Utilization of aquatic macrophyte *Spirodela polyrhiza* as phytotoxicological assessment and phytoremediation tool in selected polluted wetland sites in Ernakulam district

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Certificate

This is to certify that the thesis entitled "**Utilisation of Aquatic macrophyte Spirodela polyrhiza as Phytotoxicological assessment and Phytoremediation tool in Selected polluted wetland sites in Ernakulam district**", is an authentic work carried out by Mr. Anil Loveson, under my supervision and guidance at the School of Environmental Studies, Cochin University of Science and Technology, in partial fulfillment of the requirements for the award of the degree of Doctor of Philosophy in Environmental Toxicology, under the faculty of Environmental Studies, Cochin University of Science and Technology, and that no part thereof has been presented before for the award of any other degree, diploma or associateship of any university.

Dr. Rajathy Sivalingam
Supervising Guide

Declaration

I hereby declare that the thesis entitled "**Utilisation of Aquatic macrophyte Spirodela polyrhiza as Phytotoxicological assessment and Phytoremediation tool in selected polluted wetland sites in Ernakulam district**" is a genuine record of the research work carried out by me under the supervision and guidance of Dr. Rajathy Sivalingam., Reader, School of Environmental Studies, Cochin University of Science and Technology. The work presented in this thesis has not been submitted for any other degree or diploma earlier.

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Anil Loveson

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Chapter 1

Introduction

Contents

Pollution is the changing of the natural environment, either by natural or artificial means, so that the environment becomes harmful to the living things normally found in it. Most often than not, this refers to the input of toxic chemicals into the environment as a consequence of human activities. Water pollution is a general term associated with unfavorable alterations in the ecology, resulting in deleterious effects on aquatic organisms and resources. Water pollution is the contamination of water bodies like lakes, ponds, rivers, wetlands, oceans, ground water etc. It is continuous and growing process, which manifests itself only when the outflow of effluents/insult exceeds the capacity of the receiving ecosystem of the environment to recover. The various causes of pollution are the explosive growth of population, increasing urbanization, rapid industrialization and indiscriminate use of fertilizers, chemicals and pesticides and lack of general awareness on environmental issues. Pollution results in human health hazards and destruction of various aquatic food resources.

1.1. Wetland pollution in Kerala

Cowardin *et* al. (1979) define wetlands as "the transitional lands between terrestrial and aquatic systems where the water table is usually at or near the surface, or the land is covered by shallow water". From the utilitarian point, wetlands can be defined as transitional areas between permanently flooded deepwater environments and well drained uplands that contribute a wide array of biological, social and economic benefits (Watzin and Gozzelink, 1992). Wetland systems directly and indirectly support lakhs of people, providing goods and services to them. They help check floods, prevent coastal erosion and mitigate the effects of natural disasters like cyclones and tidal waves. They store water for long periods. Their capacity during heavy rainfall to retain excess flood water that would otherwise cause flooding results in maintaining a constant flow regime downstream, preserving water quality and increasing biological productivity for both aquatic life as well as human communities of the region. Scientists often refer to wetlands as the "kidneys" of the earth. India by virtue of its extensive geographical stretch and varied terrain and climate, supports a rich diversity of inland and coastal wetlands. Kerala is well known for its wetlands. Due to lack of effluent treatment facilities and proper disposal system of wastewater, water bodies in Kerala are getting polluted day by day and causing adverse effects on soil, water bodies, agriculture, flora and fauna with toxic and persistence chemicals. Disposal of industrial effluents into fresh water bodies deteriorates water quality, which is necessary to sustain aquatic life, primary productivity and food chain (Rao *et* al., 2001). If environmental safety of such massive amounts of wastewater is assured by industry or by pollution control boards, then treated industrial wastewater may be potentially used for fish production, irrigation for

non-edible cash crop, aquaculture and for many other uses (Wong *et* al., 2001).

1.2. Biomonitoring of pollution using aquatic macrophytes

Previously environmental scientists have shown negative attitudes toward plant tests (Kenaga and Moolenaar, 1979; Bishop and Perry, 1981). Invertebrates and fish have been frequently used to determine the potential toxicity of effluents and wastewaters, with photosynthetic organisms being restricted to a few testing species. Recently aquatic vascular plants are receiving more attention for their potential use in screening, phytotoxicity studies of chemicals and as a useful bioindicators (USEPA, 1996). This awareness can be seen from the increase in journal articles, the continuation of conference sessions on plant toxicology at the Society of Environmental Toxicology and Chemistry, and especially the inauguration of the First Symposium on Use of Plants for Toxicity Assessment sponsored by the American Society for Testing and Materials in April 1989. Algae and aquatic plants play a key role in aquatic ecosystems as they are at the base of food webs. Also, they are a food resource and provide oxygen and shelter for many aquatic organisms. They also contribute to the stabilisation of sediments and bioconcentration of compounds (Gobas *et* al., 1991) and are used as bioremediatives (Salt *et* al., 1995). In general, phytotoxicity tests are simple, sensitive, and cost- effective. They can be used for toxicity testing of organic and inorganic pollutants and are particularly useful for monitoring heavy metal pollution. Heavy metals have been used extensively, and many of the herbicides and their residues have entered rivers, lakes, wetlands, estuaries,

and ground water, causing unacceptable environmental pollution. The use of higher plants for monitoring this class of pollutants is essential.

Duckweed *Spirodela polyrhiza*, is a common floating macrophyte coming under monocotyledons class of Angiosperms. Considerable evidence has shown that duckweeds are an excellent candidate for aquatic phytotoxicity tests. In the wild, the plants grow extremely fast in the spring and summer; in the laboratory, the plants grow continuously under favorable conditions. Duckweed is small enough that large laboratory facilities are not necessary, but large enough that adverse effects can be observed. Because duckweed is a floating macrophyte, it is especially sensitive to surface-active and hydrophobic substances that concentrate at the air-water interface.

Duckweed toxicity tests are highly versatile in aquatic environment. The tests are applicable to lake, river, ground water, wetlands, single chemical compounds or complex effluents from industrial or municipal sources; organic and inorganic compounds, rain samples; and sediment samples (Wang, 1986, 1987; Wang and Williams, 1988, 1990; Hartman and Martin,1984; Fekete *et* al., 1976) reported that duckweed was more sensitive to industrial effluents than higher plants such as cabbage and millet. Unlike algal toxicity tests, duckweed toxicity tests are especially suitable for effluent biomonitoring. Many industrial and municipal wastewaters are turbid. With these samples, filtration is required to conduct algal tests resulting in the loss of sample integrity. Duckweed tests, however can be performed on the sample 'as it is'. Duckweed tests can also reveal effects that cannot be obtained by using algal tests. Nasu *et* al. (1984) observed that Cu suppressed both frond multiplication and frond growth (fresh weight increase of each frond) in *Lemna* while Cd

suppressed only frond multiplication and not frond growth. Such comparative findings are very important in environmental toxicology and not possible with algal tests. There are indications that duckweeds are tolerant to environmental toxicity, and are commonly referred to as 'Carp 'of the plant species. Gabrielson *et* al. (1980) reported that duckweed grew under a wide range of nutrient conditions including high metal concentrations. Seto *et* al. (1979) reported that Cd caused chlorosis and death of *L.gibba*. There are other indications suggesting that duckweed is sensitive to toxicity. Wang (1986) conducted a series of duckweed toxicity test on 16 aquatic pollutants. He found that duckweeds are more sensitive to metal toxicity than fish species. On one hand duckweed plants are described as tolerant to environmental toxicity, while on the other hand the plants are considered as sensitive to toxicity. The contradiction can be explained on the basis that the plants may be highly adaptive. At sub lethal range, the duckweed plants adapt and/ or develop resistance quickly due to their fast growth rate (Duncan and Klaverkamp, 1983; Benson and Birge, 1985; Dixon and Sprague, 1981).

Many end points have been used to express duckweed test results. These end points are generally based on the population of duckweed plants: frond number, plant number, root number, dry and fresh biomass, root length, frond diameter, chlorophyll and the like Bishop and Perry, 1981; Culley *et* al., 1981; Lockhart *et* al., 1983; Glandon and McNabb, 1978; Sahai *et* al., 1977; Fekete *et* al., 1976). The most commonly used end point is frond number. Any visible, protruding bud is included in order to avoid individual bias. The frond count can be made repeatedly until accurate results are obtained. This determination is rapid and nondestructive. Blaylock and Huang, (2000) indicated that determination of biomass (constant weight at 60^{o}C) were the least time consuming and least

subjected to human error. Duckweed plants can also exhibit many symptoms when they are under stress. These symptoms include chlorosis (loss of pigment), necrosis (localized dead tissue), colony break up, root destruction, loss of buoyancy and gibbosity.

Heavy metals are present in the environment as a result of anthropogenic activities (agricultural and industrial activities). Industries such as smelters, metal refineries and mining operations have been indicated as major sources of metal release into the environment (Gardea-Torresdey *et* al., 1997; Srivastava *et* al., 2007). Most of the heavy metals are toxic or carcinogenic in nature and pose a threat to human health and the environment (Shakibaie *et* al., 2008; Vinodhini and Narayanan, 2009). Copper (Cu) and Lead (Pb) are considered as toxic since they cause deleterious effect in plants, animals and humans. The metals are responsible for many alterations in the plant photosynthesis, chlorophyll production, Protein and Carbohydrate content, growth etc. (Teisseire and Vernet, 2000; Prasad *et* al., 2001; Vaillant *et* al., 2005; Kanoun-Boulé *et* al., 2008; Zhou *et* al., 2009).

1.3. Phytoremediation using aquatic macrophytes

Macrophytes are commonly observed in water bodies throughout the world (Reddy, 1984). Macrophytes play prominent role in nutrient and heavy metal recycling of many aquatic eco-system (Pip and Stepaniuk, 1992). Heavy metals and other contaminants can be removed by microorganisms or by aquatic macrophytes. Aquatic plants are suitable for wastewater treatment because they have tremendous capacity of absorbing nutrients and other substances from the water (Boyd, 1970) and hence bring the pollution load down. Recently, emerging technology using aquatic macrophytes and microalgae

for wastewater treatment has gained great interest because of its cost-effective and environmentally sound approach (Vacca *et* al., 2005). Wastewater phytoremediation approach using macrophytes and different other water plants, floating or submerged (Noemi *et* al., 2004) is based on natural processes to remove different wastewater pollutants. Scientists and engineers from several countries have paid attention to the potential of aquatic macrophytes to treat and recycle pollutants from municipal and industrial wastewater (Brix and Schierup, 1989; Rao, 1986). These plants have the capacity to assimilate nutrients and to convert them directly into valuable biomass (Reed *et* al., 1995).

Among macrophytes, duckweeds are very small floating aquatic macrophytes belonging to the Lemnaceae family which grow on the nutrient rich surface and in fresh waters and they are known for their efficiency in nutrient uptake (Bal-Krishna and Polprasert, 2008). They have great capacity in organic matter removal and in absorbing the micro-elements such as potassium, calcium, sodium and magnesium and a large number of heavy metals than other hydrophytes. However, duckweed plants grow only in the upper water surface layer where mainly pollutant removal takes place (Dalu and Ndamba, 2003). *Spirodela polyrhiza* acts as a purifier of domestic and industrial wastewater in shallow water bodies (up to 10 cm deep). The treated wastewater can be used for irrigation purpose (Oron *et* al.,1984) and converted into a protein rich biomass, which could be used for animal feed or as soil fertilizer. Zayed (1998a) found that under experimental conditions, duckweed proved to be a good accumulator of Cd, Se and Cu, a moderate accumulator of Cr, and a poor accumulator of Ni and Pb. The toxicity effect of each trace element on plant growth was in the order: Cu > Se > Pb > Cd > Ni > Cr. He

also concluded that duckweed showed promise for the removal of Cd, Se and Cu from contaminated wastewater since it accumulated high concentrations of these elements. Further, the growth rates and harvest potential make duckweed a good material for phytoremediation. One of the objectives of the current investigation was to evaluate the effectiveness of *Spirodela polyrhiza* to remove heavy metals and other contaminants from the water samples collected from wetland sites of Eloor and Kannamaly under controlled conditions.

1.4. Objectives and scope of the study

Spirodela polyrhiza is an aquatic macrophyte coming under monocotyledons and is a true and simplest representative of Angiosperms. Being a native plant species it is found everywhere in the wetlands of the district. Owing to their settled life style, plants are constantly exposed to the pollution. The measurements of biochemical responses to the chemical contaminants present in water may serve to improve the assessment of biologically significant exposure to toxic chemicals and enhance the ability to assess the risk of effect on health and survival of toxicant exposed macrophyte populations.

1) Primary objective of the current study is the utilization of *Spirodela polyrhiza* plant to assess the toxicity of two wetland sites in Ernakulam district. One of the selected sites is Eloor, which is considered as one of the environmental hotspots in the world because of the intensity of pollutants it receives from Eloor - Edayar industrial estate. Other site selected is in Kannamaly, a coastal village located south west to Eloor. The site continuously receives effluents from nearby sea food processing factory. Due to the enormous number of potentially polluting substances contained in these waters, a chemical-specific approach is insufficient to

provide the information about water quality. Therefore, it is essential to use biological test systems with living cells or organisms that give a global response to the pool of micro pollutants present in the sample. The study was conducted in three different seasons- pre monsoon, monsoon and post monsoon. It is due to the fact that concentration of toxicants may vary in different seasons. Moreover, there was a need to assess the impact of duration of exposure in the plant body. So three exposure periods of 2 days, 4 days and 8 days were selected in every season. The study includes physico- chemical analysis of water and study of various plant parameters after the exposure periods. It includes morphological parameters, growth parameters, estimation of biomass, estimation of photosynthetic pigments and estimation of total protein and carbohydrate content.

2) Heavy metals are causing major problems in wetlands of Ernakulam district. Heavy metal stress is one of the major problems affecting agricultural productivity. Natural flora show relative differences in their heavy metal tolerance capacity. Some plants grow well in water enriched with toxic levels of heavy metals while others could not grow. The effects of toxic metals differ based on its concentration in the water. From the review of literature it was very clear that Copper and Lead are widely present in wetlands of the district. It is difficult to understand toxicity of individual metals using multi-metallic samples taken from wetland sites. So the next objective is to assess the toxicity and bio-accumulation potential of these two metals individually. The assessment involves morphological, physiological and bio chemical parameters along with bioaccumulation and BCF, NOEC and EC_{50}.

3) Physical, chemical, and biological technologies have been developed to treat polluted water and restore environmental quality. However, their costs are high and most of them are difficult to use in our conditions. So simple, and cost-effective techniques for pollution control in industrial effluents and treating such wastewater. Phytoremediation was assumed to be very useful, as it is an innovative, eco-friendly and efficient technology in which natural properties of plant is used to remediate hazardous wastes. Duckweed plats are known for its environmental sanitation potential. The final objective of the study is to find out the potential of *Spirodela polyrhiza* plant to remove pollutants from water samples collected from wetlands of Eloor and Kannamaly over three seasons under different periods of exposure.

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Chapter 2

Review of Literature

The state of Kerala has a total of 217 wetland units, of which 157 units are greater than 0.6 km^2 and has a total area of about 1279 km^2 (Anonymous,1990). Details of wetlands of Kerala have been provided by Nayar *et* al., 1997). Various threats faced by wetlands of Kerala and its impact and the need for their conservation was studied by Nair *et* al. (1998). The wetlands of Kerala are subjected to severe quality degradation (Ouseph *et* al., 2006; Nair and Unni, 1993; Nair, 1994; Remadevi and Abdul Aziz 1995; Anil Kumar and Abdul Aziz, 1995; Sreejith, 1996; Vasu *et* al. 1998; Harilal et al., 2000; Gopalan 2002; Ouseph and Pillai, 2004; Krishnakumar *et* al., 2005; Mahesh and Omana, 2006 and Sabitha and Nagaraj, 2007). The indiscriminate exploitation of wetlands beyond its supportive capacity, and input of residues exceeding its assimilative capacity, pollutes the wetland system of Kerala, the magnitude of which is very alarming. This if continued will cause harm to living resources, hazards to human health, hindrance to aquatic activities, impairment of water quality and reduction of amenities and finally ecological imbalance leading to catastrophic effects (Ajaykumar Varma *et* al., 2007).

Water quality of Periyar has been investigated particularly in its lower reaches by Jayapalan *et* al. (1976). Studies conducted by several investigators revealed that Periyar water is polluted due to effluent discharges from various industrial installations situated on the banks of the river (Joseph *et* al., 1984; Sankaranarayanan and Quasim, 1989 and Joy, 1989). The Eloor-Edayar region of the Kochi estuary provides a typical example of wetland pollution due to industrial discharge. Edayar region is identified as one of the toxic hotspots in the world by Green Peace, an international NGO campaigning against environmental destruction (Nair *et* al. 2001). Devi *et* al. (1979) also reported that the industrial effluents released into the water bodies of Eloor industrial zone affects the hydrographical features during the pre-monsoon and post-monsoon months. Jayapalan *et* al. (1976) and Joy (1989) observed that during summer the aquatic environment is characterized by low levels of dissolved oxygen, high phosphate, high nitrate content, high temperature, low pH, high CO_2 content and less plankton diversity. While during monsoon it possesses high DO, low temperature, high Co_2 content, neutral pH and low nitrates. It has been reported that the water quality of river is considerably altered during pre-monsoon so that there is occasionally increase in temperature, lowering pH, DO and high core of nutrients such as nitrates, sulphates and phosphates (Nair *et* al., 1976). Joy *et* al. (1990) have reported that the water received in the area during summer months is insufficient to effect dilution of waste water received in the industrial zone.

Temperature and variable pH along with occasional high nitrates, phosphates and COD levels especially in dry months seem to make this environment hazardous. The monsoon floods provide adequate dilution and mask the effect (Devi *et* al., 1979). High turbidity affects aquatic forms, as the

bottom conditions such as light penetrations, bacterial concentration and several other factors are usually modified by the amount of suspended solids particles in the water (David, 1956; Sreenivasan and Sundraraj, 1967; Ghosh and Basu, 1968 and Arora *et* al., 1973). Many hazardous substances including heavy metals, discharging into the aquatic environment are known to accumulate in the sediments. The heavy metal pollution has a long-term impact which is evident from Beypore estuary where considerable amounts of mercury was found retained in the sediments even after the stoppage of industrial effluent discharge (Nair 1994). Ramani *et* al. (1980) studied the levels of Cu, Mn, Co, Ni and Zn in the wetlands of the area. All metals showed some degree of variations over the area studied. Cu and Zn values vary with stations and seasons. The effluent discharge area showed significant enrichment in Cu during monsoon and in Zn during pre monsoon. The heavy metal estimation revealed the localized concentration of certain heavy metals especially Cd, Co, Zn and Cr in the vicinity of Eloor industrial belt as well as adjacent regions (Jenne, 1968). The major source of Fe is effluent of industries connected with Iron and steel and the units in which iron is one of the raw materials (Gopinathan *et* al., 1974).

The wetland of Kerala, especially along the coastal stretch are also polluted to the extent that their fishery and recreational values are fast declining. The major interventions include fishing, over harvesting, subsistence activities, effluents from industries, solid waste and effluents from human habitation, coconut husk retting, stagnation, intensive shrimp farming, lime shell mining, wetland reclamation, construction of roads, embankments, shrimp farms, mangrove cleaning, alteration of shoreline environment etc. (Nalini *et* al., 2000). Mauldin and Szabo (1974) reported that the wastewater

from seafood processing plants contains large amounts of organic matter, small particles of flesh, breading, soluble proteins, and carbohydrates. Park *et* al. (2001) reported that in processing of squid and several types of finfish in fish processing facilities creates high levels of BOD in the wastewater; typical BOD readings measured in the effluent of one seafood processing facility ranged 1000–5000 mg /l. squid ink is released into the waste stream during processing and is known to contain high concentrations of organic matters, including highly soluble proteins, which contribute significantly to excessive BOD loading (Shirai *et* al., 1997; Waldon (1991).

Concentration of dissolved and particulate trace metals (Ni, Pb, Zn, Mn) and their partitioning behavior between the dissolved and particulate phases in Vembanad lake was studied by Unnikrishnan and Nair (2004). They found that lack of proper flushing of backwaters, which receive large amount of trace metals through the application of pesticides and agro-chemicals, due to the presence of salinity barrier has significantly affected the water quality of southern half. Other studies on the trace metal distribution of the water column include those of Babukutty (1991); Babukutty and Chacko (1995); Ouseph (1987, 1995); Ouseph (1987); Nair, (1994); Shibu *et* al. (1992); Luther *et* al. (1986); Nair *et* al. (1990); Shibu *et* al. (1990) and Senthilnathan and Balasubramanian (1997).

Eloor and Kannamaly wetland surface water is highly complex since it receive large quantities of waste water from industrial and other sources and standard targeted chemical analysis is rather inadequate in evaluating toxic and genotoxic potential of surface waters because the polluting substances in such complex mixtures are frequently present in enormous number and at

concentrations too low to allow their analytical determination. On the other hand, biological monitoring can effectively defined risks for the environment and the human health as it takes into account of chronic exposure at low doses of toxic chemicals (Wadhia and Thompson, 2007). Despite their position as primary producers in the food chain in aquatic ecosystems, the macrophytes are among the first organisms exposed to pollutants in these environments. Such plants are used as *in situ* biomonitors of water pollution because of their abundance and limited mobility (Roy *et* al., 1992).

Aquatic vascular plants are receiving more attention for their potential use in screening, phytotoxicity studies of chemicals and as a useful bioindicators (USEPA, 1996). Among macrophytes, duckweed has an ability to respond in recognized patterns to various stresses, which has lead its use as an ISO standard for water quality (ISO 20079, 2005 Water quality, OECD, 2002, 2006). Duckweeds are more sensitive to aquatic pollutants than terrestrial plants. The possible reason for such phenomenon might be that tested substances are taken up directly through the leafy fronds and terrestrial plants take up the substance mostly by roots (Naumann *et* al., 2007).

Duckweed, being floating plants, can be used to test colored or turbid samples without filtration. In addition, some samples may contain labile, volatile, or sorptive materials and require either renewal or flow-through methods. Algal testing may be inappropriate for these types of tests, whereas the duckweed toxicity test as described herein can be modified easily to apply in either method (Hillman, 1961). Bishop and Perry (1981), described a flow-through growth inhibition test using common duckweed, *Lemna minor*. Growth inhibition was measured by using frond count, dry weight, and root

length. The test materials included metal ion; anionic, non-ionic, and cationic surfactants; and an aquatic herbicide. They reported that results based on frond count comprised the most useful information. According to King and Coley (1985), test with *Lemna minor* also useful for determining toxicity of metals and industrial and municipal effluents. Wang (1986) carried out toxicity tests of aquatic pollutants using common duckweed, *Lemna minor*. A comparison of duckweed toxicity test results with the fish test results reported in the literature found without exception that the duckweed sensitivity compared favorably with fish sensitivity. Duckweed toxicity test is useful, especially for determining phytotoxicity of surface waters and water rich in oil and grease and other water insoluble toxic substances (Wang, 1986). Biomonitoring in terms of hyperaccumulator plants were done by Manorama Thampatti *et* al. (2007). They found that plants like *Hydrilla verticillate*, *Eichornia crassipes* and *Cyperus pangorci* were found to poses hyper accumulation capacity for iron, manganese, zinc, copper and aluminum in the wetlands of Kuttanad.

The metals are responsible for many alterations of the plant cell (photosynthesis, chlorophyll production, pigment synthesis and enzyme activity (Teisseire and Vernet, 2000). Aquatic macrophytes take up metals from the water, producing an internal concentration several fold greater than their surroundings. Many of the aquatic macrophytes found to be the potential scavengers of heavy metals from aquatic environment and are being used in wastewater renovation systems (Abbasi *et* al., 1999; Kadlec *et* al., 2000). Susarla *et* al. (2002) Duckweed *Spirodela polyrhiza* used in wastewater treatment to remove mineral and organic contamination and radionuclides . John *et* al. (2008), studied about the effects of different concentrations of cadmium and lead on *Spirodela polyrhiza*. At lower metal concentrations, an

increase in proline, protein and sugar was observed but at higher concentrations (above 30 mg/l) their decrease was noticed. Uptake of the metal concentration was time dependent. Appenroth *et* al. (2008), found that growth rates of *S. polyrhiza* were reduced by chromate concentrations higher than 50 μM. Analysis of plant cells by transmission electron microscopy revealed the accumulation of starch grains in the chloroplasts following the application of chromate at low concentrations or for short periods (100 μM for 2 days or 500 μM for 1 day). According to Vinodhini and Narayanan (2009), Copper (Cu), nickel (Ni), Lead (Pb) and zinc (Zn) are considered as toxic since they cause deleterious effect in plants, animals and humans. Sandra Radic (2009), conducted ecotoxicological assessment of industrial effluent using duckweed (*Lemna minor* L.) as a test organism. Obtained data demonstrate the relevance of duckweed as sensitive indicators of water quality as well as the significance of selected biological parameters in the reliable assessment of phyto-genotoxic potential of complex wastewaters. N.M. Rolli *et* al. (2010), used *Spirodela polyrhiza* to study the effect of different concentration of cadmium on biochemical constituents and accumulation of Cd from the experimental pond under laboratory conditions. Recently, Appenroth *et* al. (2010) showed the effects of nickel on the chloroplasts of the duckweeds *Spirodela polyrhiza* and *Lemna minor* and their possible use in biomonitoring and phytoremediation.

Phytoremediation is a word formed from the Greek prefix "phyto" meaning plant, and the Latin suffix "remedium" meaning to clean or restore (Cunningham *et* al., 1997). The term actually refers to a diverse collection of plant-based technologies that use either naturally occurring or genetically engineered plants for cleaning contaminated environments (Flathman and Lanza, 1998). The primary motivation behind the development of phytoremediative

technologies is the potential for low-cost remediation (Ensley, 2000). Although the term, phytoremediation, is a relatively recent invention, the practice is not (Brooks, 1998; Cunningham *et* al., 1997). Research using semi-aquatic plants for treating radionuclide-contaminated waters existed in Russia at the dawn of the nuclear era (Salt *et* al. 1995; Timofeev-Resovsky *et* al., 1962). Some plants which grow on metalliferous soils have developed the ability to accumulate massive amounts of the indigenous metals in their tissues without exhibiting symptoms of toxicity (Baker and Brooks, 1989; Reeves and Brooks, 1983). Chaney (1983) was the first to suggest using these "hyperaccumulators" for the phytoremediation of metal polluted sites. By definition, a hyperaccumulator must accumulate at least 1000 μgAg-1 of Co, Cu, Cr, Pb, or Ni, or 10,000 μgAg-1 (i.e. 1%) of Mn or Zn in the dry matter (Reeves and Baker, 2000; Wantanabe, 1997). Gaur *et* al. (1994), reported that, the accumulation of Cd, Cr, Co, Cu, Ni, Pb and Zn by *Spirodela polyrhiza* was directly related to the concentration of metals in the medium during a 4 day exposure period. The hierarchy of metal accumulation was Ni>Zn>Co>Cu>Cd>Pb>Cr.

Conventional remediation technologies are used to clean the vast majority of metal-polluted sites. The reason is because they are fast, relatively insensitive to heterogeneity in the contaminated matrix, and can function over a wide range of oxygen, pH, pressure, temperature, and osmotic potentials (Cunningham *et* al., 1997). However, they also tend to be clumsy, costly, and disruptive to the surrounding environment (Cunningham and Ow, 1996). Of the disadvantages of conventional remediation methods, cost is the primary driving force behind the search for alternative remediation technologies. Some micro-organism-based remediation techniques, such as bioremediation, show

potential for their ability to degrade and detoxify certain contaminants. Although these biological systems are less amenable to environmental extremes than other traditional methods, they have the perceived advantage of being more cost-effective (Cunningham *et* al., 1997). Bioremediation is most applicable for sites that have been contaminated with organic pollutants, and as such, this condition has been the focus of the majority of bioremediation research. Because heavy metals are not subject to degradation, several researchers have suggested that bioremediation has limited potential to remediate metal-polluted environments. In contrast, plants are known to sequester certain metal elements in their tissues (Marschner, 1995) and may prove useful in the removal of metals from contaminated soils (Chaney, 1983). Over the past decade there has been increasing interest for the development of plant-based remediation technologies which have the potential to be low-cost, low-impact, visually benign, and environmentally sound concept called phytoremediation. (Cunningham and Ow, 1996).

Although plants show some ability to reduce the hazards of organic pollutants (Carman *et* al., 1998; Gordon *et* al., 1997), the greatest progress in phytoremediation has been made with metals (Blaylock and Huang, 2000; Salt *et* al., 1995; Watanabe, 1997). Biomonitoring in terms of hyperaccumulator plants were also done by Manorama Thampatti *et* al. (2007). They found that plants like *Hydrilla verticillate*, *Eichornia crassipes* and *Cyperus pangorci* were found to poses hyper accumulation capacity for iron, manganese, zinc copper and aluminum in the wetlands of Kuttanad. Macrophytes are considered as important component of the aquatic ecosystem not only as food source for aquatic invertebrates, but also act as an efficient accumulator of heavy metals (Devlin, 1967; Chung and Jeng, 1974). They are unchangeable biological

filters and play an important role in the maintenance of aquatic ecosystem. Aquatic macrophytes are taxonomically closely related to terrestrial plants, but are aquatic phanerogams, which live in a completely different environment. Their characteristics to accumulate metals make them an interesting research objects for testing and modeling ecological theories on evolution and plant succession, as well as on nutrient and metal cycling (Forstner and Whittman, 1979). Therefore, it is very important to understand the functions of macrophytes in aquatic ecosystem.

Heavy metals are metals having a density of 5 g/cc, (Nies *et* al., 1999). These metals include elements such as copper, cadmium, lead, selenium, arsenic, mercury, chromium etc. Heavy metals in surface water systems can be from natural or anthropogenic sources. Currently, anthropogenic inputs of metals exceed natural inputs. High levels of Cd, Cu, Pb, Fe can act as ecological toxins in aquatic and terrestrial ecosystems (Guilizzoni, 1991; Balsberg-Pahlsson, 1989). Excess metal levels in surface water may pose a health risk to humans and to the environment. The water, sediments and plants in wetlands receiving urban runoff contain higher levels of heavy metals than wetlands not receiving urban runoff. Large aquatic plants are known to accumulate heavy metals in their tissues. Duckweeds take up heavy metals mainly through the root, although uptake through the leaves may also be of significance. As the macrophytes die and decay, the accumulated metals in the decaying macrophytes can increase in the concentration of heavy metals in the sediments. Aquatic plants often grow more vigorously nutrient loading is high. They are capable of removing water soluble substances from solution and temporarily immobilize them within the system (Ho, 1988; Untawale *et* al., 1980). Colonization of macrophytes on the sediments polluted with heavy

metals and the role of these plants in transportation of metals in shallow coastal areas are very important. The present investigation was planned and executed considering the potentials of macrophytes as a biological filter of the aquatic environment.

Copper and Lead are reported to be widespread heavy metal pollutants in wetland areas in Ernakulam resulting from agriculture and industrial activities such as pigments, mining, smelting and electroplating, etc. Cu is an essential micronutrient and a component of several enzymes mainly participating in electron flow and catalyzing the redox reactions (Devi and Prasad, 1998). But it becomes toxic at high concentrations, whereas Pb has no known biological function and is a highly toxic metal to aquatic organism (Wang *et* al., 2001). The aim of this study was to estimate how the exposure of *Spirodela polyrhiza* to different concentrations of Cu and Pb affects various parameters .The results also shows that duckweed can bio-concentrate these heavy metals (Jain *et* al*.,* 1989; Khellaf and Zerdaoui, 2009).

In developing countries, conventional remediation technologies are used to clean the vast majority of metal-polluted sites. The reason is because they are fast, relatively insensitive to heterogeneity in the contaminated matrix, and can function over a wide range of oxygen, pH, pressure, temperature, and other physical and chemical conditions. Now more countries turn their attention towards greener technologies.

Phytoremediation has recently become a subject of intense public and scientific interest and a topic of many recent researches (Raskin *et* al*.,* 1994; Cunningham *et* al.,1995; Salt *et* al*.,* 1995; Cunningham and Ow, 1996; Kumar and Jaiswal, 2007; Muneer *et* al., 2007; Sun *et* al., 2007). Phytoremediation of

heavy metals is a cost-effective green technology; there are more advantages, when it comes to the use of native and naturally growing plants. Plants used for phytoextraction must be fast growing and have the ability to accumulate large quantities of environmentally important metal contaminants in their shoot tissue (Blaylock *et* al., 1997; Cunningham and Ow, 1996; Kumar *et* al., 1995; McGrath, 1998). Although plants show some ability to reduce the hazards of organic pollutants (Carman *et* al., 1998; Cunningham *et* al., 1995; Gordon *et* al., 1997), the greatest progress in phytoremediation has been made with metals (Blaylock and Huang, 2000; Salt *et* al., 1995; Watanabe, 1997). Duckweed systems are one of the options that have been widely applied for combined handling of wastewater with the nutrients used for poultry and aqua-cultural projects (Gijzen and Kondker, 1997) and (Naphi *et* al., 2003). Aquatic plants have shown their efficiency in absorbing nutrients from various sources of polluted water, (Janjit *et* al., 2007). Floating plants are of well performers to treat wastewater (Zirschky and Reed, 1988).

The potential of duck weed was investigated by Zayed *et* al. (1998) for the removal of Cd, Cr, Cu, Ni, Pb and Se from nutrient-added solution and the results indicate that duck weed is a good accumulator for Cd, Se and Cu, a moderate accumulator for Cr, but a poor accumulator of Ni and Pb. Duckweeds have been used successfully in the United States to phytoremediate municipal, industrial and septic waste (Iqbal, 1999). Many small-scale phytoremediation efforts are found in other locations also. For example, in one village in Bangladesh, duckweed, cultivated on raw sewage, is fed to fish (Iqbal,1999). For our study in phytoremediation, we chose to use duckweed *Spirodela polyrhiza* based on their growth patterns, nutrient uptake rates and the fact that they are ethnic to the study region. Recently, Dipu, *et* al. (2012), conducted

study to determine the efficiency of an emergent wetland plant species *Typha* and floating wetland macrophytes such as *Pistia, Azolla*, *Lemna, Salvinia* and *Eichhornia* used in phytoremediation of various heavy metals with addition of a chelating agent such as EDTA. EDTA addition to the treatment systems increased the uptake of heavy metals by plants, which was much pronounced with lead and copper. However, the pattern of uptake by plants was similar as that of heavy metals without EDTA amendments.

From the review of literature it is clear that most biomonitoring and bioremediation studies using duckweeds are limited to *Lemna minor.* The macrophyte *Spirodela polyrhiza*, is a duckweed has the same potential as *Lemna minor* in all above mentioned aspects plus starch rich fronds. So this plant species are highly recommendable for phytoremediation studies in severely polluted sites. In Kerala, duckweed research is still at infancy despite having numerous polluted wetlands. The macrophyte is an attractive phytoremediation agent worth further studies and application trials, mainly in the enhancement of natural attenuation and phytoextraction. Therefore, it is imperative to study *Spirodela polyrhiza* further in the context of phytoremediation.

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Chapter 3

Materials and Methods

Contents

3.1 Area of the study

Two wetland stations in Ernakulam district namely Eloor and Kannamaly were selected for the study (*Fig: 1*). Eloor, an island with 11.21 sq/km, on the Periyar River is land of more than 247 chemical industries and large number of wetlands. Most of these units have been here for the last fifty years and use extremely obsolete and polluting technologies. Toxic pollution from heavy metals to chemicals and radioactivity is found in air, soil and in the water bodies, which spreads the contamination to the Vembanad Lake, Cochin and to the Arabian Sea. This leads to a large-scale devastation of aquatic life in the area, the agricultural land and it is also affecting the health of the population.

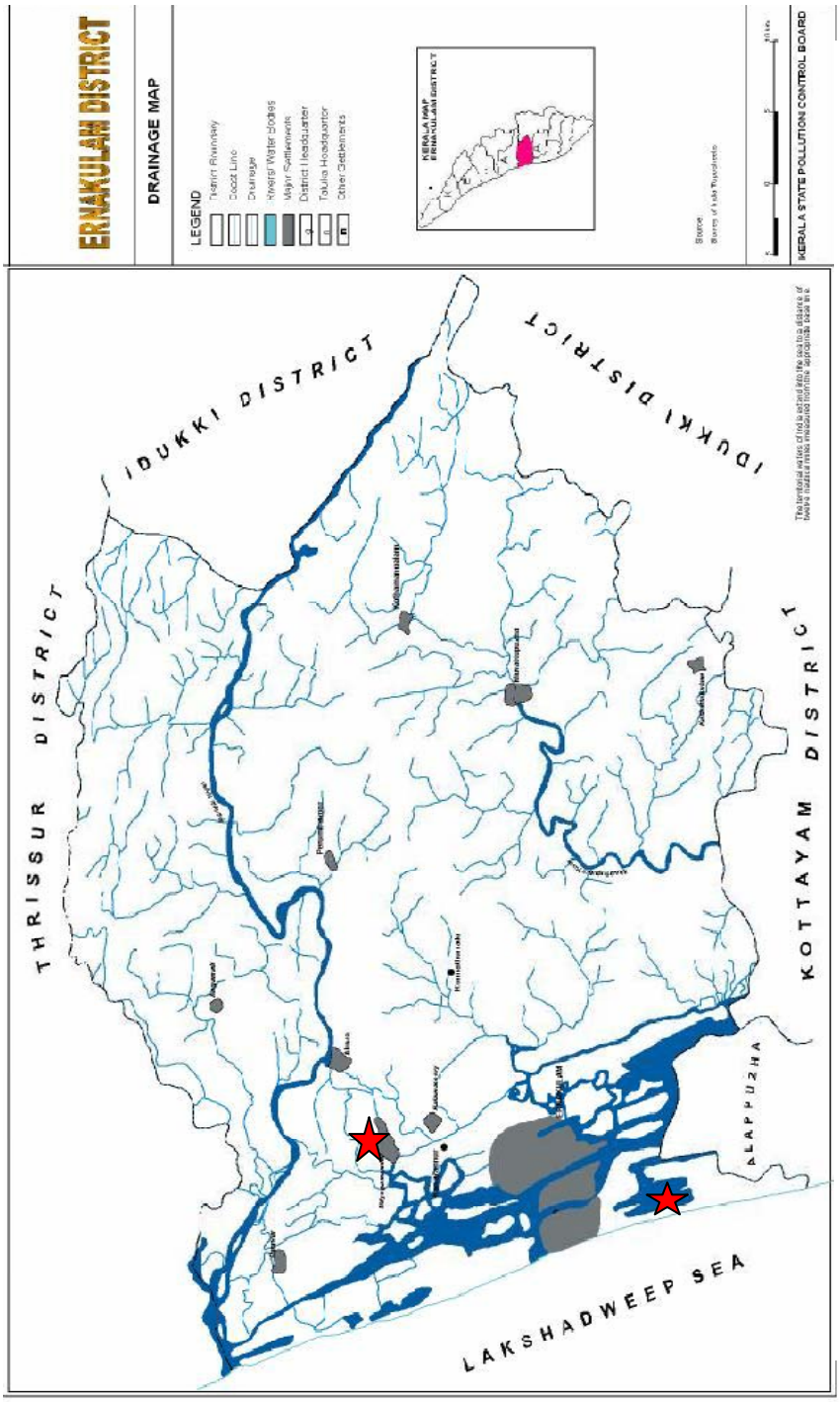

***Fig. 1**: Drainage map of Ernakulam prepared by Central Pollution Control Board. Two wetlands stations selected for present study are marked by star symbol. The upper one marks Eloor and lower mark denotes position of Kannamaly.*

The soil, water bodies and the wetlands in and around Eloor have been contaminated with heavy metals like zinc, lead, cadmium, chromium and persistent organic pollutants like DDT. Since aquatic plants are present in these waters in large quantities, they are constantly exposed to these pollutants all the time.

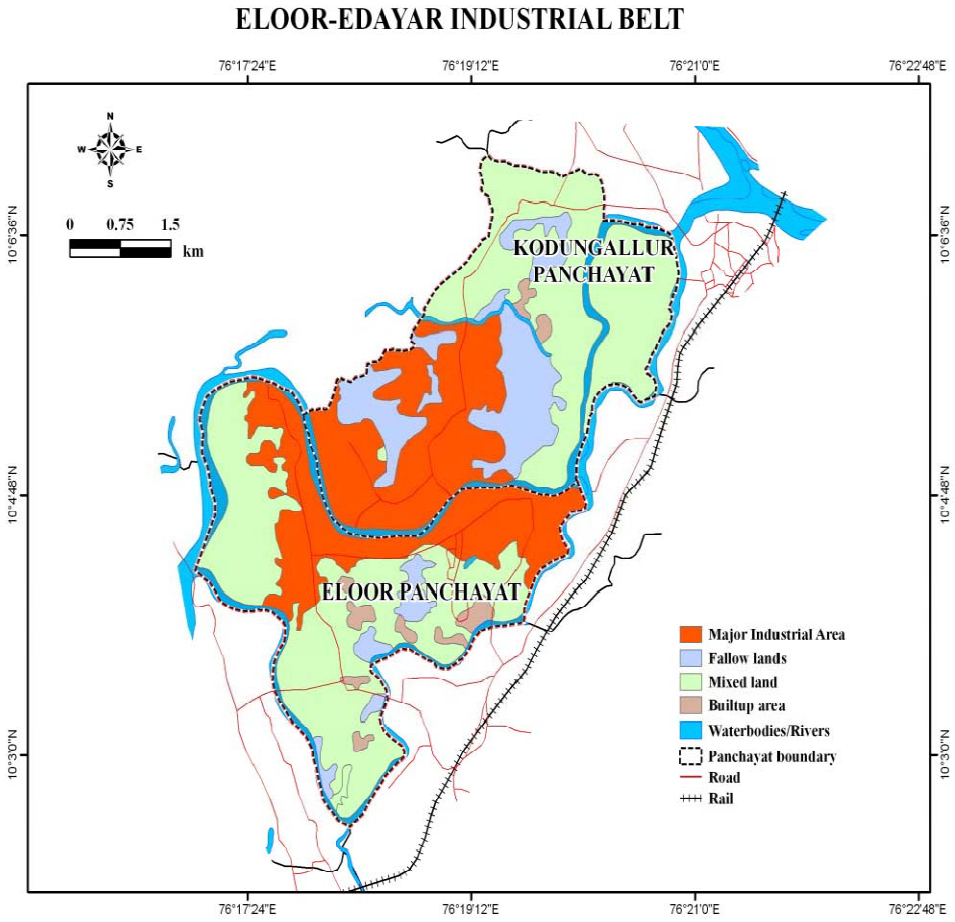

***Fig.2**: Eloor - Edayar Industrial belt in Ernakulam marked by Central Pollution Control Board.*

The chemical industries mainly fertilizers require large quantities of fresh water for their processing. To have access to large quantities of fresh water these factories are established along the sides of rivers. In Kerala there are 44 small rivers with plenty of freshwater. Periyar river open to cochin backwaters. This backwater is one of the most productive estuarine systems with an estimated annual gross production of nearly 300g C/m^2 (Qasim *et* al. 1969). Unfortunately a large number of small and large industries comprising industrial estate established on the banks of Periyar and creating enormous environmental problems.

3.2. Sampling stations in the district

The Eloor-Edayar region on the banks of River Periyar, near the river-estuary confluence region, houses Kerala's largest industrial cluster including Fertilizers and Chemicals Travancore Ltd. (FACT), Hindustan Insecticides Ltd (HIL), Indian Rare Earths Ltd., Travancore Cochin Chemicals etc. Some of the major industries, their products, pollutants and permitted effluent discharge to the Kochi estuary. In Eloor one sample (W1) was collected from a location approximately 10 m northwest (W1- Lat.10^0 04'51.76"N, Long. 76^0 17'32.55"E) of the HIL site boundary (*Fig.3*). The second sample (W 2) was collected from the wetlands approximately 40 meters south (Lat.10^0 04'48.13"N Long. 76^0 17'22.75"E) of the Kuzhikandam creek (*Fig. 4*).

Fig. 3: *Eloor Wetland 1 sampling location*

Fig.4: *Eloor Wetland 2 sampling location*

From Kannamaly, one sample (W1) was collected approximately 100 metres south of the Kannamaly St. Joseph pilgrim centre and close to *India Seafood Factory*, at a location approximately at Lat. 9.8704^{0}N and Long.

76.2665^0 E .The second sample (W2) was collected from the wetlands south to the wetland I area, approximately 1.8 km away and located at Lat. 9.8612^0 N Long. 76.2642^0 E (*Fig. 5 and 6*).

Fig.5: *Kannamaly Wetland 1 sampling location*

Fig.6: *Kannamaly Wetland 2 sampling location*

3.3. Collection of surface water samples from sampling stations

Depending upon the location of Kuzhikkandam creek which carries waste discharges from industries mentioned above, two sampling stations each (W1 and W2) from Eloor. In Kannamaly two sampling stations (W1 and W2) were selected on the basis of proximity to *India seafood Company*. For a sample of water to be the true representative of water quality, water must be well mixed. Therefore a due care was taken in selecting the distances between each sampling station so that the maximum mixing of the waste discharge with the wetland water ensured the true water quality of the river.

Water collected from both wetlands (W1 and W2) located in Eloor and Kannamaly during pre-monsoon, monsoon and post monsoon period as parameters vary during different seasons. Wetland water samples were transferred to the laboratory and carried out preliminary sieving step to get rid of large suspended solids. The transferred water was immediately collected into 3 opaque tanks. (For treatment for 2days, 4 days and 8 days). The opaque tanks were used to prevent light entering except at the top. These aquariums were arranged in such a way that light availability is maximum.

3.4. Physico chemical analysis of surface water samples

Various physico-chemical parameters studied for water quality assessment of Eloor and Kannamaly wetland water samples. For assessing physico-chemical characteristics, samples were collected from all the sampling stations every season in triplicates. During the pre-monsoon and post-monsoon period samples were collected in the first week of every month. To avoid floating material, samples were collected at about 5 cm depth from three points of the

site using the dip and grab sampling method and stored in clean polythene bottles. Samples were transferred to the laboratory and carried out preliminary sieving step to get rid of large suspended solids and later analyzed for various parameters using CPCB standard methods (CPCB, 2008). Temperature, pH, DO (Dissolved oxygen) and conductivity were measured in the field at the time of collection of samples by using portable star series Orion (USA) meter. The parameters of study were temperature, pH, BOD (Biological oxygen demand), COD (Chemical oxygen demand), EC (Electrical conductivity), Total alkalinity, TDS (Total dissolved solids), TSS (Total suspended solids), Nitrate, Phosphate, Ammonia, and Turbidity along with analysis for heavy metals before and after the experiment. Heavy metals were analyzed using AAS (Atomic absorption spectroscopy). Each parameter was determined in triplicate and the average of three values was recorded. All the measured data are presented as average values for premonsoon, monsoon and post-monsoon seasons.

Physico-chemical analysis of water was carried out as per CPCB guidelines (2008). Temperature, pH and TDS were measured *in situ.* BOD was measured respirometric method provides direct measurement of O_2 consumed by microorganisms from air in a closed vessel under conditions of constant temperature and agitation. Alkalinity of sample was estimated by titrating with standard sulphuric acid (0.02N) at room temperature using phenolphthalein and methyl orange as indicator. Conductivity meter was used to measure the conductance (EC) generated by various ions in the solution/water. The open reflux method is suitable to find out COD for a wide range of wastes with a large sample size. The dichromate reflux method was used. Turbidity is measured by its effect on the scattering light, which is termed as Nephelometry.

Phosphorous occurs in natural waters and in wastewater almost solely in the form of various types of phosphates. Stannous chloride method was used to determine phosphate content. Ammonia is produced by the microbiological degradation of organic nitrogenous matter or by leakage of ammonia. Nesslerisation method was used for the determination of ammonia (CPCB, 2008). UV spectrophotometer method was useful for the measurement of nitrates (CPCB, 2008). The ultraviolet absorption at 220 nm enables rapid determination of nitrate (CPCB, 2008). Turbidimetric method used for the determination of sulphate ions (CPCB, 2008).

3.5. Plant material for bioassay and remediation studies

Test organism is an aquatic macrophyte *Spirodela polyrhiza*. It is found worldwide in many types of freshwater and backwater habitat. It is a perennial aquatic plant usually growing in dense colonies, forming a mat on the water surface (*Fig. 7*). The plant is smooth, round with flat disc shaped leaves called fronds. It produces several minute roots. *Spirodela* species has a free-floating thallus; 2-5 plants may remain connected to each other. Plants are green, but may have a red or brown underside. Due to anthocyanin pigmentation (*Fig. 8*). Multiple roots (7 - 12) emerge from each frond (*Fig.9*).

***Fig.7**: The plant forming dense colonies in control*

***Fig.8**: The abaxial side with anthocyanin pigmentation*

Fig.9: *Multiple roots from the fronds*

Fig. 10*: The plants are easy to remove from water*

Spirodela polyrhiza is a species of duckweed known by the common names greater duckweed, giant duckweed, and duck meat. It is found to be worldwide in distribution in freshwater habitat. It is a perennial aquatic plant usually growing in dense colonies, forming a mat on the water surface. Each plant is a smooth, round, flat disc one half to one centimeter wide. It produces several minute roots. It also produces a pouch containing male and female flowers. The top part dies in the fall and the plant often overwinters as a turion. *Spirodela* is largest among duckweeds. Its fronds are measuring as much as 20 mm across. An individual frond may produce as many as 20 daughter fronds during its lifetime, which lasts for a period of 10 days. The bulk of the frond is composed of chlorenchymatous cells separated by large intracellular spaces that are filled with air and provide buoyancy. The plant can be easily removed from the water surface (*Fig. 10*).

Systematic position

Kingdom	: Plantae
Phylum	: Angiosperms
Class	: Monocotyledons
Order	: Alismatales
Family	: Araceae
Genus	: *Spirodela*
Species	: *polyrhiza*

Some cells of have needle like raphides which are presumably composed of calcium oxalate. The upper epidermis is highly cutinized and is unwettable. Stomata are on the upper side. Anthocyanin pigments found on abaxial side of the leaf. *Spirodela polyrhiza* has greatly reduced vascular bundles. Roots are adventitious type. They are usually short but this depends on species and environmental conditions and vary from a few millimetres up to 14cm.

Duckweed (*Spirodela polyrhiza* L.) is used in water quality studies to monitor heavy metals and other aquatic pollutants, because duckweed, like other water plants, may selectively accumulate certain chemicals. *Spirodela* plant is the smallest available representative of angiosperms. The plants possess same physiological and biochemical properties of terrestrial macrophytes. By assessing the plant, we can assess the toxicity of surrounding media. They shows rapid growth between pH 5 - 9, and vegetatively propagated. which make them an ideal test system. The L*emna* and *Spirodela* are among the most standardized test organisms in aquatic ecotoxicology studies (EPA, 1996; DIN, 2000 & 2001; Eberius, 2001; OECD, 2002).

