambica* Peters

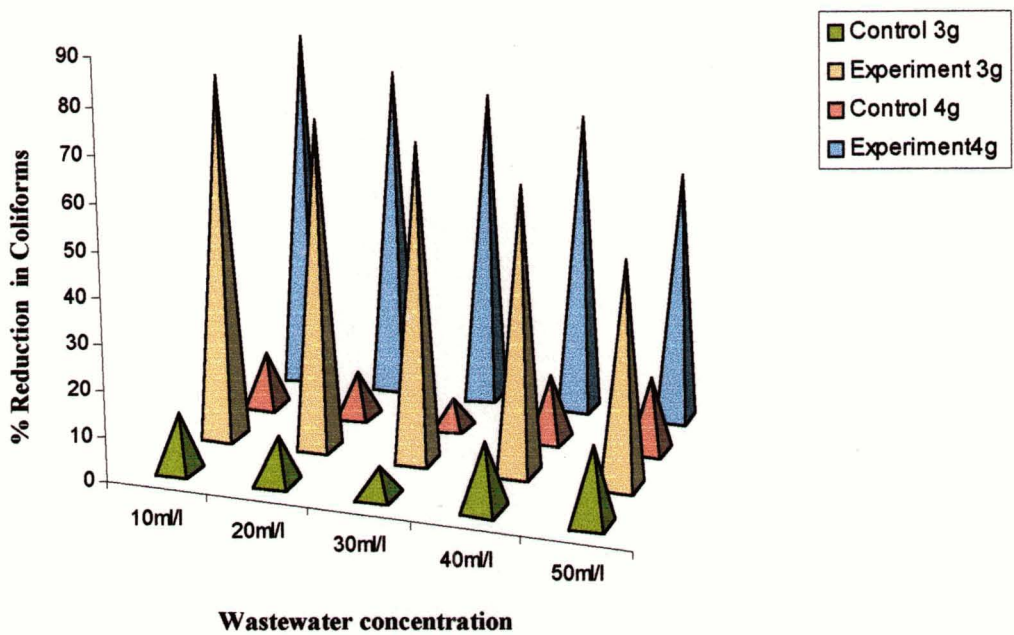


Fig. 10 Percentage Reduction of Coliforms in Experimental Sets Treated with *Tilapia mossambica* Peters

In the experiments with concentrations (20-50ml/l) there was a progressive decrease in removal of coliforms. This may be due to the greater coliform counts in higher wastewater concentrations. When 3g/l *Tilapia* was introduced, 73, 69.56, 62.6 and 48.8 percent reduction in coliforms was observed in 20-50 ml wastewater experimental sets (Fig. 10). Relatively higher reductions were obtained with 4g/l *Tilapia*, 75, 71, 68 and 56.66 percent while control had only 10.25, 6.52, 14.6, and 16.66 percent removal. Previous studies by Matena and Simek (1997) using fluorescently labelled bacteria to quantify the ingestion of bacteria by juvenile fish, revealed that *Tilapia* could ingest bacteria of size $3.92 \pm 1.55 \times 1.53 \pm 0.34$ μm .

Tilapia was efficient in reducing the BOD of experimental wastewaters. Removal upto 59.1 percent for 4g/l and 56.2 percent for 3g/l was obtained with 10 ml/l wastewater experimental sets (Fig. 11). Control for this set had a maximum of only 18.25 percent reduction. In 20-50 ml wastewater/litre experimental sets 55.2, 48.3, 42.1 and 40.6 percent reduction in BOD was recorded with 4g/l while slightly lower reduction 53.1, 45.1, 39.4 and 37.2 percent was obtained with 3g/l *Tilapia*. In the control, 6.29, 5.88, 11.87 and 13.95 percent reduction in BOD was obtained. Miravite - Casalmir (1980) reported BOD removal ranging from 23-63 percent in cane sugar processing wastewater with *Tilapia* culture.

Concentration of COD showed a maximum of 57 percent removal with 4g/l *Tilapia* treated wastewater (10ml wastewater /l) and 55.3 percent reduction with 3g/l *Tilapia*. Control for this set had only 5.90 percent reduction. In higher wastewater concentrations (20-50 ml/l) 51.6, 49.3, 36.2 and 30.1 percent reduction was noticed with 3g/l and 52.2, 51.4, 41.2 and 32.7 percent reduction with 4g/l

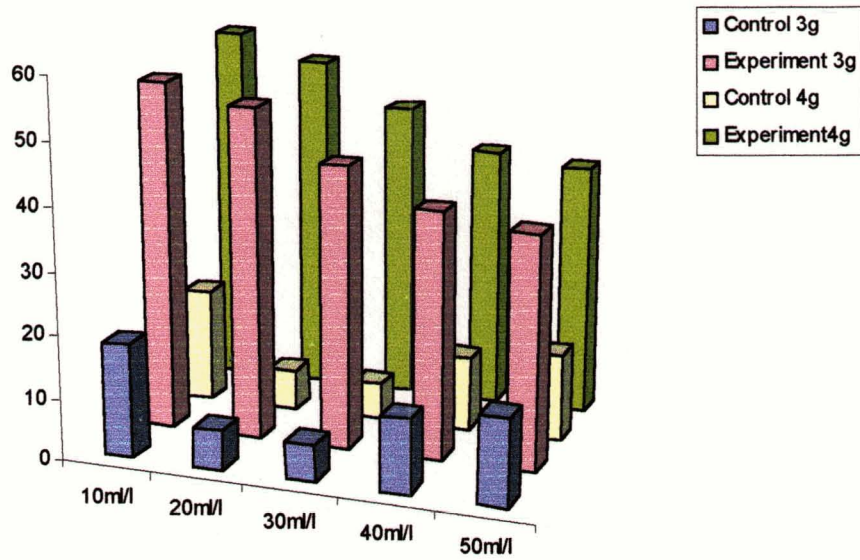


Fig. 11 Percentage Reduction of BOD in Experimental Sets Treated with *Tilapia mossambica* Peters

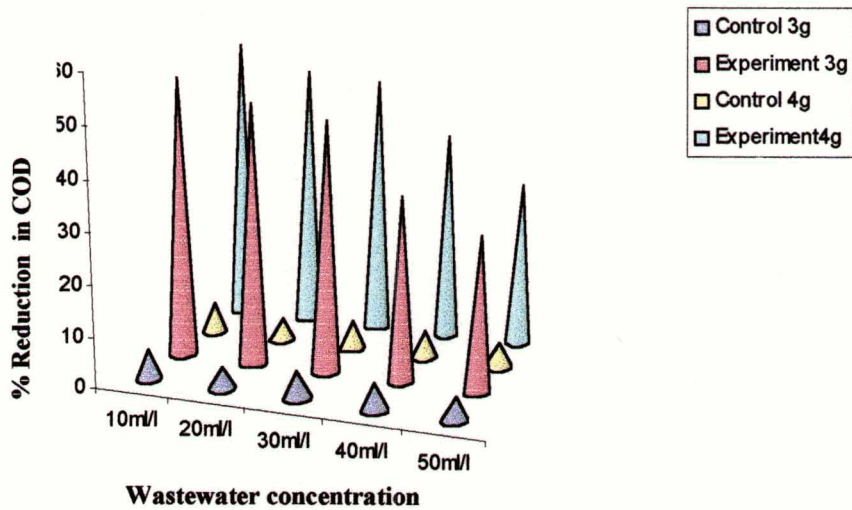


Fig. 12 Percentage Reduction of COD in Experimental Sets Treated with *Tilapia mossambica* Peters

Tilapia (Fig. 12). The results were appreciably greater than the controls 4.25, 5.53, 5.20 and 4.61 percent.

Among other physicochemical parameters studied temperature and pH did not show considerable variation. There was a small increase in electrical conductivity, which may be due to the faecal matter from fish. Dissolved oxygen showed a slight increase when compared to controls in both the biomass experimented.

Nitrate nitrogen and phosphate in the experimental media increased slightly following detention of *Tilapia* for seven days. Phosphate increase could be due to the wastes dissolved in water. Fish excrete ammonia and lesser amount of urea in to the water as wastes. Ammonia is converted to nitrates and then nitrates bacterial activity. The levels of nitrate therefore increased in the experimental sets when compared to control.

2. *Cyprinus carpio* L.

Cyprinus was treated at two varying biomass 3g/l and 4g/l. The observed variations in the physicochemical and bacteriological parameters in experimental sets are presented in Table 15 and 16. The wastewater treatment capacity of *Cyprinus* was evidenced by pronounced decrease in BOD, COD, TSS and coliforms. Better removal was obtained with higher biomass of fish in experimental waters.

When 4g/l *Cyprinus* was introduced for detention period of seven days, BOD reduction was upto a maximum of 61.48 percent in 10 ml wastewater experimental sets and 59.37 percent when 3g/l was used. This is greater than

Table 15 Experimental Sets Treated with *Cyprinus carpio* L.

Period of Treatment - 7 days
Weight -3 g/l

E- Experiment
C- Control

Parameters	10 ml Wastewater/l			20 ml Wastewater/l			30 ml Wastewater/l			40ml Wastewater/l			50 ml Wastewater/l		
	Before	After		Before	After		Before	After		Before	After		Before	After	
		E	C		E	C		E	C		E	C			
Temp	30.20	29.90	30.10	30.10	30.00	30.10	30.50	30.20	30.40	30.40	30.10	30.20	29.80	29.70	29.80
pH	7.36	7.40	7.41	7.43	7.47	7.39	7.46	7.48	7.42	7.53	7.57	7.51	7.61	7.63	7.60
EC	90.40	94.50	91.20	93.60	94.80	93.80	103.20	106.20	104.10	104.50	107.60	103.20	115.60	120.30	118.20
TSS	75.00	11.25	69.00	90.00	17.82	85.60	115.00	34.27	114.00	127.00	43.56	125.10	136.00	55.49	134.30
DO	4.30	4.80	4.60	4.40	4.90	3.70	4.20	4.61	3.80	3.20	3.10	2.70	1.90	2.10	2.00
BOD	6.35	2.58	3.80	6.46	3.26	4.60	6.89	3.58	5.20	7.25	4.20	5.80	8.39	5.10	6.90
COD	15.80	7.87	14.30	13.18	6.82	12.50	14.35	8.66	13.70	14.50	9.92	14.00	17.48	12.36	17.20
NO ₃ -N	0.26	0.33	0.28	0.38	0.49	0.40	0.46	0.59	0.49	0.50	0.65	0.54	0.78	0.86	0.80
PO ₄ -P	0.02	0.03	0.19	0.03	0.04	0.02	0.05	0.06	0.06	0.07	0.09	0.06	0.09	0.12	0.08
SO ₄	0.42	0.52	0.48	0.84	0.96	0.90	1.20	1.32	1.24	1.28	1.38	1.28	1.32	1.40	1.36
TH	40.00	48.00	48.00	52.00	56.00	54.00	56.00	62.00	60.00	60.00	64.00	62.00	68.00	74.00	72.00
Ca	9.60	9.40	9.60	12.80	12.00	13.20	16.00	13.20	17.60	16.00	19.20	14.10	15.30	16.65	16.20
Mg	3.80	4.20	4.20	3.90	3.80	3.90	3.80	3.13	3.80	3.50	3.80	4.20	3.80	4.13	5.83
T.C	3900	1100	2100	4300	1500	2100	4600	1500	2400	6400	2100	3900	7500	2800	4300
F.C	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
E.Coli	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve

Table 16 Experimental Sets Treated with *Cyprinus carpio* L.

Period of Treatment - 7 days
Weight - 4 g/l

E- Experiment
C- Control

Parameters	10 ml Wastewater/l			20 ml Wastewater/l			30 ml Wastewater/l			40ml Wastewater/l			50 ml Wastewater/l		
	Before	After		Before	After		Before	After		Before	After		Before	After	
		E	C		E	C		E	C		E	C			
Temp	30.20	30.00	30.10	30.10	30.20	29.70	30.50	30.20	29.60	30.40	30.10	30.60	29.80	29.80	29.70
pH	7.36	7.46	7.35	7.43	7.57	7.41	7.46	7.62	7.42	7.53	7.64	7.47	7.61	7.81	7.57
EC	90.40	96.20	90.20	93.60	97.30	94.20	103.20	109.40	102.30	104.50	112.40	109.30	115.60	121.40	117.50
TSS	75.00	12.75	70.00	90.00	17.55	83.60	115.00	26.91	107.00	127.00	36.45	123.00	136.00	44.74	131.00
DO	4.30	4.20	4.70	4.40	4.00	4.50	4.20	4.00	4.20	3.20	3.30	3.40	1.90	1.70	2.00
BOD	6.62	2.25	4.10	6.81	2.75	4.30	7.20	3.20	4.51	7.83	3.72	4.96	8.43	4.38	5.15
COD	11.03	5.50	10.60	14.18	7.28	13.20	15.00	8.73	13.80	22.37	14.21	21.50	20.07	13.57	19.82
NO ₃ -N	0.26	0.39	0.28	0.38	0.52	0.40	0.46	0.54	0.49	0.50	0.57	0.54	0.78	0.93	0.80
PO ₄ -P	0.02	0.03	0.19	0.03	0.04	0.02	0.05	0.07	0.06	0.07	0.09	0.06	0.09	0.12	0.08
SO ₄	0.42	0.54	0.48	0.84	1.00	0.90	1.20	1.38	1.24	1.28	1.40	1.28	1.32	1.42	1.36
TH	40.00	44.00	48.00	52.00	58.00	54.00	56.00	60.00	60.00	64.00	68.00	62.00	68.00	76.00	72.00
Ca	9.60	10.60	9.60	12.80	14.30	13.20	16.00	17.10	17.60	19.20	20.40	14.10	15.30	17.10	16.20
Mg	3.80	4.20	4.20	3.90	4.30	3.90	3.80	4.00	3.80	3.80	4.00	4.20	3.80	4.24	5.83
T.C	4300	1100	2300	6400	2300	4300	7500	2800	4600	9300	3900	6400	12000	7500	9300
F.C	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve
E.Coli	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve

control sets maintained for experiments with biomass 4g/l and 3g/l. Higher waste water concentrations showed decrease in percentage reduction in BOD 59.61, 55.55, 52.49 and 48 percent in 20, 30, 40 and 50 ml wastewater experimental sets with 4 g/l *Cyprinus* (Fig. 13). Comparatively lower reduction was seen with 3 g/l *Cyprinus*, 49.53, 48.04, 42.06 and 39.21 percent. However controls had only upto a maximum of 38 percent reduction for these experimental sets.

Maximum COD reduction of 50.2 and 51.6 percent were obtained in experiments with 10 ml wastewater with biomass 3 g/l and 4 g/l *Cyprinus*. Control for this set had only 9.49 percent reduction in COD. In 20 ml wastewater experimental set, 48.3 and 48.7 percent reduction was observed with 3 and 4 g/l fish respectively. Higher wastewater concentrations upto 50 ml/l showed 39.7, 31.6 and 29.3 percent reduction with 3 g/l fish and 41.8, 36.5 and 32.4 percent with 4 g/l *Cyprinus*. Fig. 14 represents the removal of COD by *Cyprinus*.

Total suspended solids reduction of 85 and 83 percent was obtained with 3 g/l and 4 g/l *Cyprinus* respectively while control had only a maximum of 8 percent removal, in 10 ml wastewater experimental set (Fig. 15). In 20-50 ml wastewater experimental sets, 80.2 , 70.2 , 65.7 and 59.2 percent reduction was obtained with 3 g/l and 80.5, 76.6, 71.3 and 67.1 percent reduction was recorded with 4 g/l.

Coliforms in the experiments with 10 ml wastewater and 3 g/l *Cyprinus* showed removal of 71.7 and 74.41 percent with 4g/l fish. With greater wastewater concentrations and consequent increase in coliforms, a progressive decrease in their removal was obtained. Coliform removal in the experimental set with 3 g/l was 65.11, 67.39, 67.18 and 62.66 percent in 20-50 ml wastewater concentration (Fig. 16). With 4g/l, 64.06, 62.66, 58.06 and 37.5 percent reduction was recorded.

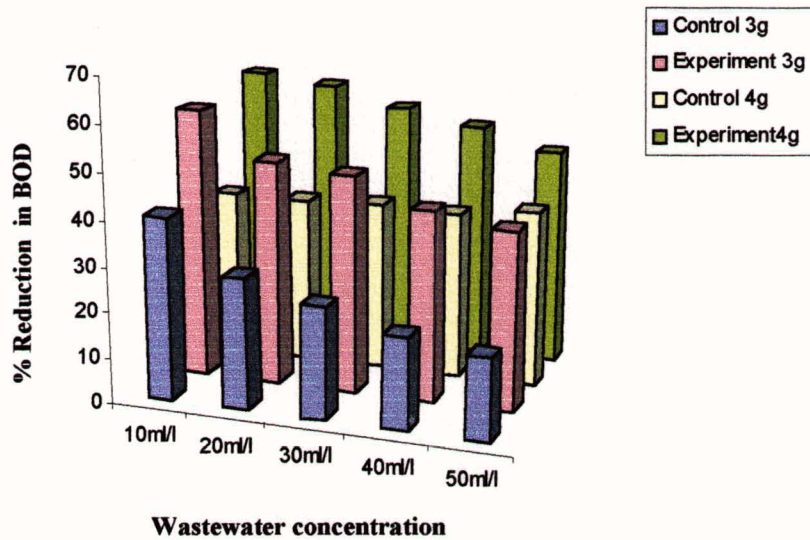


Fig. 13 Percentage Reduction of BOD in Experimental Sets Treated with *Cyprinus carpio* L.

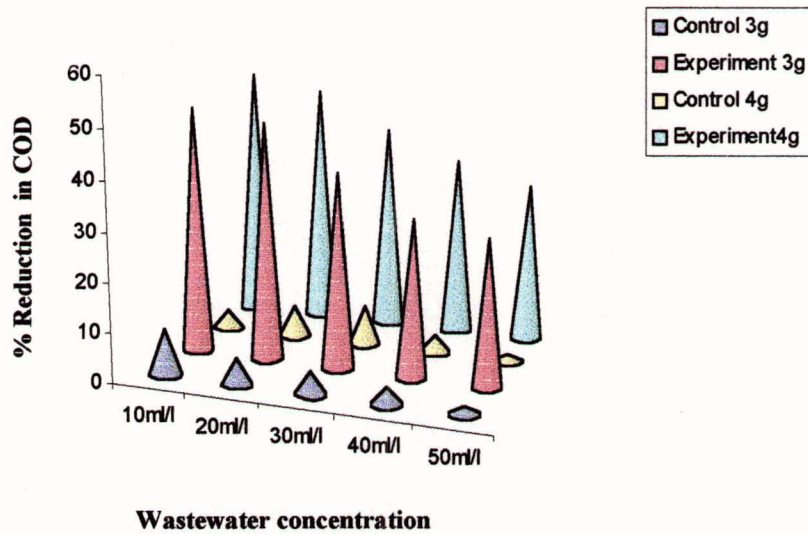


Fig. 14 Percentage Reduction of COD in Experimental Sets Treated with *Cyprinus carpio* L.

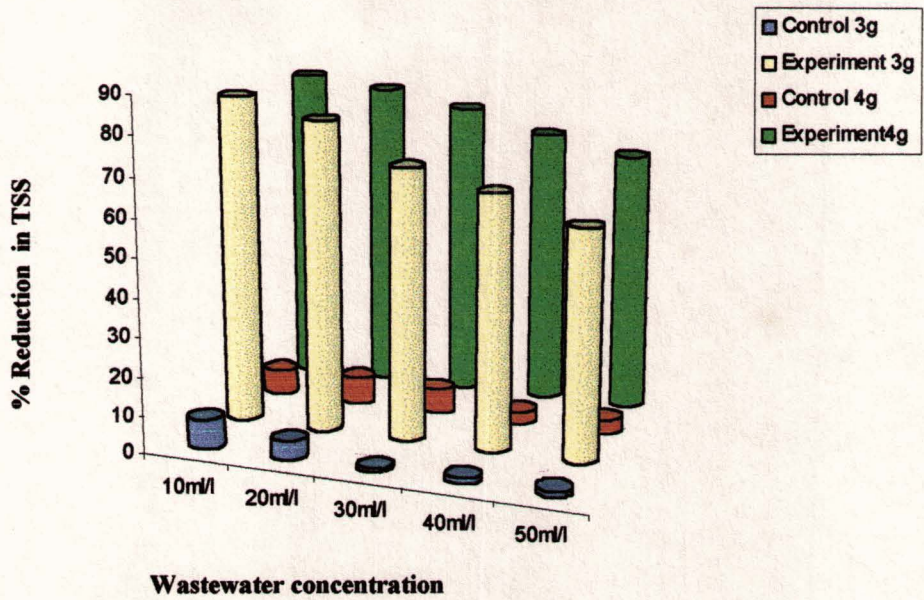


Fig. 15 Percentage Reduction of TSS in Experimental Sets Treated with *Cyprinus carpio* L.

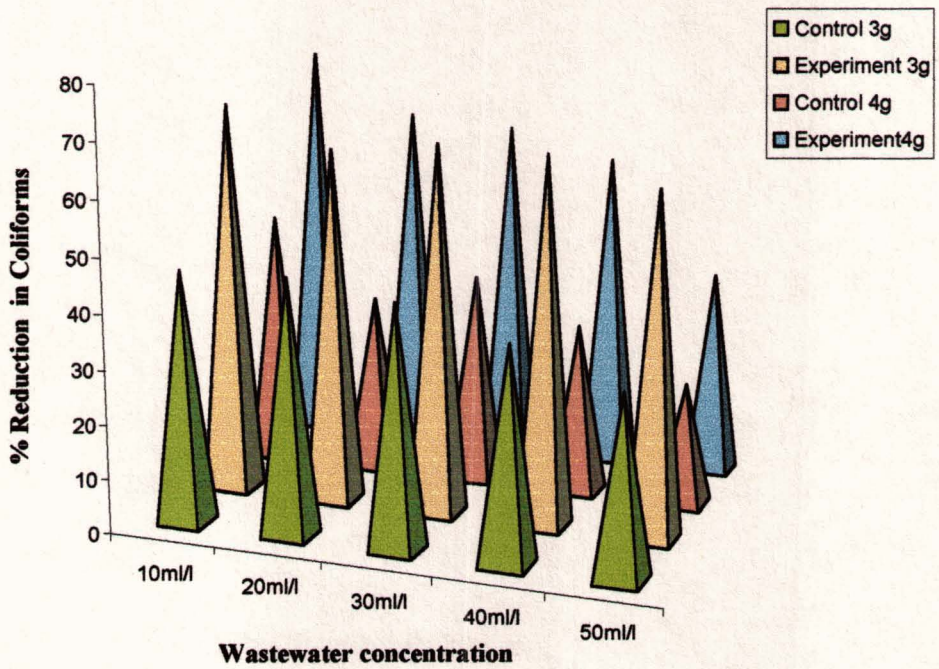


Fig. 16 Percentage Reduction of Coliforms in Experimental Sets Treated with *Cyprinus carpio* L.

The results of the present study are in confirmation with that of Matena and Simek (1997) who observed that the clearance rate in bacteria and suspended microparticles in *Cyprinus* was two orders of magnitude lower than *Tilapia*.

Temperature and pH did not show much variation during the course of the experiment. Electrical conductivity increased slightly in experimental sets. Dissolved oxygen showed a negligible increase in 3 g/l treated experimental sets while it decreased with 4 g/l *Cyprinus* presumably due to greater oxygen requirement of the increased biomass of fish.

The nutrients, phosphate and nitrate nitrogen showed an increase from pre experimental stage with both biomass of fish used. This may be due to the faecal and metabolic wastes of the fish. There was an increase in hardness in *Cyprinus* treated experimental sets. Increase in calcium and magnesium was also observed.

3. *Lebistes reticulatus* Peters

Lebistes was used in the experiments with two varying biomass 3 and 4 grams per litre for a treatment period of seven days. The physicochemical and bacteriological characteristics of the experimental wastewater are presented in Tables 17 and 18.

Lebistes treated experimental sets showed reduction in BOD upto 38.5 percent with a biomass of at 4 g/l and 32.7 percent with 3 g/l for the lowest wastewater concentration (10 ml/l), while its control recorded 23.4 percent reduction. In higher wastewater concentrations, the percentage reduction decreased gradually. 31.7, 26.4, 24.7 and 19.3 percent reduction was obtained for 20-50 ml wastewater/litre using *Lebistes* at 3 g/l.

Table 17 Experimental Sets Treated with *Lebistes reticulatus* Peters

Period of Treatment -7 days
Weight -3 g/l

E- Experiment
C- Control

Parameters	10 ml Wastewater/l			20 ml Wastewater/l			30 ml Wastewater/l			40ml Wastewater/l			50 ml Wastewater/l		
	Before	After		Before	After		Before	After		Before	After		Before	After	
		E	C		E	C		E	C		E	C			
Temp	30.20	29.40	30.10	30.10	29.80	30.60	30.50	30.10	30.40	30.40	30.70	29.70	29.80	29.70	29.40
pH	7.36	7.40	7.39	7.43	7.46	7.41	7.46	7.51	7.40	7.53	7.60	7.42	7.61	7.68	7.52
EC	90.40	91.20	90.90	93.60	95.00	93.40	103.20	110.70	104.20	128.20	132.40	128.00	115.60	120.40	116.10
TSS	75.00	45.60	69.00	90.00	57.24	82.00	115.00	75.33	108.00	127.00	88.14	121.00	136.00	95.61	130.00
DO	4.30	4.90	4.40	3.80	4.20	3.90	3.20	3.60	3.00	2.50	2.90	2.70	1.90	2.30	2.00
BOD	6.35	4.27	4.86	6.46	4.41	4.85	6.89	4.97	5.29	7.25	5.46	5.62	8.39	6.77	6.92
COD	15.80	10.73	14.90	13.18	9.40	13.14	14.31	11.30	14.39	15.42	12.37	15.38	17.48	14.79	17.36
NO ₃ -N	0.26	0.40	0.28	0.38	0.52	0.40	0.46	0.61	0.49	0.50	0.69	0.54	0.78	0.92	0.80
PO ₄ -P	0.02	0.03	0.19	0.03	0.04	0.04	0.05	0.06	0.06	0.07	0.09	0.06	0.09	0.12	0.08
SO ₄	0.42	0.72	0.48	0.84	0.10	0.90	1.20	1.32	1.24	1.28	1.36	1.28	1.32	1.40	1.36
TH	40.00	46.00	48.00	52.00	60.00	54.00	56.00	64.00	60.00	60.00	64.00	62.00	68.00	76.00	72.00
Ca	9.60	9.60	9.60	12.80	14.40	13.20	16.00	19.20	17.60	16.00	19.20	14.10	15.30	22.40	20.10
Mg	3.80	3.80	4.20	3.90	5.80	3.90	3.80	3.80	3.80	3.50	3.80	4.20	3.80	5.83	5.83
T.C	3900	2633	3500	4300	3027	3900	4600	3900	2400	6400	5261	5600	7500	6570	6400
F.C	-ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve
E.Coli	-ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve

Table 18 Experimental Sets Treated with *Lebistes reticulates* Peters

Period of Treatment - 7 days
Weight - 4 g/l

E- Experiment
C- Control

Parameters	10 ml Wastewater/l			20 ml Wastewater/l			30 ml Wastewater/l			40ml Wastewater/l			50 ml Wastewater/l			
	Before	After		Before	After		Before	After		Before	After		Before	After		
		E	C		E	C		E	C		E	C				
Temp	30.20	30.10	30.00	30.10	30.30	29.80	30.50	30.40	30.10	30.40	30.70	30.30	29.80	29.50	29.80	
pH	7.36	7.42	7.38	7.43	7.48	7.40	7.46	7.51	7.39	7.53	7.60	7.43	7.61	7.69	7.53	
EC	90.40	93.20	91.50	93.60	97.40	94.10	103.20	110.90	103.60	128.20	136.50	130.60	115.60	124.10	116.30	
TSS	75.00	48.53	69.00	90.00	56.52	82.00	115.00	73.37	108.00	127.00	84.46	121.00	136.00	94.79	130.00	
DO	4.30	4.60	4.50	4.40	4.30	3.80	4.20	4.30	3.50	3.20	3.10	2.80	1.90	1.82	2.10	
BOD	6.35	3.91	4.86	6.46	4.22	4.85	6.89	4.97	5.29	7.25	5.42	5.62	8.39	6.56	6.92	
COD	15.80	10.73	14.92	13.18	8.90	13.02	14.35	10.71	14.13	15.42	12.17	14.92	17.48	14.06	17.03	
NO ₃ -N	0.26	0.42	0.28	0.38	0.55	0.40	0.46	0.67	0.49	0.50	0.73	0.54	0.78	0.96	0.80	
PO ₄ -P	0.02	0.04	0.19	0.03	0.05	0.04	0.05	0.08	0.06	0.07	0.10	0.06	0.09	0.13	0.08	
SO ₄	0.42	0.76	0.48	0.84	0.14	0.90	1.20	1.36	1.24	1.28	1.40	1.28	1.32	1.44	1.36	
TH	40.00	48.00	42.00	52.00	56.00	54.00	56.00	59.00	60.00	60.00	66.00	62.00	68.00	74.00	72.00	
Ca	9.60	14.40	10.60	12.80	13.60	13.20	16.00	18.20	17.60	16.00	14.10	15.30	18.20	19.30	20.10	
Mg	3.80	2.91	4.20	3.90	4.30	3.90	3.80	4.80	3.80	3.50	4.80	3.80	4.80	5.62	5.83	
T.C	3900	2012	3500	4300	2611	3900	4600	3096	3900	6400	4551	5600	7500	6285	6400	
F.C	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
E.Coli	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

Similarly when 4 g/l fish was used, the percentage reduction was found to decrease with increase in wastewater concentration. 34.6, 27.9, 25.2 and 21.8 percent reduction was obtained for these experimental sets. Fig. 17 represents the percentage reduction in BOD using *Lebistes*.

COD removal followed a trend similar to BOD, with the percentage reduction decreasing with increase in wastewater concentration. Slightly higher removal was obtained when 4g/l *Lebistes* was used for treatment. Maximum reduction in COD was 36.4 percent for 4g/l and 32.1 percent for 3g/l fish in 10 ml wastewater experimental sets. The COD reductions were from 28.7, 21.3, 19.8 and 15.4 percent for 3g/l and 32.5, 25.4, 21.1 and 19.6 percent for 4g/l *Lebistes* in 20-50 ml wastewater experimental sets. Control for both the biomass tested had maximum reduction of only 5.69 percent. Fig. 18 represents the percentage reduction in COD.

Lebistes was capable of reducing the total suspended solids upto 39 percent in 3 g/l wastewater experimental sets. The reduction in TSS obtained when 3 g/l *Lebistes* was used is 39.2, 36.4, 34.5, 30.6 and 29.7 percent. 35.3, 37.2, 36.2, 33.5 and 30.3 percent reduction was recorded for the same experiments with higher biomass of fish, 4 g/l. Control values were 8, 8.8, 6.08, 4.72 and 4.41 percent thereby confirming the TSS removal due to the presence of *Lebistes*. Fig. 19 represents the reduction in TSS using *Lebistes*.

Among the parameters studied, *Lebistes* recorded a maximum reduction in coliforms, 48.4 percent with 4g/l and 32.48 percent with 3 g/l biomass of fish. The percentage reduction using 3g/l fish are 32.48, 29.6, 21.1, 17.79 and 12.4 percent for 10-50 ml wastewater/ litre. With 4g/l fish, 48.4, 39.27, 32.69, 28.88 and 16.2

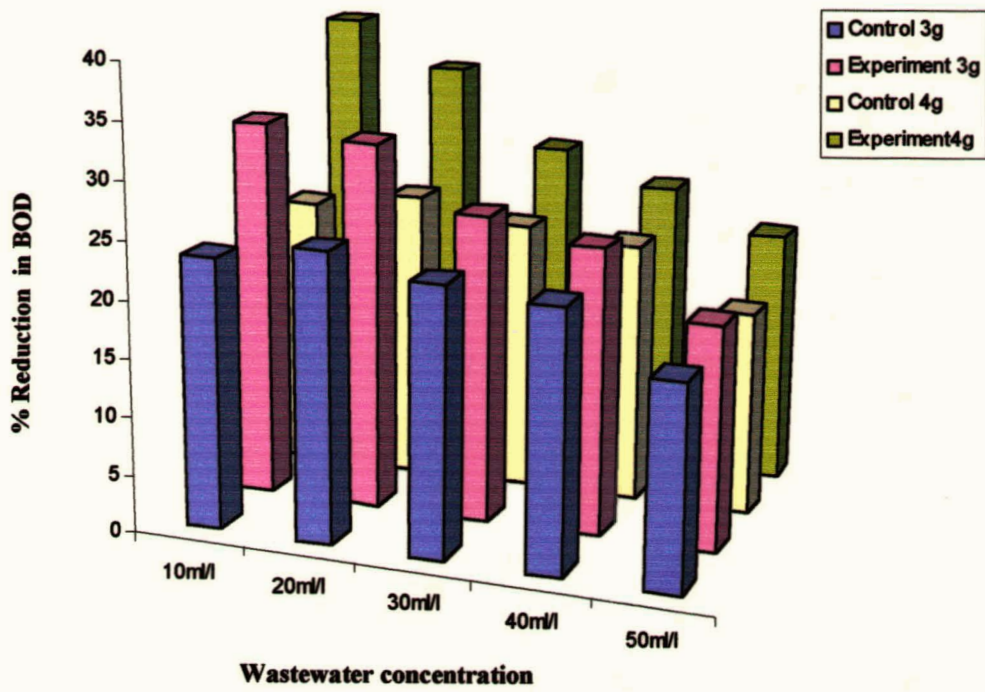


Fig. 17 Percentage Reduction of BOD in Experimental Sets Treated with *Lebistes reticulatus* Peters

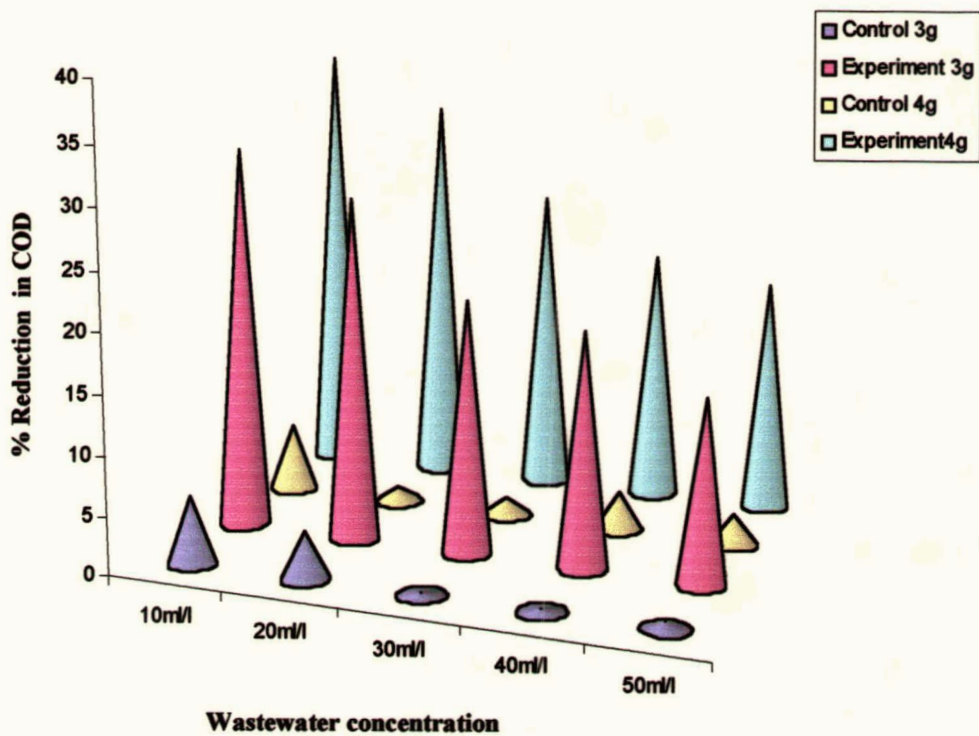


Fig. 18 Percentage Reduction of COD in Experimental Sets Treated with *Lebistes reticulatus* Peters

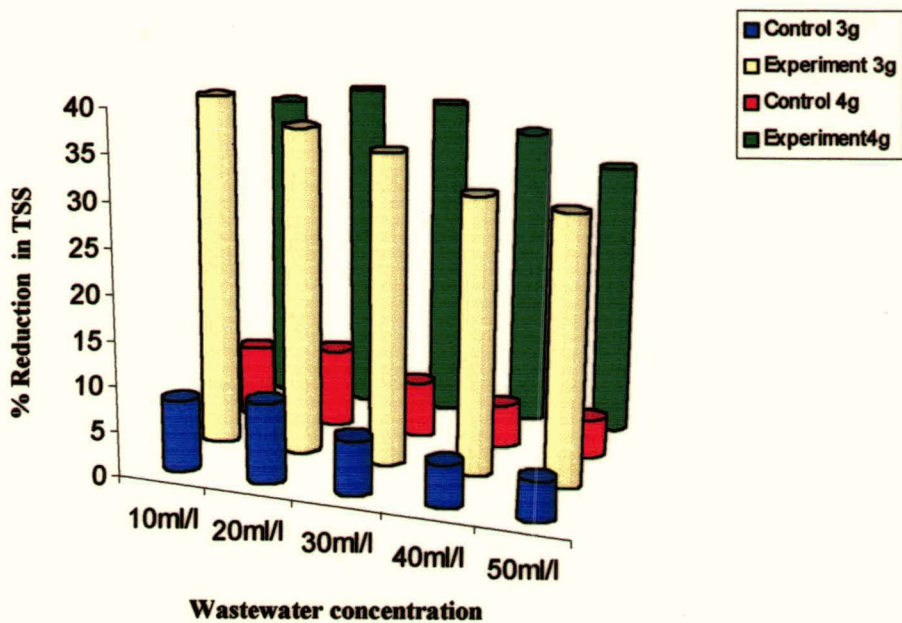


Fig. 19 Percentage Reduction of TSS in Experimental Sets Treated with *Lebistes reticulatus* Peters

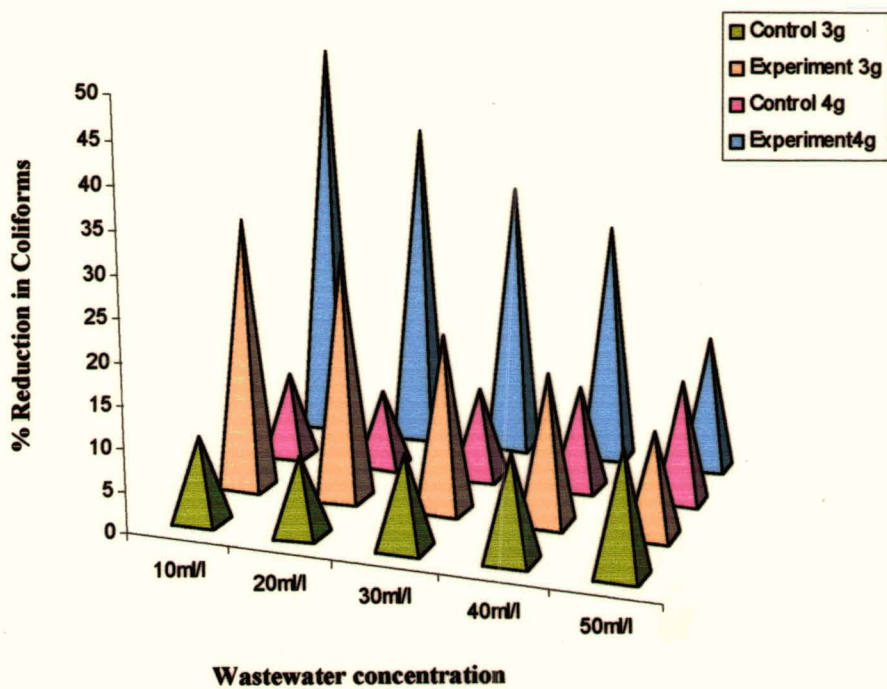


Fig. 20 Percentage Reduction of Coliforms in Experimental Sets Treated with *Lebistes reticulatus* Peters

percent reduction was recorded. Controls for these experiments had maximum removal 14.66 percent. Fig. 20 represents the reduction in coliforms using *Lebistes*.

When the physicochemical parameters studied were analysed, temperature and pH showed negligible variation for the treatment period. Dissolved oxygen and conductivity values increased slightly, with respect to controls for both the biomass experimented.

There was a small increase in nitrate nitrogen and phosphate concentrations in the experiments with *Lebistes*, when compared to controls and this could be due to excretions by the fish. Hardness, calcium and magnesium increased in the *Lebistes* treated experiments.

4. *Catla catla* Hamilton

Catla is widely used in polyculture practices and is in demand as an edible fish. *Catla* has been used in several wastewater fed ponds, with success. In the present study, its potential to improve water quality of polluted waters was examined. *Catla* at 3g/l and 4g/l were used to treat 10-50 ml wastewater experimental sets for seven days. Results indicate that *Catla* is capable of reducing BOD, COD, TSS and coliforms to considerably lower levels. The physicochemical and bacteriological characteristics of the parameters studied are presented in Tables 19 and 20.

Experiments conducted with *Catla* achieved 48.5 percent reduction in BOD for 4 g/l fish and 41.2 percent reduction for 3g/l fish, in lowest wastewater concentration (20ml/l). In higher wastewater concentrations, a decline in the

Table 19 Experimental Sets Treated with *Catla catla* Hamilton

Period of Treatment -7 days
Weight- 3 g/l

E- Experiment
C- Control

Parameters	10 ml Wastewater/l			20 ml Wastewater/l			30 ml Wastewater/l			40ml Wastewater/l			50 ml Wastewater/l		
	Before	After		Before	After		Before	After		Before	After		Before	After	
		E	C		E	C		E	C		E	C			
Temp	29.50	29.20	29.00	29.60	29.30	29.40	29.70	29.80	30.00	30.00	31.20	29.60	29.80	29.90	29.90
pH	7.86	8.00	7.92	7.84	7.97	7.90	7.88	8.01	7.91	7.89	8.04	7.96	7.92	8.12	7.96
EC	105.50	109.30	106.20	112.00	114.20	112.60	146.80	152.60	147.10	161.50	167.20	161.70	194.80	203.10	195.00
TSS	92.10	46.61	91.20	105.00	53.97	104.20	120.00	64.56	118.30	138.00	78.10	136.00	147.80	88.30	146.20
DO	5.80	6.40	5.82	5.40	5.52	5.50	5.00	5.50	5.13	4.60	5.02	4.56	4.20	4.43	4.18
BOD	11.20	6.59	8.40	13.40	8.78	11.30	15.60	10.91	13.30	19.30	14.40	16.84	20.40	15.18	18.30
COD	22.40	13.20	21.90	27.91	17.01	27.32	33.19	23.07	33.00	39.38	28.32	38.40	40.80	31.26	40.12
NO ₃ -N	0.58	0.69	0.63	0.86	1.02	0.93	1.17	1.40	1.20	1.49	1.78	1.52	1.82	2.03	1.97
PO ₄ -P	0.07	0.08	0.06	0.08	0.09	0.07	0.12	0.14	0.10	0.14	0.16	0.11	0.16	0.19	0.12
SO ₄	0.80	0.12	0.10	1.62	1.84	1.68	2.40	3.20	2.80	2.82	3.64	3.00	3.06	3.82	3.24
TH	56.00	60.00	52.00	68.00	84.00	72.00	92.00	100.00	92.00	104.00	112.00	100.00	120.00	148.00	122.00
Ca	16.00	12.80	12.80	19.30	24.00	19.90	14.40	34.40	14.40	35.20	28.80	34.40	20.80	43.20	24.00
Mg	3.88	3.88	4.80	6.12	5.83	6.29	3.49	3.40	3.49	6.40	9.72	3.40	3.88	7.77	13.60
T.C	43x10 ³	11x10 ³	41x10 ³	46 x10 ³	15x 10 ³	43 x10 ³	93 x10 ³	43 x10 ³	75 x10 ³	11 x10 ⁴	57 x10 ³	93 x10 ³	15 x10 ⁴	75 x10 ³	11 x10 ⁴
F.C	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
E.Coli	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

Table 20 Experimental Sets Treated with *Catla catla* Hamilton

Period of Treatment- 7 days
Weight - 4 g/l

E- Experiment
C- Control

Parameters	10 ml Wastewater/l			20 ml Wastewater/l			30 ml Wastewater/l			40ml Wastewater/l			50 ml Wastewater/l		
	Before	After		Before	After		Before	After		Before	After		Before	After	
		E	C		E	C		E	C		E	C			
Temp	29.50	29.60	29.30	29.60	29.80	29.50	29.70	30.00	29.40	30.00	30.20	29.60	29.80	29.30	29.00
pH	7.86	8.20	7.92	7.84	7.96	7.90	7.88	8.10	8.01	7.89	8.19	7.96	7.92	8.23	7.96
EC	105.50	111.20	106.20	112.00	117.30	112.60	146.80	155.30	152.60	161.50	167.40	161.70	194.80	206.70	195.00
TSS	92.10	45.78	91.20	105.00	51.14	104.20	120.00	60.60	118.30	138.00	70.10	136.00	147.80	84.10	146.20
DO	5.80	6.20	5.82	5.40	5.86	5.50	5.50	5.49	5.20	4.60	4.98	4.13	4.20	4.49	4.20
BOD	11.20	5.77	8.40	13.40	8.09	11.60	15.60	9.90	12.62	19.30	13.28	17.56	20.40	14.18	17.35
COD	22.40	12.93	21.90	27.91	17.01	27.32	33.19	22.34	33.00	39.38	27.93	38.40	40.80	30.56	40.12
NO ₃ -N	0.58	0.72	0.63	0.86	1.06	0.93	1.17	1.44	1.20	1.49	1.82	1.52	1.82	2.24	1.97
PO ₄ -P	0.07	0.08	0.06	0.08	0.09	0.07	0.12	0.15	0.10	0.14	0.17	0.11	0.16	0.19	0.12
SO ₄	0.80	0.16	0.10	1.62	1.72	1.68	2.40	3.84	2.80	2.82	3.96	3.00	3.06	4.00	3.24
TH	52.00	54.00	60.00	68.00	64.00	74.00	92.00	98.00	102.00	104.00	108.00	112.00	120.00	128.00	112.00
Ca	12.80	15.00	12.80	19.30	19.20	19.00	14.40	15.30	25.00	30.20	30.20	28.8	20.80	22.10	28.80
Mg	4.80	3.88	3.88	6.12	3.80	6.00	3.49	3.40	9.40	6.40	6.40	5.83	3.88	4.80	5.83
T.C	43 x10 ³	9 x10 ³	41x10 ³	46 x10 ³	14x10 ³	43x10 ³	93 x10 ³	39x10 ³	75x10 ³	11 x10 ⁴	53x10 ³	93x10 ³	15 x10 ⁴	82x10 ³	11x10 ⁴
F.C	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
E.Coli	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

percentage reduction was observed, for both the biomass of fish experimented. 34.5, 30.1, 25.4 and 25.6 percent reduction in BOD for 3 g/l and 39.7, 36.5, 31.2 and 30.5 percent reduction in BOD for 4 g/l was recorded in the present study. Only a maximum of 25 percent reduction was observed in control for the same treatment time. The results suggest the role of *Catla* in attaining the decreased BOD levels. Fig. 21 represents the reduction in BOD.

Reduction of COD was a maximum of 42.3 percent for 4 g/l and 40.7 percent for 3 g/l biomass in 10 ml/l wastewater experiments. 36.2, 30.5, 27.6 and 23.4 percent reduction for 3 g/l and 39.1, 32.7, 29.1 and 25.1 percent reduction for 4 g/l was observed in the present study in 20-50 ml experimental wastewaters. Control for these experiments achieved only a maximum of 2.23 percent reduction. Fig. 22 represents the reduction in COD.

Maximum reductions in total suspended solids of 50.3 percent was obtained using 4 g/l and 49.4 percent for 3 g/l *Catla* in the experiments, whereas control had maximum of 1.44 percent reduction among the various wastewater concentrations studied. With 3 g/l fish, 49.4, 48.6, 46.2, 43.4 and 40.3 percent reduction was achieved, while with 4 g/l fish, 50.3, 51.3, 49.5, 49.2 and 43.1 percent reduction was obtained. Fig. 23 represents the percentage reduction in TSS in experiments using *Catla*.

Coliform bacteria showed a drastic decline of 79.06 percent and 74.41 with 3g/l and 4 g/l respectively in *Catla* treated experimental wastewaters. The reductions obtained with 3 g/l *catla* are 74.41, 67.39, 53.76, 48.18 and 50 percent for 10-50 ml wastewater/ litre respectively. When 4 g/l *Catla* was used, slightly better reductions of 79.06, 69.5, 58.06, 51.54 and 45.33 percent was recorded for

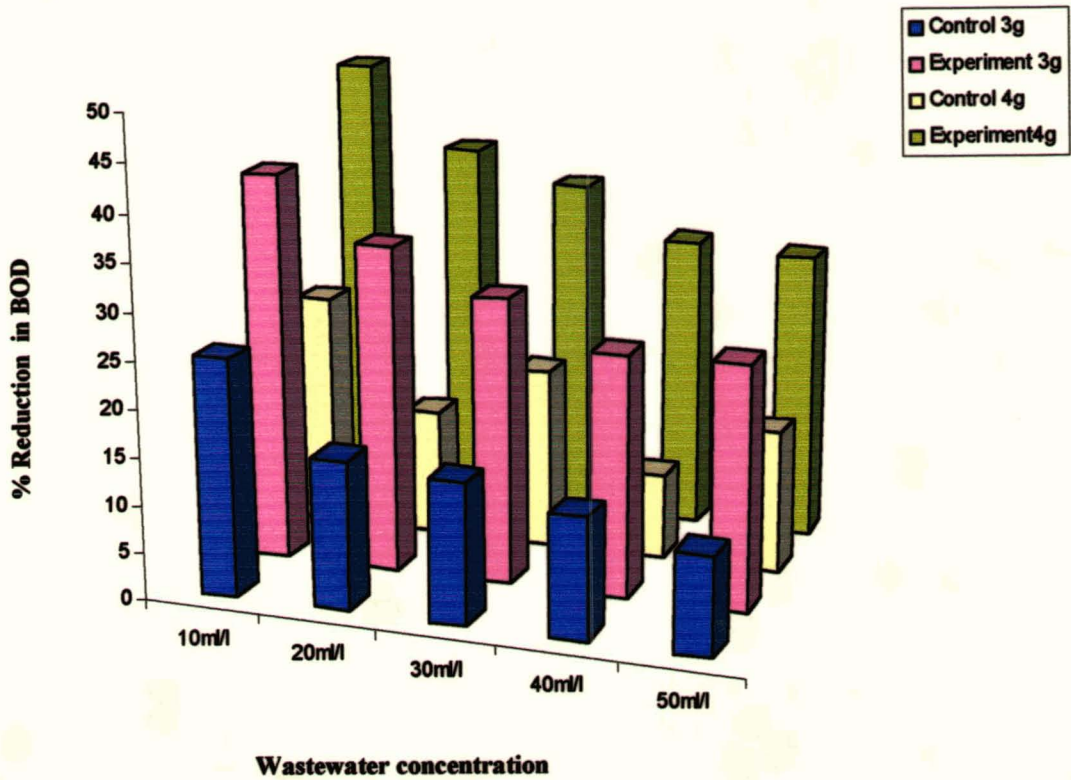


Fig. 21 Percentage Reduction of BOD in Experimental Sets Treated with *Catla catla* Hamilton

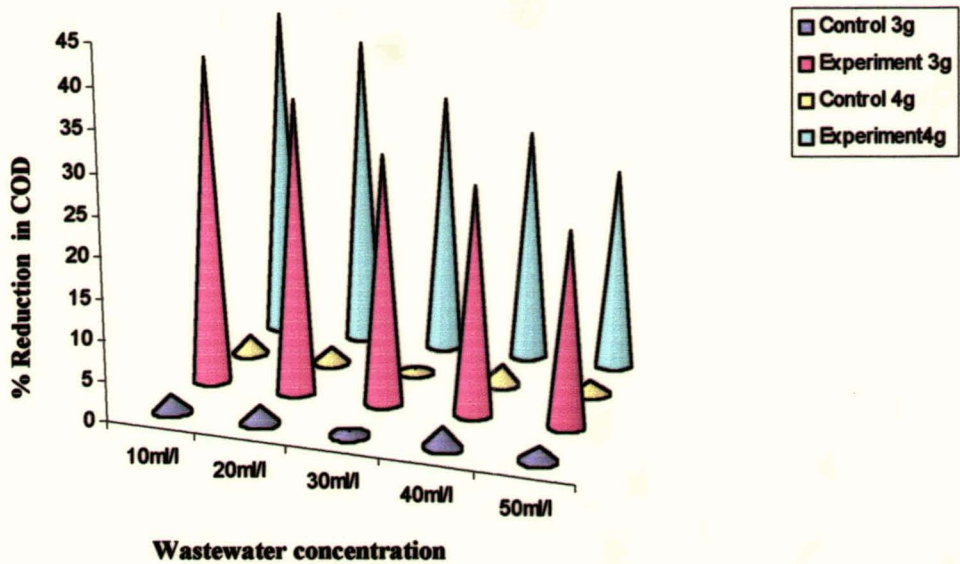


Fig. 22 Percentage Reduction of COD in Experimental Sets Treated with *Catla catla* Hamilton

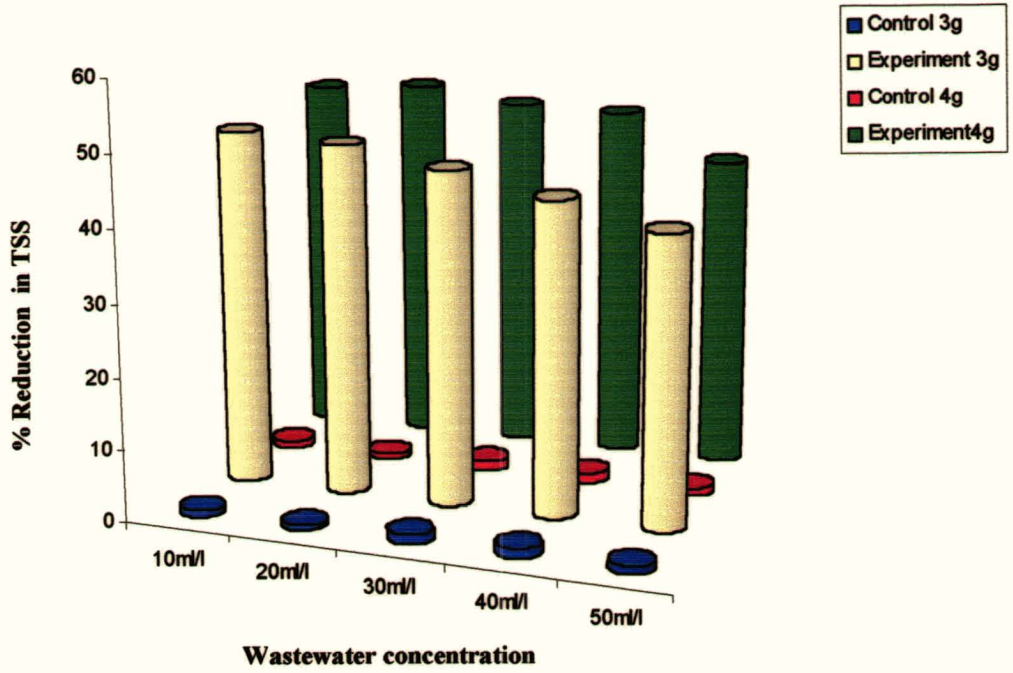


Fig. 23 Percentage Reduction of TSS in Experimental Sets Treated with *Catla catla* Hamilton

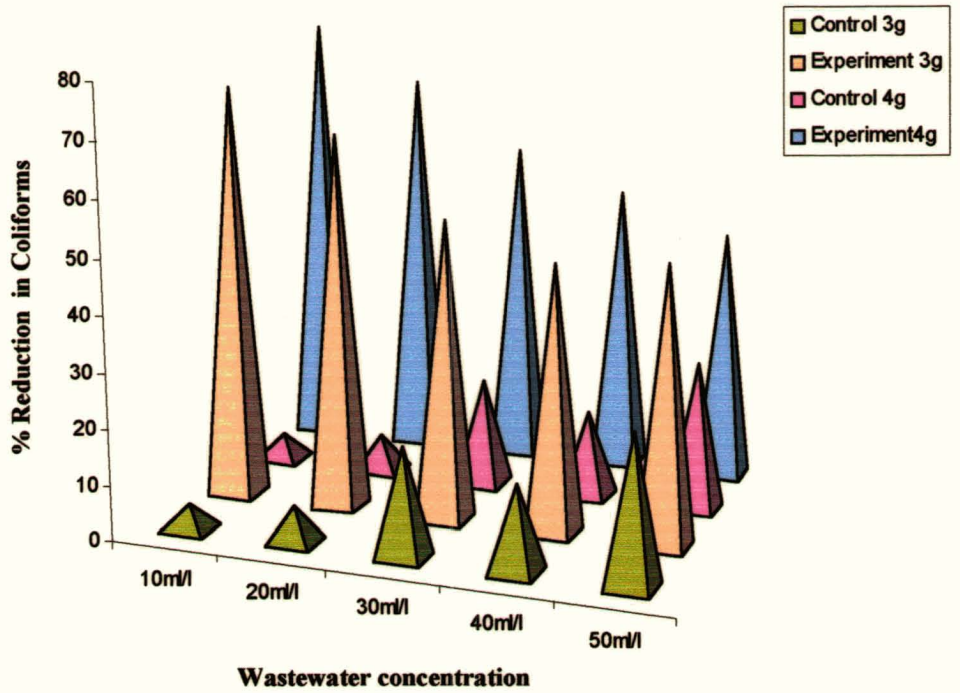


Fig. 24 Percentage Reduction of Coliforms in Experimental Sets Treated with *Catla catla* Hamilton

the same treatment conditions. Control had a maximum of only 26.6 percent reduction in various wastewater concentrations. Results indicate that higher biomass of fish result in better removal of coliforms. Fig.24 represents the percentage reduction in coliforms obtained with *Catla* as test species.

Comparison of other physicochemical parameters revealed no significant decline in nitrates, phosphates, electrical conductivity, hardness, calcium and magnesium. Dissolved oxygen and pH showed a negligible increase in experimental sets with fish. Fish excreta contribute to the nitrate and phosphate content of experimental waters.

5. *Labeo rohita* Hamilton

Labeo was introduced into the experimental sets with 10-50 ml wastewater/l with two biomass, 3g/l and 4 g/l for a detention period of seven days. It was noticed that the concentrations of BOD, COD, coliforms and suspended solids in the experiments with *Labeo* were lowered from that of the pre experimental stage. Higher reductions were obtained when 4 g/l *Labeo* was used for the experiments. Tables 21 and 22 present the changes in the physicochemical and bacteriological characteristics of experimental wastewaters for the treatment period.