The plant duckweed has several other advantages such as

1) It is the world's "greenest" feedstock. Fast growing, high in protein and dietary minerals, and easily harvested, the plant is cultivated as a feed supplement for chicken, livestock, and farmed fish, especially in developing countries. The growth rate of duckweed under ideal light, temperature and pH would be exponential if there were no limitation in terms of mineral deficiencies or excesses. In ideal conditions it may reach about 1.2kg/m^2 duckweed (fresh).

2) An inexpensive, earth-friendly source of the biofuel ethanol. Unlike corn, potato etc duckweed requires minimal human-made energy to grow and it doesn't deplete the world's food supply. *Spirodela polyrhiza* is an ideal system for biofuels since it has more starch content than potato.

3) Bioremediation efficiency coupled with other aspects of fast-growth, direct contact with media enable duckweed-based wastewater treatment systems provide genuine solutions to the problems of urban and rural human waste management with simple infrastructure at low cost.

4) A natural wastewater treatment option. The plant feeds on organic pollutants like nitrogen, phosphate and other metallic pollutants the very stuff treatment plants aim to remove from wastewater. The recycling of water through waste water treatment works or purification of water for human use from presently polluted surface water. Duckweed will remain an underutilized resource unless governments accepts that polluted water cannot be released into water bodies without removal of minerals .There is a vast need for research support for this little plant with such a great potential.

Duckweed (*Spirodela polyrhiza.)* plants were collected from JNTBG, Trivandrum and maintained in the local outdoor conditions for 3 months before the experiment for acclimatization. The stocks were cleaned by tap water then washed by distilled water. Five healthy and fresh, wet *Spirodela polyrhiza* plants were stocked into each of the three aquariums. Each aquarium was supplied sequentially with 5 liters of wetland water. Each of the three aquariums was filled with same amount of wetland water. An aquarium is kept with distilled water with nutrients and macrophyte is considered as control.

The experiment was kept under outdoor local environmental conditions for 2, 4 and 8 days retention time.

3.6. Exposure to surface water samples

In the present study, the test plant was exposed to the water samples for phytotoxicological assessment and phytoremediation studies. In phytotoxicological assessment, the sample water collected from all sampling sites during different seasons were immediately transferred to the lab aquarium (as replicates) and treated with *Spirodela polyrhiza* plants for 2 days, 4 days and 8 days. In the second part of current research, toxicity and bioaccumulation of Copper and Lead were studied. In this part, *Spirodela polyrhiza* plants were exposed with different concentrations of Copper and Lead mixed with Hoagland's 10% nutrient media as per OECD guidelines (2002, 2006). The concentration of exposure were 1 mcg/L, 10 mcg/L, 20 mcg/L, 40 mcg/Land 80 mcg/L for a duration of 8 days. The concentration ranges were selected on the basis of its concentration in the wetland water samples. During phytoremediation studies, water samples collected over 3 seasons from both wetlands of Eloor and Kannamaly were brought to the laboratory and treated with *Spirodela polyrhiza* plants for 2days, 4 days and 8 days of intervals and monitor the changes in the water parameters after each interval.

The treatment system for growing duckweed in small glass aquarium tanks (Fig. 11) were constructed in laboratory set up. After preliminary work, the entire set up was shifted to outside the laboratory for exposure to natural conditions. Each aquarium tank was 18 inches long, 10 inches deep and 9 inches wide. The stocks were cleaned by tap water and distilled water. Five

healthy fronds of Spirodela *polyrhiza* were stocked into the aquariums initially. All parameters were measured after 2, 4 and 8 days of exposure. The sides of each exposure chamber were covered with black chart paper to avoid light entry through the sides consequently preventing algal growth.

Fig. 11: *Spirodela polyrhiza* treatment tanks for the exposure

3.7. Phytotoxicity assessment end points

The phytotoxicity assessment was carried out by measuring the changes in morphological parameters, average specific growth rate, frond doubling time, estimation of biomass, estimation of photosynthetic pigments and estimation of protein and carbohydrates.

3.7.1. Changes in morphological parameters of the plant

At the start of the test, frond and colony numbers in the test vessels are counted and recorded, taking care to ensure that overlapping but distinctly visible fronds are accounted for. Frond and colony numbers (normal and abnormal) and their appearance were determined at the beginning and end of

the test when effects are assessed in terms of the average specific growth rate over the full duration of the test. Counts of frond numbers after intermediate exposure periods were taken since average specific growth rate need to be determined at intervals during the period of the test. Changes in plant development (e.g. frond size, appearance, necrosis or chlorosis, colony break-up or loss of buoyancy, root length, morphology or breakdown) were noted. .Other morphological parameters observed were root number, root length and leaf size measurements. Total frond area was measured in cm^2 by image analysis with adobe Photoshop software. Root lengths were measured with a simple millimeter scale.

3.7.2. Changes in growth and biomass

Duckweed growth was determined measuring ASGR (Average Specific Growth Rate), Frond doubling time (Td) fresh weight (FW, biomass) and dry weight (DW) and DW/FW ratio (as per OECD, 2006 test protocol). The frond number was scored at the start of the experiments (t_0) and 2, 4 and 8 days after (t_0). All visible fronds were counted. ASGR is determined by the following formula.

$$\mu i\text{-}j = \frac{\ln(Nj) - \ln(Ni)}{t}$$

where - μi-j : average specific growth rate from time i to j, Ni : measurement variable in the test or control vessel at time I, Nj : measurement variable in the test or control vessel at time j, t : time period from i to j.

T(d) can be calculated by equation $Td = \ln 2 / \mu$ where μ is the average specific growth rate .

Dry weight to Fresh weight ratio can be calculated by measuring frond weight by calculating dry weight (gms)/ Fresh weight (gms).

$$\text{Growth Index can be calculated by} = \frac{\text{Biomass (t = 8 days)}}{\text{Biomass (t = 0)}}$$

Plants were surface-dried between layers of paper towels, and the fresh weight was determined. To measure dry weight, plants were dried at 80^0 C over night. The growth parameters were measured according to OECD guidelines (2006).

3.7.3. Estimation of photosynthetic pigment concentration

Chlorophyll content was determined by the acetone method by Arnon, (1949). For estimation of photosynthetic pigments plant material (100 mg) was ground in chilled 80% acetone in dark. After centrifugation at 10,000 × g for 10 min at 4^0C, absorbance of supernatant was taken at 750, 663, 645,510 and 480 nm. Chlorophylls and carotenoid content was calculated using the formula given by Arnon (1949). Photosynthetic pigments Chlorophyll-a, Chlorophyll-b and Carotenoids were estimated using spectrophotometer (Hitachi-U-2000 spectrophotometer).

3.7.4. Biochemistry

1. *Estimation of protein content*

Proteins were estimated by the method of Bradford, (1976). Fresh leaves (0.5 g) were homogenized in 1 ml phosphate buffer (pH 7.0). The crude homogenate was centrifuged at 5000 × g for 10 min. 0.5 ml of freshly prepared trichloroacetic acid (TCA) was added and centrifuged at 8000 × g for 15 min. The debris was dissolved in 1 ml of 0.1N NaOH

and 5 ml Bradford reagent was added. Absorbance was recorded photometrically at 595 nm (Beckman 640 D, USA) using bovine serum albumin as a standard.

2) ***Estimation of soluble carbohydrates***

Total soluble carbohydrate was estimated as per Dey, (1990). Leaves (0.5g) were extracted twice with 90% ethanol. The extracts were combined. The final volume of the pooled extract was made to 25 ml with double distilled water. A suitable aliquot was taken from the extract and 1 ml 5% phenol and 5 ml concentrated sulphuric acid were added. Final volume of this solution was made to 10 ml by addition of double distilled water. Absorbance was measured at 485 nm using UV-Vis spectrophotometer.

3.7.5. Metal analysis by Atomic Absorption Spectroscopy (AAS)

All sample containers and glass wares used were washed with detergent, rinsed with water and immersed in concentrated nitric acid (AR grade) and kept for 12 hours. Then it was taken out and washed with distilled water in order to remove the unwanted traces of metallic and non metallic contents.

For determining the accumulation of metals in the samples, the samples were taken at the end of the exposure period and thoroughly washed with double distilled water to remove metal content smeared on the root and leaf surfaces. Four ml of conc. nitric acid and 1 ml of perchloric acid in the ratio of 4:1 was added into it. The digestion was carried out in small 100 ml beaker covered with small glass funnels kept in sand bath. The samples were evaporated to dryness and it was washed with double distilled water and made

up to 10 ml. Care was taken to prevent the solution from boiling. The samples were subjected to spectroscopy using Atomic Absorption Spectrophotometer of the model Hitachi Z. 8000 polarized Zeeman AAS and expressed as µg/100 mg of dry weight. AAS method was helpful in water analysis of wetland samples from Eloor and Kannamaly during three different seasons (Pre monsoon, monsoon and post monsoon). It was also helpful in determining phytoremediation capacity of the plant after different periods of exposure (8 days) to water samples in different seasons. The experiments on the accumulation of metals in *Spirodela* plant helped in determining the uptake of metals (Copper and Lead) with reference to the increasing concentration (1 mcg/L, 10 mcg/L, 20 mcg/L, 40 mcg/L and 80 mcg/L) of the metal in the medium and time duration of 8 days.

3.7.6. Bioaccumulation, Elimination and BCF

During the bio accumulation studies, the test material is collected from the treatment chamber after 8 days of exposure and analysed using AAS to find out percentage of removal or removal efficiency. BCF is the Bio concentration factor, determined to find out the efficiency of an organism to take up a metal from the surroundings in which it is living.

$$BCF = \frac{\text{Metal concentration in the plant (mg/KgDW)}}{\text{Metal concentration in the solution(mg/L)}}$$

During phytoremediation studies, the metallic and non metallic parameters were measured according to the guidelines issued by Central Pollution Control Board (CPCB). Some of the parameters were measured *in situ*. All others were estimated in the laboratory before the exposure and after

2, 4 and 8 days of exposures. The percentage of elimination of metallic and non metallic pollutants were estimated by following equation (Kellaf and Zerdaoui, 2009).

Elimination (%) = $\frac{C_o - C_f}{C_o} \times 100$ in which C_0 and C_f are initial and remaining concentration.

3.7.7. Tolerance: Determination of NOEC and EC_{50}

Tolerance was calculated by determining NOEC and EC_{50} based on biomass after exposing the plants for 8 days in various concentrations of Copper and Lead. NOEC and EC_{50} were determined Dunnet method.

3.8. Statistical analysis

The statistical analysis was done using 'R' software. All the experiments were conducted in triplicates and average values were taken. The relative standard deviations of means of triplicate measurement were less than 5%. Analysis of variance (ANOVA) for each test was conducted using "R" software, Zigmaplot and Microsoft Excel.

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Chapter 4

Results and Discussion

Contents

4.1. Phytotoxicological assessment of wetland sites in Ernakulam district

4.1. 1. Wetlands in Eloor

4.1.1.1. Physico – chemical analysis of water sample

The results of physico-chemical analysis of water samples collected from Eloor wetlands are presented Table-1. The pH levels of the surface water samples collected from three stations were found to be alkaline (between 7.6 and 8.4) which indicates the presence of carbonates, hydroxides and bicarbonate ions. In periods of high surface runoff, overland flow contributes dissolved materials to waters. In addition suspended solids load in the form of municipal and industrial effluents, agricultural runoff, and aerosol fallout are also added. High concentrations of suspended solids, limit the suitability of water as a drinking source. The highest values of TSS were measured in the sample of W2 during pre monsoon period. However, in general, the values of suspended solids in water samples collected from monitoring stations over 3 seasons were

relatively low. Salts, minerals, and even dissolved gases contribute uniformly to the conductivity of water. Electrical conductivity of water is a simple and useful indicator of the amount of dissolved materials in a solution. The slightly higher conductivity values were observed in W2 sample during pre monsoon season. COD, BOD, total nitrates, phosphates and sulphates were higher in W2 than W1. Heavy metals like Pb, Cu, Zn, Co, Cr, Fe, Cd, Mn, Hg, Ni were also detected in the water samples and the quantity is presented in table 1.

Table 1: *Seasonal variation in physico chemical parameters studied in two wetland water samples collected from Eloor*

Sl. No	Physico- chemical parameters	CPCB standard	Seasons					
			W1 Pre monsoon	W2 Pre monsoon	W1 monsoon	W2 monsoon	W1 Post monsoon	W2 Post monsoon
1	Temp(^{o}C)	25-40	33	28.8	27	26.2	33	28.8
2	PH	6.5-8.5	8.2	8.4	7.6	7.6	8.2	8
3	BOD (mg/L)	5	110	341	43	127	68	218.3
4	COD(mg/L)	250	320	679	78.5	178	169.6	298.3
5	Nitrate(mcg/L)	45	12	27	16	8.3	10.3	22
6	EC(µs/Cm)	700	952	1185	896.2	912	991	1021
7	Alkalinity(mg/L)	400	342	441	210	268	218	220
8	Phosphate(mcg/L)	5	11	13.1	6	10.8	10.3	13.6
9	Sulphate(mg/L)	400	500.12	133	89.6	327.5	410.6	101
10	TDS (mg/L)	2100	593.1	3210.3	521	2718	566	2889
11	TSS (mg/L)	100	218.41	359	210	327	216	357.6
12	Turbidity(NTU)	5	29	382	215.6	27	29	362.3
13	Copper(mcg/L)	1.5	25	43	15	27	13	25.2
14	Lead(mcg/L)	0.01	16	24.4	12.8	8.6	12	11
15	Zinc(mcg/L)	15	112	201	65.4	86	62	91.2
16	Chromium(mcg/L)	0.01	78	81	59.3	66	61	78.2
17	Cobalt(mcg/L)	0.01	7.2	8	4.2	3	6.8	3
18	Manganese(mcg/L)	0.5	8	7.3	4.8	4	4.6	6.6
19	Mercury(mcg/L)	0.001	2	3.4	1.5	1.5	3.2	2.7
20	Nickel(mcg/L)	5	19.3	22.3	11.1	7.8	16	16.3
21	Iron(mcg/L)	50	ND	5.3	ND	4.3	ND	8.6
22	Cadmium(mcg/L)	0.01	ND	3	ND	1.2	ND	4

Biomonitoring provides the direct evidences of alterations occurred in the ecosystem due to environmental pollution. Integrated information on the water quality can be reflected based on the biomonitoring of aquatic pollution, which offers the potential effects and actual toxicities. *Spirodela polyrhiza* can be an ideal biomonitoring macrophyte due to its sensitive responses, which may provide the precaution of toxic effects induced by current pollution, and also explain the potential toxicological mechanisms

4.1.1.2 Inhibition of growth parameters

Table 2(a): *Seasonal variation in ASGR and frond doubling of time in S. polyrhiza in Eloor W1sampling site. (P<0.05)*

Season/ Treatment	2 days			4 days			8 days		
	Mean N(j)	ASGR	Td	Mean N(j)	ASGR	Td	Mean N(j)	ASGR	Td
Control	8	4.09	1.32	10	3.4	1.59	16	3.74	1.45
Pre monsoon	6.3	1.46	4	7.6	1.46	3.96	10	1.7	3.17
Monsoon	8	4.07	1.33	9.3	2.71	2.05	14	3.06	3.14
Pos t monsoon	8	4.06	1.33	8.6	2.4	2.25	12.3	2.29	2.37
				Mean N(i)=5			Mean T(i)=0		

Table 2(b): *Seasonal variation in ASGR and frond doubling of time in S. polyrhiza in Eloor W2 sampling site. (P<0.05)*

Season/ Treatment	2 days			4 days			8 days		
	Mean N(j)	ASGR	Td	Mean N(j)	ASGR	Td	Mean N(j)	ASGR	Td
Control	8	4.097	1.32	10	3.4	1.59	16	3.74	1.45
Pre monsoon	6	1.36	3.96	7	1.36	3.98	8	1.07	5.07
Monsoon	8	4.59	1.18	8.6	2.41	2.24	10	1.69	3.18
Post monsoon	7.6	3.52	1.53	8	2.03	2.66	9.6	1.53	3.53
				Mean N(i)=5			Mean T(i)=0		

The result shows that maximum growth rate in monsoon and minimum growth rate in pre monsoon season. During pre monsoon season, the ASGR was reduced by 55% after 8 days of exposure with W1 water sample (*Table 2a & Fig. 1a*). At the same time there was 73% decrease of ASGR in W2 sample. Monsoon season favors growth rate with maximum reduction of 18% in W1 and 55% reduction in W2 after 8 days of exposure. During post monsoon, there was 34% of decrease of ASGR in W1 sample and 58% of decrease in W2 sample at the end of exposure period (*Table 2b & Fig. 1b*).

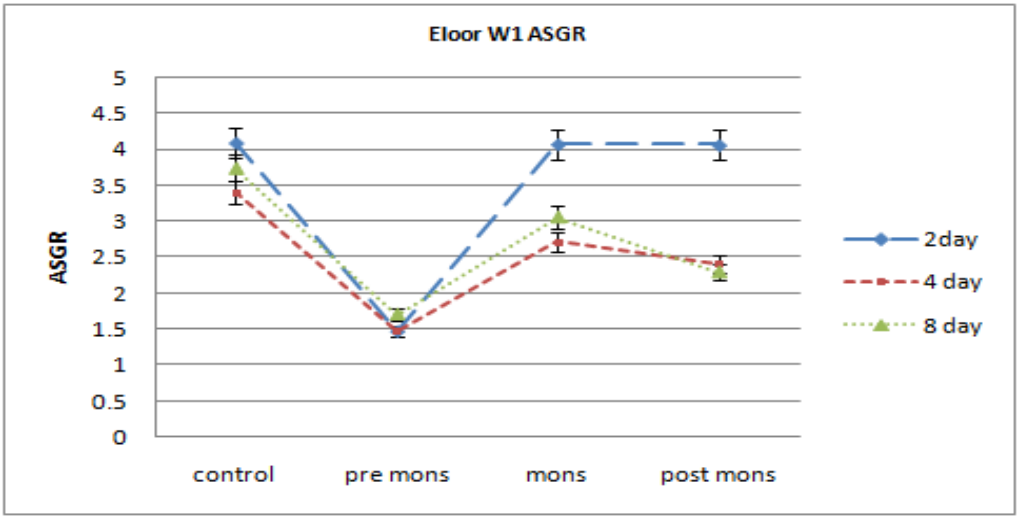

Fig. 1(a): *Graphical representation of changes in Average Specific Growth Rate (ASGR) in Spirodela culture exposed for 8 days to wetland 1 water samples collected over 3-seasons. Values are mean of three replicates ($P<0.05$).*

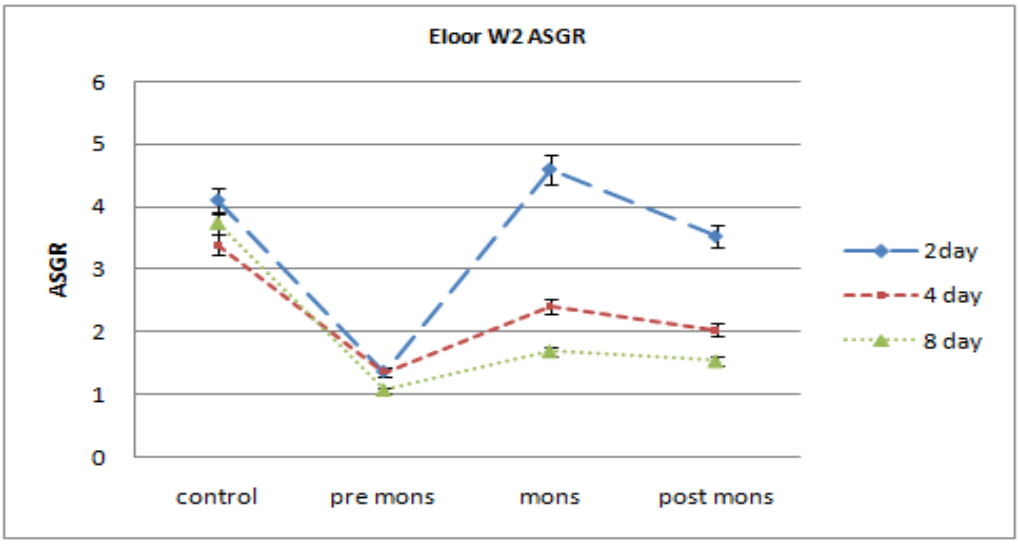

Fig. 1(b): *Graphical representation of changes in Average Specific Growth rate (ASGR) in Spirodela culture exposed for 8 days to wetland 2 water samples collected over 3-seasons ($P<0.05$).*

Frond doubling time (Td) value will be 3 or more is an indication of stress and the water is said to be toxic. During the present study 2, 4, and 8 days of treatments during pre monsoon period gives Td value more than 3.0 in both W1 and W2 samples. 8 days of treatment with W2 samples during monsoon and post monsoon yield Td values 3.19 and 3.46 respectively. The least Td value is obtained during monsoon period *(Table 2 a & b, Fig. 2a &b).*

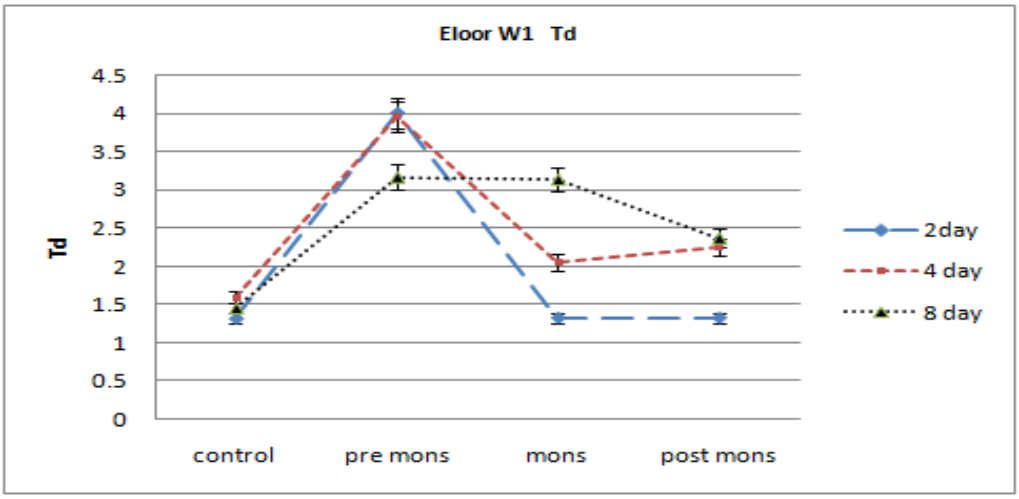

Fig. 2(a): *Graphical representation of changes in frond doubling time (Td) in Spirodela culture exposed for 2 days, 4days and 8 days to wetland water samples (W1 and W2) collected over 3-seasons. Values are mean of three replicate (P<0.05).*

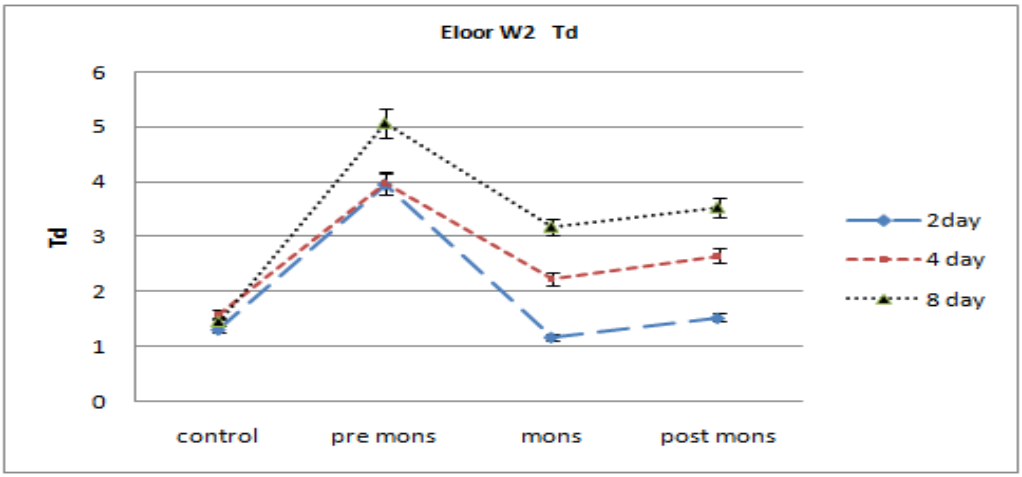

Fig. 2(b): *Graphical representation of changes in Frond doubling time(Td) in Spirodela culture exposed for 2 days, 4days and 8 days to wetland water samples(W1 and W2) collected over 3-seasons. Values are mean of three replicates (P<0.05).*

4.1.1.3. Seasonal variation in inhibition of dry weight/ fresh weight ratio

***Table 3(a)**: Variation in biomass DW/FW ratio of S. polyrhiza after 2, 4 and 8 days of exposure in water from Wetland 1. Each value is means of triplicates (P<0.05).*

Season/ Treatment	Wetland 1 - Eloor				
	2 days				
	FW initial	FW final	DW initial	DW final	DW/FW ratio
Control	21.5	34.4	3.01	4.8	139.5
Pre Monsoon	21.3	24.08	2.34	2.648	109.6
Monsoon	21.3	28.38	2.55	3.405	120.7
Post monsoon	21.2	35.69	3.18	5.35	150.1
Season/ Treatment	4 days				
	FW initial	FW final	DW initial	DW final	DW/FW ratio
Control	21.5	77.4	3	10.83	139.5
Pre Monsoon	21.3	64.79	3.01	7.23	111.2
Monsoon	21.2	71.22	2.55	9.25	129.9
Post monsoon	21.3	89.41	3.1	12.51	140.2
Season/ Treatment	8 days				
	FW initial	FW final	DW initial	DW final	DW/FW ratio
control	21.5	163.4	3.01	22.87	139.9
pre Monsoon	21.3	109.1	2.34	11	100.7
Monsoon	21.2	137.6	2.55	15.13	109
Post monsoon	21.3	162.5	3.18	19.5	120.4

***Table 3(b)**: Variation in biomass DW/FW ratio of S. polyrhiza after 2, 4 and 8 days of exposure in water from Wetland 2. Each values are means of triplicates (P<0.05).*

Season/ Treatment	Wetland 2 Eloor				
	2 days				
	FW initial	FW final	DW initial	DW final	DW/FW ratio
Control	21.5	34.41	3.01	4.81	139.7
Pre Monsoon	21.3	25.8	2.34	2.06	79.54
Monsoon	21.2	31.51	2.55	3.46	109.6
Post monsoon	21.3	28.63	3.18	3.43	120.3
Season/ Treatment	4 days				
	FW initial	FW final	DW initial	DW final	DW/FW ratio
Control	21.5	77.	3	10.83	139.9
Pre Monsoon	21.3	53.01	3.01	3.71	70.14
Monsoon	21.2	83.11	2.55	8.31	99.78
Post monsoon	21.3	78.2	3.1	8.6	109.5
Season/ Treatment	8 days				
	FW initial	FW final	DW initial	DW final	DW/FW ratio
Control	21.5	163.4	3.01	22.8	139.5
Pre Monsoon	21.3	90.3	2.34	5.41	60.31
Monsoon	21.2	154.8	2.55	13.93	89.85
Post monsoon	21.3	144.7	3.18	11.57	80.38

During pre monsoon season, 2 days exposure in water sample from W1 shows DW/FW ratio reduced by 21% from the control values (*Table 3(a), Fig. 3(a)*). In W2 sample, there was a heavy reduction in growth rate (43%) (*Table 3(b), Fig. 3(b)*). After 4 days of treatment there was a reduction of 20%

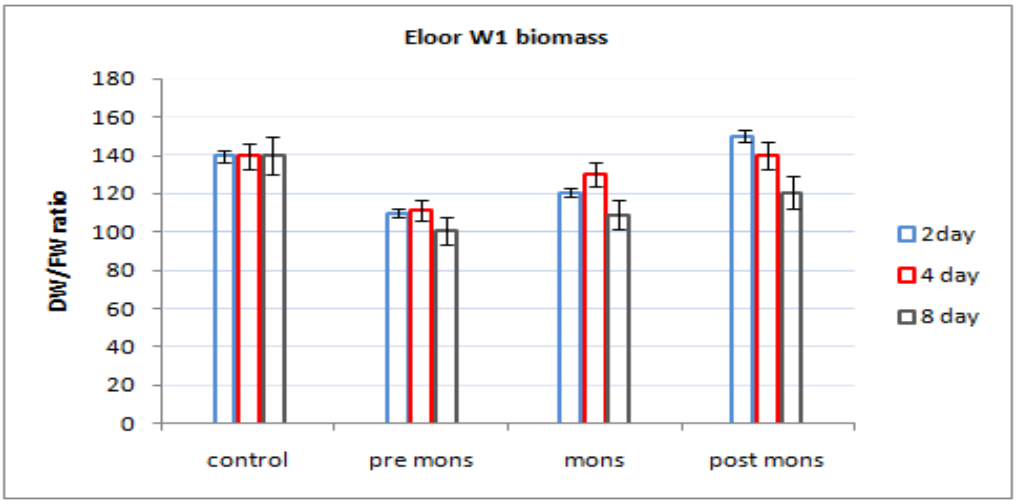

Fig. 3(a): *Graphical representation of changes in DW/FW ratio in Spirodela culture exposed for 2 days, 4days and 8 days to wetland water samples (W1 and W2) collected during 3 seasons. Values are mean of three replicates (P<0.05).*

in W1 and reduction of 50% in W2. After 8 days of treatment, 28% reduction occurred in W1 sample and 57% of reduction occurred in W2 sample.

During monsoon season, 2 days exposure with water sample from W1, shows DW/FW ratio reduced by 14% than control values. In W2 water sample, there was a reduction in growth rate (21%). After 4 days of treatment there was only a reduction of 7% in W1 sample and reduction of 29% in W2. After 8 days of treatment, 21% reduction occurred in W1 and 35% of reduction occurred in W2 sample.

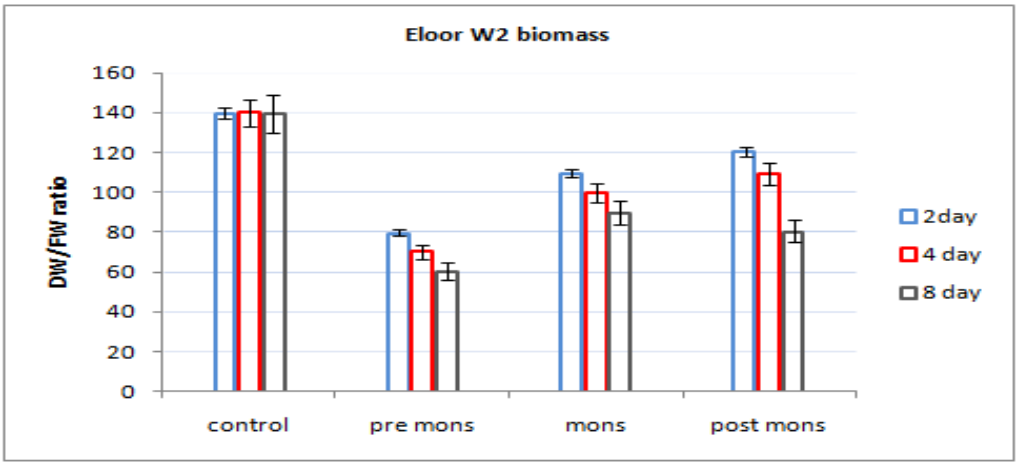

Fig. 3(b): *Graphical representation of changes in DW/FW ratio in Spirodela culture exposed for 2, 4 and 8 days to wetland water samples (W1 and W2) collected during 3-seasons. Values are mean of three replicates (P<0.05).*

During post monsoon season, 2 day exposure in water sample from W1, shows DW/FW ratio increased by 7% from the control values. In W2 sample, there was a reduction in growth rate (14%). After 4 days of treatment there was a slight increase of 0.3% in W1 sample and reduction of 21% in W2. After 8 days of treatment, 14% reduction occurred in W1 and 43% of reduction occurred in W2 sample (*Table 3b & Fig. 3b*).

4.1.1.4. Seasonal variation in photosynthetic pigment content

In control, chlorophyll –a content was 0.458 mg/g FW. During Pre monsoon season, in W1 sample after 2 days of treatment, 17% of reduction of chlorophyll- a was noted. At the same time there occurred massive decrease of 26% in W2. . After 4 days of treatment 36% of reduction occurred in W1 compared to 41% in W2 . After 8 days 38% and 46% of reduction in W1 and W2 sample respectively. The final concentration after 8 days of exposure was 0.284 mg/g and 0.247 mg/g FW (*Table 4, Fig. 4a&b*).

Table 4: *Relative photosynthetic pigment concentrations after2, 4 and 8 days of exposure in different dilutions of wetland 1and durin pre monsoon, monsoon and post monsoon seasons.. Each values are means of 3 replicates. The significant difference between treatments is P<0.05.*

Eloor Weland 1-Post Monsoon			
Exposure	**Chl a (mg/g)FW**	**Chl b (mg/g) FW**	**Carotenoids (mg/gFW**
Control	0.458	0.286	0.24
2 days	0.459	0.253	0.24
4 days	0.429	0.248	0.23
8 days	0.341	0.204	0.212

Eloor Weland 1- Monsoon			
Exposure	**Chl a (mg/g)FW**	**Chl b (mg/g) FW**	**Carotenoids (mg/gFW)**
Control	0.458	0.286	0.24
2 days	0.459	0.281	0.24
4 days	0.456	0.274	0.24
8 days	0.43	0.248	0.22

Eloor Weland 1-Pre Monsoon			
Exposure	**Chl a (mg/g)FW**	**Chl b (mg/g) FW**	**Carotenoids (mg/gFW)**
Control	0.458	0.286	0.24
2 days	0.381	0.274	0.232
4 days	0.291	0.221	0.218
8 days	0.284	0.181	0.209

Eloor Weland 2 -Post Monsoon			
Exposure	**Chl a (mg/g)FW**	**Chl b (mg/g) FW**	**Carotenoids (mg/gFW)**
Control	0.458	0.286	0.24
2 days	0.46	0.251	0.232
4 days	0.381	0.221	0.218
8 days	0.291	0.172	0.21

Eloor Weland 2- Monsoon			
Exposure	**Chl a (mg/g)FW**	**Chl b (mg/g) FW**	**Carotenoids (mg/gFW)**
Control	0.458	0.286	0.24
2 days	0.46	0.257	0.24
4 days	0.449	0.253	0.22
8 days	0.412	0.221	0.21

Eloor Weland 2- Pre monsoon			
Exposure	**Chl a (mg/g)FW**	**Chl b (mg/g) FW**	**Carotenoids (mg/gFW)**
Control	0.458	0.286	0.24
2 days	0.341	0.223	0.228
4 days	0.27	0.204	0.186
8 days	0.247	0.172	0.158

Chlorophyll-b content in control was 0.286 mg/g FW. During pre monsoon season, reduction of Chlorophyll - b by 4.1%, 23% and 36% after 2, 4 and 8 days of exposure in W1 water sample. At the same season in W2 sample ,the reduction by 22%, 29% and 40% observed after 2, 4 and 8 days of exposure. (*Table 4 & Fig. 4a and 4b*). Carotenoid content in control was 0.24 mg/g FW. After 2 days of treatment, Carotenoid content reduced by 17% in W1 and W2. After 4 days, there occurred 25% of reduction in W1 and 35.4% in W2 sample. After 8 days of treatment 33% of reduction occurred in W1 sample compared to 49% in W2.

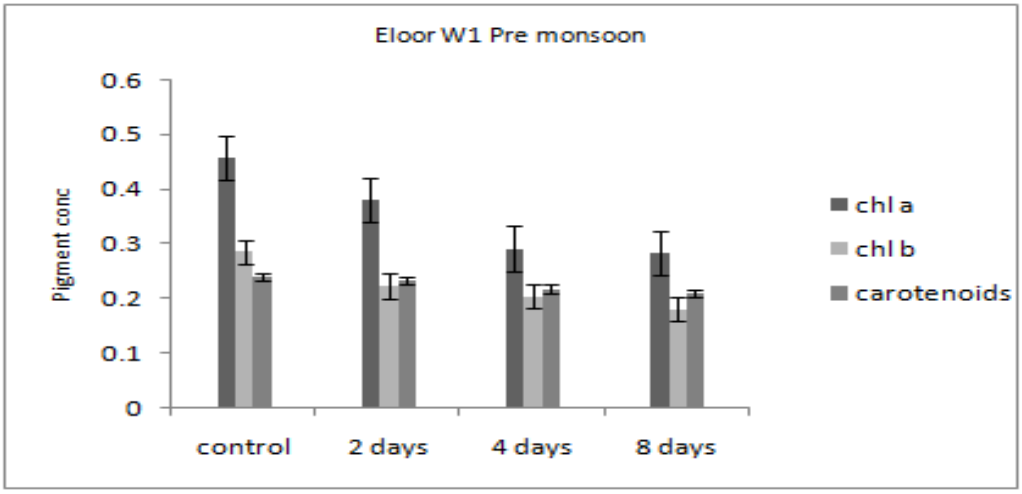

Fig.4(a): *Relative changes in concentration of photosynthetic pigments Chlorophyll -a, Chlorophyll- b and Carotenoid content after2, 4 and 8 days of exposure in water from Wetland 1 during Pre monsoon season (P<0.05).*

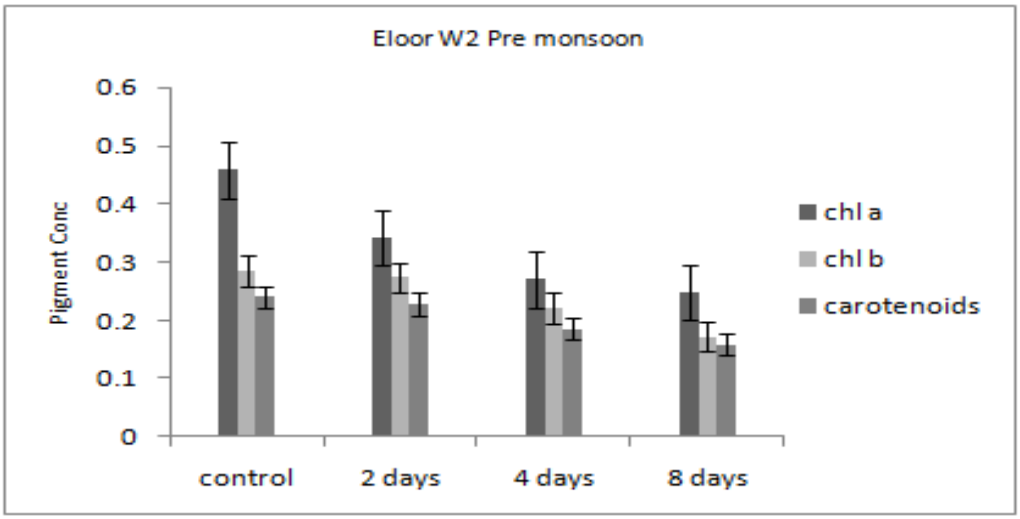

***Fig. 4(b)**: Relative changes in concentration of photosynthetic pigments Chlorophyll a, Chlorophyll b and Carotenoid content after2, 4 and 8 days of exposure in water from Wetland 2 during Pre monsoon season (P<0.05).*

During monsoon season, in W1 sample after 2 days of treatment, there occurred slight increase in chlorophyll-a by 0.21%. In W2 an increase of 0.43% was noted in the same season. After 4 and 8 days of treatment 0.43% and 10% of reduction of chlorophyll-a occurred in W1 and 2% and 6% occurred in W2. The final concentration after 8 days of exposure was 0.412 mg/g and 0.43 mg/g FW respectively. (*Table 4, Fig. 4c&d*). Chlorophyll- b content in control was 0.286 mg/g FW. During monsoon season, the reduction occurred by 1.7%, 4% and 13% in W1 sample and 10%, 12% and 23% in W2 sample after the exposure regime.

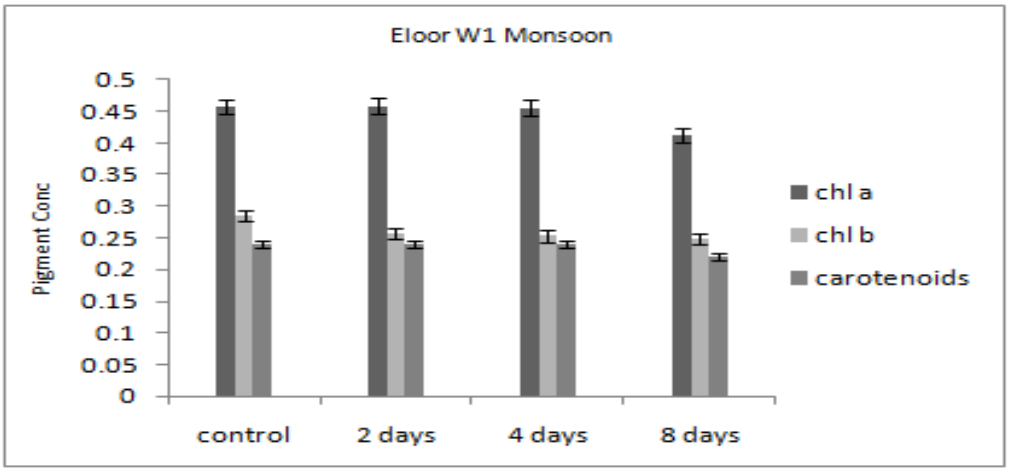

***Fig. 4(c)**: Relative changes in concentration of photosynthetic pigments Chlorophyll a, Chlorophyll b and Carotenoid content after2, 4 and 8 days of exposure in water from Wetland 1 during Monsoon season (P<0.05).*

After 2 days and 4 days of treatment, carotenoid content remains unchanged in W1 sample. But in W2 water, reduction of 5% and 8.3% occurred after 2 days and 4 days respectively. After 8 days of treatment 8.3% of reduction occurred in W1 sample compared to 12% in W2.

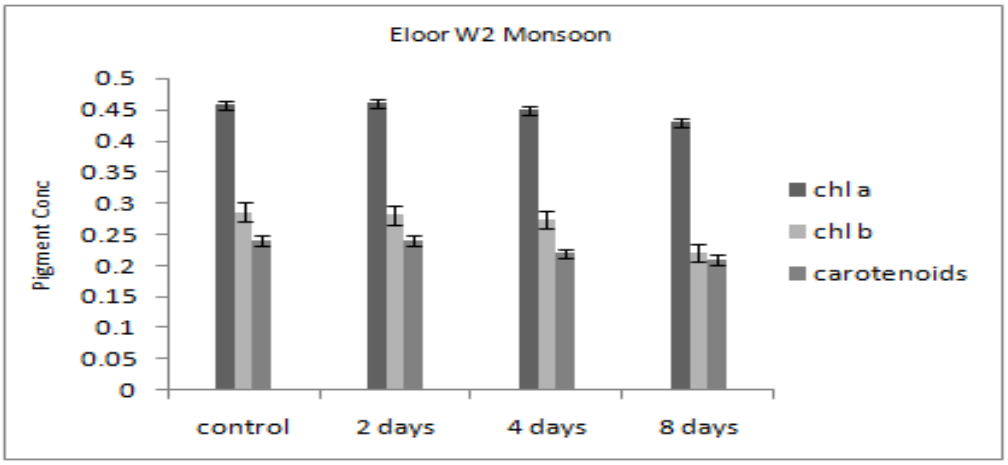

***Fig. 4(d)**: Relative changes in concentration of photosynthetic pigments Chlorophyll-a, Chlorophyll -b and Carotenoid content after2, 4 and 8 days of exposure in water from Wetland 2 during Monsoon season (P<0.05).*

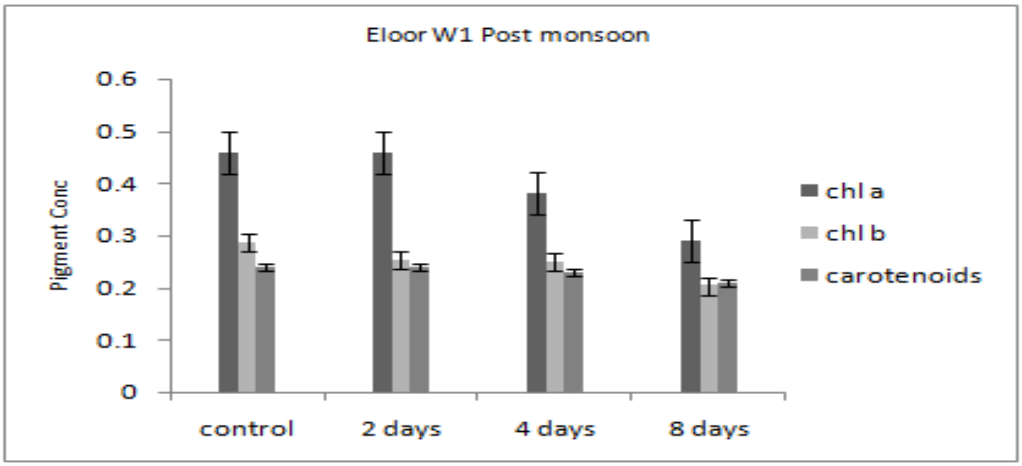

***Fig. 4(e)**: Relative changes in concentration of photosynthetic pigments Chlorophyll -a, Chlorophyll- b and Carotenoid content after2, 4 and 8 days of exposure in water from Wetland 1 during post monsoon season. Standard deviations were presented by error bars (P<0.05).*

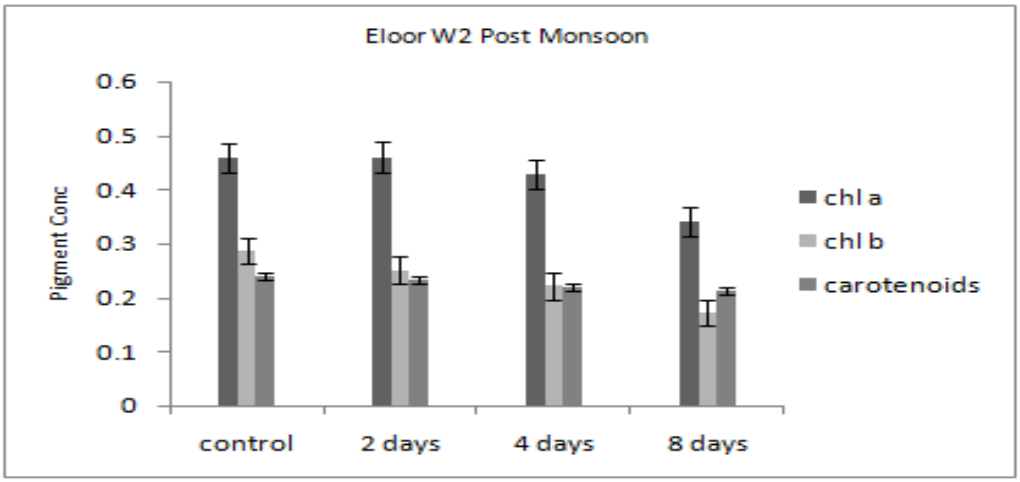

***Fig.4(f)**: Relative changes in concentration of photosynthetic pigments Chlorophyll- a, Chlorophyll -b and Carotenoid content after2, 4 and 8 days of exposure in water from Wetland 2 during post monsoon season (P<0.05).*

***Fig. 4(g)**: Comparison of Spirodela plant culture after 8 days of exposure in water sample from Wetland 1(left) and Wetland 2 (right) during pre monsoon season. W2 exposure shows necrotic patches on the fronds.*

During post monsoon season, in W1 sample after 2 days of treatment, there occurred slight increase of chlorophyll- a by 0.2% while reduction of 0.25% was noted in W2. By the end of 8 days the pigment reduced by 26% in W1 sample and 36 % in W2 sample. The final concentration was 0.291 mg/g and 0.341 mg/g FW respectively (*Table 4 & Fig. 4e&f*). Chlorophyll -b content in control was 0.286 mg/g FW. During post monsoon season there occurred a gradual reduction from this concentration with the duration of exposure. After 8 days there occurred 29% and 40% reduction in W1 and W2 samples respectively. (*Table 4 & Fig. 4e&f*). Carotenoid content in control was 0.24 mg/g FW. After 2 days of treatment, carotenoids content remain unchanged in W1 sample. But in W2 water, reduction of 5% occurred. After 4 days, there occurred 4.1% of reduction in W1 and 9.2% in W2. After 8 days of treatment 12.5% of reduction occurred in W1 compared to 17.6% in W2 sample.

4.1.1.5. Seasonal variation in inhibition of protein and carbohydrate content in *S. polyrhiza*

Pre monsoon period shows maximum reduction in protein content (14%) after 8 days of treatment in W1 water sample. In W2 water sample there was

24% reduction. In Monsoon W1 sample shows reduction between 4% to 8%. While in wetland 2 it was between 1 to 10 % of inhibition after 8 days. In Post monsoon period protein content decreased between 7 % to 10 % in W1 sample and it was 6% to 24% in W2 after 8 days. Soluble protein content reduces with exposure time in all samples. After 8 days of treatment reduction occurred between 8% to 14% in W1 and 10 to 24% in W2 *(Table 5, Fig.5 a&b).*

Table 5: *Soluble protein and Total carbohydrate content after2, 4 and 8 days of exposure in different dilutions of wetland 1and 2 samples during pre monsoon , monsoon and post monsoon seasons. Each values are means of 3 replicates. The significant difference between treatments is P<0.05.*

Eloor Weland 1-Pre Monsoon			**Eloor Weland 1- Monsoon**			**Eloor Weland 1-Post Monsoon**		
Exposure	**Soluble protein mg/g FW**	**Total carbo - hydrate mg/gmFW**	**Exposure**	**Soluble protein mg/g FW**	**Total carbo- hydrate mg/gmFW**	**Exposure**	**Soluble protein mg/g FW**	**Total carbo- hydrate mg/gmFW**
Control	7.2	32.44	**Control**	7.2	32.44	**Control**	7.2	32.44
2 days	7.2	31.2	**2 days**	7.2	32.2	**2 days**	7.2	32.6
4 days	6.8	28.2	**4 days**	6.9	30.1	**4 days**	6.7	31.5
8 days	6.2	25.8	**8 days**	6.6	29	**8 days**	6.5	29.3
Eloor Weland 2- Pre monsoon			**Eloor Weland 2- Monsoon**			**Eloor Weland 2 -Post Monsoon**		
Exposure	**Soluble protein mg/g FW**	**Total carbo - hydrate mg/gmFW**	**Exposure**	**Soluble protein mg/g FW**	**Total carbo - hydrate mg/gmFW**	**Exposure**	**Soluble protein mg/g FW**	**Total carbo- hydrate mg/gmFW**
Control	7.2	32.44	**Control**	7.2	32.44	**Control**	7.2	32.44
2 days	6.8	31.6	**2 days**	7.1	29.4	**2 days**	7.1	29.8
4 days	6.1	27.5	**4 days**	6.9	28.2	**4 days**	6.4	28
8 days	5.5	23.6	**8 days**	6.5	24.6	**8 days**	6	25.1

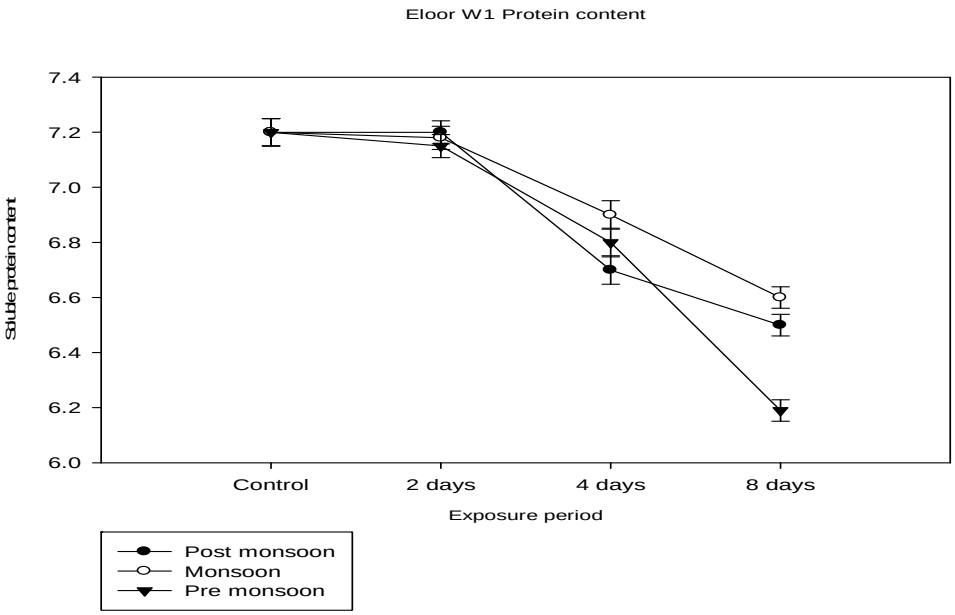

***Fig. 5(a)**: Graphical representation of soluble protein content in S. polyrhiza after2, 4 and 8 days of exposure in water from Wetland 1 (P<0.05).*

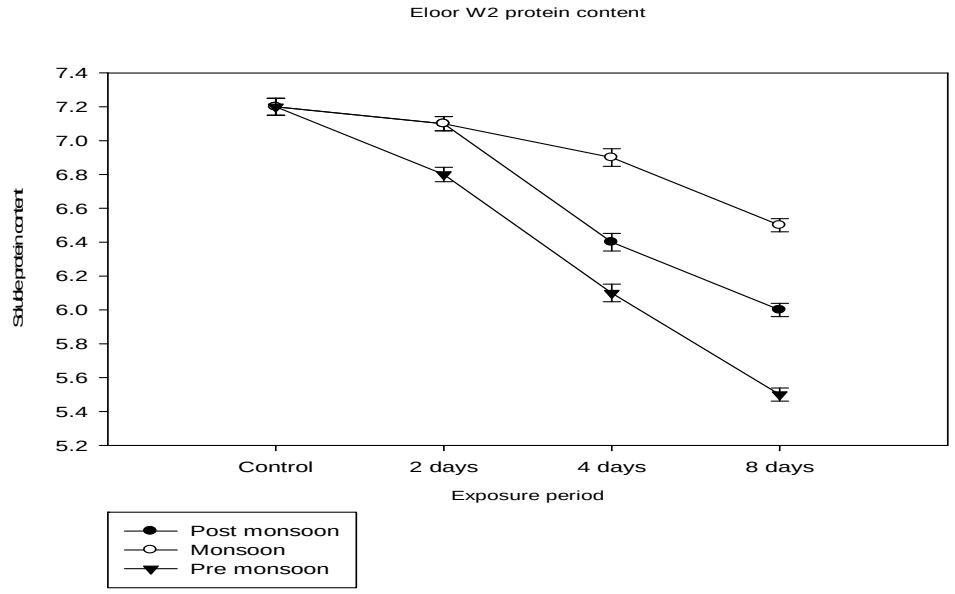

***Fig. 5(b)**: Graphical representation of soluble protein content in S. polyrhiza after2, 4 and 8 days of exposure in water from Wetland 2. Standard deviations were presented by error bars (P<0.05).*

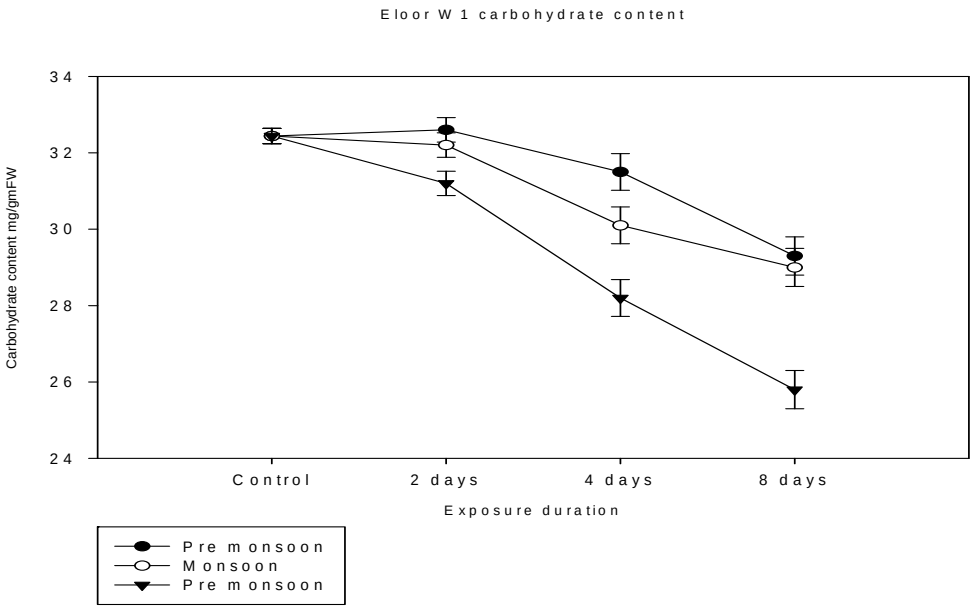

***Fig. 6(a)**: Graphical representation of Carbohydrate content in S. polyrhiza after2, 4 and 8 days of exposure in water from Wetland 1. Standard deviations were presented by error bars. Each values are means of 3 replicates. The significant difference between treatments is P<0.05.*

Pre monsoon period shows maximum reduction in carbohydrate content (10%) after 8 days of treatment in W1. In W2 it was 23% reduction. 0.5% increase in carbohydrate content in pre monsoon period was observed after 8 days of treatment in W1 water. In monsoon W1 sample shows reduction between 1% to 11 %. While in wetland 2 sample it is between 1 to 11 % of inhibition after 8 days. In Post monsoon period protein content decreased between 3 % to 20 % in W1 and it was 2% to 27% in W2 after 8 days *(Table 5, Fig.6a&b).*

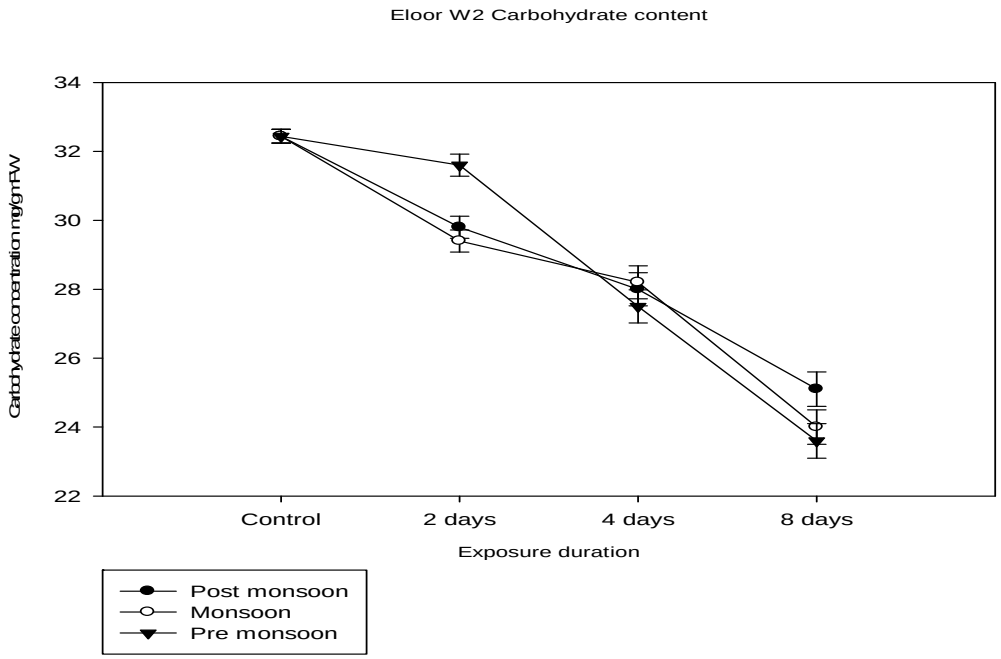

***Fig. 6(b)**: Graphical representation of Carbohydrate content in S. polyrhiza after2, 4 and 8 days of exposure in water from Wetland 2. (P<0.05).*

4.1.1.6. Seasonal variation in morphology of *Spirodela polyrhiza* in Eloor water samples

Duckweed exhibited minor effect with samples from Wl and W2. The results were not significantly different from those for the control samples. It can be concluded from the duckweed test that the monsoon effluent samples contained little or no phytotoxicity. The average root length of *Spirodela polyrhiza* plant is 2- 2.5 cm in control. Visible inhibition in root elongation occurs after 4 days of exposure, the length has been reduced to 1.8 to 2 cm and after 8 days it ends up to 1.5 to 1.7 cm. Meanwhile, root number remains the same as control up to 4 days. After 8 days it reduced to 6 to 10. Leaf area reduced to 0.6 cm^2 from 0.7-0.8 cm^2 after 8 days of treatment *(Table 6).*

Table 6: *Changes in morphological parameters after 2, 4 and 8 days of exposure in different dilutions of wetland 1and 2. Each values are means of 3 replicates. The significant difference between treatments is P<0.05.*

Eloor Weland 1-Post Monsoon			
Exposure	**Root length (Cm)**	**Root number**	**Leaf area (cm^2)**
Control	2-2.5	7 - 12	0.7 - 0.8
2 days	2-2.5	7 - 12	0.7 - 0.8
4 days	2-2.5	7 - 12	0.7 - 0.8
8 days	2	7 - 12	0.7

Eloor Weland 2 -Post Monsoon			
Exposure	**Root length (Cm)**	**Root number**	**Frond area (cm^2)**
Control	2-2.5	7 - 12	0.7 - 0.8
2 days	2-2.5	7 - 12	0.7 - 0.8
4 days	2	7 - 12	0.7 - 0.8
8 days	2	7	0.7

Eloor Weland 1- Monsoon			
Exposure	**Root length (Cm)**	**Root number**	**Leaf area (cm^2)**
Control	2-2.5	7 - 12	0.7 - 0.8
2 days	2-2.5	7 - 12	0.7 - 0.8
4 days	2-2.5	7 - 12	0.7 - 0.8
8 days	2-2.5	7 - 12	0.7 - 0.8

Eloor Weland 2- Monsoon			
Exposure	**Root length (Cm)**	**Root number**	**Frond area (cm^2)**
Control	2-2.5	7 - 12	0.7 - 0.8
2 days	2-2.5	7 - 12	0.7 - 0.8
4 days	2-2.5	7 - 12	0.7 - 0.8
8 days	2	7 - 12	0.7 - 0.8

Eloor Weland 1-Pre Monsoon			
Exposure	**Root length (Cm)**	**Root number**	**Leaf area (cm^2)**
Control	2-2.5	7 - 12	0.7 - 0.8
2 days	2-2.5	7 - 12	0.7 - 0.8
4 days	2-2.5	7 - 12	0.7 - 0.8
8 days	2	7 - 10	0.7

Eloor Weland 2- Pre monsoon			
Exposure	**Root length (Cm)**	**Root number**	**Frond area (cm^2)**
Control	2-2.5	7 - 12	0.7 - 0.8
2 days	2-2.5	7 - 12	0.7 - 0.8
4 days	2	7 - 10	0.7 - 0.8
8 days	2	7 - 10	0.6

In monsoon sample there was no adverse effect as the length was always around 2 cm even after the end of the exposure. Leaf area and root number seems to be unaffected during this season. Root number has been reduced to 7 to 10 range and leaf area remains same as control. During post monsoon period, minor inhibition was noticed.

Root length in control was 2 to 2.5 cm. In W1 sample, during pre monsoon and post monsoon season, it was slightly reduced to 2 cm after 8 days. No inhibition was noticed during monsoon season. Root number was affected more during pre monsoon, as it reduced to 7-10 cm range. Frond area seems to be unaffected in W1sample except in 8 day pre monsoon sample. In W2 sample root length, root number and frond are affected in after 8 days pre monsoon and post monsoon season sample while monsoon season seems to be normal for the plant growth.

4.1.1.7. Analysis of Variance (ANOVA) of seasonal variation in bioassay parameters of *S.polyrhiza* in Eloor W1 and W2 water samples

Table 7: *Two way ANOVA table showing the significance of the effect of wetland water samples from W1 and W2 in Eloor, on various parameters studied on macrophyte Spirodela polyrhiza.*

ASGR					
	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Day	1	17.672	17.672	34.887	0.004 ***
Season	1	13.78	13.78	27.204	0.00425***
Stations	1	0.886	0.886	1.749	0.19235
Day: Season	1	4.217	4.217	8.325	0.00593**
Day: Stations	1	0.419	0.419	0.828	0.36759
Season: Stations	1	0.172	0.172	0.339	0.56315
Day: Season: Stations	1	0.034	0.034	0.068	0.79585
Residuals	46	23.302	0.507		

Td					
	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Day	1	12.087	12.087	21.324	0.003526***
Season	1	27.37	27.37	48.288	0.00193***
Stations	1	2.331	2.331	4.113	0.0484*
Day: Season	1	2.454	2.454	4.33	0.0430*
Day: Stations	1	2.618	2.618	4.619	0.0369*
Season: Stations	1	0.003	0.003	0.005	0.9456
Day: Season: Stations	1	0.446	0.446	0.787	0.3798
Residuals	46	26.073	0.567		
DW FW ratio					
	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Day	1	0.06334	0.06334	12.462	0.000956***
Season	1	0.08047	0.08047	15.831	0.000243***
Stations	1	0.00112	0.00112	0.22	0.640889
Day: Season	1	0.0048	0.0048	0.944	0.336354
Day: Stations	1	0.00183	0.00183	0.36	0.551499
Season: Stations	1	0.00769	0.00769	1.512	0.225076
Day: Season: Stations	1	0.0011	0.0011	0.216	0.644679
Residuals	46	0.23381	0.00508		
Chlorophyll a					
	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Day	1	0.06334	0.06334	12.462	0.000956***
Season	1	0.08047	0.08047	15.831	0.000243***
Stations	1	0.00112	0.00112	0.22	0.640889
Day: Season	1	0.0048	0.0048	0.944	0.336354
Day: Stations	1	0.00183	0.00183	0.36	0.551499
Season: Stations	1	0.00769	0.00769	1.512	0.225076
Day: Season: Stations	1	0.0011	0.0011	0.216	0.644679
Residuals	46	0.23381	0.00508		

Chlorophyll b					
	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Day	1	0.028331	0.028331	72.609	0.00531***
Season	1	0.00093	0.00093	2.384	0.12943
Stations	1	0.000017	0.000017	0.043	0.83718
Day: Season	1	0.000001	0.000001	0.002	0.9678
Day: Stations	1	0.004517	0.004517	11.577	0.00139**
Season: Stations	1	0.004117	0.004117	10.552	0.00217**
Day: Season: Stations	1	0.000476	0.000476	1.221	0.27493
Residuals	46	0.017949	0.00039		
Carotenoids					
	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Day	1	0.008011	0.008011	63.406	0.00336***
Season	1	0.003442	0.003442	27.24	0.00420***
Stations	1	0.00322	0.00322	25.486	0.00742***
Day: Season	1	0.000438	0.000438	3.469	0.06893
Day: Stations	1	0.000316	0.000316	2.503	0.12045
Season: Stations	1	0.00094	0.00094	7.443	0.00899**
Day: Season: Stations	1	0.000983	0.000983	7.783	0.00765**
Residuals	46	0.005812	0.000126		
Protein					
	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Day	1	6.539	6.539	120.055	0.00206***
Season	1	0.444	0.444	8.16	0.00641**
Stations	1	1.325	1.325	24.334	0.0153***
Day: Season	1	0.102	0.102	1.868	0.1784
Day: Stations	1	0.115	0.115	2.114	0.15275
Season: Stations	1	0.197	0.197	3.609	0.06377
Day: Season: Stations	1	0.003	0.003	0.054	0.81691
Residuals	46	2.505	0.054		

Carbohydrates					
	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Day	1	221.46	221.46	575.938	0.0216***
Season	1	17.22	17.22	44.791	0.0264***
Stations	1	86.94	86.94	226.116	0.0216***
Day: Season	1	9	9	23.406	0.0151***
Day: Stations	1	10.66	10.66	27.735	0.0358***
Season: Stations	1	15.81	15.81	41.127	0.0694***
Day: Season: Stations	1	0.41	0.41	1.071	0.306
Residuals	46	17.69	0.38		

4.1.2. Wetlands in Kannamaly

4.1.2.1. Physico – chemical analysis of water sample

The analysis of the physicochemical parameters of water samples collected from wetland 1 and 2 of Kannamaly, Chellanam panchayath, Ernakulam district during different seasons are presented in Table 8. A pH range from 7.8 to 9.0 pH units is acceptable range of pH for the growth of duckweeds. . The pH levels of the surface water samples collected from three stations were alkaline which indicates the presence of ammonia, carbonates, hydroxides and bicarbonates from shellfish wastes. Significant contributions to the suspended solids load are anthropogenic in the form of domestic waste disposal and industrial effluents, agricultural runoff. High concentrations of suspended solids limit the suitability of water as a drinking source. The highest values of TSS were measured in the sample of W2 during monsoon period is due to flow of rain water and turbulence over the muddy bottom. However, in general, the values of TSS in water samples collected from monitoring stations over 3 seasons period were relatively low. Electrical conductivity of water is a simple and useful indicator of the amount of dissolved materials in a solution. The

slightly higher conductivity values, in comparison to other samples, were detected in W1sample of pre monsoon season. The highest concentrations of the other chemical indicators (COD, BOD, total nitrates, phosphates and sulphates) was also observed in W1 sample. Heavy metals like Pb, Cu, Zn, and Cd were also detected in the water samples probably due to the less dilution of water. The analysis of physio chemical parameters are shown in Table 8. The concentrations of Cu, Pb, Zn and Cd and other physio chemical parameters over 3- monitoring periods were, presented here. When compared to other water samples, the concentrations of all measured parameters including heavy metals were the highest in water sample W1collected at the pre monsoon of monitoring period.

Table 8: *Seasonal variation in physico chemical parameters studied in two wetland water samples collected from Kannamaly.*

Sl. No	Parameter (Unit)	CPCB standard	Seasons					
			W1 pre monsoon	W2 pre monsoon	W1 monsoon	W2 monsoon	W1 post monsoon	W2 post monsoon
1	Temp(^{o}C)	29-40	31	28.8	26.3	26.3	28.8	28.8
2	pH	6.5-8.5	9	7.8	7.8	7.23	8.1	7.8
3	Total akalinity(mg/L)	200	250	177	195	168	213	177
4	COD(mg/L)	250	178	711	658	611	638	633
5	EC(µs/Cm)	700	912	261	197	186	228	219
6	BOD(mg O_2/L)	5	15	6	13.52	1.83	3.7	2.02
7	Nitrate(mg/L)	45	12.28	0.91	3.81	0.28	4.01	0.3
8	Phosphate(mg/L)	5	14.42	1.57	1.44	0.06	3.41	0.31
9	Ammonia	0.5	28.09	11	3.06	0.7	5.23	0.75
10	TDS	2100	2811	1722	1722	1533	1318	1113.5
11	TSS	100	55.42	124.89	12.02	188.3	8	152
12	Turbidity	5	50	13.8	180	118.3	88.5	2.22
13	Cu(mcg/L)	1.5	3.41	2.2	2.1	1.8	2.43	1.02
14	Pb(mcg/L)	0.01	4.3	2.68	1.82	2.2	1.8	2.4
15	Zn(mcg/L)	15	60	23.2	44.39	11.13	3.71	58.2
16	Cd(mcg/L)	0.01	2.33	0.47	0.48	0.2	2.33	0.25

4.1.2.2. Inhibition of Growth parameters

During Pre monsoon season 2 day treatment in W1 sample revealed 32% reduction in growth rate than control *(Table 9 & Fig.7a)* while in W2 water sample, there was 22.2% reduction. After 4 days the growth rate reduction in W1 sample was 40% and in W2 reduction occurred by 18%. After 8 days of treatment maximum reduction occurred. In W1 sample it was 56% and in W2 growth rate has been reduced by 40% (*Table 10 & Fig.7b)*.

During monsoon season 2 days of growth in water sample collected from W1 sample reveals reduction of 13%. At the same time W2 sample shows no reduction. 4 day treatment reduced relative growth rate by 20 % and in W2 water it was 14 %. 8 days of treatment shows ASGR reduced by 13 % in W1 sample and 0% in W2.

Table 9: *Relative growth rate based on frond number (ASGR) and Frond doubling time (Td) in Spirodela exposed for 2 days, 4days and 8 days to wetland water samples(W1) collected over 3-seasons. Values are mean of three replicates. Values with significance at P<0.05*

Season/ Treatment	2 days			4 days			8 days		
	Mean N(j)	ASGR	Td	Mean N(j)	ASGR	Td	Mean N(j)	ASGR	Td
control	8	4.09	1.3	10	3.4	1.6	16	3.74	1.4
Pre monsoon	7	2.78	1.89	11.6	2.24	2.4	7.3	1.5	3.57
Monsoon	7.6	3.56	1.52	9	2.7	2	14.6	3.27	1.63
Post monsoon	8	4.04	1.3	8.6	2.45	2.2	14	3.06	1.74
				Mean N(i)=5			Mean T(i)=0		

During Post monsoon season 2 day treatment in W1 and W2 sample revealed no reduction in growth rate compared to control. After 4 days the growth rate reduction in W1 sample was 28% and in W2 water sample reduction occurred by 20%. After 8 days of treatment in W1 sample it was 18% and in W2 sample growth rate has been reduced by 6.4%.

Table 10: *Relative growth rate based on frond number (ASGR) and Frond doubling time (Td) in Spirodela exposed for 2 days, 4days and 8 days to wetland water samples(W2) collected over 3-seasons. Values are mean of three replicates. Values with significance at P<0.05*

Season/ Treatment	2 days			4 days			8 days		
	Mean N(j)	ASGR	Td	Mean N(j)	ASGR	Td	Mean N(j)	ASGR	Td
control	8	4.09	1.3	10	3.4	1.6	16	3.74	1.44
Pre monsoon	7.33	3.15	1.7	14	3.01	1.8	8	1.98	2.63
Monsoon	8	4.06	1.29	9.3	2.9	1.8	16	3.7	1.44
Post monsoon	8	4.01	1.3	9	2.7	2	15.3	3.5	1.51
				Mean N(i)=5			Mean T(i)=0		

Frond doubling time (Td) value will be three or more is an indication of stress and the water is said to be toxic. During the present only 8 days of treatments during pre monsoon period in W1 sample (*Table 9, Fig. 8a)* gives Td value more than 3.0 (Td= 3.6) But in W2 sample the Td value never exceed 3 in all treatments (*Table 10, Fig. 8b)*. It shows healthy growing conditions. The least Td value is obtained during post monsoon in W1 site sample**.**

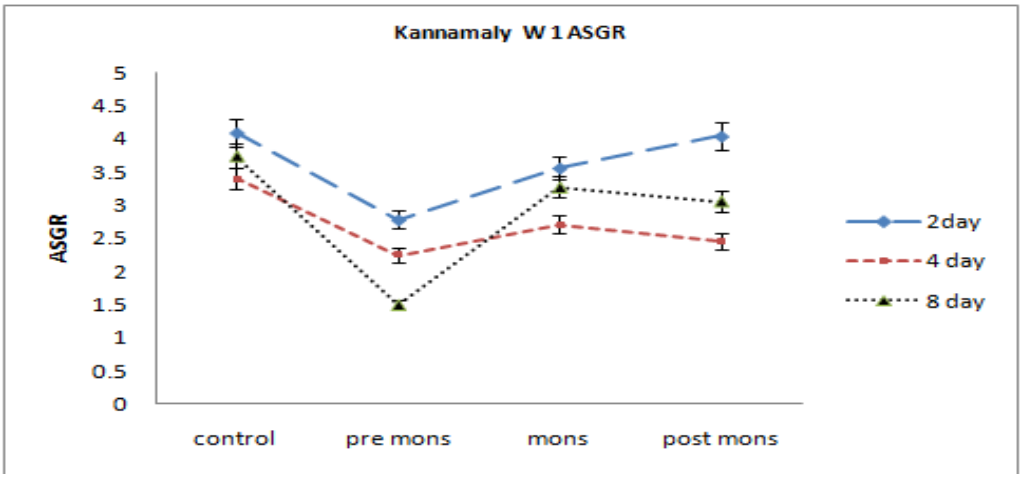

Fig.7(a): *Graphical representation of changes in Average Specific Growth rate (ASGR) in Spirodela culture exposed for 2 days, 4days and 8 days to wetland water samples(W1) collected over 3-seasons ($P<0.05$).*

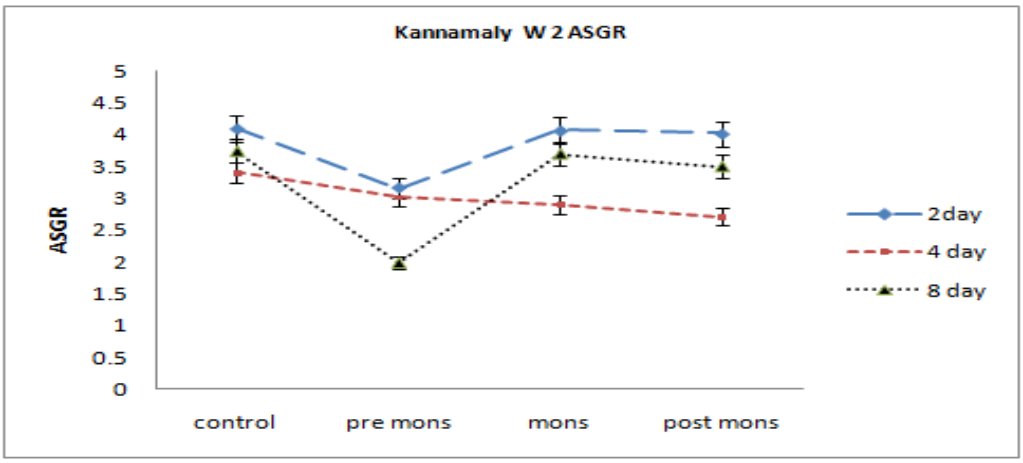

Fig.7(b): *Graphical representation of changes in Average Specific Growth rate (ASGR) in Spirodela culture exposed for 2 days, 4days and 8 days to wetland water samples (W2) collected over 3-seasons. ($P<0.05$).*

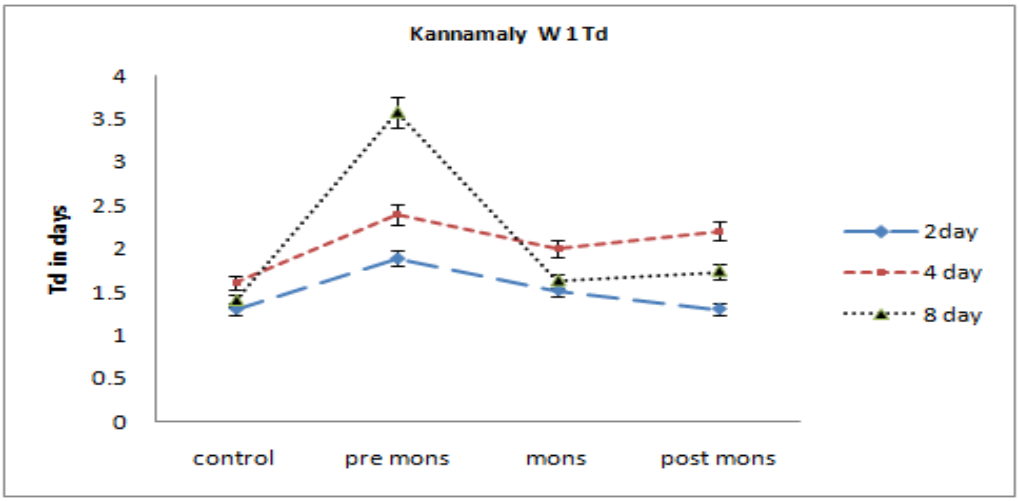

Fig. 8(a): *Graphical representation of changes in frond doubling time(Td) in Spirodela culture exposed for 2 days, 4days and 8 days to wetland water samples(W1) collected over 3-seasons (P<0.05).*

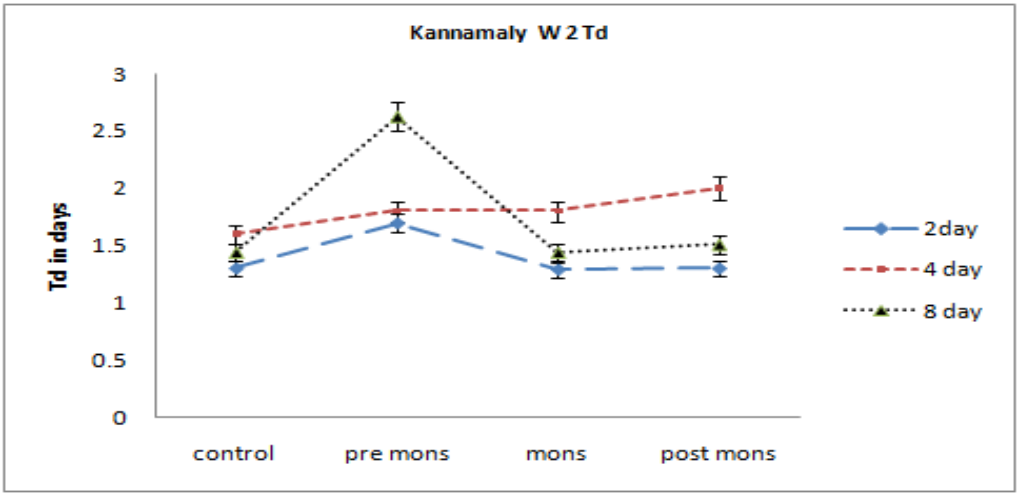

Fig.8(b): *Graphical representation of changes in frond doubling time(Td) in Spirodela culture exposed for 2 days, 4days and 8 days to wetland water samples(W2) collected over 3-seasons (P<0.05).*

4.1.2.3. Seasonal variation in inhibition dry weight/ fresh weight ratio in Kannamaly water samples

During Pre monsoon season 2 day treatment in Wetland 1(W1) water sample revealed 21.5 % reduction in DW/FW ratio than control (*Table 11 & Fig.9a)* while in Wetland 2 (W2) sample, it was 7.28% reduction. After 4 days the biomass ratio reduction in W1 sample was 21.2% and in W2 reduction occurred only by 7.2% (Table *12 & Fig. 9b)*. After 8 days of treatment maximum reduction occurred. In W1 sample it was 18% and in W2 sample DW/FW ratio has not been reduced and remains same as 4 day treatment (7.2%).

Table 11: *The changes in DW/ FW ratio in Spirodela culture exposed for 2 days, 4days and 8 days to wetland water samples of W1 collected over 3-seasons (P<0.05).*

	Wetland 1 Kannamaly				
Season/ Treatment	**2 days**				
	FW initial	**FW final**	**DW initial**	**DW final**	**DW/FW ratio**
control	21.5	34.2	34.2	4.788	139.7
pre monsoon	21.3	22.79	22.79	2.506	109.6
Monsoon	21.2	28.38	28.38	3.405	119.9
Pots Monsoon	21.3	34.4	34.4	4.644	134.8
Season/ Treatment	**4 days**				
	FW initial	**FW final**	**DW initial**	**DW final**	**DW/FW ratio**
control	21.5	77.4	3	10.83	139.9
pre monsoon	21.3	53.01	3.01	5.83	109.9
Monsoon	21.3	68.8	2.55	7.91	114.9
Pots Monsoon	21.2	87.41	3.1	10.49	119.9
Season/ Treatment	**8 days**				
	FW initial	**FW final**	**DW initial**	**DW final**	**DW/FW ratio**
control	21.5	163.4	3.01	22.87	139.9
pre monsoon	21.3	90.3	2.34	10.38	114.9
Monsoon	21.2	146.2	2.55	17.54	119.9
Pots Monsoon	21.3	144.48	3.18	18.06	124.9

Table 12: *The changes in DW/ FW ratio in Spirodela culture exposed for 2 days, 4days and 8 days to wetland water samples of W2 collected over 3-seasons. Values are mean of triplicates. Values with significance at P<0.05.*

	Wetland 2 Kannamaly				
Season/ Treatment	2 days				
	FW initial	FW final	DW initial	DW final	DW/FW ratio
control	21.5	34.2	3	5.13	140
pre monsoon	21.3	31.51	3.01	4.09	129.8
Monsoon	21.2	32.8	2.55	4.42	134.4
Pots Monsoon	21.3	32.6	3.1	4.72	145
Season/ Treatment	4 days				
	FW initial	FW final	DW initial	DW final	DW/FW ratio
control	21.5	76.8	3.01	10.75	139.97
pre monsoon	21.3	65.91	2.34	8.56	129.6
Monsoon	21.3	76.6	2.55	10.72	139.9
Pots Monsoon	21.2	76.5	3.18	10.71	140
Season/ Treatment	8 days				
	FW initial	FW final	DW initial	DW final	DW/FW ratio
control	21.5	162.5	3	22.75	140
pre monsoon	21.3	95.89	3.01	12.46	129.3
Monsoon	21.2	163.4	2.55	23.69	144.8
Pots Monsoon	21.3	154.8	3.1	22.44	144.7

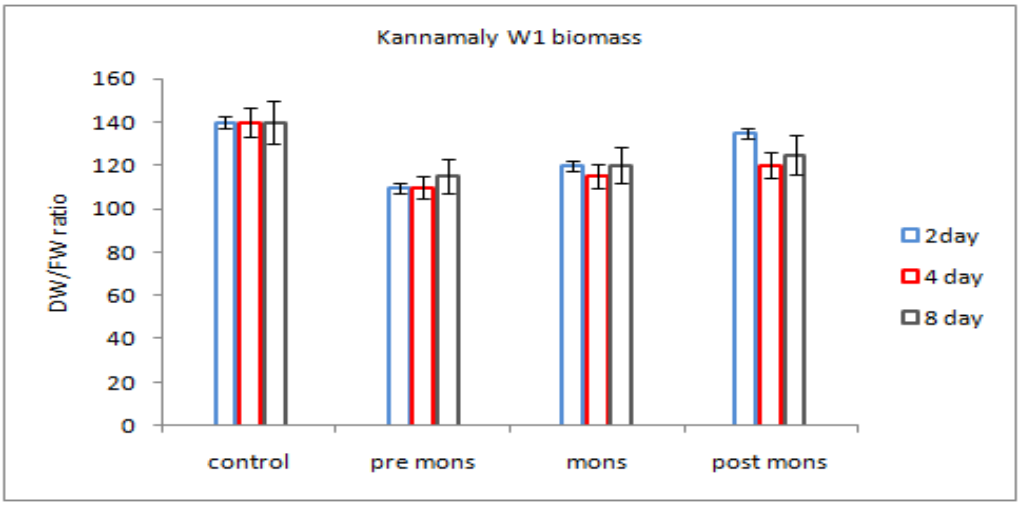

Fig. 9(a): *Graphical representation of changes in DW/FW ratio in Spirodela culture exposed for 2 days, 4days and 8 days to wetland water samples (W1) collected over 3-seasons. Values are mean of triplicates. Values with significance at P<0.05.*

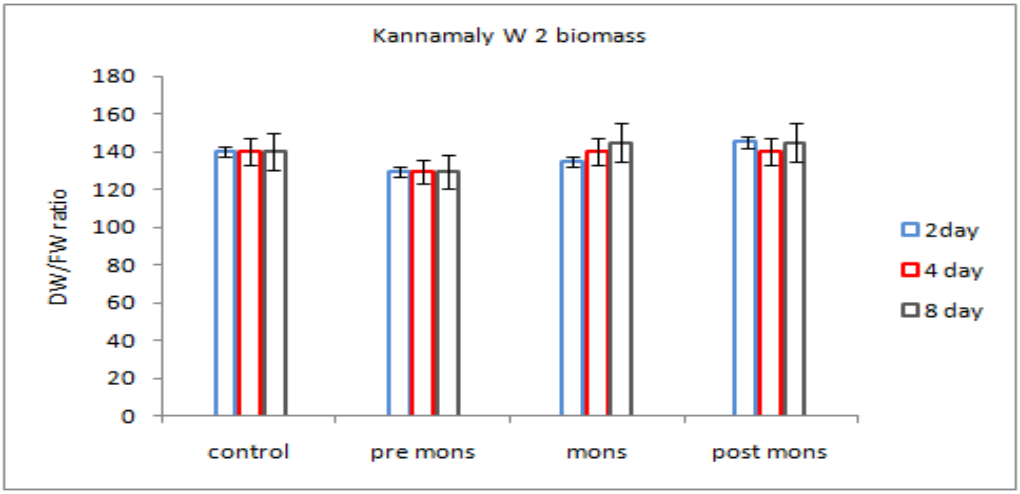

Fig. 9(b): *Graphical representation of changes in DW/FW ratio in Spirodela culture exposed for 2 days, 4days and 8 days to wetland water samples (W2) collected over 3-seasons. Values are mean of triplicates. Values with significance at P<0.05.*

During monsoon season 2 days of growth in water sample collected from W1 reveals reduction of DW/FW ratio by 14 % (*Table 11, Fig. 9a)*. At the same time W2 sample shows 4% reduction. 4 days of treatment reduced biomass ratio by 18 % in W1 and in W2 sample it was 0 %. 8 days of treatment shows ratio reduced by 14 % in W1 and 18 % in W2 sample (*Table 12, Fig. 9b)*.

During Post monsoon season 2 day treatment shows 3.3% reduction in W1 sample and in W2 there was an increase of 3.5% in DW/FW ratio compared to the control. After 4 days the biomass ratio reduction in W1 sample is 14.2 % and in W2 no reduction occurred. After 8 days of treatment in W1 sample, it was 10.6% and in W2 growth rate has been reduced by 18%.

4.1.2.4. Seasonal variation in protein and carbohydrate content in Spirodela polyrhiza plant.

During Pre monsoon season 2 day exposure in Wetland 1 and Wetland 2 water samples revealed no reduction in soluble protein content compared to control. After 4 days the protein content reduction in W1 sample was 7% and in W2 sample reduction was 4.1% (Table *13, fig. 10a &10b*). After 8 days of treatment maximum reduction occurred. In W1 it was 9.7% and in W2 water growth rate has not been reduced and remains same as 4 day treatment (8.3%). During monsoon season 2 days of growth in water sample collected from W1 sample and W2 shows no reduction or increase of soluble protein content. 4 days of treatment increase protein content by 2.7 % in W1 sample and in W2 sample it was 2.7 % of reduction. 8 days of treatment shows increase by 2.7% in W1 and 5.5% decrease in W2.

***Table 13**: Soluble protein content in S. polyrrhiza after2, 4 and 8 days of exposure in water from Wetland 1 and 2. Standard deviations were presented by error bars. Each values are means of 3 replicates. The significant difference between treatments is P<0.05.*

Kannamaly wetland 1- Post Monsoon		
Exposure	**Soluble protein mg/g FW**	**Total carbo hydrate mg/gmFW**
Control	7.2	32.44
2 days	7.28	31.6
4 days	7.15	29
8 days	6.8	26

Kannamaly wetland 1- Monsoon		
Exposure	**Soluble protein mg/g FW**	**Total carbo hydrate mg/gmFW**
Control	7.2	32.44
2 days	7.24	31
4 days	7.2	29.6
8 days	7.16	26.5

Kannamaly wetland 1- Pre Monsoon		
Exposure	**Soluble protein mg/g FW**	**Total carbo hydrate mg/gmFW**
Control	7.2	32.44
2 days	7.2	31.5
4 days	6.7	30.3
8 days	6.5	24.2

Kannamaly wetland 2-Post Monsoon		
Exposure	**Soluble protein mg/g FW**	**Total carbo hydrate mg/gmFW**
Control	7.2	32.44
2 days	7.19	32.1
4 days	6.8	31.6
8 days	6.2	28.1

Kannamaly wetland 2- Monsoon		
Exposure	**Soluble protein mg/g FW**	**Total carbo hydrate mg/gmFW**
Control	7.2	32.44
2 days	7.2	31.9
4 days	7	31.9
8 days	6.8	29.1

Kannamaly wetland 2- Pre Monsoon		
Exposure	**Soluble protein mg/g FW**	**Total carbo hydrate mg/gmFW**
Control	7.2	32.44
2 days	7.2	31.8
4 days	7	29.1
8 days	6.5	25.9

During Post monsoon season 2 day treatment shows 2.7% increase in W1 sample , But in W2 there was no or increase in protein content compared to the control. After 4 days the protein content in W1 shows 2.7 % increase while in W2 5.5% reduction occurred. After 8 days of treatment in W1 sample it was 5.5% reduction and in W2 water protein content has been reduced by 14% (*Table 13, Fig.11a &11b*).

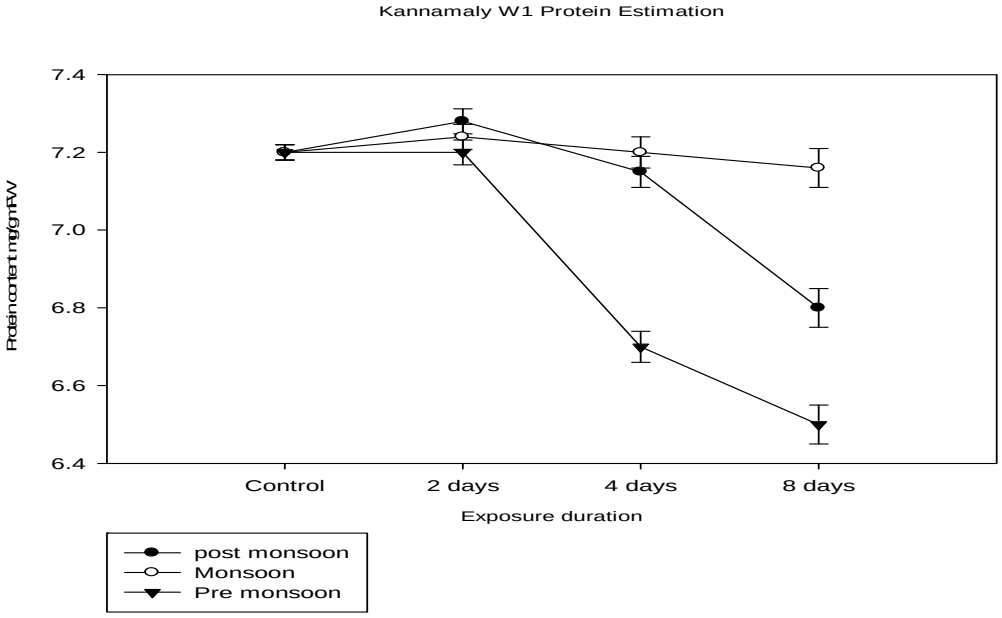

***Fig. 10(a)**: Graphical representation of soluble protein content in S. polyrhiza after2, 4 and 8 days of exposure in water from Wetland 1. Standard deviations were presented by error bars. Each value is mean of 3 replicates. The significant difference between treatments is P<0.05.*

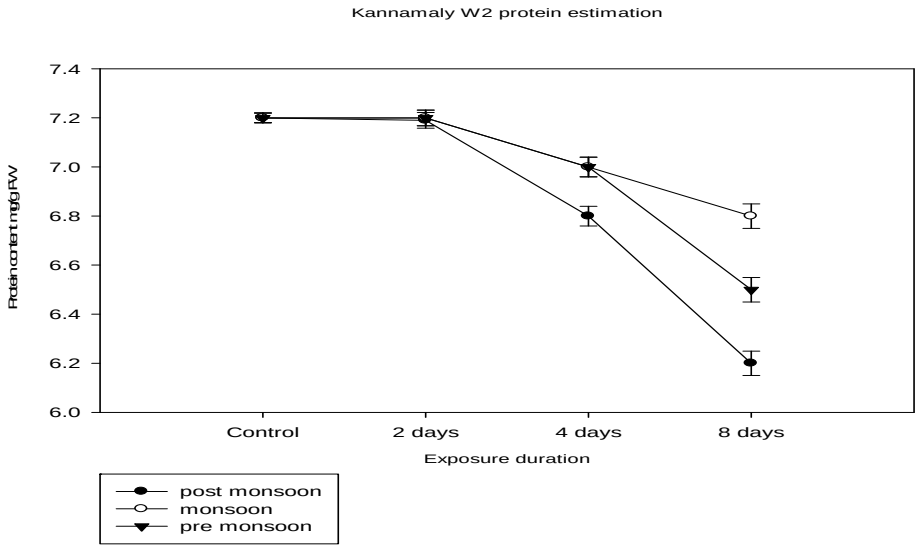

***Fig. 10(b)**: Graphical representation of soluble protein content in S. polyrhiza after2, 4 and 8 days of exposure in water from Wetland 2. Standard deviations were presented by error bars. Each value is the mean of 3 replicates. The significant difference between treatments is P<0.05.*

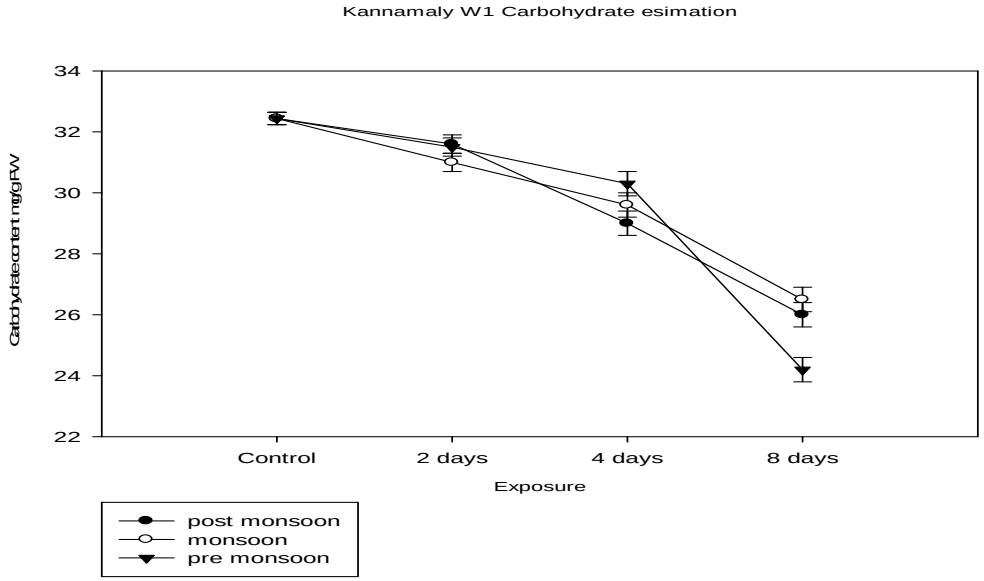

***Fig. 11(a)**: Graphical representation of Carbohydrate content in S. polyrhiza after2, 4 and 8 days of exposure in water from Wetland 1 . Standard deviations were presented by error bars. Each value is the mean of 3 replicates. The significant difference between treatments is P<0.05.*

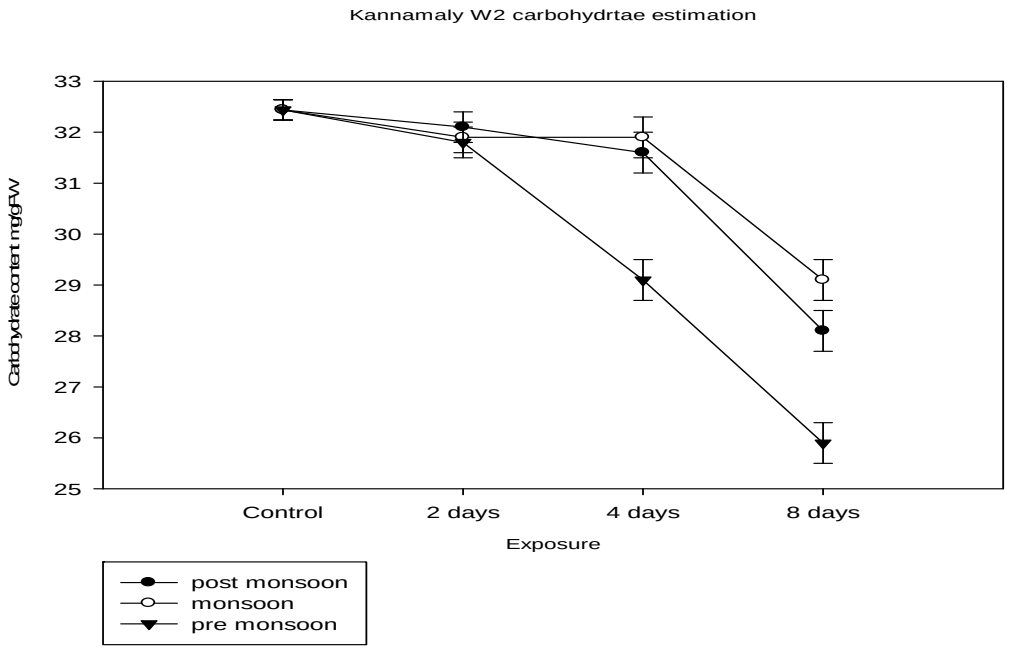

***Fig. 11(b)**: Graphical representation of Carbohydrate content in S. polyrhiza after2, 4 and 8 days of exposure in water from Wetland 2. Standard deviations were presented by error bars. Each value is the mean of 3 replicates. The significant difference between treatments is P<0.05.*

Pre monsoon period shows maximum reduction in Carbohydrate content (25.4%) after 8 days of treatment in W1. In W2 sample it was 20.1% reduction. In Monsoon W1 shows reduction upto 18.3 % and 10.3% in W1 and W2 respectively. In Post monsoon period Carbohydrate content decreased by 3 % to 19.8 % in W1 and it was 13.4% in W2 sample after 8 days *(Table 13, Fig. 11a & 11b).*