TSS reduction by *Labeo* was 48.6, 44.6, 42.5, 38.0 and 32.9 percent for 10-50 ml wastewater concentrations/litre with 4 g/l fish. With 3 g/l fish, 47.2, 43.1, 40.6, 39.5 and 32.3 percent reduction was observed. Maximum TSS removal, 48.6 percent was observed with least wastewater dilution and higher biomass of fish, 4 g/l. The TSS removal values are significant since in the

Table 21 Experimental Sets Treated with *Labeo rohita* Hamilton

Period of Treatment- 7 days
Weight-3 g/l

E- Experiment
C- Control

Parameters	10 ml Wastewater/l			20 ml Wastewater/l			30 ml Wastewater/l			40ml Wastewater/l			50 ml Wastewater/l		
	Before	After		Before	After		Before	After		Before	After		Before	After	
		E	C		E	C		E	C		E	C			
Temp	27.30	27.50	27.50	27.60	27.80	26.00	26.90	26.20	26.50	29.20	29.20	29.00	28.40	28.10	28.00
pH	6.43	6.72	6.44	6.95	7.09	7.00	7.08	7.16	7.10	7.13	7.21	7.14	7.21	7.42	7.27
EC	84.00	105.20	91.60	91.50	114.90	97.30	105.50	127.40	110.80	110.10	131.10	115.60	121.10	145.10	125.30
TSS	99.54	52.56	95.20	109.20	62.14	101.60	117.30	69.68	113.20	123.40	74.66	119.80	139.60	94.51	132.60
DO	5.93	6.52	5.90	5.40	6.00	5.40	4.86	5.23	4.91	4.32	4.62	4.41	3.98	4.31	3.95
BOD	11.60	7.19	10.30	14.20	9.60	11.60	18.30	13.45	15.40	20.20	14.66	19.30	24.60	18.81	21.60
COD	26.97	15.37	24.32	33.02	20.30	30.60	43.57	29.36	41.50	44.80	32.21	43.40	57.20	44.56	55.80
NO ₃ - N	1.02	1.26	1.06	1.23	1.53	1.27	1.39	1.73	1.42	1.52	1.89	1.53	1.69	2.08	1.72
PO ₄ - P	0.07	0.09	0.06	0.08	0.10	0.07	0.12	0.15	0.10	0.14	0.17	0.11	0.16	0.20	0.12
SO ₄	1.20	1.60	1.42	1.60	1.96	1.70	2.40	3.26	2.62	3.20	3.42	3.28	5.20	6.40	5.80
TH	46.00	52.00	48.00	60.00	56.00	58.00	72.00	60.00	72.00	84.00	72.00	82.00	102.00	92.00	98.00
Ca	11.40	12.60	11.63	17.60	16.42	17.00	19.90	12.80	19.90	24.00	19.90	22.66	25.00	14.40	15.30
Mg	4.86	5.46	4.86	3.88	3.62	3.74	6.29	3.88	6.29	5.83	6.28	7.16	9.40	3.49	3.40
T.C	43x10 ³	21 x10 ³	43 x10 ³	46 x10 ³	26 x10 ³	43 x10 ³	75 x10 ³	43 x10 ³	64 x10 ³	11 x10 ⁴	75 x10 ³	93 x10 ³	15 x10 ⁴	11 x10 ⁴	14 x10 ⁴
F.C	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
E.Coli	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

Table 22 Experimental Sets Treated with *Labeo rohita* Hamilton

Period of Treatment - 7 days
Weight- 4 g/l

E- Experiment
C- Control

Parameters	10 ml Wastewater/l			20 ml Wastewater/l			30 ml Wastewater/l			40ml Wastewater/l			50 ml Wastewater/l		
	Before	After		Before	After		Before	After		Before	After		Before	After	
		E	C		E	C		E	C		E	C			
Temp	27.30	27.40	27.50	27.60	28.00	26.00	26.90	27.30	26.50	29.20	30.10	29.00	28.40	29.60	28.00
pH	6.43	6.80	6.44	6.95	7.25	7.00	7.08	7.23	7.10	7.13	7.32	7.14	7.21	7.56	7.27
EC	84.00	106.60	91.6	91.50	117.30	97.30	105.50	130.40	110.80	110.10	137.40	115.60	121.10	149.70	125.30
TSS	99.54	51.17	95.20	109.20	60.50	101.60	117.30	67.44	113.20	123.40	76.51	119.80	139.60	93.67	132.60
DO	5.93	6.20	5.90	5.40	5.80	5.40	4.86	5.00	4.91	4.32	4.50	4.41	3.98	4.26	3.95
BOD	11.60	7.05	10.30	14.20	9.18	11.60	18.30	12.88	15.40	20.20	15.11	19.30	24.60	19.04	21.60
COD	26.97	13.94	24.32	33.02	19.41	30.60	43.57	28.15	41.50	44.80	31.54	43.40	57.20	43.30	55.80
NO ₃ -N	1.02	1.29	1.06	1.23	1.60	1.27	1.39	1.77	1.42	1.52	1.95	1.53	1.69	2.13	1.72
PO ₄ -P	0.07	0.10	0.06	0.08	0.11	0.07	0.12	0.14	0.10	0.14	0.19	0.11	0.16	0.22	0.12
SO ₄	1.20	1.82	1.42	1.60	2.04	1.70	2.40	3.48	2.62	3.20	3.64	3.28	5.20	6.80	5.80
TH	46.00	52.00	48.00	60.00	54.00	58.00	72.00	58.00	72.00	84.00	76.00	82.00	102.00	86.00	98.00
Ca	11.40	12.60	11.63	17.60	15.84	17.00	19.90	17.00	19.90	24.00	19.60	22.66	25.00	22.00	15.30
Mg	4.86	5.46	4.86	3.88	3.88	3.74	6.29	3.74	6.29	5.83	6.51	7.16	9.40	9.40	3.40
T.C	43x10 ³	20 x10 ³	43 x10 ³	46 x10 ³	24 x10 ³	43 x10 ³	75 x10 ³	43 x10 ³	64 x10 ³	11 x10 ⁴	64 x10 ³	93 x10 ³	15 x10 ⁴	93 x10 ³	14 x10 ⁴
F.C	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
E.Coli	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

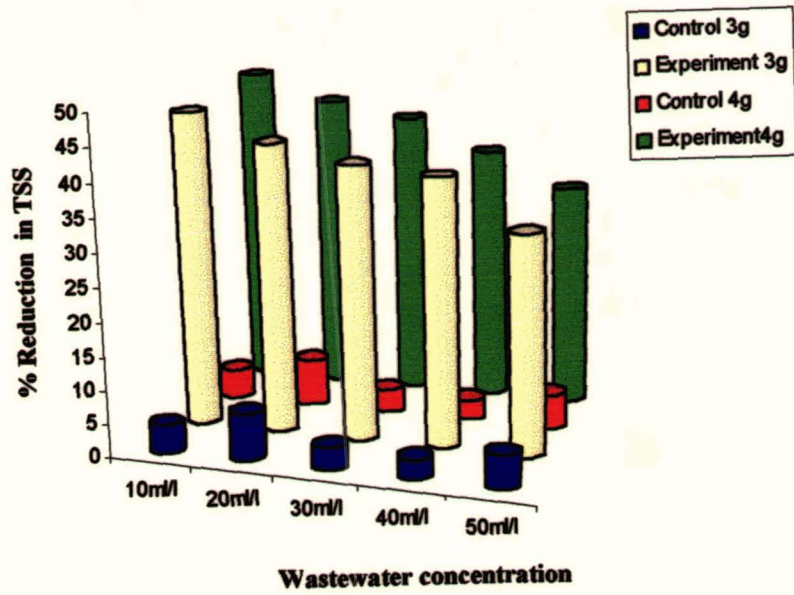


Fig. 25 Percentage Reduction of TSS in Experimental Sets Treated with *Labeo rohita* Hamilton

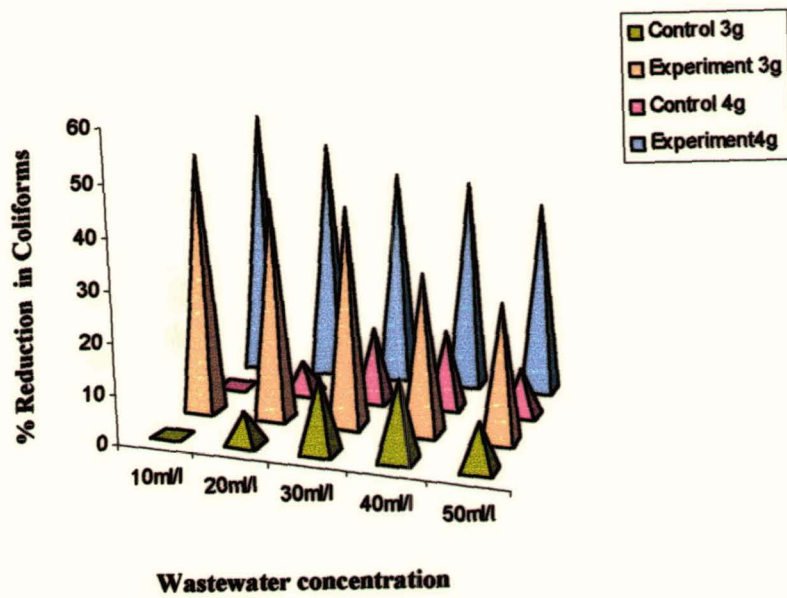


Fig. 26 Percentage Reduction of Coliforms in Experimental Sets Treated with *Labeo rohita* Hamilton

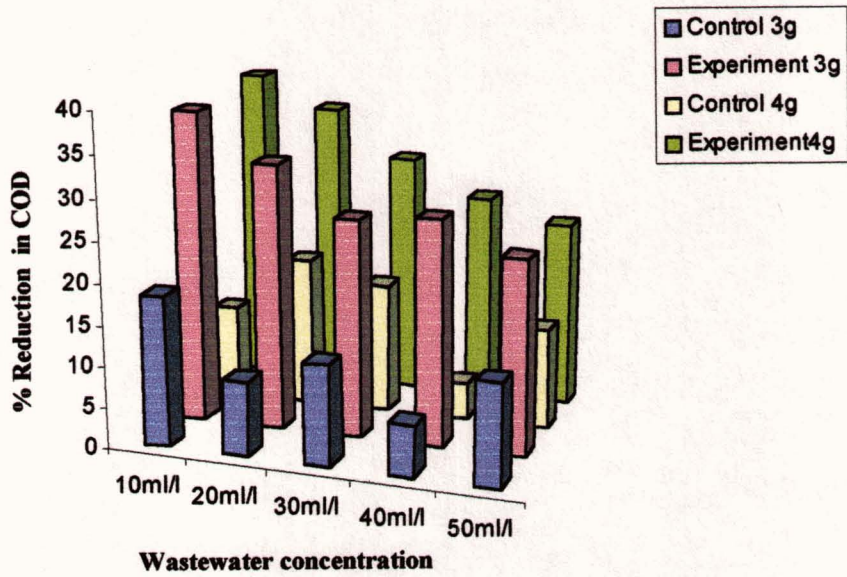


Fig. 27 Percentage Reduction of BOD in Experimental Sets Treated with *Labeo rohita* Hamilton

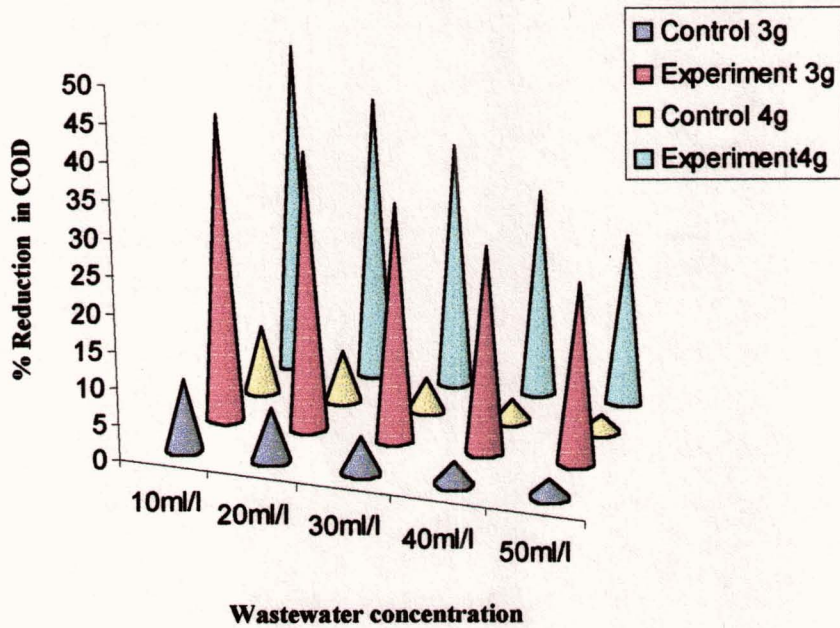


Fig. 28 Percentage Reduction of COD in Experimental Sets Treated with *Labeo rohita* Hamilton

6. *Cirrhinus mrigala* Hamilton

Experiments conducted using Mrigal as test organism at 3 and 4 g/l with 10-50 ml wastewater/l concentrations, revealed its ability to improve the water quality. Substantial reductions in TSS and coliforms were observed. BOD and COD were also reduced to a considerable extent. The changes in physicochemical and bacteriological parameters are presented in Tables 23 and 24.

Removal efficiencies for BOD upto 40 percent for 3 g/l and 43 percent for 4 g/l in the lowest wastewater concentration was obtained. The BOD removal declined gradually with increasing wastewater concentrations. 38.2, 31.6, 28.5 and 25.4 percent reduction with 3 g/l Mrigal and 41, 38.7, 32.6 and 29 percent reduction with 4 g/l Mrigal was observed (Fig.29). Control for these experimental sets had only 18.25 percent reduction. COD reduction of 47.1, 45.2, 39.4, 32.6 and 25.4 percent was obtained using 3 g/l Mrigal in 10-50 ml wastewater experimental sets. Slightly higher removal efficiency was observed with 4 g/l fish in similar experimental sets. 49.7, 47.3, 41.5, 38.5 and 29.5 percent reduction was obtained for these experiments (Fig.30). Control had a maximum of 10.66 percent reduction only.

TSS values drastically declined by 64.6 percent and 69.9 percent in 3 and 4 g/l Mrigal with 10 ml wastewater/litre. Higher wastewater concentrations showed 63.8, 60.3, 59.4 and 52.6 reduction with 4 g/l, while 61.2, 55.4, 52.3 and 48.7 percent reduction was recorded with 3 g/l Mrigal (Fig.31).

Coliform counts decreased by 64.06 percent with 4 g/l and 62.5 percent with 3 g/l Mrigal in experimental sets with 10-50 ml wastewater/l. When 4g/l of fish was used, the reduction in coliforms was 64.06, 62.66, 53.76, 41.81 and

Table 23 Experimental Sets Treated with *Cirrhinus mrigala* Hamilton

Period of Treatment - 7 days
Weight - 3 g/l

E- Experiment
C- Control

Parameters	10 ml Wastewater/l			20 ml Wastewater/l			30 ml Wastewater/l			40ml Wastewater/l			50 ml Wastewater/l		
	Before	After		Before	After		Before	After		Before	After		Before	After	
		E	C		E	C		E	C		E	C			
Temp	30.20	30.40	30.10	30.60	30.50	30.60	31.50	31.60	32.00	31.90	32.40	32.00	32.40	32.60	32.30
pH	7.53	7.60	7.54	7.57	7.63	7.58	8.06	8.27	8.10	8.25	8.39	8.27	8.32	8.46	8.32
EC	110.30	119.70	113.60	118.40	125.60	120.40	152.30	159.40	154.70	165.40	173.40	169.20	200.30	209.30	205.20
TSS	106.50	37.70	105.40	110.30	42.80	109.40	127.60	56.90	126.30	145.30	69.31	140.20	157.40	80.75	155.60
DO	5.51	5.68	5.62	5.26	5.29	5.27	5.02	5.41	5.12	4.32	4.82	4.50	4.23	4.56	4.21
BOD	12.60	7.56	10.30	14.50	8.97	13.20	17.40	11.91	15.30	20.30	14.52	18.40	24.20	18.06	22.30
COD	26.25	13.89	23.45	34.52	18.92	32.61	38.66	23.43	36.42	45.11	30.41	42.36	56.28	41.99	53.41
NO ₃ -N	0.96	1.03	0.98	1.23	1.39	1.36	1.64	1.97	1.73	2.06	2.36	2.12	2.52	2.84	2.73
PO ₄ -P	0.07	0.09	0.06	0.08	0.10	0.07	0.12	0.15	0.10	0.14	0.18	0.11	0.16	0.20	0.12
SO ₄	0.80	0.10	0.90	2.20	2.70	2.20	3.60	4.00	3.70	4.80	5.10	4.90	5.20	5.70	5.30
TH	52.00	64.00	60.00	60.00	68.00	72.00	92.00	100.00	104.00	112.00	120.00	128.00	126.00	134.00	148.00
Ca	12.80	19.20	12.80	12.80	19.30	19.90	14.40	20.30	30.20	28.80	20.80	22.18	21.83	23.21	25.63
Mg	4.80	3.80	3.88	3.88	6.12	6.29	3.49	4.91	6.40	5.83	3.88	4.13	4.06	4.31	4.76
T.C	64 x10 ³	24 x10 ³	54 x10 ³	75 x10 ³	39 x10 ³	64 x10 ³	93 x10 ³	58.x10 ³	75 x10 ³	11 x10 ⁴	75 x10 ³	93 x10 ³	15 x10 ⁴	12 x10 ⁴	14 x10 ⁴
F.C	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
E.Coli	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

Table 24 Experimental Sets Treated with *Cirrhinus mrigala* Hamilton

Period of Treatment-7 days
Weight - 4 g/l

E- Experiment
C- Control

Parameters	10 ml Wastewater/l			20 ml Wastewater/l			30 ml Wastewater/l			40ml Wastewater/l			50 ml Wastewater/l		
	Before	After		Before	After		Before	After		Before	After		Before	After	
		E	C		E	C		E	C		E	C			
Temp	30.20	30.50	30.10	30.60	30.50	30.60	31.50	31.70	32.00	31.90	32.20	32.00	32.40	32.50	32.30
pH	7.53	7.68	7.54	7.57	7.70	7.58	8.06	8.35	8.10	8.25	8.46	8.27	8.32	8.50	8.32
EC	110.30	122.30	113.60	118.40	128.40	120.40	152.30	162.30	154.70	165.40	175.60	169.20	200.30	212.30	205.20
TSS	106.50	32.06	105.40	110.30	43.06	109.40	127.60	50.66	126.30	145.30	59.00	140.20	157.40	74.61	155.60
DO	5.51	5.70	5.62	5.26	5.28	5.27	5.02	5.32	5.12	4.32	4.71	4.50	4.23	4.43	4.21
BOD	12.60	7.19	10.30	14.50	8.56	13.20	17.40	10.67	15.30	20.30	13.69	18.40	24.20	17.19	22.30
COD	26.25	13.21	23.45	34.52	18.20	32.61	38.66	22.62	36.42	45.11	27.75	42.36	56.28	39.68	53.41
NO ₃ -N	0.96	1.15	0.98	1.23	1.43	1.36	1.64	2.03	1.73	2.06	2.43	2.12	2.52	2.92	2.73
PO ₄ -P	0.07	0.09	0.06	0.08	0.12	0.07	0.12	0.16	0.10	0.14	0.18	0.11	0.16	0.22	0.12
SO ₄	0.80	0.12	0.90	2.20	2.90	2.20	3.60	4.10	3.70	4.80	5.20	4.90	5.20	5.80	5.30
TH	52.00	64.00	60.00	60.00	76.00	72.00	92.00	112.00	104.00	112.00	136.00	128.00	126.00	152.00	148.00
Ca	12.80	19.20	12.80	12.80	19.66	19.90	14.40	28.80	30.20	28.80	30.40	20.80	20.48	24.70	24.00
Mg	4.80	3.89	3.88	3.88	6.51	6.29	3.49	6.40	6.40	6.40	6.75	3.88	3.88	4.60	4.46
T.C	64x10 ³	23x10 ³	54 x10 ³	75 x10 ³	28 x10 ³	64 x10 ³	93 x10 ³	43 x10 ³	75 x10 ³	11x10 ⁴	64 x10 ³	93 x10 ³	15 x10 ⁴	11 x10 ⁴	14 x10 ⁴
F.C	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
E.Coli	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

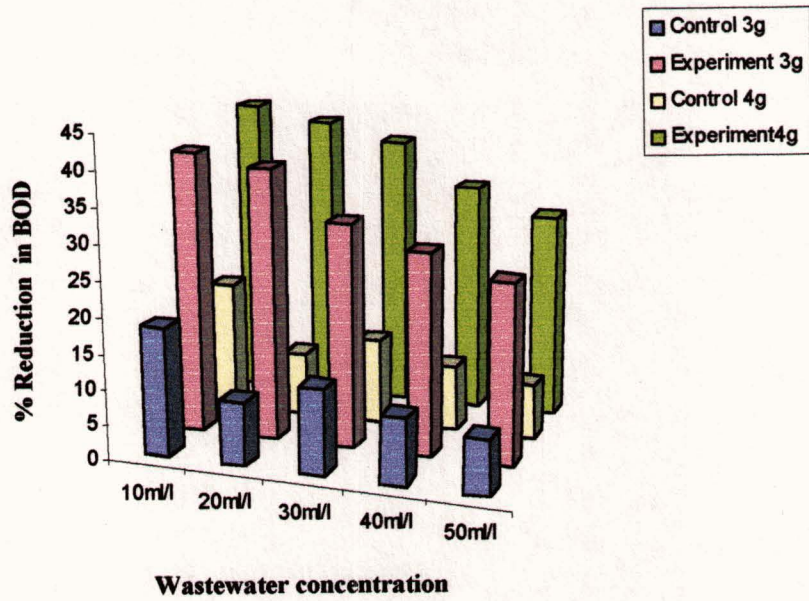


Fig. 29 Percentage Reduction of BOD in Experimental Sets Treated with *Cirrhinus mrigala* Hamilton

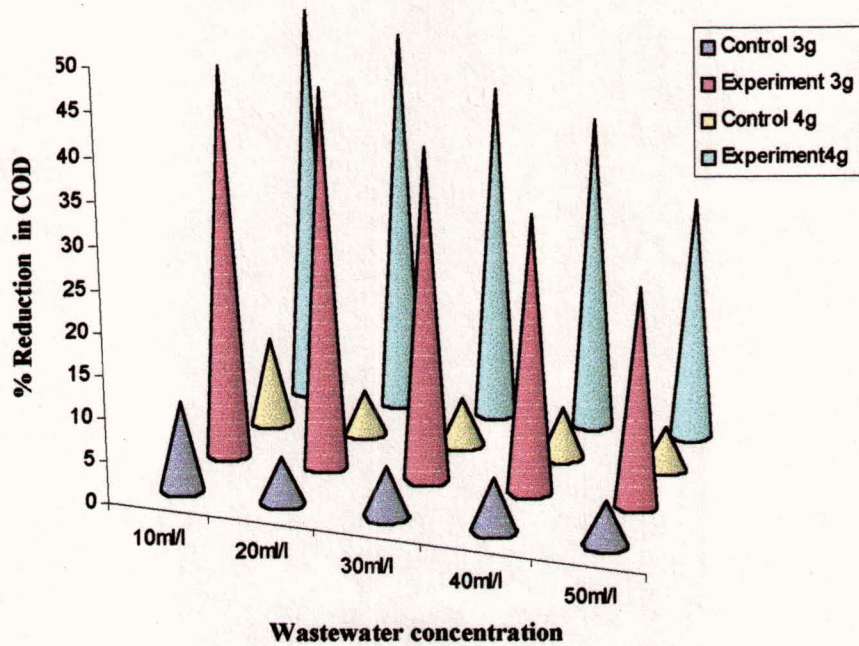


Fig. 30 Percentage Reduction of COD in Experimental Sets Treated with *Cirrhinus mrigala* Hamilton

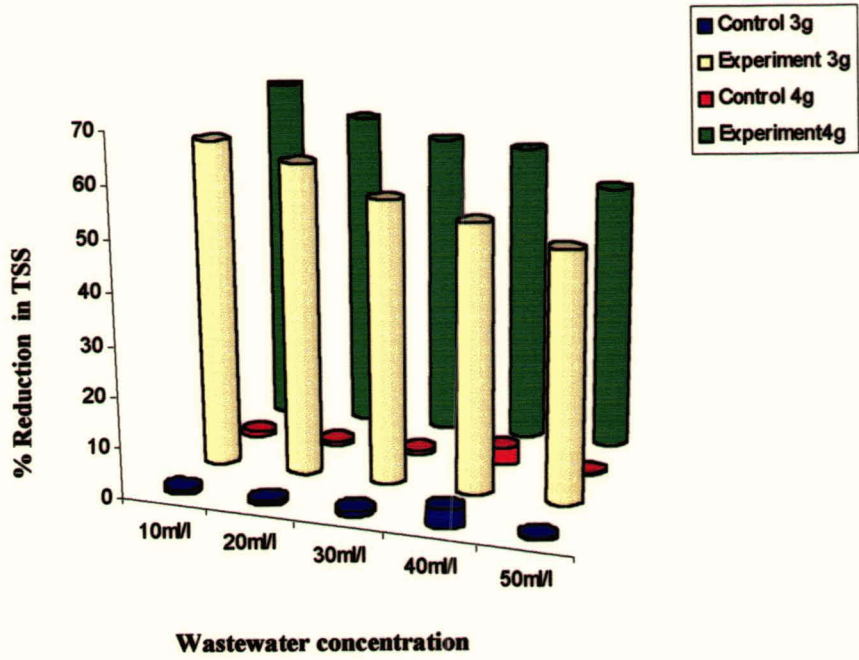


Fig. 31 Percentage Reduction of TSS in Experimental Sets Treated with *Cirrhinus mrigala* Hamilton

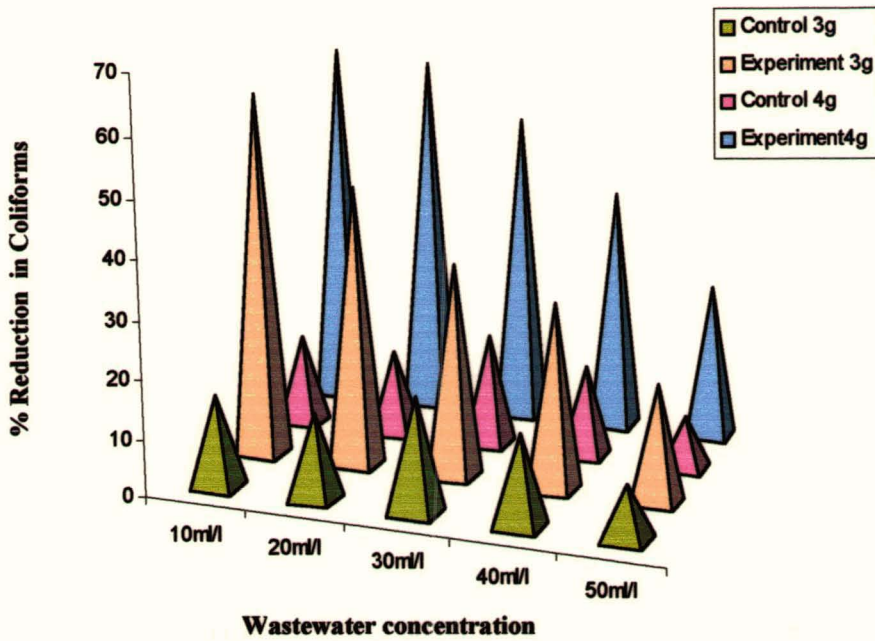


Fig. 32 Percentage Reduction of Coliforms in Experimental Sets Treated with *Cirrhinus mrigala* Hamilton

26.66 percent for the various experimental wastewaters during the treatment period, while 62.5, 48, 36.7, 31.81 and 20 percent reduction was observed with 3 g/l fish. The maximum removal in control was 19.35 percent (Fig.32).

Electrical conductivity and pH showed slightly elevated values for both the biomass in all experiments. Variations in temperature was negligible. The presence of fish increased nitrate and phosphate concentrations in the experimental wastewater. Hardness, calcium and magnesium also showed slight increase, with respect to control.

In this study the potential of two invertebrates - *Paramecium caudatum* and *Daphnia magna* as well as six fishes, *Tilapia mossambica*, *Cyprinus carpio*, *Catla catla*, *Labeo rohita*, *Cirrhinus mrigala* and *Lebistes reticulatus* for the treatment of experimental wastewaters was determined under laboratory conditions for a treatment period of seven days. It was observed that invertebrates were more efficient in the removal of coliform bacteria than fishes.

Among the invertebrates, *Daphnia magna* was found to be more efficient than *Paramecium caudatum* in the reduction in coliform count (maximum of 98.25 percent compared to 61.66 percent in *Paramecium*); BOD (54.53 compared to 24.07 percent in *Paramecium*); COD (52.7 percent compared to 36 percent in *Paramecium*) and TSS (94 percent compared to 88 percent in *Paramecium*).

BOD reductions were maximum in *Tilapia* (59.1 percent) followed by *Cyprinus* (61.48 percent), *Catla* (48.5 percent), Mrigal (43 percent), Rohu (39.2 percent) and *Lebistes* (38.5 percent).

Suspended solids removal was most efficient in *Tilapia* (91.3 percent) and *Cyprinus* (83 percent). Among other fishes suspended solids reduction was 69.9

percent in Mrigal, 50.3 percent in *Catla*, 48.3 percent in Rohu and 39.2 percent in *Lebistes*.

COD reduction was highest in *Tilapia* (57.2 percent), followed by *Cyprinus* (51.6 percent), *Cirrhinus* (49.7 percent), *Labeo* (48.3 percent), *Catla* (42.3 percent) and *Lebistes* (36.4 percent).

Ability to reduce coliform counts from the experimental wastewater was observed in the order of *Tilapia* (82.08 percent), *Catla* (79.06 percent), *Cyprinus* (74.41 percent), *Cirrhinus* (64.06 percent), *Labeo* (53.48 percent) and *Lebistes* (48.4 percent).

Among the fishes studied, ability to improve water quality in decreasing order was *Tilapia*, *Cyprinus*, *Catla*, *Cirrhinus*, *Labeo* and *Lebistes*. When the two biomass 3g/l and 4g/l were considered, 4 g/l fish gave better results. *Tilapia* was more efficient in the removal of TSS, coliforms, BOD and COD values, when compared to other fishes.

4. 2 TREATMENT STUDIES WITH AQUATIC FLORA

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4. 2 TREATMENT STUDIES USING AQUATIC FLORA

4. 2. 1 General

The capability of plants to assimilate nutrients, including nitrates and phosphates from sewage effluents has been recognized in the recent years. The utilization of aquatic plants for wastewater treatment is in various stages of experimentation. Their potential to absorb, translocate and metabolise or accumulate various chemicals is being put into use in commercial wastewater treatment. The major components of such biological systems are the aquatic macrophytes, selected on the basis of their potential to scavenge and accumulate nutrients and pollutants. Voluminous work has been done on water hyacinth, *Eichhornia crassipes* (Mart.) Solms. Hence the present study has been devoted to the exploration of the potential of lesser known aquatic flora for wastewater treatment. The efficiency of different macrophytes needs to be studied with respect to the local climatic conditions, before they can be incorporated into aquatic treatment systems.

In the present study, experiments were conducted using selected locally available aquatic flora (several of them designated as weeds) to study their potential to remediate wastewaters. The flora experimented include the floating plants *Azolla rubra* R.Br., *Salvinia molesta* Mitchell, *Pistia stratiotes* L., *Lemna perpusilla* Torrey, *Wolffia globosa* Roxb. and *Spirodela polyrrhiza* L. Schleid; submerged plants *Hydrilla verticillata* (L.f.) Royle and *Ceratophyllum demersum* L. as well as *Lagenandra toxicaria* Dalz., an emergent plant.

Aquatic plants have been used in wastewater treatment for the removal of nutrients, chemicals and metals. The mechanisms for contaminant removal in

these systems may be complex involving physiological characteristics of the plants, biological and physicochemical reactions in the aquatic environment. Although the storage of absorbed nutrients is short term, many aquatic plants have high nutrient uptake rates. Floating aquatic plants also have the capability to assimilate large quantities of trace elements, some of which are essential for plant growth, thereby improving the water quality (Reddy and De Busk, 1987). In submerged plants absorption is also facilitated by the permeability of the unthickened cellulose walls and often absence of cuticle. Even when it does occur offers little resistance to diffusing substances (Sculthorpe, 1967)

Macrophytes aid in wastewater treatment by facilitating physical sedimentation and bacterial metabolic activity. The roots and stems provide surface for bacterial growth and are media for filtration and adsorption of solids. The stem and leaves prevent growth of suspended algae, reduce the effect of wind on water and transfer oxygen from leaves to root tips (Stowell *et al.* 1980). Plants remove nutrients such as nitrates and phosphate by directly assimilating them into their tissue and on harvest remove them permanently from the water body.

Organic carbon, typically measured as BOD₅ is utilized by bacteria as an energy source and for cell synthesis. These bacteria inhabit microenvironments in the plant root zone and may also be dispersed throughout the water column. Presence of aquatic plants thus helps in reducing the BOD₅. An anatomical adaptation of aquatic plants is the development of aerenchyma cell structure which facilitates the exchange of oxygen from aerial tissue into the root zone (Moorhead and Reddy, 1988 and Armstrong, 1964). This oxygen if not consumed

during root respiration can enter the water column and be utilized by the aerobic bacteria for oxidation of organic carbon (Reddy and De Busk,1987).

Suspended and colloidal solids are removed as a result of collisions (inertial and Brownian) with an adsorption to plant parts such as stem and roots (Stowell *et al.* 1980). Particulates are filtered mechanically as water passes through the roots. Ultimate removal of suspended solids will be by bacterial metabolism, *i.e.*, aerobic decay of solids entangled in the surfaces of vegetation (Polprasert, 1996)

Nitrogen is removed from wastewater by a number of mechanisms including uptake by plants and subsequent harvesting of them, volatilisation of ammonia, adsorption as well as bacterial nitrification and denitrification (Reed *et al.* 1988). Plant uptake will not represent permanent removal, unless the plants are routinely harvested. Aquatic plants provide surfaces for the attachment, in order to maintain significant populations of the slowly reproducing nitrifying bacteria in an aquatic system. Under conditions where dissolved organic carbon is not limiting, the factor most limiting nitrogen removal is the supply of oxygen which is necessary to sustain nitrification. In this regard, the ability of an aquatic plant to translocate oxygen from the shoot to root, and thereby establish an oxidized rhizosphere, is an important factor (Wood, 1990).

Phosphorous removal mechanisms in aquatic systems are plant uptake, chemical adsorption and precipitation reactions (Polprasert, 1996). Aquatic plants supplied with sewage effluents tend to show increased growth and usually have increased tissue phosphorous concentration (Wood, 1990). Richardson (1985) suggests that between 35-75 percent of the plant phosphorous is rapidly released

on plant senescence. Thus vegetation only serves as a short-term sink for phosphorous unless the biomass is harvested as in the case of nitrogen.

The major pathway for removal of sulphates is plant uptake. The presence of sulphates or sulphites in wastewater can lead to odour problems if anaerobic conditions develop. Sulphate is quite rapidly taken up by roots, translocated to leaf chloroplast where it is reduced and thus incorporated into organic compounds or accumulated in the vacuoles.

Plant uptake of calcium and magnesium reduces its concentration, as well as the total hardness of water in aquatic systems.

Aquatic systems offer a unique combination of physical, chemical and biological factors that contribute to inactivation and removal of both pathogenic viruses and bacteria. In addition to filtration through the root substrate and attached biofilm, physical removal factors include sedimentation, aggregation and inactivation by UV radiation. Chemical factors include oxidation, exposure to biocides which may be excreted by plants (Polprasert, 1996) and adsorption to organic matter (Wood, 1990). Ingestion by nematodes or ciliates, attack by lytic bacteria are other causes (Gersberg *et al.* 1987). Aquatic macrophytes such as water hyacinth, *Pistia*, *Lagenandra* and *Ceratophyllum* have antimicrobial properties (Agarwal, 1997) and this could aid in reducing bacterial counts.

4. 2. 2 Floating plants

1. *Salvinia molesta* Mitchell

Salvinia is one of the most tenacious aquatic weed causing ecological and economic losses in Kerala. Its infestation is widespread and the biomass doubling time is 4-10 days under favourable growing conditions, making it an ideal

candidate for wastewater treatment. The studies using *Salvinia* for a detention period of seven days revealed its potential for improving water quality of experimental wastewater by bringing about noticeable decrease in BOD, COD, TSS, coliforms, nitrate and phosphate. The physicochemical and bacteriological characteristics of the experimental sets are presented in Table 25.

BOD decreased in the *Salvinia* treated experimental wastewaters upto a maximum of 67.8 percent for the seven day period. It was observed that the BOD removal was 53.4, 59.2, 62.5 and 67.8 percent respectively for 50, 100, 150 and 200 ml wastewater experimental set and then decreased to 56.8 % in the highest wastewater concentration, 250 ml/l. the reduction in BOD with the test plant *Salvinia* was comparatively greater than the control (without *Salvinia*) for all wastewater concentrations, with a removal of 7.25 percent for the control set. Fig.33 represents the percentage reduction in BOD with *Salvinia*.

COD reductions upto 26.4 percent was recorded in the study. 12.1, 17.7, 20.8, 24.5 and 26.4 percent reduction was observed in 50-250 ml experimental wastewaters, while in the control, a maximum of 5.07 percent was obtained. Fig.34 represents the percentage reduction in COD.

Total suspended solids were lower than that of control in *Salvinia* treated experimental wastewater. There was a steady increase in TSS removal in the five wastewater concentrations experimented (19.3, 21.7, 27.2, 38.8 and 42.5 percent) with maximum reduction in 250 ml wastewater/litre (Fig.35). Control had a maximum reduction of 7.24 percent for the seven day detention period. The submerged leaves, which are modified to form root like structures, are very extensive and help in the settling of suspended solids.

Table 25 Experimental Sets Treated with *Salvinia molesta* Mitchell

Period of Treatment -7 days
Weight - 5 g/l

E- Experiment
C- Control

Parameters	50 ml Wastewater/l			100 ml Wastewater/l			150 ml Wastewater/l			200 ml Wastewater/l			250 ml Wastewater/l		
	Before	After		Before	After		Before	After		Before	After		Before	After	
		E	C		E	C		E	C		E	C			
Temp	27.40	27.20	27.60	27.80	27.50	27.80	28.00	27.80	28.10	28.20	27.50	28.30	28.10	27.80	28.10
pH	7.25	8.20	7.28	7.38	8.25	7.38	7.45	8.90	7.45	7.62	8.30	7.63	7.73	8.40	7.72
EC	126.30	105.40	126.10	163.20	142.30	160.80	235.60	217.20	236.10	309.10	295.30	308.40	359	345.40	360.30
TSS	30.30	29.17	29.70	45.60	35.70	42.30	57.40	41.79	56.00	76.45	46.79	75.40	98.30	56.52	97.10
DO	2.81	4.00	2.92	1.20	3.20	3.40	0	1.98	0.24	0	1.68	0.16	0	0.53	0.10
BOD	13.10	6.10	12.60	19.30	7.87	17.90	24.60	9.22	23.80	32.20	10.37	30.20	40.30	17.40	39.60
COD	33.50	29.45	31.80	55.14	45.38	52.60	79.35	62.84	75.80	100.60	75.95	98.30	118.50	87.22	115.70
NO ₃ -N	1.06	0.58	1.01	2.41	1.21	2.32	4.87	2.32	4.80	6.37	2.78	5.93	8.12	3.06	8.00
PO ₄ -P	0.80	0.51	0.65	2.20	1.91	2.00	4.80	2.53	4.47	5.90	2.81	4.90	7.20	3.64	6.90
SO ₄	2.00	1.01	1.90	3.40	1.61	3.50	5.80	2.31	5.60	6.20	3.02	6.30	9.60	5.03	9.50
TH	56.00	48.00	52.00	72.00	52.00	74.00	96.00	84.00	92.00	104.00	96.00	100.00	120.00	108.00	112.00
Ca	13.06	9.60	12.80	22.40	12.80	22.40	27.20	22.80	14.40	23.60	27.20	20.30	38.20	22.40	28.80
Mg	5.66	4.20	3.90	3.88	3.90	3.88	7.76	6.40	3.49	10.20	7.77	4.91	12.80	3.88	6.40
T.C	15 x10 ⁵	23x10 ⁴	14x10 ⁵	24 x10 ⁵	57x10 ⁴	23x10 ⁵	64 x10 ⁵	23x10 ⁵	59x10 ⁵	93 x10 ⁵	36x10 ⁵	91x10 ⁵	19 x10 ⁶	80x10 ⁵	18x10 ⁶
F.C	+ ve	-ve	+ ve	+ ve	-ve	+ ve	+ ve	-ve	+ ve	+ ve	+ve	+ ve	+ ve	+ ve	+ ve
E.Coli	+ ve	-ve	+ ve	+ ve	-ve	+ ve	+ ve	-ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve

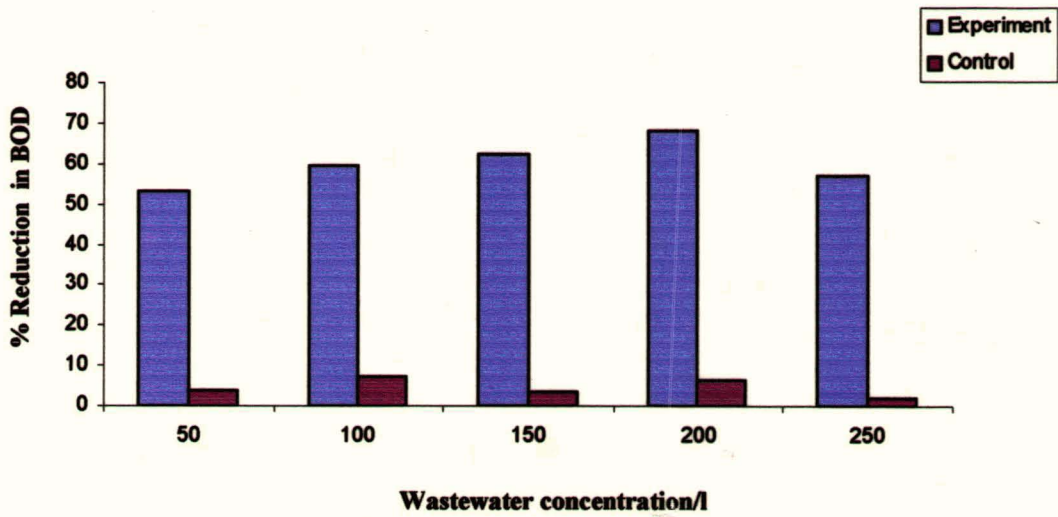


Fig. 33 Percentage Reduction of BOD in Experimental Sets Treated with *Salvinia molesta* Mitchell

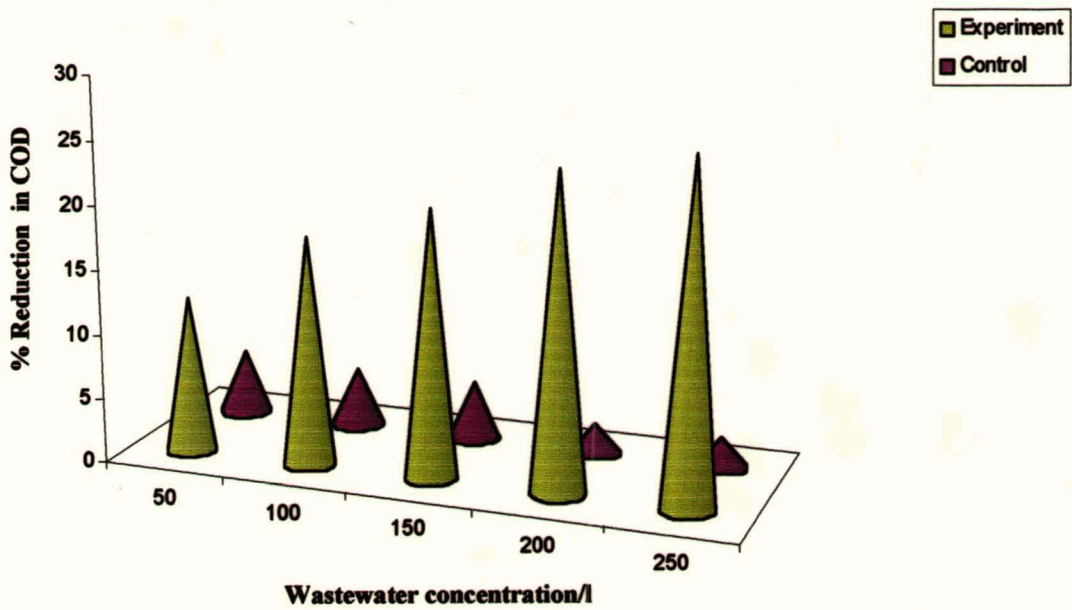


Fig.34 Percentage Reduction of COD in Experimental Sets Treated with *Salvinia molesta* Mitchell

In the studies using *Salvinia*, coliforms removal was most pronounced at 84.6 percent in 50 ml wastewater/ litre. Higher wastewater concentrations showed 76.2, 63.6, 61.2 and 57.8 percent reduction for 100-250 ml wastewater/litre. Control for these experiments had a maximum reduction of 7.8 percent only. Faecal coliforms and *E. coli* were absent in the experimental sets with *Salvinia* in 50, 100 and 150 ml wastewater. Fig.36 represents the percentage reduction in total coliforms.

Nitrate nitrogen was reduced by a maximum of 62.3 percent using *Salvinia*. The percentage reductions for various experimental wastewaters are 45.3, 49.8, 52.3 56.4 and 62.3 percent for 50-250 ml wastewater/ litre. Control for these experiments had reduction upto 6.9 percent only. Fig.37 represents the reduction in nitrate nitrogen using *Salvinia*

Phosphate attenuation upto 52.3 percent in the experimental sets. There was an increase in phosphate removal, 36.2, 40.3, 47.3 and 52.3 percent in 50-200 ml wastewater/litre. However, phosphate removal by *Salvinia* decreased to 49.4 percent in 250 ml wastewater experiment set. Fig.38 represents the reduction in phosphates.

Sulphate showed a decrease over control probably due to plant uptake. A maximum reduction of 60.2 percent was observed in 150 ml wastewater concentration. Other experimental sets had 49.5, 52.6, 51.2 and 47 percent for 50-250 ml wastewater/litre.

Hardness, calcium and magnesium showed a decrease when compared to control, due to plant uptake. There was no variation in temperature, while pH showed slightly elevated values due to the presence of *Salvinia*. Dissolved oxygen

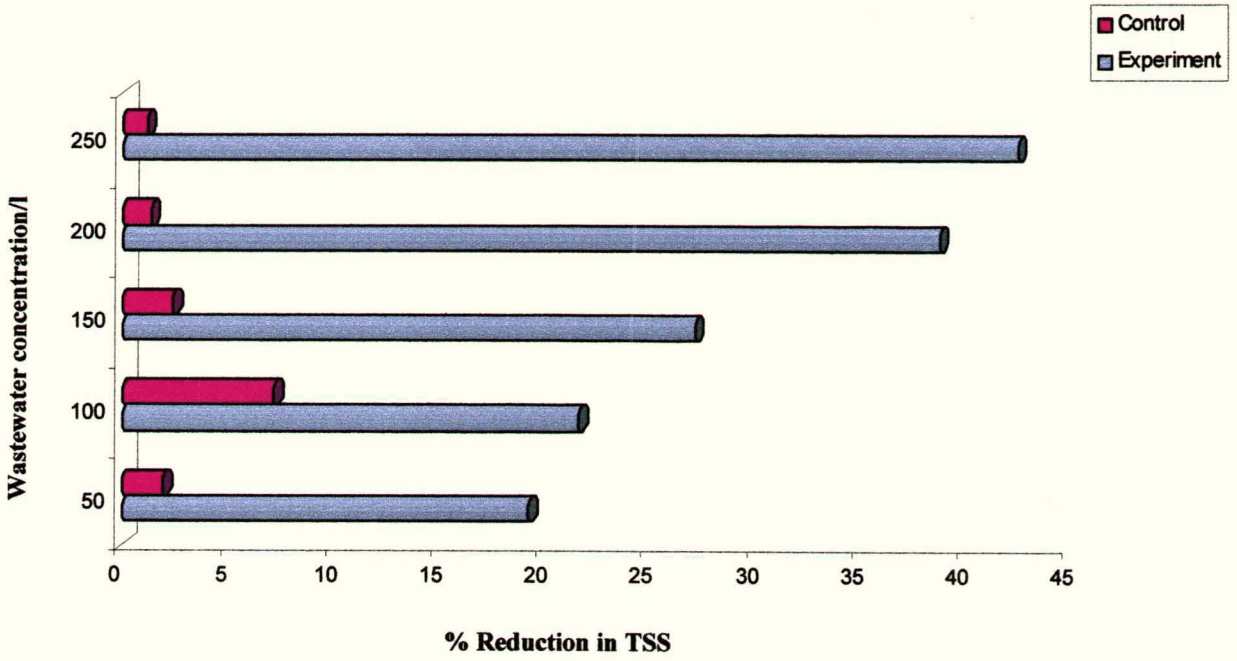


Fig. 35 Percentage Reduction of TSS in Experimental Sets Treated with *Salvinia molesta* Mitchell

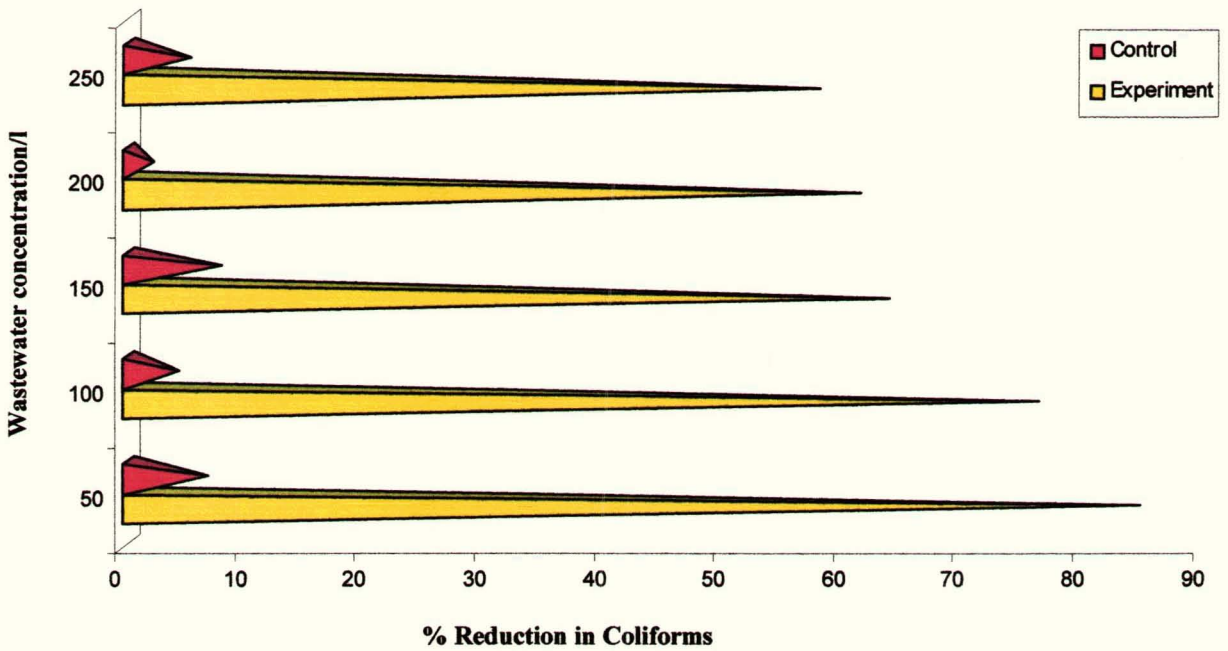


Fig. 36 Percentage Reduction of Coliforms in Experimental Sets Treated with *Salvinia molesta* Mitchell

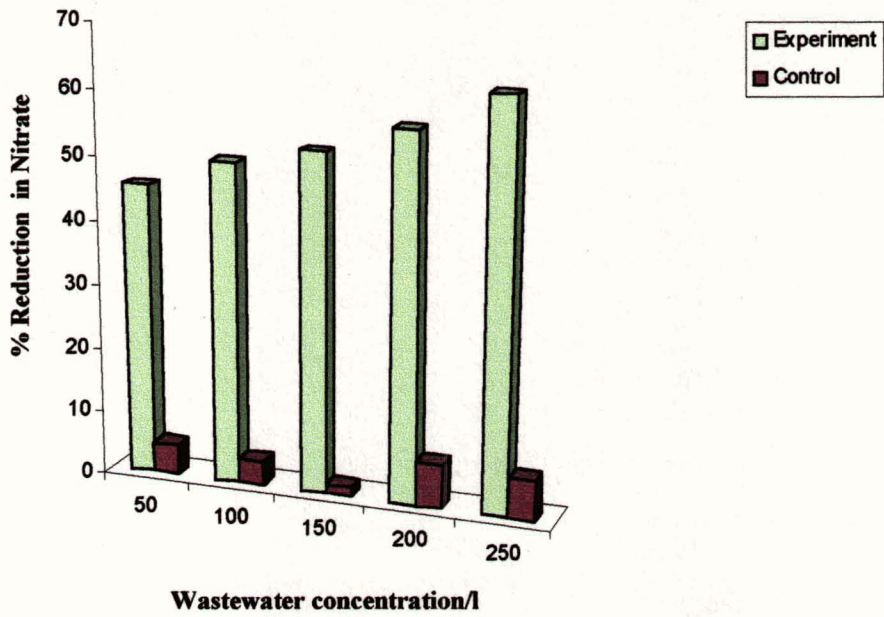


Fig. 37 Percentage Reduction of Nitrate in Experimental Sets Treated with *Salvinia molesta* Mitchell

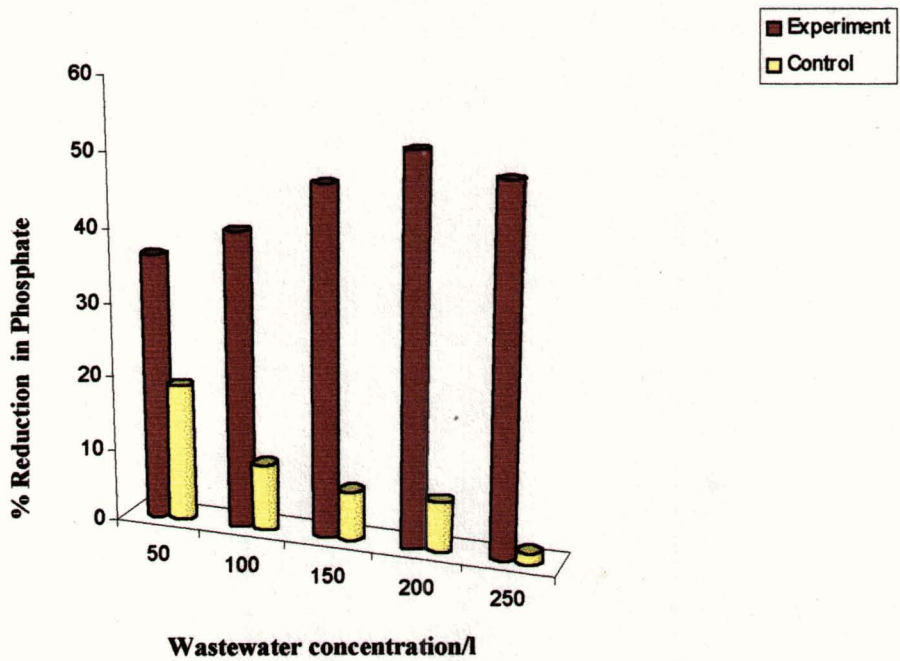


Fig. 38 Percentage Reduction of Phosphate in Experimental Sets Treated with *Salvinia molesta* Mitchell

showed a marginal increase in the experimental sets. This could be attributed to the diffusion of oxygen from the roots into water, in addition to the diffusion at the air water interface.

2. *Azolla rubra* R.Br.

Azolla, which is already in use as a biofertiliser, holds potential for bioremoval of nutrients, BOD, total suspended solids and coliforms to a good extent. Studies conducted for treatment period of seven days brought about an improvement in the quality of experimental wastewaters. Table 26 presents the variations in physicochemical and bacteriological parameters for the treatment period.

BOD decreased in *Azolla* treated experimental wastewaters to a maximum of 64 percent. It was noticed that maximum removal occurred in experimental sets with 150 ml wastewater/l experimental set. In higher concentrations above 150 ml/l a decreasing trend in BOD removal was observed. The reductions in BOD for 50-250 ml wastewater/l experimental sets are 55, 59, 64, 62 and 58.3 percent. The reduction in BOD due to presence of *Azolla* was greater than control, which had a maximum of 8.94 percent reduction, among various experimental sets. Fig.39 represents the reduction in BOD using *Azolla*.

COD reduction of 4.3, 5.4, 7.6, 8.2 and 9.8 percent was obtained for 50-250 ml wastewater/l experimental sets, while control had 2.85, 4.6, 6.68, 4.17 and 2.6 percent reduction. Fig.40 represents the reduction in COD using *Azolla*.

Total suspended solids reduced by 36.3 percent in experiments with *Azolla*. 22.1, 25.2, 29.8, 32.4 and 36.3 percent reduction for 50-250 ml wastewater

Table 26 Experimental Sets Treated with *Azolla rubra* R. Br.

Period of Treatment -7 days
Weight- 2 g/l

E- Experiment
C- Control

Parameters	50 ml Wastewater/l			100 ml Wastewater/l			150 ml Wastewater/l			200 ml Wastewater/l			250 ml Wastewater/l		
	Before	After		Before	After		Before	After		Before	After		Before	After	
		E	C		E	C		E	C		E	C			
Temp	27.40	27.00	27.60	27.80	27.90	27.80	28.00	27.90	28.10	28.20	28.10	28.30	28.10	28.00	28.10
pH	7.25	7.27	7.28	7.38	8.00	7.38	7.45	7.49	7.45	7.62	7.85	7.63	7.73	7.89	7.72
EC	126.30	104.40	126.10	163.20	144.20	160.80	235.60	219.50	236.10	309.10	300.50	308.40	359.00	347.10	360.30
TSS	30.30	23.60	29.00	45.60	34.11	42.30	57.40	40.29	56.20	76.45	51.68	75.30	98.30	62.62	97.00
DO	2.81	4.30	2.92	1.20	3.85	3.40	0	1.81	0.24	0	1.53	0.16	0	1.20	0.10
BOD	13.10	5.89	12.00	19.30	7.91	18.00	24.60	8.86	22.40	32.20	12.24	30.60	40.30	16.80	38.40
COD	33.50	32.54	30.20	55.14	52.16	52.60	79.35	73.32	74.00	100.60	92.35	96.40	118.50	106.90	115.40
NO ₃ -N	1.06	0.74	1.00	2.41	1.54	2.35	4.87	3.05	4.82	6.37	3.82	6.00	8.12	4.85	7.90
PO ₄ -P	0.80	0.58	0.73	2.20	2.11	2.13	4.80	2.95	4.60	5.90	3.46	5.78	7.20	3.72	6.80
SO ₄	2.00	1.05	1.90	3.40	1.68	3.50	5.80	2.71	5.60	6.20	3.40	6.30	9.60	5.51	9.50
TH	56.00	48.00	52.00	72.00	52.00	72.00	96.00	80.00	92.00	104.00	96.00	100.00	120.00	108.00	112.00
Ca	13.06	9.60	12.80	22.40	12.80	22.40	27.20	20.80	14.40	23.60	27.20	20.30	38.20	22.40	28.80
Mg	5.66	4.20	3.90	3.88	3.90	3.88	7.76	6.80	3.49	10.20	7.77	4.91	12.80	3.88	6.40
T.C	15 x10 ⁵	77 x10 ⁴	14 x10 ⁵	24 x10 ⁵	13 x10 ⁵	23 x10 ⁵	64 x10 ⁵	39 x10 ⁵	63 x10 ⁵	93 x10 ⁵	62 x10 ⁵	91 x10 ⁵	19 x10 ⁶	13 x10 ⁶	18 x10 ⁶
F.C	+ ve	-ve	+ ve	+ ve	-ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
E.Coli	+ ve	-ve	+ ve	+ ve	-ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve

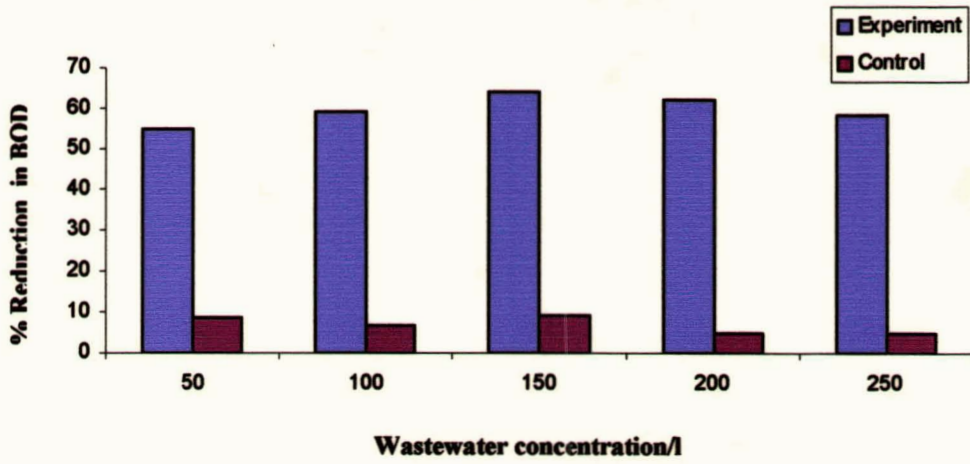


Fig. 39 Percentage Reduction of BOD in Experimental Sets Treated with *Azolla Rubra* R. Br.