4.1.2.5. Seasonal variation in photosynthetic pigment content in Kannamaly water samples

During Pre monsoon season, in W1 sample after 2 days of treatment, 2.1% of reduction of Chlorophyll-a was noted. At the same time there occurred no decrease in W2 sample. After 8 days 19% of reduction of

Chlorophyll- a occurred in W1 and 2.1% of Chlorophyll -a reduction was noted in W2 sample *(Table 14, Fig. 12 a & 12b).* The final concentration was 0.448 mg/g FW. Chlorophyll- b content in control was 0.286 mg/g FW. After 8 days 31% of reduction in Chlorophyll–b content observed in W1 compared to 3.5% of reduction in W2 sample. Carotenoids content in control was 0.24 mg/g FW. After 2 days of treatment, carotenoids content showed slight increase by 0.4% in W1 sample and in W2 sample there was no reduction. After 4 days, there occurred 7.08% of reduction in W1 sample and 3.33% in W2 sample. After 8 days of treatment 16% of reduction occurred in W1 compared to 5% in W2 water *(Table 14, Fig. 12 a & 12b).*

During monsoon season, in W1 sample, no reduction of Chlorophyll- a was noted slight increase of 2.6% initially, while no reduction in W2 sample. After 8 days 10% of reduction occurred in W1. The concentration after 8 days of exposure was 0.412 mg/g FW. In W2 0% of *Chlorophyll a* reduction was noted. Chlorophyll- b content in control was 0.286 mg/g FW. 2 days treatment in W1 water sample shows reduction of Chlorophyll- b by 7% *(Table 14 & Fig.12 c)* while no reduction in W2 sample. After 4 days of treatment Chlorophyll- b content slips down by 10.4% inW1 and 0% of reduction in W2. After 8 days 21% of reduction occurred in W1 compared to 0.3% of increase in W2 water. Carotenoids content in control was 0.24 mg/g FW. After 2 days of treatment Carotenoids show an increase of 1.6% in W1 sample. While in W2 sample there was no change noticed. 8 days of treatment yield 7.5% decrease in W1 and 0% of reduction in W2 water sample *(Table 14, Fig. 12 c & 12 d).*

***Table 14**: Relative changes in concentration of photosynthetic pigments Chlorphyll a, Chlorophyll b and Carotenoid content after2, 4 and 8 days of exposure in water from Wetland 1 and 2. Standard deviations were presented by error bars. Each values are means of 3 replicates. The significant difference between treatments is P<0.05.*

Kannamaly wetland 1- Post Monsoon			
Exposure	Chlorophyll a (mg/g)FW	Chlorophyll b (mg/g)FW	Carotenoids (mg/gFW
Control	0.458	0.286	0.24
2 days	0.475	0.241	0.23
4 days	0.46	0.225	0.208
8 days	0.407	0.204	0.191

Kannamaly wetland 2-Post Monsoon			
Exposure	Chlorophyll a (mg/g)FW	Chlorophyll b (mg/g)FW	Carotenoids (mg/gFW
Control	0.458	0.286	0.24
2 days	0.455	0.285	0.239
4 days	0.455	0.284	0.24
8 days	0.455	0.283	0.24

Kannamaly wetland 1- Monsoon			
Exposure	Chlorophyll a (mg/g)FW	Chlorophyll b (mg/g)FW	Carotenoids (mg/gFW
Control	0.458	0.286	0.24
2 days	0.471	0.284	0.244
4 days	0.446	0.255	0.236
8 days	0.411	0.225	0.222

Kannamaly wetland 2- Monsoon			
Exposure	Chlorophyll a (mg/g)FW	Chlorophyll b (mg/g)FW	Carotenoids (mg/gFW
Control	0.458	0.286	0.24
2 days	0.457	0.286	0.24
4 days	0.457	0.286	0.24
8 days	0.456	0.286	0.24

Kannamaly wetland 1- Pre Monsoon			
Exposure	Chlorophyll a (mg/g)FW	Chlorophyll b (mg/g)FW	Carotenoids (mg/gFW
Control	0.458	0.286	0.24
2 days	0.448	0.26	0.24
4 days	0.422	0.22	0.223
8 days	0.371	0.198	0.201

Kannamaly wetland 2- Pre Monsoon			
Exposure	Chlorophyll a (mg/g)FW	Chlorophyll b (mg/g)FW	Carotenoids (mg/gFW
Control	0.458	0.286	0.24
2 days	0.458	0.284	0.24
4 days	0.45	0.282	0.232
8 days	0.439	0.276	0.228

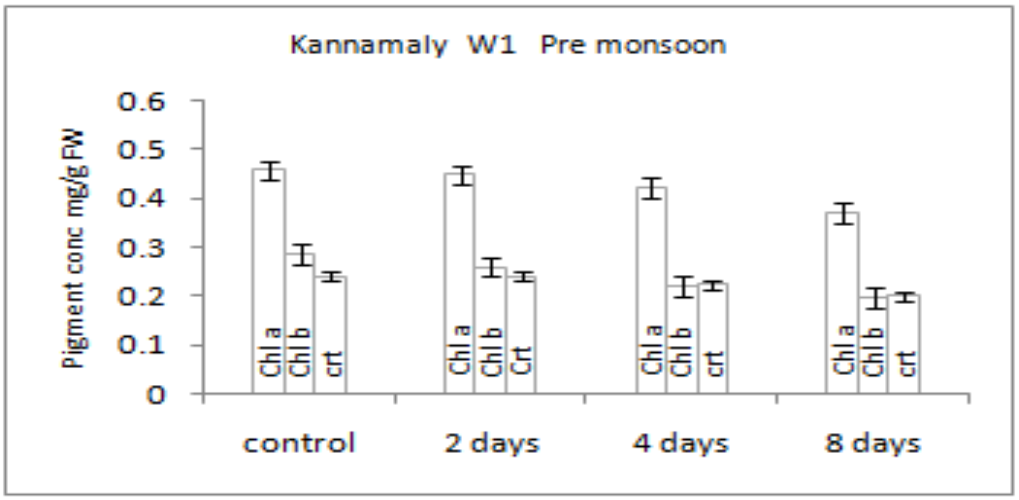

***Fig. 12(a)**: Relative changes in concentration of photosynthetic pigments Chlorphyll a, Chlorophyll b and Carotenoid content after2, 4 and 8 days of exposure in water from Wetland 1 during pre monsoon season. Standard deviations were presented by error bars. Values are means of 3 replicates. The significant difference between treatments is P<0.05.*

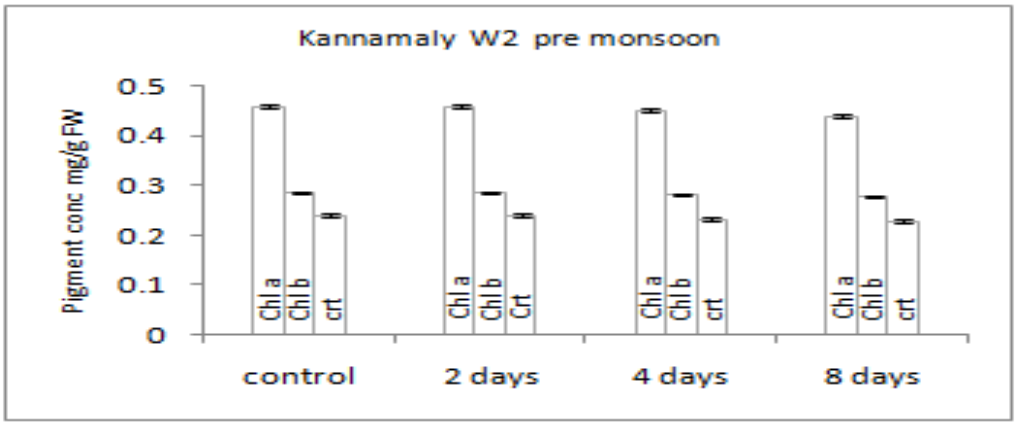

***Fig. 12(b)**: Relative changes in concentration of photosynthetic pigments Chlorphyll a, Chlorophyll b and Carotenoid content after2, 4 and 8 days of exposure in water from Wetland 2 during pre monsoon season. Standard deviations were presented by error bars. Values are means of 3 replicates. The significant difference between treatments is P<0.05.*

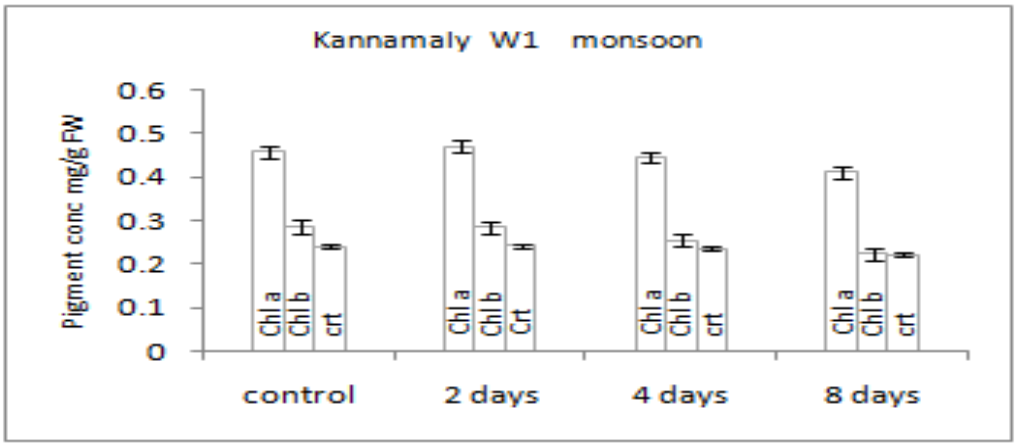

***Fig. 12(c)**: Relative changes in concentration of photosynthetic pigments Chlorphyll a, Chlorophyll b and Carotenoid content after2, 4 and 8 days of exposure in water from Wetland 1 during monsoon season. Standard deviations were presented by error bars. Values are means of 3 replicates. The significant difference between treatments is P<0.05.*

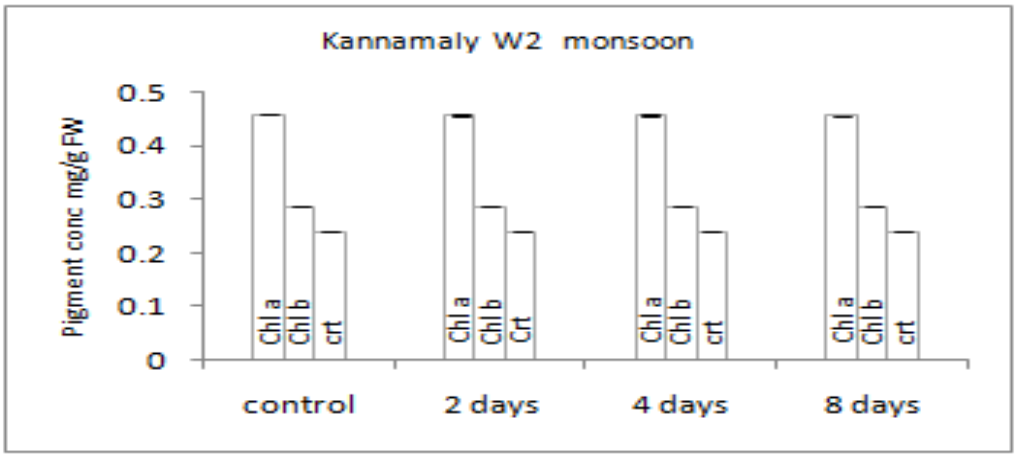

***Fig. 12(d)**: Relative changes in concentration of photosynthetic pigments Chlorphyll a, Chlorophyll b and Carotenoid content after2, 4 and 8 days of exposure in water from Wetland 2 during monsoon season. Standard deviations were presented by error bars. Values are means of 3 replicates. The significant difference between treatments is P<0.05*

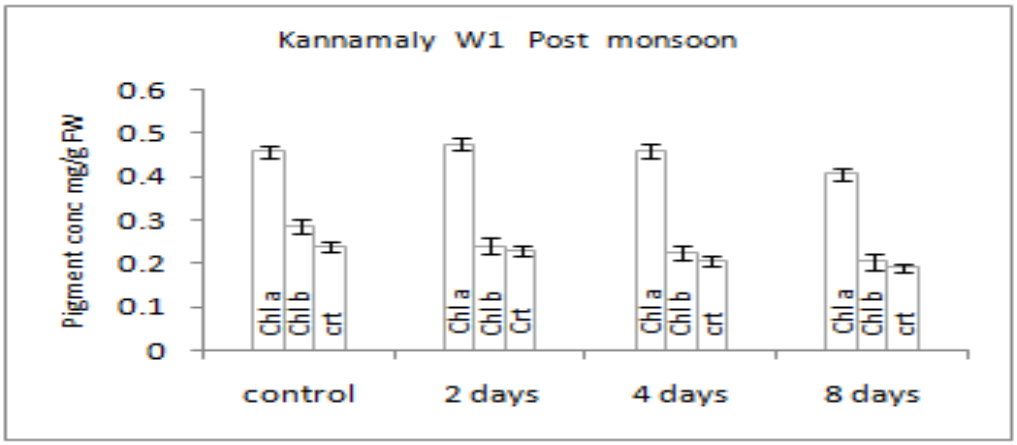

Fig. 12(e): *Relative changes in concentration of photosynthetic pigments Chlorphyll a, Chlorophyll b and Carotenoid content after2, 4 and 8 days of exposure in water from Wetland 1 during post monsoon season. Standard deviations were presented by error bars. Values are means of 3 replicates. The significant difference between treatments is P<0.05.*

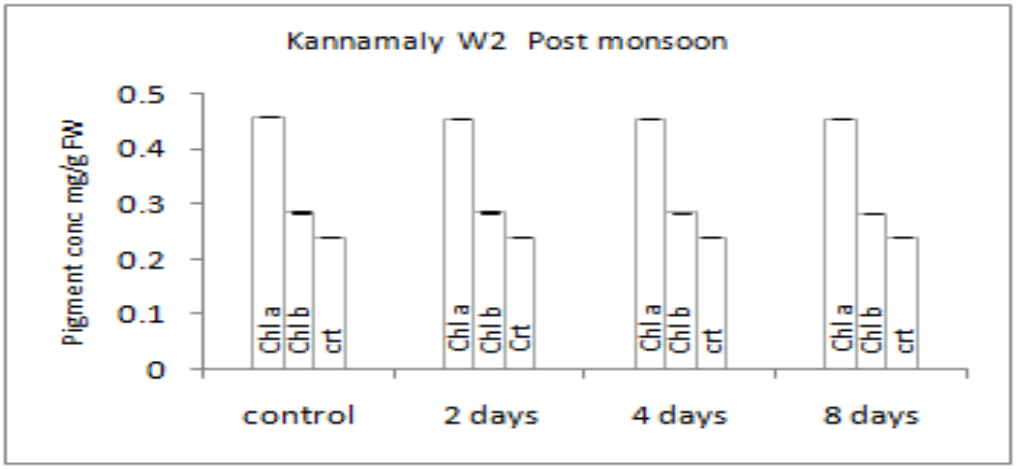

Fig. 12(f)*: Relative changes in concentration of photosynthetic pigments Chlorphyll a, Chlorophyll b and Carotenoid content after2, 4 and 8 days of exposure in water from Wetland 2 during post monsoon season. Standard deviations were presented by error bars. Values are means of 3 replicates. The significant difference between treatments is P<0.05.*

During post monsoon season, in W1 sample after 2 days of treatment, there occurred slight increase of 4% *(Table 14 & Fig. 12 e & 12 f)*. In W2 sample the reduction of 0.43% was noted in the same season. After 8 days 10% of reduction occurred in W1 sample. The concentration after 8 days of exposure was 0.412 mg/g FW. In W2 sample again 0.43% of Chlorophyll- a reduction was noted. The final concentration was 0.456 mg/g FW. Chlorophyll- b content in control was 0.286 mg/g FW. During post monsoon season, after 8 days 29% of reduction occurred in W1 compared to 1% of reduction in W2. Carotenoids content in control was 0.24 mg/g FW. After 2 days of treatment, carotenoids content reduced by 4% in W1 sample . But in W2, no reduction occurred. After 4 days, there occurred 13% of reduction in W1 and no reduction in W2. After 8 days of treatment 20% of reduction occurred in W1 compared to 0% in W2 water sample *(Table 14 & Fig. 12 e & 12f).*

***Fig.12(g)**: Comparison of Spirodela plant culture after 8 days of exposure in water sample from Kannamaly Wetland 1(left) and Wetland 2(right) during pre monsoon season. W1 sample exposure produces chlorosis on the fronds.*

4.1.2.6. Seasonal variation on the morphology of *Spirodela polyrhiza* plant in Kannamaly wetland water samples

Phytotoxicity is a measured phenomenon, but not a property of a sample. During toxicity testing, various morphological parameters can be used to measure the presence of toxicity, such as root length, root number and frond area In a single compound toxicity test, the cause-effect relationship is clear-cut, while in complex effluents, toxicity can be affected by such factors as inhibitor(s), interaction of inhibitors, matrix of constituents, speciation, temperature, and pH (Wang, 1987). In this study, phytotoxicity was used as a general term to denote the presence of adversity in a test sample in comparison with the water control.

The average root length of *Spirodela polyrhiza* plant is 2- 2.5 cm in controlled condition. In pre monsoon water sample from W1 site, the length remains around 2 cm after 2 days. Visible inhibition in root elongation occurs after 4 days to 8 days of exposure, and then the length has been reduced to 1.7 cm. Mean while root number remains the same as control up to 4 days. After 8 days it reduced to 6 to 10. Leaf area reduced to 0.6 cm^2 from 0.7-0.8 cm^2 after 8 days of treatment *(Table 15).* In monsoon sample there was no adverse effect as the length was always around 2 cm even after the end of the exposure. Leaf area and root number seems to be least affected during this season. Root number has been reduced to 7 to 10 range and leaf area remains same as control. During post monsoon period, minor inhibition was noticed. The length reduction occurred from 4 to 8 days of exposure as it reduced from 2 cm to 1.9 cm in average. Root number shows minor reduction from 7-12 range to 7-10 range. frond area reduced to 0.6-0.7 cm^2 after 8 days of treatment with W1 water sample *(Table 15).*

Table 15: *Relative changes in morphological parameters like Root length, Root number and Leaf area after2, 4 and 8 days of exposure in water from Wetland 1 and 2. Standard deviations were presented by error bars. Each values are means of 3 replicates. The significant difference between treatments is P<0.05.*

Kannamaly Weland 1-Post Monsoon			
Exposure	**Root length (Cm)**	**Root number**	**Leaf area (cm^2)**
Control	2-2.5	7 to 12	0.7 - 0.8
2 days	2	7 to 12	0.7 - 0.8
4 days	1.8 -2.0	6 to 11	0.7-0.7
8 days	1.7-1.9	6 to 10	0.6-0.7

Kannamaly Weland 1- Monsoon			
Exposure	**Root length (Cm)**	**Root number**	**Leaf area (cm^2)**
Control	2-2.5	7 to 12	0.7 - 0.8
2 days	2-2.5	7 to 12	0.7 - 0.8
4 days	2-2.5	7 to 12	0.7 - 0.8
8 days	2	7 to 10	0.7 - 0.8

Kannamaly Weland 1-Pre Monsoon			
Exposure	**Root length (Cm)**	**Root number**	**Leaf area (cm^2)**
Control	2-2.5	7 to 12	0.7 - 0.8
2 days	2	7 to 12	0.7 - 0.8
4 days	1.8 -2.0	7 to 12	06-0.7
8 days	1.5-1.7	6 to 10	0.6

Kannamaly Weland 2 -Post Monsoon			
Exposure	**Root length (Cm)**	**Root number**	**Frond area (cm^2)**
Control	2-2.5	7 to 12	0.7 - 0.8
2 days	2-2.5	7 to 12	0.7 - 0.8
4 days	2-2.5	7 to 12	0.7 - 0.8
8 days	2	7 to 10	0.7 - 0.8

Kannamaly Weland 2- Monsoon			
Exposure	**Root length (Cm)**	**Root number**	**Feaf area (cm^2)**
Control	2-2.5	7 to 12	0.7 - 0.8
2 days	2-2.5	7 to 12	0.7 - 0.8
4 days	2-2.5	7 to 12	0.7 - 0.8
8 days	2	7 to 10	0.7 - 0.8

Kannamaly Weland 2- Pre monsoon			
Exposure	**Root length (Cm)**	**Root number**	**Feaf area (cm^2)**
Control	2-2.5	7 to 12	0.7 - 0.8
2 days	2-2.5	7 to 12	0.7 - 0.8
4 days	2-2.5	7 to 12	0.7 - 0.8
8 days	1.8-2.0	7 to 10	0.7

In pre monsoon water sample from W2 site, the length remains around 2-2.5 cm after 4 days. Visible inhibition in root elongation occurs only after 8 days of exposure, and then the length has been reduced to 1.8-2.0 cm. Mean while root number remains the same as control up to 4 days. After 8 days it reduced to 7 to 10. Leaf area reduced narrowly to 0.7 cm^2 from 0.7-0.8 cm^2 after 8 days of treatment. In monsoon sample there was no adverse effect as the length was always around 2 cm even after the end of the exposure. Leaf area and root number seems to be least affected during this season. Root number has been reduced to 7 to 10 range and leaf area remains same as control *(Table 15).* During post monsoon period, minor inhibition was noticed. The length reduction occurred after 8 days of exposure as it reduced from 2-2.5 cm to 2 cm in average. Root number shows minor reduction from 7-12 range to 7-10 range. Leaf area shows no reduction even after 8 days of exposure. Studies have shown that heavy metals affect root cell elongation and decrease mitotic activity (Ouzounidou *et*.al.1995) thus inhibiting root growth and development.

4.1.2.7. Analysis of variance (ANOVA) of seasonal variation in bioassay parameters of *S. polyrhiza* in Kannamaly W1 and W2 water samples

Table 16: *Two way ANOVA table showing the significance of the effect of wetland water samples from W1 and W2 in Kannamaly, on various parameters studied on macrophyte Spirodela polyrhiza.*

ASGR					
	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Day	1	2.766	2.766	9.904	0.00356**
Season	1	4.966	4.966	17.78	0.00019***
Stations	1	1.369	1.369	4.901	0.03409*
Day: Season	1	0.39	0.39	1.396	0.24616
Day: Stations	1	0.033	0.033	0.117	0.7349
Residuals	32	8.938	0.279		

Td					
	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Day	1	2.266	2.266	10.635	0.00205**
Season	1	2.614	2.6136	12.266	0.00101**
Stations	1	0.716	0.7165	3.362	0.07291
Day: Season	1	2.356	2.3561	11.057	0.0017
Day: Stations	1	0.281	0.2808	1.318	0.25668
Residuals	48	10.228	0.2131		
DW/FW ratio					
	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Day	1	24	24	1.803	0.1857
Season	1	1859	1859	141.451	0.0017***
Stations	1	4743	4743	360.947	0.00356***
Day: Season	1	54	54	4.142	0.0474
Day: Stations	1	31	31	2.345	0.1323
Residuals	48	631	13		
Chlorophyll a					
	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Day	1	0.013017	0.013017	173.65	0.003886**
Season	1	0.00354	0.00354	47.23	0.00356***
Stations	1	0.004779	0.004779	63.75	0.00226***
Day: Season	1	0.000236	0.000236	3.15	0.0822
Day: Stations	1	0.00888	0.00888	118.46	0.00356***
Residuals	48	0.003598	0.000075		
Chlorophyll b					
	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Day	1	0.0067	0.0067	47.094	0.00356**
Season	1	0	0	0.01	0.00019***
Stations	1	0.03236	0.03236	227.408	0.03409*
Day: Season	1	0.0003	0.0003	2.077	0.23616
Day: Stations	1	0.00519	0.00519	36.474	0.4349
Residuals	48	0.00683	0.00014		

Carotenoids					
	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Day	1	0.00304	0.00304	37.488	0.003654**
Season	1	0.000047	0.000047	0.576	0.00201
Stations	1	0.003504	0.003504	43.212	0.07291
Day: Season	1	0.000075	0.000075	0.921	0.342
Day: Stations	1	0.001905	0.001905	23.489	0.00505***
Residuals	46	0.003892	0.000081		
Protein					
	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Day	1	2.6206	2.6206	41.536	0.00201
Season	1	0.1179	0.1179	1.868	0.06291
Stations	1	0.7257	0.7257	11.502	0.0014**
Day: Season	1	0.0287	0.0287	0.454	0.5036
Day: Stations	1	0.2479	0.2479	3.929	0.0532
Residuals	46	3.0284	0.0631		
Carbohydrates					
	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Day	1	239.93	239.93	354.55	0.00405***
Season	1	7.56	7.56	11.175	0.00101***
Stations	1	24.54	24.54	36.257	0.012291***
Day: Season	1	5.47	5.47	8.081	0.0607
Day: Stations	1	5.64	5.64	8.335	0.25668
Residuals	46	32.48	0.68		

Discussions:

The details of the physiochemical characteristics of water collected from Eloor and Kannamaly wetland sites are given in Table 1and 8. The samples were taken in pre monsoon, monsoon and post monsoon periods because, the nature of water changes with the season. The analysis shows most of the

parameters are beyond the standards published by Central Pollution Control Board, COPOCS, (1996). In the case of pollution caused by effluents, the degree and extent of pollution can be assessed through changes in physio-chemical parameters.

Temperature governs to a large extent the biological species present and their rates of activity. The variation in temperature in different seasons in the present study was mainly due to the climatic changes of the environment. In pre-monsoon season, the average water temperature ranged from 29.25 to 31.8^0C between stations 1 to 7. During post monsoon season, temperatures ranged from 25.15 to 26.43^0C, respectively. The values of electrical conductance reaches maximum level during summer season and there was corresponding decrease in the values during monsoon season. The present study is in accordance with a study carried out on river Ganga (Rao *et* al., 1990).

The industrial effluents and domestic wastewater which enters into wetland sites in Eloor and Kannamaly may add significant quantity of organic matter and inorganic material that contribute to turbidity. In pre-monsoon and post monsoon seasons the turbidity values at Eloor W1 were 29 NTU each and in W2 they were 382 and 362 NTU, respectively. In Kannamaly , pre monsoon and post monsoon analysis showed W1 with turbidity 50 and 88.5 NTU and W2 with 13.8 and 2.2 NTU respectively. The results showed that there was huge difference in the values may be due to the proximity of Kuzhikkanadam creek in Eloor W2 site and the waste discharge near W1 site in Kannamaly from Sea food processing plant. Turbidity values increase as a consequence of the flow of rainwater during monsoon season carrying suspended particles and the discharge of industrial effluents (Unni , **1**985).

With relatively small changes in pH, a significant change in water quality may take place. Many activities like trace metal complexation, precipitation, biological uptake and their respective reverse pathways are all highly pH dependent (Pal *et* al., 1986) . Several authors have observed alkaline pH values in the polluted water bodies (Unni, 1985). The findings in the current study are in accordance with those findings. The results show that the alkalinity values were high during pre-monsoon season in both Kannamaly and Eloor. It was reported that alkalinity higher than 50 mg/l indicates that the water body receives effluents in considerable amount (Raina *et* al., 1984). The current results are in tune with these findings since all the samples from Eloor and Kannamaly shows values exceeding 50 mg/L. As per the permissible limit fixed by CPCB (up to 200 mg/l), the alkalinity values were well below the threshold concentration. In Kannamaly W1 pre monsoon and post monsoon analysis shows alkalinity above 200 mg/L.

Higher values of TDS were found during the pre-monsoon season. This increase in TDS values can be attributed to the discharge of untreated industrial wastewater reaches all the stations. High amount of total dissolved solids were observed due to industrial pollution (Khare and Unni ,1986). All the values of TDS were well within the limits of wetland water standards of 2100 mg/l except for pre monsoon season in Kannamaly. In Eloor TDS exceeds the limit in all samples except in W1 monsoon and post monsoon seasons. The oxygen-demanding nature of biodegradable organics is of utmost importance in natural water systems. From the results it was observed that there was a sudden increase in the BOD and COD values in samples collected from W1 to W2 stations in Eloor and from W2 to W1 in Kannamaly. The results showed that the BOD and COD values were on higher side during the pre-monsoon season. The BOD

values decreased due to decomposition of organic waste in water. The maximum value of BOD and COD were recorded in W2 pre monsoon season sample from Eloor and W1 pre monsoon season sample from Kannamaly. The results showed that the BOD values were on higher side during the pre-monsoon season.

The NO_3^- concentration was high for samples taken from Eloor W1 during pre monsoon season but falls within the standards prescribed by CPCB. The presence of nitrate is probably due to mixing of effluents from FACT fertilizer plants. In Kannamaly highest value of 12.3 mg/L was obtained for nitrate during the same season from W1. The highest phosphate concentration with 13.6 mg/L obtained in Eloor during pre monsoon sample. Similar trend was visible in case of Kannamaly W1 as the PO_4^{2-} concentration was only 14.2 mg/L obtained for post monsoon season. The results are in full agreement with the study results presented by Usharani *et*.al. (2010). The sulphate content was very low in W1 monsoon sample and very high in W2 pre monsoon sample in Eloor. The observed values are in line with the reported values of Areerachakul *et* al. (2009).

The hierarchy of heavy metals in water was present in the order of Zn >Cr>Cu>Pb>Ni>Co>Mn>Hg>Fe>Cd in all stations of Eloor and in order of Zn>Pb> Cu >Cd in all the seasons (*Table 1*) W1 sample lacks Fe and cadmium as exception. In Kannamaly Zn, Cu, Pb and Cd were present in all samples. All metals are found to be above the CPCB standards in all collected samples. During monsoon, all of the metals were found beyond the CPCB standards. Even high dilution rate of wetland water during monsoon season could not bring down metal concentration within the limit. The important observation of this study is that, concentration of all heavy metals in the water sample of Eloor W2

is highest of all. Another important finding is the presence of Pb, Cu and Zn in all samples collected from Eloor and Kannamaly. Current results are in line with the findings in KSPCB Report (2000-2001).

The concept of average specific growth rate is based on the general exponential growth pattern of duckweed, where toxicity is estimated on the basis of the effects on the growth rate. The use of average specific growth rate for estimating toxicity is scientifically preferred. ASGR is determined by counting the frond number. The average specific growth rate for a specific period is calculated as the logarithmic increase in the growth variables like frond number for each replicate of control and treatments.

In all seasons of treatments the chronic exposure has tremendous influence on growth rate and frond doubling time. Growth rate comes down after 8 days of treatments. In Eloor, maximum reduction of 55% in W1 and 73% in W2 were observed after 8 days of exposure. Similarly in Kannamaly maximum reduction 56% and 40% were found in W1 and W2 respectively at the end of exposure period. Mackenzie *et* al. (2003) found that, beside frond area, growth rate based on frond number is the most sensitive end point for detecting chronic toxicity (8d) in landfill leachates.

In earlier studies, it was determined that seasonal stress causes a considerable decrease in the number of plants and dry weights of the leaves, stems, and roots of plants (Ali Dinar, 1999). Demirezen (2007) indicated that the highest growth rate was observed at monsoon and post monsoon seasons. This experimental work is in agreement with the results obtained in the present study. In Eloor and Kannamaly, Pre monsoon season samples from both W1

and W2 showed greatest reduction in growth rate and maximum frond doubling time. This may be due to the concentration of pollutants in summer season

The RGR value of *S. polyrhiza* was noted to be significantly inhibited due to the increase in the level of various contaminants in W1 water sample. This may be attributed to the high degree of sensitivity of these plants that are subjected to the physiological stress caused by various factors and the presence of heavy metals in comparison with the control plants. According to Perfus-Barbeoch *et* al. (2002), heavy metals present in the water might have affected the growth rate (ASGR) of the plant. In Eloor study, W1 and W2 water analysis revealed presence of large number of heavy metals in water sample. Eloor W2 has more heavy metal content because of its proximity to Kuzhikkandam creek. It clearly reflects in estimation of growth rate and frond doubling time as Growth inhibition was highest in W2 samples.

The nutrient concentration of the water in which duckweed resides greatly affects its growth rate. The growth rate of *Spirodela* reflects the idea that duckweed can absorb large amounts of nutrients such as nitrogen and phosphorus (Monette *et* al., 2006). The growth rate was almost similar to control or exceeds control values in some treatments. For example in Eloor study 2 days treatment with W1 and W2 samples during monsoon and post monsoon period showed no signs of growth reduction. In Kannamaly also some W2 samples during monsoon season showed no reduction. In post monsoon season also it is quite clear that growth of *Spirodela* was unaffected during this season in W1 sample after 2 days of exposure.

At higher temperature during pre monsoon season growth rate was lower. In Kannamaly W2 sample, because of less pollutants ASGR reduced to

1.98 during the same season compared to 1.5 in W1. Td outside 2.5 days means hostile growth conditions for the plant. In the present study highest Td were observed during 8 day treatment of pre monsoon season in W1 and W2 samples (F value: 3.362, Pr (>F): 0.07291***). The fastest doubling rate was observed during monsoon season in both wetlands. It can be concluded that W1 is much polluted than W2 may be due to the proximity to seafood processing industry. It is clear that there is high growth rate occurs during monsoon followed by post monsoon and pre monsoon season except in 2 day treatments. The treatment of 2 days during post monsoon shows high growth rate than any other treatment period. The less dilution of toxicants during pre monsoon may affect the growth rate in pre monsoon period.

Currently frond number is considered to be the less reliable in comparison with other growth end points observed in (final biomass, frond area and dry weight) toxicity assay. It is probably due to the fact that frond count is irrelevant to frond size or biomass. It has frequently been observed that under toxic stress small buds may protrude and be counted as individual fronds (Mohan and Hosetti, 1999). However, in the present study frond number proved to be more sensitive parameter than biomass.

Changes in Dry weight- fresh weight ratio indicate that in exposed *Spirodela* plants, growth retardation takes place in comparison to the control. Dry to fresh weight ratio (DW/FW) measured at the end of the 2, 4 and 8 days exposure has shown inverse relation in comparison with ASGR. The parameter significantly decreased in all samples but most in the water sample collected during pre monsoon monitoring period in Eloor W2 (42.8%, 50% and 57% decrease in comparison to control was recorded during 2, 4 and

8 days respectively). In Kannamaly WI highest DW/FW ratio was found during post monsoon season after 2 days of exposure (F value: 141.451 Pr (>F): 6.45e-16***). The least ratio was found during monsoon sample after 2 and 4 days of exposure. In the treatment with W2 samples, *Spirodela* shows highest DW/FW ratio during monsoon season after 8 days of exposure. The least ratio was found during pre monsoon sample after 4 days and 8 days of exposure.

The results of the tests were consistent with the physio chemical changes in the water. It has been shown that accumulation of heavy metals may disturb the plant water status which eventually results in osmotic stress and growth reduction (Perfus-Barbeoch *et* al., 2002; Poschenrieder and Barcelo, 2004). During 2day and 4 day post monsoon monitoring DW/FW ratio showed 7.4% and 0.3% increase respectively with respect to the control. The low value of dissolved oxygen can be attributed to the high organic matter content, coupled with high summer temperatures. It could thus change of the plant's water status and accumulation of compatible solutes. The findings of Garnczarska and Ratajczak (2000) and John *et* al. (2008) corroborate the hypothesis.

Eloor Wetland water analysis shows that a large number of heavy metals in W1 and W2 water samples. Eloor W2 samples consists more heavy metals compared to W1. In Kannamly W1 water analysis reveals presence of heavy metals than W2. Duckweeds are known for its affinity towards metals and tolerate metallic pollution at low concentrations. It has been shown that chronic exposure of plants to toxic metal concentrations generally causes the fast inhibition of cell elongation and expansion (Poschenrieder and Barcelo., 2004; Srivastava *et* al., 2006). Although the interactions in our multimetallic

samples considerably limit possibility to explain the results for single metals, the growth reduction observed in our study could be due to the suppression of the elongation growth rate of cells exerted by Cu, Pb, Zn and Ni.

The increase in biomass ratio (Hormesis) in wetland 1 of Eloor and Kannamaly, may be due the presence of excess nutrients present in wetland I as the result of constant discharge of wastewater from the industries. Untreated waste input in high amounts of nutrients, such as nitrogen and phosphorus, which contribute to the eutrophication of wetland waters. This increase in primary productivity is one probable cause for the persistent hypoxic zone affecting much of the Gulf of Mexico during the summer months (Rabalais *et* al., 1994, 1996). It has shown to double in mass every two days (Skillicorn *et* al., 1993) and can remove 75% of total phosphorus and nitrogen in a eutrophicated water body (Cheng *et* al., 2002).

Accumulation of heavy metals by plants in natural conditions is influenced by a number of abiotic factors, pH, presence of cations in the waters of the reservoir, temperature, intensity of photosynthetic light and the exposure period, as well as biotic factors, specific features (*e.g.* hyper accumulation capacity and resistance to high concentration of pollution), metal storage and detoxification forms by a plant as well as interaction with other compounds present in a cell (Kabziński, 2007). The research showed that temperature influences sorption of metals by macrophytes. It was confirmed that with the increase of temperature within the range from 278 to 293 K, sorption efficiency increases. It is supposed that temperature influences the change of cell membrane lipids composition, which may support metals sorption (Fritioff *et* al., 2005) The current study

reveals that maximum biomass reduction in pre monsoon season when temperature is high, supports this findings.

Chlorophylls and carotenoids are the central part of the energy manifestation of every green plant system and therefore, any significant alteration in their levels is likely to cause a marked effect on the entire metabolism of the plant. The productivity of plants is directly related with changes in the content of photosynthetic pigments. Chlorophyll-a, chlorophyll-b and Carotenoids content showed changes when compared to control in W1 and W2 sample of Eloor and Kannamaly. Industrial wastewater not only affects the chlorophyll content but the chlorophyll activity also (Song and Huang, 2001; Baron *et* al., 1995; Lewis, 1995). In the presence of Co, Cu, Fe, Mn, Ni, Pb and Zn, a decrease in photosynthetic pigments quantity in chlorophyll -a and b and damage of its structure were observed (Kupper *et* al., 1996). It was stated that the phenomenon may be caused by replacement of magnesium ions by heavy metals in chlorophyll particles, which results in reduced catchment of photons and limited photosynthesis process. Some heavy metals, even at low concentrations, can cause oxidative stress, which stops chlorophyll biosynthesis and accelerates lipids pre-oxidation, which leads to damage of cellular membranes. The influence of heavy metals on the changes of physical and chemical parameters of the tested organisms was also emphasized (Harguinteguy *et* al., 2013).

In Eloor, at the end of the 8 days of exposure, duckweed grown on pre monsoon and post monsoon especially wetland 2 water samples showed signs of necrosis (Fig. 4g). Accordingly, a marked decrease in chlorophyll -a and b contents compared to the control was detected in plants exposed to pre

monsoon water samples. The carotenoids content was much less affected in comparison with those of chlorophylls. Significant decrease of carotenoids was observed only in the water sample collected during pre monsoon season in W2 (34%). The decline in total chlorophyll and carotenoids contents as well as growth inhibition can be regarded as general responses associated with metal toxicity. The loss of photosynthetic pigment content has been reported in duckweed plants following exposure to Cu, Pb and Ni (Axtell et al., 2003; Hou *et* al., 2007; Kanoun-Boule *et* al., 2008). The degradation of chlorophyll or the inhibition of its biosynthesis, has been proposed as being responsible for photosynthesis and growth reduction caused by Zn, Ni, Cu and Pb (Kupper *et* al., 1996). The destruction of photosynthetic pigments by heavy metals could be due to: impairment of the electron transport chain, the replacement of Mg^{2+} ions associated with the tetrapyrrole ring of chlorophyll molecules, inhibition of important enzymes (Van Assche and Clijsters,1990) associated with chlorophyll biosynthesis or peroxidation processes in chloroplast membrane lipids by the reactive oxygen species (Sandalio *et* al., 2001).

In Kannamaly, on contrary to Eloor, photosynthetic pigments were not inhibited to greater extent. Duckweed leaves didn't show any signs of chlorosis (pigment loss) following 8days of exposure to monsoon and post monsoon surface water samples. In pre monsoon season treatment mild chlorosis could be observed *(Fig.12 g)*. The duckweed grown in the nutrient rich medium had a higher growth rate than the nutrient lacking population. These findings were in agreement with previous findings reported by Lacoul *et* al. (2006), in which the productivity of aquatic plants is not likely to be limited by the abundance of phosphate and inorganic nitrogen.

Brief exposure with W1 and W2 sample during monsoon and post monsoon season in Eloor and Kannamaly treatment yielded increase in Chlorophyll-a content. The tolerances of macrophytes to mild industrial pollutants including heavy metals were studied earlier. The plant *Elodea canadensis* is, incubated briefly in the solutions with higher lead concentrations (0.24 and 0.48 mcg/L), did not shown reduction in chlorophyll- a and chlorophyll- b, and the "ageing" symptoms occurred only after 168 hours (Temel, 2005). It was also confirmed that after 5 days of incubation in Cu and Ni solution with concentration of 0.01 mcg/L, there was no slowing down of photosynthetic dyes; at greater concentrations of this metal, the plant defense mechanisms fails thus, inhibits photo assimilation capacity. (Malec *et* al., 2009). Chlorophyll-*b* concentration decrease due to greater damage of pollutants present in water samples.

The fluctuations of protein content in *Spirodela polyrhiza* at different stations recorded here may be due to the influence of physicochemical factors along with the variations in vegetative growth, metabolism and development of the plant. During monsoon and post monsoon season high protein content were detected in all the treatments in Eloor and Kannamaly samples. During this period, the environmental parameters such as pH and high amount of nutrients and low heavy metal concentration created a congenial atmosphere for the luxuriant and healthy growth of the plant (Al-Sabunji, 2002). The weather conditions which were quite unfavorable for the growth and development during non-monsoon period became conducive by the onset of monsoon. During this season the plants were in the juvenile and growing stage with lots of meristematic tissue (Environnement Canada, 1999). This may be the reason for high levels of protein content during that period. This rapid growth of plant took place

accompanied by the formation of new tissues and the protein content also increased to the maximum and it was in agreement with the reports of Gangadevi (1997) who reported maximum protein content during monsoon period in different macroalgae collected from the south west coast of India. The increase in protein content may also be due to the nitrate content in the sampling site.

During pre monsoon conditions, both in Eloor and Kannamaly, the elevated amount of toxic constituents, particularly metal ions negatively affected amino acid level. Similar effect of Pb and chromium ions on amino acid biosynthesis via inhibition of nitrate reductase and limiting reduced nitrogen availability is reported in aquatic macrophytes (Sinha *et* al., 2002). The higher concentrations of heavy metals in industrial wastewater exposed plants after 72 and 96 hrs also indicate tissue injury and lowering of protein content. Decrease in protein content was recorded in Cu and Pb exposed *L. minor* plants (Singh et al., 1994). Heavy metal cadmium, which is known for its harmful effect on protein metabolism in plant, present in Eloor and Kannamaly sampling sites (*Table 8*). The stress caused by cadmium cations activated proteins induction - HSP 70 antibodies, belonging to chaperones, the so called chaperone proteins (Garnczarska and Ratajczak, 2000). At a concentration of Cd with 0.01 mcg/L, there was a 25% increase of antibodies and at the concentration of Cd 0.1 mcg/L, the antibodies increase was 70%. Chaperones can be responsible for the resistance of *Lemna minor* on the toxic influence of Pb and Cd. This gives it an advantage over other macrophytes and the possibility to survive in the conditions lethal to other water plant species (Sergio *et* al., 2007).

*Spirodel*a plants grown in water collected from different stations showing varying carbohydrate content may be attributed to the input of environmental variations that affect the different factors which influence carbohydrate synthesis. Vegetative growth as well as development might have also influenced the fluctuations in the carbohydrate concentration. The climatic factors, hydrological conditions and sediment characteristics may have influenced the vegetative growth and metabolism altering the concentration of soluble carbohydrates in the plant tissue (Hagemeyer, 1999). In Eloor and Kannamaly total carbohydrate content reduces with exposure time in all samples. After 8 days of treatment reduction occurred between 10% to 20 % in W1 and 22 to 27 % in W2 sample. The low content of soluble carbohydrate suggested that plants consumed a large amount of soluble sugar to maintain the basic physiological functions, such as photosynthesis and respiration. Our results were in agreement with the report of Samarakoon and Rauser (1979) that changes of carbohydrate and photosynthesis in leaves of *Phaseolus vulgaris* under excess Ni stress. The carbohydrate content shows slight increase during post monsoon season in W1 sample. The lower concentrations of pollutants are known to enhance photosynthesis whereas highly concentrated water of pre monsoon season turns to be inhibitory (Borah and Yadav, 1996). In the present study, low exposure favoured starch, free sugar and reducing sugar biosynthesis. Since reducing sugars act as substrate for oxidative pathway, the extra energy requirement of plants under stress is fulfilled by a rapid increase in their level. This might be achieved either by not permitting the conversion of total sugars of dark reaction into starch or by enhancing the hydrolysis of starch into reducing sugars with increase in stress quantum.

Reduced root development, root number and frond area are indicative of stress in the immediate environment of the plant. The result suggests that the hygrophyte is an ideal material for assessment of sub-lethal perturbations, in agreement with reported references for a decreased plant growth in terms of number and biomass, and a reduced morphological development with an increased pollution stress under controlled conditions (Roshon, *et* al., 2000).

4.2. Toxicity and Bioaccumulation of Copper and Lead in aquatic macrophyte *Spirodela polyrhiza*

Aquatic plants are known to accumulate heavy metals. In this study, duckweed plant *Spirodela polyrhiza* were exposed to different concentrations of Cu and Pb. Various physio-biochemical parameters (growth and biomass, photosynthetic pigment content, soluble protein, soluble sugars, metal absorption and bio concentration factor (BCF) were studied. At lower metal concentrations, an increase in protein and sugar was observed but at higher concentrations their decrease was noticed. Uptake of the metals was concentration dependent. The results suggest that the *S.polyrhiza* can be effectively used as a phytoremediator for wastewater polluted with heavy metals like Cu and Pb at moderate concentrations.

4.2.1. Growth rate, Frond doubling time and Percentage of inhibition

The growth rate is similar to control at 1 mcg/L concentration. At 10 mcg/L, there was 18% decrease in growth rate. At 20 mcg/L there was 27% decrease and at 40mcg/L there was sharp decline in growth rate (64%). Finally there was 73% decrease at 80 mcg/L exposure. Lead is toxic even at 1 mcg/L when compared to Copper.

Table 17: *Relative growth rate based on frond number (ASGR) , Frond doubling time (Td) and Percentage of Inhibition of growth in duckweed Spirodela polyrhiza exposed to different concentrations of Cu and Pb. Values are mean of three replicates. Different letters indicate significantly different values at P<0.05. (N(i)=5, T(j)=8 and T(i)=0).*

Metal	Medium	mean Nj	mean ASG (μ)	Td	%Ir
mcg/L	control	16	3.72	1.45	0
Cu	1	16	3.72	1.45	0
	10	14	3.04	1.77	18.17
	20	13	2.71	2	27.26
	40	9	1.35	4	63.62
	80	8	1.01	5.33	72.72
Pb	1	15	3.38	1.6	9.08
	10	14	3.04	1.77	18.17
	20	12	2.37	2.28	36.35
	40	10	1.69	3.2	54.53
	80	9	1.35	4	63.62

Table 18: *ANOVA of Relative growth rate based on frond number (ASGR) , Frond doubling time (Td) and Percentage of Inhibition of growth in duckweed Spirodela polyrhiza exposed to different concentrations of Copper. Values are mean of three replicates. ASGR and Td values are highly significant with P <0.05.*

ANOVA- Average Specific Growth rate:						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	2299.398	3	766.466	3.247444	0.043505	3.098391
Within Groups	4720.426	20	236.0213			
Total	7019.824	23				
ANOVA- Frond doubling time						
Between Groups	2257.805	3	752.6015	3.158562	0.04726	3.098391
Within Groups	4765.469	20	238.2734			
Total	7023.273	23				
ANOVA- Percentage of Inhibition						
Between Groups	2355.002	3	535.3568	3.310711	0.04863	3.098391
Within Groups	4744.0051	20	751.9966			
Total	7095.112	23				

Table 19:*ANOVA of Relative growth rate based on frond number (ASGR) , Frond doubling time (Td) and Percentage of Inhibition of growth in duckweed Spirodela polyrhiza exposed to different concentrations of Lead. Values are mean of three replicates. ASGR and Td values are highly significant with P <0.05.*

ANOVA- Average Specific Growth rate:						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	2295.893	3	765.2978	3.246483	0.043544	3.098391
Within Groups	4714.627	20	235.7314			
Total	7010.521	23				
ANOVA- Frond doubling time						
Between Groups	2345.002	3	781.6674	3.310708	0.04103	3.098391
Within Groups	4722.055	20	236.1028			
Total	7067.057	23				
ANOVA- Percentage of Inhibition						
Between Groups	2355.002	3	525.3568	3.21041	0.04933	3.072191
Within Groups	4235.001	20	722.9966			
Total	7115.112	23				

There was a reduction of 9% in growth rate at 1mcg/L exposure. Growth rate further reduced by 18%, 36%, 55% and 64% in exposure with 10 mcg/L, 20 mcg/L, 40 mcg/L and 80 mcg/L concentration (*table 17*).

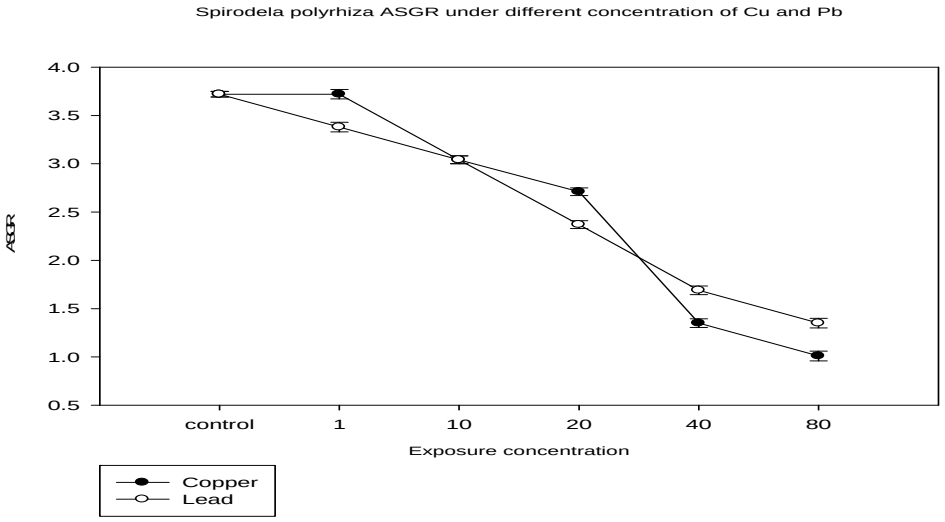

Fig.13: *Graphical representation of relative growth rate based on frond number (ASGR) in duckweed Spirodela polyrhiza exposed to different concentrations of Cu and Pb. Values are mean of three replicates. Values are different with significance P<0.05. (N(i)=5, T(j)=8 and T(i)=0).*

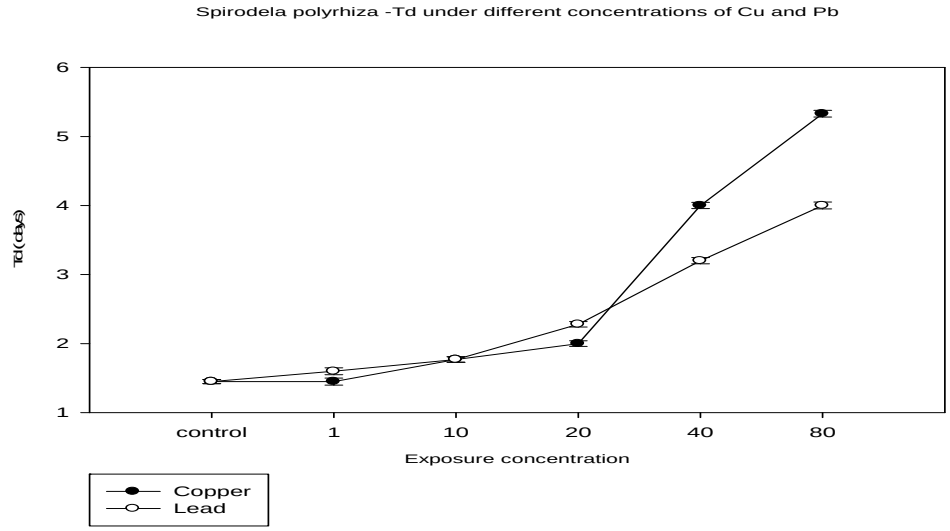

Fig.14: *Graphical representation of Frond doubling time (Td) in duckweed Spirodela polyrhiza exposed to different concentrations of Cu and Pb. Values are mean of three replicates. Values are different with significance P<0.05. (N(i)=5, T(j)=8 and T(i)=0).*

At 1 mcg/L of Copper there were zero inhibition (*Table 17 & Fig.15)*. There was a linear increase in inhibition rate from 10mcg/L to 80mcg/L exposure. At 80 mcg/L Ir% (inhibition rate percentage) was 73%. Against Lead Ir% is 9% even at I mcg/L concentration. Ir% doubled when concentration changes from 10 to 20mcg/L. Here also there was a linear increase in inhibition. At 80 mcg/L Ir% was 63. It means Pb is much toxic to plants (*Table 17 & Fig. 16)*.

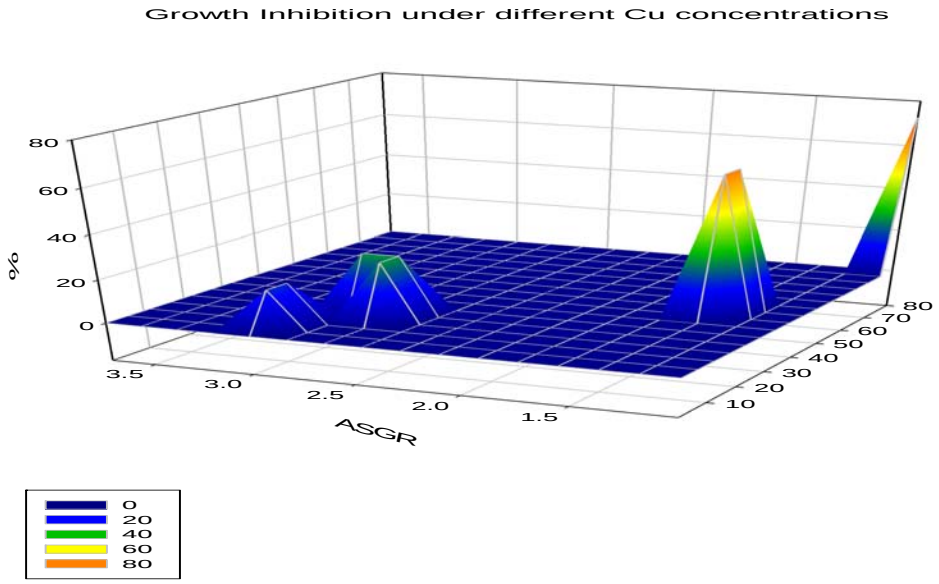

Fig. 15: *A 3D representation of percentage of inhibition of growth rate in duckweed Spirodela polyrhiza exposed to different concentrations of Cu. Values are mean of three replicates. Values are different with significance P<0.05. (N(i)=5, T(j)=8 and T(i)=0).*

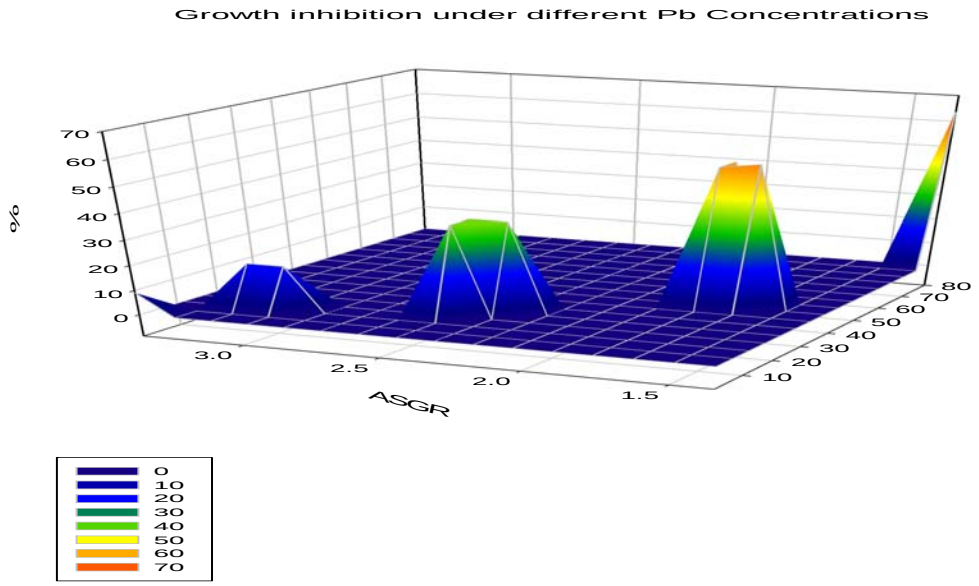

Fig. 16: *A 3D representation of percentage of inhibition of growth rate in duckweed Spirodela polyrhiza exposed to different concentrations of Pb. Values are mean of three replicates. Values are different with significance P<0.05. (N(i)=5, T(j)=8 and T(i)=0).*

In control Td value was 1.45. At 1mcg/L Cu it resembles control. An increase in Td had noticed from 10mcg/L onwards. Td values were 1.77% (10 mcg/L), 2% (20 mcg/L), 4% (40 mcg/L) and 5.3(80 mcg/L). At 1 mcg/L Pb takes more time for doubling than Copper. At 10 mcg/L Td values were same as Cu. At 20 mcg/L Td values were 2.28. At 40 mcg/L and 80 mcg/L Td values further decreased by 3.2% and 4% respectively (*Table 17 & Fig.13 and 14*).

4.2.2. The inhibition of morphological parameters

The average root length of *Spirodela polyrhiza* plant is 2- 2.5 cm in control. No visible inhibition in root elongation occurred after 8 days of exposure with 1 mcg/L Copper sulphate (*Fig. 17*) . But the length has been

reduced to 2 cm after 40 mcg/L treatment. It further reduced to 1.5 cm in average length after treatment with 80 mcg/L. Meanwhile root number follows the same trend as root length. Root number reduces to 7 around in average as the concentration reaches 80 mcg/L. Average frond area of the plant in control is 0.7-0.8 cm^2. Frond area remains the same at upto 10 mcg/L treatment. It remains 0.7-0.6 cm^2 average till the end (80 mcg/L) *(Table 20).*

When the plant is exposed to various concentration of Pb, again inhibition in morphological parameters observed. Root length seems to be unaffected till 20 mcg/L exposure. Then it reduced to 2 cm but remains the same upto 80 mcg/L exposure. Meanwhile root number remains 7-12 range upto 40 mcg/L and slips down to 7-10 at the end. Average frond of the plant is 0.7-0.8 cm^2. Leaf length remains the same until the end. *(Table 20).*

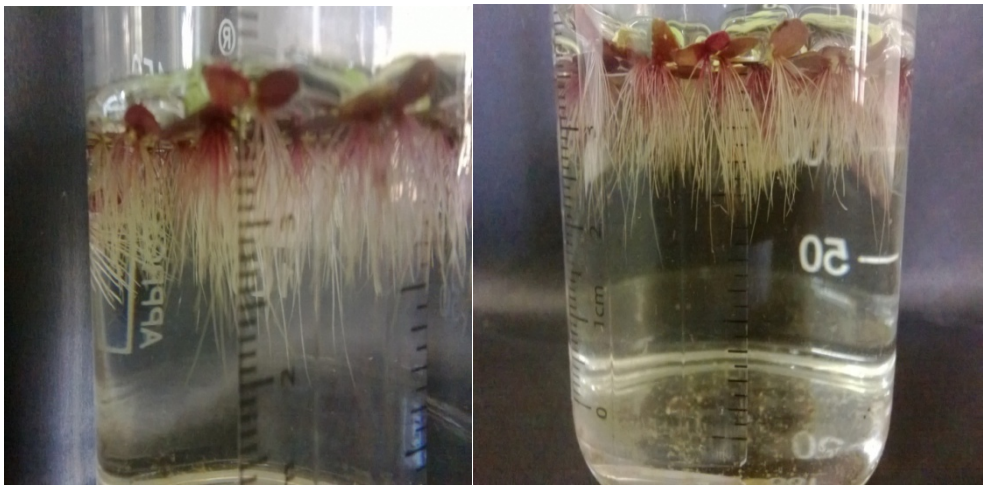

Fig. 17: *Images showing inhibition of root length and root number in duckweed Spirodela polyrhiza exposed to 80 mcg/L concentrations of Pb and Cu. Lead (left) is less inhibitory on the plan root than copper (right).*

Table 20: *Morphological parameters measured in duckweed Spirodela polyrhiza exposed to different concentrations of Cu and Pb. Values are mean of three replicates. Values are different with significance P<0.05.*

Conc. of Copper	Root length	Root number	Frond area (cm sq.)
Control	2-2.5cm	7 to 12	0.7-0.8
1mcg/L	2-2.5cm	7 to 12	0.7-0.8
10 mcg/L	2.0 cm	7 to 10	0.7-0.8
20 mcg/L	0.9 cm	7 to 9	0.7-0.6
40 mcg/L	0.8cm	6 to 7	0.6-0.5
80 mcg/L	0.7	6 to 7	0.4

Conc. of Lead	Root length	Root number	Frond area (cm sq.)
Control	2-2.5cm	7 to 12	0.7-0.8
1mcg/L	2.0cm	7 to 12	0.7-0.8
10 mcg/L	2.0 cm	7 to 10	0.7
20 mcg/L	0.8 cm	7 to 9	0.6-0.5
40 mcg/L	0.7cm	6 to 9	0.5
80 mcg/L	0.6cm	6 to 8	0.5

4.2.3. Inhibition of biomass and growth index (G.I.)

In control *Spirodela polyrhiza* plant shows Growth index one (0 % reduction). At 1 mcg/L of Pb exposure GI shows slight increment by 0.89% because of stimulatory effect of low Lead concentration. At 10 mcg/L exposure it shows 6.3 % reduction. It was followed by 12%, 18% and 45% of reduction in GI during 20%, 40% and 80% of exposure respectively. At lower concentration, Copper is more stimulatory than Lead. An increase in GI upto 74%, 21% and 16 % noticed during the exposure with 1mcg /L, 10 mcg/L and 20 mcg/L. after that there was a reduction of 20% and 58% after the exposure of 40 mcg/L and 80 mcg/L of copper respectively (*Table 21 & fig. 18*).

Table 21: *Biomass and Growth Index measured in duckweed Spirodela polyrhiza exposed to different concentrations of Cu and Pb. Values are mean of three replicates. Values are different with significance P<0.05.*

Pb mcg/L	Biomass (mg DW)	Growth Index
Control	7.773	1
1	7.8933	1.008
10	7.3067	0.93
20	6.87	0.87
40	6.41	0.822
80	4.1767	0.552

Cu mcg/L	Biomass (mg DW)	Growth Index
Control	7.7867	1
1	13.64	1.744
10	8.8367	1.205
20	10.0267	1.16
40	6.18	0.796
80	3.3067	0.419

Table 22: *ANOVA of Growth Index (GI) based on biomass in duckweed Spirodela polyrhiza exposed to different concentrations of Copper. Values are mean of triplicates. ASGR and Td values are highly significant with P <0.05.*

ANOVA- Growth Index with Pb treatment						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	2651.856	3	883.9521	3.760089	0.027289	3.098391
Within Groups	4701.762	20	235.0881			
Total	7353.618	23				
ANOVA- Growth Index with Cu treatment						
Between Groups	2615.443	3	871.8143	3.706777	0.02862	3.098391
Within Groups	4703.894	20	235.1947			
Total	7319.337	23				

At a concentration higher than 0.3 mcg/L, copper decreased considerably the biomass (70%) and caused the photosystem alteration by reducing electron transport. This effect was manifest by a rapid development of chlorosis; after

exposing *Lemna* fronds to the cupric ions (2 or 3 hours), the fronds colour changed from green to yellow and some fronds were separated from the colonies. These results are very different from those reported by Zayed *et* al. (1998). The authors used *L. minor* for the phytoaccumulation of copper in quarter-strength Hoagland's solution at pH = 6; the lowest copper concentration causing > 10% growth reduction was 5 mcg/L.

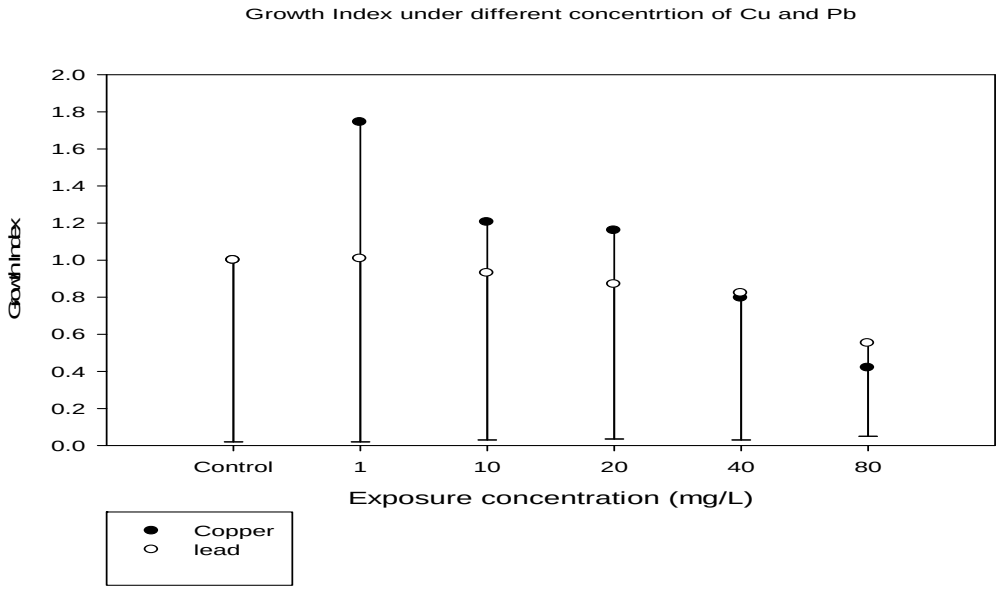

Fig. 18. *Growth index measured in duckweed Spirodela polyrhiza exposed to different concentrations of Cu and Pb. Values are mean of three replicates. Different letters indicate significantly different values at P<0.05.*

4.2.4. Photosynthetic pigment estimation

Chlorophyll-a content shows great increase at 1mcg/L exposure (22.4%). But at 10 mcg/L it shows a narrow increase (0.2%).From 20 mcg/L onwards chlorophyll- a content reduced by 18.8%, 26% and 41% for 20 mcg/L, 40 mcg/L and 80 mcg/L respectively.

Table 23. *Chlorophyll a, Chlorophyll b and Carotenoid content measured in duckweed Spirodela polyrhiza exposed to different concentrations of Cu and Pb. Values are mean of three replicates. Values are different with significance P<0.05.*

Metal	Exposure mcg/L	Chlorophyll a (mg/g)FW	Chlorophyll b (mg/g)FW	Carotenoids (mg/g)FW
	control	0.458	0.286	0.24
Cu	1	0.561	0.281	0.24
	10	0.459	0.274	0.155
	20	0.374	0.251	0.123
	40	0.341	0.221	0.111
	80	0.27	0.195	0.102
Pb	**control**	0.458	0.286	0.24
	1	0.458	0.253	0.24
	10	0.456	0.257	0.18
	20	0.456	0.246	0.158
	40	0.38	0.223	0.128
	80	0.292	0.203	0.114

Chlorophyll-b did not shown any signs of hormesis. From 1 mcg/L onwards reduction in Chlorophyll b noticed. The reduction rates were 1.7% (1 mcg/L), 4.19% (10 mcg/L), 12.2% (20 mcg/L), 23% (40 mcg/L) and 32% (80 mcg/L). Carotenoid seems to be less affected and seems to be resistant to the lower concentrations of Copper. Carotenoid content remains unchanged even at 10mg/L exposure. But it reduces by 1.1% at 20 mcg/L. It is followed by 2.3% reduction at 40 mcg/L and 3.8 % reduction in 80mcg/L exposure (*Table 23 & fig. 19*).

Table 24: *ANOVA of relative concentration of photosynthetic pigments in duckweed Spirodela polyrhiza exposed to different concentrations of Copper and Lead . Values are mean of three replicates. ASGR and Td values are highly significant with P <0.05.*

ANOVA- Chlorophyll a (Copper)						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	2757.983	3	919.3275	3.911198	0.023868	3.098391
Within Groups	4701.002	20	235.0501			
Total	7458.985	23				
ANOVA- Chlorophyll b (Copper)						
Between Groups	2793.458	3	931.1526	3.961632	0.022832	3.098391
Within Groups	4700.854	20	235.0427			
Total	7494.311	23				
ANOVA- Carotenoids (Copper)						
Between Groups	2813.563	3	937.8542	3.990111	0.022269	3.098391
Within Groups	4700.893	20	235.0446			
Total	7514.455	23				

ANOVA- Chlorophyll a (Lead)						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	2756.63	3	918.8768	3.909362	0.023906	3.098391
Within Groups	4700.904	20	235.0452			
Total	7457.535	23				
ANOVA- Chlorophyll b (Lead)						
Between Groups	2797.964	3	932.6546	3.967997	0.022705	3.098391
Within Groups	4700.884	20	235.0442			
Total	7498.848	23				
ANOVA- Carotenoids (Lead)						
Between Groups	2810.363	3	936.7876	3.985586	0.022357	3.098391
Within Groups	4700.878	20	235.0439			

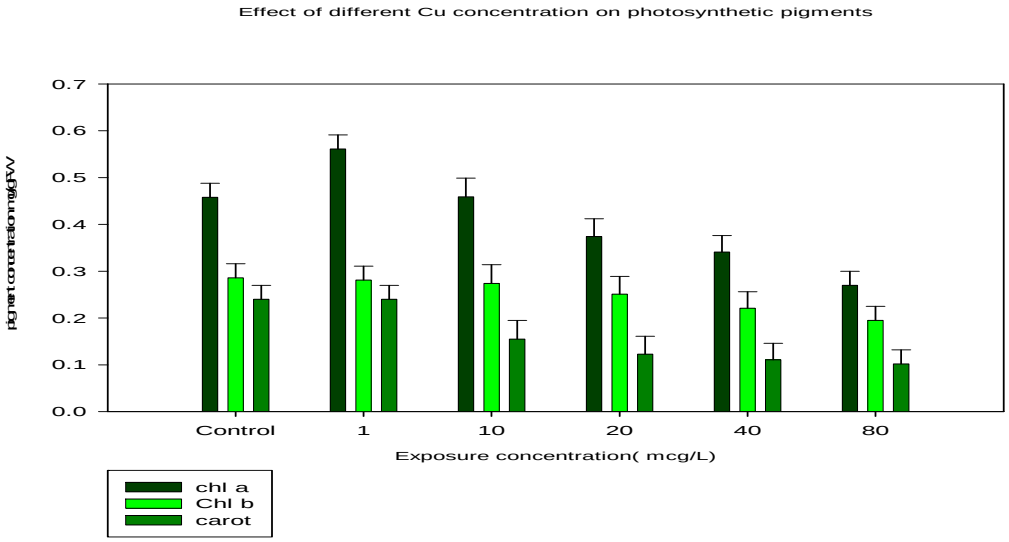

Fig. 19: *Diagramatic representation of relative concentration of photosynthetic pigment content measured in duckweed Spirodela polyrhiza exposed to different concentrations of Cu . Values are mean of three replicates. Different letters indicate significantly different values at P<0.05.*

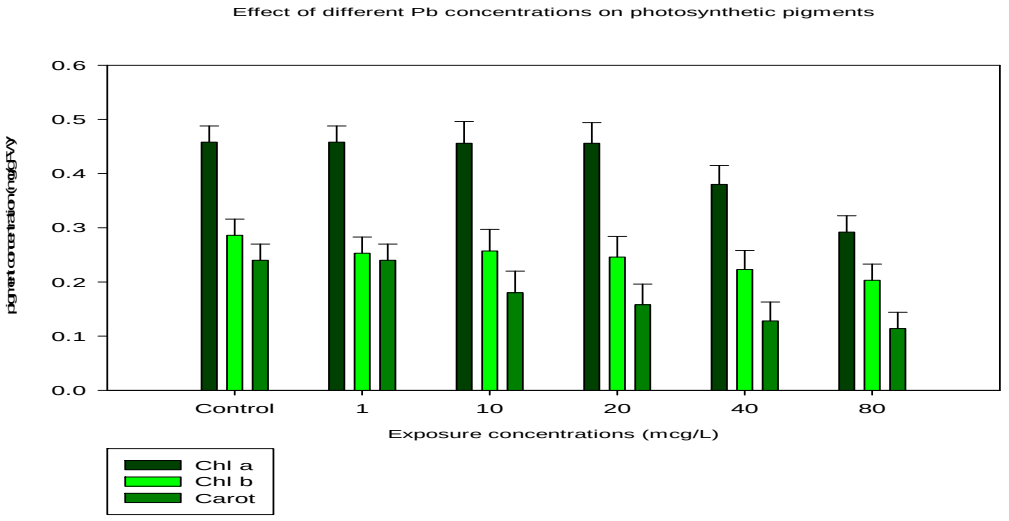

Fig.20: *Diagramatic representation of relative concentration of photosynthetic pigment content measured in duckweed Spirodela polyrhiza exposed to different concentrations of Pb. Values are mean of three replicates. Different letters indicate significantly different values at P<0.05*

Copper and Lead caused visible damage to duckweed at concentration of 20 and 10 mcg/L respectively. Chlorosis (a progression of green to yellow colour on the frond) and frond disconnection (detachment of fronds from colonies) were toxicity signs observed at the start of exposing *S. polyrhiza* to copper and Lead. These signs progressed to necrosis at the end of the treatment. Visibly, Lead was toxic at concentrations ≥ 10 mcg/L; fronds were chlorotic and some fronds separated from the others (necrosis was observed after 8 days of exposure plants to ≥ 20 mcg/L of Cu).

Chlorophyll- a remains unchanged at 1mcg/L of Pb exposure (*table 23 & fig. 20*). But reduced by 0.43% at 10 mcg/L concentration. It remains the same at 20 mcg/L. at 40 mcg/L and 80 mcg/L exposure, Chlorophyll a was reduced by 17% and 36% respectively. Chlorophyll- b content was reduced by 11.5% even at 1 mcg/L. There occurred 10%, 14%, 22% and 29% reduction in Chlorophyll- b content at 10 mcg/L, 20mcg/L, 40 mcg/L and 80mcg/L exposure respectively. Overall reduction at the end (80mcg/L) was high for Cu (32%) than Pb(29%). The Carotenoid content was not affected at 1mcg/L. Only 0.18% reduction at 10mcg/L. It was followed by 1.8% reduction at 20mcg/L and 2.1 % reduction at 40 mcg/L exposure. Final exposure of 80 mcg/L yield 3.5% reduction of Chlorophyll -b.

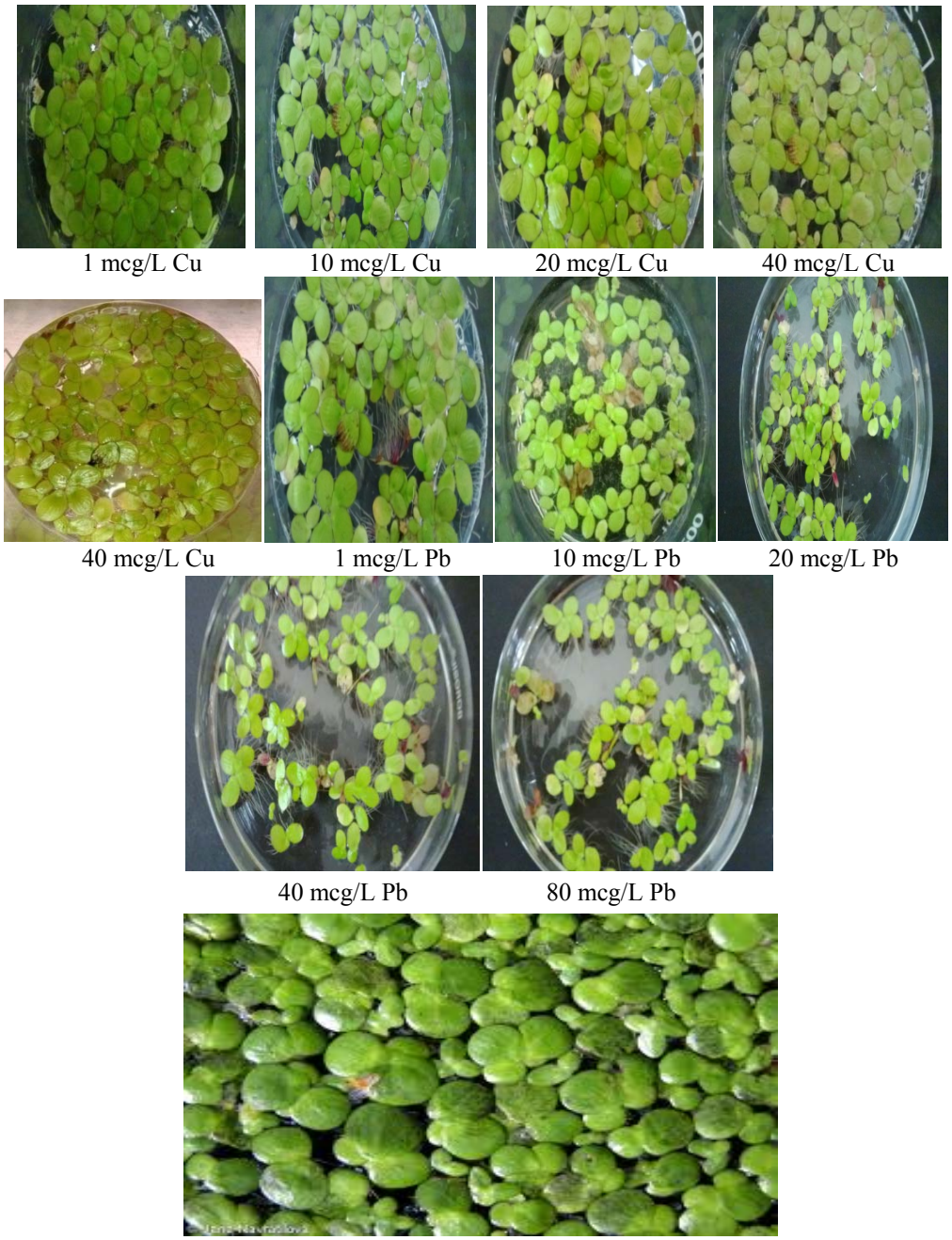

Fig. 21: *Images showing effect of different concentrations of Cu and Pb on Spirodela polyrhiza*

4.2.5. Biochemical parameters

1. ***Soluble protein content***

Under treatment with copper Protein content shows an increase in 5.5% in 1 mcg/L and 8.6% in 10 mcg/L. Higher concentrations shows reduction in protein content. At 80 mcg/L protein content were reduced by 28%. Treatment with Lead at 1 mcg/L shows 3% of increase in protein content. At 10 mcg/L there was 7% of increase. At 80 mcg/L protein content reduced by 47% than control (*table 25 & fig.22*).

Table 25: *Effect of different concentrations of Cu and Pb on soluble sugars (mg/g fw) and total protein content. Values are means ± SE (n = 3)*

Conc of Pb (mcg/L)	Soluble protein content (mg/g FW)	Total carbohydrate content (mg/g FW)
Control	7.2	32.44
1	7.42	33.12
10	7.7	32.8
20	7.1	32.5
40	5.3	25.3
80	3.8	21.4

Conc of Cu (mcg/L)	Soluble protein content (mg/g FW)	Total carbohydrate content (mg/g FW)
Control	7.2	32.44
1	7.6	33.12
10	7.82	33.05
20	7.16	32.5
40	6	25.3
80	5.2	23.6

Table 26: *ANOVA of relative concentration of photosynthetic pigments in duckweed Spirodela polyrhiza exposed to different concentrations of Copper and Lead . Values are mean of three replicates. ASGR and Td values are highly significant with P <0.05.*

ANOVA- Protein content						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	1744.474	3	581.4914	2.450302	0.043296	3.098391224
Within Groups	4746.283	20	237.3142			
Total	6490.757	23				
ANOVA- Carbohydrate content						
Between Groups	1893.23	3	631.0765	2.433099	0.044893	3.098391224
Within Groups	5187.43	20	259.3715			
Total	7080.66	23				

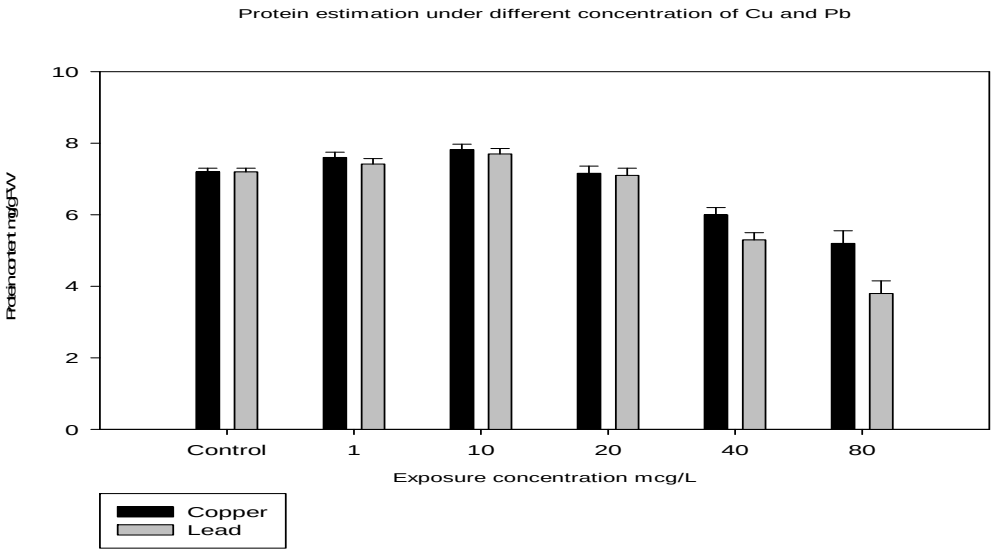

Fig. 22: *Soluble protein content in duckweed Spirodela polyrhiza exposed to different concentrations of Cu and Pb. Values are mean of three replicates. Values are different with significance P<0.05. (N(i)=5, T(j)=8 and T(i)=0).*

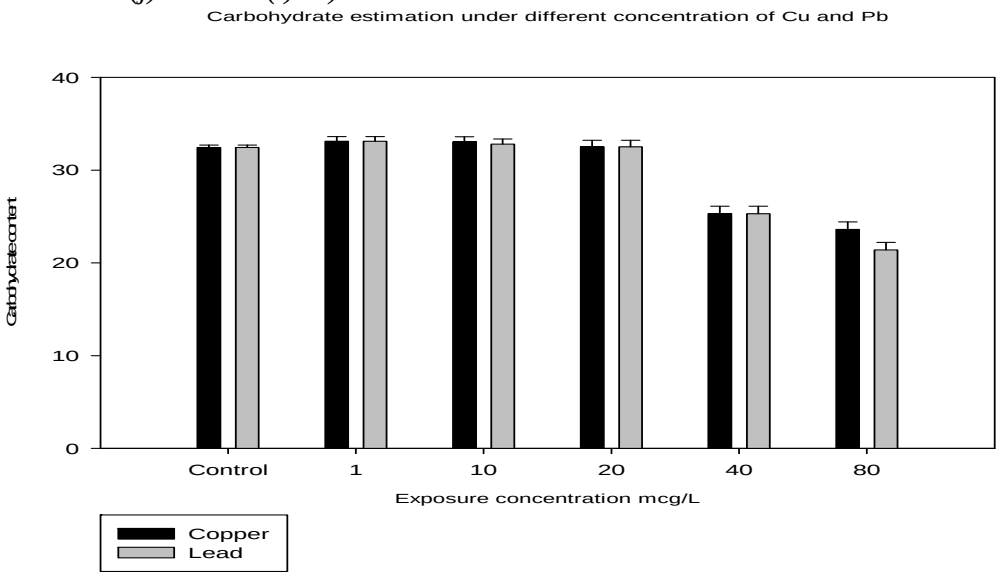

Fig. 23: *Carbohydrate content in duckweed Spirodela polyrhiza exposed to different concentrations of Cu and Pb. Values are mean of three replicates. Values are different with significance P<0.05. (N(i)=5, T(j)=8 and T(i)=0).*

2) *Soluble carbohydrate content*

Carbohydrate content in control was 32.44 mg/g DW. Carbohydrate shows an increase of 3% during 1 mcg/L Copper exposure followed by 2% increase in 10 mg/L exposure. An increase of 0.1% was noted during 20 mcg/L followed by decrease of 22% and 27% during exposure with 40 mcg/L and 80 mcg/L respectively. Under the treatment with Lead, increase in carbohydrate content was observed at 1 mcg/L, 10 mcg/L and 20 mcg/L by 2%, 1.8%, and 0.18% respectively. But there was a reduction in carbohydrate content after that. During 20 mcg/L exposure there was 22% reduction in carbohydrate content. And finally another decrease by 34% was observed at 80 mcg/L exposure (*Table 25 & fig. 23*).

4.2.6. Bioaccumulation and BCF

Plants have developed a range of mechanisms to obtain metals from the substrate and transport these metals within the plant. Mechanisms operating at deficiency and sufficiency levels of metals and at excess metal supply are well studied. The main pathway by which plants accumulate metals is through root uptake (Sharma and Dubey, 2005; Uzu *et* al., 2009). The BCF was calculated for quantifying the metal removal potential of the plants. The factor is defined as the ratio of the metal concentration in the dry plant biomass (ppm) to the initial concentration of metal in the feed solution (ppm).

Table 27: *Accumulation , percentage of removal and BCF of Copper and Lead by duckweed Spirodela polyrhiza exposed to different concentrations of Cu and Pb for 8 days. Values are mean of three replicates. Values are different with significance P<0.05. (N(i)=5, T(j)=8 and T(i)=0).*

Removal of Cu and Pb and BCF							
Lead (mcg/L)	**Accumulation after 8 days**	**% of removal**	**BCF**	**Cu (mcg/L)**	**accumulation after 8 days**	**% of removal**	**BCF**
1	1	100	1000	1	0.83	83	830
10	9.11	91.1	911	10	7.6	76	760
20	17.6	88	880	20	13.6	68	680
40	30	75	750	40	24	60	600
80	52	65	650	80	38.2	47.7	382

After 8 days of treatment 100% removal of Pb has occurred in 1 mcg/L exposure hence the BCF is 1000. From 10 to 80 mcg/L exposure of Lead accumulation becomes lesser and lesser. At 10 mcg/L it was observed that the removal of Pb from the solution was 91%. At 20 mcg/L exposure the removal rate was reduced to 88%. At 40 and 80 mcg/L exposure accumulation was further reduced to 75% and 65% respectively after 8 days (*Table 27 & fig. 25*).

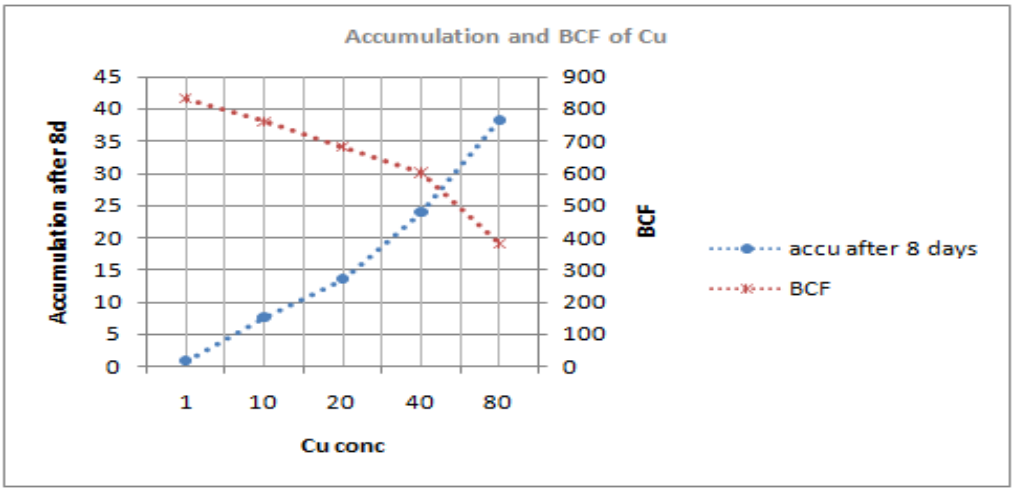

Fig. 24: *Accumulation and Bio Concentration Factor (BCF) after 8 days of exposure under different concentration Cu by Spirodela polyrhiza .(p<0.05).*

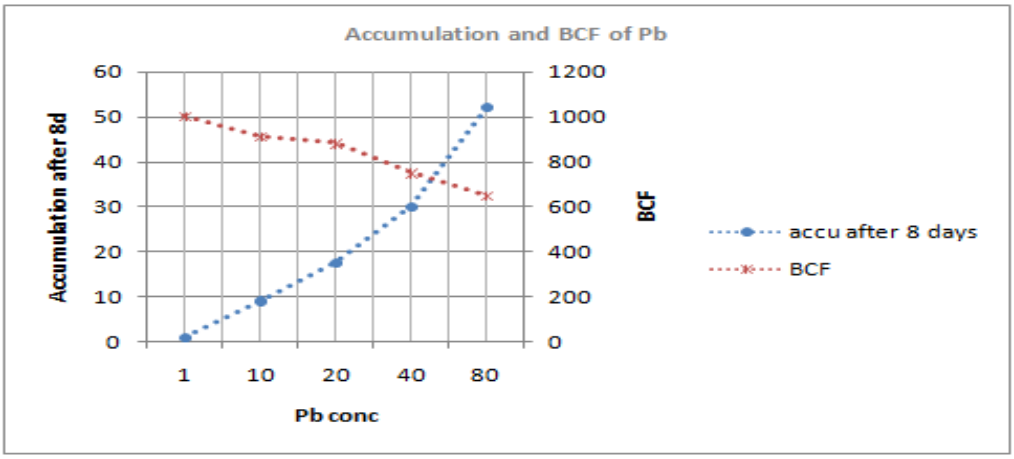

***Fig. 25**: Accumulation and Bio Concentration Factor (BCF) after 8 days of exposure under different concentration of Pb ($p<0.05$).*

Spirodela plant is a not good accumulator of Copper at lower concentrations when compared to Pb. Only 83% of removal was observed in 1mcg/L exposure (BCF=830). The removal reduced as the exposure concentration increases. It was 76%, 68%, 60% and 48% removal at exposure of 10 mcg/L, 20 mcg/L, 40 mcg/L and 80 mcg/L respectively. This indicates that at low concentration, copper accumulated by specific sites while with increasing copper concentration the specific sites are saturated and the exchange sites are filled (*table 27& fig.24*)

4.2.7. Tolerance- EC_{50} and NOEC

The tolerance is defined as the ability of the plants to survive to concentrations of metals in their environment that are toxic to other plants (Kamal *et* al., 2004). Lead inhibited duckweed growth even at low concentrations. The inhibition consisted on the reduction of the biomass which might be explained by an excessive absorption of the metal. Copper when present in the nutrient solution at concentrations ≤ 20 mcg/ L was an

essential element for the development of *Spirodela* fronds because of its important role in cellular metabolism. Copper when present in the culture media at concentrations ≤ 20 mcg/L (NOEC) was essential to develop the fronds of *S.polyrhiza*. At a concentration higher than 20 mcg/L, Cu caused the photosystem alteration by reducing electron transport. This effect was manifest by a rapid development of chlorosis; after exposing *Spirodela* to the cupric ions (2 or 3 hours), the fronds colour changed from green to yellow and some fronds were separated from the colonies (*table 28& 29*)

Table 28: *EC_{50} for Copper and Lead calculated from biomass of duckweed Spirodela polyrhiza exposed to different concentrations of Cu and Pb. Values are mean of three replicates. Values are different with significance P<0.05.*

Conc. (Pb) mcg/L	Mean Biomass	SD
ctrl	7.7733	0.0737
1	7.8933	0.0153
10	7.3067	0.0153
20	6.87	0.02
40	6.41	0.02
80	4.1767	0.2113
Metal	**EC_{50}**	**Std Error**
Pb	91.889	4.6621

Conc. (Cu) mcg/L	Mean Biomass	SD
ctrl	7.7867	0.0321
1	13.64	0.0755
10	8.8367	0.7321
20	10.0267	0.0643
40	6.18	0.1997
80	3.3067	0.1124
Metal	**EC_{50}**	**Std Error**
Cu	52.52	10.2

Table 29: *NOEC for Copper and Lead calculated from biomass of Spirodela polyrhiza exposed to different concentrations of Cu and Pb. Values are mean of three replicates. Values are different with significance P<0.05.*

NOEC Pb				
conc. (mcg/L)	**n**	**Mean**	**SD**	**CV%**
control	3	7.7733	0.0737	0.9
1	3	7.8933	0.0153	0.2
10*	3	7.3067	0.0153	0.2
20*	3	6.87	0.02	0.3
40*	3	6.41	0.02	0.3
80*	3	4.1767	0.2113	5.1
* the mean for this conc. is significantly less than the control mean at alpha = 0.05 (1-sided) by Dunnett's test				
Between concentrations sum of squares = 28.246583 with 5 degrees of freedom. Error mean square = .008556 with 12 degrees of freedom.				

NOEC Cu				
conc. (mcg/L)	**n**	**Mean**	**SD**	**CV%**
control	3	7.7867	0.0321	0.4
1	3	13.64	0.0755	0.6
10	3	8.8367	0.07321	8.3
20	3	10.0267	0.0643	0.6
40*	3	6.18	0.1997	3.2
80*	3	3.3067	0.1124	3.4
* the mean for this conc. is significantly less than the control mean at alpha = 0.05 (1-sided) by Dunnett's test				
Between concentrations sum of squares = 184.428561 with 5 degrees of freedom. Error mean square = .099906 with 12 degrees of freedom.				

The results shows that Lead has much higher EC 50 value (91.88 mcg/L) than Copper which means *Spirodela* has much tolerance towards elevated levels of Copper. Lead if present in the exposure solution at concentrations ≤ 10 mcg/L inhibits growth which is clear from estimation of biomass (*Table 23*) and estimation of NOEC (*Table 28*).

Discussions:

1. *Morphological parameters:*

Root length was observed to be decreased with an increase in concentrations of Copper and Lead showing perfect negative correlation. Literature survey showed that the heavy metals accumulations in

duckweed increased linearly with the solution concentration in the order of leaves<stems<roots (Mane *et* al., 2011). Munns (2003) concluded that the reduction might be attributed to the inhibition of hydrolysis of reserved foods and their translocation to the growing parts. Growth changes are often the first and most obvious reactions of plants under heavy metal stress (Hagemeyer, 1999). Some metals are accumulated in roots, probably due to some physiological barriers against metal transport to the aerial parts, while others are easily transported in plants. Translocation of trace elements from roots to shoots could be a limiting factor for the bioconcentration of elements in shoots (Zhu, *et* al., 1999). Morphological toxicity symptoms were observed at designed metal concentrations except for 1 mcg /L. Excess Lead and copper caused plants necrosis or death and colonies disintegration as well as roots length reduction. The results are in line with the findings of Li and Xiong (2004).

At low concentrations, lead inhibits the growth of roots and leaves (Islam *et* al., 2007; Kopittke *et* al., 2008). This inhibition is stronger for the root, which may be correlated to its higher lead content (Liu *et* al., 2008). Recently, Jiang and Liu (2010), reported mitochondrial swelling, loss of cristae, vacuolization of endoplasmic reticulum and dictyosomes, injured plasma membrane and deep colored nuclei, after 48–72 hrs of lead exposure in roots.

At 1 mcg/L Copper concentration root length were not affected. It may be due to some tolerance mechanisms, e.g., binding to metalloproteins (Rauser, 1984) or the precipitation of Cu complexes in globular bodies (Sela *et* al., 1988). In some species, high Cu sensitivity of root growth is

related to disturbances of mitosis (Eleftheriou and Karataglis, 1989) and especially to damage to the cell membrane, which is often the first target of Cu toxicity (Meharg 1993).

2. *ASGR and Td*

Heavy metals in growth media can function as stressors, causing physiological constrains that decrease plant vigor and inhibit plant growth (Wu and Lin, 1990). *Spirodela polyrhiza* growth retardation from lead exposure starts at 1 mcg/L exposure. It may be attributed to nutrient metabolic disturbances (Kopittke *et* al., 2008; Gopal and Rizvi., 2008) and disturbed photosynthesis (Islam *et* al., 2008). Under severe lead toxicity stress (40 and 80 mcg/L exposure), plants displayed obvious symptoms of growth inhibition, with fewer, smaller, and more brittle leaves having dark purplish abaxial surfaces (Islam *et* al., 2007; Gupta *et* al., 2009). The purplish abaxial colouration is not detectable in *Spirodela* due to its anthocyanin pigmentation on the abaxial surface.

Copper is considered to be a micro nutrient for plants (Thomas *et* al., 1998) and plays an important role in Co_2 assimilation and ATP synthesis. Cu is also an essential component of various proteins like plastocynin and Cytochrome oxidase of ETS (Demirevska-kepova *et* al., 2004). In the current study Copper is less toxic in lower concentration but more toxic than Pb in higher concentrations. Excess Cu in the medium plays a cytotoxic role, induces stress and causes injury to the plants. This leads to retardation of growth rate and causes leaf chlorosis (Lewis *et* al., 2001). Copper may alter the energy storage via photosynthesis which causes the decrease of biomass growth.

3. *Biomass and Growth Index*

In duckweed *Spirodela polyrhiza* heavy metals retard growth at higher concentrations. (Oron *et* al., 1984; Banerjee and Sarker, 1997). The present study underlines the findings. Both Cu and Pb were found to inhibit biomass in many species of aquatic plants (Huebert and Shay, 1993; Miranda and Hangovan, 1978; Mohan and Hosetti, 1997), Cu was shown to interfere with the increased tissue permeability, hence the increase in toxicity (Krupa, and Baszynsk, 1995) the increased cross linking of pectins in the middle lamella of cell wall which might inhibit cell expansion and the direct and indirect effect on the growth hormone, auxin metabolisms or auxin carriers. Similarly, Pb also causes severe impairment of plant metabolic processes including the photosynthetic activity and the induction of enzyme peroxidase that is involved in the degradation of indoleacetic acid which stimulates plant growth and multiplication (Hoffman *et* al., 1985).

4. *Photosynthetic pigment estimation:*

The most apparent effect of Cu toxicity on PSII is the inhibition of oxygen evolution accompanied by quenching of variable fluorescence (Hsu and Lee, 1988; Samson *et* al., 1988; Mohanty *et* al., 1989). Both the acceptor and the donor sides of PSII were suggested as the main targets of Cu toxic action. A side effect of Cu inhibiting photosynthesis is an increase in the production of free radicals and therefore an increase in rate of leaf senescence due to oxidative damage (Luna *et* al., 1994). High concentrations of Cu are known to activate oxidative damage and alter cell-membrane properties by lipid peroxidation, thereby demonstrating the

inhibitory effect on the enzymes involved in chlorophyll production. An increase in photosynthetic pigments occurs because of a Cu induced reduction in CO_2 fixation and, as such, photosynthesis does not decrease, at least initially (Romeu-Moreno and Mas, 1999).

Lead inhibits important enzymes such as δ-aminolevulinic acid dehydratase (ALA dehydratase) and protochlorophyllide reductase (Van Assche and Clijsters, 1990) associated with chlorophyll biosynthesis; (b) impairment in the supply of Mg^{2+} and Fe^{2+} required for the synthesis of chlorophylls; (c) Zn2+ deficiency resulting in inhibition of enzymes, such as carbonic anhydrase (Van Assche and Clijsters 1990); (d) the replacement of Mg^{2+} ions associated with the tetrapyrrole ring of chlorophyll molecule. Lead decrease ferredoxin $NADP^{+}$ reductase and delta-aminolevulinic acid dehydratase (ALAD) activity as the origin of chlorophyll synthesis inhibition (Gupta et al. 2009; Cenkci *et* al., 2010), Pb cause inhibition of plastoquinone and carotenoid synthesis (Kosobrukhov *et* al., 2004; Chen *et* al., 2007; Liu *et* al., 2008; Cenkci *et* al., 2010). Lead causes obstruction of the electron transport system (Qufei and Fashui, 2009). Lead cause stomatal closure and there by blocking entry of carbon dioxide (Romanowska *et* al., 2008). Lead affect uptake of essential elements such as Mn and Fe (Chatterjee *et* al., 2004;Gopal and Rizvi 2008) and substitution of divalent cations by lead (Gupta *et* al.2009; Cenkci *et* al., 2010). Finally Lead cause inhibition of Calvin cycle enzymatic catalysis (Mishra *et* al., 2006; Liu *et* al., 2008), and increased chlorophyllase activity (Liu *et* al., 2008)

5. *Biochemistry*

My studies coincide with Costa and Spitz (1997) who also reported a decrease in soluble protein content under heavy metal stress in *Lupinus albus.* The decrease in protein content in *L. polyrhiza* at higher concentration may be caused by enhanced protein degradation process as a result of increased protease activity (Palma *et* al., 2002) that is found to increase under stress conditions. Cu-induced damage to integral proteins, through the formation of disulfide links, resulted in increased cell membrane permeability and ion efflux.