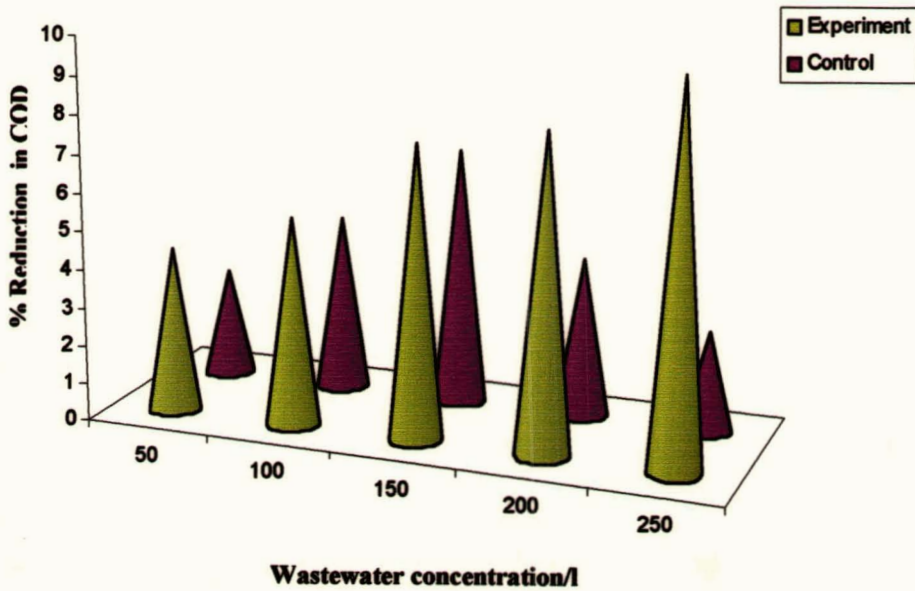


Fig. 40 Percentage Reduction of COD in Experimental Sets Treated with *Azolla Rubra* R. Br.

concentration was recorded. Control sets had a maximum of 7.23 percent reduction. Fig.41 represents the reduction in TSS using *Azolla*.

Coliform count declined by 48.6, 45.8, 39.1, 33.3 and 31.6 percent in the experimental sets with 50-250 ml wastewater/l. Maximum reduction was recorded in least wastewater concentration, 48.6 percent, while maximum reduction for control was 7.14 percent. Fig.42 represents the reduction in coliforms using *Azolla*. Fecal coliforms and *E.coli* were absent in the experimental sets with *Azolla* upto 100 ml wastewater per liter. *E.coli* was absent in all the experimental wastewaters with *Azolla* with the exception of 250ml/l wastewater concentration.

Nitrate nitrogen removal for the various experimental sets was 30.18, 36.1, 37.3, 40 and 40.2 percent. Control had a maximum removal of 5.8 percent. Phosphate removal was 27.5, 34, 38.5, 41.3, and 48.6 percent. Control had maximum reduction of 8.75 percent. Fig 43 and 44 represent the reduction in nitrate nitrogen and phosphate using *Azolla*.

There was a decrease in electrical conductivity, calcium, magnesium and hardness in the experiments using *Azolla*. Temperature and pH did not show much variation. Dissolved oxygen levels increased in the experimental sets, when compared to controls.

3. *Pistia stratiotes* L.

Pistia is a beautiful aquatic macrophyte that propagates by offsets and buds, covering the surface of water bodies rapidly. *Pistia* is seen in various localities of Kerala in the form of dense mats. *Pistia* revealed excellent potential for removal of nutrients, reducing BOD, COD and suspended solids as well as coliforms in the seven day treatment study. pH showed an increase while

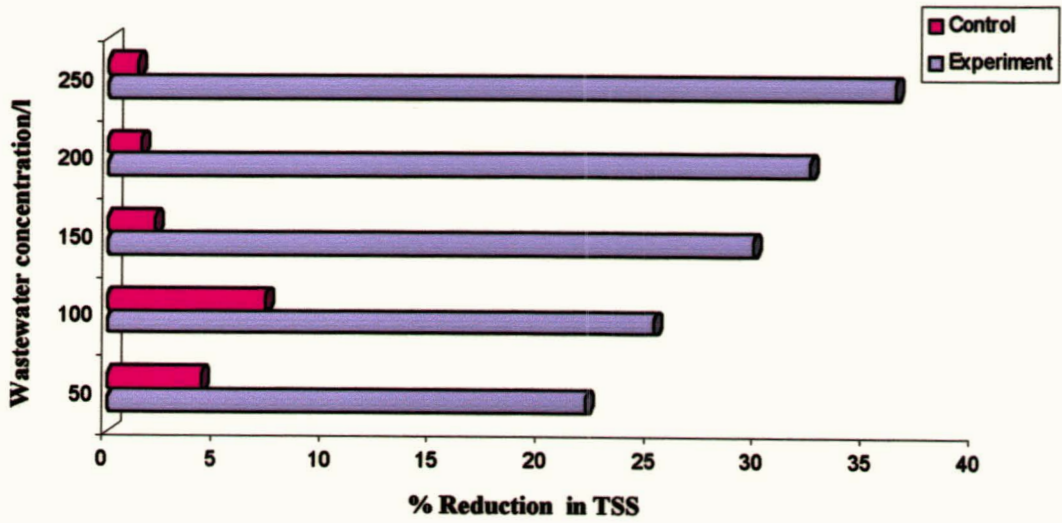


Fig. 41 Percentage Reduction of TSS in Experimental Sets Treated with *Azolla Rubra* R. Br.

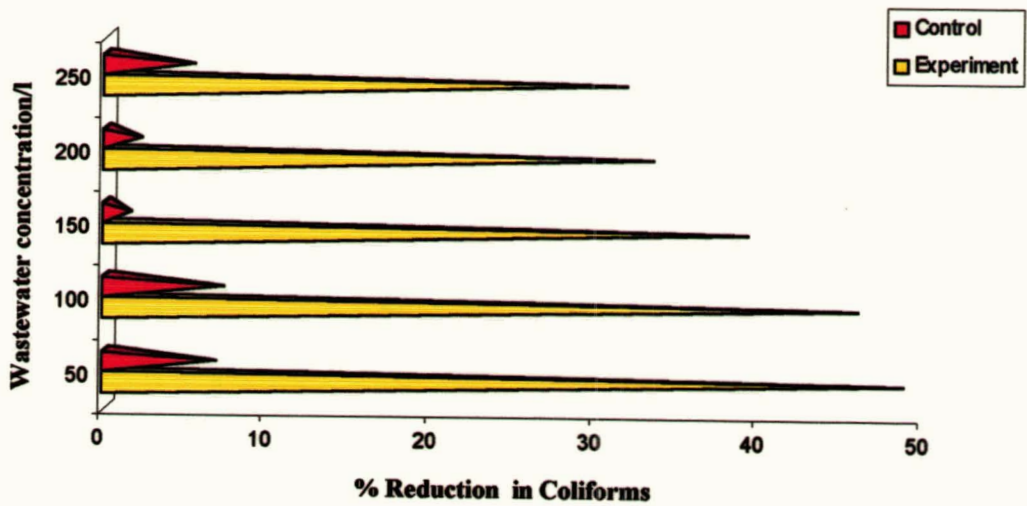


Fig. 42 Percentage Reduction of Coliforms in Experimental Sets Treated with *Azolla Rubra* R. Br.

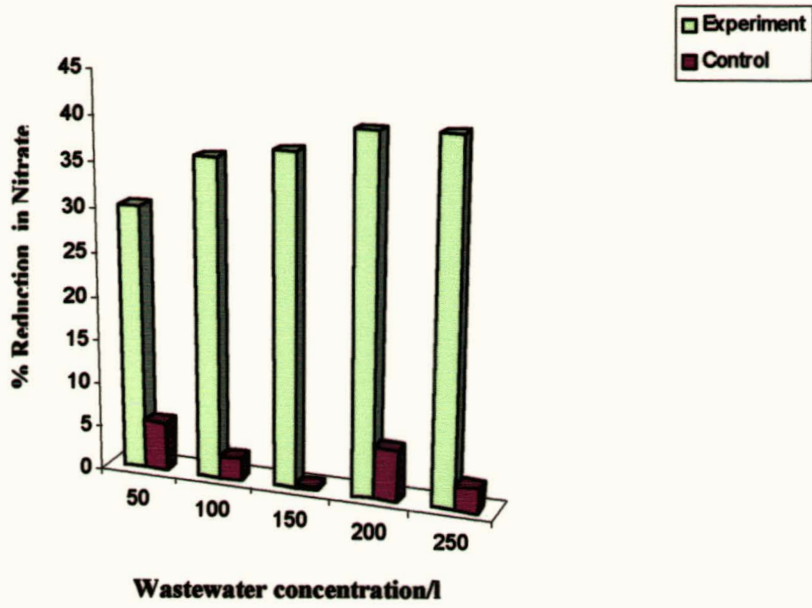


Fig. 43 Percentage Reduction of Nitrate in Experimental Sets Treated with *Azolla Rubra* R. Br.

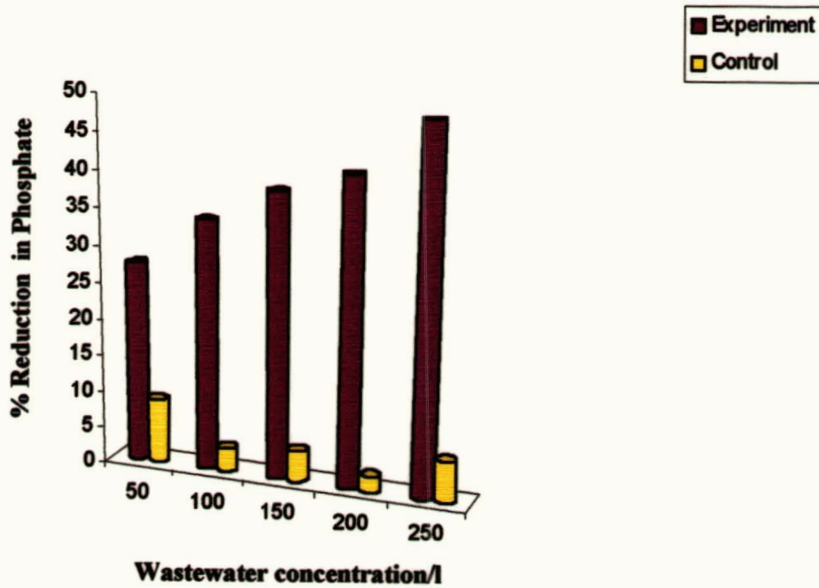


Fig. 44 Percentage Reduction of Phosphate in Experimental Sets Treated with *Azolla Rubra* R. Br.

electrical conductivity decreased in all the wastewater experimental sets, when compared to the respective control. Dissolved oxygen improved from the initial concentration, which could be due to transport of oxygen to the plant roots and reduction in BOD. The physicochemical and bacteriological characteristics of the experimental waters is presented in Table 27.

Pistia was capable of reducing the BOD of experimental wastewater upto a maximum of 75 percent, while control had only 7.25 percent reduction. The reduction in BOD was 62, 64, 72, 75 and 70 percent for 50-250 ml wastewater concentrations. The extensive root system of *Pistia* helps in adsorption of colloidal and soluble matter contributing to BOD and COD. The metabolic activity of microorganisms and physicochemical interactions within the root zone also reduce BOD to a great extent. Fig.45 represents the percentage reduction in BOD in experiments with *Pistia*.

COD reduction was in the range of 28-42 percent for various wastewater concentrations experimented. 28.8, 31.7, 36.6, 39.2 and 42 percent reductions were obtained for 50-250 ml wastewater/litre. Fig.46 represents the percentage reduction in COD.

Substantial reduction in total suspended solids was observed in *Pistia* treated experimental sets. An increasing trend in the TSS removal with increasing wastewater concentrations was observed (29.5, 45.4, 49.1 and 57.6), reaching a maximum of 66.4 percent in 250 ml wastewater/litre (Fig.47). Control had a maximum of 2.1 percent reduction. The removal was probably due to the effect of the root network, which helps in adsorption of suspended solids.

Table 27 Experimental Sets Treated with *Pistia stratoites* L.

Period of Treatment -7 days
Weight -5 g/l

E- Experiment
C- Control

Parameters	50 ml Wastewater/l			100 ml Wastewater/l			150 ml Wastewater/l			200 ml Wastewater/l			250 ml Wastewater/l		
	Before	After		Before	After		Before	After		Before	After		Before	After	
		E	C		E	C		E	C		E	C			
Temp	27.40	27.20	27.60	27.80	28.50	27.80	28.00	27.30	28.10	28.20	27.60	28.30	28.00	27.70	28.10
pH	7.25	8.13	7.28	7.38	8.17	7.38	7.45	8.20	7.45	7.62	8.29	7.63	7.73	8.35	7.72
EC	126.30	106.81	126.10	163.20	145.70	163.60	235.60	214.80	236.10	309.10	292.40	308.40	359.00	340.40	360.30
TSS	30.30	21.36	29.70	45.60	24.90	44.60	57.40	29.21	56.90	76.45	32.41	75.91	98.30	33.04	97.81
DO	2.80	4.60	2.90	1.20	3.30	1.05	0	2.00	0	0	1.96	0.10	0	0.84	0.10
BOD	13.10	4.98	12.60	19.30	6.95	17.90	24.60	6.89	23.80	32.20	8.05	30.20	40.30	12.09	39.60
COD	33.50	23.85	30.20	55.14	37.66	53.40	79.35	50.31	74.60	100.60	6.16	95.30	118.50	68.73	114.80
NO ₃ -N	1.06	0.42	1.00	2.41	0.84	2.35	4.87	1.47	4.82	6.37	1.45	6.00	8.12	2.23	7.90
PO ₄ -P	0.80	0.48	0.72	2.20	1.26	2.10	4.80	2.48	3.92	5.90	2.63	4.80	7.20	3.48	6.80
SO ₄	2.00	0.95	1.90	3.40	1.21	3.50	5.80	2.19	5.60	6.20	2.80	6.30	9.60	5.51	9.50
TH	56.00	40.00	52.00	72.00	54.00	68.00	96.00	76.00	96.00	104.00	92.00	100.00	120.00	104.00	112.00
Ca	13.06	9.60	12.80	22.40	13.20	15.30	27.20	22.40	27.20	23.60	14.40	20.30	38.20	22.40	28.80
Mg	5.66	3.80	3.90	3.88	3.90	3.80	7.77	3.88	7.76	10.20	3.49	4.91	12.80	4.91	6.40
T.C	15 x10 ⁵	11 x10 ³	14 x10 ⁵	24 x10 ⁵	7 x10 ³	23 x10 ⁵	64 x10 ³	43 x10 ⁵	59 x10 ³	93 x10 ⁵	75 x10 ⁵	91 x10 ⁴	19 x10 ⁵	20 x10 ⁵	18 x10 ⁶
F.C	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
E.Coli	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve

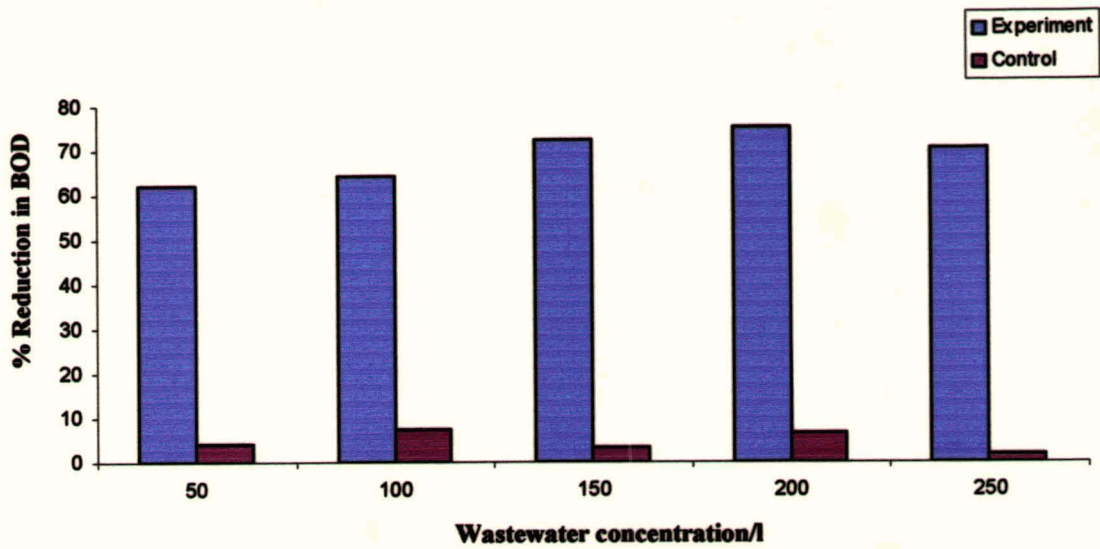


Fig. 45 Percentage Reduction of BOD in Experimental Sets Treated with *Pistia stratiotes* L.

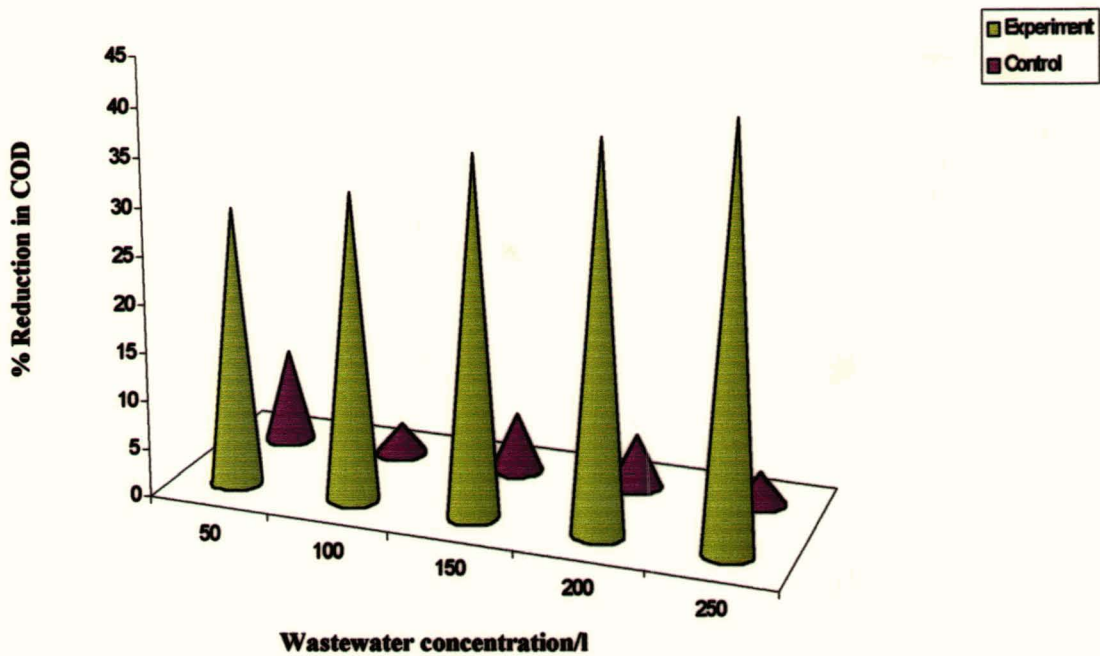


Fig. 46 Percentage Reduction of COD in Experimental Sets Treated with *Pistia stratiotes* L.

Coliform removal was excellent in *Pistia* treated experimental wastewaters, with a maximum of 99.7 percent reduction in 100 ml wastewater /litre. Reduction in the range of 99 percent was consistently obtained in all experimental sets upto 200 ml wastewater /litre. A reduction in the rate of removal of coliforms was noted in experiments with higher wastewater concentrations. Control had a maximum of only 7.81 percent reduction. Fig.48 represents the percentage reduction in coliforms. Fecal coliforms and *E. coli* were absent in *Pistia* treated experimental sets in 50 and 100 ml wastewater concentrations. *E. coli* was absent in all experimental wastewaters except highest concentration of wastewater (250 ml/l).

Removal efficiency for nitrate nitrogen was in the range of 60-77 percent, while control had only a maximum of 5.7 percent reduction. The percentage reductions for various wastewater concentrations studied are 60.2, 65.3, 69.8 77.2 and 72.5 percent for 50-250 ml wastewater/litre. Fig.49 represents the reductions in nitrate nitrogen.

Phosphate uptake was observed to increase with increasing wastewater concentrations, recording maximum at 200 ml wastewater/litre (55.4 percent). With increase in wastewater concentration, there was a decrease in the phosphate removal. Fig.50 represents the percentage reduction in phosphate in *Pistia* treated experiments.

There was a reduction in sulphate upto a maximum of 64 percent in experimental sets with 100 ml wastewater. The percentage reduction in sulphate was 52.6, 64.3, 62.3, 54.8 and 42.6 percent for 50-250 ml wastewater /litre. Hardness, calcium and magnesium also showed decrease with respect to control

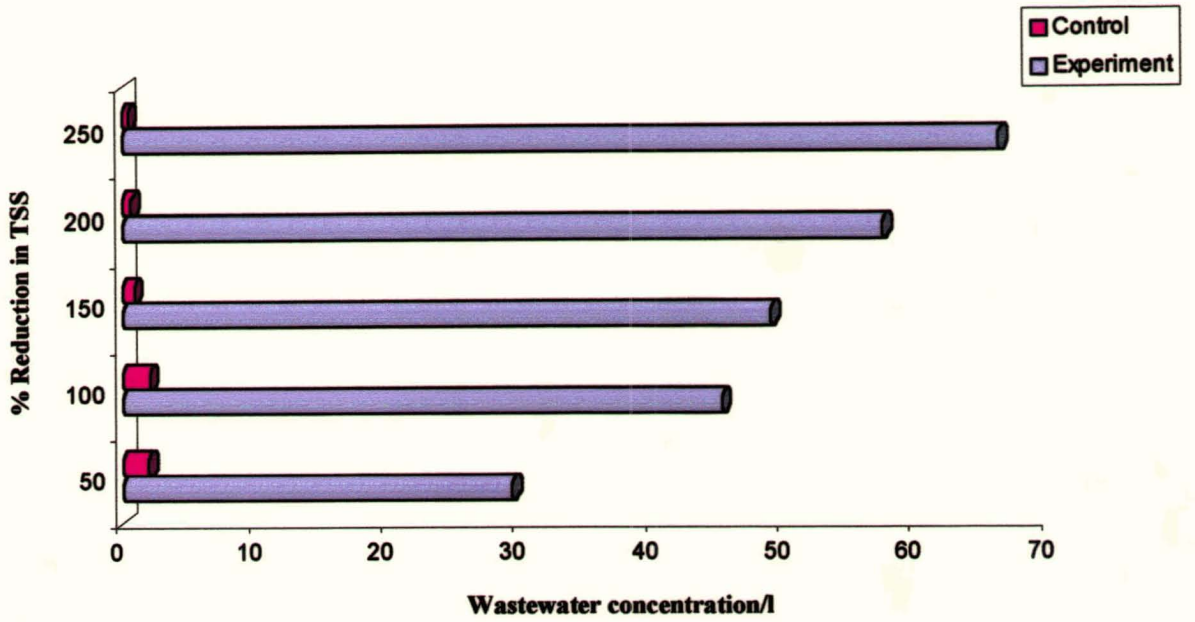


Fig. 47 Percentage Reduction of TSS in Experimental Sets Treated with *Pistia stratiotes* L.

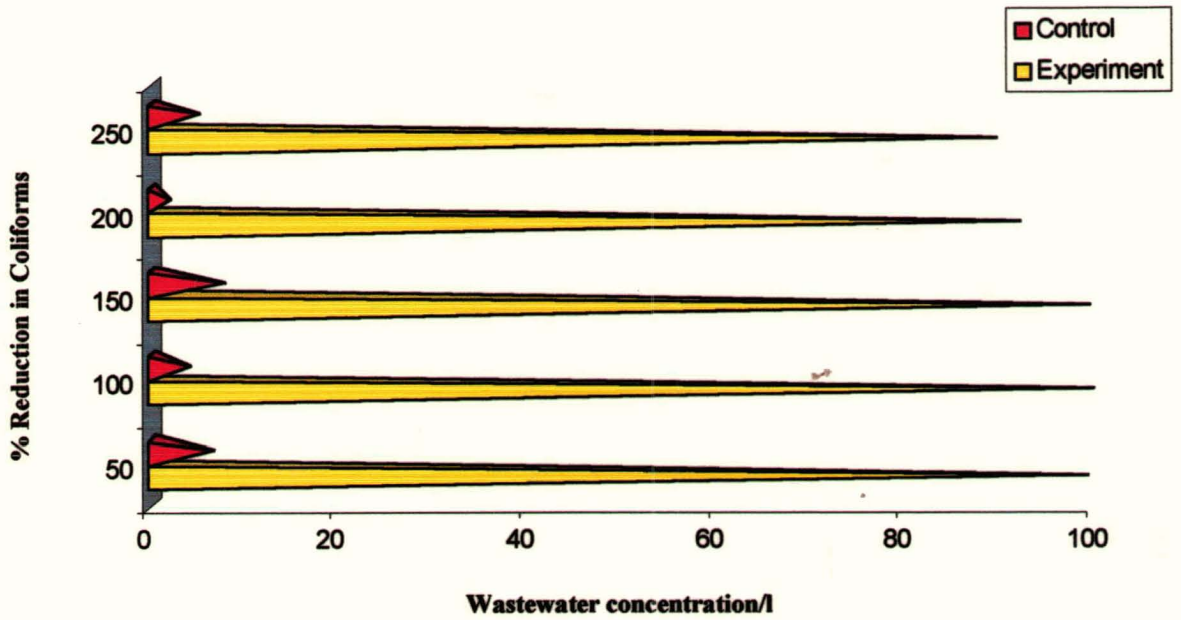


Fig. 48 Percentage Reduction of Coliforms in Experimental Sets Treated with *Pistia stratiotes* L.

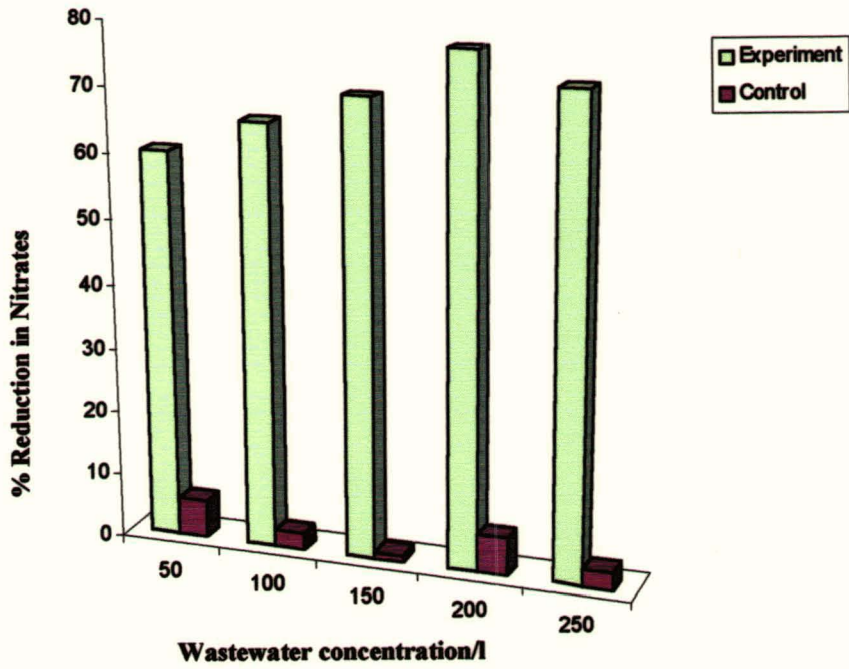


Fig. 49 Percentage Reduction of Nitrate in Experimental Sets Treated with *Pistia stratoites* L.

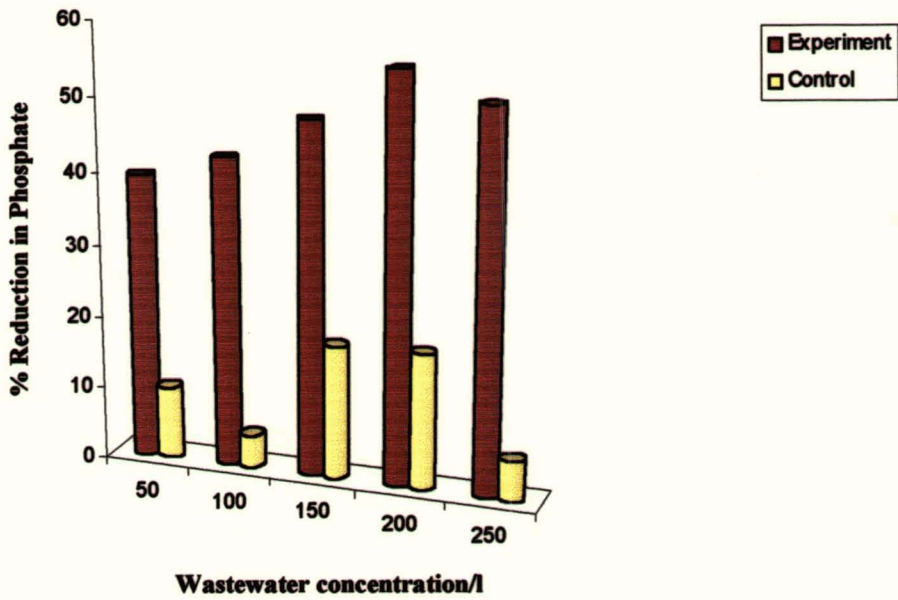


Fig. 50 Percentage Reduction of Phosphate in Experimental Sets Treated with *Pistia stratoites* L.

for all the experiments and this could be due to the plant uptake of calcium and magnesium.

4. *Lemna perpusilla* Torrey

Lemna is the most commonly seen duckweed forming dense mats in stagnant water bodies. *Lemna* holds good potential for use in wastewater treatment systems due to its rapid doubling time and uptake of nutrients. In the present study, results revealed that *Lemna* was efficient in removing nutrients and reducing BOD of experimental wastewaters. The physicochemical and bacteriological characteristics of the experimental waters is presented in Table 28.

Maximum reduction of BOD in the experimental sets with *Lemna* was 82.6 percent in various wastewater concentrations. 56.3, 67.8, 72.7, 79.4 and 82.6 percent reduction in BOD was obtained for 50-250 ml wastewater /litre experimental sets. Fig.51 represents the percentage reduction in BOD. *Lemna gibba* has been utilized to treat municipal wastewater in Israel and a BOD removal 97 percent has been reported (Oron and Porath, 1987).

COD removal upto 46 percent was obtained in the treatment study. There was a gradual increase in the percentage removal of COD with increasing wastewater concentrations upto 200 ml/l (Fig.52). The reductions obtained are 34.5, 39.2, 42.6, 46 and 40 percent for 50-250 ml wastewater concentrations. Significant COD removal using duckweed has been reported in studies by Korner *et al.* b (1998).

The effect of *Lemna* on the removal of suspended solids was apparent by the reductions obtained in the study. 62.1, 55.4, 50.3, 42.6 and 42 percent

Table 28 Experimental Sets Treated with *Lemna perpusilla* Torrey

Period of Treatment-7 days
Weight-2 g/l

E- Experiment
C- Control

Parameters	50 ml Wastewater/l			100 ml Wastewater/l			150 ml Wastewater/l			200 ml Wastewater/l			250 ml Wastewater/l		
	Before	After		Before	After		Before	After		Before	After		Before	After	
		E	C		E	C		E	C		E	C			
Temp	27.60	27.80	27.60	27.80	27.20	27.70	27.90	27.50	27.80	27.80	27.60	27.70	27.70	27.10	27.60
pH	7.60	7.75	7.40	7.77	7.77	7.53	7.82	7.91	7.50	7.93	8.02	7.01	8.00	8.30	8.00
EC	98.10	94.60	97.40	107.30	105.30	103.40	129.50	124.60	127.80	150.60	143.50	148.70	197.20	182.60	193.40
TSS	29.60	11.22	29.10	40.20	17.93	39.50	52.30	26.00	51.70	73.40	42.13	72.70	84.60	49.10	83.90
DO	3.60	5.20	3.73	2.40	4.30	2.52	1.12	2.80	1.25	0	1.60	0.20	0	1.20	0
BOD	10.30	4.50	9.68	16.40	5.28	15.80	21.90	5.98	21.30	31.60	6.51	31.01	42.30	7.36	41.60
COD	25.75	16.87	24.10	39.04	23.74	37.18	50.93	29.23	49.29	79.00	42.66	77.38	88.12	52.87	86.48
NO ₃ -N	0.96	0.33	0.81	1.32	0.41	1.17	2.74	0.71	2.57	3.92	0.81	3.78	4.56	0.85	4.41
PO ₄ -P	0.37	0.23	0.36	1.06	0.61	1.02	1.84	0.91	1.79	2.10	0.90	1.97	2.40	0.74	2.23
SO ₄	0.80	0.42	0.79	2.52	1.05	2.50	4.80	1.79	4.76	5.90	2.58	5.83	7.20	3.58	7.08
TH	28.00	20.00	28.00	36.00	24.00	32.00	48.00	40.00	46.00	60.00	52.00	56.00	76.00	68.00	72.00
Ca	6.40	6.20	6.40	9.60	6.40	8.00	12.80	8.00	9.60	14.40	12.80	13.06	19.20	15.30	22.40
Mg	2.91	2.80	2.92	2.91	2.91	3.88	3.88	4.86	3.80	5.83	3.80	3.88	6.80	3.80	3.88
T.C	20 x10 ⁵	11x10 ⁴	19x10 ⁵	28 x10 ⁵	42x10 ⁴	26x10 ⁵	39 x10 ⁵	12x10 ⁵	38x10 ⁵	43 x10 ⁵	16x10 ⁵	41x10 ⁵	64 x10 ⁵	30x10 ⁵	63x10 ⁵
F.C	+ ve	- ve	+ ve	+ ve	- ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
E.Coli	+ ve	-ve	+ ve	+ ve	- ve	+ ve	+ ve	- ve	+ ve	+ ve	- ve	+ ve	+ ve	+ ve	+ ve

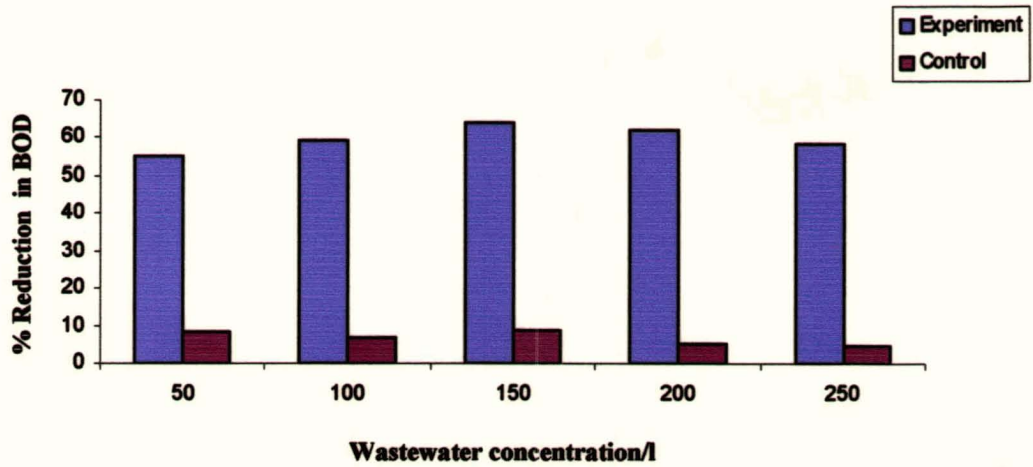


Fig. 51 Percentage Reduction of BOD in Experimental Sets Treated with *Lemna perpusilla* Torrey

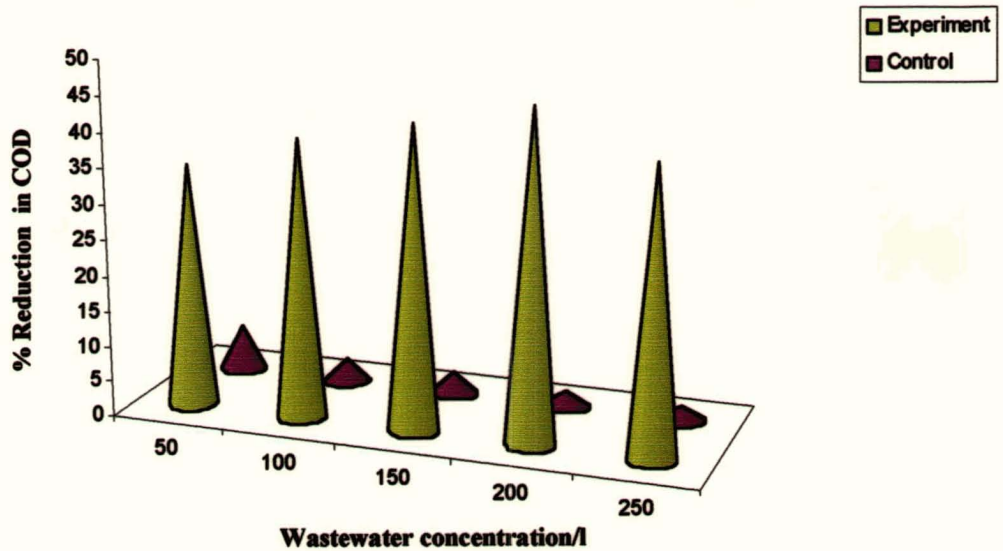


Fig. 52 Percentage Reduction of COD in Experimental Sets Treated with *Lemna perpusilla* Torrey

reduction was recorded for 50-250 ml wastewater concentrations (Fig.53). In addition to sedimentation, a minor fraction is absorbed by the roots of duckweed, where organic particles undergo aerobic biodegradation and the plants assimilate part of the degraded products.

93 percent removal of coliforms was observed in the experiments using *Lemna* in 50 ml wastewater concentration. Reduction in the removal was noted for concentrations above 50 ml/l. Control had a maximum of 7.14 reduction in the experiments conducted. Fig.54 represents the percentage reduction in coliforms. *Lemna* marginally concentrate pathogens on their surfaces. As such, pathogens will, inevitably be harvested along with duckweed on their removal from the system (Skillicorn *et al.* 1993). Fecal coliforms were absent in *Lemna* treated experimental sets upto 100 ml wastewater/litre. E.coli was absent in all experimental sets with *Lemna* except the highest wastewater concentration.

Nitrate nitrogen uptake was observed to increase with a maximum at 250 ml/l. The various percentage reductions obtained for 50-250 ml experimental wastewater are 65.6, 69, 74.1, 79.3 and 81.3 percent respectively. Control for these sets had only 15.62 percent reduction. Fig.55 represents the percentage reduction in nitrate nitrogen using *Lemna*.

Reduction of phosphate upto 69 percent in *Lemna* treated experimental wastewaters was observed. 38, 42, 50.3, 57 and 69 percent reduction of phosphate was observed for 50-250 ml wastewater experimental sets, while control for these experimental sets had maximum of 7.08 percent reduction only. Fig.56 represents the percentage reduction of phosphate using *Lemna*. Earlier studies by

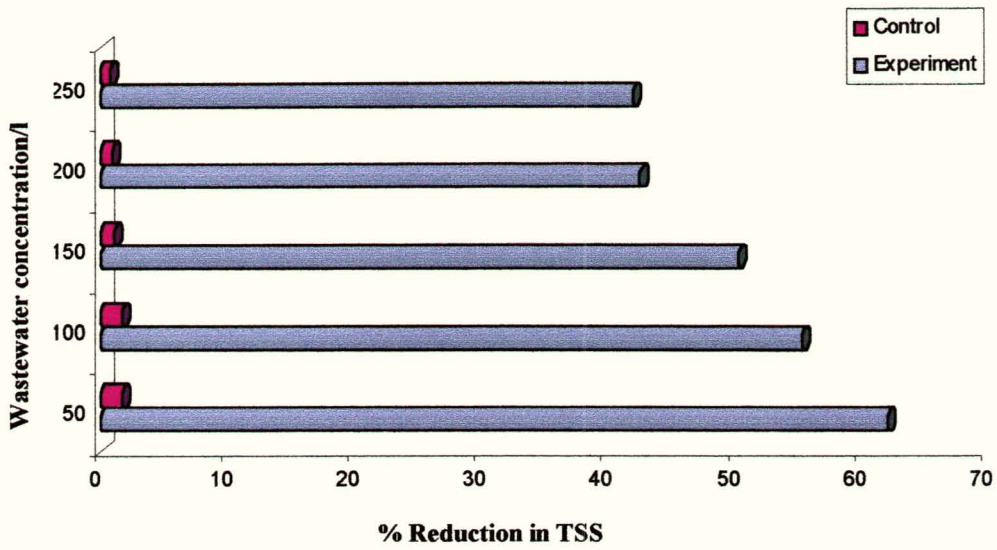


Fig. 53 Percentage Reduction of TSS in Experimental Sets Treated with *Lemna perpusilla* Torrey

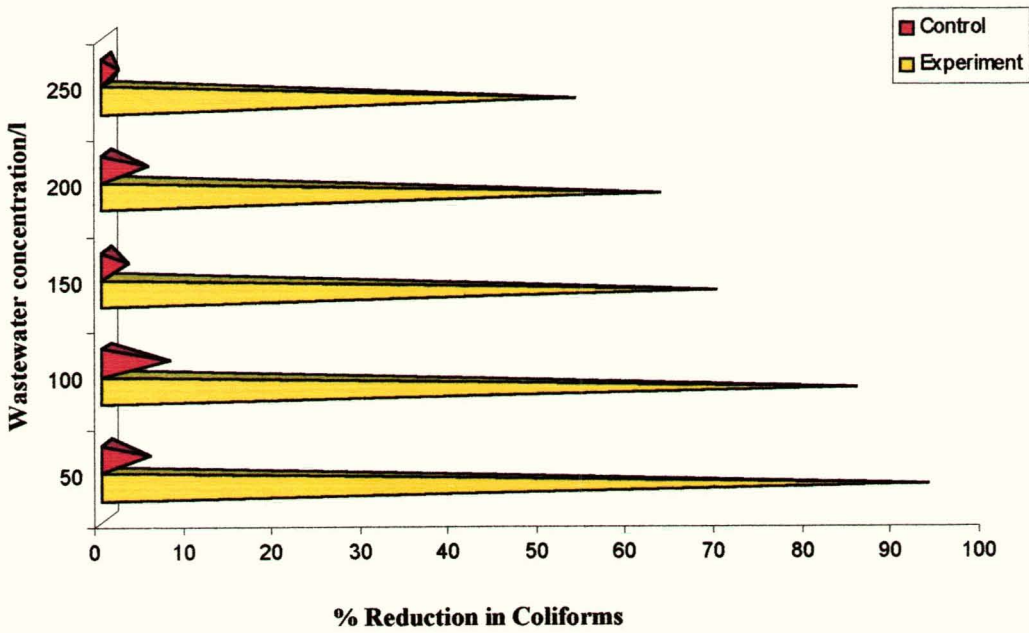


Fig. 54 Percentage Reduction of Coliforms in Experimental Sets Treated with *Lemna perpusilla* Torrey

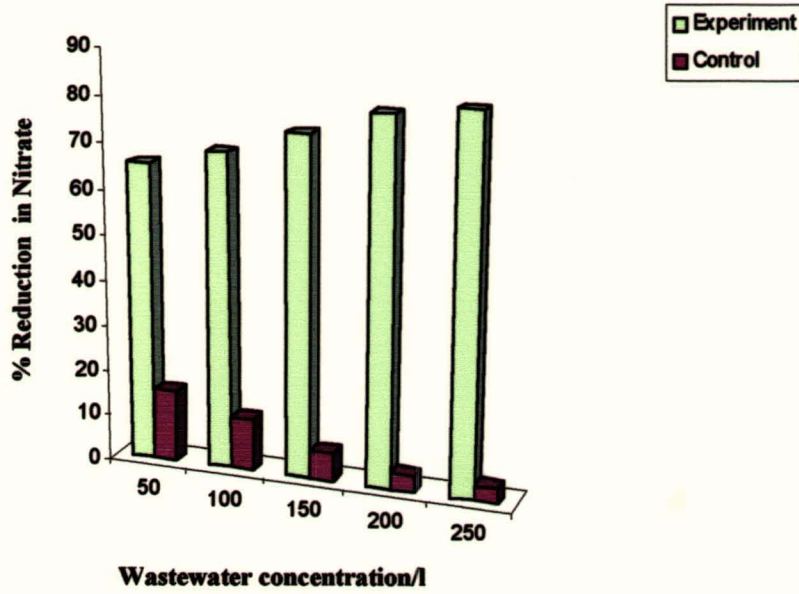


Fig. 55 Percentage Reduction of Nitrate in Experimental Sets Treated with *Lemna perpusilla* Torrey

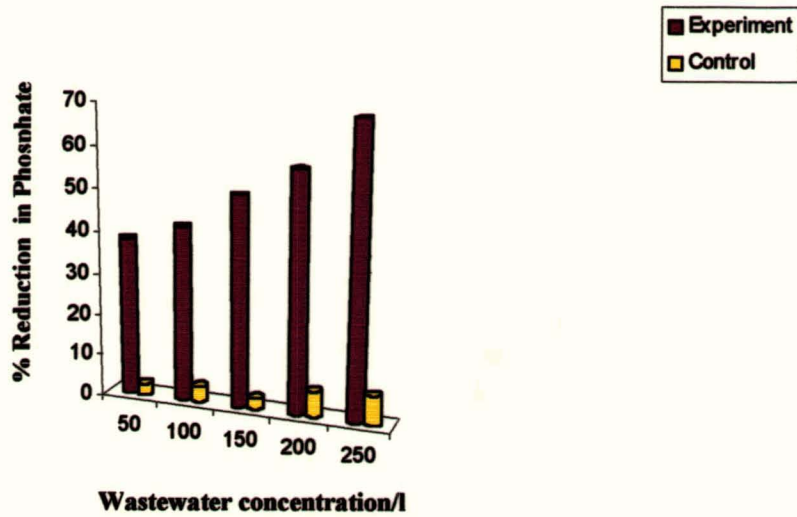


Fig. 56 Percentage Reduction of Phosphate in Experimental Sets Treated with *Lemna perpusilla* Torrey

Korner and Vermaat (1998) attributed approximately three fourth of the total nitrogen and phosphorous loss to the duckweed mat.

During the experiments, temperature and pH did not show much variation. Electrical conductivity decreased to a small extent compared to controls. Dissolved oxygen levels increased in the experimental sets, probably due to the diffusion of oxygen from the root zone (Moorhead and Reddy, 1988).

Sulphate also showed reduction of 62.7 percent while in control only 1.25 percent reduction was obtained. Total hardness, calcium and magnesium showed a decrease when compared to control due to plant uptake.

5. *Spirodela polyrrhiza* Schleid

Spirodela is the largest of duckweeds and it has the fastest doubling rate among duckweeds. Experiments conducted using *Spirodela* for wastewater treatment revealed its ability to reduce nitrates, phosphates, coliforms and BOD to a considerable extent. Table 29 presents the variations in physicochemical and bacteriological parameters for the seven day study.

Spirodela was capable of reducing BOD of experimental wastewaters by a maximum of 46.1 percent (in 200 ml/l wastewater). The percentage reduction obtained for other wastewater concentrations are 35.7, 33.4, 39.6 and 42.3 percent for 50, 100, 150 and 250 ml wastewater/litre respectively. The maximum reduction in control was 6.02 in the lowest concentration of experimental wastewater. Fig.57 represents the reduction in BOD.

COD removal upto 25 percent was recorded in the studies conducted using *Spirodela*. Control had maximum of 6.41 percent reduction. The reduction in

Table 29 Experimental Sets Treated with *Spirodela polyrrhiza* (L.) Schleid

Period of Treatment -7 days
Weight-2 g/l

E- Experiment
C- Control

Parameters	50 ml Wastewater/l			100 ml Wastewater/l			150 ml Wastewater/l			200 ml Wastewater/l			250 ml Wastewater/l		
	Before	After		Before	After		Before	After		Before	After		Before	After	
		E	C		E	C		E	C		E	C		E	C
Temp	27.60	27.50	27.60	27.80	27.50	27.70	27.90	27.60	27.80	27.80	27.50	27.70	27.70	27.70	27.60
pH	7.62	7.83	7.40	7.77	7.89	7.53	7.82	7.99	7.50	7.93	8.30	7.40	8.00	8.40	7.80
EC	98.10	95.20	97.40	107.30	104.60	103.40	129.50	128.40	127.80	150.60	143.20	148.70	197.20	187.60	193.40
TSS	29.60	23.56	29.10	40.20	29.55	39.50	52.30	37.55	51.70	73.40	51.10	72.70	84.60	57.02	83.90
DO	3.60	5.61	3.73	2.40	4.75	2.52	1.12	3.00	1.25	0	1.75	0.20	0	1.32	0.10
BOD	10.30	6.62	9.68	16.40	10.92	15.80	21.90	13.23	21.30	31.60	17.03	31.01	42.30	24.41	41.60
COD	25.75	20.29	24.10	39.04	29.00	37.38	50.93	41.61	49.29	79.00	63.83	77.38	88.12	69.97	86.48
NO ₃ -N	0.96	0.39	0.81	1.32	0.48	1.17	2.74	0.95	2.57	3.92	1.16	3.78	4.56	1.26	4.41
PO ₄ -P	0.37	0.23	0.36	1.06	0.60	1.02	1.84	0.95	1.79	2.10	1.04	1.97	2.40	0.79	2.23
SO ₄	0.80	0.49	0.79	2.52	1.46	2.50	4.80	2.64	4.76	5.90	3.52	5.83	7.20	5.21	7.08
TH	28.00	24.00	28.00	36.00	24.00	32.00	48.00	44.00	46.00	60.00	56.00	56.00	76.00	70.00	72.00
Ca	6.40	6.40	6.40	9.60	6.40	8.00	12.80	11.80	9.60	14.40	13.06	13.06	19.20	17.68	22.40
Mg	2.91	2.91	2.92	2.91	2.91	3.88	3.88	3.80	3.80	5.83	3.88	3.88	6.80	6.26	3.88
T.C	20 x10 ⁵	24 x10 ⁴	19 x10 ⁵	28 x10 ⁵	66 x10 ⁴	26 x10 ⁵	39 x10 ⁵	11 x10 ⁵	38 x10 ⁵	43 x10 ⁵	14 x10 ⁵	41 x10 ⁵	64 x10 ⁵	29 x10 ⁵	63 x10 ⁵
F.C	+ ve	- ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
E.Coli	+ ve	-ve	+ ve	+ ve	- ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve

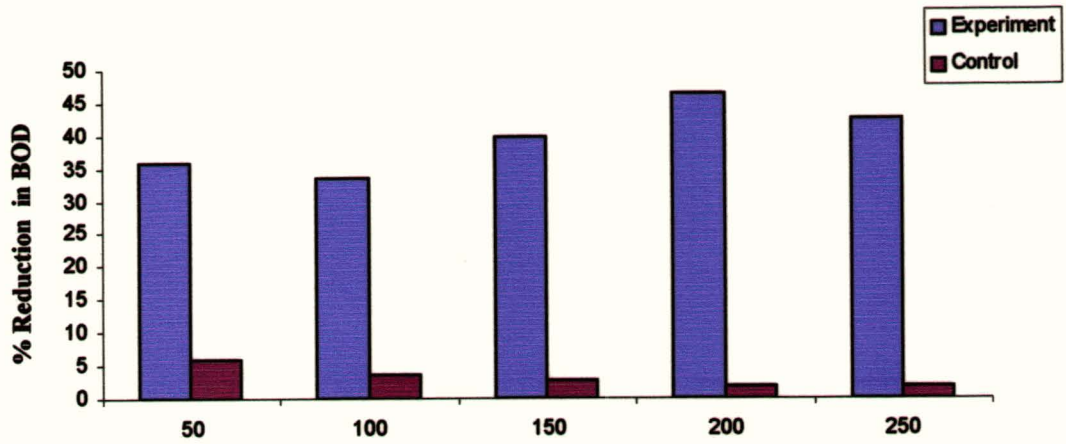
COD obtained for the wastewater concentrations are 21.2, 25.7, 18.3, 19.2 and 20.6 percent for 50-250 ml wastewater concentrations. Fig.58 represents the reduction in COD.

Total suspended solids were reduced by a maximum of 32.6 percent in the various wastewater concentrations experimented. 20.4, 26.5, 28.2, 30.4 and 32.6 percent reduction was observed in 50-250 ml wastewater concentrations. Fig.59 represents the reduction in TSS.

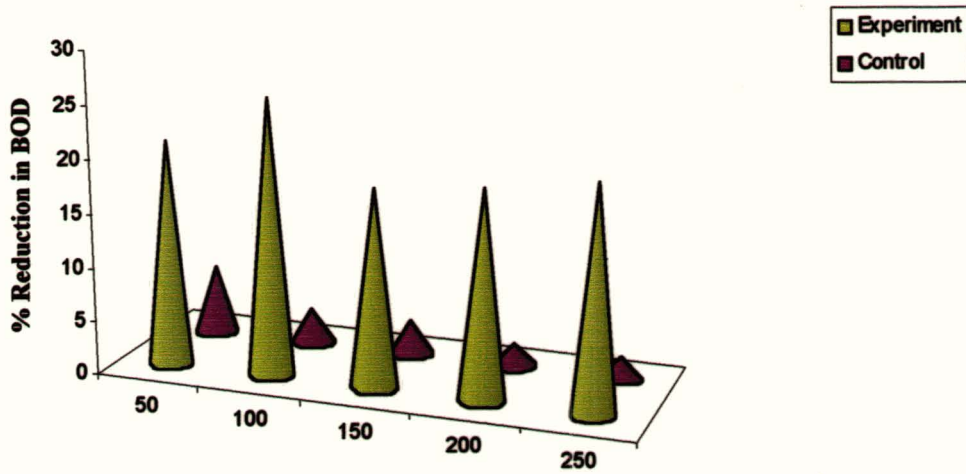
Coliforms removal was pronounced in studies with *Spirodela*. Upto 88 percent reduction was obtained in the lowest wastewater concentration. 76.4, 71.8, 67.4 and 54.7 percent reduction in coliforms count was recorded for other wastewater concentrations (100-250 ml wastewater/l). There was a gradual decrease in the removal of coliforms with higher wastewater concentrations. Control for these experiments had only 7.14 percent reduction. Fig.60 represents the reduction in coliforms.

Nitrate nitrogen reduced by a maximum of 72.4 percent in the treatment studies with *Spirodela*. 59.4, 63.2, 65.4, 70.3 and 72.4 percent reduction was obtained with 50-250 ml wastewater concentrations and could be attributed to plant uptake. Control for these experiments had only 15.62 percent reduction. Fig.61 represents the reduction in nitrate nitrogen.

Phosphate uptake of *Spirodela* increased with increasing concentrations of wastewater. 37.4, 42.6, 48.2, 50.3 and 67 percent reduction was obtained with 50-250 ml wastewater experimental sets. The control had 7.08 decrease in phosphate for the seven day detention period. Fig.62 represents the reduction in phosphate in experiments with *Spirodela*.



Wastewater concentration/l
Fig. 57 Percentage Reduction of BOD in Experimental Sets Treated with *Spirodela polyrrhiza* (L.) Schleid



Wastewater concentration/l
Fig. 58 Percentage Reduction of COD in Experimental Sets Treated with *Spirodela polyrrhiza* (L.) Schleid

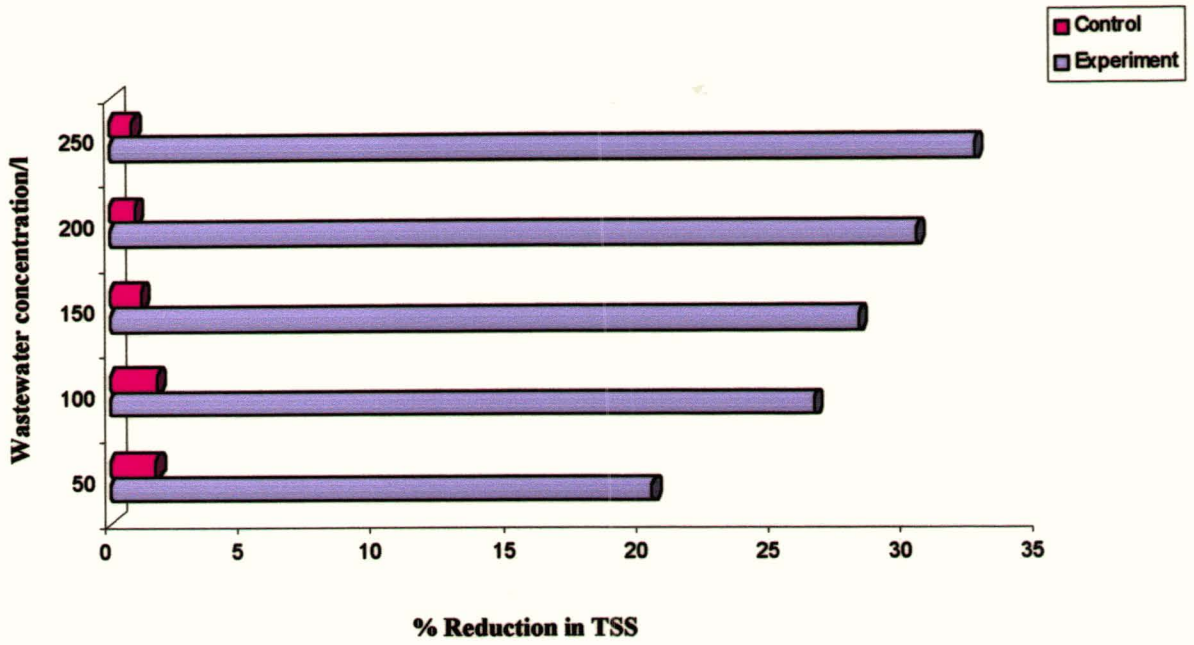


Fig. 59 Percentage Reduction of TSS in Experimental Sets Treated with *Spirodela polyrrhiza* (L.) Schleid

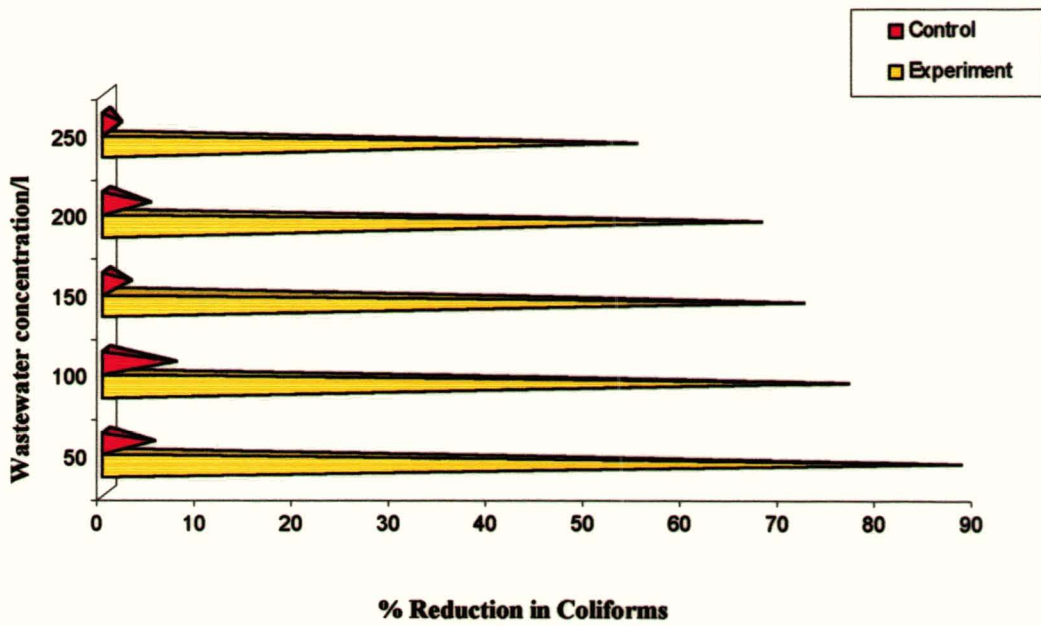


Fig. 60 Percentage Reduction of Coliforms in Experimental Sets Treated with *Spirodela polyrrhiza* (L.) Schleid

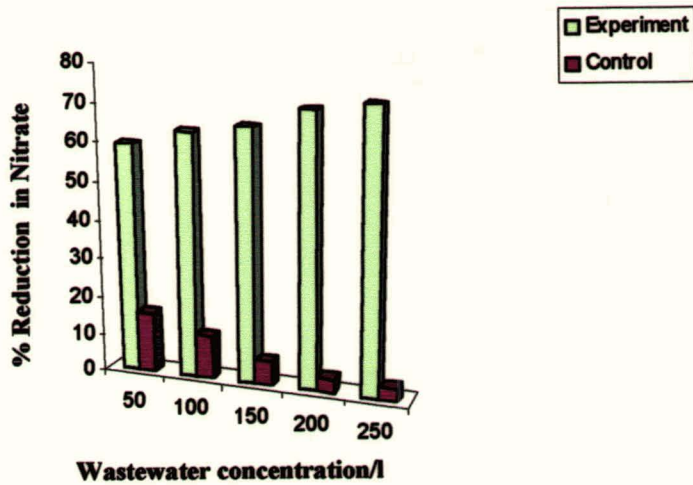


Fig.61 Percentage Reduction of Nitrate in Experimental Sets Treated with *Spirodela polyrrhiza* (L.) Schleid

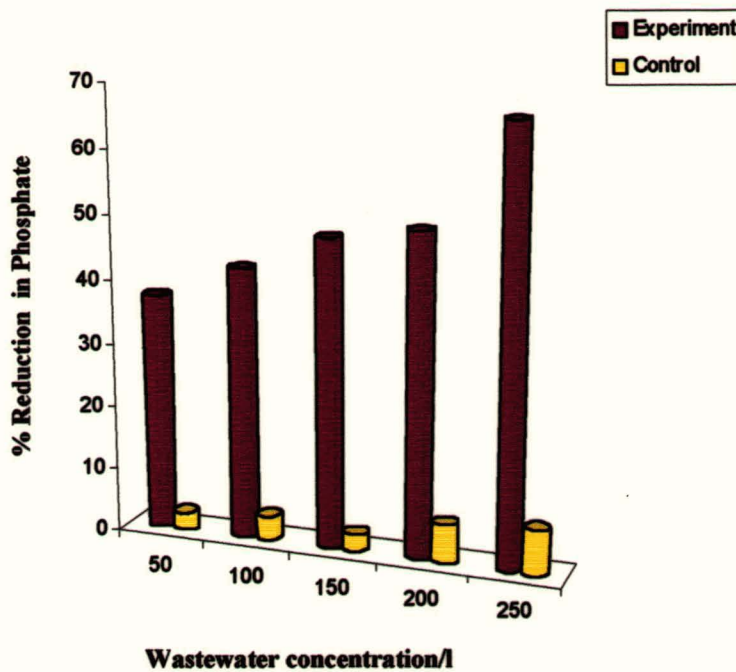


Fig.62 Percentage Reduction of Phosphate in Experimental Sets Treated with *Spirodela polyrrhiza* (L.) Schleid

Sulphate also showed a decrease in the experimental wastewaters upto 45 percent. Temperature and pH showed negligible variation at the end of treatment period. Values of electrical conductivity declined with respect to control. Dissolved oxygen values showed slightly elevated levels, probably due to the presence of *Spirodela*. Hardness, calcium and magnesium decreased in the experimental sets when compared to control.