The decrease in total sugar content of stressed leaves probably corresponded with the photosynthetic inhibition or stimulation of respiration rate. Higher starch accumulation in damaged leaves of *Tilia argentea* and *Quercus cerris* may result both in the higher resistance of their photosynthetic apparatus (Prokopiev, 1978) and low starch export from the mesophyll. The negative effect of heavy metals on carbon metabolism is a result of their possible interaction with the reactive centre of ribulose bisphosphate carboxylase (Stiborova *et*. al., 1987). The decrease in total sugar content of stressed leaves probably corresponded with the photosynthetic inhibition or stimulation of respiration rate. Higher starch accumulation in damaged leaves of *Tilia argentea* and *Quercus cerris* may result both in the higher resistance of their photosynthetic apparatus (Prokopiev, 1978) and low starch export from the mesophyll. The negative effect of heavy metals on carbon metabolism is a result of their possible interaction with the reactive centre of ribulosebisphosphate carboxylase (Stiborova *et*. al., 1987).

6. *Metal Accumulation and BCF*

Metal uptake by plants is regulated by the electrochemical potential gradient for each metal ion that exists across the plasma membrane of root cells (Welch, 1995). The exact nature of the membrane transporter, which controls influx across the plasma membrane into the cytoplasm, is not yet known. It has been suggested that the transporter may be a metal specific or nonspecific channel protein (Kochian, 1993; Welch, 1995; Grusak *et* al., 1999) although conclusive evidence does not exist. It appears that the capability of the root membrane transport mechanism is far in excess of the plant metal requirements (Welch, 1995; Grusak *et* al., 1999) indicating the mechanism by which toxic concentrations of metals may enter the plant roots.

Through phytoextraction, duckweed plants can sequester excessive amounts of Pb in their biomass without incurring damage to basic metabolic functions (Arshad *et* al., 2008; Zaier *et* al., 2010). *Spirodela* can extract huge amounts of lead from contaminated water without showing morphophytotoxicity symptoms. Indeed, these plants have efficient natural detoxification mechanism to alleviate lead toxicity. Part of the lead present in the solution is adsorbed onto the roots, and then becomes bound to carboxyl groups of mucilage uronic acid, or directly to the polysaccharides of the rhizoderm cell surface (Seregin and Ivanov, 2001). Once adsorbed onto the rhizoderm roots surface, lead may enter the roots passively and follow translocating water streams. The highest lead concentrations can be found in root apices. The low rate of lead translocation from root to shoot is due to endodermis, which acts as a

physical barrier. Lead is blocked in the endodermis by the casparian strips. Wong *et* al., (1976) reported in rooted macrophytes a greater accumulation of Pb than in plants without roots. In general, concentrations of metals are higher in roots compared to leaves and other aerial parts (Garcia *et* al., 1979; Sela *et* al., 1989). As far as the extent of metal accumulation is concerned, duckweeds surpasses algae and angiosperms (Taylor *et* al., 1979).

Several studies demonstrated that duckweed species were able to accumulate elevated amount of Cu in their tissues (Jain *et* al., 1989; Zayed *et* al., 1998b; Ater *et* al., 2006; Megateli *et* al., 2009) inducing an abatement of Cu concentration in water. Copper in xylem sap has been shown to be almost 100% bound to amino acids and this high percentage of complex formation is retained under conditions of excess Cu supply (Graham, 1979; White *et* al., 1981; Pich and Scholz, 1996; Liao *et* al., 2000). In addition, in both *Lycopersicon esculentum* (tomato) and *Cichorium intybus* (chicory) increasing the supply of Cu increased the production of amino acids, particularly nicotianamine and histadine (Liao *et* al., 2000). This suggests that even under toxic conditions plants have mechanisms to regulate complexation of Cu within the xylem sap and hence minimize potential damage caused from high concentrations of free Cu ions (Welch, 1995).

The bioconcentration factor is more significant than the amount accumulated in plants since it indicates the plant's ability to accumulate trace elements relative to their concentration in the external nutrient solution (Del-Campo Marin and Oron, 2007). At copper concentration

tolerated by duckweed, the BCF values were approximately 1000 and the removal percentage was high. Based on these results, we can conclude that *S. polyrhiza* could be a good candidate for the phytoremediation of low concentrations of copper from polluted water. If BCF is 1000, the treatment is considered to be good for accumulation (100%). At higher concentration of Cu, the plant is better accumulator than Pb. The BCF value was 650 for Pb and 382 for Cu. Generally, plants having a BCF greater than 1000 are categorized as hyperaccumulators, whereas those with transfer factor less than 1 are termed as non-accumulators (Arshad *et* al. 2008). Our result confirmed that *Spirodela polyrhiza* showed a potential of phytoremediation of contaminated waters charged with low concentrations of Cu.

According to Teisseire and Vernet (2000), CuSO4 at 10 μM was inhibitory for *L. minor*; at this concentration, activities of glutathione S-transferase and glutathione reductase were inhibited. The toxicity metal in *Spirodela* tissues was in decreasing order of damage: Cu > Pb at higher concentrations. It can be concluded that *S. polyrhiza*, could be a good candidate for the phytoremediation of water polluted with Cu or Pb. Miranda and Hangovan (1978), studied the Pb influence on specific growth rate of *Lemna gibba.* They found that high Pb concentrations (20-50 mcg/L) in the media significantly inhibited the specific growth rate of *L. gibba* under continuous and discontinuous illumination. This might be due to the fact that Pb induces the activity of the enzyme peroxidase that is involved in the degradation of indoleacetic acid (IAA), the hormone which stimulates plant growth and multiplication.

7. *NOEC and EC_{50}*

The present study investigates the effect of Cu and Pb on the duckweed (*Spirodela polyrhiza*) to assess tolerance of this aquatic plant to metal pollution. This effect is determined from the concentration that results in a 50% reduction in the growth of *Spirodela* (EC_{50}) and the no-observed effect concentration (NOEC). The tolerance is defined as the ability of the plants to survive to concentrations of metals in their environment that are toxic to other plants (Kamal *et* al., 2004). As far as biomass is concerned Pb slightly enhances growth at low concentrations (1 mcg/L). The inhibition follows and the reduction of the biomass which might be explained by an excessive absorption of the metal. Copper when present in the nutrient solution at concentrations 1 mcg/L to10 mcg/L was seemingly an essential element for the development of *Spirodela* fronds because of its important role in cellular metabolism. At a concentration higher than 10 mcg/L, Cu found to be inhibitory may be it caused the photosystem alteration by reducing electron transport (Kamal *et* al., 2004). This effect was explained by a rapid development of chlorosisat higher concentrations. The calculated NOEC for Pb using Dunnett test is 1mcg/L and NOEC for Cu is 20 mcg/L with the significance of $P>0.05$ (*Table 23*). The EC_{50} values of Lead (91.88 mcg/L) and Copper (52.52 mcg/L) clearly shows the tolerance of *Spirodela polyrhiza* to these metals. Higher EC_{50} value for Pb means the plants can tolerate the metal to a greater extent even though it is toxic at lower concentrations.

4.3. Utilization of *Spirodela polyrhiza* as phytoremediation agent in selected wetlands of Ernakulam district:

Spirodela polyrhiza of family *Lemnaceae* have been rarely studied for their potential application in phytoremediation. The plant has many unique properties ideal for phytoremediation: they have fast growth and primary production; high bio accumulation capacity for heavy metals; ability to transform or degrade contaminants; ability to regulate chemical speciation and resilient to extreme contaminant concentration; and can be applied to multiple pollutants. In the present study two wetland sites each from Eloor and Kannamaly were selected and study the phytoremediation potential of this plant in those sites.

4.3.1. Studies on wetlands of Eloor:

4.3.1.1. Analysis of variations in physico chemical parameters

In physio-chemical analysis different parameters (Temperature, pH, Total alkalinity, BOD, COD, EC, Nitrate, Phosphate, Sulphates, TDS, TSS ,turbidity and analysis for heavy metals of wetland I and II were studied.

1) **pH**: During pre monsoon season, the pH of water from wetland I was alkaline 8.2 and for wetland II was 8.4 were found to be in the optimum range for duckweed growth (Dalu & Ndamba, 2002). After 2 days of treatment it has reduce to 7.4 and 8 for WI and W2. After 4 days, theW1 sample showed 7.3 and Sample from W2 showed 7.4. After 8 days, both the sample treatment chambers showed the pH 7.2 (*Tables 30 and 31*). During monsoon season pH was measured 7.6 before treatment in W1 sample. After 2 days it came down to 7.4 and became 7.3 after 4 to 8 days *(Fig.26a & 26b*). In W2 sample initial pH was 7.6. After 4 days of treatment it came down to 7.4 and remains the same even after 8 days of treatment. During post monsoon

season pH was measured 8.2 before treatment in W1 water. After 2 days it came down to 7.4. After 4 days it further reduced to 7.3 and finally becomes 7.2 the same after 8 days. In W2 sample initial pH was 8. After 2 days it came down to 7.8 and reduced 7.2 after 8 days.

***Table 30**:Physiochemical analysis of water sample collected from Wetland 1 sampling site during pre monsoon season before and after 2, 4 and 8 days of exposure is given. CPCB standards were included for comparison. The values are means of triplicates.*

Sl. No	Physiochemical parametrs	CPCB standard	Before treatment	After 2 days of treatment	Removal efficiency	After 4 days of treatment	Removal efficiency	After 8 days of treatment	Removal efficiency
1	Temp(^{o}C)	29-40	33	30	9.1	29.5	10.1	29	12.1
2	PH	6.5-8.5	8.2	7.4	10	7.3	10	7.2	12.1
3	BOD (mg/L)	5	110	96	13	87	21	69	37.2
4	COD (mg/L)	250	320	196	39	178	44.3	162	49.3
5	Nitrate (mcg/L)	45	12	6.1	48	1.1	91	0	100
6	EC (µs/Cm)	700	952	878	7.8	812	15	692	27.3
7	Alkalinity (mg/L)	400	342	315	8	298	13	243	29
8	Phosphate (mg/L)	5	11	9.2	16.36	8.2	25.4	7	36.3
9	Sulphate (mg/L)	400	500.12	469	6.3	444.2	11.1	421	16
10	TDS (mg/L)	2100	593.1	477	20	266.3	55.1	158	73.3
11	TSS (mg/L)	100	218.41	99	55	65.12	70.1	32	85.4
12	Turbidity (NTU)	5	29	22.4	23	19.7	32.06	14	52
13	Copper (mcg/L)	1.5	25	15.75	37	8.25	67	5.25	79
14	Lead (mcg/L)	0.01	16	10.65	33.4	5.76	64	0.8	95
15	Zinc (mcg/L)	15	112	93.6	16.4	48.8	56.4	38.08	66
16	Chromium (mcg/L)	0.01	78	66.9	14.2	53.82	31	36.66	53
17	Cobalt (mcg/L)	0.01	7.2	5.87	18.4	4.9	31.2	3.45	52
18	Manganese(mcg/L)	0.5	8	7.6	5	7.2	10	6.4	20
19	Mercury (mcg/L)	0.001	2	2	0	1.8	10	1.1	45
20	Nickel (mcg/L)	5	19.3	19.1	1.03	19	4	18	9

***Table 31**: Physiochemical analysis of water sample collected from Wetland 2 sampling site during pre monsoon season before and after 2,4 and 8 days of exposure is given. CPCB standards were included for comparison. The values are means of triplicates.*

Sl. No	Physiochemical parameters	CPCB standard	Before treatment	After 2 days of treatment	Removal efficiency	After 4 days of treatment	Removal efficiency	After 8 days of treatment	Removal efficiency
1	Temp (^{o}C)	25-40	28.8	27.5	4.51	27	6.25	27	0
2	PH	6.5-8.5	8.4	8	6	7.4	12	7.2	14.2
3	BOD (mg/L)	5	341	292.1	14.3	271.2	20.4	205.1	40
4	COD (mg/L)	250	679	511.2	25	464.4	32	268.3	60.4
5	Nitrate (mcg/L)	45	27	15	46	4.3	84	0	100
6	EC (µs/Cm)	700	1185	1112	6	942	20.5	853	28
7	Alkalinity (mg/L)	400	441	402	9	373	15.2	312	29.2
8	Phosphate(mcg/L)	5	13.1	12	10	10.4	21	8.1	38
9	Sulphate (mg/L)	400	133	46.2	65.1	27.4	79.3	15	89.04
10	TDS (mg/L)	2100	3210.3	3111	3.09	2928.11	9	1522	53
11	TSS (mg/L)	100	359	181	50	126	65	53	85.2
12	Turbidity (NTU)	5	382	286	25.1	262	31.4	189	51
13	Copper (mcg/L)	1.5	43	26.6	38	14.6	66	11.18	74
14	Lead (mcg/L)	0.01	24.4	16.11	34	9.03	63	2.2	91
15	Zinc (mcg/L)	15	201	172.4	14.2	96.48	52	75.5	62.4
16	Chromium (mcg/L)	0.01	81	70.4	13	56.7	30	41.31	49
17	Cobalt (mcg/L)	0.01	8	7.2	10	6.3	21.2	5	40
18	Manganese(mcg/L)	0.5	7.3	7.1	3	6.4	12.3	5.1	30.1
19	Mercury (mcg/L)	0.001	3.4	3.1	9	3	18	2	53
20	Nickel (mcg/L)	5	22.3	21	6	18.1	19	18	22
21	Iron (mcg/L)	50	5.3	5.1	4	4.2	21	0.1	98.1
22	Cadmium (mcg/L)	0.01	3	3	0	2.49	17	0	100

***Table 32**:Physiochemical analysis of water sample collected from Wetland 1 sampling site during monsoon season before and after 2,4 and 8 days of exposure is given. CPCB standards were included for comparison. The values are means of triplicates.*

Sl. No	Physiochemical parametrs	CPCB standard	Before treatment	After 2 days of treatment	Removal efficiency	After 4 days of treatment	Removal efficiency	After 8 days of treatment	Removal efficiency
1	Temp (^{o}C)	29-40	27	27.2	0.74	27.4	1.48	27.4	1.48
2	PH	6.5-8.5	7.6	7.42	2.36	7.34	3.42	7.34	3.42
3	BOD (mg/L)	5	43	36.76	14.5	33.97	21	8.85	79.4
4	COD (mg/L)	250	78.5	51.02	35	42.23	46.2	24.72	68.5
5	Nitrate (mcg/L)	45	16	8.32	48	2.56	84	0	100
6	EC (µs/Cm)	700	896.2	792.1	11.6	710	20.7	654	27
7	Alkalinity (mg/L)	400	210	188	10.4	158.1	24.7	133.2	36.5
8	Phosphate (mcg/L)	5	6	4.77	20.5	4.38	27	3.22	46.3
9	Sulphate (mg/L)	400	89.6	65.4	27	51.96	42	31.27	65.1
10	TDS (mg/L)	2100	521	410.02	21.3	218.82	58	93.78	82
11	TSS (mg/L)	100	210	109.2	48	58.38	72.2	24.36	88.4
12	Turbidity (NTU)	5	215.6	163.85	24	137.98	36	105.64	51
13	Copper(mcg/L)	1.5	15	6.45	57	3.99	73.4	0.9	94
14	Lead (mcg/L)	0.01	12.8	8.16	36.2	4.6	64	0	100
15	Zinc (mcg/L)	15	65.4	53.5	18.2	24.33	62.8	13.61	79.2
16	Chromium (mcg/L)	0.01	59.3	46.4	21.7	40.9	31	20.7	65
17	Cobalt (mcg/L)	0.01	4.2	3.69	12	3.23	23	0.51	87.8
18	Manganese (mcg/L)	0.5	4.8	4.53	5.5	4.14	13.7	2.97	38
19	Mercury (mcg/L)	0.001	1.5	1.5	0	1.35	10	0	100
20	Nickel (mcg/L)	5	11.1	10.98	1	10.65	4	7.32	34

***Table 33**:Physiochemical analysis of water sample collected from Wetland 2 sampling site during monsoon season before and after 2,4 and 8 days of exposure is given. CPCB standards were included for comparison. The values are means of triplicates.*

Sl. No	Physiochemical parameters	CPCB standard	Before treatment	After 2 days of treatment	Removal efficiency	After 4 days of treatment	Removal efficiency	After 8 days of treatment	Removal efficiency
1	Temp (^{o}C)	25-40	26.2	26.4	0.76	26.8	0.76	27.2	3.81
2	PH	6.5-8.5	7.6	7.4	2.63	7.4	2.63	7.4	2.63
3	BOD (mg/L)	5	127	105.66	16.8	96.13	24.3	43.18	66
4	COD (mg/L)	250	178	131.72	26	113.92	36	56.07	68.5
5	EC (µs/Cm)	700	912	811	11	721	30	681	25.3
6	Alkalinity (mg/L)	400	268	203	24.2	181.8	32.1	152.4	43.1
7	Nitrate (mcg/L)	45	8.3	4.31	48	0.58	93	0	100
8	Phosphate (mcg/L)	5	10.8	9.5	12	7.99	26	5.61	48
9	Sulphate (mg/L)	400	327.5	301.3	8	287.54	12.2	244.64	25.3
10	TDS (mg/L)	2100	2718	2424.45	10.8	2419.02	11	679.5	75
11	TSS (mg/L)	100	327	160.23	51	114.45	65	48.39	85.2
12	Turbidity (NTU)	5	27	20.52	24	18.09	33	9.01	66.6
13	Copper (mcg/L)	1.5	27	18.36	32	12.96	52	3.24	88
14	Lead (mcg/L)	0.01	8.6	5.68	34	3.05	64.5	0	100
15	Zinc (mcg/L)	15	86	73.79	14.2	41.28	52	24.08	72
16	Chromium (mcg/L)	0.01	66	56.1	15	46.2	30	27.39	58.5
17	Cobalt (mcg/L)		3	2.63	12.3	2.39	20.2	1.56	48
18	Manganese(mcg/L)	0.5	4	3.86	3.5	3.52	12	2.6	35
19	Mercury(mcg/L)	0.001	1.5	1.36	9	1.17	21.4	0.67	55
20	Nickel(mcg/L)	5	7.8	7.3	6.3	6.23	20.1	5.36	31.2
21	Iron(mcg/L)	50	4.3	4.12	4	3.35	22	0	100
22	Cadmium (mcg/L)	0.01	1.2	0.98	18	0.67	43.5	0	100

***Table 34**: Physiochemical analysis of water sample collected from Wetland 1 sampling site during post monsoon season before and after 2,4 and 8 days of exposure is given. CPCB standards were included for comparison. The values are means of triplicates.*

Sl.No	Physiochemical parametrs	BIS standard	Before treatment	After 2 days of treatment	Removal efficiency	After 4 days of treatment	Removal efficiency	After 8 days of treatment	Removal efficiency
1	Temp(^{o}C)	29-40	33	30	9.1	29.5	10.1	29	12.1
2	PH	6.5-8.5	8.2	7.42	10	7.34	10.4	7.2	12.1
3	BOD (mg/L)	5	68	58.48	14	54.4	20	21.55	68.3
4	COD(mg/L)	250	169.6	111.93	34	94.63	44.2	57.32	66.2
5	EC(µs/Cm)	700	991	938	5.3	891	10	772	22
6	Alkalinity (mg/L)	400	218	182	16.5	161	26.1	118	46
7	Nitrate (mcg/L)	45	10.3	5.53	46.3	0.72	93	0	100
8	Phosphate(mcg/L)	5	10.3	8.24	20	7.67	25.5	6.02	41.5
9	Sulphate (mg/L)	400	410.6	377.75	8	365.43	11	320.2	22
10	TDS (mg/L)	2100	566	445.4	21.3	275.07	51.4	125.65	77.8
11	TSS (mg/L)	100	216	146.88	32	64.36	70.2	40.39	81.3
12	Turbidity (NTU)	5	29	22.33	23	20.01	31	10.15	65
13	Copper (mcg/L)	1.5	13	5.83	55.2	3.94	69.7	1.4	89.2
14	Lead (mcg/L)	0.01	12	7.68	36	4.44	63	0	100
15	Zinc (mcg/L)	15	62	50.84	18	24.18	61	17.17	72.3
16	Chromium (mcg/L)	0.01	61	47.76	21.7	43	29.5	23.79	61
17	Cobalt (mcg/L)	0.01	6.8	6.01	11.5	5.3	22	0.88	87
18	Manganese(mcg/L)	0.5	4.6	4.37	5	4.04	12	3.05	33.6
19	Mercury (mcg/L)	0.001	3.2	3.2	0	2.89	9.5	0.24	92.2
20	Nickel (mcg/L)	5	16	15.84	1	15.44	3.5	10.88	32

Table 35: *Physiochemical analysis of water sample collected from Wetland 2 sampling site during post monsoon season before and after 2, 4 and 8 days of exposure is given. CPCB standards were included for comparison. The values are means of triplicates.*

Sl. No	Physiochemical parameters	BIS standard	Before treatment	After 2 days of treatment	Removal efficiency	After 4 days of treatment	Removal efficiency	After 8 days of treatment	Removal efficiency
1	Temp (^{o}C)	25-40	28.8	27.5	4.51	27	6.25	27	6.25
2	PH	6.5-8.5	8	7.8	2.5	7.4	7.5	7.2	10
3	BOD (mg/L)	5	218.3	181.6	16.8	165.9	24	78.5	64
4	COD (mg/L)	250	298.3	220.7	26	195.38	34.5	100.82	66.2
5	EC (µs/Cm)	700	1021	892	13	809	21	712	30.2
6	Alkalinity (mg/L)	400	220	185	16	162	26.3	123	44
7	Nitrate (mcg/L)	45	22	11.81	46.3	3.74	83	0	100
8	Phosphate (mcg/L)	5	13.6	12	11.4	10.13	25.5	7.14	47.5
9	Sulphate (mg/L)	400	101	74.5	26.2	58.58	42	35.35	65
10	TDS (mg/L)	2100	2889	2605.8	9.8	2585.6	10.5	947.5	67.2
11	TSS (mg/L)	100	357.6	180.5	49.5	127.66	64.3	67.22	81.2
12	Turbidity (NTU)	5	362.3	277.15	23.5	239.11	34	190.93	47.3
13	Copper (mcg/L)	1.5	25.2	17.41	30.9	13.02	48.3	4.788	81.3
14	Lead (mcg/L)	0.01	11	7.3	33.6	4.48	59.2	1.518	86.2
15	Zinc (mcg/L)	15	91.2	78.25	14.2	45.88	49.7	31.29	65.7
16	Chromium(mcg/L)	0.01	78.2	67.01	14.3	54.74	30	37.85	51.6
17	Cobalt(mcg/L)	0.01	3	2.64	12	2.4	19.7	1.61	46.2
18	Manganese(mcg/L)	0.5	6.6	6.38	3.2	5.87	11	4.54	31.2
19	Mercury(mcg/L)	0.001	2.7	2.47	8.5	2.21	17.8	1.51	43.9
20	Nickel(mcg/L)	5	16.3	15.32	6	13.04	20	10.8	33.2
21	Iron (mcg/L)	50	8.6	8.27	3.8	6.89	19.8	0	100
22	Cadmium (mcg/L)	0.01	4	3.44	14	2.32	42	0	100

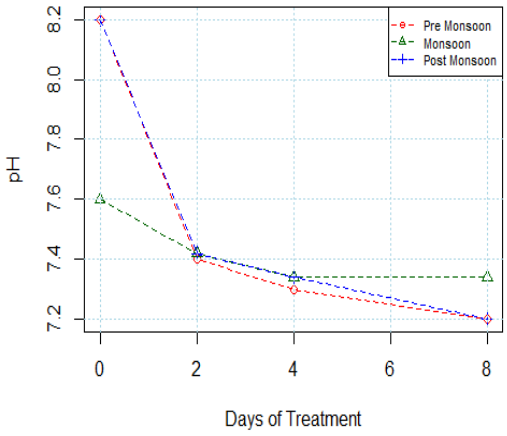

Fig.26(a): *Variation in pH after 2, 4 and 8 days of treatment in three seasons in Eloor W1*

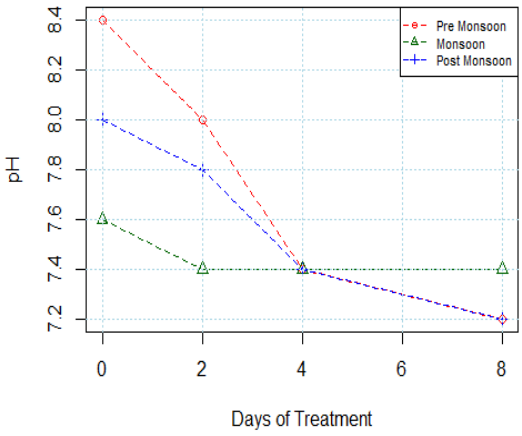

Fig.26(b): *Variation in pH after 2, 4 and 8 days of treatment in three seasons in Eloor W2*

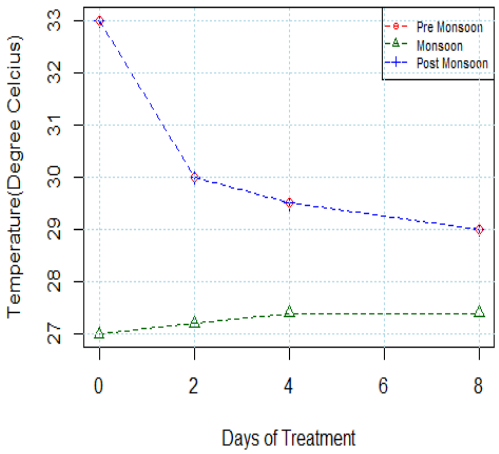

Fig.27(a): *Variation in Temperature after 2, 4 and 8 days of treatment in three seasons in Eloor W1 sample.*

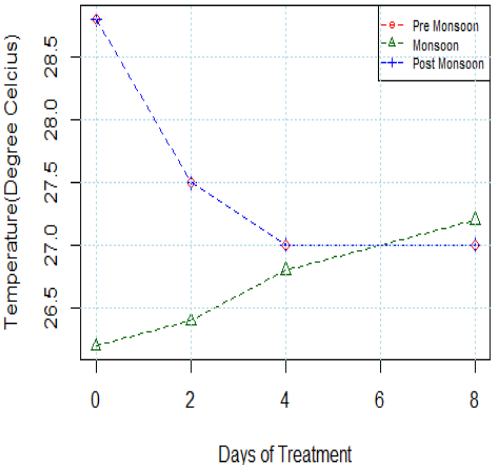

Fig.27(b): *Variation in Temperature after 2, 4 and 8 days of treatment in three seasons in Eloor W2 sample.*

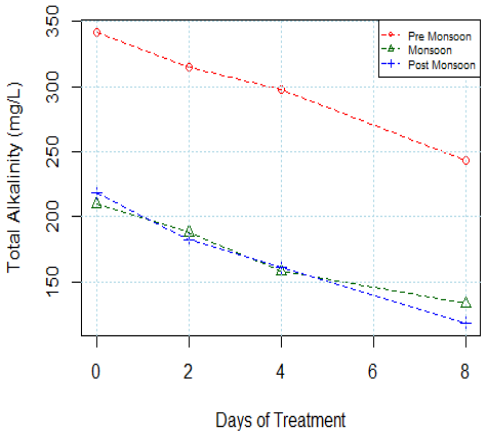

Fig.28(a): *Variation in total alkalinity after 2, 4 and 8 days of treatment in three seasons in Eloor W1 sample.*

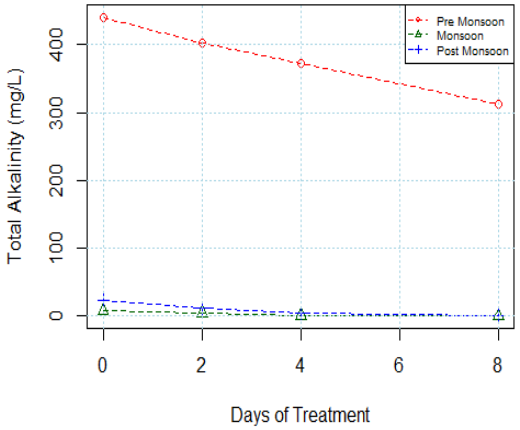

Fig.28(b): *Variation in total alkalinity after 2, 4 and 8 days of treatment in three seasons in Eloor W2 sample.*

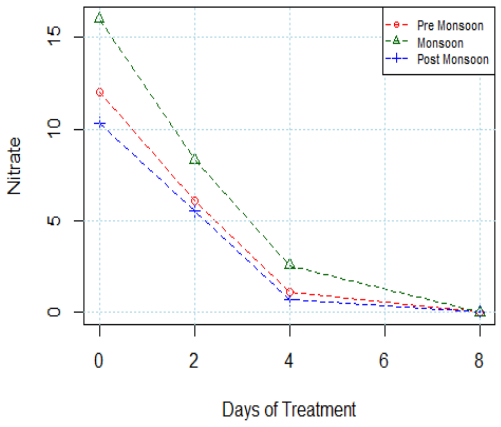

Fig.29(a): *Variation in nitrates after 2, 4 and 8 days of treatment in three seasons in Eloor W1 sample.*

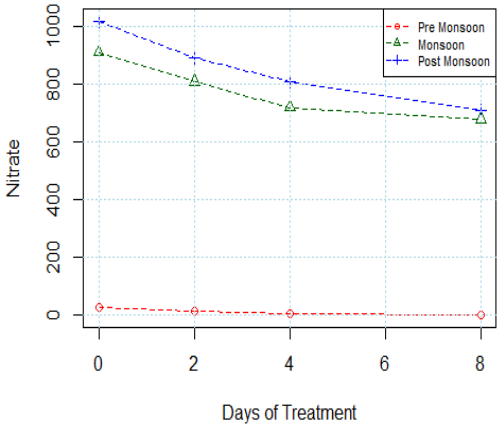

Fig.29(b): *Variation in nitrates after 2, 4 and 8 days of treatment in three seasons in Eloor W2 sample.*

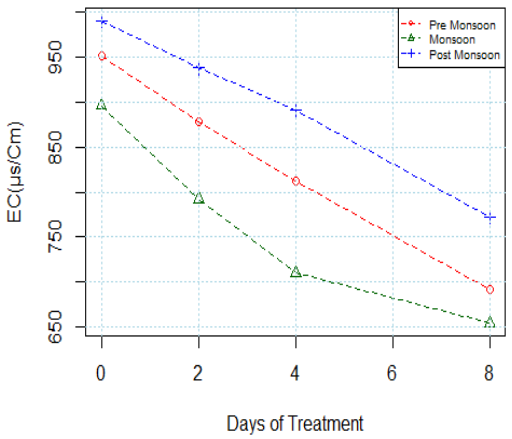

Fig.30(a): *Variation in EC after 2, 4 and 8 days of treatment in three seasons in Eloor W1 sample.*

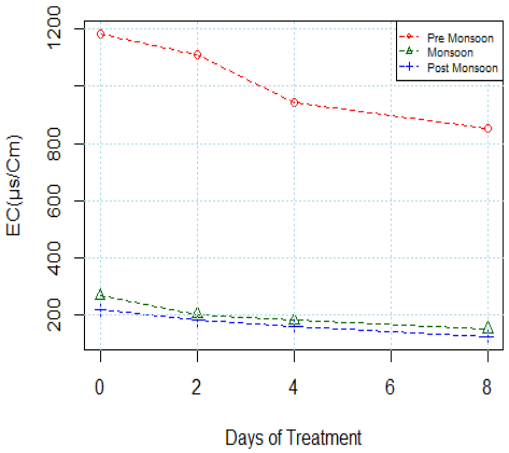

Fig.30(b): *Variation in EC after 2, 4 and 8 days of treatment in three seasons in Eloor W2 sample.*

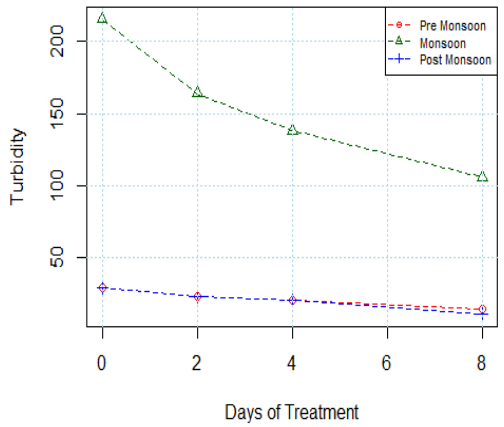

Fig. 31(a): *Variation in turbidity after 2, 4 and 8 days of treatment in three seasons in Eloor W1 sample.*

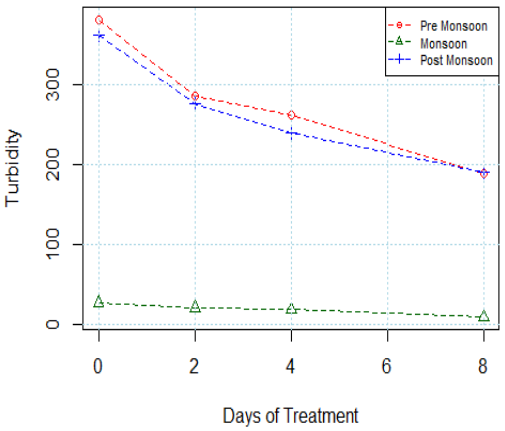

Fig. 31(b): *Variation in turbidity after 2, 4 and 8 days of treatment in three seasons in Eloor W2 sample.*

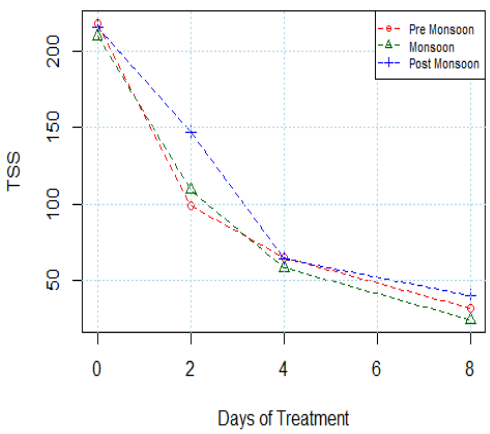

Fig.32(a): *Variation in TSS after 2, 4 and 8 days of treatment in three seasons in Eloor W1 sample.*

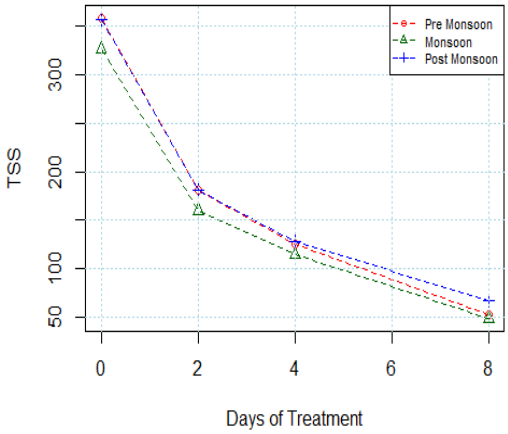

Fig.32(b): *Variation in TSS after 2, 4 and 8 days of treatment in three seasons in Eloor W2 sample.*

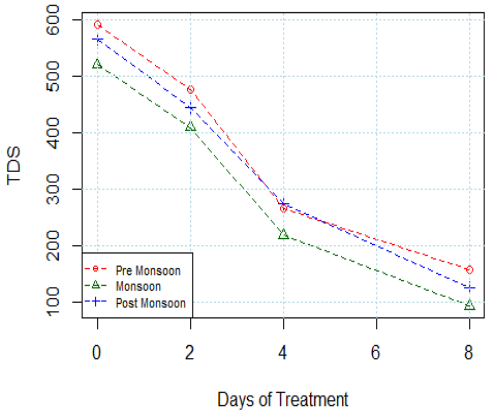

Fig.33(a): *Variation in TSS after 2, 4 and 8 days of treatment in three seasons in Eloor W1 sample.*

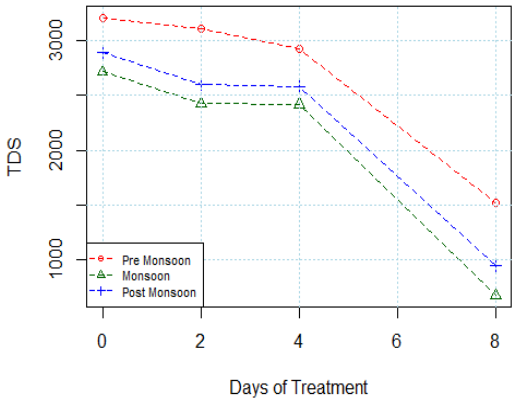

Fig.33(b): *Variation in TSS after 2, 4 and 8 days of treatment in three seasons in Eloor W2 sample.*

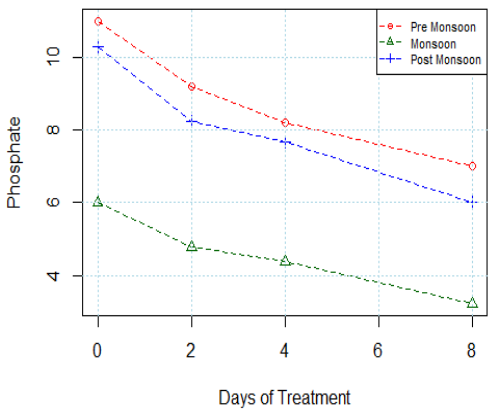

Fig.34(a): *Variation in phosphates after 2, 4 and 8 days of treatment in three seasons in Eloor W1 sample.*

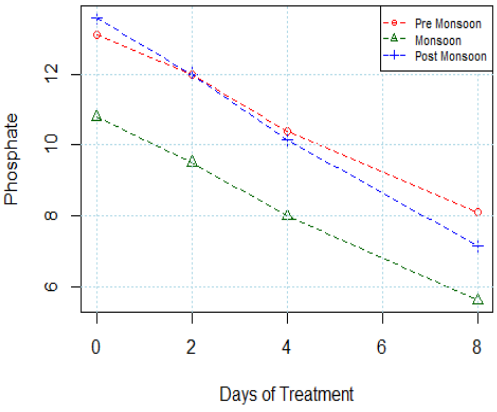

Fig.34(b): *Variation in phosphates after 2, 4 and 8 days of treatment in three seasons in Eloor W2 sample.*

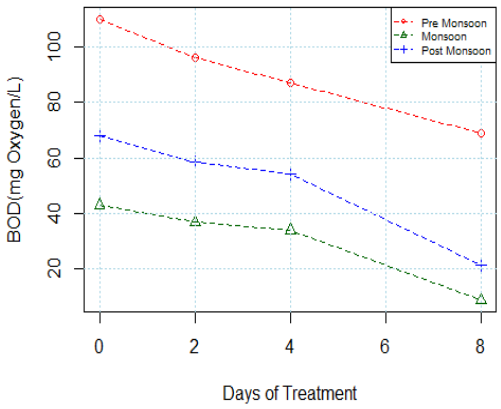

Fig.35(a): *Variation in BOD after 2, 4 and 8 days of treatment in three seasons in Eloor W1 sample.*

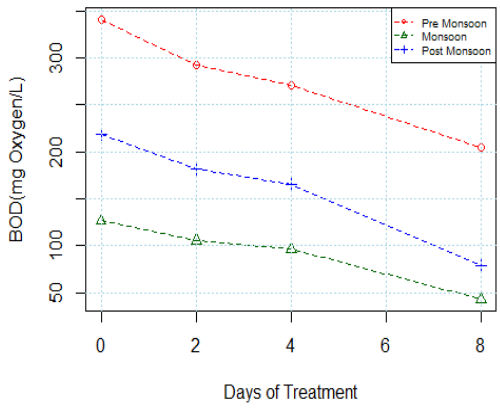

Fig.35(a): *Variation in BOD after 2, 4 and 8 days of treatment in three seasons in Eloor W2 sample.*

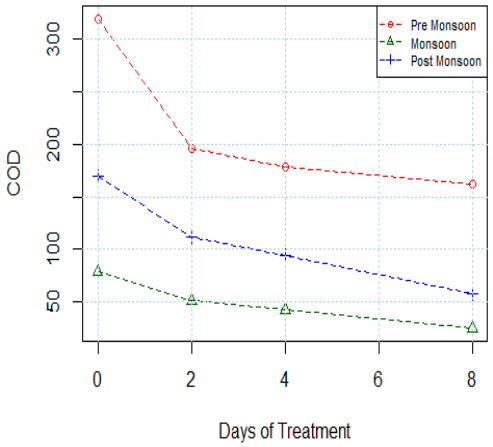

Fig.36(a): *Variation in COD after 2, 4 and 8 days of treatment in three seasons in Eloor W1*

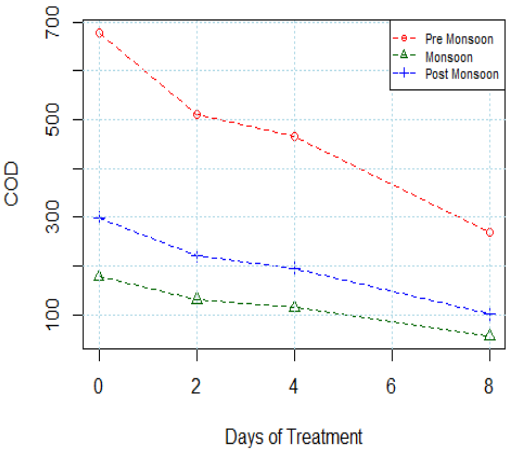

Fig.36(b): *Variation in COD after 2, 4 and 8 days of treatment in three seasons in Eloor W2*

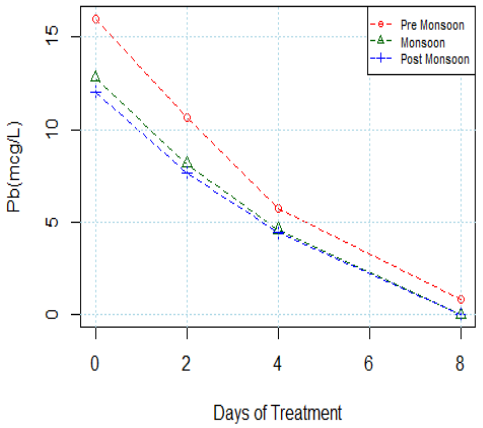

Fig.37(a): *Variation in Pb concentration after 2, 4 and 8 days of treatment in three seasons in Eloor W1 sample.*

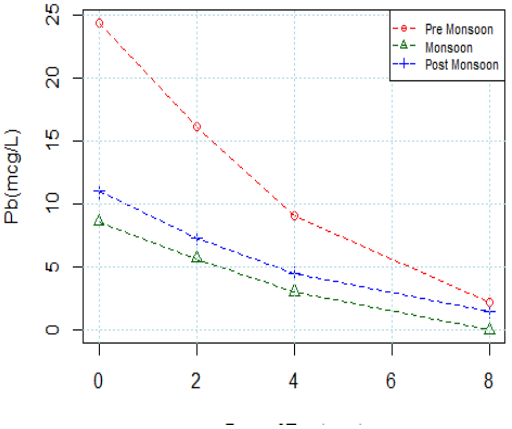

Fig.37(b): *Variation in Pb concentration after 2, 4 and 8 days of treatment in three seasons in Eloor W2 sample.*

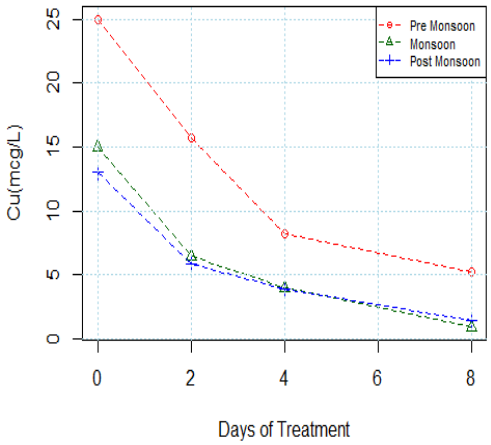

Fig.38(a): *Variation in Cu concentration after 2, 4 and 8 days of treatment in three seasons in Eloor W1 sample.*

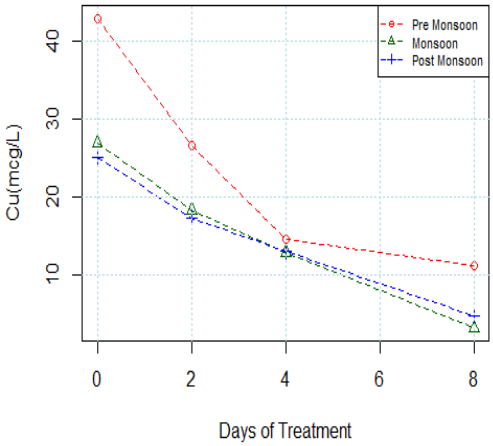

Fig.38(b): *Variation in Cu concentration after 2, 4 and 8 days of treatment in three seasons in Eloor W2 sample.*

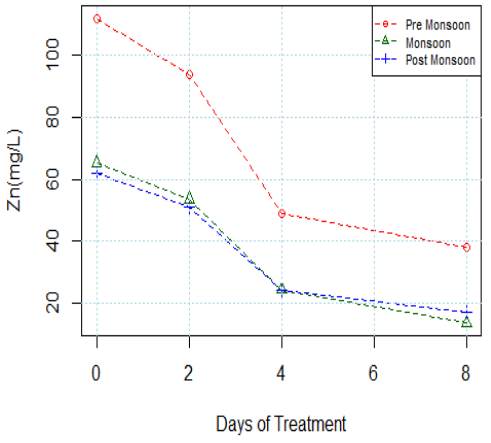

Fig.39(a): *Variation in Zn concentration after 2, 4 and 8 days of treatment in three seasons in Eloor W1 sample.*

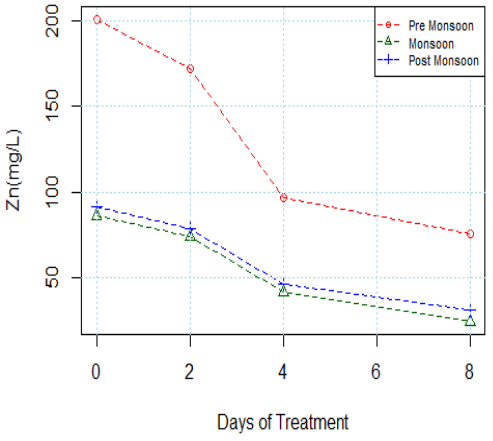

Fig.39(b): *Variation in Zn concentration after 2, 4 and 8 days of treatment in three seasons in Eloor W2 sample.*

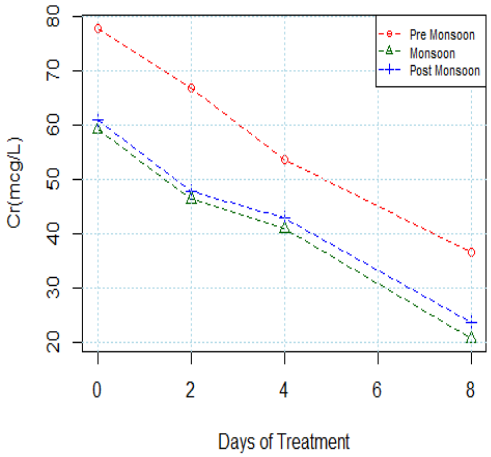

Fig.40(a): *Variation in Cr concentration after 2, 4 and 8 days of treatment in three seasons in Eloor W1 sample.*

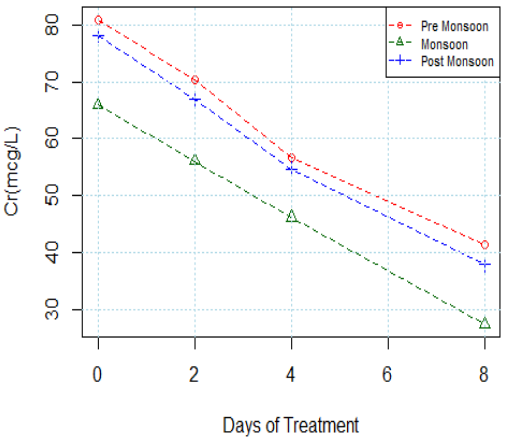

Fig.40(b): *Variation in Cr concentration after 2, 4 and 8 days of treatment in three seasons in Eloor W2 sample.*

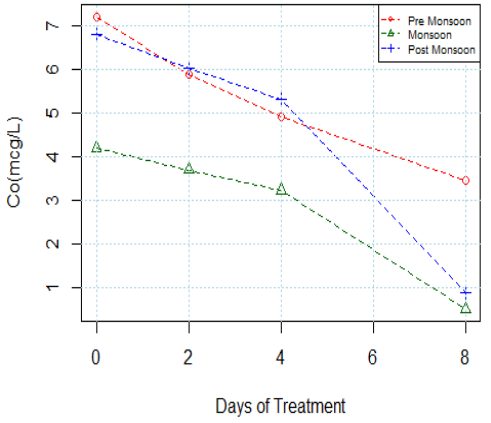

Fig.41(a): *Variation in Co concentration after 2, 4 and 8 days of treatment in three seasons in Eloor W1 sample.*

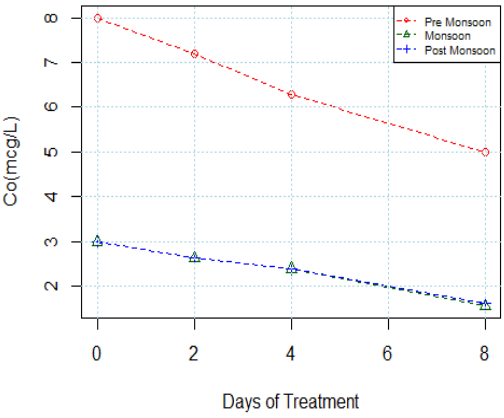

Fig.41(b): *Variation in Co concentration after 2, 4 and 8 days of treatment in three seasons in Eloor W2 sample.*

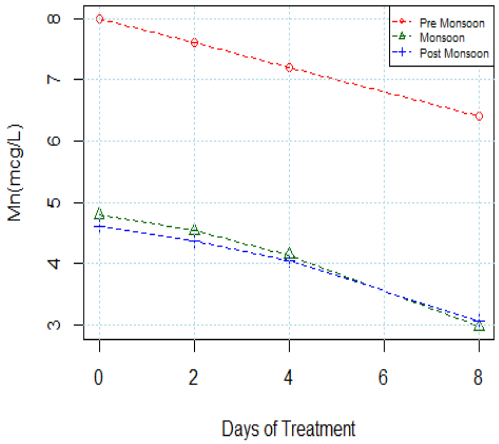

Fig.42(a): *Variation in Mn concentration after 2, 4 and 8 days of treatment in three seasons in Eloor W1 sample.*

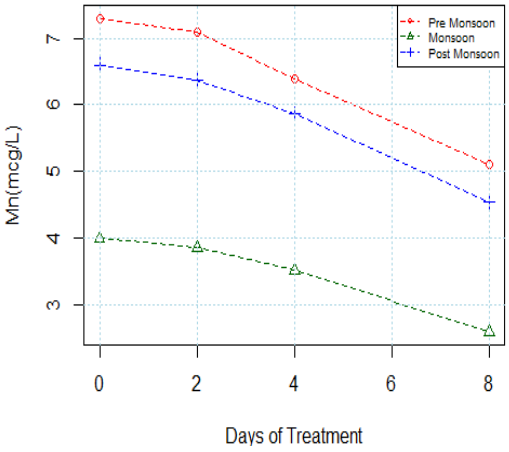

Fig.42(b): *Variation in Mn concentration after 2, 4 and 8 days of treatment in three seasons in Eloor W2 sample.*

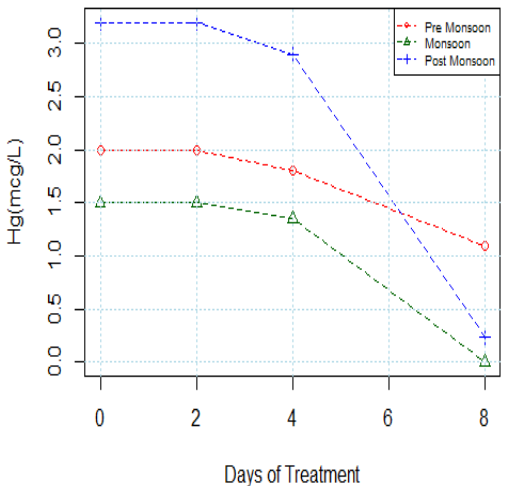

Fig.43(a): *Variation in Hg concentration after 2, 4 and 8 days of treatment in three seasons in Eloor W1 sample.*

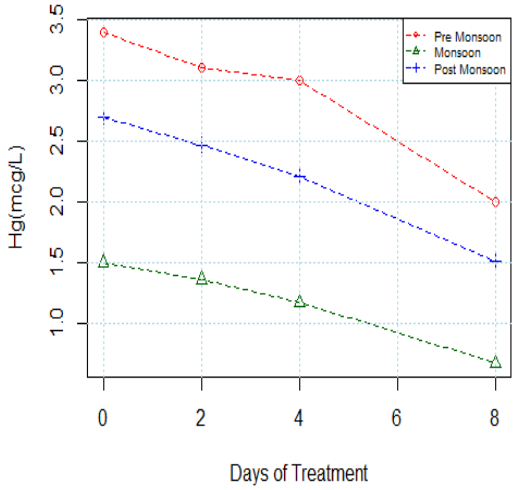

Fig.43(b): *Variation in Hg concentration after 2, 4 and 8 days of treatment in three seasons in Eloor W2 sample.*

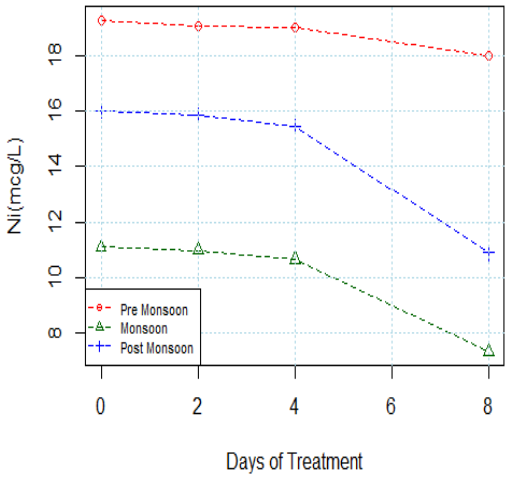

Fig.44(a): *Variation in Ni concentration after 2, 4 and 8 days of treatment in three seasons in Eloor W1 sample.*

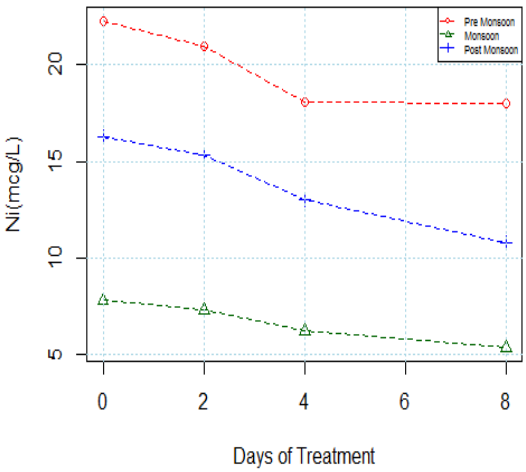

Fig.44(b): *Variation in Ni concentration after 2, 4 and 8 days of treatment in three seasons in Eloor W2 sample.*

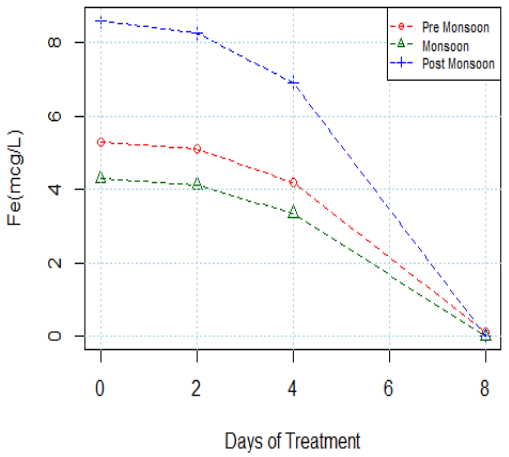

Fig. 45: *Variation in Fe concentration after 2, 4 and 8 days of treatment in three seasons in Eloor W2 sample.*

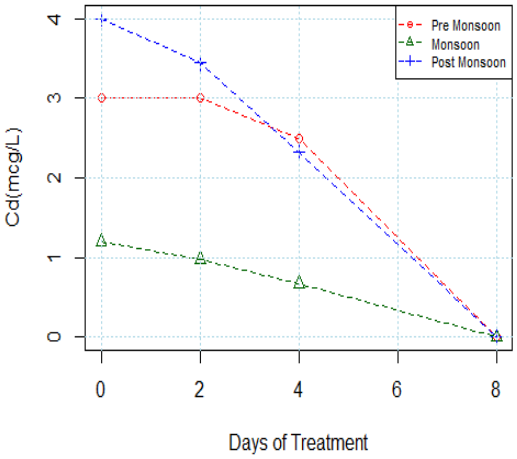

Fig.46: *Variation in Cd concentration after 2, 4 and 8 days of treatment in three seasons in Eloor W2 sample.*

b) **Temperature:** Water sample collected during pre monsoon, monsoon and post monsoon seasons, showed the temperature (*Fig. 27 a & b*) ranged between 26.2°C and 33°C which was within temperature tolerance limit for duckweed growth as mentioned by Culley *et* al., (1981) ,who found that the upper temperature tolerance limit for duckweed growth was around 34°C. Duckweed tolerance allows it to be used for year-round wastewater treatment in areas where tropical macrophyte, such as water hyacinths, can only grow in summer (Cheng *et* al., 2002).

c) Total alkalinity: Water sample collected during pre monsoon season revealed that total alkalinity of WI sample was 342 mg/L before treatment. After two days of treatment it reduces to 315 mg/L with an efficiency of 8%. After 4 days it came down further to 298 mg/L (13% removal). After 8 days alkalinity measurement was 243 mg/L with a removal efficiency of 29% (*Table 30, Fig. 28a*). In W2 sample, during the same season, water sample analysis shows initial total alkalinity of 441 mg/L. After 2 days 9% of reduction took place and concentration came down to 402 mg/L. After 4 days it came down further to 373 mg/L (15% removal). After 8 days total alkalinity measurement was 312 mg/L with a removal efficiency of 29% (*Table 31, Fig. 28b*).