6. *Wolffia globosa* (Roxb.) Hartog & Plas.

Wolffia is commonly seen growing along with other duckweeds. It has not been given importance as other duckweeds owing to its small size. *Wolffia* has good potential for use in wastewater treatment. In the present study, *Wolffia* could reduce coliforms and nitrate nitrogen to a good extent. Considerable removal for BOD, COD, TSS and phosphates were also recorded. Table 30 presents the variations in physicochemical and bacteriological parameters of the experimental water treated with *Wolffia*.

BOD was reduced by 6.31, 6.6, 7.1, 10.2 and 8.7 percent for 50-250 ml wastewater concentrations. Maximum removal obtained for experimental sets was 10.2 percent while control had 6.02 percent reduction for these wastewater concentrations. Fig. 63 represents the reduction in BOD.

COD decreased in the experimental wastewaters upto a maximum of 12.8 percent in the lowest wastewater concentration, 50ml/l. 10.2, 9.8, 4.6 and 5.2 percent reduction in experimental wastewaters was obtained for higher concentrations. Control had a maximum reduction of 9.8 percent in the various experimental sets. Fig. 64 represents the reduction in COD.

Table 30 Experimental Sets Treated with *Wolffia globosa* (Roxb.) Hartog & Plas.

Period of Treatment -7 days
Weight-2 g/l

E- Experiment
C- Control

Parameters	50 ml Wastewater/l			100 ml Wastewater/l			150 ml Wastewater/l			200 ml Wastewater/l			250 ml Wastewater/l		
	Before	After		Before	After		Before	After		Before	After		Before	After	
		E	C		E	C		E	C		E	C		E	C
Temp	27.60	27.30	27.60	27.80	27.60	27.70	27.90	27.90	27.80	27.80	27.50	27.70	27.70	27.40	27.60
pH	7.60	7.72	7.40	7.77	7.80	7.53	7.82	7.87	7.50	7.93	8.00	7.40	8.00	8.20	7.80
EC	98.10	95.30	97.40	107.30	102.80	103.40	129.50	125.40	127.80	150.60	142.20	148.70	197.20	188.80	193.40
TSS	29.60	27.50	29.10	40.20	34.90	39.50	52.30	43.10	51.70	73.40	59.16	72.70	84.60	67.43	83.90
DO	3.60	4.90	3.73	2.40	3.80	2.52	1.12	2.50	1.25	0	1.20	0.20	0	0.89	0.10
BOD	10.30	9.65	9.68	16.40	15.32	15.80	21.90	20.34	21.30	31.60	28.38	31.01	42.30	38.62	41.60
COD	25.75	22.45	24.10	39.04	35.06	37.38	50.93	50.839	49.29	79.00	75.37	77.38	88.12	83.54	86.48
NO ₃ -N	0.96	0.57	0.81	1.32	1.80	1.17	2.74	1.16	2.57	3.92	1.68	3.78	4.56	2.25	4.41
PO ₄ -P	0.37	0.30	0.36	1.06	0.85	1.02	1.84	1.39	1.79	2.10	1.51	1.97	2.40	1.77	2.23
SO ₄	0.80	0.77	0.79	2.52	2.23	2.50	4.80	3.62	4.76	5.90	3.82	5.83	7.20	5.21	7.08
TH	28.00	26.00	28.00	36.00	34.00	32.00	48.00	46.00	46.00	60.00	54.00	56.00	76.00	74.00	72.00
Ca	6.40	5.80	6.40	9.60	9.20	8.00	12.80	9.60	9.60	14.40	12.96	13.06	19.20	18.60	22.40
Mg	2.91	2.64	2.92	2.91	2.80	3.88	3.88	3.80	3.80	5.83	5.24	3.88	6.80	6.64	3.88
T.C	20 x10 ⁵	11x10 ⁵	19x10 ⁵	28 x10 ⁵	16x10 ⁵	23x10 ⁵	39 x10 ⁵	23x10 ⁵	38x10 ⁵	43 x10 ⁵	29x10 ⁵	41x10 ⁵	64 x10 ⁵	48x10 ⁵	63x10 ⁵
F.C	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
E.Coli	+ ve	-ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve

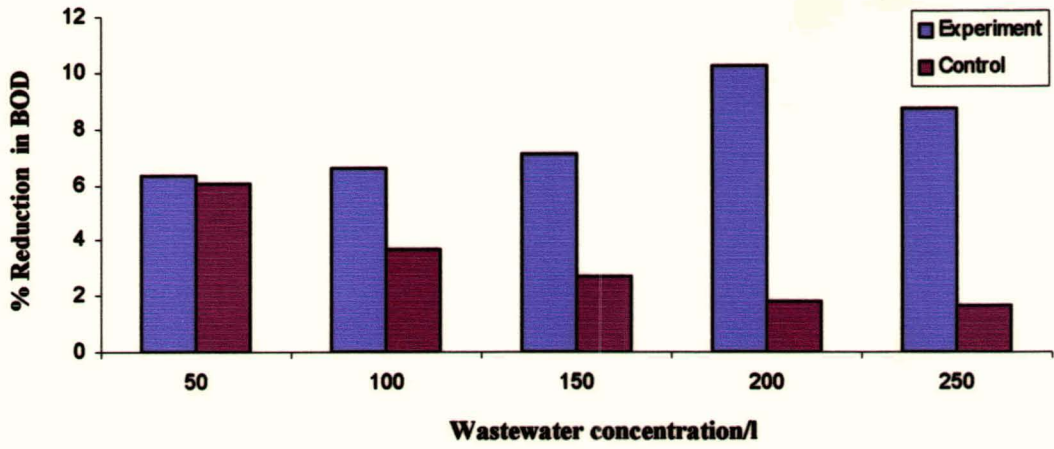


Fig. 63 Percentage Reduction of BOD in Experimental Sets Treated with *Wolffia globosa* (Roxb.) Hartog & Plas.

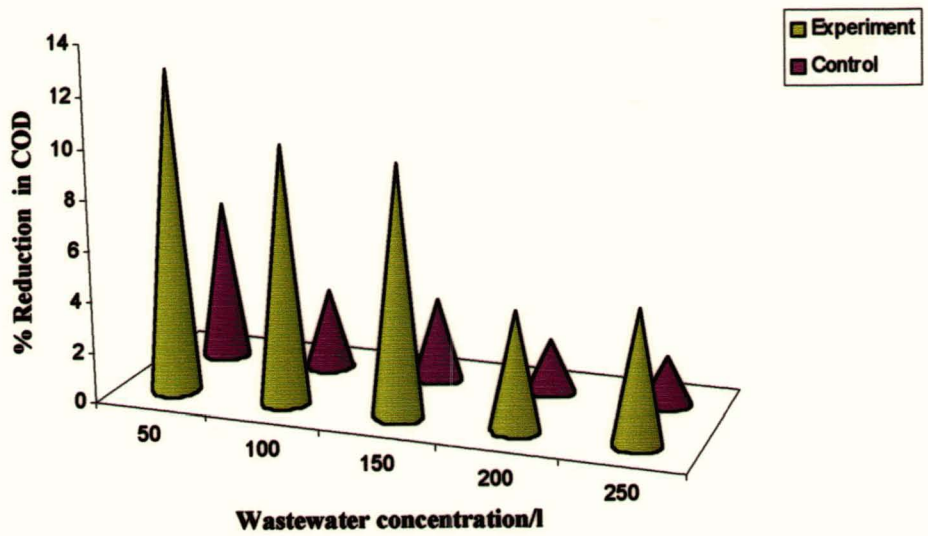


Fig. 64 Percentage Reduction of COD in Experimental Sets Treated with *Wolffia globosa* (Roxb.) Hartog & Plas.

Total suspended solids reduced upto 20.3 percent in experiments with *Wolffia*. 7.1, 13.2, 17.6, 19.4 and 20.3 percent reduction for 50-250 ml wastewater concentration was recorded. Control sets had 1.69, 1.74, 1.14, 0.95 and 0.82 percent reduction. Fig. 65 represents the reduction in TSS using *Wolffia*.

In the experiments with *Wolffia*, removal efficiency for coliforms was maximum in 100 ml wastewater/litre. 45, 45.8, 39.1, 33.3 and 31.6 percent reduction was recorded with 50, 150, 200 and 250 ml wastewater/litre concentrations. In control sets removal reported was 7.14 percent. Fig.66 represents the reduction in coliforms. Fecal coliforms were not removed in *Wolffia* treated experimental wastewaters.

Among the parameters studied, *Wolffia* was able to reduce the nitrate nitrogen content of experimental wastewaters upto 57.7 percent. The percentage reductions in various concentrations are 40.6, 54.2, 57.7, 57.2 and 50.7 percent respectively. An increasing trend in the reduction of nitrate nitrogen was found upto the concentration of increase till 200 ml wastewater/l. in further higher concentrations the removal rate was observed to be low. Fig. 67 represents the reduction in nitrate nitrogen.

Phosphate reduction by *Wolffia* in the experimental wastewaters was 18.6, 19.4, 24.3, 28.03 and 26.09 percent whereas control had a maximum of 7.08 percent for 50-250 ml wastewater concentrations. Fig. 68 represents the reduction in phosphates using *Wolffia*.

As in the case of other plants, pH and temperature did not show considerable variations, while electrical conductivity, calcium, magnesium,

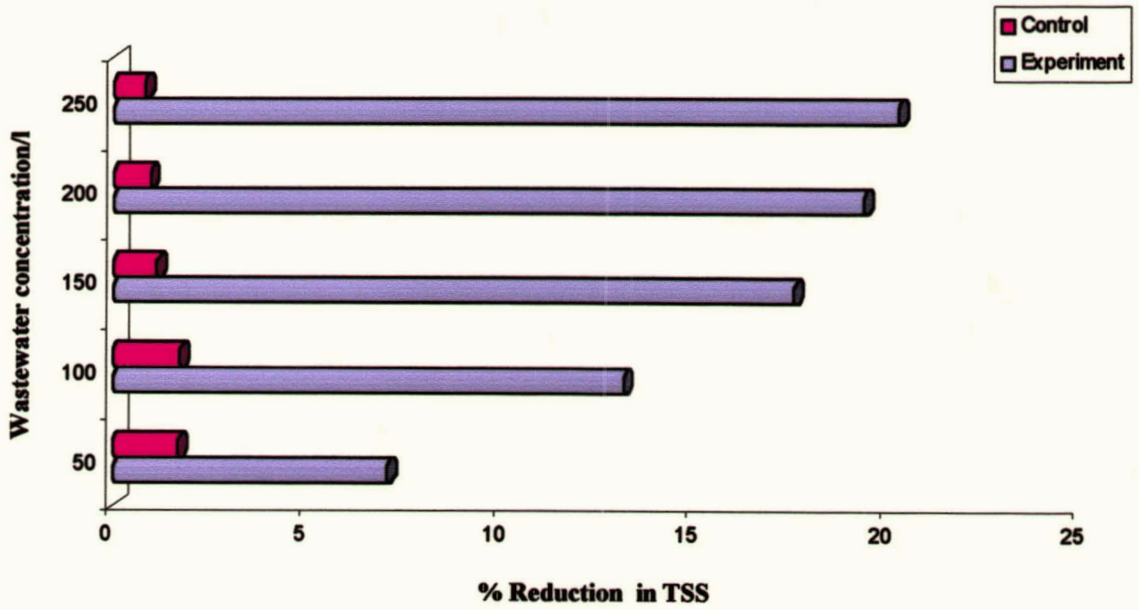


Fig. 65 Percentage Reduction of TSS in Experimental Sets Treated with *Wolffia globosa* (Roxb.) Hartog & Plas.

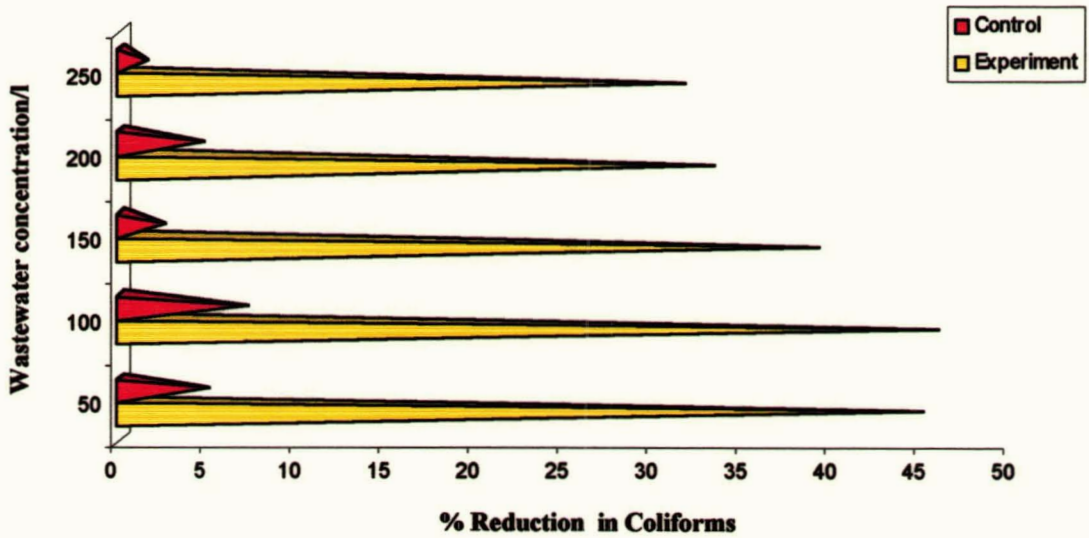


Fig. 66 Percentage Reduction of Coliforms in Experimental Sets Treated with *Wolffia globosa* (Roxb.) Hartog & Plas.

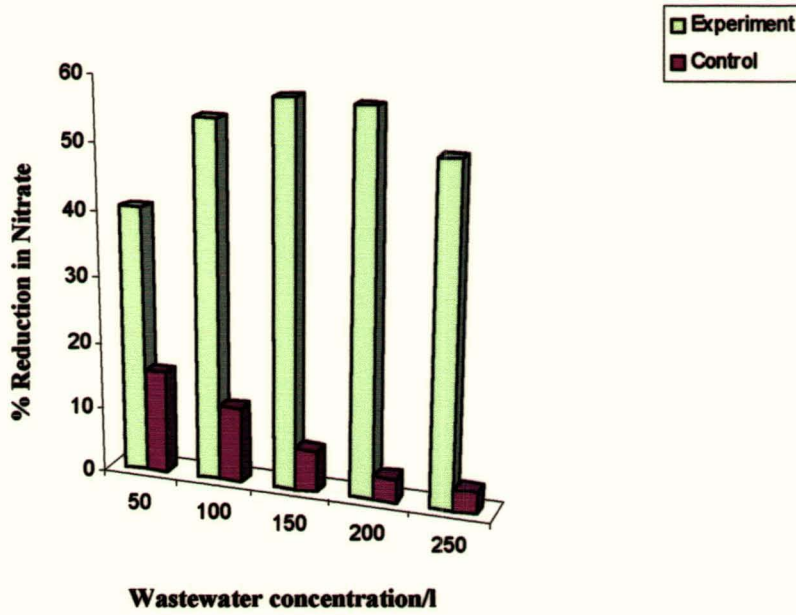


Fig. 67 Percentage Reduction of Nitrate in Experimental Sets Treated with *Wolffia globosa* (Roxb.) Hartog & Plas.

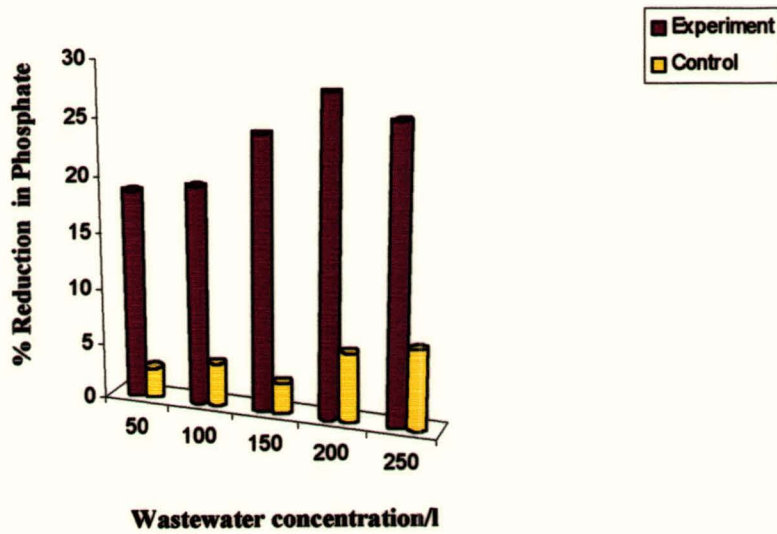


Fig. 68 Percentage Reduction of Phosphate in Experimental Sets Treated with *Wolffia globosa* (Roxb.) Hartog & Plas.

hardness and sulphates showed slight decrease with respect to controls. Dissolved oxygen increased with respect to controls and pre experimental stage.

4. 2. 3 Submerged Plants

1. *Hydrilla verticillata* (L.f.) Royle

Hydrilla is a ubiquitous submerged weed seen in ponds and canals, forming dense mats underwater. So far it has been considered as a weed and methods to eradicate are under research. In this study, the pollutant mitigation potential of this weed has been attempted. Results indicate substantial reduction in nutrients, coliforms, total suspended solids and BOD. Table 31 presents the variations in physicochemical and bacteriological parameters for the seven day study.

In experiments with *Hydrilla*, 42.3, 49.7, 51.3, 53.6 and 51.4 percent reduction in BOD was obtained for 50-250 ml wastewater concentrations. Maximum reduction with *Hydrilla* was 53.6 percent, while control had only 11.68 percent reduction. Fig.69 represents the reduction in BOD using *Hydrilla*.

Similar to the reduction pattern in wastewater BOD, COD reductions of 18.6, 22.9, 28.3, 31.4 and 35 percent was recorded in the various experimental wastewaters, while control had 5.54 percent reduction. Fig.70 represents the reduction in COD using *Hydrilla*.

Reduction in total suspended solids upto 63.4 percent was recorded in experiments with *Hydrilla*. The reductions obtained for 50-250 ml wastewater concentrations were 45.3, 52.1, 59.6, 61.2 and 63.4 percent respectively. Controls

Table 31 Experimental Sets Treated with *Hydrilla verticillata* (L. f.) Royle

Period of Treatment 7 days
Weight 5 g/l

E- Experiment
C- Control

Parameters	50 ml Wastewater/l			100 ml Wastewater/l			150 ml Wastewater/l			200 ml Wastewater/l			250 ml Wastewater/l		
	Before	After		Before	After		Before	After		Before	After		Before	After	
		E	C		E	C		E	C		E	C			
Temp	29.80	29.70	29.40	30.50	30.10	30.40	30.20	29.40	30.10	30.10	29.80	30.60	30.40	30.70	29.70
pH	7.43	7.72	7.44	7.95	8.09	8.00	8.08	8.15	8.12	8.16	8.23	8.17	8.25	8.47	8.32
EC	142.10	138.60	141.30	168.50	165.00	167.40	200.80	197.20	198.80	232.70	229.30	232.00	256.30	252.80	255.90
TSS	38.40	21.00	36.20	42.50	20.35	40.10	56.40	22.80	53.50	77.80	30.19	72.60	89.20	32.65	87.30
DO	2.50	4.52	2.76	2.20	4.65	2.50	0	2.50	1.30	0	2.20	1.10	0	1.95	0.76
BOD	15.40	8.88	13.60	20.40	10.26	19.20	26.30	12.80	24.80	32.30	14.99	30.80	45.60	22.16	44.00
COD	32.08	26.11	30.30	44.35	34.19	42.50	59.77	42.85	58.10	78.80	54.06	77.30	108.60	97.09	106.50
NO ₃ -N	1.85	0.96	1.86	2.63	1.30	2.64	5.42	2.48	5.37	7.64	2.66	7.60	8.34	3.30	8.28
PO ₄ -P	0.64	0.43	0.61	1.03	0.63	0.98	1.88	0.99	1.82	2.34	1.10	2.10	2.62	1.47	2.58
SO ₄	3.32	1.81	3.30	7.04	3.42	7.00	8.80	5.13	8.50	10.52	6.43	10.60	13.28	8.96	13.30
TH	76.00	68.00	72.00	96.00	84.00	92.00	112.00	100.00	108.00	116.00	108.00	112.00	128.00	116.00	120.00
Ca	22.40	19.30	18.60	27.20	24.00	26.80	28.80	25.80	24.20	28.80	24.20	28.80	32.80	28.80	38.20
Mg	4.86	6.12	6.17	6.40	5.83	6.10	7.76	6.92	9.72	7.80	9.72	9.70	11.08	7.80	12.80
T.C	15 x10 ⁵	70x10 ⁴	14x10 ⁵	23 x10 ⁵	11x10 ⁵	21x10 ⁵	46 x10 ⁵	29x10 ⁵	45x10 ⁵	75 x10 ⁵	51x10 ⁵	73x10 ⁵	93 x10 ⁵	64x10 ⁵	92x10 ⁵
F.C	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
E.Coli	+ ve	-ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve

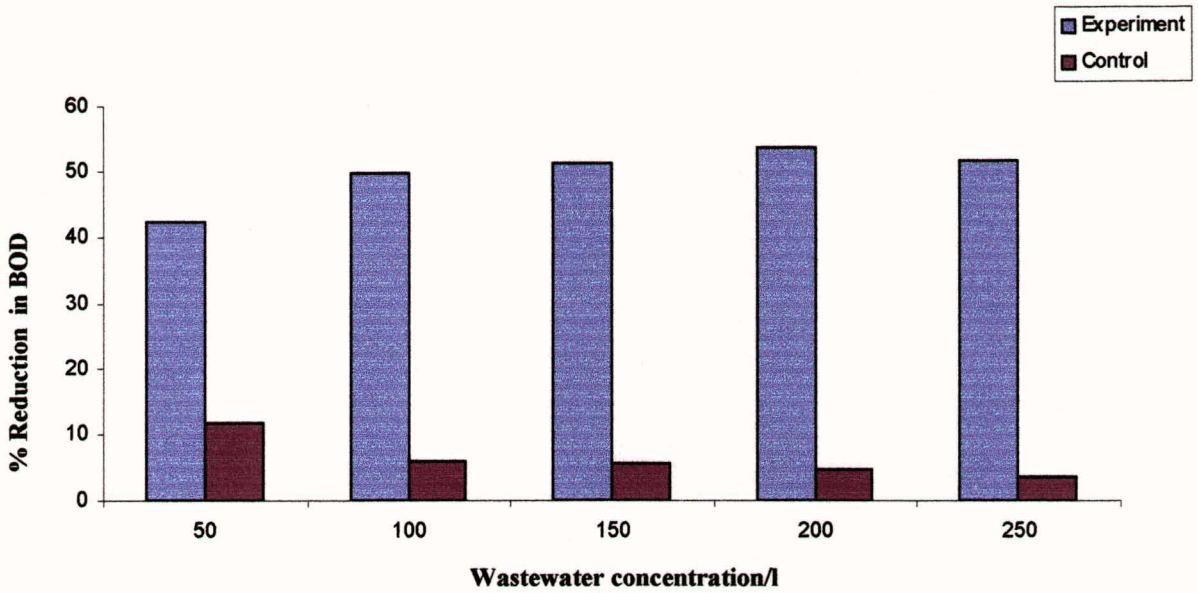


Fig. 69 Percentage Reduction of BOD in Experimental Sets Treated with *Hydrilla verticillata* (L.f.) Royle

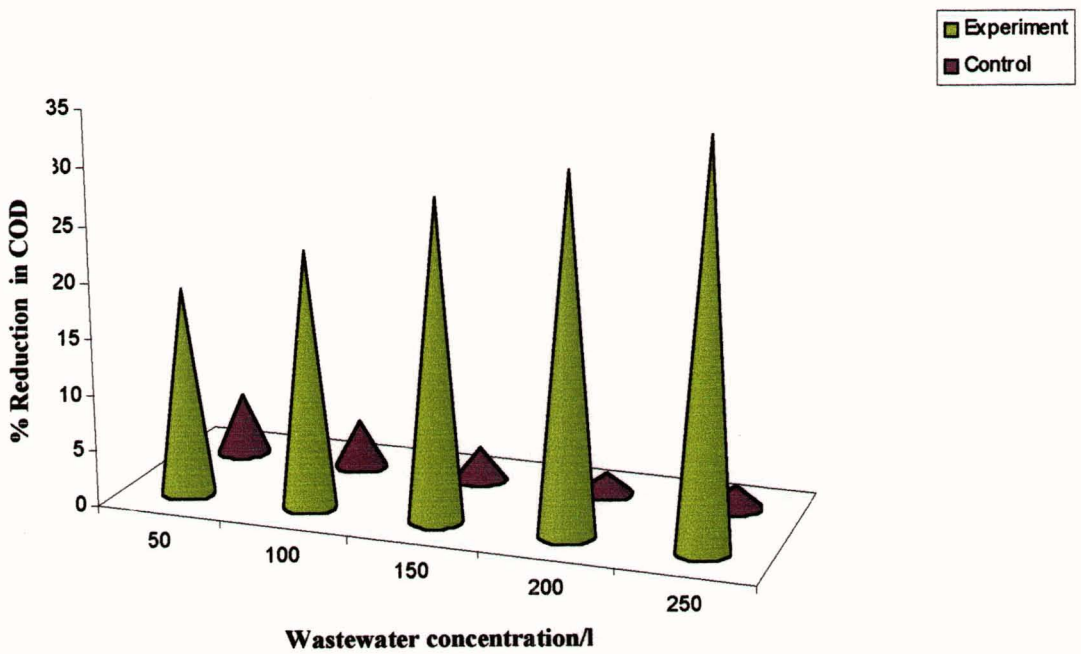


Fig. 70 Percentage Reduction of COD in Experimental Sets Treated with *Hydrilla verticillata* (L.f.) Royle

had maximum reduction of 6.68 percent for these experimental sets. Fig.71 represents the reduction in TSS using *Hydrilla*.

It was noticed that the coliforms counts declined sharply in lowest wastewater concentration (50 ml/l) and was 53.3 percent. With increasing wastewater concentrations (100-250 ml/l), 52.17, 37, 32 and 31.2 percent reduction was recorded. Control for these experimental sets had 8.7 percent removal in coliforms. Fig.72 represents the reduction in coliforms using *Hydrilla*. *Hydrilla* was not very efficient in removal of fecal coliforms. *E.coli* was absent only in the lowest wastewater concentration (50 ml/l).

Reductions in nitrate nitrogen concentration in all the wastewater experimental sets with *Hydrilla* was better than the control set. Maximum of 65.2 percent reduction in nitrate nitrogen was observed in the studies. 48.3, 50.6, 54.3, 65.2 and 60.4 percent reduction was obtained for 50-250 ml wastewater/litre. Fig.73 represents the reduction in nitrate nitrogen using *Hydrilla*.

Phosphate removal followed a trend similar to nitrate nitrogen removal with reduction of 32.8, 40, 47.3, 52.9 and 43.9 percent in the various concentrations experimented (Fig.74). Controls for these experiments had maximum reduction of 4.8 percent only.

There was no appreciable change in temperature and pH, while a small decrease in electrical conductivity, sulphate, hardness, calcium and magnesium were observed in experiments with *Hydrilla*. Dissolved oxygen levels increased considerably with respect to controls.

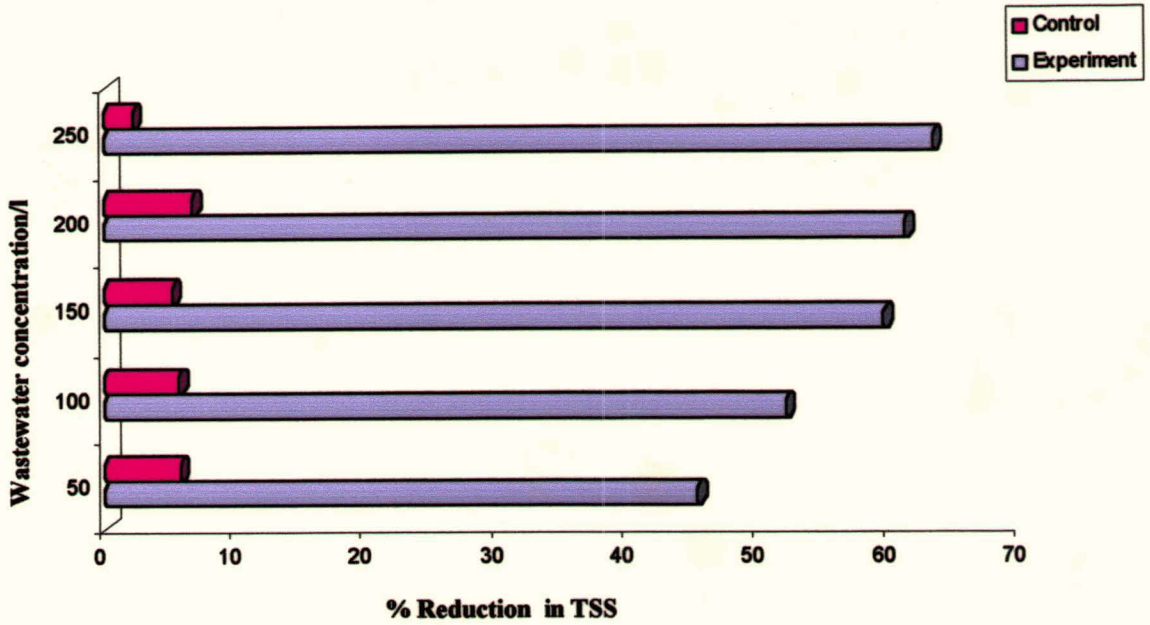


Fig. 71 Percentage Reduction of TSS in Experimental Sets Treated with *Hydrilla verticillata* (L.f.) Royle

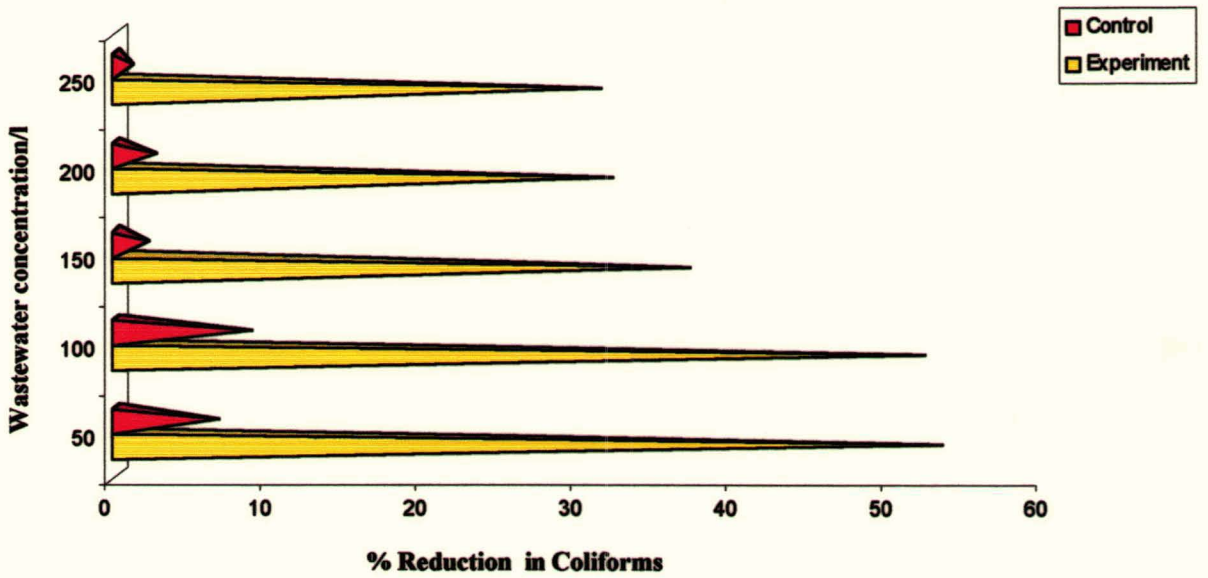


Fig. 72 Percentage Reduction of Coliforms in Experimental Sets Treated with *Hydrilla verticillata* (L.f.) Royle

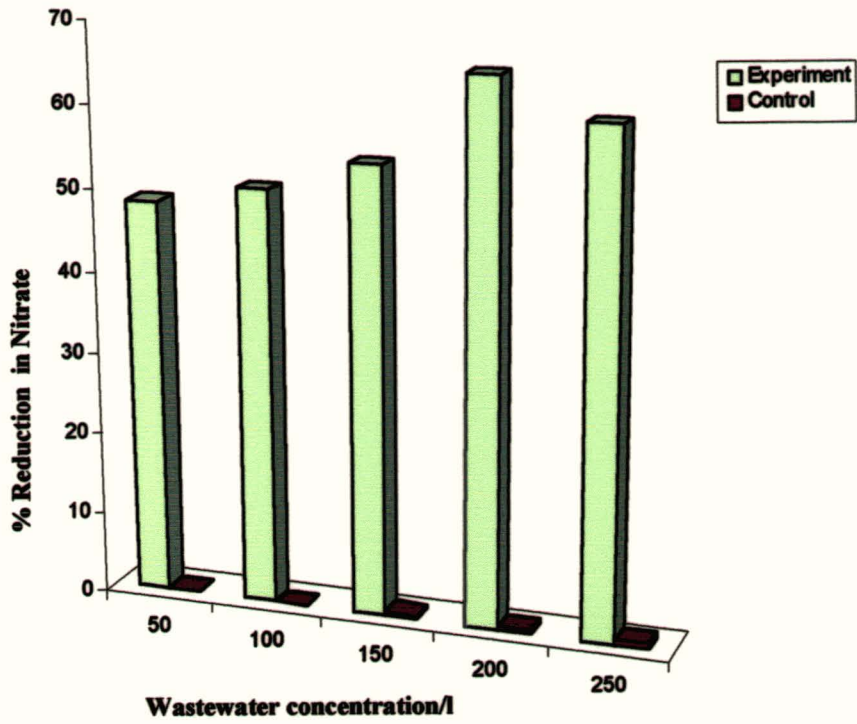


Fig. 73 Percentage Reduction of Nitrate in Experimental Sets Treated with *Hydrilla verticillata* (L.f.) Royle

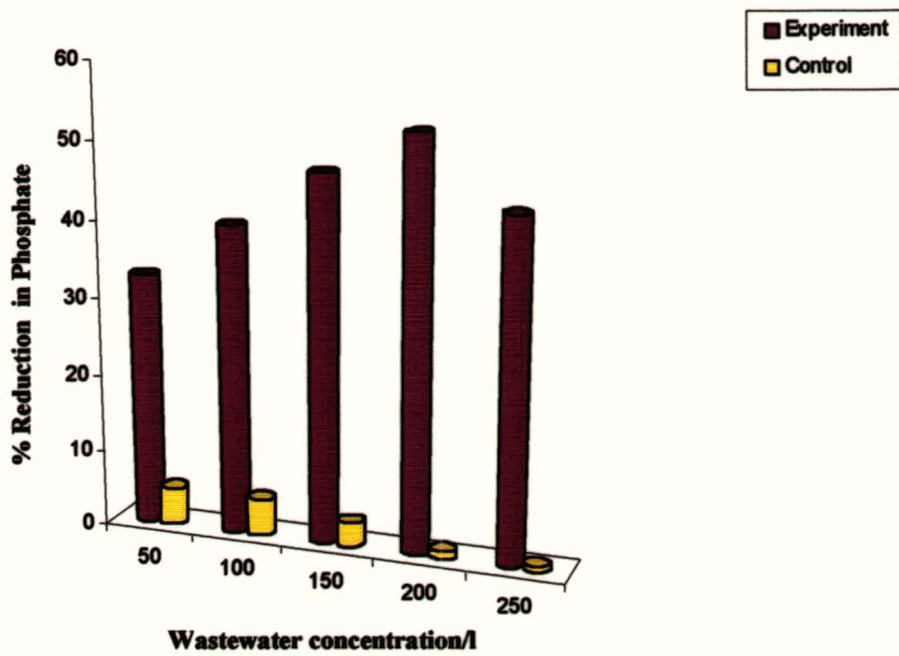


Fig.74 Percentage Reduction of Phosphate in Experimental Sets Treated with *Hydrilla verticillata* (L.f.) Royle

2. *Ceratophyllum demersum* L.

Ceratophyllum is a submerged aquatic plant that can survive in polluted waters. In this study, *Ceratophyllum* was able to bring down the concentrations of coliforms, nutrients (nitrate nitrogen and phosphate), suspended solids and BOD. Table 32 presents the variations in physicochemical and bacteriological parameters for the seven day experimental period.

BOD reductions upto 80.7 percent was recorded in the study in highest wastewater concentration. 63.6, 68.5, 72.2, 78.6 and 80.7 percent reduction in BOD was obtained for 50-250 ml wastewater concentration. The greatest removal for control however was only 11.68 percent. Fig.75 represents the reduction in BOD using *Ceratophyllum*. Studies in Tamil Nadu have indicated that *Ceratophyllum demersum* could remove 95 percent of BOD₅ from raw sewage (Pescod, 1992).

As for BOD, COD removal increased with increase in wastewater concentrations. 25.6, 27.1, 31.9, 36.8 and 38 percent reduction was recorded with 50-250 ml wastewater/l. Highest removal with *Ceratophyllum* was 38 percent, while control had only 5.54 percent reduction. Fig.76 represents the reduction in COD using *Ceratophyllum*.

Total suspended solids reduced upto 57.4 percent in experiments with *Ceratophyllum*. 47.2, 49.8, 52.6, 56.42 and 57.4 percent reduction for 50-250 ml wastewater concentration was recorded. Control sets had a maximum 6.68 percent reduction in these experimental sets. Fig.77 represents the reduction in total suspended solids using *Ceratophyllum*.

Table 32 Experimental Sets Treated with *Ceratophyllum demersum* L.

Period of Treatment -7 days
Weight-5 g/l

E- Experiment
C- Control

Parameters	50 ml Wastewater/l			100 ml Wastewater/l			150 ml Wastewater/l			200 ml Wastewater/l			250 ml Wastewater/l		
	Before	After		Before	After		Before	After		Before	After		Before	After	
		E	C		E	C		E	C		E	C			
Temp	29.80	29.70	29.40	30.50	30.10	30.40	30.20	29.40	30.10	30.10	29.80	30.60	30.40	30.70	29.70
pH	7.43	7.72	7.44	7.95	8.09	8.00	8.08	8.15	8.12	8.16	8.23	8.17	8.25	8.47	8.32
EC	142.10	137.60	141.30	168.50	165.30	167.40	200.80	197.10	198.80	232.70	229.00	232.00	256.30	253.40	255.90
TSS	38.40	20.27	36.20	42.50	21.33	40.10	56.40	35.31	53.50	77.80	33.90	72.60	89.20	38.0	87.30
DO	2.50	4.60	2.76	2.20	4.50	2.50	0	2.30	1.30	0	2.00	1.10	0	1.80	0.76
BOD	15.40	5.60	13.60	20.40	6.43	19.20	26.30	7.31	24.80	32.30	6.91	30.80	45.60	8.80	44.00
COD	32.08	23.87	30.30	44.35	32.33	42.50	59.77	40.70	58.10	78.80	49.80	77.30	108.60	67.33	106.50
NO ₃ -N	1.85	0.74	1.86	2.63	0.86	2.64	5.42	1.61	5.40	7.64	1.83	7.60	8.34	1.98	8.28
PO ₄ -P	0.64	0.37	0.61	1.03	0.53	0.98	1.88	0.90	1.82	2.34	0.90	2.10	2.62	0.83	2.58
SO ₄	3.32	1.91	3.30	7.04	3.53	7.00	8.80	4.13	8.50	10.52	3.77	10.60	13.28	6.63	13.30
TH	76.00	64.00	72.00	96.00	88.00	92.00	112.00	102.00	108.00	116.00	104.00	112.00	128.00	116.00	120.00
Ca	22.40	19.20	18.60	27.20	25.60	26.80	28.80	23.60	24.20	28.80	23.60	28.80	32.80	28.80	38.20
Mg	4.86	3.89	6.17	6.40	5.83	6.10	7.76	4.37	9.72	7.80	10.20	9.70	11.08	7.80	12.80
T.C	15 x10 ⁵	50x10 ⁴	14x10 ⁵	23 x10 ⁵	11x10 ⁵	21x10 ⁵	46 x10 ⁵	23x10 ⁵	45x10 ⁵	75 x10 ⁵	43x10 ⁵	73x10 ⁵	93 x10 ⁵	56x10 ⁵	92x10 ⁵
F.C	+ ve	-ve	+ ve	+ ve	-ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
E.Coli	+ ve	-ve	+ ve	+ ve	-ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve

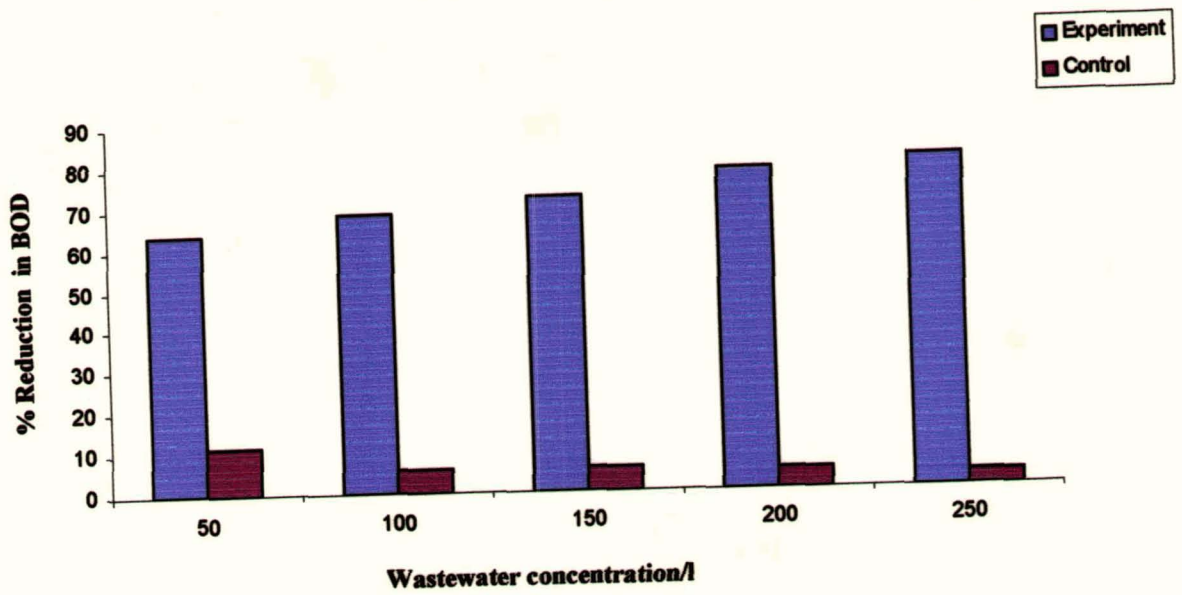


Fig. 75 Percentage Reduction of BOD in Experimental Sets Treated with *Ceratophyllum demersum* L.

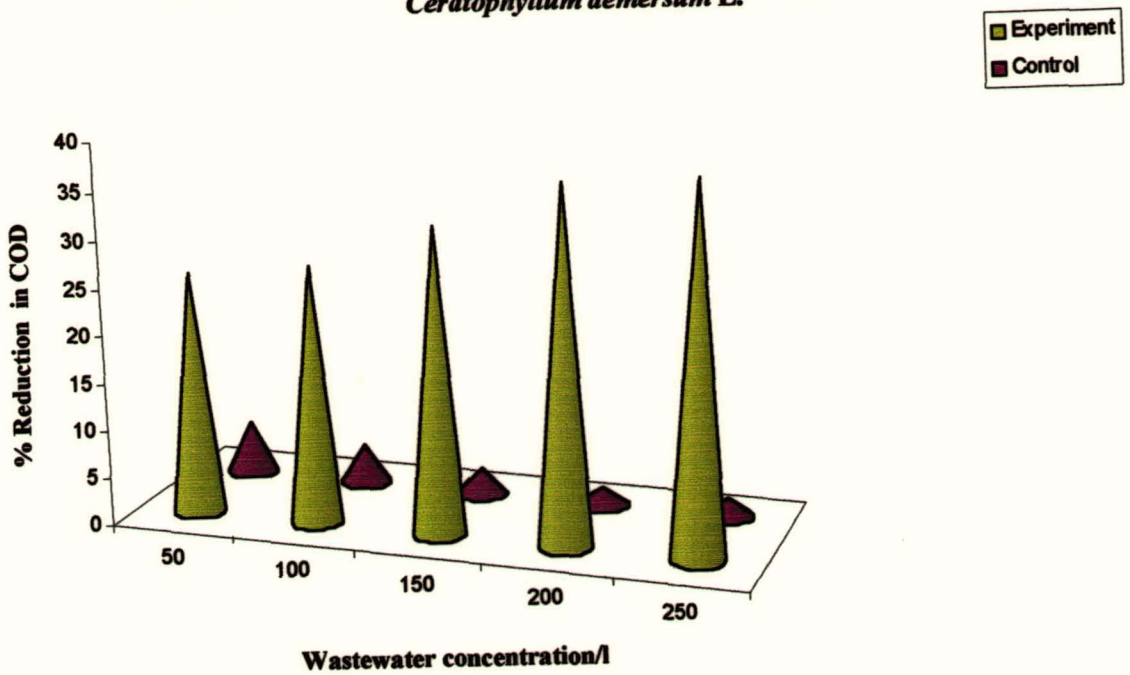


Fig.76 Percentage Reduction of COD in Experimental Sets Treated with *Ceratophyllum demersum* L.

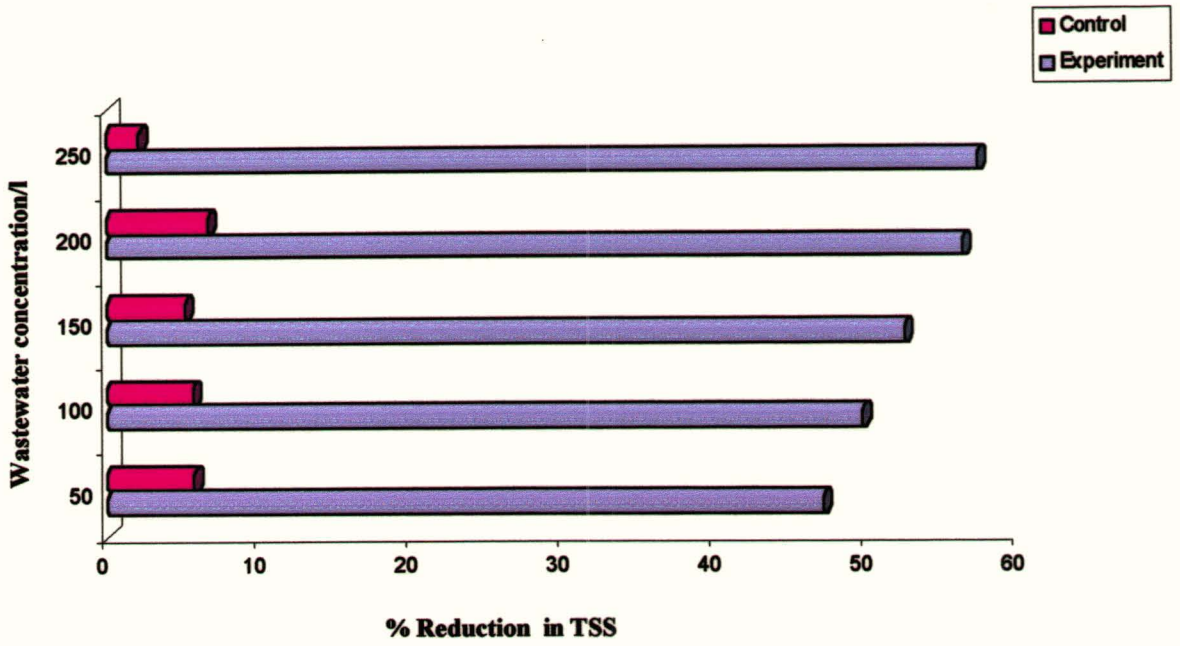


Fig.77 Percentage Reduction of TSS in Experimental Sets Treated with *Ceratophyllum demersum* L.

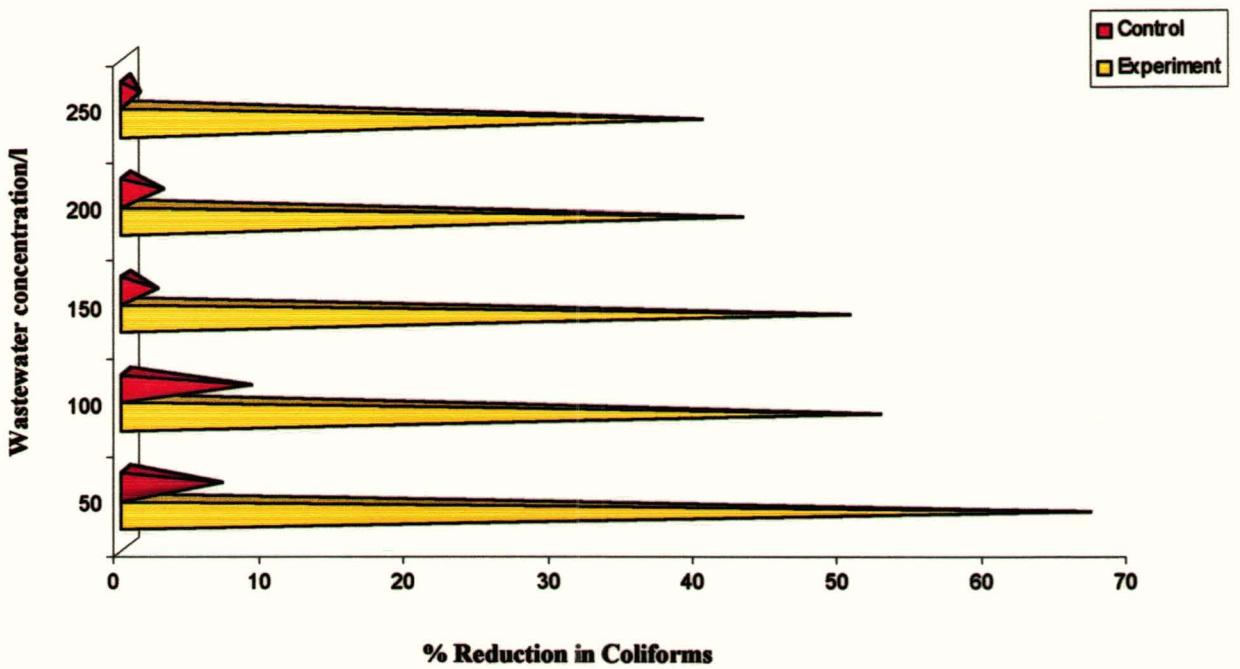


Fig.78 Percentage Reduction of Coliforms in Experimental Sets Treated with *Ceratophyllum demersum* L.

Decrease in coliforms counts of 66.7 percent was achieved in experiments with *Ceratophyllum*. 66.7, 52.5, 50, 42.6 and 39.8 percent reduction was obtained for 50-250 ml wastewater concentrations. There was a decline in coliforms removal with increasing wastewater concentrations. Fig.78 represents the reduction in total coliforms using *Ceratophyllum*. Fecal coliforms were absent in 50 and 100 ml wastewater experimental sets. *E.coli* removal was recorded in the same experimental sets.

Ceratophyllum was capable of uptake of the nutrients, nitrate and phosphate to a considerable extent in the experiments. Nitrate nitrogen removal upto 76.3 percent was noticed in 250 ml wastewater/l experimental set. Lower wastewater concentrations (50-200 ml/l) had 60.2, 67.4, 70.3 and 76 percent reduction of nitrate nitrogen. Fig.79 represents the reduction in nitrate nitrogen using *Ceratophyllum*.

The efficiency of phosphate uptake by *Ceratophyllum* was 42.3, 48.5, 52.2, 61.4 and 68.3 percent for 50-250 ml wastewater experimental sets. Control had only 4.8 percent reduction in phosphate. Fig.80 represents the reduction in phosphate using *Ceratophyllum*.

Temperature and pH showed negligible variation for the treatment period. Electrical conductivity, hardness, calcium and magnesium showed lower values compared to controls after detention period of seven days. Dissolved oxygen values increased in the experimental wastewaters at the end of treatment period.

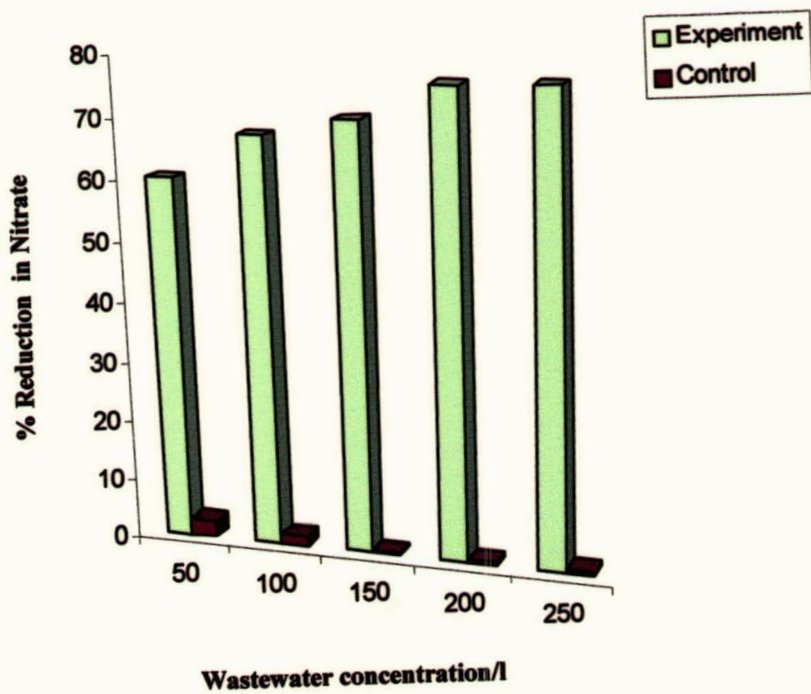


Fig. 79 Percentage Reduction of Nitrate in Experimental Sets Treated with *Ceratophyllum demersum* L.

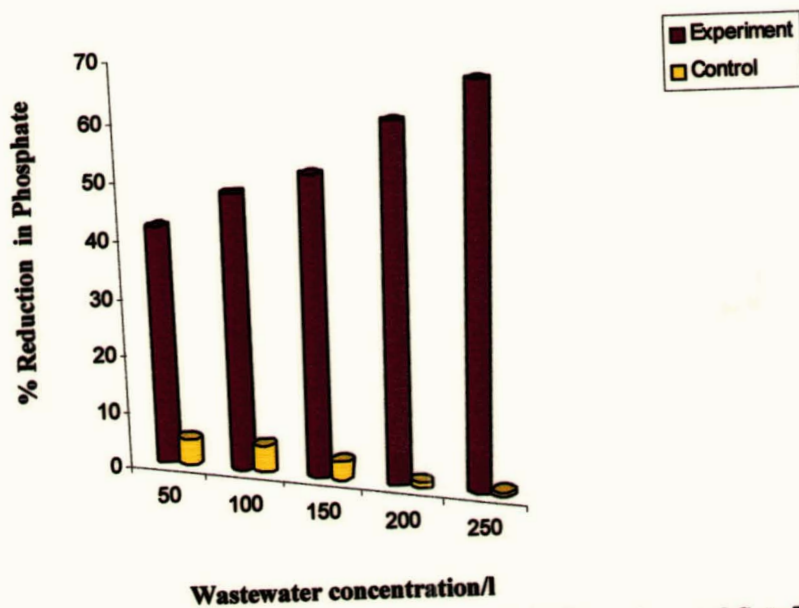


Fig. 80 Percentage Reduction of Phosphate in Experimental Sets Treated with *Ceratophyllum demersum* L.

4. 2. 4 Emergent Plants

1. *Lagenandra toxicaria* Dalz

This is an emergent plant seen in wetlands and occasionally in domestic wells. The potential of this plant for wastewater treatment was therefore examined with respect to removal of nutrients, BOD and coliforms. Results indicated that the plant was excellent in removal of coliforms, nitrate, phosphate and BOD (Table 33).

Treatment studies using *Lagenandra* showed a maximum reduction of BOD, 78.5 percent in highest wastewater concentration (250 ml/l). The reductions in BOD for other wastewater concentrations were 54.8, 62.1, 67.7 and 70.1 respectively for 50-200 ml experimental wastewaters (Fig.81). This was significant as control (without *Lagenandra*) had only 15.27 percent reduction in BOD.

Similarly, in the case of COD, reduction upto 44 percent was obtained. 30.1, 36.5, 39.3, 41.9 and 44 percent reductions in COD was recorded with 50-250 ml wastewater, while control for these sets had a maximum reduction of 19.53 percent. Fig.82 represents the reductions in COD using *Lagenandra*.

Total suspended solids concentration in the experimental sets after seven days reduced considerably. 31.2, 42.3, 56.6, 63.8 and 65.1 percent reduction was recorded with 50-250 ml wastewater concentrations respectively. The control had a maximum of 6.74 percent reduction. Fig.83 represents the reductions in TSS using *Lagenandra*.

Removal efficiencies for coliforms were excellent in studies using *Lagenandra*. 94.4 percent reduction was observed in the lowest wastewater

Table 33 Experimental Sets Treated with *Lagenandra toxicaria* Dalz.

Period of Treatment-7 days
Weight-300g/l

E- Experiment
C- Control

Parameters	50 ml Wastewater/l			100 ml Wastewater/l			150 ml Wastewater/l			200 ml Wastewater/l			250 ml Wastewater/l		
	Before	After		Before	After		Before	After		Before	After		Before	After	
		E	C		E	C		E	C		E	C			
Temp	27.60	27.80	26.00	27.30	27.50	27.80	26.90	26.40	26.30	29.40	29.30	29.10	28.60	28.30	28.20
pH	7.12	7.36	7.20	7.23	7.46	7.28	7.46	7.80	7.48	8.02	8.25	8.10	8.30	8.92	8.38
EC	145.40	139.30	142.60	164.80	178.40	161.20	203.10	197.30	200.60	235.40	230.60	234.20	259.80	255.60	258.70
TSS	35.60	24.90	33.20	43.50	25.10	41.80	57.90	25.13	55.20	78.20	28.30	76.50	88.40	30.85	86.70
DO	2.30	4.20	2.50	1.90	3.10	2.00	0	1.23	0.01	0	1.10	0.10	0	0.98	0.16
BOD	16.90	7.64	14.32	21.60	8.19	20.60	27.80	8.98	26.50	33.80	10.10	32.70	46.10	9.91	45.20
COD	33.80	23.63	27.20	45.70	29.02	44.20	58.90	35.75	57.00	79.20	46.02	74.50	109.30	61.21	107.20
NO ₃ -N	1.85	0.64	1.79	2.63	0.84	2.60	5.42	1.54	5.38	7.64	1.67	7.62	8.34	1.29	8.30
PO ₄ -P	0.64	0.38	0.62	1.03	0.53	1.00	1.88	0.76	1.85	2.34	0.85	2.23	2.62	0.78	2.54
SO ₄	3.82	2.00	3.80	7.32	3.32	7.31	8.56	2.35	8.52	10.36	3.49	10.28	13.80	6.57	13.72
TH	96.00	80.00	92.00	112.00	96.00	106.00	136.00	122.00	132.00	148.00	134.00	142.00	16.00	148.00	156.00
Ca	27.20	20.80	14.40	28.80	27.20	30.00	38.00	34.0	36.80	41.20	38.00	40.20	46.00	42.60	44.00
Mg	7.80	6.80	3.49	9.72	7.80	8.60	10.80	9.60	10.40	11.60	10.60	11.20	12.80	11.80	12.20
T.C	15 x10 ⁵	84x10 ³	14x10 ⁵	23 x10 ⁵	27x10 ⁴	21x10 ⁵	46 x10 ⁵	89x10 ⁴	44x10 ⁵	75 x10 ⁵	13x10 ⁵	73x10 ⁵	93 x10 ⁵	23x10 ⁵	92x10 ⁵
F.C	+ ve	-ve	+ ve	+ ve	-ve	+ ve	+ ve	-ve	+ ve	+ ve	+ve	+ ve	+ ve	+ ve	+ ve
E.Coli	+ ve	-ve	+ ve	+ ve	-ve	+ ve	+ ve	-ve	+ ve	+ ve	-ve	+ ve	+ ve	+ve	+ ve

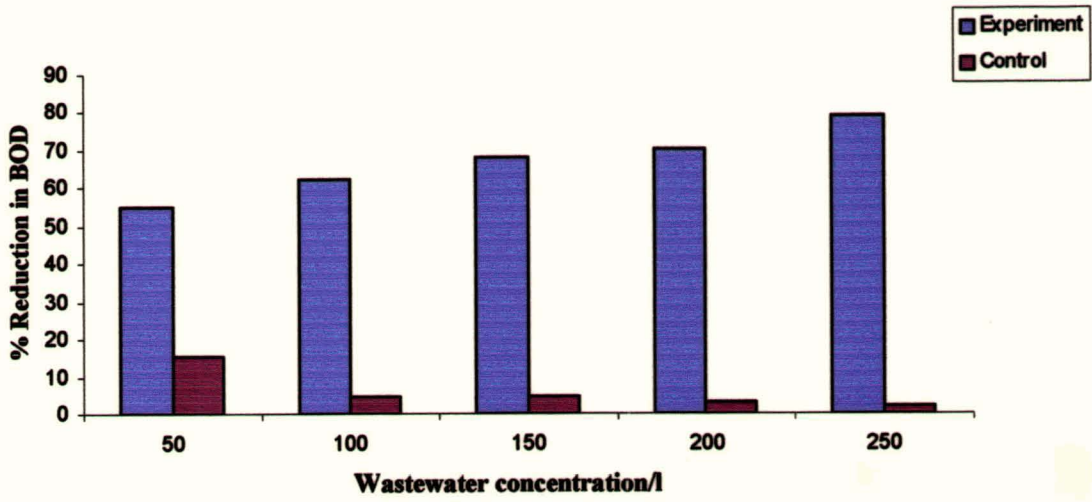


Fig. 81 Percentage Reduction of BOD in Experimental Sets Treated with *Lagenandra toxicaria* Dalz.

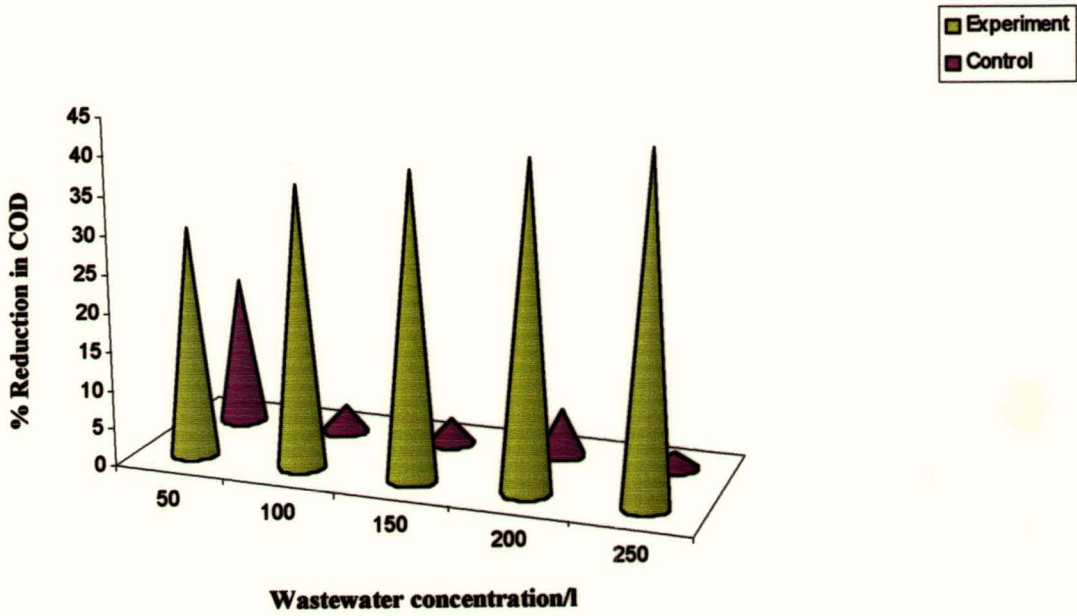


Fig. 82 Percentage Reduction of COD in Experimental Sets Treated with *Lagenandra toxicaria* Dalz.

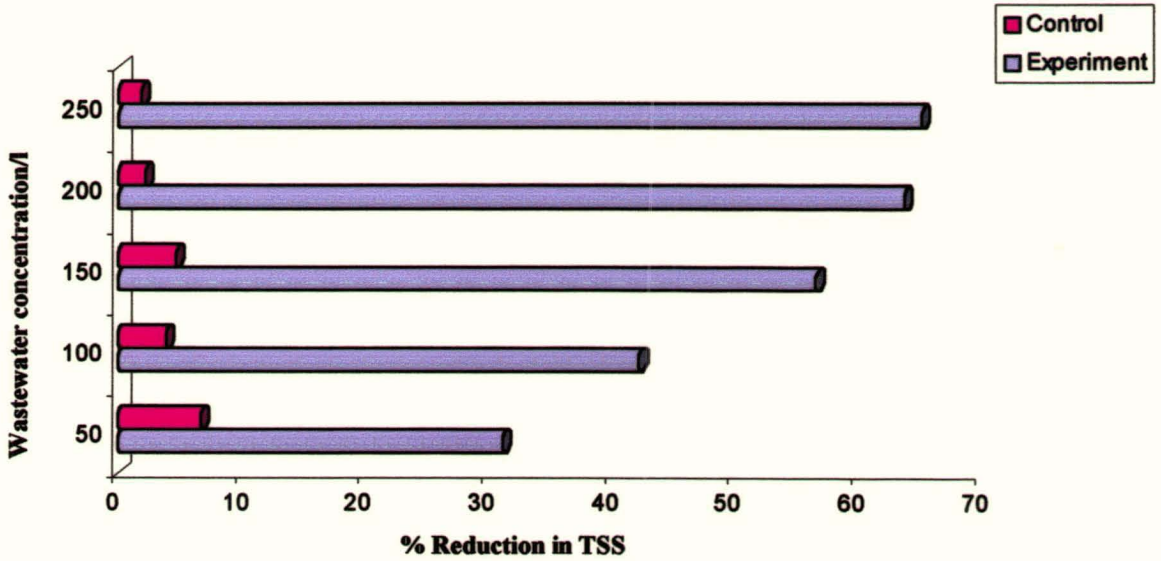


Fig. 83 Percentage Reduction of TSS in Experimental Sets Treated with *Lagenandra toxicaria* Dalz.

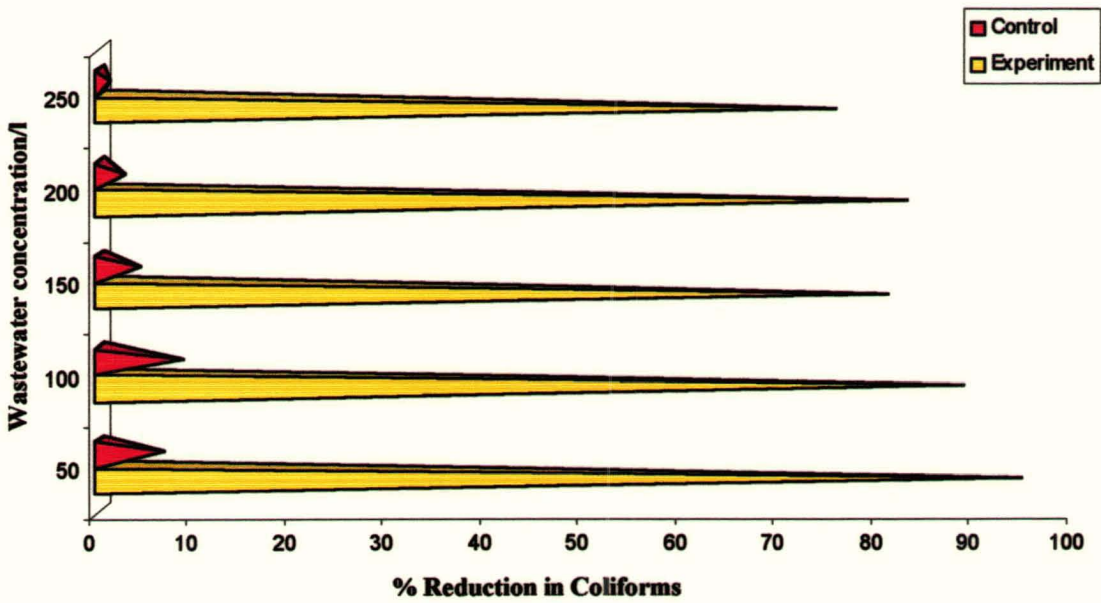


Fig.84 Percentage Reduction of Coliforms in Experimental Sets Treated with *Lagenandra toxicaria* Dalz.

concentration. Higher concentrations recorded gradually decreasing removal efficiencies - 88.3, 80.6, 82.7 and 75.3 percent for 100-250 ml/l. Control without *Lagenandra* had maximum reduction of 6.67 percent only. Fig.84 represents the reduction in coliforms using *Lagenandra*. Fecal coliforms were absent in wastewater experimental sets with 50, 100 and 150 ml wastewater/l. *E.coli* was completely removed in all experimental sets except the highest wastewater concentration.

Nitrates were reduced by a maximum of 84.5 percent in studies using *Lagenandra*. The percentage reduction obtained for nitrate nitrogen are 65.4, 68, 71.6, 78.14 and 84.5 percent for 50-250 ml/l wastewater respectively. *Lagenandra* was found to substantially reduce nitrate nitrogen from experimental wastewaters since control for these experiments resulted in 11.4 percent reduction only. Fig. 85 represents the reductions in nitrate nitrogen using *Lagenandra*.

Phosphate uptake upto 70.2 percent was recorded with *Lagenandra* treated experimental wastewaters. The reductions obtained for various concentrations of wastewater are 40.6, 48.3, 59.6, 63.5 and 70.2 percent in 50-250 ml experimental wastewaters respectively. Fig. 86 represents the reductions in phosphate using *Lagenandra*.

There was a negligible variation in temperature, while pH showed slightly elevated values. Electrical conductivity decreased to some extent when compared to control in all the concentrations of experimental wastewaters. Hardness, calcium and magnesium showed a decline probably due to plant uptake. Dissolved oxygen showed an increase from the pre experimental values and when compared to control.

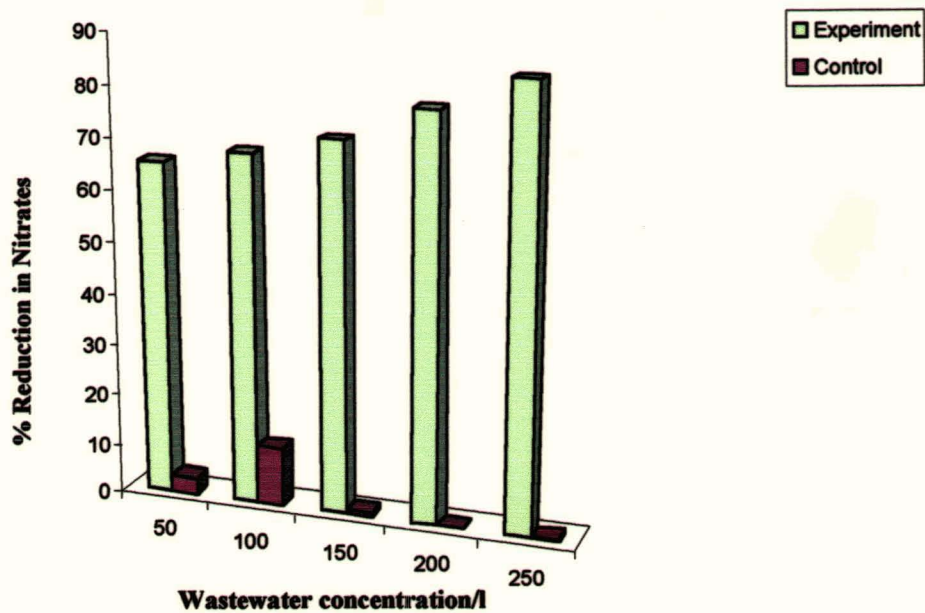


Fig. 85 Percentage Reduction of Nitrate in Experimental Sets Treated with *Lagenandra toxicaria* Dalz.

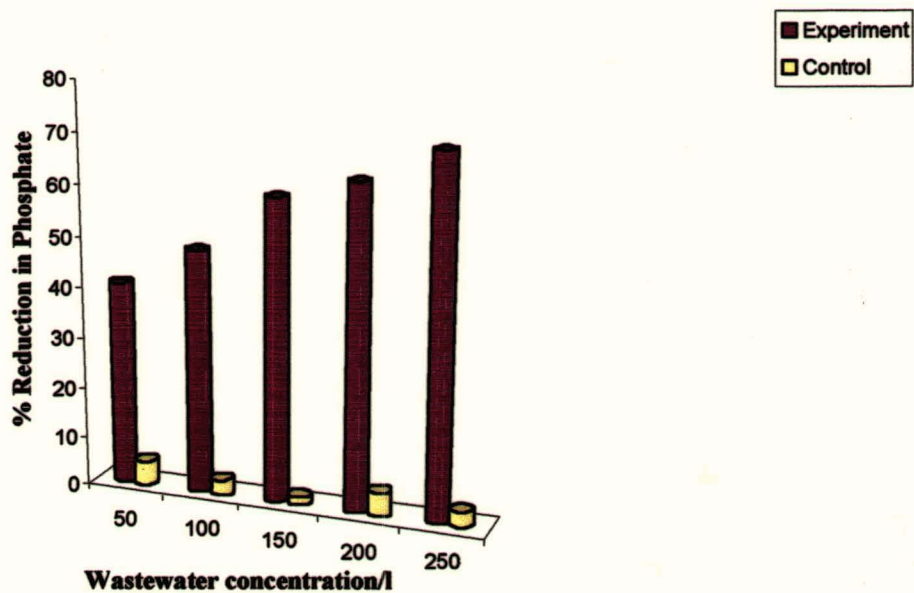


Fig. 86 Percentage Reduction of Phosphate in Experimental Sets Treated with *Lagenandra toxicaria* Dalz.

In this study the role of nine aquatic flora in improving the quality of experimental wastewaters was evaluated under laboratory conditions. All the plants had natural ability to uptake nutrients, reduce BOD, COD, TSS and coliform count. However, some macrophytes showed excellent performance with respect to selected parameters. The removal efficiency increased with increasing nutrient concentration in the wastewater. This is in conformity with observations of Tripathy *et al.* (1991).

Total Suspended Solids removal was greatest in *Pistia* (66.4%), followed by *Lagenandra* (65.1%), *Hydrilla* (63.4 %), *Lemna* (62%), *Ceratophyllum* (57.4 %), *Salvinia* (42.5 %), *Azolla* (36.3%), *Spirodela* (32.6%) and *Wolffia* (20.3%).

BOD reductions were maximum in *Lemna* (82.6 %), followed by *Ceratophyllum* (80.7 %), *Lagenandra* (78.5%), *Pistia* (75%), *Salvinia* (67.8%), *Azolla* (64%), *Hydrilla* (53.6%), *Spirodela* (46.1%) and *Wolffia* (10.2%).

COD reductions by macrophytes was in the order of 46% in *Lemna*, 46% in *Lagenandra*, 42% in *Pistia*, 38% in *Ceratophyllum*, 35% in *Hydrilla*, 26.4% in *Salvinia*, 10.6% in *Spirodela*, 9.8 % in *Azolla* and 5.2 % in *Wolffia*.

Nitrate removal was best in *Lagenandra* (84.5%) followed by *Lemna* (81.3 percent) *Pistia* (77.2%), *Ceratophyllum* (76.3%), *Spirodela* (72.4%), *Hydrilla* (65.2%), *Salvinia* (62.3%), *Wolffia* (57.7%) and *Azolla* (40.2%).

Phosphate removal potential of experimented macrophytes are *Lagenandra* (70.2%), *Lemna* (69%), *Ceratophyllum* (68.3%), *Spirodela* (67%), *Pistia* (55.4%), *Salvinia* (52.9%), *Hydrilla* (52.3%), *Azolla* (48.6%) and *Wolffia* (28.03%). In earlier studies by Tripathy *et al.* (1991) using four macrophytes, nitrogen and phosphorous removal efficiency was in decreasing order-*Pistia*,

Lemna and *Salvinia*. However in this study, *Lemna* was found to be more efficient. Removal of phosphate is limited to the plants need and usually does not exceed 50-70 percent of the phosphorous contained in the wastewater (Richardson and Daigger, 1984). The present study is in conformity with the above finding.

Sulphate removal efficiencies of the macrophytes experimented are *Lagenandra* (72.5%), *Pistia* (64.3%), *Ceratophyllum* (64.2%), *Lemna* (62.7%), *Salvinia* (60.2%), *Azolla* (53.2 %), *Hydrilla* (51.4%), *Spirodela* (45%) and *Wolffia* (35.2 %).

Excellent coliforms reduction was obtained in experiments with *Pistia* (99.75), *Lagenandra* (94.4%) and *Lemna* (93%). Percentage reductions for other plants are *Spirodela* (88%), *Salvinia* (84.6%), *Ceratophyllum* (66.7%), *Hydrilla* (53.3%), *Azolla* (48.6 %) and *Wolffia* (45.8 %).

Filtration through the root substrate and attached biofilm, sedimentation, aggregation, oxidation, exposure to biocides secreted by plants (Polprasert, 1996), adsorption to organic matter (Wood, 1990), ingestion by ciliates (Gersberg *et al.* 1987) are causes for coliform removal. Aquatic macrophytes such as Water Hyacinth, *Pistia*, *Lagenandra* and *Ceratophyllum* have antimicrobial properties (Agarwal, 1997).

Increase in dissolved oxygen levels was greatest in *Ceratophyllum* treated experimental wastewater, followed by *Hydrilla*, *Lagenandra* and *Pistia*.

Comparison of the aquatic macrophytes indicates that *Lagenandra*, *Pistia*, *Ceratophyllum* and *Lemna* have good potential for wastewater treatment, followed by *Spirodela*, *Salvinia*, *Hydrilla*, *Azolla* and *Wolffia*.

4. 3 STATISTICAL ANALYSIS

4.3 STATISTICAL ANALYSIS

The mean and 95% confidence intervals were estimated in respect of selected water quality parameters and are presented in Figures 87 to 103. The data generated from the experiments were analysed for all parameters for significance between dilutions, stages of experiments and presence or absence of test species using Analysis of Variance. The results of selected parameters are presented in Tables 34 to 50. The functional relation of different water quality parameters were assessed by fitting appropriate polynomial regression equations with dilution as independent variable. Tables 51 and 52 represent the regression equations derived for the test species used in the study.

Aquatic fauna

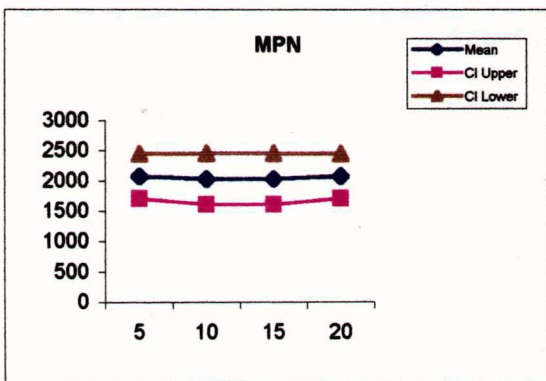
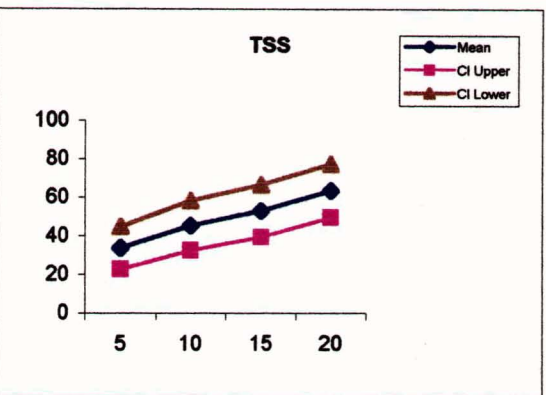
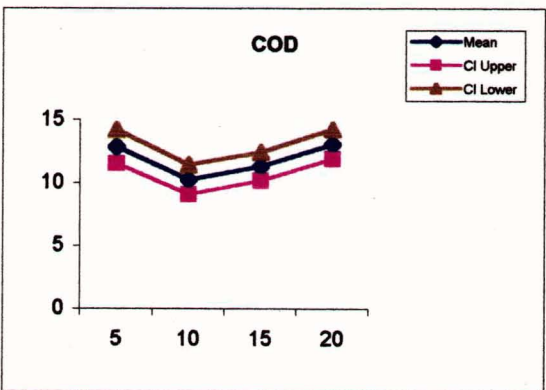
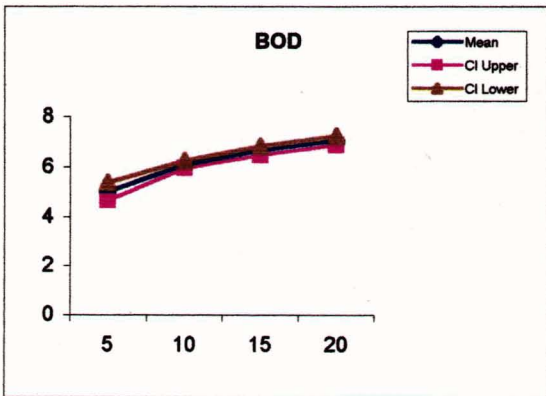
1. *Paramecium caudatum* Ehrenberg

Mean values and 95 % confidence interval of water quality parameters are presented in Fig. 87.

All the parameters studied showed significant differences ($p < 0.01$) between dilution as well as pre/post experimental stages (Table 34). Conductivity, hardness, magnesium, calcium, pH, TSS, BOD, COD, NO_3 , SO_4 , PO_4 , and MPN showed significant difference ($p < 0.05$) between presence/ absence of test species. Two way interactions between dilution and pre/post experimental stages was significant ($p < 0.05$) for pH, temperature, conductivity, magnesium, calcium, TSS, BOD, SO_4 , and MPN. Interactions between dilution and presence/absence of *Paramecium* was

Fig 87 & 88 Mean and Confidence Intervals for experiments with *Paramecium* and *Daphnia*

PARAMECIUM



DAPHNIA

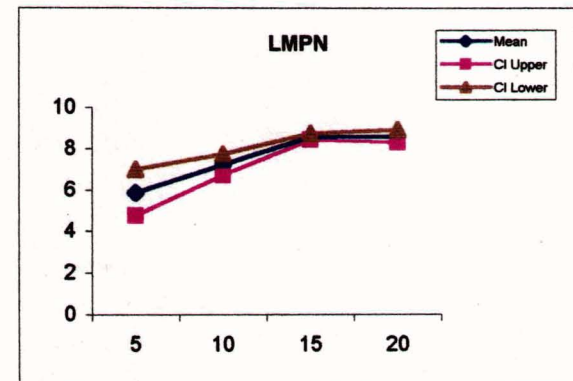
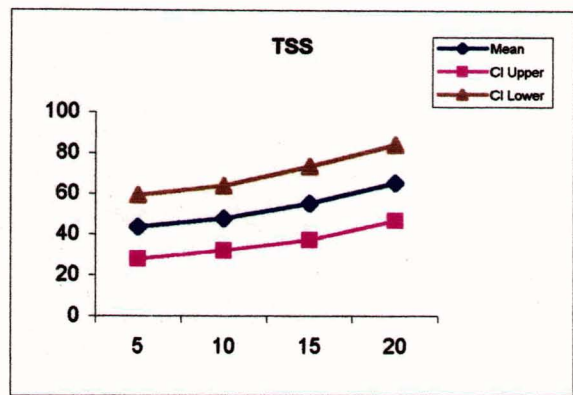
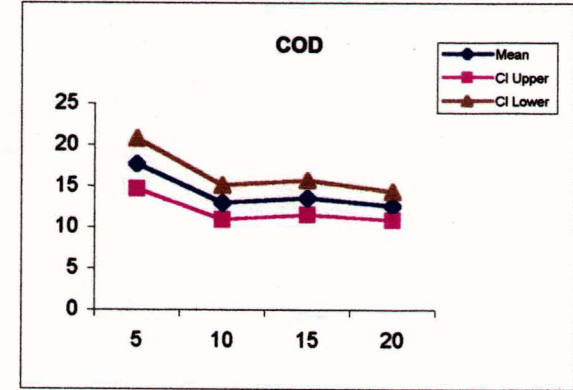
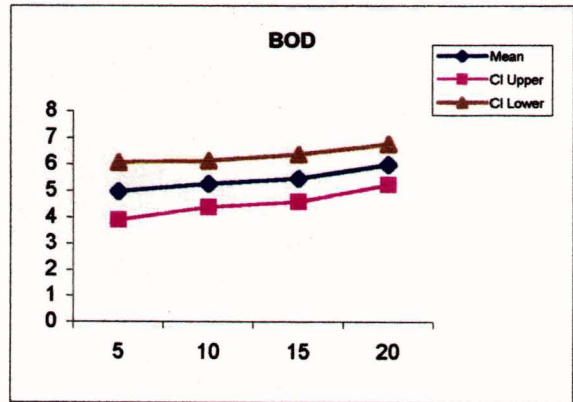


Table 34 Anova for Experiments with Paramecium

TSS		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	5693.3166	3	1897.772	813.324	<0.01
	beaf	8465.2032	1	8465.203	3627.91	<0.01
	wwof	4949.9532	1	4949.953	2121.39	<0.01
2-Way Interactions	dil*beaf	70.5966	3	23.532	10.0851	<0.01
	dil*wwof	37.5066	3	12.502	5.35804	<0.01
	beaf*wwof	4949.9532	1	4949.953	2121.39	<0.01
Residual		81.6674	35	2.333		
Total		24248.1968	47	515.919		

BOD		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	27.9818	3	9.327266667	350.699	<0.01
	beaf	1.794133333	1	1.794133333	67.4583	<0.01
	wwof	1.178133333	1	1.178133333	44.2971	<0.01
2-Way Interactions	dil*beaf	0.5658	3	0.1886	7.09124	<0.01
	dil*wwof	0.2298	3	0.0766	2.88011	>0.05
	beaf*wwof	1.178133333	1	1.178133333	44.2971	<0.01
Residual		0.930866667	35	0.02659619		
Total		33.85866667	47	0.720397163		

COD		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	64.103756	3	21.367919	804.25	<0.01
	beaf	88.210519	1	88.210519	3320.09	<0.01
	wwof	34.629019	1	34.629019	1303.37	<0.01
2-Way Interactions	dil*beaf	0.178706	3	0.059569	2.24206	>0.05
	dil*wwof	0.799106	3	0.266369	10.0256	<0.01
	beaf*wwof	34.629019	1	34.629019	1303.37	<0.01
Residual		0.929906	35	0.026569		
Total		223.480031	47	4.754894		

MPN		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	24300	3	8100	11.6667	<0.01
	beaf	5796300	1	5796300	8348.58	<0.01
	wwof	5796300	1	5796300	8348.58	<0.01
2-Way Interactions	dil*beaf	24300	3	8100	11.6667	<0.01
	dil*wwof	24300	3	8100	11.6667	<0.01
	beaf*wwof	5796300	1	5796300	8348.58	<0.01
Residual		24300	35	694.2857143		
Total		17486100	47	372044.6809		

dil - Dilution

beaf- Before/After

wwof- With fish/without fish

significant ($p < 0.05$) for temperature, COD, pH, TSS, calcium and MPN. Conductivity, COD, PO_4 , hardness, TSS, BOD, NO_3 , SO_4 , calcium and MPN showed significant interactions between presence/absence of *Paramecium* and pre/post experimental stage.

First degree polynomial equation fitted well for BOD and TSS while for COD, second degree equation was found to fit best.

2. *Daphnia magna* Straus

Mean values and 95 % confidence interval of water quality parameters are presented in Fig. 88.

Significant differences ($p < 0.01$) were observed between dilutions for all the parameters analyzed (Table 35). In the case of pre/post experimental stage significant difference ($p < 0.05$) was seen for all the parameters except temperature and phosphate. The significant differences for presence/ absence of *Daphnia* was ($p < 0.05$) for all parameters except phosphate.

The two way interactions between dilution and pre/post experimental stage was significant ($p < 0.05$) for temperature, pH, DO, COD, BOD, TSS, calcium, magnesium, SO_4 and log MPN. All the parameters except PO_4 showed significant difference ($p < 0.05$) for the interaction between pre/post experimental stage and presence/absence of *Daphnia*.

Table 35 Anova for Experiments with Daphnia

TSS		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	3285.229825	3	1095.076608	379.84	<0.01
	beaf	10645.15901	1	10645.15901	3692.4	<0.01
	wwof	10526.35568	1	10526.35568	3651.19	<0.01
2-Way Interactions	dil*beaf	368.379825	3	122.793275	42.5923	<0.01
	dil*wwof	33.249825	3	11.083275	3.84436	<0.05
	beaf*wwof	10526.35568	1	10526.35568	3651.19	<0.01
Residual		100.9047583	35	2.882993095		
Total		35485.63459	47	755.013502		

BOD		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	6.5103	3	2.1701	86.0565	<0.01
	beaf	55.47	1	55.47	2199.69	<0.01
	wwof	16.9932	1	16.9932	673.875	<0.01
2-Way Interactions	dil*beaf	0.3042	3	0.1014	4.02107	<0.01
	dil*wwof	0.7962	3	0.2654	10.5246	<0.01
	beaf*wwof	16.9932	1	16.9932	673.875	<0.01
Residual		0.8826	35	0.025217143		
Total		97.9497	47	2.08403617		

COD		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	198.89886	3	66.29962	297.888	<0.01
	beaf	219.43577	1	219.43577	985.936	<0.01
	wwof	162.61922	1	162.61922	730.657	<0.01
2-Way Interactions	dil*beaf	11.07696	3	3.69232	16.5898	<0.01
	dil*wwof	7.68501	3	2.56167	11.5097	<0.01
	beaf*wwof	162.61922	1	162.61922	730.657	<0.01
Residual		7.78981	35	0.22257		
Total		770.12483	47	16.38563		

LMPN		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	61.2215	3	20.4072	163.184	<0.01
	beaf	13.7656	1	13.7656	110.075	<0.01
	wwof	8.2314	1	8.2314	65.8214	<0.01
2-Way Interactions	dil*beaf	6.3337	3	2.1112	16.8824	<0.01
	dil*wwof	4.3770	3	1.4590	11.6667	<0.01
	beaf*wwof	8.2314	1	8.2314	65.8214	<0.01
Residual		4.3770	35	0.1251		
Total		106.5375	47	2.2668		

dil - Dilution

beaf- Before/After

wwof- With fish/without fish

First degree polynomial equation was fitted for BOD, TSS and MPN. Second degree equation fitted well for COD.

3. *Tilapia mossambica* Peters

Mean values and 95 % confidence interval of water quality parameters are presented in Fig. 89.

All the parameters except temperature showed significant difference ($p < 0.01$) between dilutions (Table 36). Conductivity, TSS, DO, BOD, COD, NO_3 , PO_4 , SO_4 , hardness, calcium, magnesium and log MPN showed significant difference ($p < 0.05$) between pre/post experimental stage. Significant differences were observed between presence and absence of fish for pH, conductivity, TSS, DO, BOD, COD, NO_3 , PO_4 , magnesium and log MPN.

Two way interactions between dilution and pre/post experimental stages was significant ($p < 0.05$) for conductivity, DO, BOD, COD, NO_3 , SO_4 , hardness, calcium, magnesium and log MPN. Interactions between dilution and presence and absence of fish was significant ($p < 0.05$) for conductivity, DO, BOD, COD, NO_3 , SO_4 , hardness, calcium, magnesium and log MPN. Significant differences were observed ($p < 0.05$) between presence and absence of fish and pre/post experimental stage for pH, conductivity, TSS, DO, BOD, COD, NO_3 , PO_4 , magnesium and log MPN.

First-degree polynomial equation fitted well for the parameters BOD, COD, TSS and log MPN.

Fig.89 & 90 Mean and Confidence Intervals for experiments with *Tilapia* and *Cyprinus*

TILAPIA

CYPRINUS

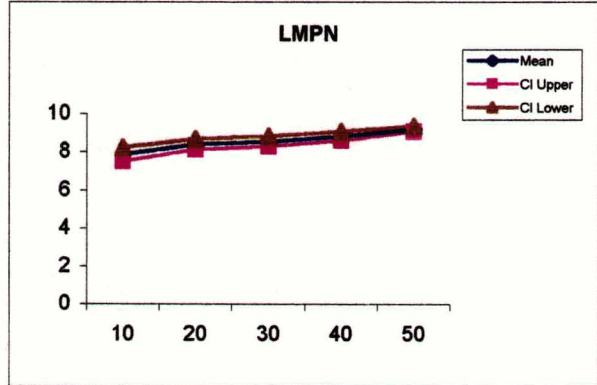
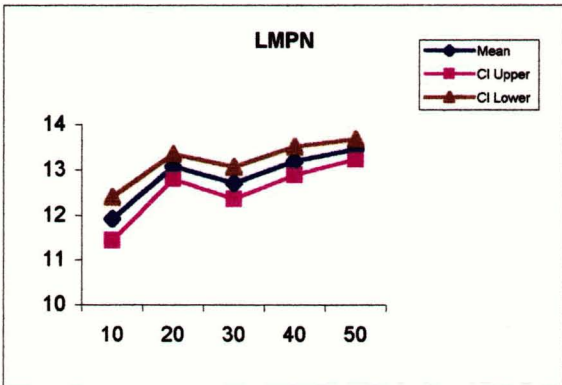
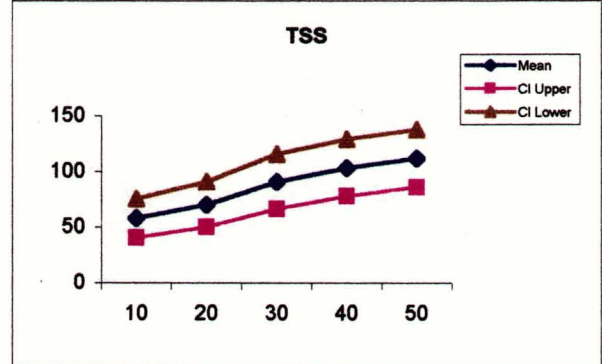
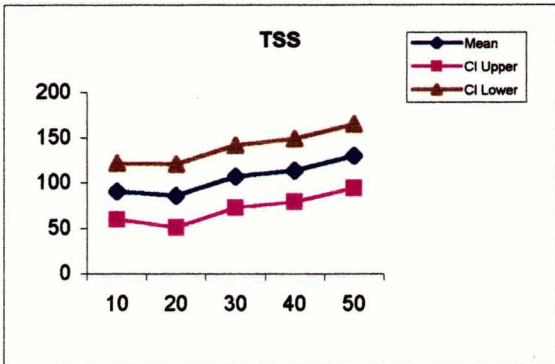
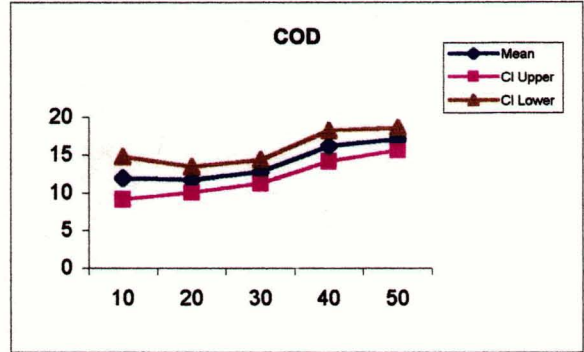
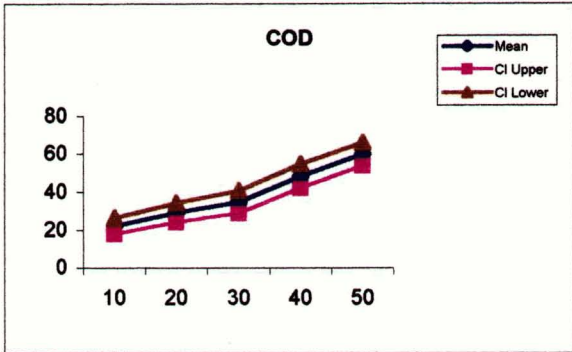
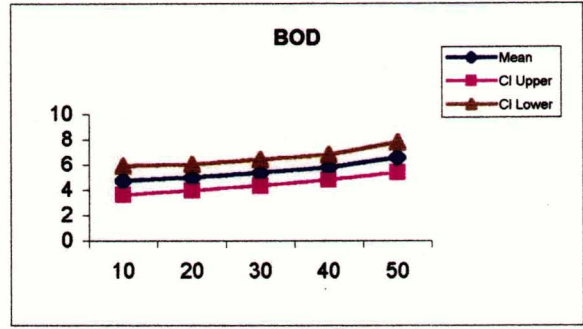
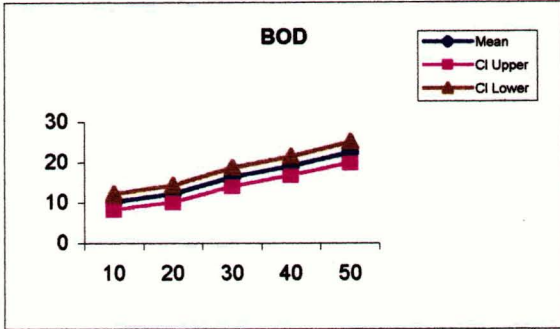


Table 36 Anova for Experiments with Tilapia

TSS		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	15066.285	4	3766.5712	18.3632	<0.01
	beaf	62975.880	1	62975.8804	307.028	<0.01
	wwof	42233.107	1	42233.1070	205.9	<0.01
2-Way Interactions	dil*beaf	623.067	4	155.7667	0.75941	>0.05
	dil*wwof	1166.596	4	291.6491	1.42188	>0.05
	beaf*wwof	42233.107	1	42233.1070	205.9	<0.01
Residual		9025.050	44	205.1148		
Total		173323.092	59	2937.6795		

BOD		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	1194.33122	4	298.58280	4617.02	<0.01
	beaf	402.74504	1	402.74504	6227.69	<0.01
	wwof	142.45004	1	142.45004	2202.72	<0.01
2-Way Interactions	dil*beaf	10.45922	4	2.61480	40.433	<0.01
	dil*wwof	2.70022	4	0.67505	10.4384	<0.01
	beaf*wwof	142.45004	1	142.45004	2202.72	<0.01
Residual		2.84548	44	0.06467		
Total		1897.98126	59	32.16917		

COD		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	11112.225	4	2778.05625	5930.03	<0.01
	beaf	1783.785	1	1783.785375	3807.66	<0.01
	wwof	1128.834	1	1128.834375	2409.61	<0.01
2-Way Interactions	dil*beaf	42.873	4	10.71825	22.8791	<0.01
	dil*wwof	18.669	4	4.66725	9.96269	<0.01
	beaf*wwof	1128.834	1	1128.834375	2409.61	<0.01
Residual		20.613	44	0.468473		
Total		15235.834	59	258.2344733		

LMPN		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	16.5630146	4	4.1407536	43.1928	<0.01
	beaf	3.8811339	1	3.8811339	40.4847	<0.01
	wwof	1.3024898	1	1.3024898	13.5865	<0.01
2-Way Interactions	dil*beaf	2.1267072	4	0.5316768	5.546	<0.01
	dil*wwof	4.2181344	4	1.0545336	11	<0.01
	beaf*wwof	1.3024898	1	1.3024898	13.5865	<0.01
Residual		4.2181344	44	0.0958667		
Total		33.6121041	59	0.5696967		

dil - Dilution

beaf- Before/After

wwof- With fish/without fish

4. *Cyprinus carpio* L.

Mean values and 95 % confidence interval of water quality parameters are presented in Fig. 90.

All the parameters showed significant differences ($p < 0.01$) between dilutions, while for pre/post experimental stage, pH, COD, BOD, TSS, SO_4 , hardness, calcium, magnesium and log MPN showed $p < 0.05$. Presence/absence of fish was significant at $p < 0.05$ for pH, DO, COD, BOD, TSS, NO_3 , SO_4 , calcium and log MPN (Table 37).

In the two-way interactions between dilution and pre/post experimental stage, significant difference ($p < 0.05$) was observed for DO, COD, BOD, TSS, PO_4 , magnesium and log MPN. The interaction between dilution and presence or absence of *Cyprinus* was significant ($p < 0.05$) for COD, BOD, TSS, PO_4 , NO_3 , pH, calcium, magnesium and log MPN. The parameters DO, COD, BOD, TSS, pH, NO_3 , SO_4 , calcium and log MPN showed significant differences ($p < 0.05$) for the interactions between pre/post experimental stages and presence/ absence of *Cyprinus*.

COD, log MPN and TSS fitted the first-degree polynomial equation while for BOD the second-degree polynomial fitted well.

5. *Lebistes reticulates* Peters

Mean values and 95 % confidence interval of water quality parameters are presented in Fig. 91.

Significant differences ($p < 0.01$) was observed between dilutions for all the parameters in the experiments conducted using *Lebistes* (Table 38). Temperature,

Table 37 Anova for Experiments with Cyprinus

TSS		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	24177.1374	4	6044.28435	451.467	<0.01
	beaf	28123.35	1	28123.35	2100.62	<0.01
	wwof	21228.966	1	21228.966	1585.66	<0.01
2-Way Interactions	dil*beaf	510.7974	4	127.69935	9.53828	<0.01
	dil*wwof	510.7314	4	127.68285	9.53704	<0.01
	beaf*wwof	21228.966	1	21228.966	1585.66	<0.01
Residual		589.0762	44	13.38809545		
Total		96369.0244	59	1633.373295		

BOD		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	24.789	4	6.1973	472.794	<0.01
	beaf	147.517	1	147.5174	11254.2	<0.01
	wwof	6.774	1	6.7738	516.776	<0.01
2-Way Interactions	dil*beaf	1.027	4	0.2567	19.5869	<0.01
	dil*wwof	0.480	4	0.1200	9.15376	<0.01
	beaf*wwof	6.774	1	6.7738	516.776	<0.01
Residual		0.577	44	0.0131		
Total		187.938	59	3.1854		

COD		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	302.7460	4	75.68650	978.842	<0.01
	beaf	75.3312	1	75.33122	974.247	<0.01
	wwof	131.6905	1	131.69054	1703.13	<0.01
2-Way Interactions	dil*beaf	194.9555	4	48.73888	630.332	<0.01
	dil*wwof	2.5354	4	0.63385	8.19745	<0.01
	beaf*wwof	131.6905	1	131.69054	1703.13	<0.01
Residual		3.4022	44	0.07732		
Total		842.3514	59	14.27714		

LMPN		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	11.939126	4	2.984782	1148.5	<0.01
	beaf	7.042204	1	7.042204	2709.73	<0.01
	wwof	0.990870	1	0.990870	381.271	<0.01
2-Way Interactions	dil*beaf	0.621732	4	0.155433	59.8081	<0.01
	dil*wwof	0.114350	4	0.028587	11	<0.01
	beaf*wwof	0.990870	1	0.990870	381.271	<0.01
Residual		0.114350	44	0.002599		
Total		21.813501	59	0.369720		

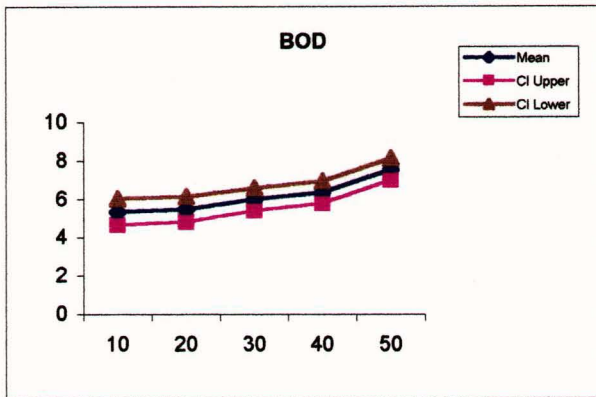
dil - Dilution

beaf- Before/After

wwof- With fish/without fish

Fig.91& 92 Mean and Confidence Intervals for experiments with *Lebistes* and *Catla*

LEBISTES



CATLA

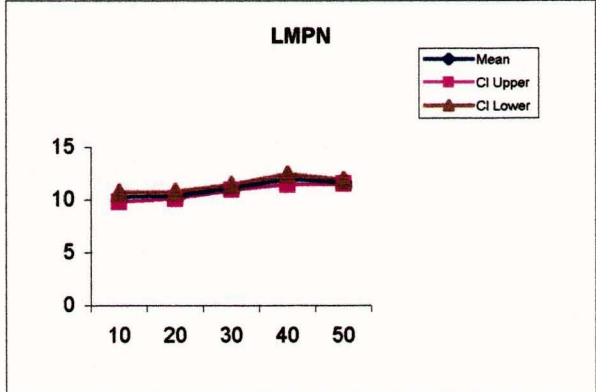
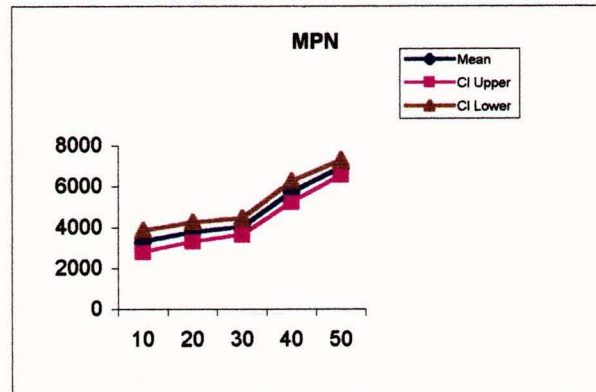
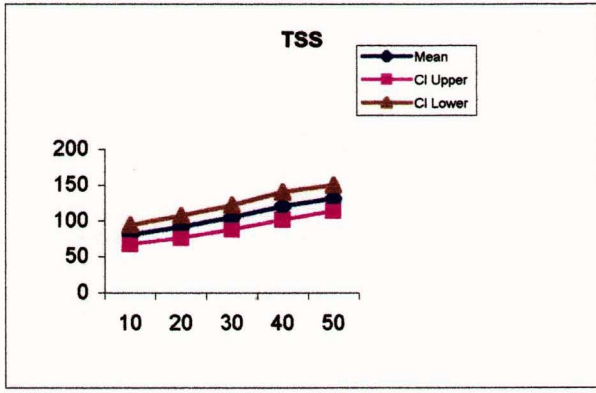
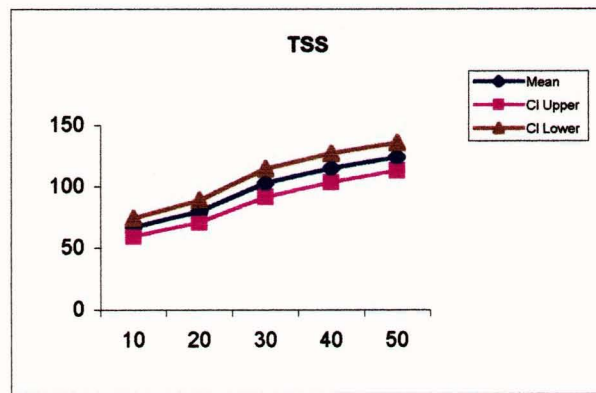
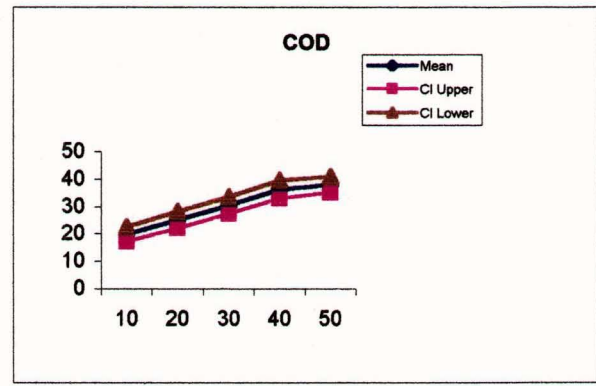
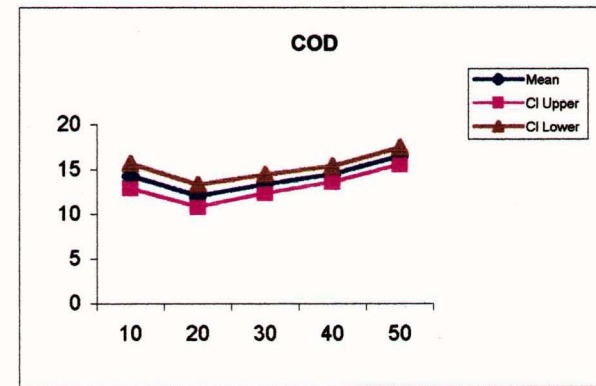
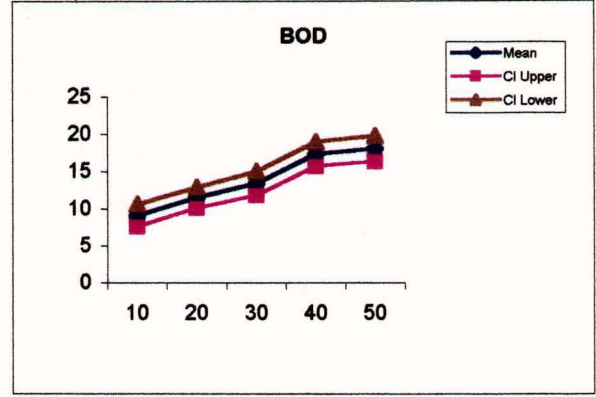


Table 38 Anova for Experiments with Lebistes

TSS		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	27728.2456	4	6932.061	1242.91	<0.01
	beaf	7019.8003	1	7019.800	1258.65	<0.01
	wwof	3572.6623	1	3572.662	640.576	<0.01
2-Way Interactions	dil*beaf	129.4576	4	32.364	5.80292	<0.01
	dil*wwof	167.3956	4	41.849	7.50348	<0.01
	beaf*wwof	3572.6623	1	3572.662	640.576	<0.01
Residual		245.3996	44	5.577		
Total		42435.6234	59	719.248		

BOD		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	37.3679	4	9.34196	1447.3	<0.01
	beaf	48.9245	1	48.92454	7579.59	<0.01
	wwof	0.9077	1	0.90774	140.631	<0.01
2-Way Interactions	dil*beaf	0.2150	4	0.05375	8.32756	<0.01
	dil*wwof	0.2708	4	0.06770	10.4888	<0.01
	beaf*wwof	0.9077	1	0.90774	140.631	<0.01
Residual		0.2840	44	0.00645		
Total		88.8777	59	1.50640		

COD		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	127.5596	4	31.8899	947.982	<0.01
	beaf	71.7445	1	71.7445	2132.73	<0.01
	wwof	45.6754	1	45.6754	1357.78	<0.01
2-Way Interactions	dil*beaf	2.5468	4	0.6367	18.9269	<0.01
	dil*wwof	1.2824	4	0.3206	9.53001	<0.01
	beaf*wwof	45.6754	1	45.6754	1357.78	<0.01
Residual		1.4802	44	0.0336		
Total		295.9641	59	5.0163		

MPN		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	109775026.5	4	27443756.63	1421.41	<0.01
	beaf	19993053.75	1	19993053.75	1035.51	<0.01
	wwof	3377253.75	1	3377253.75	174.92	<0.01
2-Way Interactions	dil*beaf	131626.5	4	32906.625	1.70435	>0.05
	dil*wwof	849526.5	4	212381.625	11	<0.01
	beaf*wwof	3377253.75	1	3377253.75	174.92	<0.01
Residual		849526.5	44	19307.42045		
Total		138353267.3	59	2344970.631		

dil - Dilution

beaf- Before/After

wwof- With fish/without fish

DO, COD, BOD, TSS, hardness, calcium, PO₄, NO₃ and MPN showed differences (p<0.05) between pre/post experimental stages. Temperature, DO, COD, pH, TSS, BOD, NO₃ and MPN showed significant differences (p<0.05) between presence/absence of *Lebistes*.

The two way interactions between dilution and pre/ post experimental stages was significant for DO, COD, PO₄, magnesium, TSS, BOD, NO₃, SO₄ and calcium. All parameters except hardness, calcium and nitrate showed significance at p<0.05 for the interactions between dilution and presence/absence of *Lebistes*. Temperature, DO, COD, pH, TSS, BOD, NO₃, calcium and MPN showed significant interactions (p<0.05) between pre/post experimental stage and presence/absence of *Lebistes*.

BOD, MPN and TSS fitted first degree polynomial equation while for COD, second degree polynomial equation was found to fit best.

6. *Catla catla* Hamilton

Mean values and 95 % confidence interval of water quality parameters are presented in Fig. 92.

Significant difference (p<0.01) was observed between dilutions, pre and post-experimental stage as well as with and without test species, for all parameters except dissolved oxygen and phosphate (Table 39).

The two way interactions between dilution and pre/ post experimental stage was significant for all parameters (p<0.01) except temperature, pH, DO, PO₄ and SO₄.

Table 39 Anova for Experiments with Catla

TSS		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	20806.585	4	5201.646	1039.61	<0.01
	beaf	13372.502	1	13372.502	2672.64	<0.01
	wwof	12147.866	1	12147.866	2427.88	<0.01
2-Way Interactions	dil*beaf	245.123	4	61.281	12.2476	<0.01
	dil*wwof	196.931	4	49.233	9.8397	<0.01
	beaf*wwof	12147.866	1	12147.866	2427.88	<0.01
Residual		220.153	44	5.003		
Total		59137.028	59	1002.323		

BOD		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	695.7456	4	173.9364	2969.09	<0.01
	beaf	247.8227	1	247.8227	4230.33	<0.01
	wwof	39.9187	1	39.9187	681.412	<0.01
2-Way Interactions	dil*beaf	2.8184	4	0.7046	12.0273	<0.01
	dil*wwof	1.3492	4	0.3373	5.75752	<0.01
	beaf*wwof	39.9187	1	39.9187	681.412	<0.01
Residual		2.5776	44	0.0586		
Total		1030.1509	59	17.4602		

COD		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	2773.477	4	693.369	16262.5	<0.01
	beaf	467.883	1	467.883	10973.9	<0.01
	wwof	374.550	1	374.550	8784.8	<0.01
2-Way Interactions	dil*beaf	2.407	4	0.602	14.1139	<0.01
	dil*wwof	1.505	4	0.376	8.82579	<0.01
	beaf*wwof	374.550	1	374.550	8784.8	<0.01
Residual		1.876	44	0.043		
Total		3996.249	59	67.733		

LMPN		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	26.9836	4	6.7459	61.4213	<0.01
	beaf	1.7923	1	1.7923	16.3189	<0.01
	wwof	0.5080	1	0.5080	4.62516	<0.05
2-Way Interactions	dil*beaf	4.3403	4	1.0851	9.87963	<0.01
	dil*wwof	4.8325	4	1.2081	11	<0.01
	beaf*wwof	0.5080	1	0.5080	4.62516	<0.05
Residual		4.8325	44	0.1098		
Total		43.7973	59	0.7423		

dil - Dilution

beaf- Before/After

wwof- With fish/without fish

The interaction between pre/post experimental stage and presence or absence of test species was significant in all cases except PO₄ and total hardness.

7. *Labeo rohita* Hamilton

Mean values and 95 % confidence interval of water quality parameters are presented in Fig. 93.

Significant difference ($p < 0.01$) between dilution as well as presence/absence of *Labeo* was observed in experiments conducted (Table 40). All parameters except temperature and PO₄ showed significant difference ($p < 0.05$) between pre/post experimental stage.

Interactions between dilution and pre/post experimental stage was significant ($p < 0.05$) for temperature, pH, BOD, COD, SO₄, hardness, calcium, magnesium and log MPN. Temperature, pH, BOD, COD, NO₃, SO₄, hardness, calcium, magnesium and log MPN showed significant difference ($p < 0.05$) between dilution and presence/absence of *Labeo*. Interactions between presence/absence of *Labeo* and pre/post experimental stage was significant ($p < 0.05$) for all parameters studied.

Regression equation fitted first degree polynomial for BOD, COD, log MPN and TSS.

8. *Cirrhinus mrigala* Hamilton

Mean values and 95 % confidence interval of water quality parameters are presented in Fig. 94.