Water sample collected during monsoon season revealed that total alkalinity of WI sample was 210 mg/L before treatment. After two days of treatment it reduces to 188 mg/L with an efficiency of 10%. After 4 days it came down further to 158 mg/L (25% removal). After 8 days alkalinity measurement was 133.2 mg/L with a removal efficiency of

37% (*Table 32, Fig. 28a*). In W2, during the same season, water sample analysis shows initial total alkalinity of 268 mg/L. After 2 days 24% of reduction took place and concentration came down to 203 mg/L. After 4 days it came down further to 181.8 mg/L (32% removal). After 8 days alkalinity measurement was 152.4 mg/L with a removal efficiency of 43 % (Table *33, Figure 28b*).

Water ample collected during post monsoon season revealed that total alkalinity of WI sample was 218 mg/L before treatment. After two days of treatment it reduces to 182 mg/L with an efficiency of 17%. After 4 days it came down further to 161 mg/L (26% removal). After 8 days alkalinity measurement was 118 mg/L with a removal efficiency of 46 % (*Table 30, Fig.28a)*. In W2 sample, during the same season, water sample analysis shows initial total alkalinity of 220 mg/L. After 2 days 16% of reduction took place and concentration came down to 185 mg/L. After 4 days it came down further to 162 mg/L (26% removal). After 8 days total alkalinity measurement was123 mg/L with a removal efficiency of 44% (*Table 35, Fig. 28b*).

d) Nitrates:

Water sample collected during pre monsoon season revealed that Nitrate concentration of WI sample was 12 mg/L before treatment (*Table 30, Fig.29a*) after two days of treatment it reduces to 6.1 mg/L with an efficiency of 48%. After 4 days it came down further to 1.1 mg/L (91% removal). After 8 days nitrate content was not detected which means removal efficiency of 100%. In W2 sample, during the same season, water sample analysis shows initial nitrate content of 27 mg/L. After

2 days 46% of reduction took place and concentration came down to 15 mg/L. After 4 days it came down further to 4.3 mg/L (84% removal). After 8 days nitrate measurement was 0 mg/L with a removal efficiency of 100% (*Table 31, Fig. 29b*).

In W1 sample, during the monsoon season, water sample analysis shows initial nitrate content of 8.3 mg/L (*Table 32, Fig. 29a*). After 2 days 48% of reduction took place and concentration came down to 4.31 mg/L. After 4 days it came down further to 0.58 mg/L (93% removal). After 8 days nitrate measurement was 0 mg/L with a removal efficiency of 100%. During monsoon season water sample collected from W2 revealed that Nitrate concentration was 16 mg/L before treatment (*Table 33, Fig. 29b*). After two days of treatment it reduces to 8.32 mg/L with an efficiency of 48%. After 4 days it came down further to 2.56 mg/L (84% removal). After 8 days nitrate was not detected in the sample.

Water sample collected during post monsoon season revealed that Nitrate concentration of WI sample was 10.3 mg/L before treatment. After two days of treatment it reduces to 5.53 mg/L with an efficiency of 46%. After 4 days it came down further to 0.72 mg/L (93% removal). After 8 days nitrate measurement was 0 mg/L with a removal efficiency of 100% (*Table 34, Fig. 29a*). In W2 sample, during the same season, water sample analysis shows initial nitrate content of 22 mg/L. After 2 days it drops down to 11.81 mg/L with an efficiency of 46% (*Table 35, Fig. 29b*). After 8 days 100% of reduction took place and concentration came down to 0 mg/L.

e) Electrical Conductivity (EC):

Water sample collected during Pre monsoon season revealed that Electrical conductivity (EC) of WI sample was 952 µs/Cm before treatment. After two days of treatment it reduces to 878 µs/Cm with an efficiency of 7.8% (*Table 30, Fig. 30a*). After 4 days it came down further to 812 µs/Cm (15% removal). After 8 days Electrical conductivity measurement was 692 µs/Cm with a removal efficiency of 27.3%. In W2 sample, during the same season, water sample analysis shows initial Electrical conductivity of 1185 µs/Cm. After 2 days 6% of reduction took place and concentration came down to 1112 µs/Cm. After 4 days it came down further to 942 µs/Cm (21% removal) . After 8 days Electrical conductivity measurement was 853 µs/Cm with a removal efficiency of 28% (*Table 31, Fig. 30b*).

During monsoon season water sample analysis revealed that Electrical conductivity (EC) of WI was 896.2 µs/Cm before treatment. After two days of treatment it reduces to 792.1 µs/Cm with an efficiency of 12%. After 4 days it came down further to 710 µs/Cm (21% removal) . After 8 days Electrical conductivity measurement was 654 µs/Cm with a removal efficiency of 27% (*Table 32, Fig.30a*). In W2, during the same season, water sample analysis shows initial Electrical conductivity of 912 µs/Cm. After 2 days 11% of reduction took place and concentration came down to 811 µs/Cm. After 4 days it came down further to 721 µs/Cm (30% removal). After 8 days Electrical conductivity measurement was 681 µs/Cm with a removal efficiency of 25% (*Table 33, Fig.30b*).

Electrical conductivity (EC) of water sample collected during Post monsoon season from W1revealed 991 µs/Cm before treatment. After two days of treatment it reduces to 938 µs/Cm with an efficiency of 5.3%. After 4 days it came down further to 891 µs/Cm (10% removal) . After 8 days Electrical conductivity measurement was 772 µs/Cm with a removal efficiency of 22% *(Table 34, Fig.30a*). In W2, during the same season, water sample analysis shows initial Electrical conductivity of 1021 µs/Cm. After 2 days 13% of reduction took place and concentration came down to 892 µs/Cm. After 4 days it came down further to 809 µs/Cm (21% removal). After 8 days Electrical conductivity measurement was 712 µs/Cm with a removal efficiency of 30.2% *(Table 31, Fig.30b*).

g) **Turbidity**: Pre monsoon water sample treatment shows turbidity reduced by 23% from 29 NTU to 22.4 NTU after 2 days for WI. It further reduced to 19.7(32%) after 4 days of treatment. After 8 days it was 14 NTU which means almost half of the turbidity has been removed (*Table 30, Fig. 31a*). In W2 sample turbidity was reduced by 25% from 382 NTU to 286 NTU after 2 days. It further reduced to 262 (31.4%) and to 189 NTU (51%) after 4 days and 8 days of treatment respectively (*Table 31, Fig. 31b*). During monsoon season turbidity was reduced by 24% from 215.6 NTU to 163.8 NTU after 2 days for WI. It further reduced to 138 (36%) after 4 days of treatment. After 8 days it was 105.6 NTU which means almost half of the turbidity has been removed (*Table 32, Fig.31a*). In WII turbidity was reduced by 24% from 27NTU to 20.5 NTU after 2 days. It further reduced to 18.1(33%) and to 9.01 NTU (66%) after 4 days and 8 days of treatment respectively *(Table 33, Fig. 31b*).

During post monsoon season turbidity reduced by 23% from 29 NTU to 22.3 NTU after 2 days for WI sample. It further reduced to 20.01 NTU (31%) after 4 days of treatment. After 8 days it was 10.15 NTU which means majority of the turbidity has been removed. In WII turbidity was reduced by 23.5% from 362.3NTU to 277.1 NTU after 2 days. It further reduced to 239.1 (34%) and to 191 NTU (47.3%) after 4 days and 8 days of treatment respectively *(Table 35, Fig.31b*).

h) **Total Suspended Solids**: Total suspended solids (TSS) values decreased by increasing treatment periods. During pre monsoon season TSS showed maximum concentration of 218.4 and 359 for WI sample and WII sample respectively before treatment. The concentration sides down to 99 and 181 mg.L-1 after 2 days (55% and 50% respectively) and further reduced to 65.12 mg/L (70%) and 126 (65%) after 4 days and finally decreased by 85.4% and 85.2% (32 mg/L and 53mg/L respectively) (*Fig. 32a & b)*. During monsoon season initial TSS measured was 210 mg/L in W1 sample. After 2 days of treatment it came down to 109.2 mg/L (48%) removal and after 4 days it shows 58.3 mg/L which means 72% removal. After 8 days 88% of TSS was removed and final concentration falls down to 24.3 mg/L *(Table 32, Fig. 32a*). In W2 sample , after 2 days of treatment initial concentration of 327 mg/L came down to 160.23 mg/L (51%) removal and after 4 days it shows 114.4 mg/L which means 65% removal . After 8 days 85% of TSS was removed and final concentration falls down to 48.3mg/L (*Table 33, Fig. 32b*) .During post monsoon season initial TSS measured was 216 mg/L in W1 sample. After 2 days of treatment it came down to 146.8 mg/L (32%) removal and after 4 days it shows 64.3mg/L which means 70.2 %

removal. After 8 days 81 % of TSS was removed and final concentration falls down to 40.39 mg/L *(Table 34, Fig. 32a*). In W2 sample, after 2 days of treatment initial concentration of 357.6mg/L came down to 180.5 mg/L (50%) removal and after 4 days it shows 127.6 mg/L which means 64.3% removal . After 8 days 81% of TSS were removed and final concentration falls down to 67.2 mg/L (*Table 35, Fig. 32b*).

i**)** **TDS**: Analysis of water sample collected during pre monsoon season revealed that total dissolved solids (TDS) of WI and WII recorded their minimum values of and 158 mg/L (73.3%) 1522 mg/L (53%) after 8 days treatment. It was 477 mg/L (20% reduction) and 3111 mg/L (3% reduction) after 2 days of treatment. It was 266.3mg/L (55% reduction) and 2928.1 mg/L (9%) after 4 days *(Fig.33 (a and b)*).

Water sample collected during monsoon season revealed that total dissolved solids (TDS) of WI sample was 521 mg/L before treatment. After two days of treatment it reduces to 410 with an efficiency of 21%. After 4 days it came down further to 219 mg/L (58% removal). After 8 days TDS measurement was 93.7 mg/L with a removal efficiency of 82% (*Table 32, Fig. 33a*). In W2, during the same season, water sample analysis shows initial TDS of 2718 mg/L. After 2 days 11% of reduction took place and concentration came down to 2424.4 mg/L. After 4 days it came down further to 2419 mg/L (11% removal). After 8 days TDS measurement was 679.5mg/L with a removal efficiency of 75%. *(Table 33, Fig. 33b*).

Water sample collected during Post monsoon season revealed that total dissolved solids (TDS) of WI was 566 mg/L before treatment. After two

days of treatment it reduces to 445.4 with an efficiency of 21.3%. After 4 days it came down further to 275.1 mg/L (51% removal). After 8 days TDS measurement was 125.6 mg/L with a removal efficiency of 78% (*Table 34, Fig. 33a*). In W2, during the same season, water sample analysis shows initial TDS of 2889 mg/L. After 2 days 10% of reduction took place and concentration came down to 2605.8 mg/L. After 4 days it came down further to 2585.6 mg/L (11% removal). After 8 days TDS measurement was 947 mg/L with a removal efficiency of 67 % (*Table 35, Fig.33b*).

j) **Phosphate**: The phosphate content of the sample collected during pre monsoon period from the wetland water I and II were 11mcg/L and 13.1 mcg/L respectively. After 2 days of treatment, it has been reduced by 16.3% and 10% and after 4 days it has been reduced by 25.4% and 21% respectively. After 8 days it has been reduced by 36.3% to 7 mcg/L for WI sample and reduced by 38% to 8.1 mcg/L after 8 days of growth in W2 (*Fig. 34(a& b).*

Water sample collected during monsoon season revealed that phosphate content of WI was 6 mg/L before treatment. After two days of treatment it reduces to 4.77 mg/L with an efficiency of 21.5%. After 4 days it came down further to 4.38 mg/L (27% removal). After 8 days phosphate content was 3.22 mg/L with a removal efficiency of 46% *(Table 32, Fig.34a*). In W2, during the same season, water sample analysis shows initial phosphate concentration of 10.8 mg/L. After 2 days 12% of reduction took place and concentration came down to 9.5 mg/L. After 4 days it came down further to 7.9 mg/L (26% removal). After 8 days

phosphate measurement was 5.61 mg/L with a removal efficiency of 48% *(Table 33, Fig.34b*).

Water sample collected during post monsoon season revealed that phosphate content of WI was 10.3 mg/L before treatment. After two days of treatment it reduces to 8.24 mg/L with an efficiency of 20%. After 4 days it came down further to 7.67 mg/L (26 % removal). After 8 days phosphate content was 6.02 mg/L with a removal efficiency of 42% (*Table 34, Fig. 34a*). In W2, during the same season, water sample analysis shows initial phosphate concentration of 13.6 mg/L. After 2 days 11.4% of reduction took place and concentration came down to 12 mg/L. After 4 days it came down further to 10.13 mg/L (26% removal). After 8 days phosphate measurement was 7.14 mg/L with a removal efficiency of 48% *(Table 35, Fig. 34b*).

k) **BOD:** Tables 1 and 2 of pre monsoon sample treatment reveals the gradual reduction of BOD with time means that *Spirodela polyrhiza* mat effectively reduced BOD by 37% for WI and 40% for WII at the end of exposure period i.e., reduced from initial concentration of 110 mg O_2 L^{-1} at zero days and 341 mg O_2 L^{-1} for WI sample and W2 respectively. After 4 days it was reduced by 21% (reduced to 87 mg/L) and 20.4% (reduced to 271.2 O_2/L) for WI and WII samples respectively. After 8 days BOD stands at 3.5 O_2/L (reduced by77%) for WI and stands at 2.8 O_2/L (reduced by 53.3%) for WII sample (*Table 30&31, Fig.35 a& b).*

Water sample collected during monsoon season revealed that BOD of WI was 43 O_2/L before treatment. After two days of treatment it reduces to 36.76 O_2/L with an efficiency of 14.5%. After 4 days it came down

further to 33.97 O_2/L (21% removal). After 8 days BOD was 8.85 O_2/L with a removal efficiency of 79.4% (*Table 32, Fig. 35a*). In W2, during the same season, water sample analysis shows initial BOD of 178 O_2/L. After 2 days 26% of reduction took place and concentration came down to 131.7 O_2/L. After 4 days it came down further to 96.13 O_2/L (24.3% removal). After 8 days BOD measurement was 43.18 O_2/L with a removal efficiency of 66% *(Table 33, Fig. 35b*).

Water sample collected during post monsoon season revealed that BOD of WI was 68 O_2/L before treatment. After two days of treatment it reduces to 58.48 O_2/L with an efficiency of 14%. After 4 days it came down further to 54.4 O_2/L (20% removal). After 8 days BOD was 21.55 O_2/L with a removal efficiency of 68% *(Table 34, Fig. 35a*). In W2, during the same season, water sample analysis shows initial BOD of 218.3 O_2/L. After 2 days 17% of reduction took place and concentration came down to 181.6 O_2/L. After 4 days it came down further to 165.9 O_2/L (24% removal). After 8 days BOD measurement was 78.5 O_2/L with a removal efficiency of 64% *(Table 35, Fig. 35b*).

l) **COD:** During pre monsoon season, the sample analysis after treatment with *Spirodela* reveals that the COD has been reduced by 39% for WI sample and 25 % immediately after 2 days of phytoremediation (reduced from the initial concentration of 320 mg/L to 196 mg/L and 679 mg/L to 511.2 mg/L). After 4 days it further reduced by 44.3% (reduced to 178 mg/L) for WI and reduced by 32% (reduced to 464.4 mg/L) and finally after 8 days, reduced to mere 162 mg/L (49%) for WI and reduced to 268.3 mg/L (60.4%) for WII (*Fig. 36 a& b*).

Water sample collected during monsoon season revealed that COD of WI was 78.5 mg/L before treatment. After two days of treatment it reduces to 51 mg/L with an efficiency of 35%. After 4 days it came down further to 42.23 mg/L (46% removal). After 8 days COD was 24.72 mg/L with a removal efficiency of 68.5% *(Table 32, Fig. 36a*). In W2, during the same season, water sample analysis shows initial COD of 178 mg/L. After 2 days 26% of reduction took place and concentration came down to 131.7 mg/L. After 4 days it came down further to 113.9 mg/L (36% removal). After 8 days COD measurement was 56.07mg/L with a removal efficiency of 68.5 %.(*Table 33, Fig.36b*).

Water sample collected during post monsoon season revealed that COD of WI was 169.6 mg/L before treatment. After two days of treatment it reduces to 111.9 mg/L with an efficiency of 34%. After 4 days it came down further to 94.63 mg/L (44.2% removal). After 8 days COD was 57.32 mg/L with a removal efficiency of 66.2% *(Table 34, Fig.36a*). In W2, during the same season, water sample analysis shows initial COD of 298.3 mg/L. After 2 days of exposure 26% reduction took place and concentration came down to 220.7 mg/L. After 4 days it came down further to 195.38 mg/L (34.5% removal). After 8 days COD measurement was 100.8 mg/L with a removal efficiency of 66.2% *(Table 35, Fig. 36b*). .

m) Heavy metals:

(a) Lead: During pre monsoon season Pb concentration was 16 mcg/L in the W1 sample solution. After 2 days of treatment it has been reduced by 33.4% to 10.65 mcg/L. After 4 days it further reduced by 64% to

5.76 mcg/L. Finally after 8 days Lead concentration is only 0.8 mcg/L, which means 95% removal *(Table 30, Figure 37a)*. Pb concentration was 24.4 mcg/L in the W2 sample solution. After 2 days of treatment it has been reduced by 34% to 16.11 mcg/L. After 4 days it further reduced by 63% to 9.03 mcg/L. Finally after 8 days Lead concentration became 2.2mcg/L, which means 91% removal (*Table 31, Fig.37b*).

Water sample collected during monsoon season revealed that Pb content of WI was 12.8 mcg/L before treatment. After two days of treatment it reduces to 8.16 mg/L with an efficiency of 36.2%. After 4 days it came down further to 4.60 mcg/L (64% removal). After 8 days Pb concentration was 0 mcg/L with a removal efficiency of 100% (*Table 32, Fig. 37a*). In W2, during the same season, water sample analysis shows initial Pb content of 8.6 mcg/L. After 2 days 34% of reduction took place and concentration came down to 5.68 mcg/L. After 4 days it came down further to 3.05 mg/L (64.5% removal). After 8 days Pb measurement was 0 mcg/L with a removal efficiency of 100% (*Table 33, Fig. 37b*). .

During Post monsoon season, sample analysis revealed that Pb content of WI was 12 mcg/L before treatment. After two days of treatment it reduces to 7.68 mcg/L with an efficiency of 36%. After 4 days it came down further to 4.44 mcg/L (63% removal). After 8 days Pb concentration was 0 mcg/L with a removal efficiency of 100% *(Table 34, Fig. 37a*). In W2, during the same season, water sample analysis shows initial Pb content of 11 mcg/L . After 2 days 33.6% of reduction took place and concentration came down to 7.30 mcg/L. After 4 days it came down

further to 4.48 mcg/L (59.2% removal). After 8 days Pb measurement was 1.51 mcg/L with a removal efficiency of 86.2% (*Table 35, Fig. 37b*).

(b) Copper: After 8 days of treatment on sample collected during pre monsoon season Copper content in the water has been removed by 79% in W1 sample which means a reduction from initial concentration of 25 mcg/L to final concentration of 5.25 mcg/L. Copper shows removal by 37% after 2 days and removal by 67% after 4 days of treatment with *Spirodela polyrhiza* plant (*Table 30, Fig. 38a*). Cu concentration was 43 mcg/L in the W2 sample solution. After 2 days of treatment it has been reduced by 38% to 26.6 mcg/L. After 4 days it further reduced by 66% to 14.6 mcg/L. Finally after 8 days Cu concentration became 11.1 mcg/L, which means 74 removal *(Table 31, Fig. 38b*).

Water sample collected during monsoon season revealed that Cu content of WI was 15 mcg/L before treatment. After two days of treatment it reduces to 6.45 mcg/L with an efficiency of 57%. After 4 days it came down further to 3.99 mcg/L (73.4% removal). After 8 days Cu concentration was 0.9 mcg/L with a removal efficiency of 94% (*Table 32, Fig. 38a*). In W2, during the same season, water sample analysis shows initial Cu content of 27 mcg/L. After 2 days 32% of reduction took place and concentration came down to 18.36 mcg/L. After 4 days it came down further to 12.96 mcg/L (52% removal). After 8 days Cu measurement was 3.24 mcg/L with a removal efficiency of 88 (*Table 33, Fig.38b)*.

During post monsoon season, sample analysis revealed that Cu content of WI was 13 mcg/L before treatment. After two days of treatment it reduces to 5.83 mcg/L with an efficiency of 55.2%. After 4 days it came down further to 3.94 mcg/L (70% removal). After 8 days Cu concentration was 1.40 mcg/L with a removal efficiency of 89.2% (*Table 34, Fig.38a*). In W2, during the same season, water sample analysis shows initial Cu content of 25.2 mcg/L . After 2 days 31% of reduction took place and concentration came down to 17.41 mcg/L. After 4 days it came down further to 13.02 mcg/L (48.3% removal). After 8 days Cu measurement was 4.78 mcg/L with a removal efficiency of 81.3% (*Table 35*, *Fig. 38b*).

(c) Zinc: Initial concentration of Zn was 112 mcg/L in W1 sample before treatment during premonsoon season. Two days of treatment is only enough to remove 16.4% of Zn from the wetland water which means reduction to 93.6 mcg/L. Four days of treatment was enough to remove 56.4% of Zn (reduced to 48.8 mcg/L). After 8 days 66% of reduction occurred. Treatment with *Spirodela* was not successful since the concentration of Zn did not came down to the standard proposed by CPCB (*Table 30, Fig. 39a*). Zn concentration was 201 mcg/L in the W2 sample solution. After 2 days of treatment it has been reduced by 14.2% to 172.4 mcg/L. After 4 days it further reduced by 52% to 96.48 mcg/L. Finally after 8 days Zn concentration became 75.5 mcg/L, which means 62.4% removal. The final concentration was not within the range of CPCB standards (*Table 31, Fig. 39b*).

Water sample collected during monsoon season revealed that Zn content of WI was 65.4 mcg/L before treatment. After two days of treatment it reduces to 53.5 mcg/L with an efficiency of 18.2%. After 4 days it came down further to 24.33 mcg/L (63% removal). After 8 days Zn concentration was 13.61 mcg/L with a removal efficiency of 79.2% (*Table 32, Fig. 39a*). In W2, during the same season, water sample analysis shows initial Zn content of 86 mcg/L. After 2 days 14.2% of reduction took place and concentration came down to 73.79 mcg/L. After 4 days it came down further to 41.28 mcg/L (52% removal). After 8 days Zn measurement was 24.08 mcg/L with a removal efficiency of 72% (*Table 33*, *Fig. 39b*).

During Post monsoon season, sample analysis revealed that Zn content of WI was 62 mcg/L before treatment. After two days of treatment it reduces to 50.84 mcg/L with an efficiency of 18%. After 4 days it came down further to 24.18 mcg/L (61% removal). After 8 days Zn concentration was 17.17 mcg/L with a removal efficiency of 72.3% (*Table 34, Fig. 39a*). In W2, during the same season, water sample analysis shows initial Zn content of 91.2 mcg/L. After 2 days 14.2% of reduction took place and concentration came down to 78.25 mcg/L. After 4 days it came down further to 45.88 mcg/L (50% removal). After 8 days Zn measurement was 31.29 mcg/L with a removal efficiency of 66% (*Table 35*, *Fig. 39b*).

(d) Chromium: Cr concentration was 78 mcg/L in the W1 sample collected during pre monsoon period. After 2 days of treatment it has been reduced by 14.2% to 66.9 mcg/L. After 4 days it further reduced by

31% to 53.82 mcg/L. Finally after 8 days Cr concentration became 36.66 mcg/L, which means 53 removal (*Table 30, Fig. 40a*). Cr concentration was 81 mcg/L in the W2 sample solution. After 2 days of treatment it has been reduced by 13% to 70.4 mcg/L. After 4 days it further reduced by 30% to 56.7 mcg/L. Finally after 8 days Cr concentration became 41.31 mcg/L that means 49 % removal (*Table 31, Fig. 40b*).

Water sample collected during monsoon season revealed that Cr content of WI was 59.3 mcg/L before treatment. After two days of treatment it reduces to 46.4 mcg/L with an efficiency of 21.7%. After 4 days it came down further to 40.9 mcg/L (31% removal). After 8 days Cr concentration was 20.7 mcg/L with a removal efficiency of 65% (*Table 32, Fig. 40a*) . In W2, during the same season, water sample analysis shows initial Cr content of 66 mcg/L. After 2 days 15% of reduction took place and concentration came down to 56.1 mcg/L. After 4 days it came down further to 46.2 mcg/L (30% removal). After 8 days Cr measurement was 27.39 mcg/L with a removal efficiency of 59% (*Table 33, Fig. 40b*) .

During post monsoon season, sample analysis revealed that Cr content of WI was 61 mcg/L before treatment. After two days of treatment it reduces to 47.76 mcg/L with an efficiency of 22%. After 4 days it came down further to 43 mcg/L (30% removal). After 8 days Cr concentration was 23.79 mcg/L with a removal efficiency of 61%(*Table 34, Fig. 40a*). In W2, during the same season, water sample analysis shows initial Cr content of 78.2 mcg/L. After 2 days 14.3% of reduction took place and

concentration came down to 67.01 mcg/L. After 4 days it came down further to 54.74 mcg/L (30% removal). After 8 days Cr measurement was 37.85 with 52% removal efficiency (*Table 35, Fig. 40b*) .

(e) Cobalt: Cobalt concentration was 7.2 mcg/L in the W1 sample collected during pre monsoon period. After 2 days of treatment it has been reduced by 18.4 % to 5.87 mcg/L. After 4 days it further reduced by 31.2% to 4.9 mcg/L. Finally after 8 days Cobalt concentration became 3.45 mcg/L, which means 52% removal (*Table 30, Fig. 41a*). Cobalt concentration was 8 mcg/L in the W2 sample solution. After 2 days of treatment it has been reduced by 10% to 7.2 mcg/L. After 4 days it further reduced by 21.2% to 6.3 mcg/L. Finally after 8 days Cobalt concentration became 5 mcg/L that means 40 % removal *(Table 31, Fig. 41b*).

Water sample collected during monsoon season revealed that Cobalt content of WI was 4.2 mcg/L before treatment. After two days of treatment it reduces to 3.69 mcg/L with an efficiency of 12%. After 4 days it came down further to 3.23 mcg/L (23% removal). After 8 days Cobalt concentration was 0.51mcg/L with a removal efficiency of 88% (*Table 32, Fig. 41a*). In W2, during the same season, water sample analysis shows initial Cobalt content of 3 mcg/L. After 2 days 12.3% of reduction took place and concentration came down to 2.63 mcg/L. After 4 days it came down further to 2.39 mcg/L (20.2% removal). After 8 days Cobalt measurement was 1.56mcg/L with a removal efficiency of 48% *(Table 33, Fig. 41b*).

During post monsoon season, sample analysis revealed that Cobalt content of WI was 6.8 mcg/L before treatment. After two days of

treatment it reduces to 6.01 mcg/L with an efficiency of 11.5%. After 4 days it came down further to 5.3 mcg/L (22% removal). After 8 days Cobalt concentration was 0.88 mcg/L with a removal efficiency of 87% (*Table 34, Fig. 41a*). In W2, during the same season, water sample analysis shows initial Cobalt content of 3 mcg/L. After 2 days 12% of reduction took place and concentration came down to 2.64 mcg/L. After 4 days it came down further to 2.4 mcg/L (20% removal). After 8 days Cobalt measurement was 1.61mg/L with a removal efficiency of 46.2% (*Table 35, Fig. 41b*).

(f) Manganese: Mn concentration was 8 mcg/L in the W1sample collected during pre monsoon period. After 2 days of treatment it has been reduced by 5% to 7.6 mcg/L. After 4 days it further reduced by 10% to 7.2 mcg/L. Finally after 8 days Mn concentration became 6.4mcg/L, which means 20% removal (*Table 30, Fig. 43a*). Mn concentration was 7.3 mcg/L in the W2 sample solution. After 2 days of treatment it has been reduced by 3% to 7.1 mcg/L. After 4 days it further reduced by 12.3% to 6.4 mcg/L. Finally after 8 days Mn concentration became 5.1 mcg/L that means 30 % removal *(Table 31, Fig.42b*).

Water sample collected during monsoon season revealed that Mn content of WI was 4.8 mcg/L before treatment. After two days of treatment it reduces to 4.53 mcg/L with an efficiency of 5.5%. After 4 days it came down further to 4.14 mcg/L (14% removal). After 8 days Mn concentration was 2.97 mcg/L with a removal efficiency of 38% (*Table 32, Fig. 42a*). In W2, during the same season, water sample analysis shows initial Mn content of 4 mcg/L. After 2 days 3.5% of reduction took place and concentration came

down to 3.86 mcg/L. After 4 days it came down further to 3.52 mcg/L (12% removal). After 8 days Mn measurement was 2.6mcg/L with a removal efficiency of 35% (*Table 33, Fig.42b*).

During Post monsoon season, sample analysis revealed that Mn content of WI was 4.6 mcg/L before treatment. After two days of treatment it reduces to 4.37 mcg/L with an efficiency of 5%. After 4 days it came down further to 4.04mcg/L (12% removal). After 8 days Mn concentration was 3.05mcg/L with a removal efficiency of 34% (*Table 34, Fig.42a*). In W2, during the same season, water sample analysis shows initial Mn content of 6.6 mcg/L . After 2 days 3.2% of reduction took place and concentration came down to 6.38 mcg/L. After 4 days it came down further to 5.87 mcg/L (11% removal). After 8 days Mn measurement was 4.54mcg/L with a removal efficiency of 31% *(Table 35, Fig. 42b*).

(g) Mercury: Hg concentration was 2.33 mcg/L in the W1sample collected during pre monsoon period. After 2 days of treatment it has been reduced by 3% to 2.26 mcg/L. After 4 days it further reduced by 15% to 1.98 mcg/L. Finally after 8 days Hg concentration became 0 mcg/L, which means 100 removal (*Table 30, Fig.43a*). Hg concentration was 0.47 mcg/L in the W2 sample solution. After 2 days of treatment it has been reduced by 4.5% to 0.44 mcg/L. After 4 days it further reduced by 58% to 0.19 mcg/L. Finally after 8 days Hg concentration became 0 mcg/L that means 100 % removal *(Table 31*, *Fig.43b*).

During monsoon season, sample analysis revealed that Hg content of WI was 1.5 mcg/L before treatment. After two days of treatment it remains the same. After 4 days it came down further to 1.35 mcg/L (10%

removal). After 8 days Hg concentration was 0 mcg/L with a removal efficiency of 100% *(Table 32, Fig. 43a*). In W2, during the same season, water sample analysis shows initial Hg content of 1.5 mcg/L. After 2 days 9% of reduction took place and concentration came down to 1.36 mcg/L. After 4 days it came down further to 1.17 mcg/L (21.4% removal). After 8 days Hg measurement was 0.67 with a removal efficiency of 55% (*Table 33, Fig.43b*)

Water sample collected during post monsoon season revealed that Hg content of WI was 3.2 mcg/L before treatment. After two days of treatment it remains 3.2 mcg/L . After 4 days it came down further to 2.89 mcg/L (9.5% removal). After 8 days Hg concentration was 0.24 mcg/L with a removal efficiency of 92.2 %.(*Table 34, Fig. 43a).* In W2, during the same season, water sample analysis shows initial Hg content of 2.7 mcg/L. After 2 days 8.5% of reduction took place and concentration came down to 2.47 mcg/L. After 4 days it came down further to 2.21 mcg/L (18% removal). After 8 days Hg measurement was 1.51 mcg/L with a removal efficiency of 44% (*Table 35, Fig.43b*).

(h) Nickel: Ni concentration was 19.3 mcg/L in the W1 sample collected during pre monsoon period. After 2 days of treatment it has been reduced by 1.03% to 19.1 mcg/L. After 4 days it further reduced by 4% to 19 mcg/L. Finally after 8 days Ni concentration became 18 mcg/L, which means only 9% removal (*Table 30, Fig.44a*) . Ni concentration was 22.3 mcg/L in the W2 sample solution. After 2 days of treatment it has been reduced by 6% to 21 mcg/L. After 4 days it further reduced by

19% to 18.1 mcg/L. Finally after 8 days Ni concentration became 18 mcg/L that means 22 % removal (*Table 31, Fig. 44b*) .

Water sample collected during monsoon season revealed that Ni content of WI was 11.1 mcg/L before treatment. After two days of treatment it reduces to 10.9 mg/L with an efficiency of 1%. After 4 days it came down further to 10.65 mcg/L (4% removal). After 8 days Ni concentration was 7.32 mcg/L with a removal efficiency of 34% (*Table 32, Fig.44a*). In W2, during the same season, water sample analysis shows initial Ni content of 7.8 mcg/L. After 2 days 6.3% of reduction took place and concentration came down to 7.3 mcg/L. After 4 days it came down further to 6.23 mcg/L (20% removal). After 8 days Ni measurement was 5.36 mcg/L with a removal efficiency of 31.2 % (*Table 33, Fig. 44b*).

During Post monsoon season, sample analysis revealed that Ni content of WI was 16 mcg/L before treatment. After two days of treatment it reduces to 15.8 mcg/L with an efficiency of 1%. After 4 days it came down further to 15.44 mcg/L (3.5% removal). After 8 days Ni concentration was 10.88 mcg/L with a removal efficiency of 32% (*Table 34, Fig.44a*). In W2, during the same season, water sample analysis shows initial Ni content of 16.3 mcg/L. After 2 days 6% of reduction took place and concentration came down to 15.32 mcg/L. After 4 days it came down further to 13.04 mcg/L (20% removal). After 8 days Ni measurement was 10.8 mcg/L with a removal efficiency of 33.2 % (*Table 35, Fig. 44b*).

(i) Iron: Iron content was detected only in the water sample taken from W2. Fe concentration was 5.3 mcg/L in the W2 sample collected during pre monsoon period. After 2 days of treatment it has been reduced by

4% to 5.1 mcg/L. After 4 days it further reduced by 21% to 4.2 mcg/L. Finally after 8 days Fe concentration became 0.1 mcg/L, which means only 98.1% removal (*Table 31, Fig. 45*).

Water sample collected during monsoon season revealed that Fe content of W2 was 4.3 mcg/L before treatment. After two days of treatment it reduces to 4.12 mcg/L with an efficiency of 4%. After 4 days it came down further to 3.35 mcg/L (22% removal). After 8 days Fe concentration was 0 mcg/L with a removal efficiency of 100% *(Table 33, Fig. 45*).

Water sample collected during post monsoon season revealed that Fe content of W2 was 8.6 mcg/L before treatment. After two days of treatment it reduces to 8.27 mcg/L with an efficiency of 3.8%. After 4 days it came down further to 6.89 mcg/L (20% removal). After 8 days Fe concentration was 0 mcg/L with a removal efficiency of 100% (*Table 35, Fig.45*).

(j) Cadmium: Cd content was detected only in the water sample taken from W2. Cd concentration was 3 mcg/L in the W2 sample collected during pre monsoon period. After 2 days of treatment it has been seen unchanged in concentration.. After 4 days it was reduced by 17% to 2.49mcg/L. Finally after 8 days Cd concentration became 0 mcg/L, which means only 100% removal (*Table 31, Fig. 46*).

Water sample collected during monsoon season revealed that Cd content of W2 was 1.2 mcg/L before treatment. After two days of treatment it reduces to 0.98 mcg/L with an efficiency of 18%. After 4 days it came down further to 0.67 mcg/L (43.5% removal). After 8 days Cd concentration was 0 mcg/L with a removal efficiency of 100% (*Table 33, Fig. 46*).

Water sample collected during post monsoon season revealed that Cd content of W2 was 4 mcg/L before treatment. After two days of treatment it reduces to 3.44 mcg/L with an efficiency of 14%. After 4 days it came down further to 2.32 mcg/L (42% removal). After 8 days Cd concentration was 0 mcg/L with a removal efficiency of 100% (*Table 35, Fig. 46*).

4.3.1.2 Bio Concentration Factor (BCF) of Heavy metals:

After 8 days of exposure period the maximum accumulation (BCF) value of heavy metals has been shown by Cadmium (1000) in all samples collected over pre monsoon, monsoon and Post monsoon periods. Other BCF values for all treatments are given in table (7).

***Table 36**: The values indicate the BCF values after 8 days of treatment period. Values are mean of three replicates of experiments and expressed with significance P<0.05 by one-way ANOVA with Dunnett multiple comparisons test.*

Sample	Cu	pb	zn	Cr	Co	Mn	Hg	Ni	Fe	Cd
W1 pre monsoon	784.6	950	658.96	525.42	520.83	200	450	67.35	ND	ND
W2 Pre monsoon	740.91	912.79	624.25	485.12	375	301.36	411.76	192.82	981.13	1000
W1 monsoon	942.02	1000	792.01	650.55	880.95	395.83	966.66	342.34	ND	ND
W2 Monsoon	881.4	932.3	720.3	585.8	500	350	600	320.5	1000	1000
W1 Post monsoon	893.5	1000	723	608.4	882.3	347.8	925	312.5	ND	ND
W2 post monsoon	813.4	860.1	657.3	514	467	318.1	444.4	325.1	1000	1000

The BCF values were increased serially as per the retention time. Monsoon and post monsoon samples showed higher accumulation as the BCF values were more than 1000.

In Pre monsoon season W1 water treatment results in highest accumulation of Pb (BCF=950). The remaining metals shows accumulation in order Cu>Zn>Cr>Co>Hg>Mn>Ni. Nickel shows least accumulation hence lowest BCF value. In W2 the BCF value decreases in order Cd>Fe>Pb>Cu>Zn>Cr>Hg>Co>Mn>Ni. Cd has accumulated completely (BCF=1000). Nickel again least absorbed in this station also. In Monsoon season W1, the BCF value decreases in order Pb>Hg>Cu>Co>Zn>Cr>Mn>Ni. Cd has accumulated completely (BCF=1000). Ni again least absorbed in this season too (BCF=342.34). In W2 Fe and Cd were absorbed completely (BCF=1000) It is followed by Pb>Cu>Hg>Zn> Cr> Co>Mn>Ni. In Post monsoon season , W1 water treatment shows BCF in following order. Pb> Hg>Cu> CO>Zn> Cr> Mn>Ni. Pb has 1000 as BCF. Nickel is poor accumulator (BCF=312). In W2 Cd and Fe were completely removed (BCF=1000). It is followed by Pb> Cu> Zn> Cr> Co>Hg> Co> Ni. In all seasons, for Cu, Pb, Hg, Cd, and Zn, the BCF values were approximately 1000 and the removal percentage was high; however the BCF values were very low for Ni. Based on these results, we can conclude that *Spirodela* could be a good candidate for the phytoremediation of low concentrations of these metals from polluted water.

4.3.2. Studies on wetlands of Kannamaly:

4.3.2.1. Analysis of variations in physico chemical parameters

The results of efficiency of *Spirodela* in scavenging contaminants indicate that the presence of this macrophyte was an important element for contaminant removal in wastewater. Hydrophytes can supply required oxygen by oxygen leakage from the roots into the rhizosphere to accelerate aerobic degradation of organic compounds in wetlands. This assumption was

confirmed in the present study, since the accumulation of heavy metals was higher in plants than water. In physio-chemical analysis different parameters Temperature, pH, Total alkalinity, BOD, COD, EC, Nitrate, Phosphate, Ammonia, TDS, TSS, turbidity and analysis for heavy metals of wetland I and II were studied.