Fig.93 & 94 Mean and Confidence Intervals for experiments with *Labeo* and *Cirrhinus*

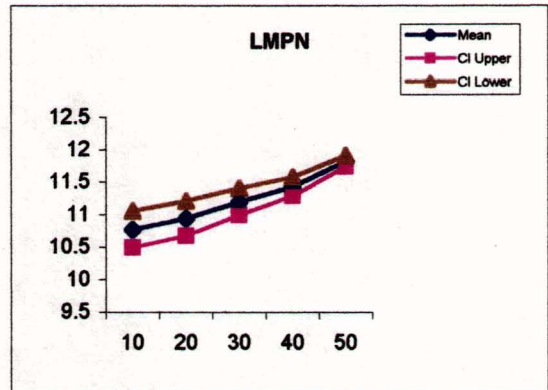
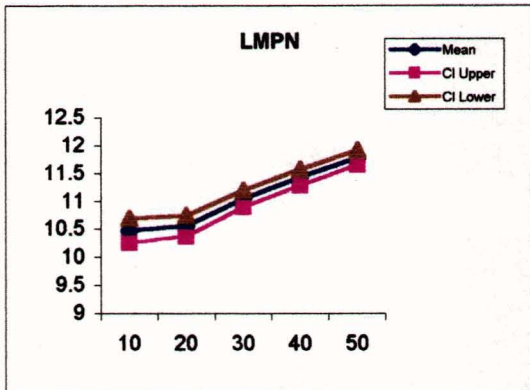
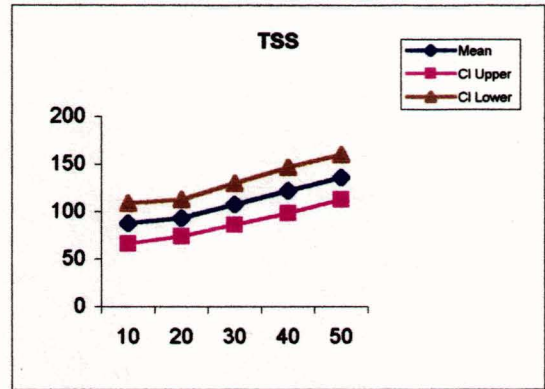
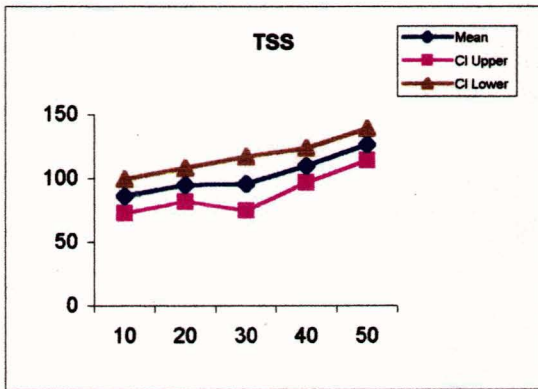
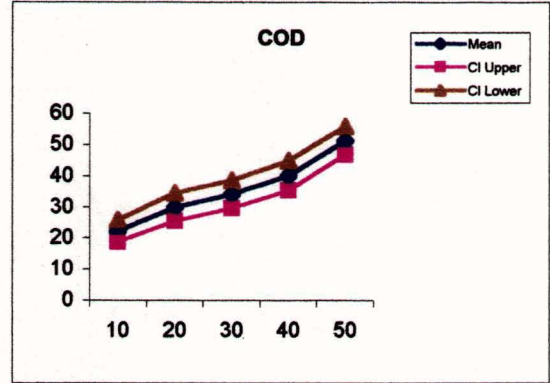
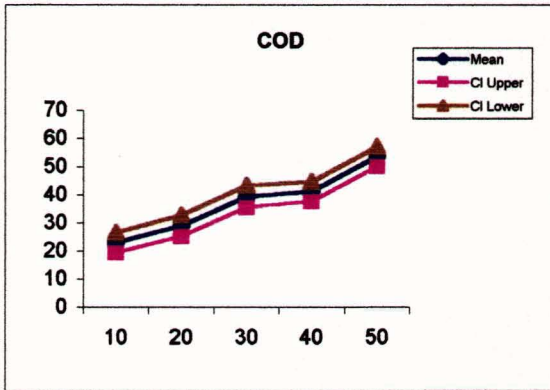
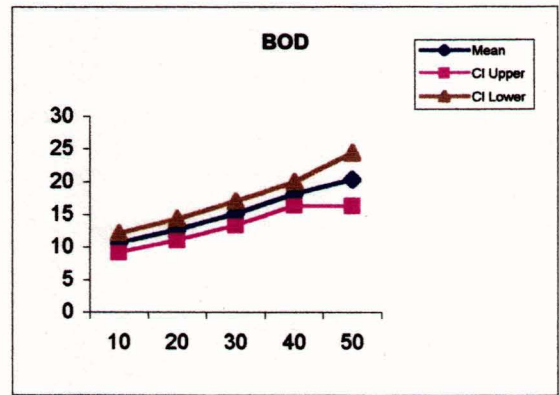
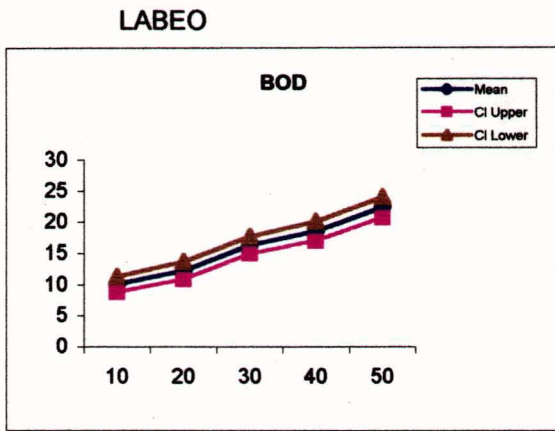


Table 40 Anova for Experiments with Labeo

TSS		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	11959.3227	4	2989.8307	17.9009	<0.01
	beaf	13365.0390	1	13365.0390	80.0202	<0.01
	wwof	4782.0154	1	4782.0154	28.6312	<0.01
2-Way Interactions	dil*beaf	611.4319	4	152.8580	0.9152	>0.05
	dil*wwof	633.2402	4	158.3100	0.94785	>0.05
	beaf*wwof	4782.0154	1	4782.0154	28.6312	<0.01
Residual		7348.9187	44	167.0209		
Total		43481.9833	59	736.9828		

BOD		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	1151.0210	4	287.75524	3616.72	<0.01
	beaf	199.2904	1	199.29038	2504.83	<0.01
	wwof	33.9754	1	33.97538	427.027	<0.01
2-Way Interactions	dil*beaf	4.0249	4	1.00624	12.6471	<0.01
	dil*wwof	3.1760	4	0.79399	9.97942	<0.01
	beaf*wwof	33.9754	1	33.97538	427.027	<0.01
Residual		3.5008	44	0.07956		
Total		1428.9637	59	24.21972		

COD		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	6734.8399	4	1683.7100	26050.7	<0.01
	beaf	866.8561	1	866.8561	13412.2	<0.01
	wwof	472.7549	1	472.7549	7314.56	<0.01
2-Way Interactions	dil*beaf	3.6715	4	0.9179	14.2015	<0.01
	dil*wwof	1.1654	4	0.2914	4.50786	<0.01
	beaf*wwof	472.7549	1	472.7549	7314.56	<0.01
Residual		2.8438	44	0.0646		
Total		8554.8865	59	144.9981		

LMPN		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	15.009518	4	3.75238	1967.16	<0.01
	beaf	1.790447	1	1.79045	938.631	<0.01
	wwof	0.959419	1	0.95942	502.97	<0.01
2-Way Interactions	dil*beaf	0.020937	4	0.00523	2.74403	<0.05
	dil*wwof	0.083930	4	0.02098	11	<0.01
	beaf*wwof	0.959419	1	0.95942	502.97	<0.01
Residual		0.083930	44	0.00191		
Total		18.907600	59	0.32047		

dil - Dilution

beaf- Before/After

wwof- With fish/without fish

Table 41 Anova for Experiments with *Cirrhinus*

TSS		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	19536.13511	4	4884.033777	1877.75	<0.01
	beaf	23750.25313	1	23750.25313	9131.17	<0.01
	wwof	21377.44753	1	21377.44753	8218.91	<0.01
2-Way Interactions	dil*beaf	235.3775067	4	58.84437667	22.6237	<0.01
	dil*wwof	113.4431067	4	28.36077667	10.9038	<0.01
	beaf*wwof	21377.44753	1	21377.44753	8218.91	<0.01
Residual		114.4443733	44	2.601008485		
Total		86504.54827	59	1466.178784		

BOD		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	745.763	4	186.4408	27.5072	<0.01
	beaf	339.864	1	339.8640	50.1429	<0.01
	wwof	34.961	1	34.9607	5.15804	<0.05
2-Way Interactions	dil*beaf	32.621	4	8.1553	1.20322	>0.05
	dil*wwof	23.650	4	5.9124	0.87231	>0.05
	beaf*wwof	34.961	1	34.9607	5.15804	<0.05
Residual		298.228	44	6.7779		
Total		1510.047	59	25.5940		

COD		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	5776.0835	4	1444.0209	6268.06	<0.01
	beaf	1267.6687	1	1267.6687	5502.57	<0.01
	wwof	669.1356	1	669.1356	2904.52	<0.01
2-Way Interactions	dil*beaf	7.9987	4	1.9997	8.67998	<0.01
	dil*wwof	9.5474	4	2.3869	10.3606	<0.01
	beaf*wwof	669.1356	1	669.1356	2904.52	<0.01
Residual		10.1366	44	0.2304		
Total		8409.7061	59	142.5374		

LMPN		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	8.300358	4	2.07509	415.349	<0.01
	beaf	2.920276	1	2.92028	584.521	<0.01
	wwof	1.219517	1	1.21952	244.098	<0.01
2-Way Interactions	dil*beaf	0.344274	4	0.08607	17.2274	<0.01
	dil*wwof	0.219825	4	0.05496	11	<0.01
	beaf*wwof	1.219517	1	1.21952	244.098	<0.01
Residual		0.219825	44	0.00500		
Total		14.443590	59	0.24481		

dil - Dilution

beaf- Before/After

wwof- With fish/without fish

Significant difference ($p < 0.01$) was observed between dilutions for all the parameters. pH, electrical conductivity, COD, BOD, TSS, NO_3 , PO_4 , SO_4 , hardness calcium, magnesium and log MPN showed $p < 0.05$ between pre and post experimental stage (Table 41). BOD, COD, TSS, pH, PO_4 , SO_4 , NO_3 , hardness calcium, magnesium and MPN showed significant differences ($p < 0.05$) for presence or absence of test species.

DO, COD, TSS, hardness, pH, NO_3 , SO_4 , calcium, magnesium, and MPN showed significant interactions ($p < 0.05$) between dilutions and pre/post experimental stages. COD, TSS, NO_3 , SO_4 , calcium, magnesium and log MPN showed significant interactions ($p < 0.05$) between dilution and presence or absence of fish. The interaction between pre/post experimental stage and presence or absence of fish was significant ($p < 0.05$) for COD, TSS, BOD, NO_3 , SO_4 , calcium, magnesium, hardness, pH and log MPN.

The first-degree polynomial regression equation was fitted for COD, TSS and log MPN. Second-degree polynomial equation fitted well for BOD.

Aquatic flora

1. *Azolla rubra* R. Br.

Mean values and 95 % confidence interval of water quality parameters are presented in Fig. 95.

Significant difference ($p < 0.01$) was obtained between dilutions for all the parameters studied (Table 42). All parameters except temperature showed significant

Fig.95 Mean and Confidence Intervals for experiments with *Azolla*

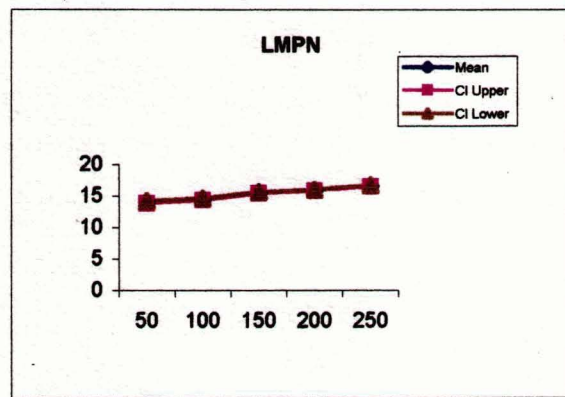
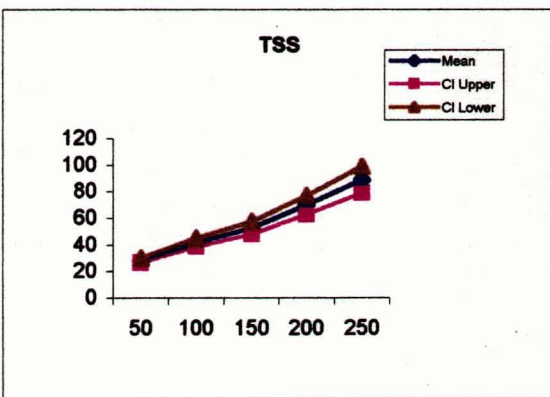
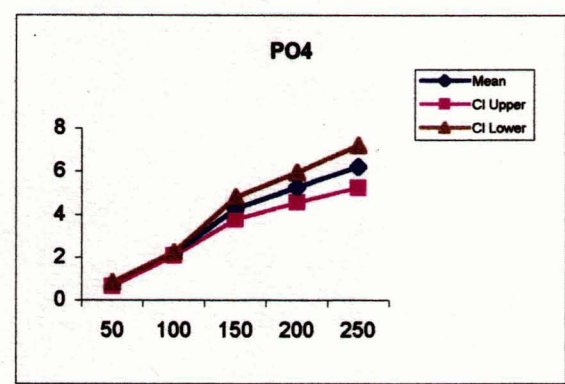
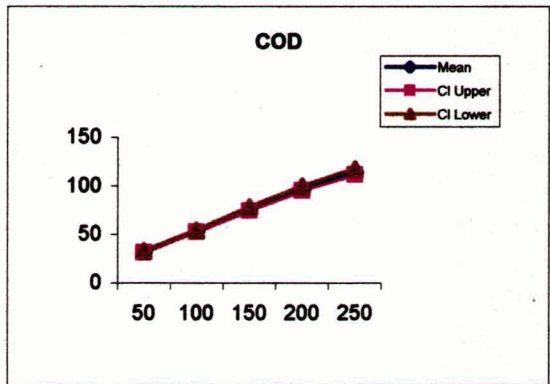
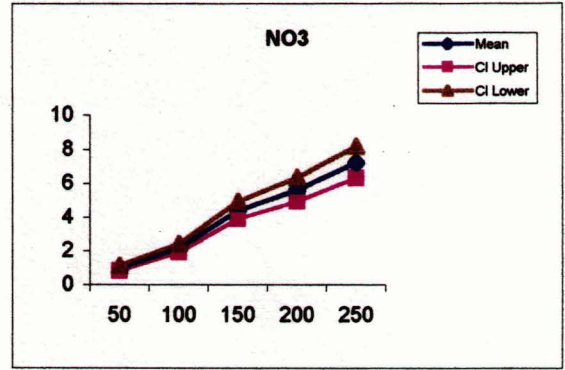
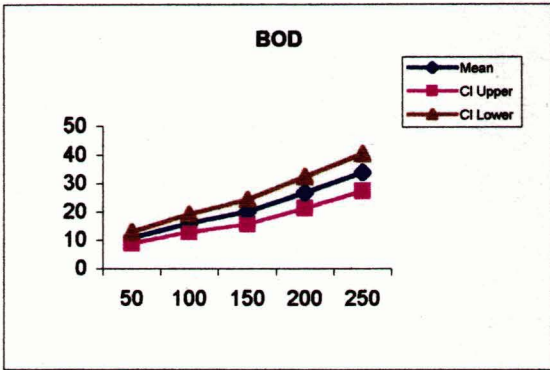


Table 42 Anova for Experiments with Azolla

TSS		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	27156.95325	4	6789.238313	711.3930213	<0.01
	beaf	1622.4	1	1622.4	169.9990462	<0.01
	wwof	1148.4375	1	1148.4375	120.3360945	<0.01
2-Way Interactions						
	dil*beaf	370.62525	4	92.656313	9.708755395	<0.01
	dil*wwof	418.50225	4	104.6255625	10.96292273	<0.01
	beaf*wwof	1148.4375	1	1148.4375	120.3360945	<0.01
Residual		419.91765	44	9.543583		
Total		32285.2734	59	547.2080237		

BOD		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	3885.64422	4	971.41105	362.1207718	<0.01
	beaf	1103.38817	1	1103.38817	411.3189497	<0.01
	wwof	728.71350	1	728.71350	271.6484375	<0.01
2-Way Interactions						
	dil*beaf	139.48422	4	34.87105	12.99916549	<0.01
	dil*wwof	115.87755	4	28.96939	10.79915337	<0.01
	beaf*wwof	728.71350	1	728.71350	271.6484375	<0.01
Residual		118.03268	44	2.68256		
Total		6819.85383	59	115.59074		

COD		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	52282.8012	4	13070.7003	10219.62474	<0.01
	beaf	350.078415	1	350.078415	273.7167826	<0.01
	wwof	19.255335	1	19.255335	15.05522225	<0.01
2-Way Interactions						
	dil*beaf	61.83276	4	15.45819	12.08633795	<0.01
	dil*wwof	51.83274	4	12.958185	10.13165209	<0.01
	beaf*wwof	19.255335	1	19.255335	15.05522225	<0.01
Residual		56.27514	44	1.278980		
Total		52841.33093	59	895.6157784		

NO3		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	310.16947	4	77.542367	814.7475822	<0.01
	beaf	13.72817	1	13.728167	144.2436062	<0.01
	wwof	9.84150	1	9.841500	103.4059015	<0.01
2-Way Interactions						
	dil*beaf	5.18367	4	1.295917	13.61636246	<0.01
	dil*wwof	3.60450	4	0.901125	9.468235837	<0.01
	beaf*wwof	9.84150	1	9.841500	103.4059015	<0.01
Residual		4.18763	44	0.095173		
Total		356.55643	59	6.043329		

PO4		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	244.21832	4	61.05458	444.3033122	<0.01
	beaf	12.06017	1	12.06017	87.76363818	<0.01
	wwof	7.87713	1	7.87713	57.32302992	<0.01
2-Way Interactions						
	dil*beaf	7.52232	4	1.88058	13.68525611	<0.01
	dil*wwof	5.44946	4	1.36236	9.91412798	<0.01
	beaf*wwof	7.87713	1	7.87713	57.32302992	<0.01
Residual		6.04632	44	0.13742		
Total		291.05083	59	4.93306		

LMPN		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	53.74699	4	13.43675	14364.09017	<0.01
	beaf	1.14539	1	1.14539	1224.438939	<0.01
	wwof	0.83339	1	0.83339	890.9065384	<0.01
2-Way Interactions						
	dil*beaf	0.05686	4	0.01421	15.19592167	<0.01
	dil*wwof	0.04116	4	0.01029	11	<0.01
	beaf*wwof	0.83339	1	0.83339	890.9065384	<0.01
Residual		0.04116	44	0.00094		
Total		56.69834	59	0.96099		

difference ($p < 0.05$) between pre/post experimental stage. DO, COD, TSS, BOD, NO_3 , PO_4 , SO_4 , hardness, pH, and log MPN showed significant differences ($p < 0.05$) between presence or absence of *Azolla*.

Two way interactions between dilution and pre/post experimental stage was significant ($p < 0.05$) for DO, COD, TSS, BOD, NO_3 , PO_4 , SO_4 , calcium, magnesium and log MPN. Interactions between dilution and presence or absence of *Azolla* was significant ($p < 0.05$) for DO, COD, TSS, BOD, NO_3 , PO_4 , SO_4 , hardness, calcium, magnesium and log MPN. In the interactions between presence or absence of *Azolla* and pre/post experimental stage, significant difference ($p < 0.05$) was observed for DO, COD, TSS, BOD, NO_3 , PO_4 , SO_4 , hardness and log MPN.

The regression equations for BOD, COD, TSS, log MPN, NO_3 , and PO_4 fitted the first degree polynomial.

2. *Salvinia molesta* Mitchell

Mean values and 95 % confidence interval of water quality parameters are presented in Fig. 96.

Significant difference ($p < 0.01$) was observed between dilutions as well as for pre/post experimental stage for all parameters studied using *Salvinia* (Table 43). Temperature, pH, conductivity, TSS, DO, BOD, COD, NO_3 , hardness, calcium and log MPN showed significant difference ($p < 0.05$) between presence/absence of test species.

Fig.96 Mean and Confidence Intervals for experiments with *Salvinia*

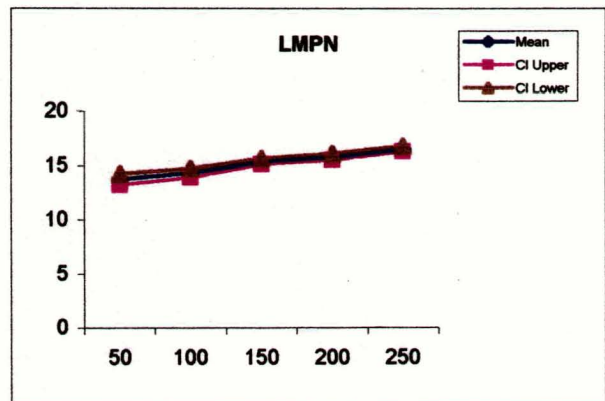
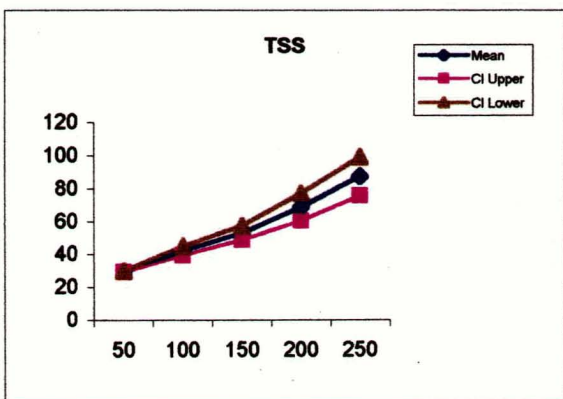
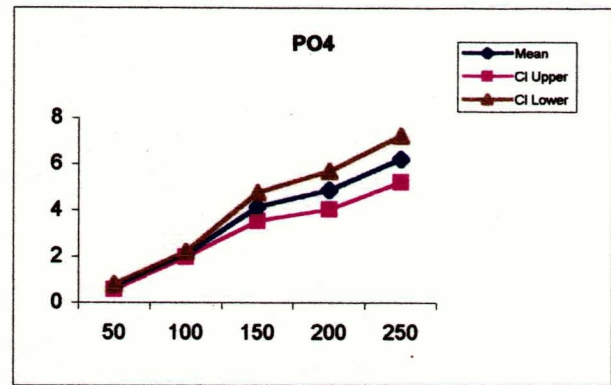
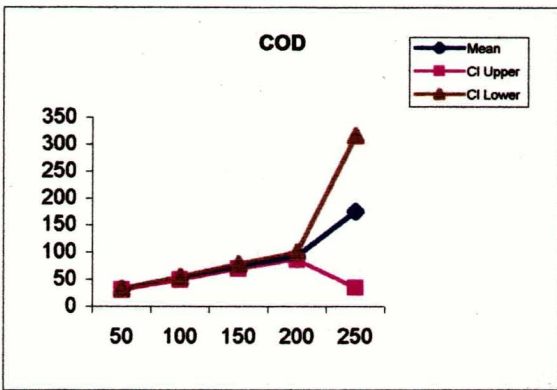
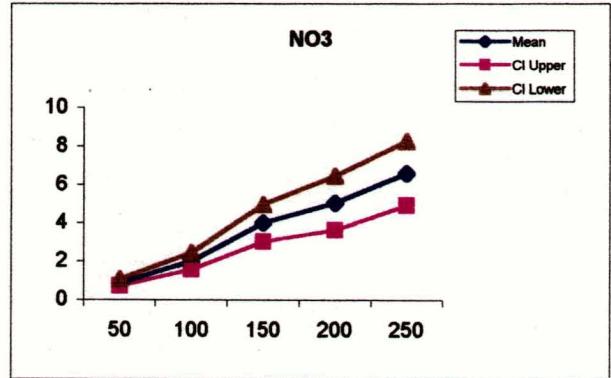
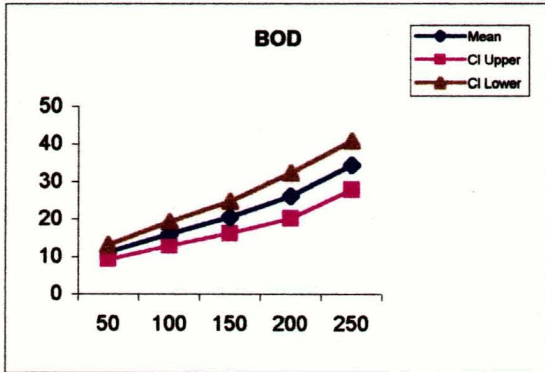


Table 43 Anova for Experiments with Salvinia

		Sum of Squares	df	Mean Square	F	p
TSS						
Main Effects	dil	926.15950	1	926.1595	1065.410482	<0.01
	beaf	83.21650	1	83.2165	95.72836582	<0.01
	wwof	18.88600	1	18.8860	21.72557396	<0.01
2-Way Interactions						
	dil*beaf	49.62250	1	49.6225	57.08340286	<0.01
	dil*wwof	13.96900	1	13.9690	16.06928763	<0.01
	beaf*wwof	18.88600	1	18.8860	21.72557396	<0.01
Residual		14.77807	17	0.8693		
Total		1125.51760	23	48.9355		

		Sum of Squares	df	Mean Square	F	p
BOD						
Main Effects	dil	142.15534	1	142.15534	417.4944858	<0.01
	beaf	154.99084	1	154.99084	455.190928	<0.01
	wwof	102.46534	1	102.46534	300.9293505	<0.01
2-Way Interactions						
	dil*beaf	10.65334	1	10.65334	31.28767262	<0.01
	dil*wwof	4.67284	1	4.67284	13.72360633	<0.01
	beaf*wwof	102.46534	1	102.46534	300.9293505	<0.01
Residual		5.78844	17	0.34050		
Total		523.19146	23	22.747455		

		Sum of Squares	df	Mean Square	F	p
COD						
Main Effects	dil	4166.72554	1	4166.7255	441.2377175	<0.01
	beaf	707.96344	1	707.9634	74.97018184	<0.01
	wwof	458.58784	1	458.5878	48.56241402	<0.01
2-Way Interactions						
	dil*beaf	133.24594	1	133.2459	14.11015264	<0.01
	dil*wwof	158.05534	1	158.0553	16.73735784	<0.01
	beaf*wwof	458.58784	1	458.5878	48.56241402	<0.01
Residual		160.53554	17	9.4433		
Total		6243.70146	23	271.4653		

		Sum of Squares	df	Mean Square	F	p
NO3						
Main Effects	dil	7.4928375	1	7.4928375	384.6687177	<0.01
	beaf	2.0358375	1	2.0358375	104.5162131	<0.01
	wwof	1.6380375	1	1.6380375	84.09388094	<0.01
2-Way Interactions						
	dil*beaf	0.3243375	1	0.3243375	16.65090031	<0.01
	dil*wwof	0.3243375	1	0.3243375	16.65090031	<0.01
	beaf*wwof	1.6380375	1	1.6380375	84.09388094	<0.01
Residual		0.3311375	17	0.019478676		
Total		13.7845625	23	0.599328804		

		Sum of Squares	df	Mean Square	F	p
PO4						
Main Effects	dil	11.55094	1	11.55094	571.9326829	<0.01
	beaf	0.32434	1	0.32434	16.05923472	<0.01
	wwof	0.01984	1	0.01984	0.982233225	>0.05
2-Way Interactions						
	dil*beaf	0.00094	1	0.00094	0.04641934	>0.05
	dil*wwof	0.00094	1	0.00094	0.04641934	>0.05
	beaf*wwof	0.01984	1	0.01984	0.982233225	>0.05
Residual		0.34334	17	0.02020		
Total		12.26016	23	0.53305		

		Sum of Squares	df	Mean Square	F	p
LMPN						
Main Effects	dil	2.06038	1	2.0604	552.6205367	<0.01
	beaf	4.39714	1	4.3971	1179.370363	<0.01
	wwof	3.84282	1	3.8428	1030.695865	<0.01
2-Way Interactions						
	dil*beaf	0.08073	1	0.0807	21.65321499	<0.01
	dil*wwof	0.06338	1	0.0634	17	<0.01
	beaf*wwof	3.84282	1	3.8428	1030.695865	<0.01
Residual		0.06338	17	0.0037		
Total		14.35066	23	0.6239		

pH, TSS, DO, BOD, COD, NO₃, hardness, calcium, magnesium and log MPN showed significant interactions ($p < 0.05$) between dilution and pre/post experimental stage. Two way interactions between dilution and presence/absence of test species showed significance at $p < 0.05$ for TSS, DO, BOD, COD, NO₃, hardness, calcium, and log MPN. All the parameters except PO₄ and magnesium showed significant interactions ($p < 0.05$) between presence/absence of test species and pre/post experimental stage.

BOD, TSS, log MPN, NO₃ and PO₄ fitted first degree polynomial equation while COD fitted second degree polynomial equation.

3. *Lemna perpusilla* Torrey

Mean values and 95 % confidence interval of water quality parameters are presented in Fig. 97.

Significant difference between dilutions were observed for all parameters ($p < 0.01$) except temperature in the studies with *Lemna* (Table 44). There were significant differences between pre/post experimental stages for all parameters except pH. All parameters except temperature and magnesium revealed significant differences ($p < 0.05$) between presence/absence of *Lemna*.

Two way interactions between dilution and pre/post experimental stages was significant for conductivity, COD, PO₄, calcium, magnesium, TSS, BOD, NO₃, SO₄ and log MPN. Conductivity, COD, PO₄, calcium, magnesium, TSS, BOD, NO₃, SO₄ and log MPN showed significant interactions ($p < 0.05$) between dilution and

Fig.97 Mean and Confidence Intervals for experiments with *Lemna*

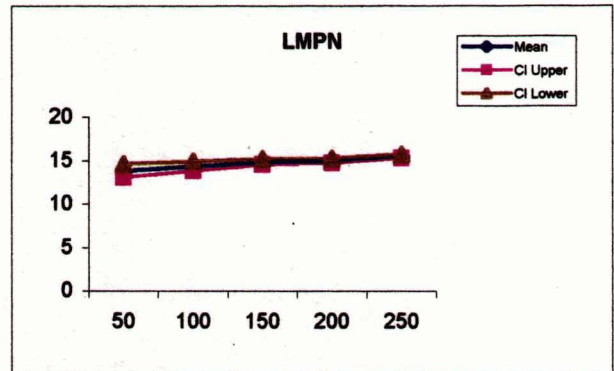
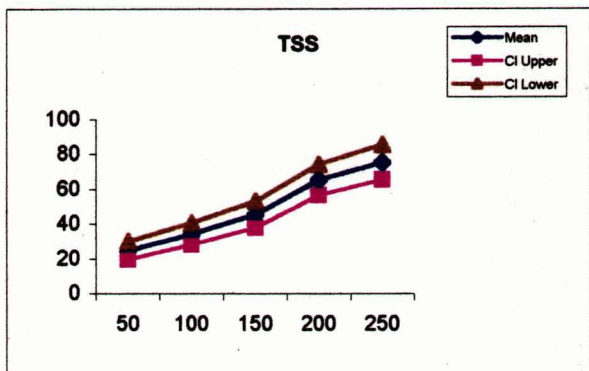
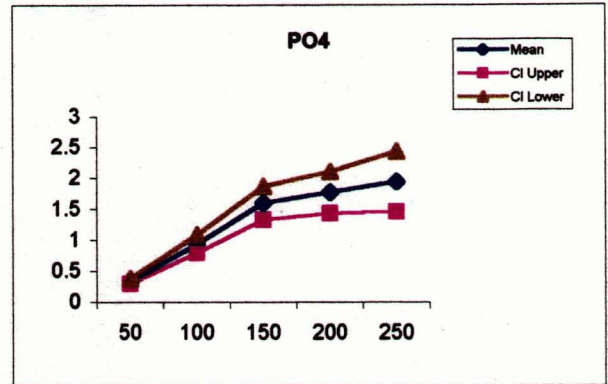
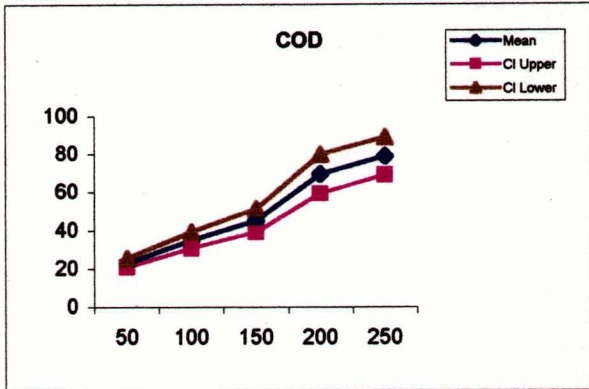
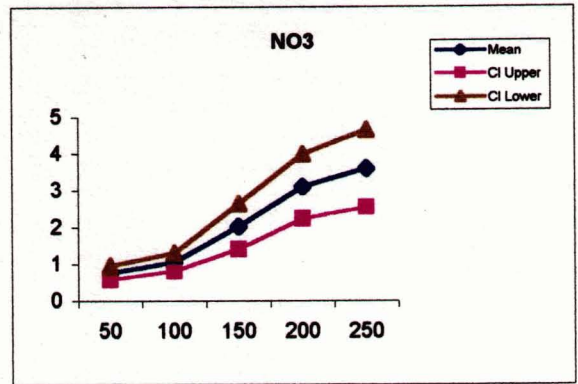
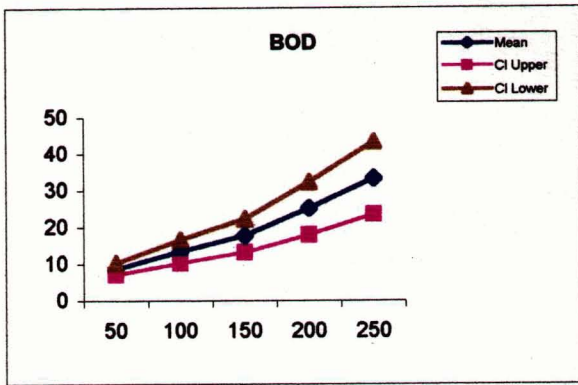


Table 44 Anova for Experiments with Lemna

TSS		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	21382.7737	4	5345.6934	1681.505677	<0.01
	beaf	2795.6566	1	2795.6566	879.3830854	<0.01
	wwof	2555.3206	1	2555.3206	803.7845959	<0.01
2-Way Interactions						
	dil*beaf	145.8361	4	36.4590	11.468307	<0.01
	dil*wwof	137.9401	4	34.4850	10.84737872	<0.01
	beaf*wwof	2555.3206	1	2555.3206	803.7845959	<0.01
Residual		139.8809	44	3.1791		
Total		29712.7284	59	503.6056		

BOD		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	4570.832427	4	1142.708107	125.1011883	<0.01
	beaf	1381.152282	1	1381.152282	151.2053559	<0.01
	wwof	1208.618402	1	1208.618402	132.3169034	<0.01
2-Way Interactions						
	dil*beaf	403.41896	4	100.85474	11.0413567	<0.01
	dil*wwof	400.1287067	4	100.0321767	10.95130427	<0.01
	beaf*wwof	1208.618402	1	1208.618402	132.3169034	<0.01
Residual		401.9079067	44	9.134270606		
Total		9574.677085	59	162.2826625		

COD		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	26277.9281	4	6569.4820	660.1454206	<0.01
	beaf	2353.5101	1	2353.5101	236.4964145	<0.01
	wwof	1804.4554	1	1804.4554	181.3237239	<0.01
2-Way Interactions						
	dil*beaf	444.7712	4	111.1928	11.17339529	<0.01
	dil*wwof	436.1476	4	109.0369	10.95675556	<0.01
	beaf*wwof	1804.4554	1	1804.4554	181.3237239	<0.01
Residual		437.8690	44	9.9516		
Total		33559.1367	59	568.7989		

NO3		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	73.55747	4	18.38937	90.58828359	<0.01
	beaf	16.48504	1	16.48504	81.20734425	<0.01
	wwof	13.91054	1	13.91054	68.52500754	<0.01
2-Way Interactions						
	dil*beaf	5.67387	4	1.41847	6.987541387	<0.01
	dil*wwof	5.41104	4	1.35276	6.66386226	<0.01
	beaf*wwof	13.91054	1	13.91054	68.52500754	<0.01
Residual		8.93197	44	0.20300		
Total		137.88046	59	2.33696		

PO4		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	21.424257	4	5.356064	126.2557203	<0.01
	beaf	3.432042	1	3.432042	80.90173668	<0.01
	wwof	2.380042	1	2.380042	56.10348676	<0.01
2-Way Interactions						
	dil*beaf	1.326017	4	0.331504	7.814375642	<0.01
	dil*wwof	0.865317	4	0.216329	5.099415152	<0.01
	beaf*wwof	2.380042	1	2.380042	56.10348676	<0.01
Residual		1.866583	44	0.042422		
Total		33.674298	59	0.570751		

LMPN		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	19.337438	4	4.834359	121.7851711	<0.01
	beaf	8.884459	1	8.884459	223.8135984	<0.01
	wwof	7.920475	1	7.920475	199.5293194	<0.01
2-Way Interactions						
	dil*beaf	1.882515	4	0.470629	11.8558851	<0.01
	dil*wwof	1.746615	4	0.436654	11	<0.01
	beaf*wwof	7.920475	1	7.920475	199.5293194	<0.01
Residual		1.746615	44	0.039696		
Total		49.438594	59	0.837942		

presence/absence of *Lemna*. Conductivity, DO, COD, PO₄, hardness, pH, calcium, TSS, BOD, NO₃, SO₄ and log MPN had significant interaction between pre/post experimental stage and presence/absence of *Lemna*.

BOD fitted second degree polynomial equation while for COD, TSS, log MPN, NO₃ and PO₄ first degree polynomial equation was found to fit best.

4. *Spirodela polyrrhiza* (L.) Schleid

Mean values and 95 % confidence interval of water quality parameters are presented in Fig. 98.

Significant difference was observed between dilutions for all parameters except temperature ($p < 0.01$). Except pH all parameters showed significant differences ($p < 0.05$) between pre/post experimental stage (Table 45). All parameters except temperature showed significant difference ($p < 0.05$) between presence/absence of *Spirodela*.

Two way interactions between dilution and pre/post experimental stage was significant ($p < 0.05$) for temperature, pH, BOD, COD, SO₄, hardness, calcium, magnesium and log MPN. Temperature, pH, BOD, COD, NO₃, SO₄, hardness, calcium, magnesium and log MPN showed significant difference ($p < 0.05$) between dilution and presence/absence of *Spirodela*. Interaction between presence/absence of *Spirodela* and pre/post experimental stage was significant for all parameters studied.

First degree polynomial equation was found to fit well for BOD, COD, log MPN, NO₃, PO₄ and TSS.

Fig.98 Mean and Confidence Intervals for experiments with *Spirodela*

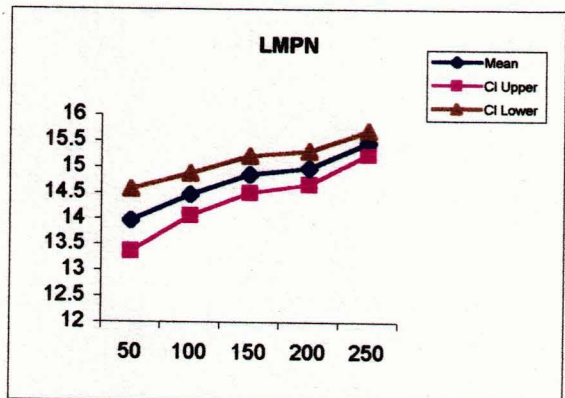
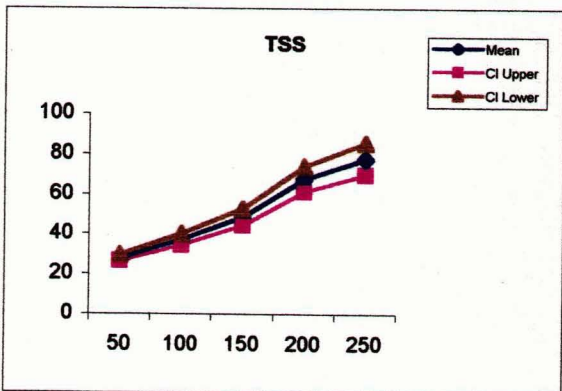
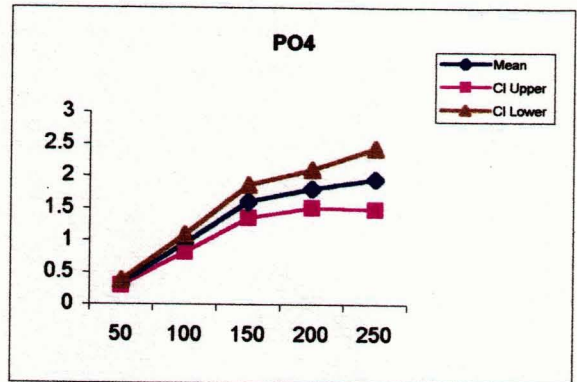
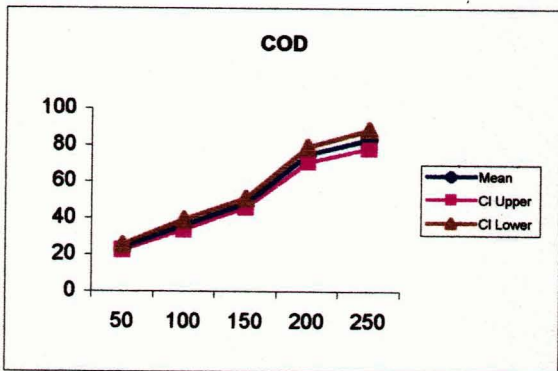
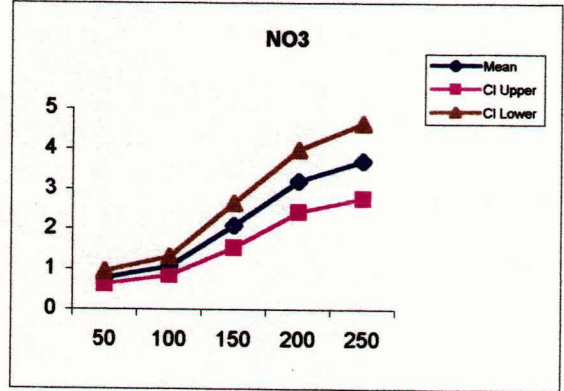
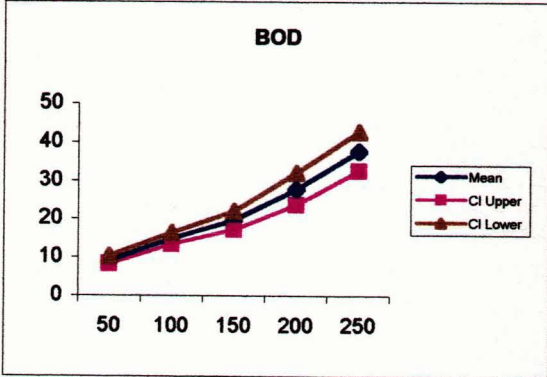


Table 45 Anova for experiments with Spirodela

TSS		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	20446.04469	4	5111.511173	996.0619336	<0.01
	beaf	1061.42616	1	1061.42616	206.8363264	<0.01
	wwof	915.41016	1	915.41016	178.3827098	<0.01
2-Way Interactions	dil*beaf	233.86509	4	58.4662725	11.39311379	<0.01
	dil*wwof	223.84809	4	55.9620225	10.90511954	<0.01
	beaf*wwof	915.41016	1	915.41016	178.3827098	<0.01
Residual		225.79569	44	5.131720227		
Total		24021.80004	59	407.1491532		

BOD		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	5977.68908	4	5981.689	600.4807698	<0.01
	beaf	427.30691	1	427.307	171.6981777	<0.01
	wwof	333.89286	1	333.893	134.1630446	<0.01
2-Way Interactions	dil*beaf	109.55228	4	27.388	11.00492759	<0.01
	dil*wwof	108.03669	4	27.009	10.85268135	<0.01
	beaf*wwof	333.89286	1	333.893	134.1630446	<0.01
Residual		109.50322	44	2.489		
Total		7399.87389	59	125.422		

COD		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	30392.02713	4	7598.006782	4253.555039	<0.01
	beaf	652.0806667	1	652.0806667	365.0511358	<0.01
	wwof	380.3187267	1	380.3187267	212.9119758	<0.01
2-Way Interactions	dil*beaf	76.56936667	4	19.14234167	10.71636367	<0.01
	dil*wwof	75.06050667	4	18.76512667	10.50518924	<0.01
	beaf*wwof	380.3187267	1	380.3187267	212.9119758	<0.01
Residual		78.59597333	44	1.786272121		
Total		32034.97109	59	542.9656118		

NO3		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	78.33485667	4	19.58371417	113.3632386	<0.01
	beaf	13.12272667	1	13.12272667	75.96285266	<0.01
	wwof	10.8375	1	10.8375	62.73447864	<0.01
2-Way Interactions	dil*beaf	4.490056667	4	1.122514167	6.497840001	<0.01
	dil*wwof	4.21035	4	1.0525875	6.093059103	<0.01
	beaf*wwof	10.8375	1	10.8375	62.73447864	<0.01
Residual		7.601083333	44	0.172751894		
Total		129.4340733	59	2.193797853		

PO4		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	21.92421	4	5.4810525	133.1755711	<0.01
	beaf	3.024015	1	3.024015	73.47583785	<0.01
	wwof	2.049801667	1	2.049801667	49.80494306	<0.01
2-Way Interactions	dil*beaf	1.23261	4	0.3081525	7.48731839	<0.01
	dil*wwof	0.791356667	4	0.197839167	4.806986252	<0.01
	beaf*wwof	2.049801667	1	2.049801667	49.80494306	<0.01
Residual		1.81089	44	0.041156591		
Total		32.882685	59	0.557333644		

LMPN		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	15.345519	4	3.8363798	243.2097992	<0.01
	beaf	7.265296	1	7.2652962	460.5881905	<0.01
	wwof	6.396217	1	6.3962166	405.4923228	<0.01
2-Way Interactions	dil*beaf	0.768892	4	0.1922230	12.18610511	<0.01
	dil*wwof	0.694054	4	0.1735135	11	<0.01
	beaf*wwof	6.396217	1	6.3962166	405.4923228	<0.01
Residual		0.694054	44	0.0157740		
Total		37.560249	59	0.6366144		

5. *Wolffia globosa* (Roxb.) Hartog & Plas.

Mean values and 95 % confidence interval of water quality parameters are presented in Fig. 99.

All parameters except temperature showed significant difference ($p < 0.01$) between dilutions in experiments conducted using *Wolffia* (Table 46). Conductivity, DO, COD, hardness, magnesium, calcium, TSS, BOD, NO_3 , SO_4 and log MPN showed significant difference ($p < 0.05$) between pre/post experimental stage. Conductivity, DO, COD, magnesium, pH, TSS, BOD, NO_3 , SO_4 , calcium and log MPN showed significant difference between presence/absence of *Wolffia*.

Two way interactions between pre/post experimental stages was significant for conductivity, COD, PO_4 , magnesium, TSS, BOD, NO_3 , SO_4 , calcium and log MPN. Interactions between dilution and presence/absence of *Wolffia* was significant for conductivity, COD, PO_4 , magnesium, TSS, BOD, NO_3 , SO_4 , calcium and log MPN. conductivity, DO, COD, pH, TSS, BOD, NO_3 , SO_4 , calcium and log MPN showed significant difference ($p < 0.05$) between presence/absence of *Wolffia* and pre/post experimental stages.

Regression equations for COD, log MPN, NO_3 , PO_4 , TSS fitted first degree polynomial equation while second degree polynomial equation fits well for BOD.

6. *Pistia stratiotes* L.

Mean values and 95 % confidence interval of water quality parameters are presented in Fig. 100.

Fig.99 Mean and Confidence Intervals for experiments with *Wolffia*

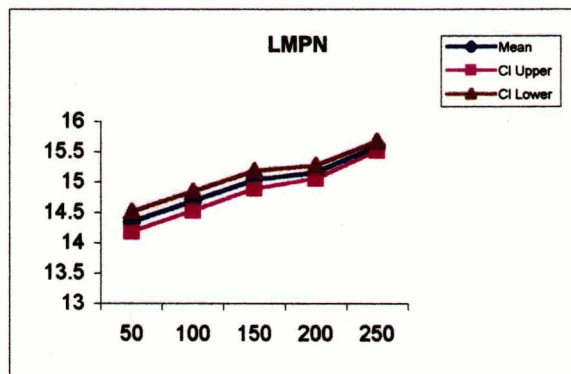
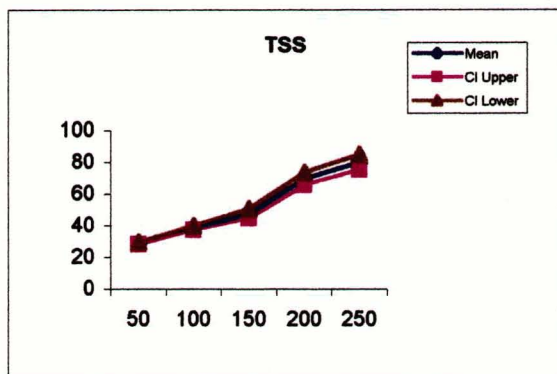
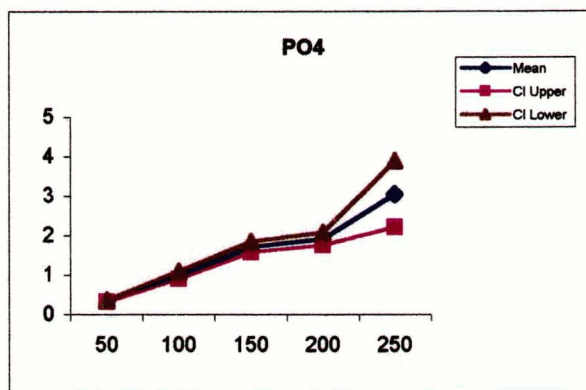
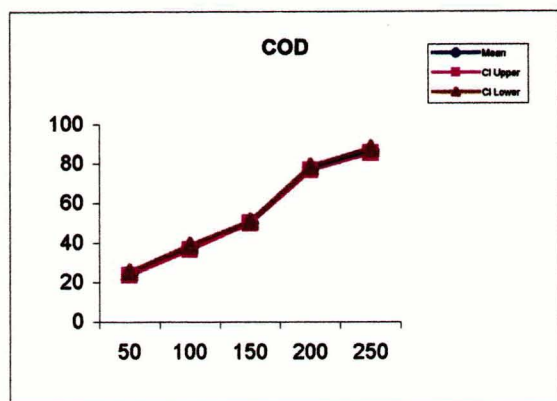
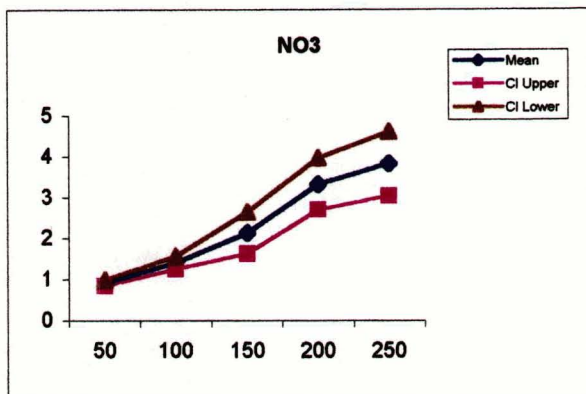
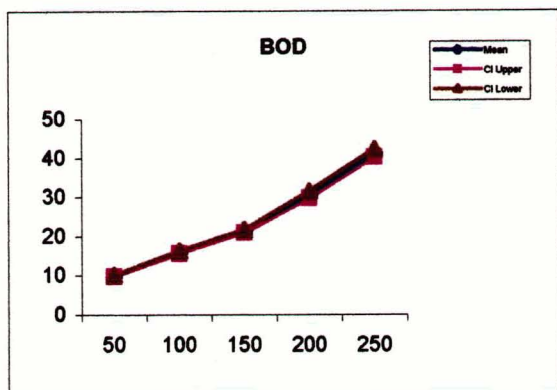


Table 46 Anova for Experiments with Wolffia

TSS		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	21932.9620	4	5483.2405	1466.264259	<0.01
	beaf	529.4322	1	529.4322	141.5745919	<0.01
	wwof	196.6746	1	196.6746	52.5924331	<0.01
2-Way Interactions	dil*beaf	159.9832	4	39.9958	10.69520797	<0.01
	dil*wwof	162.0598	4	40.5149	10.83403301	<0.01
	beaf*wwof	196.6746	1	196.6746	52.5924331	<0.01
Residual	164.5424	44	3.7396			
Total	23342.3287	59	395.6327			

BOD		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	7291.36678	4	1822.8417	12032.99934	<0.01
	beaf	26.42721	1	26.4272	174.4520993	<0.01
	wwof	7.51896	1	7.5190	49.63439281	<0.01
2-Way Interactions	dil*beaf	5.49918	4	1.3748	9.07533405	<0.01
	dil*wwof	5.19369	4	1.2984	8.571187026	<0.01
	beaf*wwof	7.51896	1	7.5190	49.63439281	<0.01
Residual	6.66542	44	0.1515			
Total	7350.19019	59	124.5795			

COD		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	33097.66539	4	8274.4163	31985.02276	<0.01
	beaf	81.99366	1	81.9937	316.9491322	<0.01
	wwof	9.126	1	9.1260	35.27684678	<0.01
2-Way Interactions	dil*beaf	8.77839	4	2.1946	8.483287284	<0.01
	dil*wwof	9.83805	4	2.4595	9.507324744	<0.01
	beaf*wwof	9.126	1	9.1260	35.27684678	<0.01
Residual	11.38265	44	0.2587			
Total	33227.91014	59	563.1849			

NO3		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	73.24508	4	18.31127	85.64195765	<0.01
	beaf	5.80948	1	5.80948	27.1709939	<0.01
	wwof	4.32554	1	4.32554	20.23056304	<0.01
2-Way Interactions	dil*beaf	5.90028	4	1.47507	6.898910718	<0.01
	dil*wwof	6.01479	4	1.50370	7.03280567	<0.01
	beaf*wwof	4.32554	1	4.32554	20.23056304	<0.01
Residual	9.40772	44	0.21381			
Total	109.02842	59	1.84794			

PO4		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	49.98141	4	12.4953525	74.47391834	<0.01
	beaf	0.17496	1	0.17496	1.042784247	>0.05
	wwof	0.53016	1	0.53016	3.159822226	>0.05
2-Way Interactions	dil*beaf	5.64069	4	1.4101725	8.404810637	<0.01
	dil*wwof	6.38859	4	1.5971475	9.51920584	<0.01
	beaf*wwof	0.53016	1	0.53016	3.159822226	>0.05
Residual	7.38239	44	0.167781591			
Total	70.62836	59	1.197090847			

LMPN		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	10.836777	4	2.709194	2951.284422	<0.01
	beaf	0.999900	1	0.999900	1089.249765	<0.01
	wwof	0.694894	1	0.694894	756.9886258	<0.01
2-Way Interactions	dil*beaf	0.062820	4	0.015705	17.10845811	<0.01
	dil*wwof	0.040391	4	0.010098	11	<0.01
	beaf*wwof	0.694894	1	0.694894	756.9886258	<0.01
Residual	0.040391	44	0.000918			
Total	13.370066	59	0.226611			

All the parameters except temperature showed significant difference ($p < 0.05$) between dilutions (Table 47). Similar result was obtained for pre/post experimental stage. Significant difference was obtained between presence/ absence of test species for all parameters except temperature.

DO, COD, PO₄, pH, TSS, BOD, NO₃, SO₄, calcium, magnesium and log MPN showed significant interactions ($p < 0.05$) between dilutions and pre/post experimental stages. DO, COD, PO₄, TSS, BOD, pH, NO₃, SO₄, calcium, magnesium and log MPN showed significant interactions ($p < 0.05$) between dilution and presence/ absence of *Pistia*. The two way interactions between pre/post experimental stage and presence/absence of *Pistia* were significant ($p < 0.05$) for all parameters studied except temperature.

7. *Hydrilla verticillata* (L. f.) Royle

Mean values and 95 % confidence interval of water quality parameters are presented in Fig. 101.

All parameters showed significant difference ($p < 0.01$) between dilutions (Table 48). All parameters except magnesium showed significant difference ($p < 0.05$) between pre/post experimental stage. Significant differences were observed between presence or absence of *Hydrilla* except for the parameters temperature and pH.

Two way interactions between dilution and pre/post experimental stage was significant ($p < 0.05$) for conductivity, TSS, COD, PO₄, magnesium, DO, BOD, NO₃, SO₄, calcium and log MPN. Interactions between dilution and presence or absence of

Fig.100 Mean and Confidence Intervals for experiments with *Pistia*

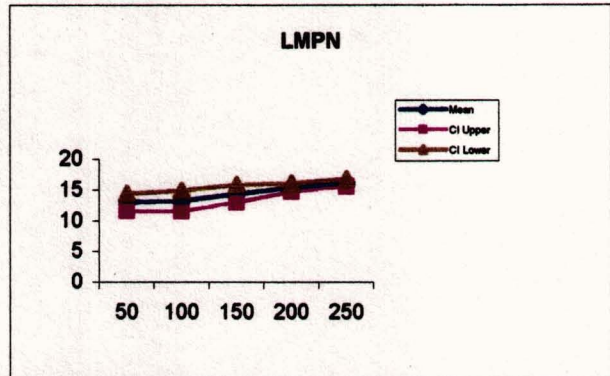
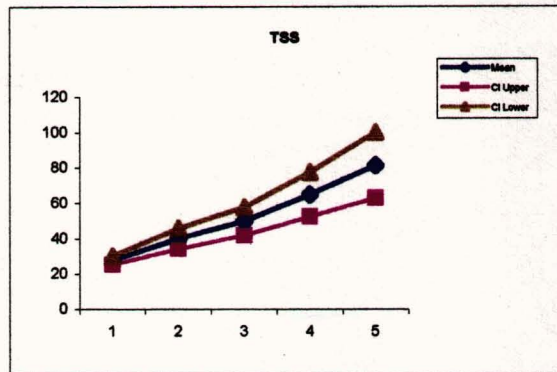
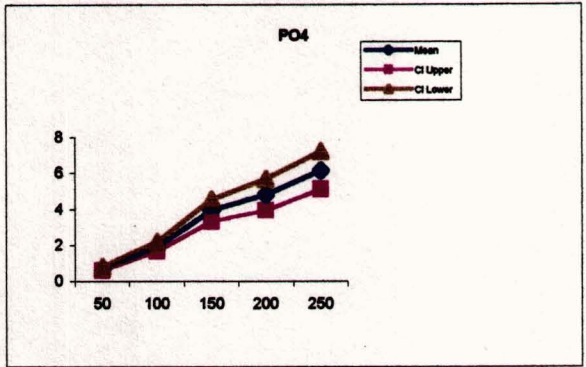
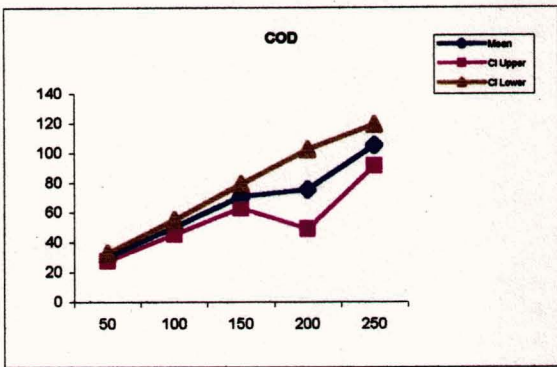
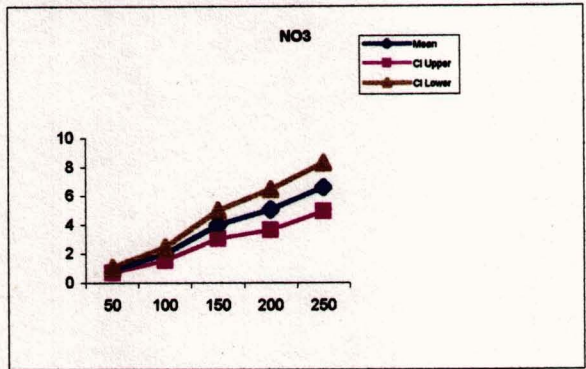
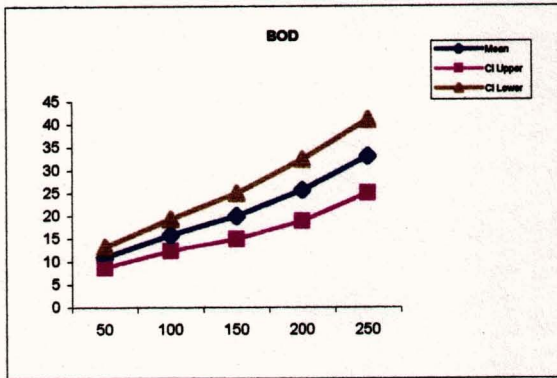


Table 47 Anova for Experiments with Pistia

TSS		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	21284.39661	4	5321.0992	165.3029783	<0.01
	beaf	4457.746815	1	4457.7468	138.4824458	<0.01
	wwof	3931.255815	1	3931.2558	122.1267028	<0.01
2-Way Interactions	dil*beaf	1458.88221	4	364.7206	11.33025186	<0.01
	dil*wwof	1415.08521	4	353.7713	10.99010717	<0.01
	beaf*wwof	3931.255815	1	3931.2558	122.1267028	<0.01
Residual		1416.35901	44	32.1900		
Total		37894.98149	59	642.2878		

BOD		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	3551.80566	4	887.951415	197.4363517	<0.01
	beaf	1380.67254	1	1380.67254	306.9930907	<0.01
	wwof	1087.32294	1	1087.32294	241.7666899	<0.01
2-Way Interactions	dil*beaf	213.52566	4	53.381415	11.86937894	<0.01
	dil*wwof	196.18026	4	49.045065	10.90518979	<0.01
	beaf*wwof	1087.32294	1	1087.32294	241.7666899	<0.01
Residual		197.88586	44	4.497405909		
Total		7714.71586	59	130.7578959		

COD		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	38061.69528	4	9515.423819	128.6375782	<0.01
	beaf	7161.556002	1	7161.556002	96.81599451	<0.01
	wwof	4982.623882	1	4982.623882	67.3593401	<0.01
2-Way Interactions	dil*beaf	3669.474957	4	917.3687392	12.4017695	<0.01
	dil*wwof	3251.843477	4	812.9608692	10.99029527	<0.01
	beaf*wwof	4982.623882	1	4982.623882	67.3593401	<0.01
Residual		3254.714943	44	73.97079417		
Total		65364.53242	59	1107.873431		

NO3		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	252.9762667	4	63.24406667	182.3335201	<0.01
	beaf	44.376	1	44.376	127.9366226	<0.01
	wwof	36.72272667	1	36.72272667	105.8721296	<0.01
2-Way Interactions	dil*beaf	16.002	4	4.0005	11.53349691	<0.01
	dil*wwof	13.22820667	4	3.307051667	9.534275759	<0.01
	beaf*wwof	36.72272667	1	36.72272667	105.8721296	<0.01
Residual		15.26180667	44	0.346859242		
Total		415.2897333	59	7.03880904		

PO4		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	231.7463067	4	57.93657667	288.1578298	<0.01
	beaf	25.10360167	1	25.10360167	124.857211	<0.01
	wwof	7.413135	1	7.413135	36.87054046	<0.01
2-Way Interactions	dil*beaf	9.661106667	4	2.415276667	12.01280647	<0.01
	dil*wwof	5.11644	4	1.27911	6.361880231	<0.01
	beaf*wwof	7.413135	1	7.413135	36.87054046	<0.01
Residual		8.846573333	44	0.201058485		
Total		295.3002983	59	5.005089802		

LMPN		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	91.3963	4	22.8491	130.3249837	<0.01
	beaf	64.8535	1	64.8535	369.9070517	<0.01
	wwof	61.5445	1	61.5445	351.0332671	<0.01
2-Way Interactions	dil*beaf	7.9181	4	1.9795	11.29073996	<0.01
	dil*wwof	7.7142	4	1.9286	11	<0.01
	beaf*wwof	61.5445	1	61.5445	351.0332671	<0.01
Residual		7.7142	44	0.1753		
Total		302.6853	59	5.1303		

Hydrilla was significant ($p < 0.05$) for all the parameters except hardness and pH. Conductivity, TSS, COD, PO_4 , hardness, magnesium, DO, BOD, NO_3 , SO_4 , calcium and log MPN showed significant differences ($p < 0.05$) between presence or absence of *Hydrilla* and pre/post experimental stage.

The regression equation for BOD, TSS, NO_3 , PO_4 and LMPN fitted first degree polynomial well while for COD second degree polynomial equation was found fit.

8. *Ceratophyllum demersum* L.

Mean values and 95 % confidence interval of water quality parameters are presented in Fig. 102.

All parameters showed significant difference ($p < 0.01$) between dilutions. All parameters except magnesium showed significant differences ($p < 0.05$) between pre/post experimental stage (Table 49). Significant differences were observed between presence/ absence of *Ceratophyllum* except for the parameters, temperature and pH.

Two way interactions between dilution and pre/post experimental stages was significant for conductivity, TSS, COD, PO_4 , Interactions between dilution and presence/absence of *Ceratophyllum* was significant ($p < 0.05$) for all parameters except hardness and pH. Conductivity, TSS, COD, PO_4 , hardness, DO, BOD, NO_3 , SO_4 , calcium, magnesium and log MPN showed significant differences between pre/post experimental stages and presence/ absence of *Ceratophyllum*.

Fig.101 Mean and Confidence Intervals for experiments with *Hydrilla*

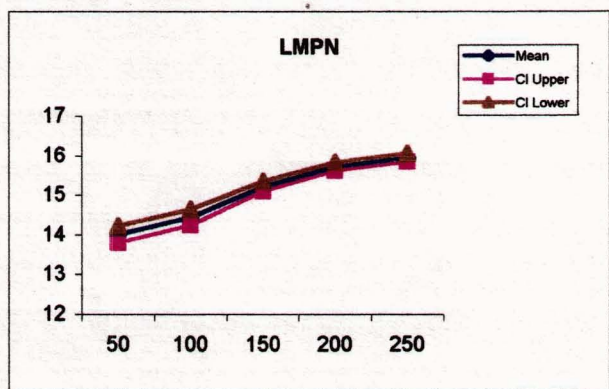
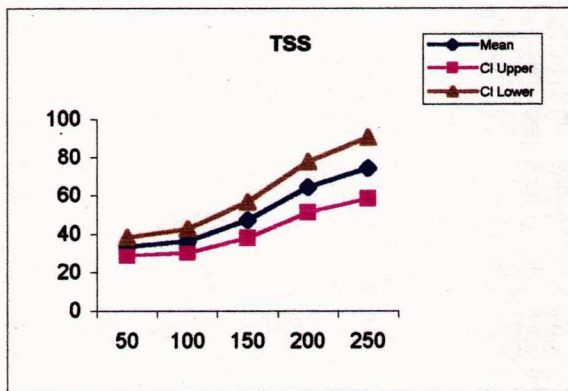
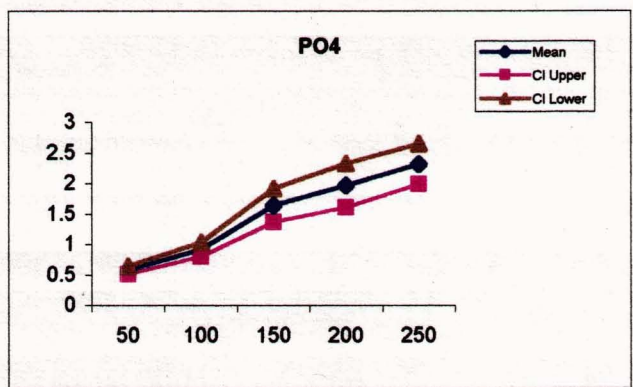
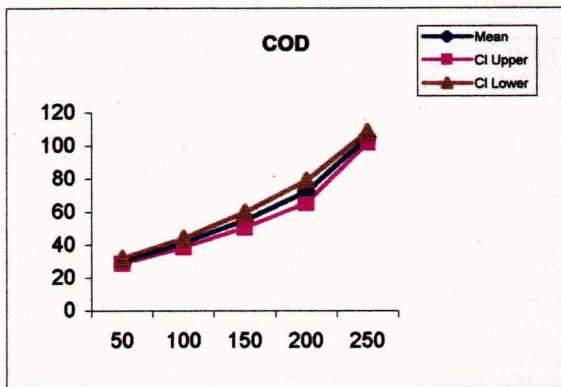
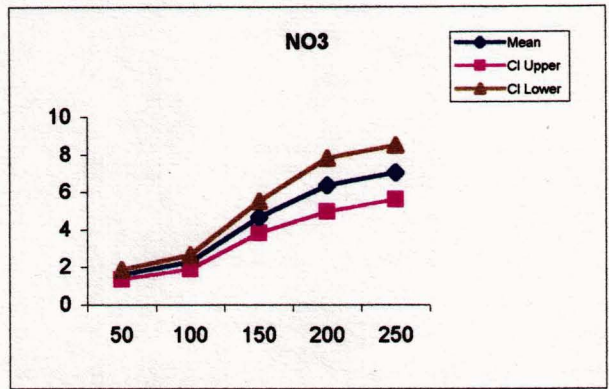
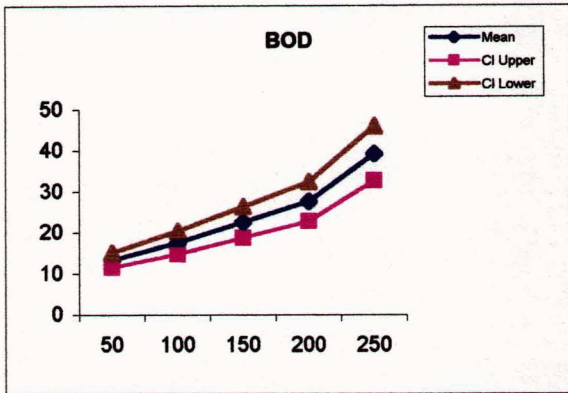


Table 48 Anova for Experiments with Hydrilla

TSS		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	15301.6216	4	3825.405	212.341359	<0.01
	beaf	5519.8123	1	5519.812	306.3948342	<0.01
	wwof	3975.0876	1	3975.088	220.6499491	<0.01
2-Way Interactions	dil*beaf	869.1208	4	217.280	12.06083215	<0.01
	dil*wwof	790.2185	4	197.555	10.96590131	<0.01
	beaf*wwof	3975.0876	1	3975.088	220.6499491	<0.01
Residual		792.6757	44	18.015		
Total		31223.6242	59	529.214		

BOD		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	4870.07797	4	1217.5195	409.2720313	<0.01
	beaf	924.73004	1	924.7300	310.8501713	<0.01
	wwof	601.35004	1	601.3500	202.1452262	<0.01
2-Way Interactions	dil*beaf	128.07997	4	32.0200	10.76359526	<0.01
	dil*wwof	128.55497	4	32.1387	10.80351335	<0.01
	beaf*wwof	601.35004	1	601.3500	202.1452262	<0.01
Residual		130.89303	44	2.9748		
Total		7385.03606	59	125.1701		

COD		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	41430.3261	4	10357.58153	2735.487511	<0.01
	beaf	917.286	1	917.286	242.2596811	<0.01
	wwof	547.224	1	547.224	144.5245122	<0.01
2-Way Interactions	dil*beaf	150.4593	4	37.614825	9.9342577	<0.01
	dil*wwof	163.6413	4	40.910325	10.80461523	<0.01
	beaf*wwof	547.224	1	547.224	144.5245122	<0.01
Residual		166.6005	44	3.786375		
Total		43922.7612	59	744.4535797		

NO3		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	280.0256	4	70.00639	255.4805998	<0.01
	beaf	35.6665	1	35.66646	130.160817	<0.01
	wwof	33.4805	1	33.48054	122.1835427	<0.01
2-Way Interactions	dil*beaf	11.5168	4	2.87920	10.50731412	<0.01
	dil*wwof	11.4638	4	2.86595	10.45897796	<0.01
	beaf*wwof	33.4805	1	33.48054	122.1835427	<0.01
Residual		12.0568	44	0.27402		
Total		417.6905	59	7.07950		

PO4		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	25.23009	4	6.30752	202.1656553	<0.01
	beaf	2.786415	1	2.78642	89.30882364	<0.01
	wwof	1.806135	1	1.80614	57.889364	<0.01
2-Way Interactions	dil*beaf	0.79041	4	0.19760	6.333459597	<0.01
	dil*wwof	0.50079	4	0.12520	4.012769615	<0.01
	beaf*wwof	1.806135	1	1.80614	57.889364	<0.01
Residual		1.37279	44	0.03120		
Total		34.292765	59	0.58123		

LMPN		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	32.881838	4	8.220459	4762.614318	<0.01
	beaf	1.296758	1	1.296758	751.2908374	<0.01
	wwof	0.938010	1	0.938010	543.4466656	<0.01
2-Way Interactions	dil*beaf	0.150059	4	0.037515	21.73464043	<0.01
	dil*wwof	0.075946	4	0.018986	11	<0.01
	beaf*wwof	0.938010	1	0.938010	543.4466656	<0.01
Residual		0.075946	44	0.001726		
Total		36.356567	59	0.616213		

Fig.102 Mean and Confidence Intervals for experiments with *Ceratophyllum*

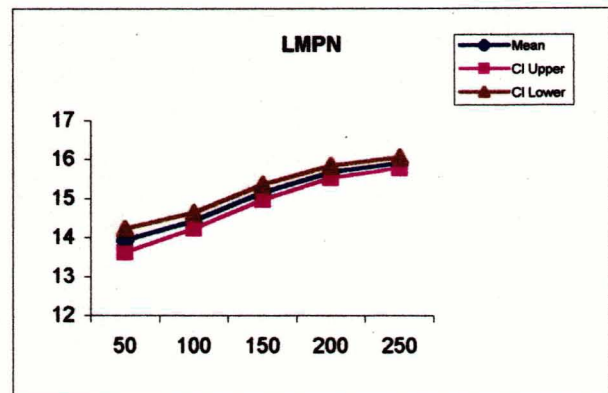
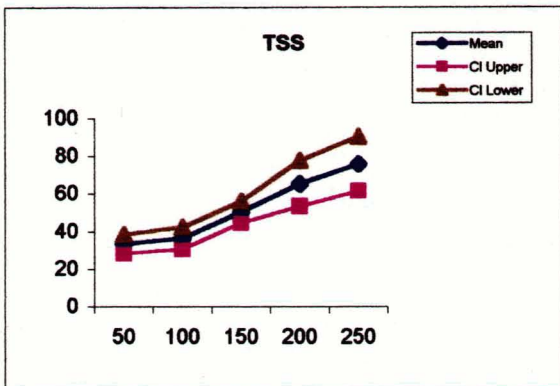
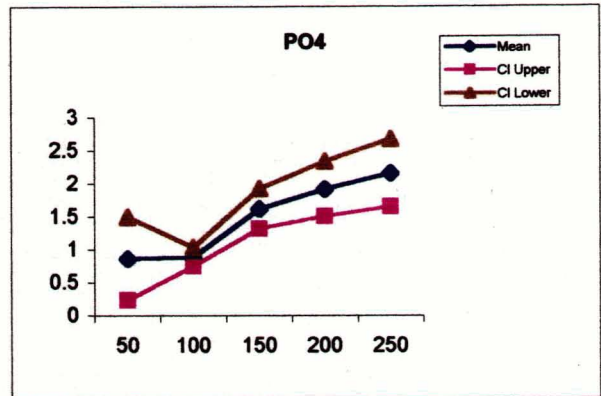
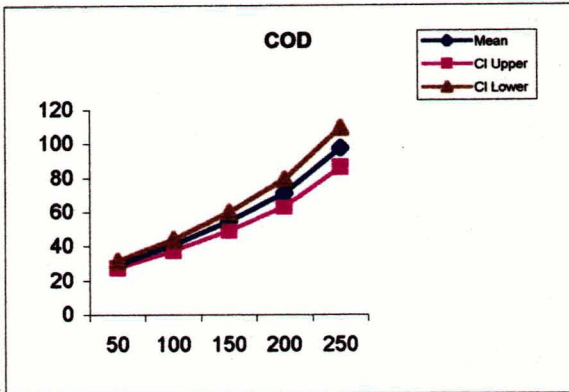
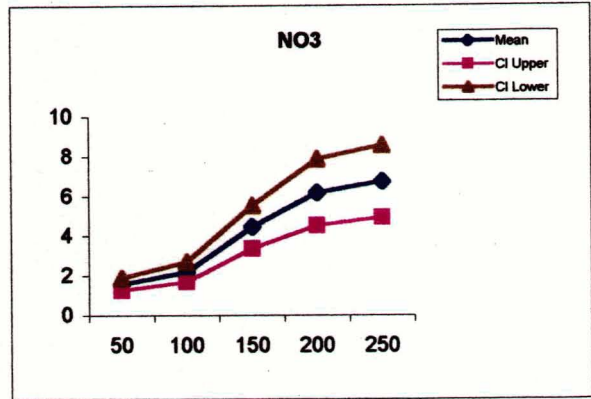
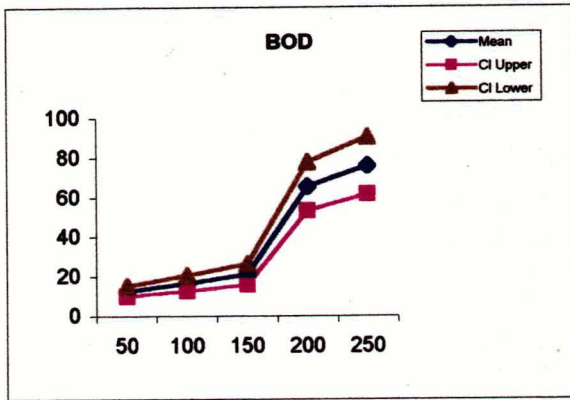


Table 49 Anova for Experiments with Ceratophyllum

TSS		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	16117.6610	4	4029.4153	263.7463421	<0.01
	beaf	4335.5100	1	4335.5100	283.7818482	<0.01
	wwof	2980.8811	1	2980.8811	195.1142899	<0.01
2-Way Interactions						
	dil*beaf	742.7114	4	185.6779	12.15358838	<0.01
	dil*wwof	670.1569	4	167.5392	10.96632001	<0.01
	beaf*wwof	2980.8811	1	2980.8811	195.1142899	<0.01
Residual		672.2151	44	15.2776		
Total		28500.0167	59	483.0511		

BOD		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	3937.3952	4	984.3488	128.2659902	<0.01
	beaf	1900.4630	1	1900.4630	247.6406463	<0.01
	wwof	1421.8454	1	1421.8454	185.274176	<0.01
2-Way Interactions						
	dil*beaf	336.4652	4	84.1163	10.96081064	<0.01
	dil*wwof	335.4758	4	83.8690	10.9285796	<0.01
	beaf*wwof	1421.8454	1	1421.8454	185.274176	<0.01
Residual		337.6682	44	7.6743		
Total		9691.1584	59	164.2569		

COD		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	34358.9282	4	8589.7321	704.5770265	<0.01
	beaf	2105.1527	1	2105.1527	172.6761902	<0.01
	wwof	1520.0667	1	1520.0667	124.6842213	<0.01
2-Way Interactions						
	dil*beaf	544.9460	4	136.2365	11.17486674	<0.01
	dil*wwof	533.4557	4	133.3639	10.93924235	<0.01
	beaf*wwof	1520.0667	1	1520.0667	124.6842213	<0.01
Residual		536.4186	44	12.1913		
Total		41119.0345	59	696.9328		

NO3		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	255.94764	4	63.98691	170.9431874	<0.01
	beaf	54.093015	1	54.093015	144.5113133	<0.01
	wwof	52.621935	1	52.621935	140.5812735	<0.01
2-Way Interactions						
	dil*beaf	16.89156	4	4.22289	11.28159301	<0.01
	dil*wwof	16.03554	4	4.008885	10.70987144	<0.01
	beaf*wwof	52.621935	1	52.621935	140.5812735	<0.01
Residual		16.46994	44	0.374316818		
Total		464.681565	59	7.875958729		

PO4		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	16.8200	4	4.20500	14.34430101	<0.01
	beaf	2.6460	1	2.64600	9.026158835	<0.01
	wwof	1.6934	1	1.69344	5.776741655	<0.05
2-Way Interactions						
	dil*beaf	3.6281	4	0.90701	3.094043421	<0.05
	dil*wwof	3.1613	4	0.79033	2.696002096	<0.05
	beaf*wwof	1.6934	1	1.69344	5.776741655	<0.05
Residual		12.8985	44	0.29315		
Total		42.5408	59	0.72103		

LMPN		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	33.476472	4	8.369118	2732.280777	<0.01
	beaf	2.180476	1	2.180476	711.8639638	<0.01
	wwof	1.706682	1	1.706682	557.1834154	<0.01
2-Way Interactions						
	dil*beaf	0.196934	4	0.049233	16.07330837	<0.01
	dil*wwof	0.134774	4	0.033694	11	<0.01
	beaf*wwof	1.706682	1	1.706682	557.1834154	<0.01
Residual		0.134774	44	0.003063		
Total		39.536794	59	0.670115		

The regression equations for BOD, log MPN, TSS, NO₃ and PO₄ fitted first degree polynomial, while the second degree polynomial equation fitted well for COD.

9. *Lagenandra toxicaria* Dalz.

Mean values and 95 % confidence interval of water quality parameters are presented in Fig. 103.

Dilutions showed significant differences ($p < 0.01$) for all parameters; pre/post experimental stage had significant differences ($p < 0.05$) for all parameters except conductivity (Table 50). Presence/absence of *Lagenandra* showed significant difference for all parameters except conductivity, magnesium and calcium.

The two way interactions between pre/post experimental stage was significant ($p < 0.05$) for all parameters except hardness. The interactions between dilution and presence/ absence of *Lagenandra* was significant for all parameters except hardness and pH. All parameters except calcium and magnesium showed significant interactions for pre/post experimental stage and presence/ absence of *Lagenandra*.

The first degree polynomial equation fitted well for the parameters BOD, COD, TSS, LMPN, NO₃ and PO₄.

Fig.103 Mean and Confidence Intervals for experiments with *Lagenandra*

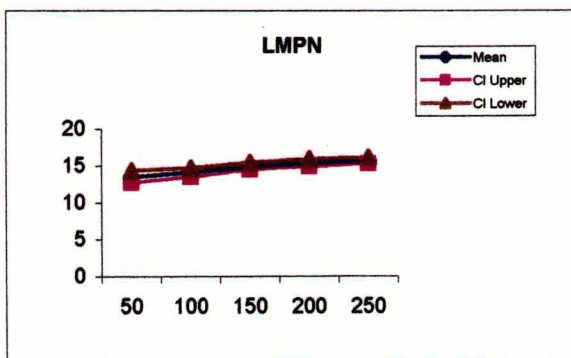
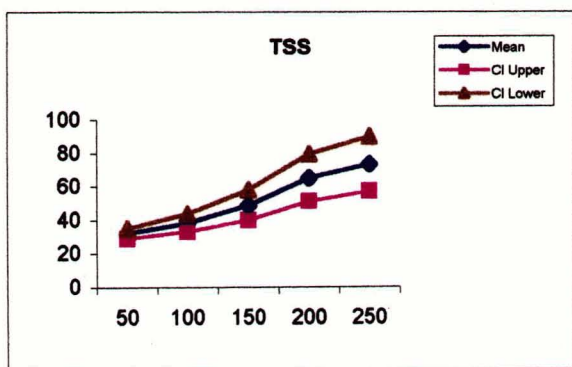
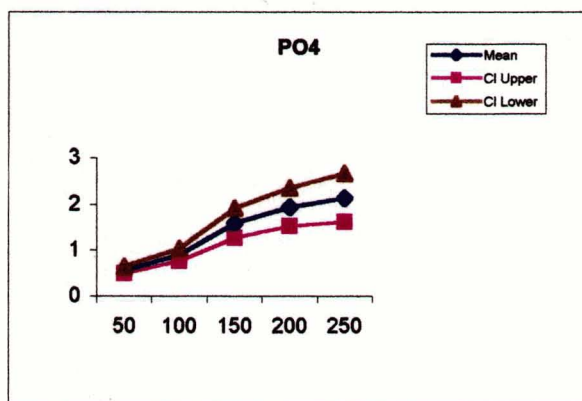
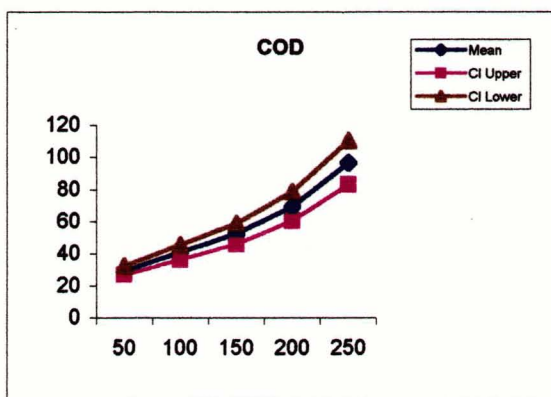
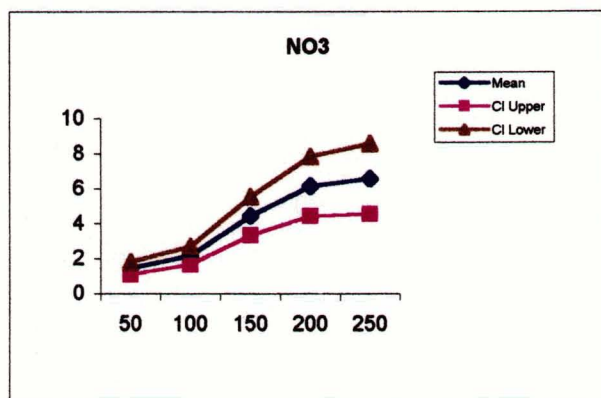
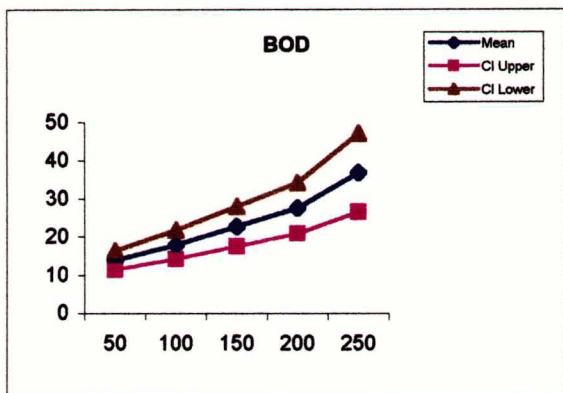


Table 50 Anova for Experiments with Lagenandra

TSS		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	14704.9090	4	3676.2272	132.7862758	<0.01
	beaf	4856.2207	1	4856.2207	175.4079447	<0.01
	wwof	3817.4731	1	3817.4731	137.8881137	<0.01
2-Way Interactions	dil*beaf	1157.1094	4	289.2773	10.44877236	<0.01
	dil*wwof	1208.5930	4	302.1482	10.91367258	<0.01
	beaf*wwof	3817.4731	1	3817.4731	137.8881137	<0.01
Residual	1218.1530	44	27.6853			
Total	30779.9314	59	521.6938			

BOD		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	3793.2194	4	948.3049	116.1625656	<0.01
	beaf	1758.0341	1	1758.0341	215.3503228	<0.01
	wwof	1339.5375	1	1339.5375	164.0865934	<0.01
2-Way Interactions	dil*beaf	299.4830	4	74.8708	9.171289882	<0.01
	dil*wwof	356.7563	4	89.1891	10.9252107	<0.01
	beaf*wwof	1339.5375	1	1339.5375	164.0865934	<0.01
Residual	359.1985	44	8.1636			
Total	9245.7663	59	156.7079			

COD		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	33095.8906	4	8273.9726	484.3101818	<0.01
	beaf	3288.7087	1	3288.7087	192.5018601	<0.01
	wwof	1965.5071	1	1965.5071	115.0493431	<0.01
2-Way Interactions	dil*beaf	606.1750	4	151.5437	8.870488583	<0.01
	dil*wwof	750.1906	4	187.5476	10.97794725	<0.01
	beaf*wwof	1965.5071	1	1965.5071	115.0493431	<0.01
Residual	751.6976	44	17.0840			
Total	42423.6768	59	719.0454			

NO3		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	253.1216567	4	63.28041417	128.7201478	<0.01
	beaf	62.56688167	1	62.56688167	127.2687349	<0.01
	wwof	56.31828167	1	56.31828167	114.5583138	<0.01
2-Way Interactions	dil*beaf	17.98137667	4	4.495344167	9.144083098	<0.01
	dil*wwof	20.97127667	4	5.242819167	10.66453921	<0.01
	beaf*wwof	56.31828167	1	56.31828167	114.5583138	<0.01
Residual	21.63094333	44	0.491612348			
Total	488.9086983	59	8.286588107			

PO4		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	21.7649	4	5.44123	199.6930629	<0.01
	beaf	4.5046	1	4.50456	165.3173633	<0.01
	wwof	3.6605	1	3.66054	134.3418272	<0.01
2-Way Interactions	dil*beaf	1.4416	4	0.36040	13.22658915	<0.01
	dil*wwof	1.1909	4	0.29773	10.92659999	<0.01
	beaf*wwof	3.6605	1	3.66054	134.3418272	<0.01
Residual	1.1989	44	0.02725			
Total	37.4220	59	0.63427			

LMPN		Sum of Squares	df	Mean Square	F	p
Main Effects	dil	40.28505026	4	10.07126257	475.8954088	<0.01
	beaf	15.17793312	1	15.17793312	717.1999179	<0.01
	wwof	13.75101424	1	13.75101424	649.7739979	<0.01
2-Way Interactions	dil*beaf	1.091955911	4	0.272988978	12.89949501	<0.01
	dil*wwof	0.931161648	4	0.232790412	11	<0.01
	beaf*wwof	13.75101424	1	13.75101424	649.7739979	<0.01
Residual	0.931161648	44	0.021162765			
Total	85.91929106	59	1.456259171			

Table 51 Regression Equations for Selected Parameters - Aquatic Fauna

Test Species	Parameter	Regression Equation	r ²
Paramecium	BOD	$y = 4.5441 + 0.133 x$	0.948
	COD	$y = 14.9876 + 0.0410x - 0.8583x^2$	0.997
	MPN	$y = 2052.5$	0
	TSS	$y = 24.6875 + 1.942 x$	0.994
Daphnia	BOD	$y = 4.615 + 0.0646 x$	0.961
	COD	$y = 22.489 - 1.212 x + 0.0367x^2$	0.85
	LMPN	$y = 3.5262 + 0.5221 x - 0.0132$	0.982
	TSS	$y = 34.8141 + 1.4568x$	0.969
Cyprinus	BOD	$y = 4.744 - 0.00485x + 0.00082 x^2$	0.996
	COD	$y = 9.5037 + 0.1479 x$	0.867
	LMPN	$y = 7.64325 + 0.0310 x$	0.967
	TSS	$y = 44.779 + 1.405x$	0.980
Catla	BOD	$y = -26.8964 + 4.0910 x$	0.970
	COD	$y = -31.617 + 2.0577 x$	0.978
	LMPN	$y = -186.553 + 19.506 x$	0.855
	TSS	$y = -50.096 + 0.7581 x$	0.996
Rohu	BOD	$y = 6.71 + 0.3082 x$	0.990
	COD	$y = 15.24 + 0.7357 x$	0.964
	LMPN	$y = 10.0140 + 0.0348 x$	0.969
	TSS	$y = 74.1976 + 0.9561 x$	0.917
Mrigal	BOD	$y = 8.3765 + 0.212718 x + 0.000602 x^2$	0.990
	COD	$y = 15.058 + 0.6836 x$	0.9709
	LMPN	$y = 10.4505 + 0.0260 x$	0.978
	TSS	$y = 71.5868 + 1.2645 x$	0.98
Tilapia	BOD	$y = 6.6466 + 0.3140 x$	0.991
	COD	$y = 10.449 + 0.94895 x$	0.972
	LMPN	$y = 11.9182 + 0.03187 x$	0.718
	TSS	$y = 73.7148 + 1.0549 x$	0.886
Lebistes	BOD	$y = 5.5615 - 0.0312 x + 0.00140$	0.984
	COD	$y = 16.2725 - 0.2887 x + 0.0059 x^2$	0.902
	MPN	$y = 2018.82 + 91.465 x$	0.914
	TSS	$y = 52.749 + 1.5011 x$	0.975

Table 52 Regression Equations for Selected Parameters - Aquatic Flora

Test Species	Parameter	Regression Equation	r ²
Azolla	BOD	$y = 4.6370 + 0.1130 x$	0.987
	COD	$y = 12.4502 + 0.4170 x$	0.997
	LMPN	$y = 13.3476 + 0.0132 x$	0.984
	TSS	$y = 11.5367 + 0.2991 x$	0.988
	NO ₃	$y = -0.7154 + 0.0320 x$	0.991
	PO ₄	$y = -0.49637 + 0.0281 x$	0.975
Salvinia	BOD	$y = 4.746 + 0.113 x$	0.983
	COD	$y = 47.465 - 0.3809 x + 0.0034 x^2$	0.964
	LMPN	$y = 13.035 + 0.014 x$	0.984
	TSS	$y = 13.7809 + 0.283 x$	0.987
	NO ₃	$y = -0.631 + 0.0289 x$	0.991
	PO ₄	$y = -0.5607 + 0.027 x$	0.981
Lemna	BOD	$y = 6.3575 + 0.0367 x + 0.00028 x^2$	0.998
	COD	$y = 6.4555 + 0.2923 x$	0.975
	LMPN	$y = 13.523 + 0.0079 x$	0.971
	TSS	$y = 9.437 + 0.2647 x$	0.983
	NO ₃	$y = -0.2045 + 0.0154 x$	0.970
	PO ₄	$y = 0.0995 + 0.0081x$	0.919
Spirodela	BOD	$y = 0.897 + 0.139 x$	0.977
	COD	$y = 6.176 + 0.3139 x$	0.973
	LMPN	$y = 13.689 + 0.007 x$	0.971
	TSS	$y = 12.918 + 0.259x$	0.984
	NO ₃	$y = -0.2215 + 0.0159 x$	0.970
	PO ₄	$y = 0.101 + 0.008 x$	0.917

Wolffia	BOD	$y = 7.211 + 0.0426 x + 0.00037 x^2$	0.998
	COD	$y = 6.1817 + 0.328 x$	0.975
	LMPN	$y = 14.073 + 0.0059 x$	0.980
	TSS	$y = 12.980 + 0.2668 x$	0.974
	NO ₃	$y = 0.0039 + 0.0154 x$	0.978
	PO ₄	$y = -0.2932 + 0.0126 x$	0.964
Pistia	BOD	$y = 4.88 + 0.108 x$	0.987
	COD	$y = 15.29 + 0.327 x + 0.00007 x^2$	0.966
	LMPN	$y = 11.856 + 0.0172 x$	0.970
	TSS	$y = 13.229 + 0.2650 x$	0.990
	NO ₃	$y = -0.6313 + 0.02892 x$	0.991
	PO ₄	$y = -0.6290 + 0.0276 x$	0.987
Hydrilla	BOD	$y = 5.4514 + 0.1241 x$	0.949
	COD	$y = 6.5092 + 0.3620 x$	0.948
	LMPN	$y = 13.5228 + 0.0103 x$	0.971
	TSS	$y = 18.1455 + 0.2208 x$	0.956
	NO ₃	$y = -0.0942 + 0.0299x$	0.963
	PO ₄	$y = -0.1252 + 0.0090 x$	0.979
Ceratophyllum	BOD	$y = -14.2838 + 0.3515 x$	0.861
	COD	$y = 25.5315 + 0.0465 x + 0.00095 x^2$	0.997
	LMPN	$y = 13.4615 + 0.0104 x$	0.975
	TSS	$y = 18.1197 + 0.2282x$	0.969
	NO ₃	$y = -0.0657 + 0.0286x$	0.959
	PO ₄	$y = 0.4052 + 0.0072 x$	0.936
Lagenandra	BOD	$y = 7.2137 + 0.1107 x$	0.970
	COD	$y = 9.117 + 0.3257 x$	0.961
	LMPN	$y = 12.9995 + 0.0114 x$	0.973
	TSS	$y = 18.857 + 0.2191x$	0.979
	NO ₃	$y = -0.1064 + 0.0284 x$	0.956
	PO ₄	$y = 0.17325 + 0.0083 x$	0.964

4. 4 PHYTOREMEDIATION OF METALS

4. 4 PHYTOREMEDIATION OF METALS

Aquatic macrophytes possess the ability for uptake of metals, greater than their metabolic needs. They have fairly high potential for pollutant transformation or to act as sinks. The efficiency of metal removal by different plants depends upon the amount of biomass, nature of metal to be removed, pattern of uptake and tolerance to each metal (Hutchinson and Czyska, 1975).

Plants like all living organisms have evolved mechanisms that control and respond to the uptake and accumulation of both essential and non-essential metals. Exposure of plants to high levels of metals results in reduction and inhibition of plant growth caused by the denaturation of proteins such as enzymes. It is considered that the plant cells have the physiological mechanism to excess metals. These include cellular exclusion of metals, adsorption of the ions in cell wall, synthesis of heavy metal insensitive enzymes, chelation of the metal ions by chelators (Cobbett, 2000). Organic acids such as citrate and malate (Delhaize and Ryan, 1995) and some amino acids, particularly histidine, also have roles in the chelation of metal ions both within the cells and in the xylem sap (Kramer, *et al.* 1996 and Rauser, 1999). Peptide ligands include metallothioneins, small gene coded cysteine rich polypeptides and phytochelatins, which are produced in plants by enzymes that are expressed in response to heavy metals (Rauser, 1999; Steffens, 1990).

Macrophyte based wastewater treatment using tolerant plants is considered to be ecofriendly in the long run. Selection of optimal plant species depends on the ease of growth, harvest and disposal considerations.

In this study, locally available macrophytes were screened for their ability to remove metals from solutions. The plants selected include *Lemna perpusilla* Torrey, *Azolla rubra* R.Br, *Salvinia molesta* Mitchell, *Spirodela polyrrhiza* (L.) Schleid, *Wolffia globosa* (Roxb. Hartog and Plas.), *Pistia stratiotes* L., *Hydrilla verticillata* (L.f.) Royle and *Ceratophyllum demersum* L. Lab scale studies for uptake of selected metals, copper, chromium, iron, zinc and manganese using spiked solutions were conducted for seven days.

Manganese

Manganese uptake studies were conducted using *Azolla*, *Lemna*, *Pistia*, *Salvinia*, *Hydrilla*, *Ceratophyllum*, *Wolffia* and *Spirodela*. Most of the plants studied possessed the ability to remove upto 99 percent manganese from solutions of 2-10 mg/l for the seven day treatment period. The plant uptake was maximum in low concentrations and decreased with higher metal concentrations (Table 53).

Azolla showed maximum uptake of 99 percent in 2 mg/l manganese. Higher concentrations of metal (4-10 mg/l) had 76.5, 54.16, 69.25 and 68.6 percent reduction in manganese. *Lemna* had maximum removal in 2 mg/l manganese (94 percent). Other concentrations showed 91.75, 63.66, 66.75 and 56 percent reduction. In studies with *Pistia*, 99 percent reduction was obtained for 2 and 4 mg/l manganese and plant uptake reduced in 6-10 mg/l solutions by 48.3, 30.62 and 41 percent. *Salvinia* had 99 percent removal in 2 mg/l manganese solution and 71.25, 72.83, 78.12 and 77 percent reduction in 4-10 mg/l metal concentrations. Manganese removal by *Hydrilla* was excellent and consistently in the range of 99 percent upto 8 mg/l and 97.8 percent in 10 mg/l manganese. In *Ceratophyllum* also pronounced decrease was obtained, 99 percent in 2 and 4

mg/l, 94.5, 94.25 and 80.7 percent in 6-10 mg/l manganese. The maximum uptake of *Wolffia* was 73.5 percent in 2 mg/l, while in other metal concentrations (4-10 mg/l) the uptake recorded was 43, 29.16, 20 and 28.5 percent. *Spirodela* could remove upto 82.5 percent of manganese in 2 mg/l while 64, 70.6, 65 and 38 percent reduction was obtained in higher metal concentrations. Fig. 104 represents results obtained using the aquatic plants for the manganese removal.

Zinc

Phytoremediation of zinc by the aquatic macrophytes *Azolla*, *Lemna*, *Pistia*, *Salvinia*, *Hydrilla*, *Wolffia*, *Ceratophyllum* and *Spirodela* from 2-10 ppm metal solutions was studied for a detention period of seven days. Remarkable uptake of zinc was noticed in *Ceratophyllum*, *Lemna* and *Salvinia*. As in the case of manganese, plant metal uptake decreased with increase in concentration of the metal solutions (Table 54).

Among the plants experimented, maximum removal was obtained for *Ceratophyllum*, 99 percent (in 2 mg/l zinc solution). Higher concentrations 4-10 mg/l had 80, 86, 87.12 and 81.5 percent removal of zinc. The duckweed *Wolffia* was capable of uptake upto 92.5 percent (in 2 mg/l zinc solution) and 82.2, 73.6, 59.37 and 50.5 percent reduction in 4-10 mg/l zinc. *Lemna* showed higher percentage metal removal at low concentrations, 90.5 percent at 2 mg/l and lowest percentage removal was 57 percent at 10 mg/l. *Spirodela* had upto 99 percent removal in 2 and 4 mg/l zinc solution. In higher concentrations, 80, 79.37 and 69.5 percent reduction was observed in the studies with *Spirodela*.

Azolla had a removal of 66 percent from 10 mg/l zinc and other concentrations (2-8 mg/l) had 55.5, 48.25, 57 and 63.75 percent uptake. *Pistia*

Table 53 Manganese Removal by Aquatic Macrophytes

Initial Conc	Azolla		Salvinia		Lemna		Spirodela		Wolffia		Pistia		Hydrilla		Ceratophyllum	
	Final Conc	% Removal	Final Conc	% Removal	Final Conc	% Removal	Final Conc	% Removal	Final Conc	% Removal	Final Conc	% Removal	Final Conc	% Removal	Final Conc	% Removal
2	ND	99.99	ND	99.99	0.12	94.00	0.35	82.50	0.53	73.50	ND	99.99	ND	99.99	ND	99.99
4	0.94	76.50	1.15	71.25	0.33	91.75	1.44	64.00	2.28	43.00	ND	99.99	ND	99.99	ND	99.99
6	2.75	54.16	1.63	72.83	2.18	63.66	1.76	70.60	4.25	29.16	3.10	48.30	ND	99.99	0.33	94.50
8	2.46	69.25	1.75	78.12	2.66	66.75	2.80	65.00	6.40	20.00	5.55	30.62	ND	99.99	0.46	94.25
10	3.14	68.60	2.30	77.00	4.40	56.00	6.20	38.00	7.15	28.50	5.90	41.00	0.22	97.80	1.93	80.70

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Table 54 Zinc Removal by Aquatic Macrophytes

Initial Conc	Azolla		Salvinia		Lemna		Spirodela		Wolffia		Pistia		Hydrilla		Ceratophyllum	
	Final Conc	% Removal	Final Conc	% Removal	Final Conc	% Removal	Final Conc	% Removal	Final Conc	% Removal	Final Conc	% Removal	Final Conc	% Removal	Final Conc	% Removal
2	0.89	55.5	0.21	89.50	0.19	90.50	ND	99.90	0.15	92.50	1.06	47.00	ND	99.90	ND	99.90
4	2.07	48.25	1.08	73.00	1.02	74.50	ND	99.90	0.71	82.20	1.22	69.50	ND	99.90	0.80	80.00
6	2.58	57.00	1.31	78.16	1.59	73.50	1.20	80.00	1.58	73.60	1.94	67.66	1.01	83.17	0.84	86.00
8	2.9	63.75	5.65	29.37	3.02	62.25	1.65	79.37	3.25	59.37	4.60	42.50	1.45	81.87	1.03	87.12
10	3.4	66.00	5.50	45.00	4.30	57.00	3.05	69.50	4.95	50.50	7.15	28.50	2.45	75.50	1.85	81.50

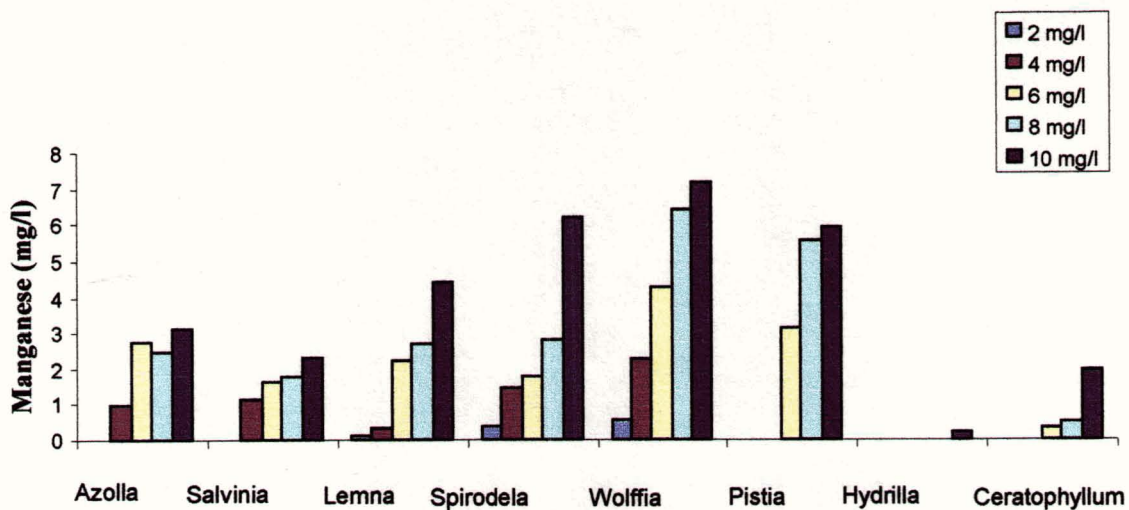


Fig. 104 Manganese Removal by Aquatic Macrophytes

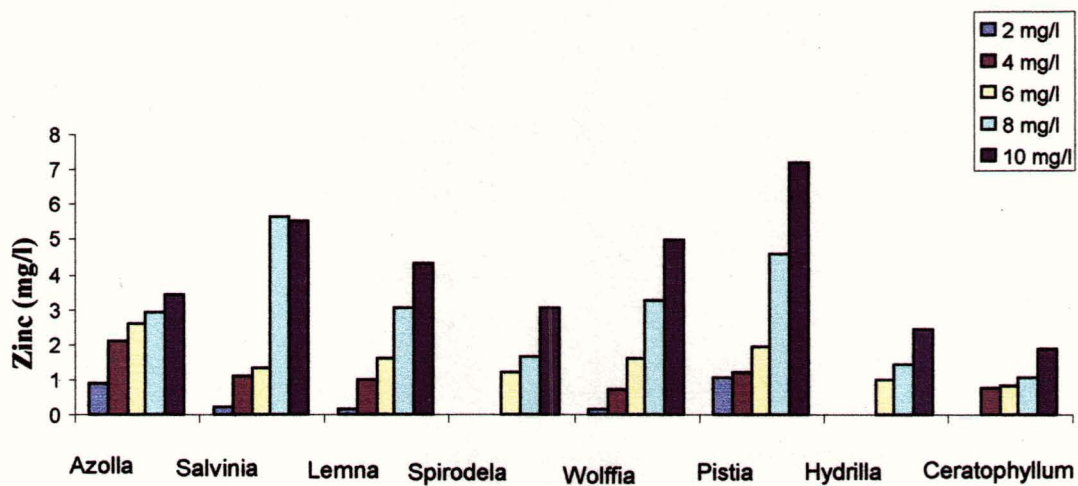


Fig.105 Zinc Removal by Aquatic Macrophytes

showed maximum removal, 69.5 percent at 4 mg/l zinc and it decreased to 28.5 percent in 10 mg/l zinc. In *Salvinia* uptake was 89.5, 73, 78.16, 45 and 29.4 percent in 2-10 mg/l zinc. *Hydrilla* had removal of 99 percent in 2 and 4 g/l zinc. Higher concentrations showed 83.17, 81.87 and 75.5 percent removal of zinc (Fig. 105).

Copper

Lab scale studies were conducted using the aquatic plants *Hydrilla*, *Ceratophyllum*, *Pistia*, *Lemna*, *Azolla* and *Salvinia*. It was observed that in this range, defoliation occurred within two days in *Hydrilla* and *Ceratophyllum*. Therefore 0.25-2 mg/l copper solution was used for these two plants. Other plants were maintained in 2-10 mg/l copper.

Considerable removal of copper was recorded with the plants under study (Table 55). *Azolla*, *Pistia* and *Salvinia* were capable of reducing the copper levels in the metal solutions of 2-10 mg/l. *Azolla* recorded uptake of 94, 95.5, 96.16, 96.12 and 95.8 percent from 2-10 mg/l copper. *Lemna* had 91.5, 91.5, 87.83, 89.87 and 93.9 percent uptake from initial copper concentrations. *Pistia* showed 90.5, 96.5, 97.5, 97.75 and 97.5 percent reduction for these concentrations, while *Salvinia* had 96.5, 97.25, 98.33, 98.25 and 99 percent reduction.

When *Hydrilla* and *Ceratophyllum* were introduced in lower copper concentrations (0.25-2 mg/l), they revealed good copper uptake. *Hydrilla* showed 96.5, 97.5, 96.83, 98 and 98 percent reduction for these concentrations. It was observed that *Ceratophyllum* had the ability to remove 96, 95.25, 96, 89.75 and 96 percent copper from the spiked solutions had 96.5, 97.25, 98.33, 98.5 and 99

Table 55 Copper Removal by Aquatic Macrophytes

Initial Conc	Azolla		Salvinia		Lemna		Pistia	
	Final Conc	% Removal	Final Conc	% Removal	Final Conc	% Removal	Final Conc	% Removal
2	0.12	94.00	0.07	96.50	0.17	91.50	0.19	90.50
4	0.18	95.50	0.11	97.25	0.34	91.50	0.14	96.50
6	0.23	96.16	0.1	98.33	0.73	87.83	0.15	97.50
8	0.31	96.12	0.12	98.50	0.81	89.87	0.18	97.75
10	0.42	95.80	0.1	99.00	0.61	93.90	0.25	97.50

Initial Conc	Hydrilla		Ceratophyllum	
	Final Conc	% Removal	Final Conc	% Removal
0.25	0.07	96.50	0.08	96.00
0.5	0.10	97.50	0.19	95.25
1.0	0.19	96.83	0.24	96.00
1.5	0.16	98.00	0.82	89.75
2.0	0.20	98.00	0.28	86.00

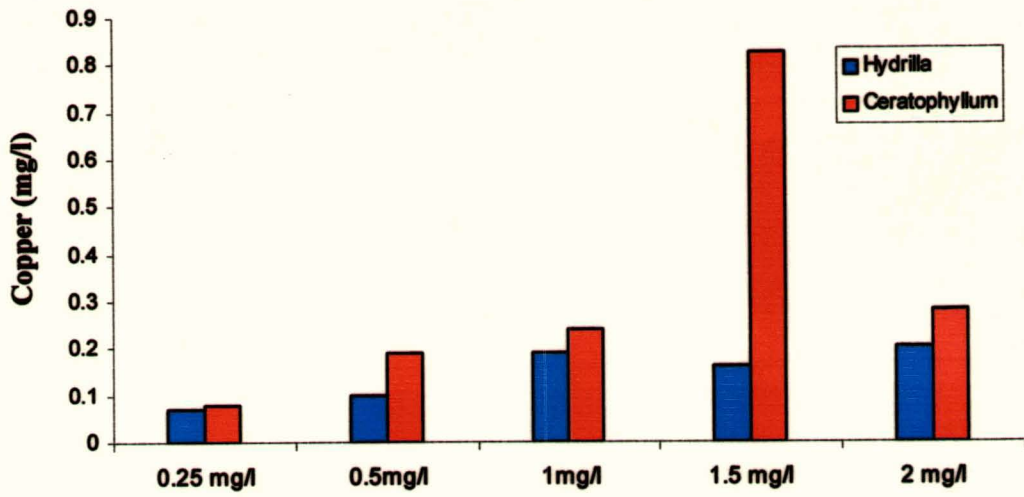


Fig. 106 (a) Copper Removal by Aquatic Macrophytes

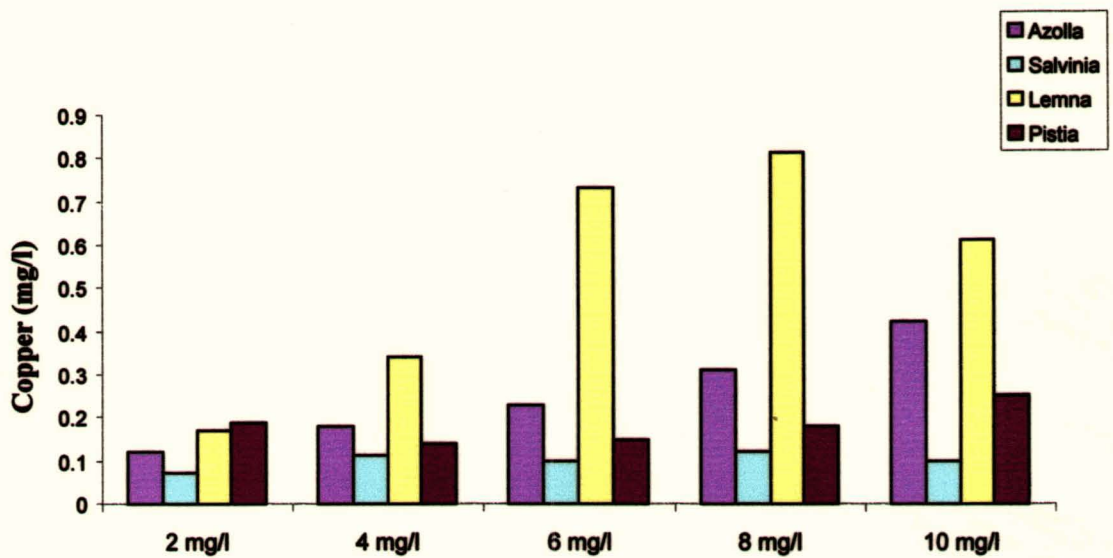


Fig. 106 (b) Copper Removal by Aquatic Macrophytes

percent reduction. Fig.106 represents the removal of copper by various macrophytes experimented.

Chromium

Studies on chromium uptake was conducted using *Azolla*, *Lemna*, *Pistia*, *Salvinia*, *Hydrilla* and *Ceratophyllum* in 2-10 mg/l metal solutions for a seven day period. The results revealed that the ferns, *Salvinia* and *Azolla* were capable of high removal of chromium (99.5 percent). Table 56 and Fig.107 represents the results obtained in experiments with chromium.

Azolla had chromium uptake rates of 99.5, 72.5, 66.6, 50 and 47 percent in 2-10 mg/l metal solutions. In *Salvinia*, 99.5, 97.5, 88.33, 70.62 and 44 percent reduction of chromium was observed on the seventh day. *Pistia* had removal of 81, 40, 6.67 and 5 percent in 2 -8 mg/l chromium. The plant did not survive in 10 mg/l concentration for seven days. *Lemna* had 59, 53, 5.8, 5.75 and 5.5 percent removal in 2 -10 mg/l chromium.

Observations with *Hydrilla* revealed 28, 18.75, 14.2, 14 and 12.5 percent uptake of chromium from 2-10 mg/l metal solutions. In the case of *Ceratophyllum*, 10.5 percent reduction was noted in 2 mg/l chromium. In other concentrations, the leaf detached from the stem, and settled to the bottom within 4 days.

Iron

Iron, although essential for plants in micro levels are found to be toxic at increasing concentrations. Experiments were conducted using the aquatic plants *Azolla*, *Salvinia*, *Lemna*, *Pistia*, *Hydrilla*, *Wolffia*, *Spirodela* and *Ceratophyllum*

Table 56 Chromium Removal by Aquatic Macrophytes

Initial Conc	Azolla		Salvinia		Lemna		Pistia		Hydrilla		Ceratophyllum	
	Final Conc	% Removal	Final Conc	% Removal	Final Conc	% Removal	Final Conc	% Removal	Final Conc	% Removal	Final Conc	% Removal
2	0.01	99.5	0.01	99.50	0.82	59.00	0.38	81.00	1.44	28.00	1.79	10.5
4	1.1	72.5	0.10	97.50	1.85	53.75	2.40	40.00	3.25	18.75	-	-
6	2.0	66.6	0.70	88.33	5.65	5.80	5.60	6.67	5.15	14.20	-	-
8	4.0	50.0	2.35	70.62	7.54	5.75	7.60	5.00	6.88	14.00	-	-
10	5.3	47.0	5.60	44.00	9.45	5.50	9.40	6.00	8.75	12.50	-	-

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Table 57 Iron Removal by Aquatic Macrophytes

Initial Conc	Azolla		Salvinia		Lemna		Wolffia		Pistia	
	Final Conc	% Removal	Final Conc	% Removal	Final Conc	% Removal	Final Conc	% Removal	Final Conc	% Removal
0.25	0.04	84	0.02	92	0.04	84	0.20	20	0.03	88.0
0.5	0.06	88	0.04	92	0.05	90	0.37	26	0.03	94.0
1.0	0.03	97	0.02	98	0.03	97	-	-	0.03	97.0
1.5	0.10	93	0.00	99	0.00	99	-	-	0.02	98.7
2.0	0.14	93	0.10	95	0.02	99	-	-	0.05	97.5

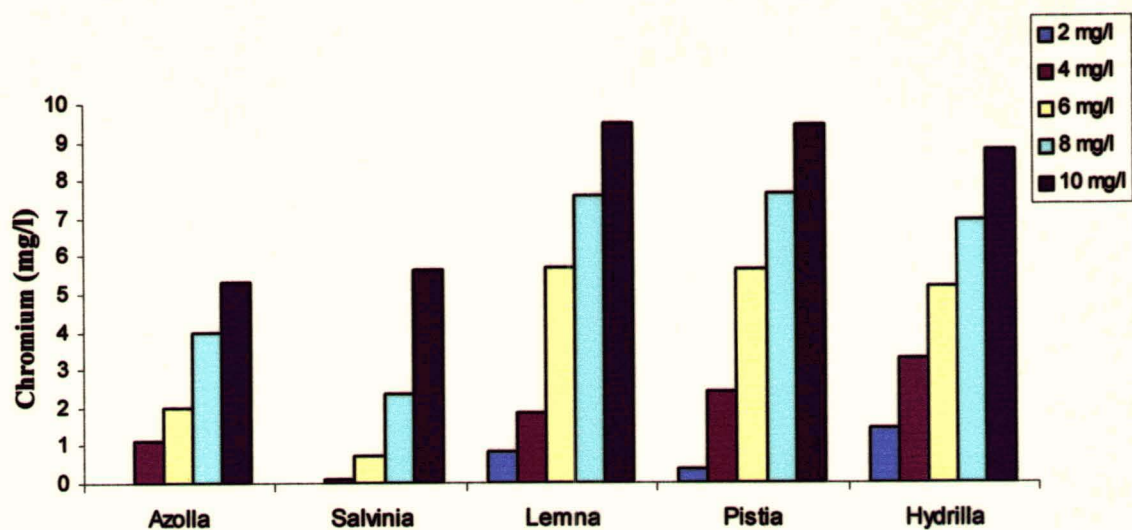


Fig. 107 Chromium Removal by Aquatic Macrophytes

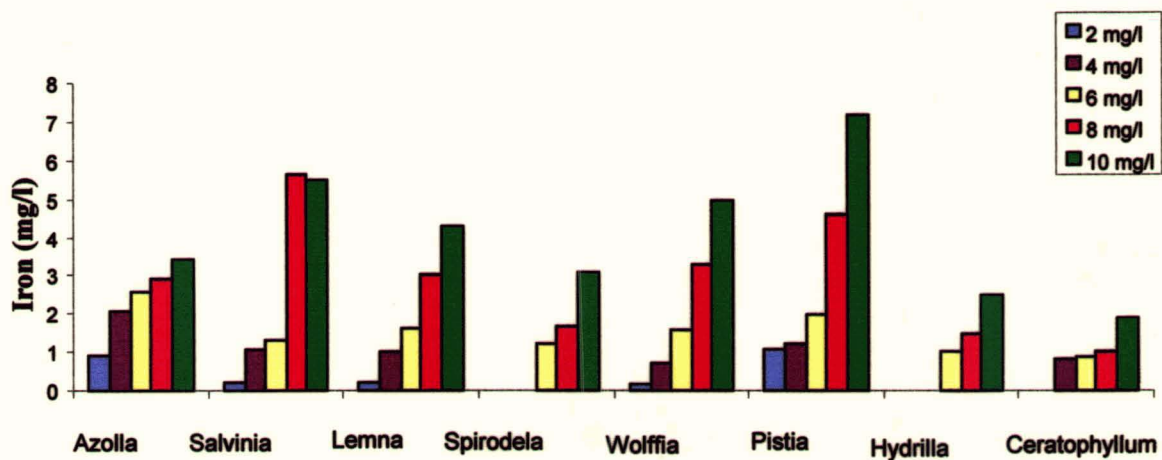


Fig. 108 Iron Removal by Aquatic Macrophytes

for seven days. Initial studies with 2- 10 mg/l iron solution revealed its toxicity to *Spirodela*, *Hydrilla* and *Ceratophyllum*. Therefore the concentration of iron solution was reduced to 0.25-2 mg/l. However it was observed that in *Spirodela*, leaching of pigments occurred within an hour after introduction into the iron solution. *Hydrilla* and *Ceratophyllum* survived only for 2 days. *Wolffia* survived upto 0.5 mg/l iron and perished in higher concentrations.

Azolla was capable of removal upto 97 percent (in 1 mg/l iron solution). The percentage uptake of iron from solution was 84, 88, 97, 93 and 93 percent for 0.25 to 2 mg/l iron. The uptake decreased in 1.5 and 2 mg/l. Table 57 and Fig.108 represents the iron removal using selected aquatic plants.

Lemna had metal uptake of 84, 90, 97, 99 and 99 percent for 0.25 to 2 mg/l iron. *Pistia* had 88, 94, 97, 98.7 and 97.5 percent uptake. *Salvinia* recorded increasing uptake upto 1.5 mg/l (92, 92, 98 and 99 percent) and then a decrease (95 percent) was noted in 2 mg/l iron solution. *Wolffia* had removal of 20 and 26 percent in 0.25 and 0.5 mg/l iron solution. The plant did not survive beyond 1 mg/l iron for the treatment period.

4. 5 COLUMN STUDIES WITH DRIED PLANTS

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4.5 COLUMN STUDIES WITH DRIED PLANTS

Aquatic plants have several uses such as feed for poultry, fish and livestock, compost, mulching, biogas generation. An attempt has been done to utilise these plants after drying for remediation/ biosorption of metals from spiked metal solutions. An advantage of using dead biomass is the easier and non-destructive recovery of adsorbed metals, which is of economic significance in the recovery of valuable metals from wastewaters.

Column studies were conducted to evaluate the biosorption of zinc, copper and manganese using dried aquatic flora including *Salvinia*, *Lemna*, *Pistia* and *Azolla*. All the four plants are ubiquitous, easily available and classified as weeds. 20 mg/l of individual metal solutions were passed through the column for 2 hours and the eluted samples were collected at 15 minute intervals and analysed for metal removal.

In the studies conducted, it was observed that in the initial stages adsorption of metal was very fast. In the first 30 minutes, more than 90 percent removal was observed in manganese, copper and zinc. Upto 80 percent removal was noticed in 60 minutes. Then the removal efficiency decreased with time.

Manganese

In the column studies with *Lemna*, maximum removal occurred within 30 minutes (99.9 percent) and then decreased to 81.3 percent in 60 minutes. The removal capacity decreased to 45.75 percent in 120 minutes. When *Azolla* was used as biosorbent, 99 percent reduction was obtained in the first 30 minutes and it decreased to 87 percent in 60 minutes. After 120 minutes, the percentage removal of manganese was 29 percent.

Salvinia showed 90 percent reduction in the first 30 minutes and decreased to 54 percent in 60 minutes. After 120 minutes, 38 percent reduction was observed. *Hydrilla* had removal efficiency of 99 percent in the first 15 minutes, 98 percent at 30 minutes, 90 percent at 60 minutes and 13.35 percent at 120 minutes. Table 58 and Fig.109 represents the results obtained for manganese removal.

Zinc

In the studies conducted for removal of zinc, 99 percent reduction was obtained within 15 minutes for all the dried plants used. *Hydrilla* showed excellent removal of zinc upto 105 minutes (99.9 percent) and at 120 minutes 95.5 percent removal was obtained. *Azolla* had 99.9 removal of zinc upto 30 minutes, 94 percent at 60 minutes and then a decreasing trend was observed with 78 percent removal at 120 minutes.

Salvinia showed uptake upto 99 percent within 30 minutes and 50 percent at 60 minutes. 23.2 percent removal was obtained at 90 minutes after which there was no sorption of zinc. *Lemna* had removal upto 89 percent in 30 minutes and then its efficiency reduced to 12.5 percent in 60 minutes. After 90 minutes, there was no sorption of zinc. Table 59 and Fig. 110 represents the results obtained for zinc removal.

Copper

In the biosorption studies for removal of copper using dried plants, considerable removal was obtained for all the plants. *Azolla* showed very high sorption capacity for copper consistently in the range of 99 percent for a time period of 120 minutes. *Hydrilla* also had good removal ability, with 99.77 percent

Table 58 Manganese Removal by Dried Plant Biosorbents

Time (in min.)	Initial Concentration	Lemna		Azolla		Salvinia		Hydrilla	
		Final Conc.	% Reduction	Final Conc.	% Reduction	Final Conc.	% Reduction	Final Conc.	% Reduction
15	20	ND	99.90	ND	99.9	0.18	99.10	0.12	99.40
30	20	0.15	99.20	0.15	99.2	1.98	90.10	0.30	98.50
45	20	1.42	92.90	0.95	95.25	5.75	71.25	1.57	92.15
60	20	3.74	81.30	2.54	87.30	9.15	54.25	1.93	90.35
75	20	4.22	78.90	8.43	57.85	9.55	52.25	4.32	78.40
90	20	4.86	75.70	11.20	44.00	9.75	51.25	7.50	62.50
105	20	6.50	67.50	12.60	37.00	12.05	39.75	9.00	55.00
120	20	10.85	45.75	14.20	29.00	12.35	38.25	13.35	33.25

Table 59 Zinc Removal by Dried Plant Biosorbents

Time (in min.)	Initial Concentration	Lemna		Azolla		Salvinia		Hydrilla	
		Final Conc.	% Reduction	Final Conc.	% Reduction	Final Conc.	% Reduction	Final Conc.	% Reduction
15	20	0.65	96.75	ND	99.00	ND	99.00	ND	99.0
30	20	2.20	89.00	ND	99.00	ND	99.00	ND	99.0
45	20	15.25	23.75	0.65	96.75	7.75	61.25	ND	99.0
60	20	17.50	12.50	1.20	94.00	10.00	50.00	ND	99.0
75	20	19.24	3.80	1.80	91.00	12.75	36.25	ND	99.0
90	20	20.00	0	2.55	87.25	15.36	23.20	ND	99.0
105	20	20.00	0	2.65	86.75	20.00	0	ND	99.0
120	20	20	0	4.25	78.75	20	0	0.9	95.5

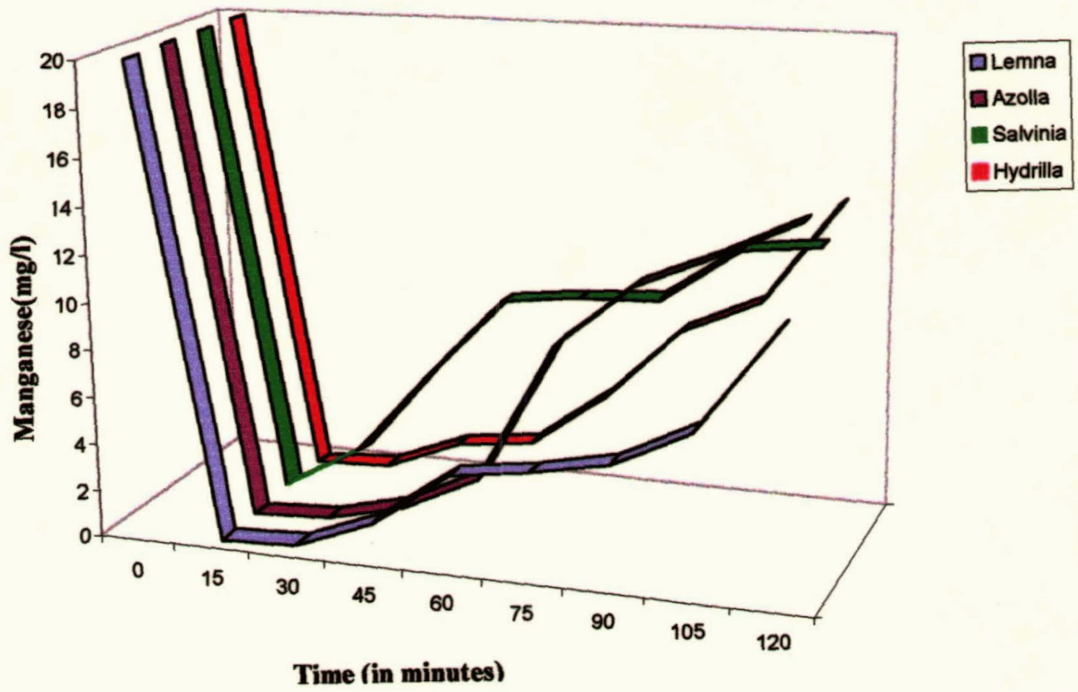


Fig. 109 Column studies for Manganese Removal

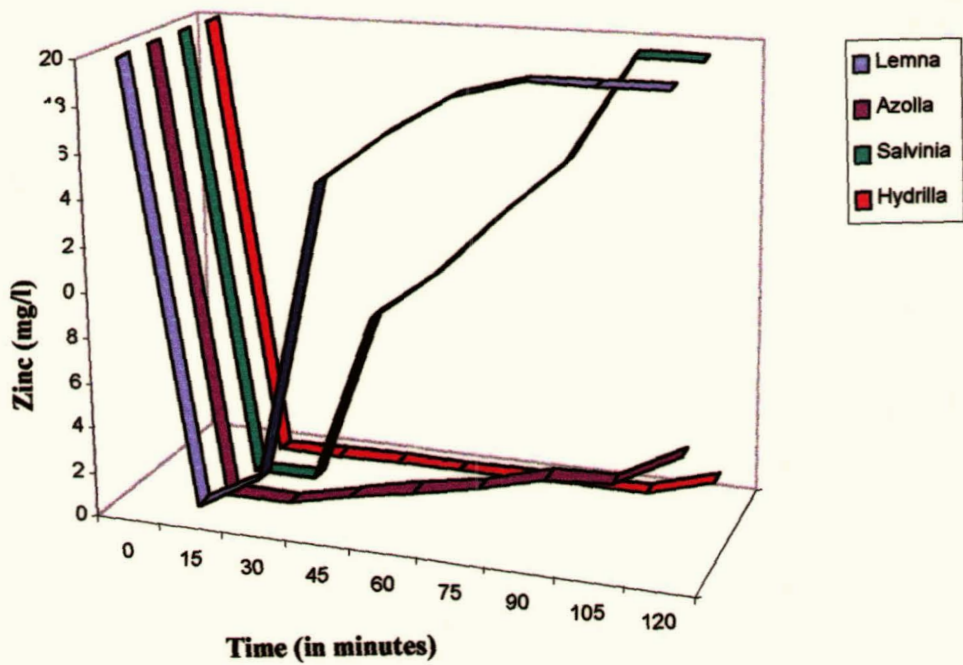


Fig. 110 Column studies for Zinc Removal

removal after 15 minutes. The removal efficiency increased with time and reached a maximum of 99.96 percent after 105 minutes. After 120 minutes, the percentage removal of copper was 94.73 percent.

Salvinia had removal efficiency in the range of 99 percent upto 75 minutes. Then the sorption of copper decreased slightly to 97 percent at 90 minutes. After 120 minutes, 83 percent removal was obtained. *Lemna* had copper removal capacity of 99 percent upto 45 minutes after which the efficiency was 98.96, 96.06 and 94.86 percent for 75, 90 and 105 minutes respectively. 83.85 percent reduction was observed after 120 minutes. Table 60 and Fig. 111 represents the results obtained for copper removal.

There are many functional groups available for metal binding in plant cell walls including carboxyl, hydroxyl, phosphoryl and sufhydril groups (Schneegurt *et al.* 2001). According to Wase *et al.* (1997) metal binding to the biomass is in essence an ion exchange mechanism, which involves electrostatic interaction between the negatively charged groups in the cell walls and metallic cations. Metal adsorption may be characterised as a passive process because the plant tissues are inactivated.

Table 60 Copper Removal by Dried Plant Biosorbents

Time (in min.)	Initial Concentration	Lemna		Azolla		Salvinia		Hydrilla	
		Final Conc.	% Reduction	Final Conc.	% Reduction	Final Conc.	% Reduction	Final Conc.	% Reduction
15	20	0.058	99.71	0.020	99.9	0.005	99.97	0.045	99.77
30	20	0.058	99.71	0.035	99.82	0.005	99.97	0.023	99.88
45	20	0.043	99.78	0.020	99.90	0.004	99.98	0.015	99.92
60	20	0.207	98.96	0.045	99.77	0.057	99.71	0.007	99.96
75	20	0.787	96.06	0.011	99.94	0.074	99.63	0.011	99.94
90	20	1.028	94.86	0.011	99.94	0.428	97.86	0.007	99.96
105	20	3.186	84.07	0.148	99.26	0.890	95.55	0.007	99.96
120	20	3.230	83.85	0.014	99.93	3.379	83.10	1.054	94.73

5. 0 SUMMARY AND CONCLUSIONS

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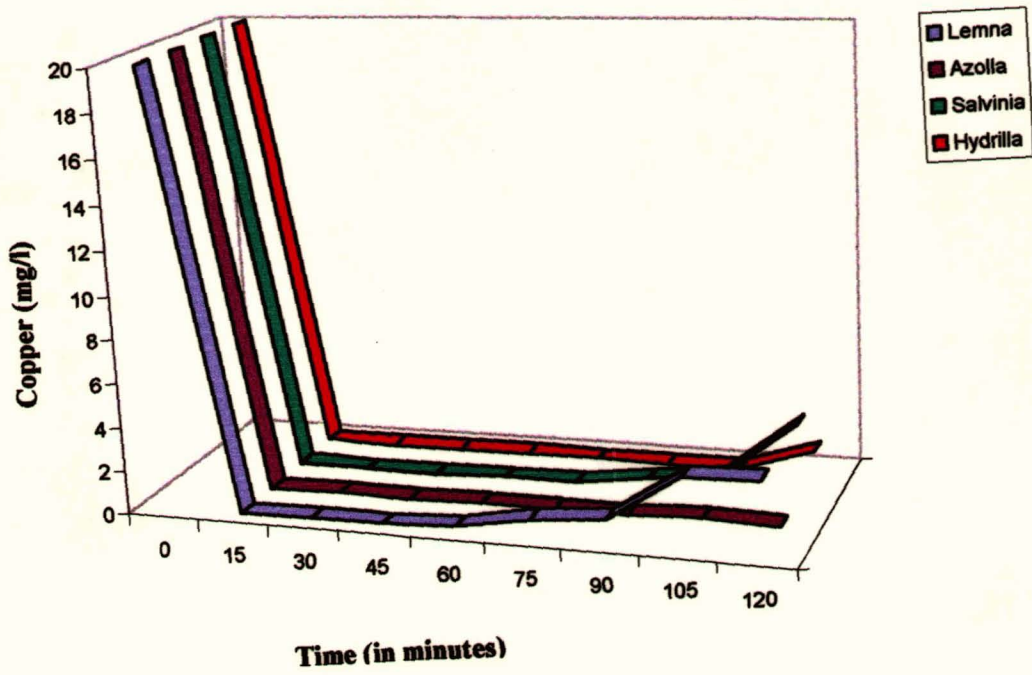


Fig. 111 Column studies for Copper Removal

SUMMARY AND CONCLUSIONS

Shiny K J “Use of aquatic organisms for water treatment” Thesis. Department of Life Science, University of Calicut, 2003

5. 0 SUMMARY AND CONCLUSIONS

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5. 0 SUMMARY AND CONCLUSIONS

Water pollution created by large scale disposal of wastewater into various water resources is considered as one of the most serious problems faced by man today. Explosive growth in human population, reckless use of natural resources, rapid industrialisation and urbanisation has resulted in generation of huge amount of wastewater. Wastes range from sewage containing organic and mineral matter to highly toxic kind containing pesticides, heavy metals and other substances. Safe collection, treatment and disposal of wastewater needs establishment cost, skilled manpower for regular maintenance, expenditure on power and other materials. In the absence of suitable technology and facilities for treatment they are inevitably discharged into nearest water body. The effect is depletion of oxygen in the ecosystem, consequent death of biota and degradation in water quality for domestic, agricultural, industrial and recreational use. The ultimate victim is man who suffers in many ways - epidemics of water borne diseases, accumulation of heavy metals in the body, loss of fishery resources and aesthetic quality of water being few of them.

Wastewater treatment basically involves the reduction of BOD, removal of nutrients which cause eutrophication and elimination of pathogens. Conventional wastewater treatment methods use chemicals to achieve these ends, with hazardous after effects on the ecosystem. Nature has the capability to ameliorate pollutants which it receives upto a certain extent. Taking cue from this, ecological systems for wastewater treatment can be opted, especially by developing countries. Natural treatment systems for upgrading quality of wastewater utilise

several species of plants, invertebrate zooplankton and fishes in monoculture and polyculture operations.

The bioremediation capacity of natural ecosystems is known, but there is no knowledge about the capability of individual animal and plant species to improve the quality of wastewaters. In this study, experiments were conducted to evaluate this ability of several locally available aquatic flora and fauna.

The aquatic fauna for experimental studies were selected based on certain criteria such as availability of species, resistance to handling and transport, ability to survive in wastewaters, species which can be cultured on a large scale, species with filter-feeding/omnivorous/ detritivorous habit. The selected species of aquatic fauna included *Paramecium caudatum* Ehrenberg, *Daphnia magna* Straus among invertebrates. Fishes selected for the experiments were *Cyprinus carpio* L., *Tilapia mossambica* Peters, *Catla catla* Hamilton, *Cirrhinus mrigala* Hamilton, *Labeo rohita* Hamilton, and *Lebistis reticulata* Peters.

Aquatic flora were selected based on rapid growth rate, hardiness, ease of harvest, useful products on harvesting, high mineral absorption ability, low water content, non toxicity to animals. The aquatic flora selected as test species for experiments in this study include the ferns *Azolla rubra* R.Br. and *Salvinia molesta* Mitchell; the duckweeds - *Lemna perpusilla* Torrey, *Spirodela polyrrhiza* (L.) Schleid and *Wolffia globosa* (Roxb.) Hartog and Plas. as well as *Pistia stratiotes* L., *Hydrilla verticillata* (L.f.) Royle, *Ceratophyllum demersum* L. and *Lagenandra toxicaria* Dalz.

Aliquots of the wastewater sample were introduced into dechlorinated tap water to prepare experimental wastewater for treatment with aquatic flora and fauna. 5, 10, 15 and 20 ml of wastewater per litre of water was used to prepare experimental wastewater for the invertebrates, *Paramecium* and *Daphnia*. 10, 20, 30, 40 and 50 ml of wastewater per litre of water was used for fishes. 50, 100, 150, 200 and 250 ml wastewater/l water was used for macrophytes. The experiments were carried out with 2 litre of experimental wastewater. *Paramecium* was introduced at 20 numbers/ml and *Daphnia* was introduced at 3 different numbers -10, 20 and 30/100 ml of experimental wastewater. Fishes were experimented with two varying biomass 3g/l and 4g/l in each wastewater experimental set. 2 gm/l of *Azolla*, *Lemna* and *Spirodela* were taken while 5 gm/l of *Pistia*, *Salvinia*, *Ceratophyllum* and *Hydrilla* were used for the experiments. Controls were maintained for each concentration of experimental wastewater to determine whether the treatment effects due to presence of the test species was significant. The physicochemical and bacteriological characteristics of experimental and control sets were analysed at pre experimental and post experimental stage.

Locally available macrophytes were screened for their ability to remove selected metals, copper, chromium, iron, zinc and manganese from solutions. The aquatic flora used in this study include *Azolla rubra* R.Br., *Salvinia molesta* Mitchell, *Lemna perpusilla* Torrey, *Spirodela polyrrhiza* (L.) Schleid, *Wolffia globosa* (Roxb.) Hartog and Plas., *Pistia stratiotes* L., *Hydrilla verticillata* (L.f.) Royle and *Ceratophyllum demersum* L. Healthy plants were selected, washed

thoroughly, blotted, weighed and placed in individual metal solutions (0.25-10 mg/l) of copper, chromium, manganese, zinc and iron for a period of seven days.

The plants after use in nutrient removal from wastewater have several alternate uses such as manure, compost, mulching and biogas. In this study the plants after harvest from wastewater treatment was dried and column studies were carried out using *Hydrilla*, *Azolla*, *Salvinia* and *Lemna*, to test its ability to remove metals from solutions. Zinc, manganese and copper (20 mg/l) were passed through the column at a flow rate of 5 ml/min and eluted samples collected at intervals of 15 minutes were analysed for metal removal by the biosorbent.

In this study the potential of two invertebrates-*Paramecium caudatum* and *Daphnia magna* as well as six fishes, *Tilapia mossambica*, *Cyprinus carpio*, *Catla catla*, *Labeo rohita*, *Cirrhinus mrigala* and *Lebistes reticulatus* for the treatment of experimental wastewaters was determined under laboratory conditions for a treatment period of seven days. It was observed that invertebrates were more efficient in the removal of coliform bacteria than fishes.

Among the invertebrates, *Daphnia magna* was found to be more efficient than *Paramecium caudatum* in the reduction in coliform count (maximum of 98.25 percent compared to 61.66 percent in *Paramecium*); BOD (54.53 compared to 24.07 percent in *Paramecium*); COD (52.7 percent compared to 36 percent in *Paramecium*) and TSS (94 percent compared to 88 percent in *Paramecium*).

Daphnia are filter feeders, filtering setae of the third and fourth pair of appendages retains food particles as small as 0.5 μ m. The efficiency of the filter allows the uptake of algae, bacteria, detritus and colloidal particles, small particles of food are collected and concentrated in the food groove and then ingested.

Daphnia feed intensively on the microbes and other suspended colloidal particles. However, they can regulate their feeding rate by rejecting the excess food from the food groove in the form of a bolus, which settles contributing to rapid flocculation. In wastewater treatment systems, this may be a very important process in removing suspended organic and inorganic matter and processing the wastes into settleable forms. *Daphnia* species can handle 5-30 ml per day, which implies that if present in reasonably high numbers may process substantial proportions of their habitat everyday.

The role of *Paramecium* in reduction of various water quality parameters such as BOD, COD, TSS and coliforms could be attributed to its feeding habit. *Paramecium* is a heterotrophic filter-feeding protozoan. It feeds chiefly on bacteria, organic particles and detritus, which are swept down the peristomial groove to the cytostome by the action of cilia. *Paramecium caudatum* secretes a soluble polysaccharide (a polymer of glucose and arabinose) into the medium, which changes the surface charge of the suspended colloidal particles present. Particles ingested during cyclosis are glued together by a mucin. The soluble organic compounds and colloidal materials present in wastewater are used by the protozoan for its nutrition. The reductions in TSS and other parameters could be attributed to this. Protozoa are known to feed on bacteria, including pathogenic strains causing diphtheria, cholera, typhus and streptococcal infections as well as faecal bacteria such as *E. coli*.

Among fishes, BOD reductions were maximum in *Tilapia* (59.1 percent) followed by *Cyprinus* (61.48 percent), *Catla* (48.5 percent), Mrigal (43 percent), Rohu (39.2 percent) and *Lebistes* (38.5 percent). Suspended solids removal was

most efficient in *Tilapia* (91.3 percent), and *Cyprinus* (83 percent). Among other fishes suspended solids reduction was 69.9 percent in Mrigal, 50.3 percent in *Catla*, 48.3 percent in Rohu and 39.2 percent in *Lebistes*.

COD reduction was highest in *Tilapia* (57.2 percent), followed by *Cyprinus* (51.6 percent), Mrigal (49.7 percent), Rohu (48.3 percent), *Catla* (42.3 percent) and *Lebistes* (36.4 percent).

Ability to reduce coliform counts from the experimental wastewater was observed in the order of *Tilapia* (82.08 percent), *Catla* (79.06 percent), *Cyprinus* (74.41 percent), Mrigal (64.06 percent), Rohu (53.48 percent) and *Lebistes* (48.4 percent).

Among the fishes studied, ability to improve water quality in decreasing order was *Tilapia*, *Cyprinus*, *Catla*, Mrigal, Rohu and *Lebistes*. When the two biomass 3g/l and 4g/l were considered, 4 g/l fish gave better results. *Tilapia* was more efficient in the removal of TSS, coliforms, BOD and COD values, when compared to other fishes.

Certain fishes and some fishes at its juvenile stage are adapted to remove even small phytoplankton (less than 20 μm). Mucous secretion from the gill surface and other specialised structures appears to entrap food particles and the gill rakers prevent the resultant slime from escaping in the outward current. Direct consumption of materials could be possible at the fairly diluted water, where concentration of wastes does not interfere with fish life.

The anatomy of gills aid in filtering water effectively, more apparently in filter feeding fishes. The gills bear gill lamellae and gill rakers, of which the latter project into the pharyngeal cavity and are arranged in 2-3 rows. The individual

filaments of gill rakers are situated very close in filter feeders and herbivores like *Labeo rohita*, *Cirrhinus mrigala* and *Tilapia* and can remove small sized particles from water. The filtering efficiency increases considerably from carnivorous to herbivorous fishes and this could result in improved water quality.

The mechanisms for contaminant removal in aquatic macrophyte systems may be complex involving physiological characteristics of the plants, biological and physicochemical reactions in the aquatic environment. Floating aquatic plants also have the capability to assimilate large quantities of elements, some of which are essential for plant growth, thereby improving the water quality. In submerged plants absorption is also facilitated by the permeability of the unthickened cellulose walls and often absence of cuticle.

Macrophytes aid in wastewater treatment by facilitating physical sedimentation and bacterial metabolic activity. The roots and stems provide surface for bacterial growth and are media for filtration and adsorption of solids. The stem and leaves prevent growth of suspended algae, reduce the effect of wind on water and transfer oxygen from leaves to root tips. Plants remove nutrients such as nitrates and phosphate by directly assimilating them into their tissue and on harvest remove them permanently from the water body.

Organic carbon, typically measured as BOD₅ is utilized by bacteria inhabiting microenvironments in the plant root zone and the water column as an energy source and for cell synthesis. Presence of aquatic plants thus helps in reducing the BOD₅. An anatomical adaptation of aquatic plants is the development of aerenchyma cell structure, which facilitates the exchange of

oxygen from aerial tissue into the root zone and be utilized by the aerobic bacteria for oxidation of organic carbon.

Suspended and colloidal solids are removed as a result of collisions (inertial and Brownian) with an adsorption to plant parts such as stem and roots. Particulates are filtered mechanically as water passes through the roots. Nitrogen is removed by plant uptake and subsequent harvest, volatilisation of ammonia, adsorption, bacterial nitrification and denitrification.

Phosphorous removal mechanisms in aquatic systems are plant uptake, chemical adsorption and precipitation reactions. Aquatic plants supplied with sewage effluents tend to show increased growth and usually have increased tissue phosphorous concentration. The major pathway for removal of sulphates is plant uptake. Sulphate is quite rapidly taken up by roots, translocated to leaf chloroplast where it is reduced and thus incorporated into organic compounds or accumulated in the vacuoles. Plant uptake of calcium and magnesium reduces its concentration, as well as the total hardness of water in aquatic systems.

Filtration through the root substrate and attached biofilm, sedimentation, aggregation, oxidation, exposure to biocides secreted by plants, adsorption to organic matter, ingestion by ciliates are causes for coliform removal. Aquatic macrophytes such as Water hyacinth, *Pistia*, *Lagenandra* and *Ceratophyllum* have antimicrobial properties.

Comparison of nine aquatic flora in improving the quality of experimental wastewaters indicated that all the plants had natural ability to uptake nutrients; reduce BOD, COD, TSS and coliform count. However, some macrophytes

showed excellent performance with respect to selected parameters. The removal efficiency increased with increasing nutrient concentration in the wastewater.

Total Suspended Solids removal was greatest in *Pistia* (66.4%), followed by *Lagenandra* (65.1%), *Hydrilla* (63.4 %), *Lemna* (62%), *Ceratophyllum* (57.4 %), *Salvinia* (42.5 %), *Azolla* (36.3%), *Spirodela* (32.6%) and *Wolffia* (20.3%).

BOD reductions were maximum in *Lemna* (82.6 %). Significant levels of BOD removal were noticed in *Ceratophyllum* (80.7 %), *Lagenandra* (78.5%), *Pistia* (75%), *Salvinia* (67.8%), *Azolla* (64%), *Hydrilla* (53.6%), *Spirodela* (46.1%) and *Wolffia* (10.2%).

COD reductions by macrophytes was in the order of 46% in *Lemna*, 46% in *Lagenandra*, 42% in *Pistia*, 38% in *Ceratophyllum*, 35% in *Hydrilla*, 26.4% in *Salvinia*, 10.6% in *Spirodela*, 9.8 % in *Azolla* and 5.2 % in *Wolffia*.

Nitrate removal was best in *Lagenandra* (84.5%) where as reductions recorded for other plants are-*Lemna* (81.3 %) *Pistia* (77.2%), *Ceratophyllum* (76.3%), *Spirodela* (72.4%), *Hydrilla* (65.2%), *Salvinia* (62.3%), *Wolffia* (57.7%) and *Azolla* (40.2%).

Phosphate removal potential of experimented macrophytes are *Lagenandra* (70.2%), *Lemna* (69%), *Ceratophyllum* (68.3%), *Spirodela* (67%), *Pistia* (55.4%), *Salvinia* (52.9%), *Hydrilla* (52.3%), *Azolla* (48.6%) and *Wolffia* (28.03%). Sulphate removal efficiencies of the macrophytes experimented were *Lagenandra* (72.5%), *Pistia* (64.3%), *Ceratophyllum* (64.2%), *Lemna* (62.7%), *Salvinia* (60.2%), *Azolla* (53.2 %), *Hydrilla* (51.4%), *Spirodela* (45%) and *Wolffia* (35.2 %).

Excellent coliforms reduction was obtained in experiments with *Pistia* (99.75%), *Lagenandra* (94.4%) and *Lemna* (93%). Percentage reductions for other plants are *Spirodela* (88%), *Salvinia* (84.6%), *Ceratophyllum* (66.7%), *Hydrilla* (53.3%), *Azolla* (48.6%) and *Wolffia* (45.8%).

Increase in dissolved oxygen levels was greatest in *Ceratophyllum* treated experimental wastewater, followed by *Hydrilla*, *Lagenandra* and *Pistia*.

Comparison of the aquatic macrophytes indicates that *Lagenandra*, *Pistia*, *Ceratophyllum* and *Lemna* have good potential for wastewater treatment, followed by *Spirodela*, *Salvinia*, *Hydrilla*, *Azolla* and *Wolffia*.

The data generated from the experiments were analysed for all parameters for significance between dilutions, stages of observation and presence or absence of test species using Analysis of Variance. Significant differences ($p < 0.05$) were obtained for most parameters between dilutions, stages of observation and also between experiments and controls (without test species). Two-way interactions were also studied and significant differences determined. The functional relation for selected water quality parameters such as BOD, COD, TSS, coliforms, nitrate and phosphate were assessed by fitting appropriate polynomial regression equations with dilution as independent variable. A relationship between wastewater concentration and rate of removal of pollutants for a given biomass of test species worked out contributes to the standardisation of biotreatment and serves the field applications of wastewater treatment using aquatic organisms.

In the studies for phytoremediation of metals, it was observed that the efficiency of metal uptake by *Azolla* was copper > manganese > chromium > zinc > iron. *Lemna* removed copper > manganese > zinc > chromium > iron, while

Spirodela showed uptake in the order zinc >manganese. In *Wolffia* uptake of metals was zinc >manganese >iron. *Pistia* showed uptake of copper > manganese >chromium >zinc > iron. In *Hydrilla* and *Ceratophyllum*, removal was manganese >zinc >chromium >copper >iron.

Results of column studies for biosorption of metals using *Hydrilla*, *Azolla*, *Salvinia* and *Lemna*, revealed that the method holds good potential for metal removal from solutions. Removal was very rapid in the initial stages. Above 90 percent reduction in metal concentration was recorded within 30 minutes. The removal efficiency decreased with time. Zinc removal by dried plants was in the order *Hydrilla* > *Azolla* > *Salvinia* > *Lemna*. Manganese removal was *Lemna* >*Salvinia* > *Hydrilla* > *Azolla* and for copper it was *Azolla* > *Hydrilla* >*Lemna* >*Salvinia*.

The studies conducted using aquatic flora and fauna indicate their excellent potential for use in wastewater treatment. An aquatic organisms based wastewater treatment system is very promising and an ecologically sound approach towards reducing pollution levels from wastewater. The employment of various aquatic organisms in an integrated system may improve the treatment process.

The ability of macrophytes as well as dried plants to remove metals from solutions can be utilized effectively to ameliorate metal pollution. Living plants can be used for metal removal in areas with low metal concentration while dried plants can be used in column applications for high metal concentrations as in industrial applications.

These systems are low cost, low energy alternatives or supplements to conventional treatment systems. Such wastewater treatment systems are inexpensive to build and maintain and if the gradient of the location permits delivery of wastewater, has virtually no energy costs other than harvesting the aquatic flora and fauna. This can be substantiated with the alternate uses of harvested flora and fauna, such as biogas, compost, mulching and animal feed.

Areas with easy availability of land and plenty of sunshine can think in terms of developing an appropriate treatment systems with components from commercially available technology but relying heavily on the locally available aquatic biota to achieve pollution control and at the same time recycle wastewater. Sustainable technologies for wastewater treatment within the economic capabilities of developing countries needs research, and wastewater treatment using natural systems is a significant step towards this direction.

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7. 0 PUBLICATIONS

301A

7.0 PUBLICATIONS

1. Shiny, K.J., Nirmala, E., Jalaja, T.K. and Remani, K.N., 2000. Improvement of Water Quality using Cyprinid Fishes. Paper presented at the 10th Swadeshi Science Congress, 7-9 Nov., 2000, Cochin.
2. Shiny, K. J., Nirmala, E., Jalaja, T. K. and Remani, K. N., 2000. Development of Biological Models for Water Purification using Aquatic Fauna. Paper presented at the National Symposium on Problems and Prospects of Environment in the New Millennium, 14-16 Dec. 2000, Mangalore.
3. Remani, K. N., Shiny, K.J. and Nirmala E., 2001. Biological Water Treatment using Aquatic Organisms, pp 87-93. In: Trivedy, R.K. and Kaul, S. (Ed.), Low Cost Wastewater Treatment Technologies. ABD Publishers, Jaipur.
4. Shiny, K.J., Nirmala, E., Jalaja, T.K. and Remani, K.N., 2003. Development of Biological Models for Water Purification using Aquatic Fauna. pp 237-243. In: Madhyastha, M. N., Sridhar, K. R. and Lakshmi, A. (Ed.), Prospects and Problems of Environment Across the Millennium, Daya Publishing House, Delhi.
5. Shiny, K. J., Remani, K. N., Nirmala, E. and Sasidharan, V.K. Phytoremediation of Metals by *Azolla Rubra* (Communicated).
6. Shiny, K. J., Remani, K. N., Jalaja, T. K. and Sasidharan, V. K. Removal of Chromium by two Aquatic Pteridophytes (Communicated).
7. Shiny, K. J., Remani, K. N., Nirmala, E., Jalaja, T. K and Sasidharan, V. K. Biotreatment of Wastewater using Aquatic Invertebrates, *Daphnia Magna* and *Paramecium Caudatum*. (Communicated).

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Abstracts

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SESSION - 6

Environmental Sciences

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302B

IMPROVEMENT OF WATER QUALITY USING CYPRINID FISHES

K. J. Shiny, E. Nirmala, T. K. Jalaja and K. N. Remani
Centre for Water Resources Development and Management
KOZHIKODE - 673 571

The basic aim of wastewater treatment is to reduce the biochemical oxygen demand loading, remove nutrients and eliminate pathogens harmful to living organisms, which alter the ecological stability of the receiving water bodies. Wastewater treatment involves the reduction of nutrients like nitrogen and phosphorus to prevent eutrophication. Traditional water treatment systems use chemicals to achieve these ends, with hazardous effects on living beings. Aquatic treatment systems, using protozoans, crustaceans and fish convert nutrients and organic matter in wastewater to useful biomass, with added advantage of improved water quality.

*The main objective of the present study is to investigate the possibility of improving the quality of sewage contaminated water by the omnivorous filter feeding fish, *Cyprinus carpio* (common carp). Laboratory scale studies were carried out and compared for various physico-chemical and biological parameters for water quality improvement with controls.*

Results show marked reduction in biochemical oxygen demand and coliform bacteria. The treatment responses may be attributed to the uptake of organic matter and bacteria by the fishes.

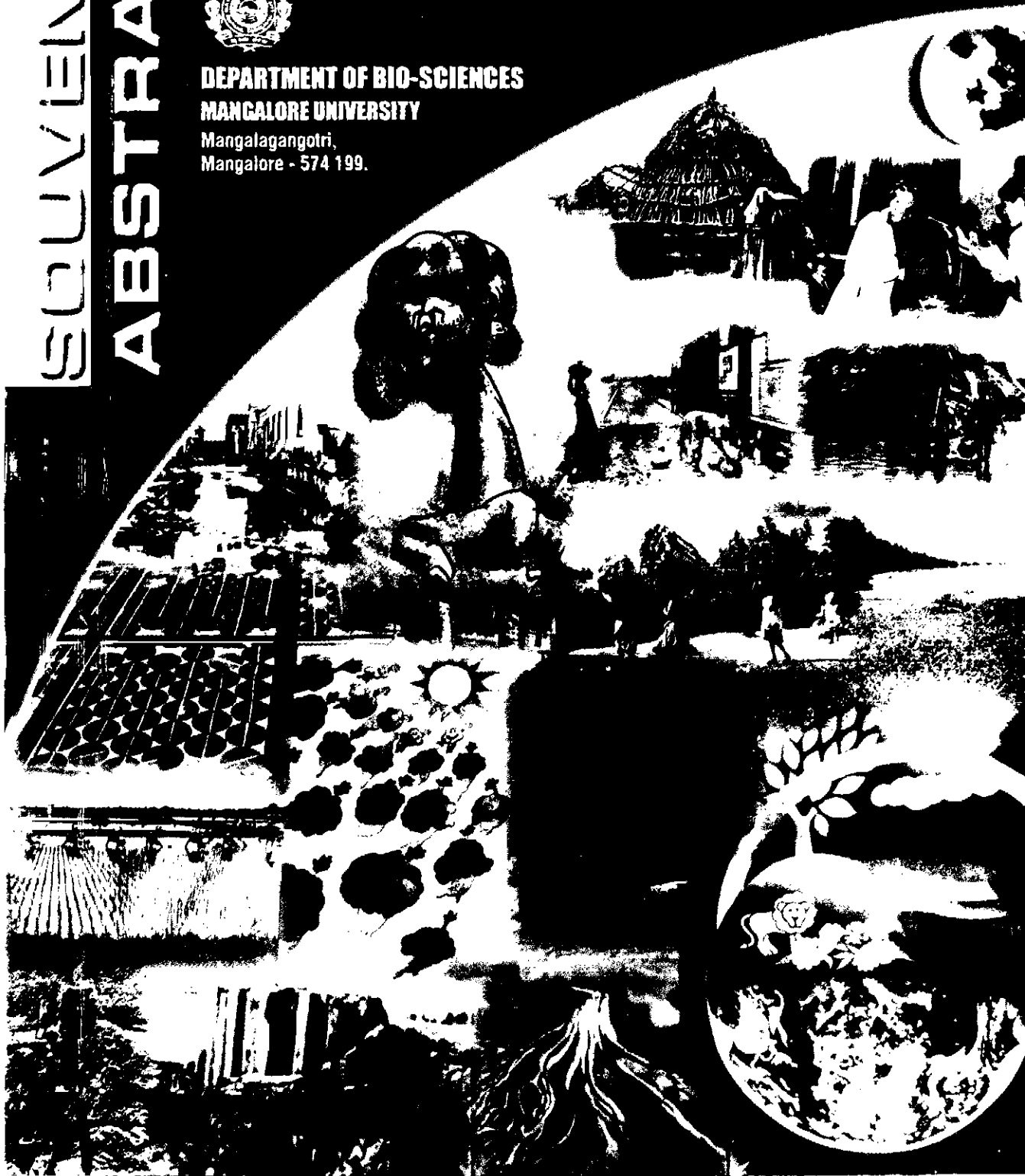


**SOUVENIR &
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BT 07

DEVELOPMENT OF BIOLOGICAL MODELS FOR WATER PURIFICATION USING AQUATIC FAUNA

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Development of low technology approaches to the recycling of wastewaters is the need of the present day taking into account the global increase in inevitable wastewater produced annually. The existing treatment methods require expensive, complicated facilities and involve several after effects of chemical treatment. It is therefore useful to develop alternative ecologically sound methods of water treatment. Bioremediation which takes cue from the food chains in the ecosystem can be used to remove pollutants from the aquatic environment. Animals and plants can be made use of to remove organic matter from wastewaters. Efforts by many workers in the use of aquatic invertebrates and fishes for water treatment has proven to be successful to a large extent. Stabilization of polluted waters occurs by the conversion of unstable organic matter. The subject of utilizing fish to cleanse wastewaters is relatively new, since the objective of most fish culturists is to produce food. Previous studies are qualitative, emphasizing on the water quality parameters. A study has not been conducted on the relation between the biomass or number of organisms and the rate of pollutant removal. In the present study, an effort has been made to find a relationship between the biomass of test species and reduction in water pollutants and to predict removal rates from wastewaters. Two aquatic filter feeders were chosen, considering the physiological structures which aid in the removal of suspended matter from water. A crustacean, *Daphnia magna* (Family Daphnidae) and a fish *Cyprinus carpio* (Family Cyprinidae) were chosen. Treatment studies were conducted varying the biomass/number of organisms in each set. Results show about 98% reduction in coliform bacteria and 48% reduction in BOD by *Daphnia magna*. Regression models were drawn based on the results. These models could be used to predict the pollution reduction ability of a given wastewater using particular biomass of the organisms.



LOW COST WASTEWATER TREATMENT TECHNOLOGIES

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Biological Water Treatment using Aquatic Organisms

Introduction

The growing global awareness on the impacts of water pollution has led to greater public concern and progressive enforcement of environmental legislations. The existing water treatment methods, mainly involves chemical methods where health problems are a matter of concern. The emergence of biological systems for wastewater treatment has received growing attention since they represent an alternative cost-effective and environmentally sound approach for the removal of pollutants.

S
Nature has evolved a number of systems to compensate for pollution generated as a result of normal events. The functioning systems in the environment offer a variety of ways in which contaminants can be altered and transported. The best way of fighting pollution is by bio-remediation using aquatic organisms. The capacity of ecosystems that are dominated by aquatic filter-feeders and plants to assimilate inputs of nutrients and organic matter have resulted in the use of such systems to different types of waste water. The use of sewage in increasing productivity of fish culture is well known but using fishes for wastewater treatment is a recent concept. (Rajan and Paul, 1996)

In any water body, the zooplankton form an important group, consuming the detritus, algae, bacteria etc. and make themselves available to be eaten by higher organisms in the food chain, including fishes. Using filter feeding invertebrates as well as fishes to consume the organic matter in wastewater can result in decreased BOD (Biochemical oxygen demand) values and bacterial populations.

The filtering mechanisms of different filter feeders are responsible for their efficiency in wastewater treatment. In *Paramecium*, protrusions of the body called cilia sweep bacteria, diatoms etc. down the peristomial groove. The physiological structures of *Daphnia* which enable them to improve water quality are five pairs of thoracic legs which are well endowed with very fine hair-like projections. These appendages permit *Daphnia* to filter suspended material (algae, bacteria, protozoa, detritus etc.) and concentrate it as they move toward the mouth region. It has been found by Gellis and Clarke (1935) that *Daphnia magna* require particulate food and bacteria to reach maturity. Loeldoff (1964) evaluated the role of cladocera in a stabilisation pond system in South Africa and reported that a significant reduction in turbidity was observed at times when cladoceran populations were high. In fish, the gills bear gill lamellae and gill rakers. The gill rakers project into the pharyngeal cavity and are arranged in two to three rows. The gill rakers prevent food from escaping out through the respiratory current of water. The individual filaments of gill rakers are situated very close in filter feeders and in the case of silver carp, can remove particles as small as four microns in diameter as per Dinges (1982).

In the present study, the removal of various organic and biological pollutants from waste waters using filter feeding aquatic organisms as well as plants has been discussed. More emphasis is laid on the important water quality parameters such as biochemical oxygen demand and coliform count.

Materials and Methods

Aquatic organisms were selected based on the following criteria for wastewater treatment studies :

- (a) Availability of the species.
- (b) Species with filter feeding/omnivorous/detritivorous food habits.
- (c) Resistance to handling and transportation.
- (d) Species which can be easily cultured.

The test organisms which were selected are *Paramecium caudatum* (Protozoa), *Daphnia magna* (Daphniidae), fishes such as *Labeo rohita*

(Cyprinidae), *Cirrhinus mrigala* (Cyprinidae), *Channa marulius* (Ophiocephalidae) and *Cyprinus carpio* (Cyprinidae).

The organisms selected were cultured as per the procedures cited in APHA (1995). *Paramecium* was cultured in the hay infusion method. *Daphnia* was cultured in the manure-soil medium developed by Banta. Fish fingerlings were collected from the hatchery of Kerala State Fisheries, Kozhikode and reared in glass aquariums.

Wastewater collected from the open sewers of Kozhikode city, Kerala, was analysed for water quality parameters. The physicochemical and biological parameters studied including pH, electrical conductivity, total hardness, calcium, magnesium, nitrate nitrogen, phosphate phosphorous, sulphate, total organic carbon, dissolved oxygen, biochemical oxygen demand, total coliforms, fecal coliforms and *Escherichia coli*.

Experiments were conducted using different test organisms. Sewage was diluted to various concentrations (5, 10, 15 and 20 ml/l). The test organisms were introduced in varying numbers/biomass. *Paramecium* was introduced at the rate of 20 numbers/ml, *Daphnia* was introduced in three experiment sets at the rate of 10, 20 and 30 numbers per 100ml respectively. Fishes were taken according to their biomass as 3 and 4 grams per litre. Each of these experiments had 10 replicates. Experimental sets of *Daphnia* and *Paramecium* were treated for 7 days, while the sets of fishes were treated for 15 days. The samples were analysed before and after treatment for various water quality parameters as per the methods cited in APHA, (1985).

Results and Discussion

The results of the experiments conducted indicates considerable reduction in water quality parameters. Significant reduction in biochemical oxygen demand and coliforms were observed. The test organism used, period of treatment, biomass/number, percentage reduction in biochemical oxygen demand and coliforms are shown in table 1.

Table 1
Percentage reduction in Biochemical
Oxygen Demand and Coliforms

Test Organism	Biomass/ No.	Period days	%Reduction In BOD	%Reduction In Coliforms
Paramecium	20/ml	7	24.07	61.53
Daphnia	30/100ml	7	54.53	98.25
Mrigal	3g/1	15	37.03	54.27
Channa	4g/1	15	32.81	98.20
Rohu	4g/1	15	24.48	92.90
Cyprinus	4g/1	15	70.00	97.00

Among the experimented invertebrates the treatment set with 30 numbers per 100 ml of *Daphnia* showed maximum percentage removal in biochemical oxygen demand and coliforms (55.43 and 98.25%) respectively. *Paramecium* at the rate of 20 numbers per ml also showed considerable reduction in biochemical oxygen demand and coliforms (24.07 and 61.53% respectively).

Among fishes *Cyprinus* showed maximum percentage reduction in BOD and coliforms (70 and 97%) respectively). *Channa* at the rate of 4 gms/1 showed percentage reduction in biochemical oxygen demand and coliforms (32.81 and 98.2%) respectively. The treatment set with *Rohu* (4 gms/1) gave a reduction in BOD of 24.48 per cent and percentage reduction in coliforms of 92.9%. In experimental sets with 4 gms/1 of *Mrigal* 37.03 per cent reduction in BOD and 54.27 per cent reduction in coliforms was observed.

Percentage reduction in biochemical oxygen demand and coliforms using test organisms is shown in Figure 1

The results obtained were statistically interpreted using ANOVA (Analysis of Variance). The interpretations show that each of the treatment were significantly different with respect to biomass and rate of removal of pollutants. Maximum biomass is to be introduced into the experimental sets for optimum removal of pollutants from water. Considering biomass/number per ml as a limiting factor biological models were developed based on the above findings. The biological model evolved using the results from

PERCENTAGE REDUCTION IN BOD AND COLIFORMS

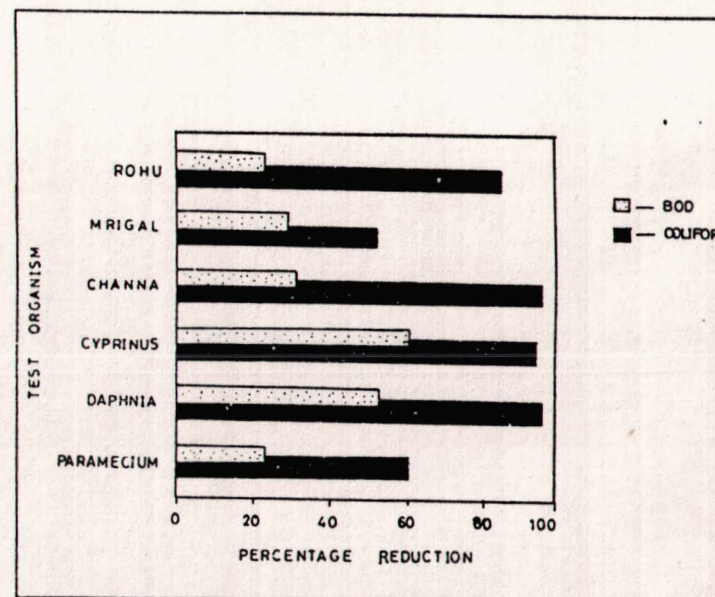


Fig. 1

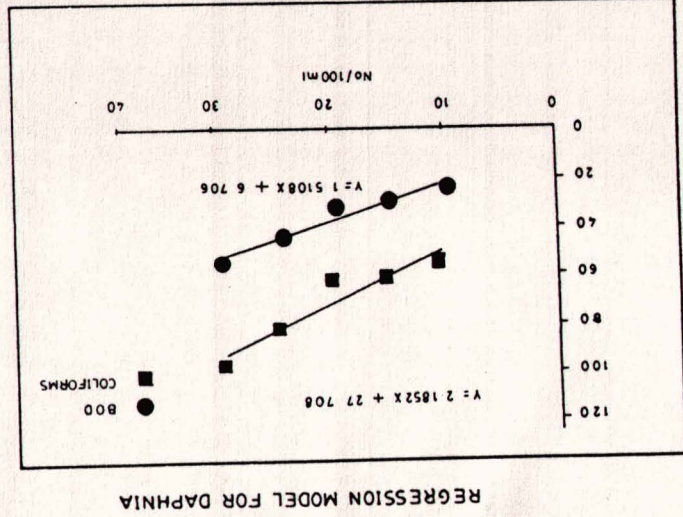
experimental set of *Daphnia* is given in Figure 2. Regression equations were obtained for BOD and coliform reduction. The regression equation for percentage reduction in coliforms for a given number of organisms is

$$y = 2.2852x + 27.708$$

The regression equation for percentage reduction in biochemical oxygen demand for a given number of organisms is

$$y = 1.510x + 6.706$$

Regression model for *Daphnia* is shown in Figure 2.



Wastewater treatment via aquatic organisms is a technically feasible method for the removal of pollutants, especially biochemical oxygen demand and coliforms. Filter feeding organisms were found to be efficient in the removal of coliforms and biochemical oxygen demand. Among the experimented invertebrates, *Daphnia* showed percentage reduction in biochemical oxygen demand and coliforms of 54.53 and 98.25 per cent respectively. Among fishes, *Cyprinus* and *Channa* (32.81 and 98.29%) were found to be efficient in the reduction BOD and coliforms. The optimum removal of pollutants has been evolved through regression modelling. The results obtained from the present study can be used to determine the number/biomass of organisms to be used for maximum percentage removal of pollutants in a large scale treatment system. The practical

Fig. 2

applications of this approach could be used for waste treatment programmes in sub-urban and rural areas which are not subject to intense competition for land use.

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**PROSPECTS AND PROBLEMS OF
ENVIRONMENT
Across the Millennium**

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Development of Biological Models for Water Purification Using Aquatic Fauna

❖ *Shiny K J, Nirmala E, Jalaja T K &
Remani K N*

Introduction

Bioremediation of pollutants is an alternative to the conventional chemical treatment methods. This low-cost technology in recycling wastewater is a boon, especially in the developing countries although they tend to be land intensive (Pescod, 1992). Aquatic treatment systems utilize several species of plants, invertebrates, zooplankton and fishes in monoculture and polyculture operations. There have been many successful efforts in the use of such methods to treat wastewater. In water body the primary consumers constitute an important segment, they feed on detritus and algae and makes themselves available to organisms at higher trophic levels. The filter-feeding invertebrates and fishes consume organic matter in wastewater results in the improvement of water quality and production of useful biomass. Among zooplanktonic Cladocerans, *Daphnia*, the filtering mechanisms enable to improve water quality. They possess five pairs of thoracic legs endowed with very fine hair-like projection help to filter and concentrate suspended material such as algae, bacteria, protozoa and detritus. A significant reduction in turbidity was observed when Cladoceran population was high in a stabilization pond system in South Africa (Loeldoff, 1964). Studies also revealed that stabilization

of polluted waters occur through the mineralization of organic matter and conversion of unstable organic matter into zooplankton (Kryutchkova, 1968). Many workers considered the mass culture of *Daphnia* for the wastewater treatments (Ehrlich, 1966; Dinges, 1972; Gram, 1973 and Froom, 1974). The role of fishes including herbivores, carnivores and omnivores in improving the quality of water has been studied extensively in stabilization ponds (Schroeder, 1975). This is possible if fish production is considered as a secondary objective. The use of biological wastes in fish culture as a measure of recycling wastes and producing protein rich food at a low-cost has been tried with success (Kaur and Dhawan, 1992). The structure of gills aid in filtering water effectively, especially in the filter-feeding fishes. The gill rakers are situated very close and remove small size particles in water.

The present study has been conducted to remove pollutants in wastewater by employing *Daphnia* and *Cyprinus* and to find a relationship between the biomass/number of organisms and reduction in water quality parameters. Regression equations have been developed to predict the reduction in biochemical oxygen demand (BOD) and coliforms in wastewater under laboratory conditions.

Materials and Methods

Zooplankter and Fish

Daphnia magna was collected from the local ponds around Kozhikode and cultured in manure-soil medium developed by Banta (APHA, 1976). Healthy fingerlings of *Cyprinus carpio*, ranging from 3.5-5 cm in length were obtained from Ullalom fish farm, Kozhikode and acclimatized to laboratory conditions in glass aquarium in dechlorinated tap water for one week. The aquaria were aerated and fishes were fed daily with commercially available fish food.

Sewage Inoculum

Wastewater used in the study was collected from the municipal drains of Kozhikode City, Kerala. The quality of wastewater was assessed according to the standard methods (APHA, 1995). Table 1 gives the characteristics of the wastewater. Experiments were conducted in ten replicates using *Daphnia* and *Cyprinus* for a treatment period of seven days.

Table 1: Characteristics of waste water used in the experiments

Parameters	Values*
Colour	Gray
Temperature	27° C
Odour	foul
pH	7.85
Electrical conductivity	315 µS
Total Hardness	236
Calcium	65
Magnesium	18
Nitrate nitrogen	2.41
Phosphate phosphorous	2.08
Sulphate	16
Suspended solids	240
Dissolved oxygen	1.2
BOD ₅ , 20° C	300
COD	390
MPN/100ml	43 × 10 ⁴

*All values except colour, temperature, odour, pH, EC and MPN are in mg/l

Experiments with *Daphnia* and Fish

The neonates of *Daphnia* were selected for experiments. Sewage was diluted to various concentrations: 0.5, 1, 1.5 and 2% with dechlorinated tap water. *Daphnia* was introduced in varying numbers: 10, 20 and 30 numbers per 100 ml. Controls were maintained for each set without *Daphnia*. Healthy fingerlings of *Cyprinus* with average length, 3.8 cm and average weight, 1.8 g were introduced in the experimental set at two varying biomass: 3 g/l and 4 g/l. Sewage was diluted with dechlorinated tap water at concentrations 1, 2, 3, 4 and 5%.

Pre- and Post-Experimental Analysis of Water Samples

The physicochemical and bacteriological parameters were assessed as per the standard methods (APHA, 1995). The water samples in each experimental set and controls were analyzed before and after the treatment period for the following water quality

parameters: pH, electrical conductivity, total hardness, calcium, magnesium, nitrate-nitrogen, phosphate-phosphorous, sulphate, dissolved oxygen, biochemical oxygen demand and bacteriological parameters such as total coliforms, fecal coliforms and *Escherichia coli*.

Statistical Analysis

The mean values of the ten experimental sets were considered for statistical analysis and to develop regression equation using SPSS software for the percentage reduction in BOD and coliforms.

Results and Discussion

Experiments with *Daphnia*

In the treatments sets using *Daphnia*, maximum reduction in BOD and coliforms was observed in sewage dilution at 0.5% with test organisms 30 per 100 ml after seven days. The maximum reduction in BOD was 54.53%, while in control it was 12.63%. The maximum reduction in coliforms was 98.25%, while in controls it was 31.18% at 0.5% dilution. Table 2 shows the percentage reduction in BOD and coliforms.

Table 2: Percentage reduction in BOD and MPN in *Daphnia* (period of treatment 7 days)

Para- meters	Number/ 100 ml	0.5%		1%		1.5%		2%	
		E	C	E	C	E	C	E	C
BOD	10	24.26	12.63	22.46	15.38	23.42	17.61	19.37	17.89
	20	32.62	12.63	30.46	15.38	29.53	17.61	23.78	17.89
	30	54.53	12.63	51.33	15.38	50.13	17.61	40.86	17.89
MPN	10	54.52	37.18	49.39	16.66	43.67	15.21	37.54	14.66
	20	61.63	37.18	52.38	16.66	43.67	15.21	49.81	14.66
	30	98.25	37.18	84.64	16.66	43.67	15.21	66.38	14.66

E-Experiment sets; C-Control sets

Experiments with *Cyprinus*

The experimental sets with *Cyprinus* at 4g/l showed maximum reduction in BOD and coliforms. The maximum reduction in BOD was seen at 1% dilution of sewage (61.48%), while in control it was only 37%. The maximum reduction of coliforms was at 1% dilution

(74.41%) with biomass 4g/l, while in control it was 46.57%. Table 3 shows the percentage reduction in BOD and coliforms at different dilutions of sewage inoculum.

Table 3: Percentage reduction in BOD and MPN in *Cyprinus* (Period of treatment 7 days)

Para- meters	Biomass	1%		2%		3%		4%		5%	
		E	C	E	C	E	C	E	C	E	C
BOD	3g/l	59.37	40.1	49.53	28.79	48.04	24.52	42.06	20	39.21	17.75
	4g/l	61.48	37.0	59.61	36.85	55.55	37.36	52.49	36.65	48.04	38.90
MPN	3g/l	71.79	46.42	65.11	46.61	67.39	44.18	67.18	39.13	62.66	32.81
	4g/l	74.41	46.57	64.06	32.81	62.66	38.66	58.06	37.11	37.5	22.5

E-Experiment sets; C-Control sets

Regression Equations

The results obtained were statistically analyzed using SPSS software to develop regression equations for maximum reduction in BOD and coliforms for a given biomass/number of organisms using data from the experimental set showing maximum reduction in the parameters.

The regression equation for reduction in BOD for a given number of *Daphnia* is:

$$y = 1.510x + 6.706$$

where, y is the reduction in BOD and x is the number of organisms.

The regression equation for reduction in coliforms for a given number of *Daphnia* is:

$$y = 2.2852x + 27.708$$

where, y is the reduction in coliforms and x is the number of organisms.

The regression equation for reduction in BOD for a given biomass of fish is:

$$y = 53.04 + 2.11x$$

where, y is the percentage reduction in BOD and x is the biomass of fish.

The regression equation for reduction in coliforms for a given biomass of fish is:

$$y = 63.93 + 2.62x$$

where, y is the reduction in coliforms and x is the biomass of fish.

Laboratory scale studies using aquatic organisms with omnivorous and herbivorous filter-feeding mechanisms were employed to improve the quality of wastewater. The Cladoceran fresh water flea, *Daphnia magna* and the Cyprinid fish, *Cyprinus carpio* were cultured in different dilutions of the sewage inoculum. Considerable reduction in the BOD and coliforms was observed. *Daphnia* was more efficient in improving in the bacteriological quality of water than *Cyprinus*. Control sets without organisms were maintained along with each experimental set in order to determine whether the removal of pollutants was due to settling of sewage or by the influence of test organisms. Purification due to settling of sewage was seen in the control sets. Higher purification in the experimental sets when compared to control could be attributed to the influence of the filter feeders. The aspect of employing fish to cleanse the wastewater is relatively new, since the objective of most fish cultures is to produce food.

Summary

Development of low-cost technology to the recycle wastewater is the need of the present day. The existing treatment methods are expensive and involve several after effects of chemical treatment. It is therefore useful to develop alternative ecologically sound methods of water treatment. Animals and plants can be used to remove organic matter from wastewater. Efforts by many workers to employ aquatic invertebrates and fishes for wastewater treatment have proven to be successful to a large scale. Stabilization of polluted waters occurs by the conversion of unstable organic matter into zooplankton biomass and mineralisation of organic matter. Regression models were drawn based on the results could be used to predict the pollution reduction ability of a given wastewater using a specific biomass of the filter feeders.

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