***Table 37**: Physiochemical analysis of water sample collected from Wetland 1 sampling site during pre monsoon season before and after 2 days, four days and 8 days of exposure is given. CPCB standards were included for comparison. The values are means of triplicates.*

Sl. No	Parameter (Unit)	CPCB standard	Before treatment	After 2 days of treatment	Removal efficiency	After 4 days of treatment	Removal efficiency	After 8 days of treatment	Removal efficiency
1	Temp(^{o}C)	29-40	31	30	3.2	27	12.1	27	12.1
2	pH	6.5-8.5	9	8.2	8.8	7.6	15.5	7.6	15.5
3	Akalinity (mg/L)	200	250	233	6.8	206	17.6	180	28
4	COD (mg/L)	250	178	131.72	26	113.92	36	56.07	68.5
5	EC (µs/Cm)	700	912	811	11	721	30	681	25.3
6	BOD (mg O_2/L)	5	15	13.2	12	8.4	44	3.5	76.6
7	Nitrate (mg/L)	45	12.28	6.53	46.8	1.86	84.8	1.34	89.2
8	Phosphate(mg/L)	5	14.42	12.86	10.8	10.61	26.4	5.4	62.5
9	Ammonia	0.5	28.09	24.7	12	22.1	21	14.88	47
10	TDS	2100	2811	1921.5	8.5	1587.6	24.4	1029	51
11	TSS	100	55.42	28.81	48	15.96	71.2	13.96	74.8
12	Turbidity	5	50	48	4	29	42	13	74
13	Cu (mcg/L)	1.5	3.41	1.31	61.7	1.11	67.5	0.1	97
14	Pb (mcg/L)	0.01	4.3	2.79	35	1.52	64.8	0.056	98.7
15	Zn (mcg/L)	15	60	55.4	7.6	48.58	56.7	30.85	72.5
16	Cd mcg/L)	0.01	2.33	2.26	3	1.98	15	0	100

Table 38: *Physiochemical analysis of water sample collected from Wetland II sampling site during Pre monsoon season before and after 2 days, four days and 8 days of exposure is given. CPCB standards were included for comparison. The values are means of triplicates*

Sl. No	Parameter (Unit)	CPCB standards	Before treatment	After 2 days of treatment	Removal efficiency	After 4 days of treatment	Removal efficiency	After 8 days of treatment	Removal efficiency
1	Temp(^{o}C)	25-40	28.8	27.5	4.51	27	6.25	27	0
2	pH	6.5-8.5	7.8	7.4	5.12	7.4	5.12	7.2	7.6
3	Akalinity(mg/L)	200	177	151	14.7	109.3	38.2	88.5	50
4	EC(µs/Cm)	700	711	675	5	618	13	516	27.4
5	COD(mg/L)	250	261	198.3	24	185.3	29	135.8	48
6	BOD(mg O_2/L)	5	6	5.2	13.3	4	33.3	2.8	53.3
7	Nitrate(mg/L)	45	0.91	0.47	48	0.11	87	0.09	89.2
8	Phosphate(mg/L)	5	1.57	1.38	12	1.11	28.8	1	36.1
9	Ammonia	0.5	11	7.9	28	6.6	40	3.74	66
10	TDS (mg/L)	2100	1722	1686	2.1	1343	22	825	52.1
11	TSS (mg/L)	100	124.89	112.3	9.6	96.27	22.4	64.7	48
12	Turbidity (NTU)	5	13.8	10.1	26.8	7.93	42.5	1.1	92
13	Cu (mcg/L)	1.5	2.2	0.83	62	0.63	71	0.004	99.8
14	Pb (mcg/L)	0.01	2.68	1.71	36	0.69	74.1	0	100
15	Zn (mcg/L)	15	23.2	18.04	22.2	9.09	60.8	5.2	77.6
16	Cd (mcg/L)	0.01	0.47	0.44	4.5	0.19	58	0	100

***Table 39**: Physiochemical analysis of water sample collected from Wetland I sampling site during monsoon season before and after 2 days, four days and 8 days of exposure is given. CPCB standards were included for comparison. The values are means of triplicates.*

Sl. No	Parameter (Unit)	CPCB standard	Before treatment	After 2 days of treatment	Removal efficiency	After 4 days of treatment	Removal efficiency	After 8 days of treatment	Removal efficiency
1	Temp(^{o}C)	29-40	26.3	26.8	1.9	27	2.66	27	0
2	pH	6.5-8.5	7.8	7.4	5.12	7.4	0	7.4	0
3	Alkalinity(mg/L)	200	195	166.4	14.7	148	24.2	115	41
4	EC(µs/Cm)	700	658	539.6	18	460.6	30	360	45.3
5	COD(mg/L)	250	197	134	32	126.1	36	63	68
6	BOD(mg O_2/L)	5	13.52	11.4	15	10.68	21	6.28	53.5
7	Nitrate(mg/L)	45	3.81	1.88	50.5	0.56	85.2	0.41	89
8	Phosphate(mg/L)	5	1.44	1.27	11.5	1.04	27.4	0.92	36.1
9	Ammonia	0.5	3.06	2.68	12.3	2.41	21	0	100
10	TDS (mg/L)	2100	1722	1686	2.1	1343	22	811	53
11	TSS (mg/L)	100	12.02	6.39	46.8	3.19	73.4	2.95	75.4
12	Turbidity (NTU)	5	180	163	9.4	109	39.4	35.46	80.3
13	Cu(mcg/L)	1.5	2.1	0.794	62.2	0.462	78.3	0	100
14	Pb(mcg/L)	0.01	1.82	1.16	36	0.62	65.7	0	100
15	Zn(mcg/L)	15	44.39	35.8	19.2	18.6	58	10.5	76.3
16	Cd(mcg/L)	0.01	0.48	0.46	3	0.4	16	0	100

***Table 40**: Physiochemical analysis of water sample collected from Wetland II sampling site during monsoon season before and after 2 days, four days and 8 days of exposure is given. CPCB standards were included for comparison. The values are means of triplicates.*

Sl. No	Parameter (Unit)	CPCB standard	Before treatment	After 2 days of treatment	Removal efficiency	After 4 days of treatment	Removal efficiency	After 8 days of treatment	Removal efficiency
1	Temp(^{o}C)	29-40	26.3	26.8	1.9	27	2.66	27	0
2	pH	6.5-8.5	7.23	7.2	0.41	7.2	0	7.2	100
3	Alkalinity (mg/L)	200	168	143.4	14.7	104	38.2	67.2	60
4	EC(µs/Cm)	700	611	489	20	428	30	318	48
5	COD(mg/L)	250	186	126	32	115.3	38	60	68
6	BOD(mg O_2/L)	5	1.83	1.53	16.1	1.39	23.7	0.85	53.5
7	Nitrate(mg/L)	45	0.28	0.14	48	0.03	87	0	100
8	Phosphate(mg/L)	5	0.06	0.05	12	0.04	28.8	0.03	36.1
9	Ammonia	0.5	0.7	0	100	0	100	0	100
10	TDS (mg/L)	2100	1533	1486	3	1343	12.3	753	51
11	TSS (mg/L)	100	188.3	164.12	13	122.09	35.2	98	48
12	Turbidity (NTU)	5	118.3	86.59	26.8	68.02	42.5	9.2	92.2
13	Cu(mcg/L)	1.5	1.8	0.63	64.5	0.39	78.2	0	100
14	Pb(mcg/L)	0.01	2.2	1.34	39	0.41	81	0	100
15	Zn(mcg/L)	15	11.13	8.65	22.2	4.36	60.8	2.1	81.14
16	Cd(mcg/L)	0.01	0.2	0.17	15	0.08	58	0	100

***Table 41**: Physiochemical analysis of water sample collected from Wetland I sampling site during post monsoon season before and after 2 days, four days and 8 days of exposure is given. CPCB standards were included for comparison. The values are means of triplicates.*

Sl. No	Parameter (Unit)	CPCB standard	Before treatment	After 2 days of treatment	Removal efficiency	After 4 days of treatment	Removal efficiency	After 8 days of treatment	Removal efficiency
1	Temp(^{o}C)	25-40	28.8	27.5	4.51	27	6.25	27	6.25
2	pH	6.5-8.5	8.1	7.9	5.12	7.8	3.7	7.5	7.4
3	Akalinity(mg/L)	200	213	198	7.04	177	17	127.5	40.1
4	EC(µs/Cm)	700	638	548	14	446	30	368	42.3
5	COD(mg/L)	250	228	155	32	150.4	34	82	64
6	BOD(mg O_2/L)	5	3.7	3.1	16.2	2.4	35.1	2	46
7	Nitrate(mg/L)	45	4.01	2	50	0.64	84	0.44	89
8	Phosphate(mg/L)	5	3.41	3.01	11.5	2.43	28.6	2.1	38.3
9	Ammonia	0.5	5.23	4.58	12.3	4.13	21	1.57	70
10	TDS (mg/L)	2100	1318	1160	12	1028	22	646	51
11	TSS (mg/L)	100	8	3.97	50.3	2.016	74.8	1.52	81
12	Turbidity (NTU)	5	88.5	64.78	26.8	50.88	42.5	7.78	91.2
13	Cu(mcg/L)	1.5	2.43	1.55	36	0.77	68.6	0	100
14	Pb(mcg/L)	0.01	1.8	1.74	3	1.512	16	0	100
15	Zn(mcg/L)	15	3.71	2.96	20.2	1.55	58	0.96	74
16	Cd(mcg/L)	0.01	2.33	2.26	3	1.98	15	0	100

***Table 42**: Physiochemical analysis of water sample collected from Wetland II sampling site during post monsoon season before and after 2 days, four days and 8 days of exposure is given. CPCB standards were included for comparison. The values are means of triplicates.*

Sl. No	Parameter (Unit)	CPCB standard	Before treatment	After 2 days of treatment	Removal efficiency	After 4 days of treatment	Removal efficiency	After 8 days of treatment	Removal efficiency
1	Temp(^{o}C)	25-40	28.8	27.5	4.51	27	6.25	27	0
2	pH	6.5-8.5	7.8	7.4	5.12	7.4	5.12	7.2	7.6
3	Akalinity(mg/L)	200	177	151	14.7	109.3	38.2	85	52
4	EC(µs/Cm)	700	633	531.7	16	443	30	346	45.3
5	COD(mg/L)	250	219	162.06	26	140.16	36	74.46	66
6	BOD(mg O_2/L)	5	2.02	1.69	16.1	1.54	23.7	0.93	53.5
7	Nitrate(mg/L)	45	0.3	0	100	ND	100	ND	100
8	Phosphate(mg/L)	5	0.31	0.27	12	0.22	28.8	0.19	36.1
9	Ammonia	0.5	0.75	0	100	0	100	0	100
10	TDS (mg/L)	2100	1113.5	1082	3	1055	5.2	1002.4	10
11	TSS (mg/L)	100	152	141	7.2	129	15.2	103	32.2
12	Turbidity (NTU)	5	2.22	1.1	50.3	0.71	68	0	100
13	Cu(mcg/L)	1.5	1.02	0.38	62.3	0.24	76	0	100
14	Pb(mcg/L)	0.01	2.4	1.51	37	0.53	77.6	0.03	98.6
15	Zn(mcg/L)	15	58.2	45.27	22.2	22.81	60.8	13.2	77.3
16	Cd(mcg/L)	0.01	0.25	0.24	4.5	0.1	58	0	100

a) **pH**: During Pre monsoon season, the pH of water from wetland I was alkaline 9 and for wetland II was 7.8 were found to be in the optimum range for duckweed growth (Dalu & Ndamba, 2002). After 2 days of treatment it has reduce to 8.2 and 7.4 for WI and WII. In the remaining two treatment chambers the pH remains 7.6 and 7.4 respectively after 4days and 7.6 and 7.2 for W1 and W2 samples after 8 days of treatment (*Tables 37 and 38*).

During monsoon season pH was measured 7.8 before treatment in W1. After 2 days it came down to 7.4 and remains the same after 4 and 8 days. In W2 initial pH was 7.2. Even after 8 days of treatment it remains the same.

During Post monsoon season pH was measured 8.1 before treatment in W1 sample. After 2 days it came down to 7.9. After 4 days it further reduced to 7.8 and finally becomes 7.5 the same after 8 days. In W2 sample initial pH was 7.8. After 2 days it came down to 7.4 and remains same after 4 days and finally becomes 7.2 the same after 8 days (*Fig.47 a&b*).

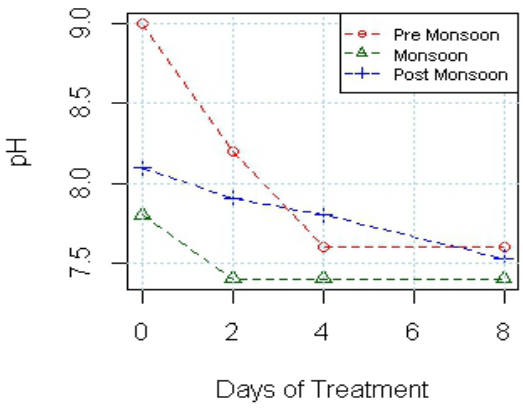

***Fig.47(a)**: Variation in pH after 2, 4 and 8 days of treatment in three seasons in Kannamaly W1 sample*

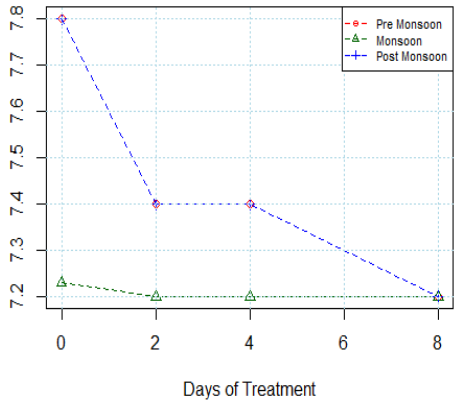

***Fig.47(b):** Variation in pH after 2, 4 and 8 days of treatment in three seasons in Kannamaly W2 sample.*

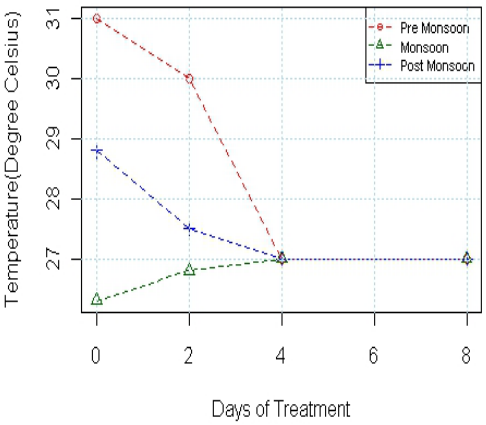

***Fig.48(a)**: Variation in temperature after 2, 4 and 8 days of treatment in three seasons in Kannamaly W1 sample.*

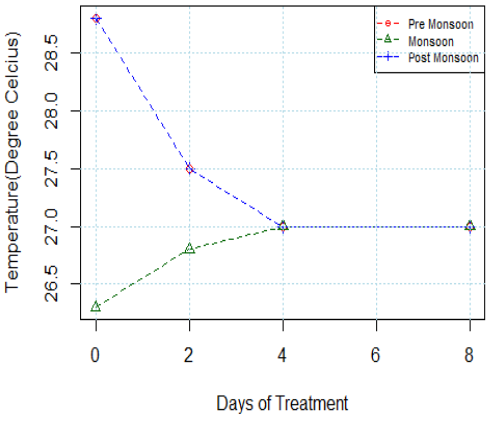

***Fig.48 (b)**: Variation in temperature after 2, 4 and 8 days of treatment in three seasons in Kannamaly W2 sample.*

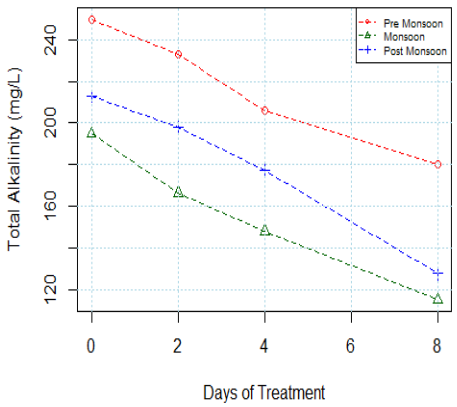

***Fig.49(a)**: Variation in alkalinity after 2, 4 and 8 days of treatment in three seasons in Kannamaly W1 sample.*

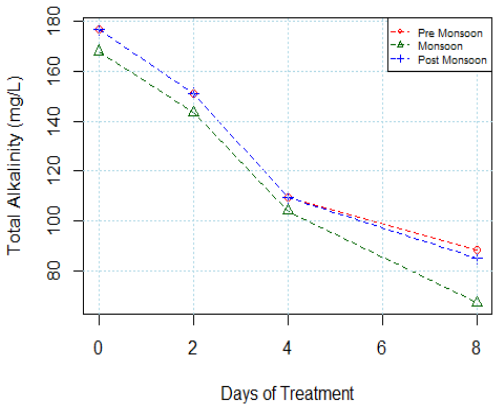

***Fig.49 (b)**: Variation in alkalinity after 2, 4 and 8 days of treatment in three seasons in Kannamaly W2 sample.*

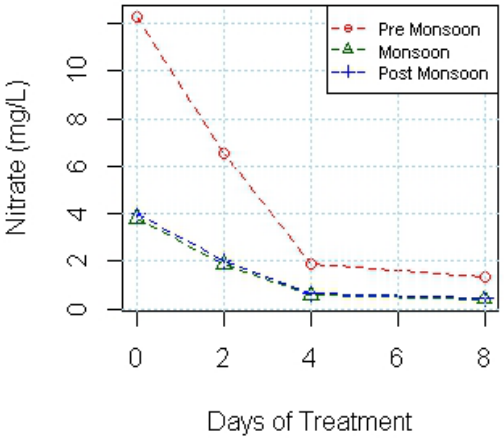

***Fig.50(a)**: Variation in nitrate content after 2, 4 and 8 days of treatment in three seasons in Kannamaly W1 sample.*

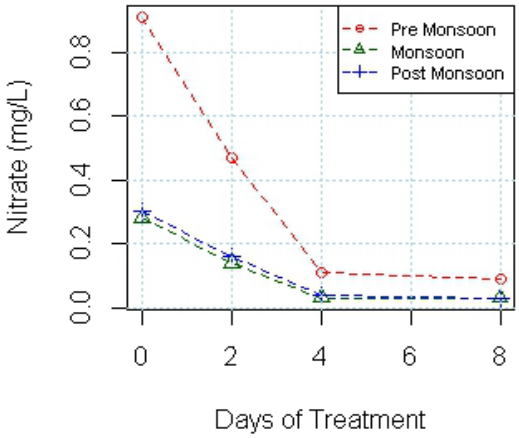

***Fig.50(b)**: Variation in nitrate content after 2, 4 and 8 days of treatment in three seasons in Kannamaly W2 sample.*

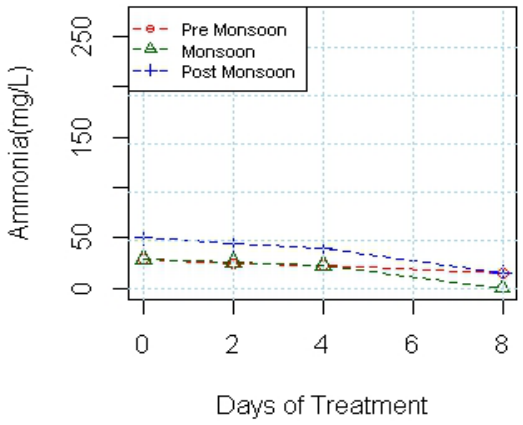

***Fig.51(a)**: Variation in ammonia content after 2, 4 and 8 days of treatment in three seasons in Kannamaly W1 sample.*

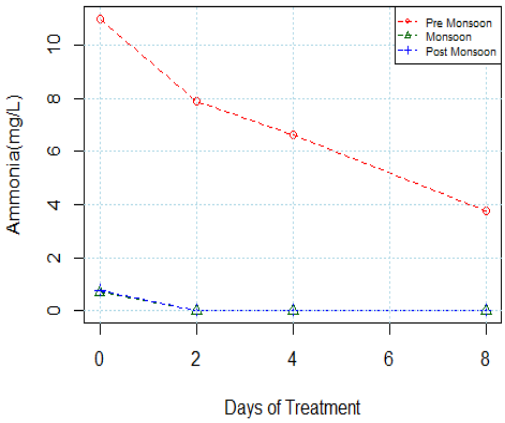

***Fig.51(b)**: Variation in ammonia content after 2, 4 and 8 days of treatment in three seasons in Kannamaly W2 sample.*

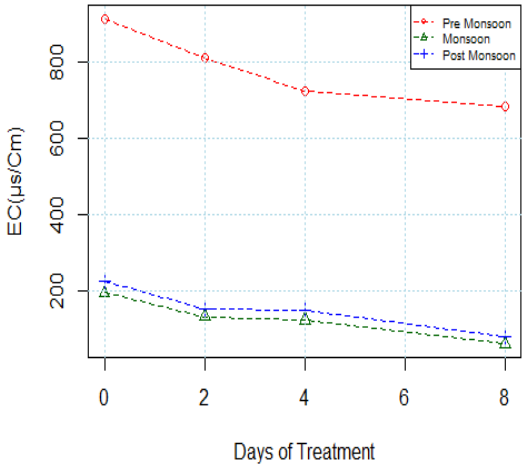

***Fig.52(a)**: Variation in EC after 2, 4 and 8 days of treatment in three seasons in Kannamaly W1 sample.*

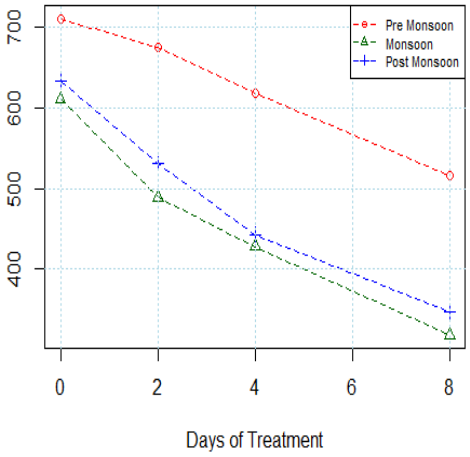

***Fig.52(b)**: Variation in EC after 2, 4 and 8 days of treatment in three seasons in Kannamaly W2 sample.*

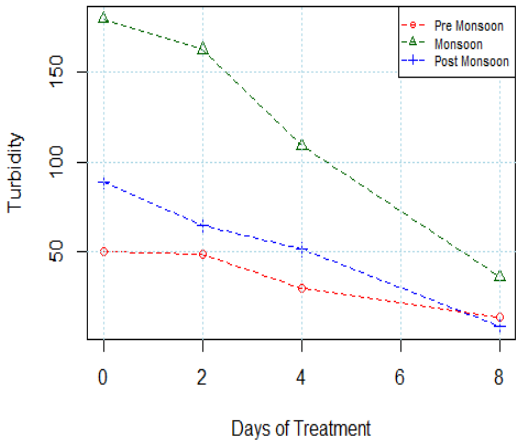

***Fig.53(a)**: Variation in turbidity after 2, 4 and 8 days of treatment in three seasons in Kannamaly W1 sample.*

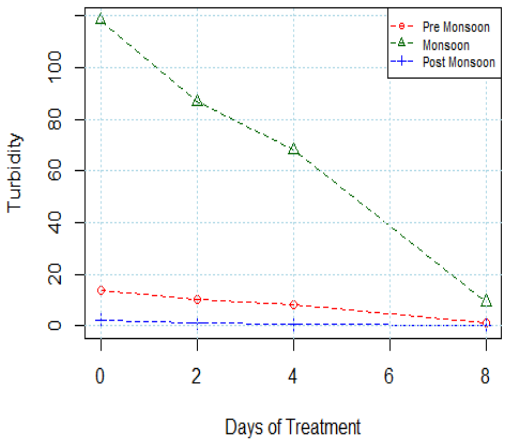

***Fig.53(b)**: Variation in turbidity after 2, 4 and 8 days of treatment in three seasons in Kannamaly W2 sample.*

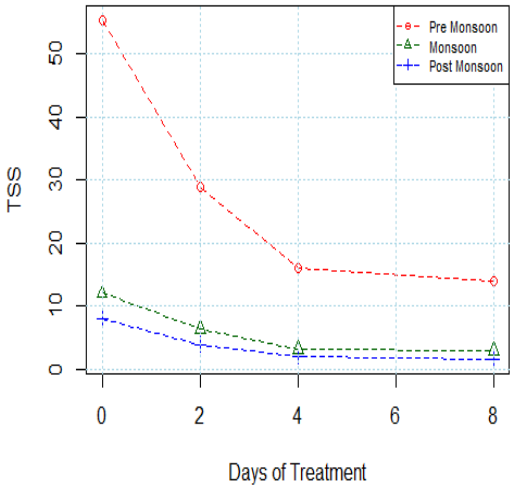

***Fig.54(a)**: Variation in TSS after 2, 4 and 8 days of treatment in three seasons in Kannamaly W1 sample.*

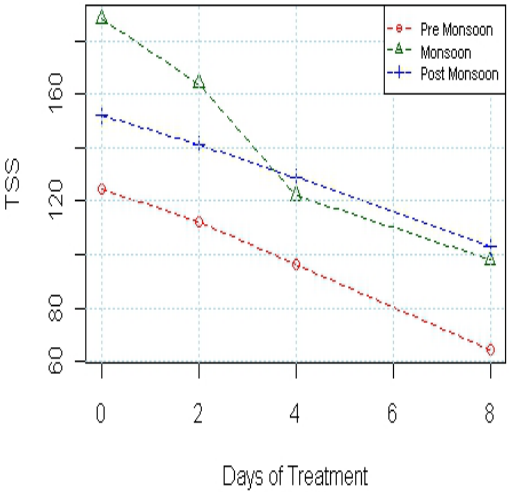

***Fig.54(b)**: Variation in TSS after 2, 4 and 8 days of treatment in three seasons in Kannamaly W2 sample.*

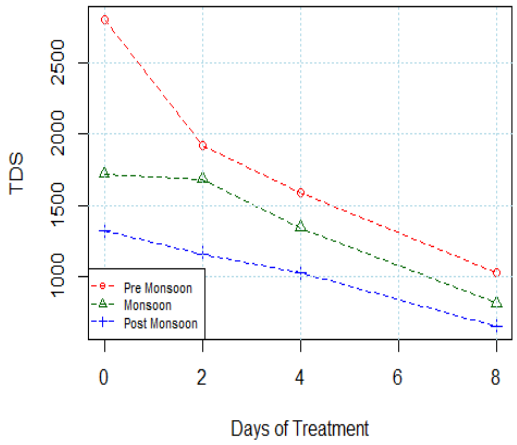

***Fig. 55(a)**: Variation in TDS after 2, 4 and 8 days of treatment in three seasons in Kannamaly W1 sample.*

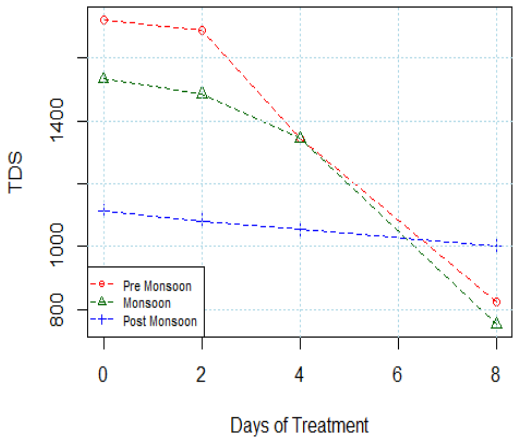

***Fig.55(b)**: Variation in TDS after 2, 4 and 8 days of treatment in three seasons in Kannamaly W2 sample.*

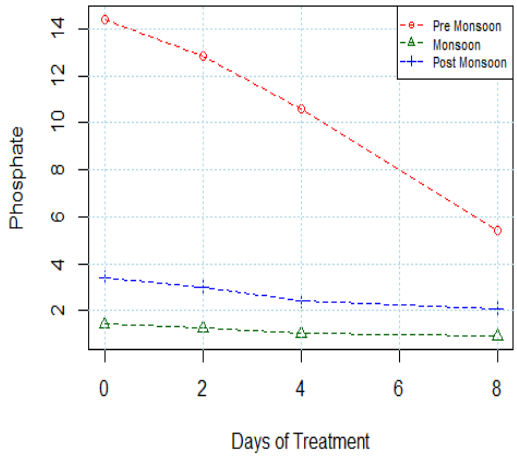

***Fig. 56(a)**:Variation in phosphate content after 2, 4 and 8 days of treatment in three seasons in Kannamaly W1 sample.*

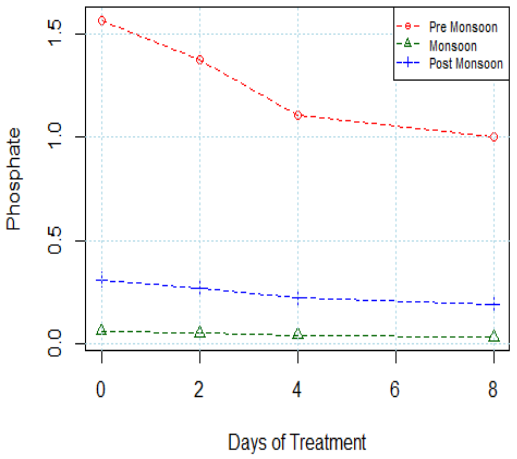

***Fig.56(b)**:Variation in phosphate content after 2, 4 and 8 days of treatment in three seasons in Kannamaly W2 sample.*

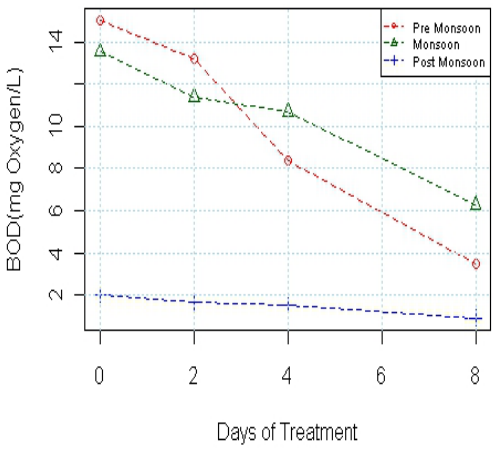

***Fig.57(a)**: Variation in BOD after 2, 4 and 8 days of treatment in three seasons in Kannamaly W1 sample.*

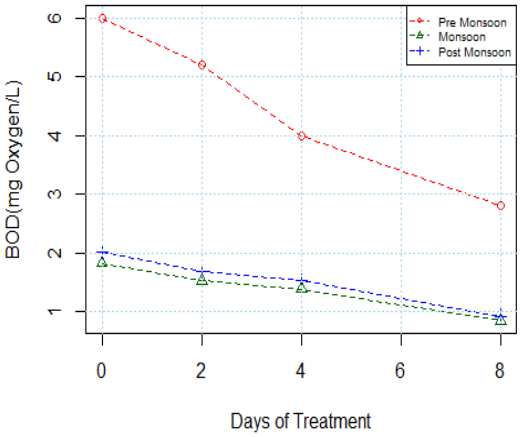

***Fig.57(b)**: Variation in BOD after 2, 4 and 8 days of treatment in three seasons in Kannamaly W2 sample.*

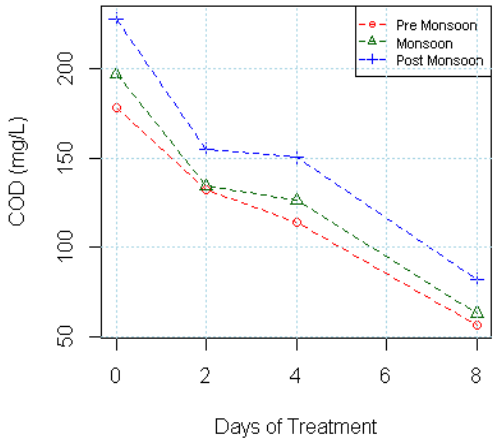

***Fig.58(a)**: Variation in COD after 2, 4 and 8 days of treatment in three seasons in Kannamalyr W1 sample.*

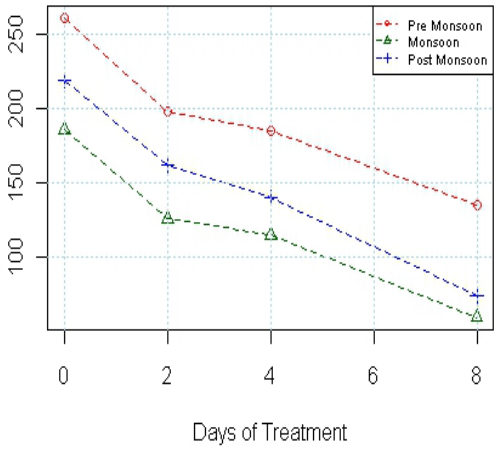

***Fig.58(b)**: Variation in COD after 2, 4 and 8 days of treatment in three seasons in Kannamaly W2 sample.*

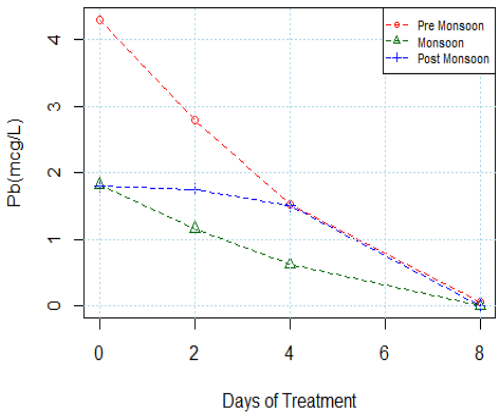

***Fig.59(a)**: Variation in Pb content after 2, 4 and 8 days of treatment in three seasons in Kannamaly W1 sample.*

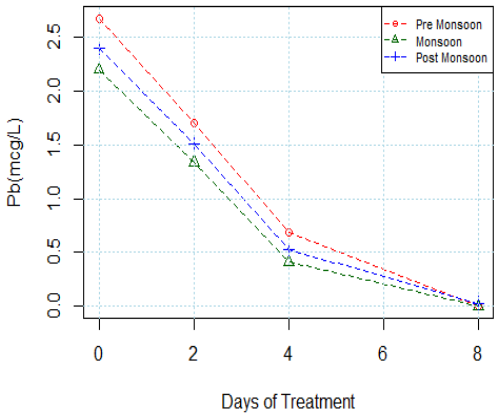

***Fig.59(b)**: Variation in Pb content after 2, 4 and 8 days of treatment in three seasons in Kannamaly W2 sample.*

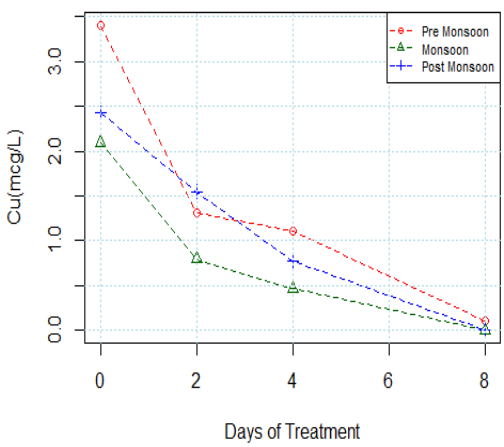

***Fig.60(a)**: Variation in copper content after 2, 4 and 8 days of treatment in three seasons in Kannamaly W1 sample.*

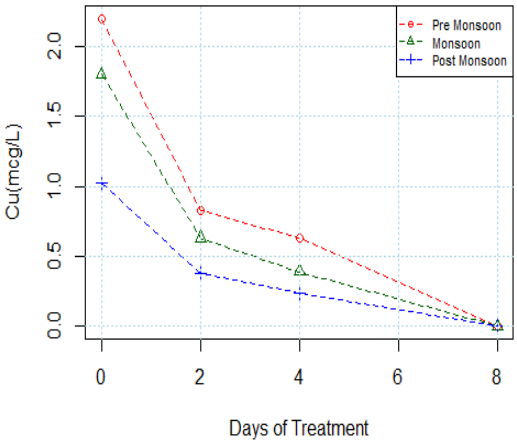

***Fig.60(b)**: Variation in copper content after 2, 4 and 8 days of treatment in three seasons in Kannamaly W2 sample.*

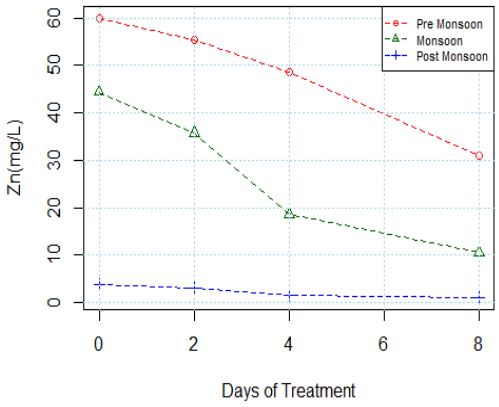

***Fig.61(a)**: Variation in zinc content after 2, 4 and 8 days of treatment in three seasons in Kannamaly W1 sample.*

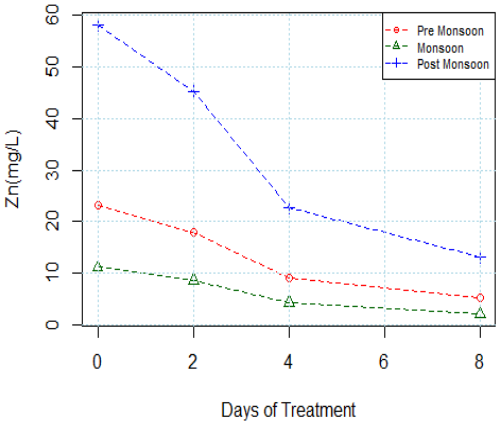

***Fig.61(b)**: Variation in zinc content after 2, 4 and 8 days of treatment in three seasons in Kannamaly W2 sample.*

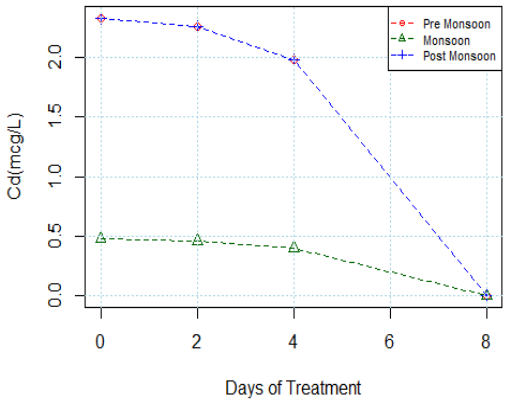

***Fig.62(a)**: Variation in cadmium content after 2, 4 and 8 days of treatment in three seasons in Kannamaly W1 sample.*

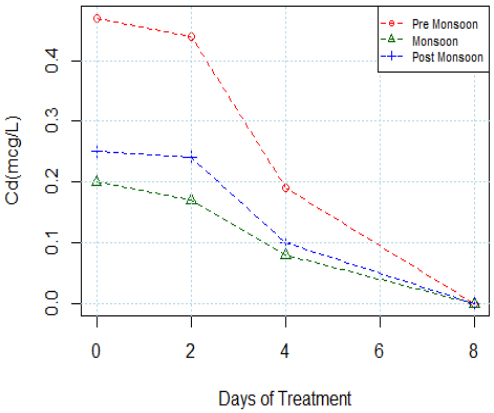

***Fig.62(b)**: Variation in cadmium content after 2, 4 and 8 days of treatment in three seasons in Kannamaly W2 sample.*

b) **Temperature:** Water sample collected during Pre monsoon, monsoon and post monsoon seasons, showed the temperature ranged between 26.3°C and 31°C which was within temperature tolerance limit for duckweed growth(*Fig.48 &b*) as mentioned by Culley *et* al., (1981) who found that the upper temperature tolerance limit for duckweed growth was around 34°C.

c) **Total alkalinity**: Water sample collected during pre monsoon season revealed that total alkalinity of WI was 250 mg/L before treatment. After two days of treatment it reduces to 233 mg/L with an efficiency of 6.8%. After 4 days it came down further to 206 mg/L (18% removal). After 8 days alkalinity measurement was 180 mg/L with a removal efficiency of 28% (*Table 37, Fig.49a*). In W2, during the same season, water sample analysis shows initial total alkalinity of 177 mg/L. After 2 days 15% of reduction took place and concentration came down to 151 mg/L. After 4 days it came down further to 109.3 mg/L (38% removal). After 8 days total alkalinity measurement was 88.5 mg/L with a removal efficiency of 50% *(Table 38, Fig. 49b*).

Water sample collected during monsoon season revealed that total alkalinity of WI was 195 mg/L before treatment. After two days of treatment it reduces to 166.4 mg/L with an efficiency of 15%. After 4 days it came down further to 148 mg/L (24% removal). After 8 days alkalinity measurement was 115 mg/L with a removal efficiency of 41%. In W2, during the same season, water sample analysis shows initial total alkalinity of 168mg/L. After 2 days 15% of reduction took place and concentration came down to 143.4 mg/L. After 4 days it came down

further to 104 mg/L (38% removal). After 8 days alkalinity measurement was 67.2 mg/L with a removal efficiency of 60% (*Table 40, Fig.49b*)

Water sample collected during post monsoon season revealed that total alkalinity of WI was 213 mg/L before treatment. After two days of treatment it reduces to 198 mg/L with an efficiency of 7%. After 4 days it came down further to 177 mg/L (17% removal). After 8 days alkalinity measurement was 128 mg/L with a removal efficiency of 40% *(Table 41, Fig. 49a*). In W2, during the same season, water sample analysis shows initial total alkalinity of 177 mg/L. After 2 days 15% of reduction took place and concentration came down to 151 mg/L. After 4 days it came down further to 109.3 mg/L (38% removal). After 8 days total alkalinity measurement was 85 mg/L with a removal efficiency of 52% (*Table 42, Fig.49b*).

d) Nitrates:

Water sample collected during pre monsoon season revealed that Nitrate concentration of WI was 12.28 mg/L before treatment. After two days of treatment it reduces to 6.53 mg/L with an efficiency of 46.8%. After 4 days it came down further to 1.86 mg/L (85% removal). After 8 days nitrate measurement was 1.34 mg/L with a removal efficiency of 89% *(Table 37, Fig. 50a).* In W2, during the same season, water sample analysis shows initial nitrate content of 0.91 mg/L. After 2 days 48% of reduction took place and concentration came down to 0.47 mg/L. After 4 days it came down further to 0.11 mg/L (87% removal). After 8 days nitrate measurement was 0.09 mg/L with a removal efficiency of 89% *(Table 38*, *Fig.50b*).

During monsoon season water sample collected revealed that Nitrate concentration of WI was 3.81 mg/L before treatment. After two days of treatment it reduces to 1.88 mg/L with an efficiency of 51%. After 4 days it came down further to 0.56 mg/L (85% removal). After 8 days nitrate measurement was 0.41 mg/L with a removal efficiency of 89% (*Table 39, Fig. 50a*). In W2, during the same season, water sample analysis shows initial nitrate content of 0.28 mg/L. After 2 days 48% of reduction took place and concentration came down to 0.14 mg/L. After 4 days it came down further to 0.03 mg/L (87% removal). After 8 days nitrate measurement was 0 mg/L with a removal efficiency of 100% (*Table 40, Fig. 50b*)

Water sample collected during post monsoon season revealed that Nitrate concentration of WI was 4.01 mg/L before treatment. After two days of treatment it reduces to 2 mg/L with an efficiency of 50%. After 4 days it came down further to 0.64 mg/L (84% removal). After 8 days nitrate measurement was 0.44 mg/L with a removal efficiency of 89% (*Table 41, Fig. 50a*). In W2, during the same season, water sample analysis shows initial nitrate content of 0.3 mg/L. After 2 days 100% of reduction took place and concentration came down to 0 mg/L (*Table 42, Fig. 50b*)

e) Ammonia:

Water sample collected during Pre monsoon season revealed that concentration of Ammonia in of WI was 28.1 mg/L before treatment. After two days of treatment it reduces to 24.7 with an efficiency of 12%. After 4 days it came down further to 22.1 mg/L (21% removal). After

8 days Ammonia measurement was 14.88mg/L with a removal efficiency of 47% (*Table 37, Fig. 51a*). In W2, during the same season, water sample analysis shows initial Ammonia content of 11 mg/L. After 2 days 28% of reduction took place and concentration came down to 7.9 mg/L. After 4 days it came down further to 6.6 mg/L (40% removal). After 8 days Ammonia concentration was 3.74 mg/L with a removal efficiency of 66% (*Table 38, Fig.51b*).

During monsoon season water sample collected from W1 revealed that concentration of Ammonia was 3.06 mg/L before treatment. After two days of treatment it reduces to 2.68 with an efficiency of 12.3%. After 4 days it came down further to 2.41 mg/L (21% removal). After 8 days Ammonia measurement was 0mg/L with a removal efficiency of 100% *(Table 39, Fig. 516a*). In W2, during the same season, water sample analysis shows initial Ammonia content of 0.7 mg/L. After 2 days 100% of reduction took place and concentration came down to 0 mg/L (*Table 40, Fig. 51 b*).

Concentration of Ammonia was 5.23 mg/L in water sample collected from W1 during Post monsoon. After two days of treatment it reduces to 4.58 with an efficiency of 12.3%. After 4 days it came down further to 4.13 mg/L (21% removal). After 8 days Ammonia measurement was 1.57mg/L with a removal efficiency of 70% (*Table 41, Fig. 51a*). In W2, during the same season, water sample analysis shows initial Ammonia content of 0.75 mg/L. After 2 days 100% of reduction took place and concentration came down to 0 mg/L (*Table 42, Fig.51b*).

f) Electrical Conductivity (EC):

Water sample collected during Pre monsoon season revealed that Electrical conductivity (EC) of WI was 912 µs/Cm before treatment. After two days of treatment it reduces to 811 µs/Cm with an efficiency of 11%. After 4 days it came down further to 721 µs/Cm (30% removal). After 8 days Electrical conductivity measurement was 681 µs/Cm with a removal efficiency of 25.3% (*Table 37, Fig. 52a*). In W2, during the same season, water sample analysis shows initial Electrical conductivity of 711 µs/Cm. After 2 days 5% of reduction took place and concentration came down to 675 µs/Cm. After 4 days it came down further to 618 µs/Cm (13% removal). After 8 days Electrical conductivity measurement was 516 µs/Cm with a removal efficiency of 27.4% (*Table 38, Fig.52b*).

During monsoon season water sample analysis revealed that Electrical conductivity (EC) of WI was 658 µs/Cm before treatment. After two days of treatment it reduces to 540 µs/Cm with an efficiency of 18%. After 4 days it came down further to 461 µs/Cm (30% removal). After 8 days Electrical conductivity measurement was 360 µs/Cm with a removal efficiency of 45.3 %(*Table 39, Fig. 52a*). In W2, during the same season, water sample analysis shows initial Electrical conductivity of 611 µs/Cm. After 2 days 20% of reduction took place and concentration came down to 489 µs/Cm. After 4 days it came down further to 428 µs/Cm (30% removal). After 8 days Electrical conductivity measurement was 318 µs/Cm with a removal efficiency of 48% (*Table 40, Fig. 52b*).

Electrical conductivity (EC) of water sample collected during Post monsoon season from W1revealed 638 µs/Cm before treatment. After

two days of treatment it reduces to 548 µs/Cm with an efficiency of 14%. After 4 days it came down further to 446 µs/Cm (30% removal) . After 8 days Electrical conductivity measurement was 368 µs/Cm with a removal efficiency of 42.3% (*Table 41, Fig. 52a*). In W2, during the same season, water sample analysis shows initial Electrical conductivity of 633 µs/Cm. After 2 days 16% of reduction took place and concentration came down to 532 µs/Cm. After 4 days it came down further to 443 µs/Cm (30% removal). After 8 days Electrical conductivity measurement was 346 µs/Cm with a removal efficiency of 45.3% (*Table 42, Fig. 52b*). *S. polyrhiza* growth decreased water EC in the treatment aquaria of both wetland samples due to salt removal from the waters by plant uptake or root adsorption. Compared to the W2, the EC of water from the W1 sample was higher (close to 900 µS cm-1 in pre monsoon).

g) **Turbidity**: Pre monsoon water sample treatment shows turbidity reduced by 4% from 50 NTU to 48 NTU after 2 days for WI. It further reduced to 29(42%) after 4 days of treatment. After 8 days it was 13 NTU which means majority of the turbidity has been removed *(Table 37, Fig. 53a*). In WII turbidity was reduced by 27% from 13.8NTU to 10.1 NTU after 2 days. It further reduced to 7.9 (42.5%) and to 1.1 NTU (92%) after 4 days and 8 days of treatment respectively (*Table 38, Fig.53b*).

During monsoon season turbidity was reduced by 9.4% from 180 NTU to 163 NTU after 2 days for WI sample. It further reduced to 109 (39.4%) after 4 days of treatment. After 8 days it was 35.4 NTU which

means majority of the turbidity has been removed (*Table 39, Fig. 53a*). In WII sample, turbidity was reduced by 27% from 118.3NTU to 86.5 NTU after 2 days. It further reduced to 68 (43%) and to 9.2 NTU (92%) after 4 days and 8 days of treatment respectively (*Table 40, Fig. 53b*). During post monsoon season turbidity reduced by 27% from 88.5 NTU to 64.7 NTU after 2 days for WI sample. It further reduced to 50.88 NTU (43%) after 4 days of treatment. After 8 days it was 7.7 NTU which means majority of the turbidity has been removed *(Table 41, Fig. 53a*). In WII sample turbidity was reduced by 50.3% from 2.22NTU to 1.1 NTU after 2 days. It further reduced to 0.71 (68%) and to 0 NTU (100%) after 4 days and 8 days of treatment respectively *(Table 42, Fig. 53b*).

h) **Total Suspended Solids**: Total suspended solids (TSS) values decreased by increasing treatment periods. During pre monsoon season TSS showed maximum concentration of 55.4 and 125 for WI sample and WII sample respectively before treatment. The concentration sides down to 28.8 and 112.3 mg.L-1 after 2 days (48% and 10% respectively) and further reduced to 16 mg/L (71%) and 96.2 (22.4%) after 4 days and finally decreased by 75% and 48% (14 mg/L and 65 mg/L respectively) (*Table 37&38, Fig. 54a & b)*). During monsoon season initial TSS measured was 12 mg/L in W1 sample. After 2 days of treatment it came down to 6.39 mg/L (47%) removal and after 4 days it shows 3.19 mg/L which means 73.4% removal. After 8 days 75.4% of TSS was removed and final concentration falls down to 2.9 mg/L *(Table 39, Fig.54a*). In W2 sample, after 2 days of treatment initial concentration of 188.3 mg/L came down to 164.1 mg/L (13%) removal and after 4 days it shows

122 mg/L which means 35% removal . After 8 days 48% of TSS was removed and final concentration falls down to 98 mg/L. (*Table 40, Fig.54b*)

During post monsoon season initial TSS measured was 8 mg/L in W1 sample. After 2 days of treatment it came down to 3.97 mg/L (51%) removal and after 4 days it shows 2 mg/L which means 74.8 % removal. After 8 days 81 % of TSS was removed and final concentration falls down to 1.52 mg/L *(Table 41, Fig. 54 a*). In W2 site, after 2 days of treatment initial concentration of 152 mg/L came down to 141 mg/L (7.2%) removal and after 4 days it shows 129 mg/L which means 15.2% removal. After 8 days 32.2% of TSS was removed and final concentration falls down to 103 mg/L. The ability of the plant for sedimentation, filtration/adsorption and biological degradation (hydrolysis) helps to remove TSS from the samples.

Total solids and turbidity in the waters of both sites varied seasonally: increasing during the monsoon and post monsoon season and decreasing during the dry season from mid-November to late May with values in the rainy season being several times higher than those in the dry pre monsoon season.

i**)** **TDS**: Analysis of water sample collected during pre monsoon season revealed that total dissolved solids (TDS) of WI and WII samples recorded their minimum values of 1029 mg/L (51%) and 825 mg/L (52.1%) after 8 days treatment. It was 1922 mg/L (8.5% reduction) and 1686 mg/L (2.1% reduction) after 2 days of treatment. It was 1588 mg/L

(24.4% reduction) and 1343 mg/L (22%) after 4 days (*Table 37 and 38*, *Fig.55a &b*).

Water sample collected during monsoon season revealed that total dissolved solids (TDS) of WI were 1722 mg/L before treatment. After two days of treatment it reduces to 1686 with an efficiency of 2.1%. After 4 days it came down further to 1343 mg/L (22% removal). After 8 days TDS measurement was 811 mg/L with a removal efficiency of 53% (*Table 39, Fig. 55a*). In W2, during the same season, water sample analysis shows initial TDS of 1533 mg/L. After 2 days 3% of reduction took place and concentration came down to 1486 mg/L. After 4 days it came down further to 1343 mg/L (12.3% removal). After 8 days TDS measurement was 753 mg/L with a removal efficiency of 51 % (*Table 40, Fig.55b*).

Water sample collected during Post monsoon season revealed that total dissolved solids (TDS) of WI was 1318 mg/L before treatment. After two days of treatment it reduces to 1160 with an efficiency of 12%. After 4 days it came down further to 1028 mg/L (22% removal). After 8 days TDS measurement was 646 mg/L with a removal efficiency of 51% (*Table 41, Fig. 55a*). In W2, during the same season, water sample analysis shows initial TDS of 1113.5 mg/L. After 2 days 3% of reduction took place and concentration came down to 1082 mg/L. After 4 days it came down further to 1055 mg/L (5.2% removal). After 8 days TDS measurement was 1002.4 mg/L with a removal efficiency of 10% (*Table 42, Fig.55b*). This decrease in TDS was due to the plant capacity to take some organic and inorganic ions. TDS is simply the sum of the

cations and anions concentration expressed in mg/l. It is obvious that TDS in the water increases with the increasing levels of various heavy metals. TDS content increased with the increase in concentration (pre monsoon season), showing a perfect positive correlation.

j) **Phosphate**: The phosphate content of the sample collected during pre monsoon period from the wetland water I and II were 14.4mcg/L and 1.57 mcg/L respectively. After 2 days of treatment, it has been reduced by 11% and 12% and after 4 days it has been reduced by 26.4% and 29% respectively. After 8 days it has been reduced by 63% to 5.4 mcg/L for WI and reduced by 36.1% to 1 after 8 days of growth (*Table 37, Fig.56a*) Similarly Nitrate content were 12.28 mcg/L for WI sample and 0.9 mcg/L for WII sample. It has been reduced to 6.53 mcg/L (47%) and 0.47 mcg/L (48%) respectively with 2 days of treatment. It was further down to mere 1.86 mcg/L and 0.11 mcg/L after 4 days (85% and 87% respectively). Eight days of treatment was enough to remove nitrate from the water almost completely from both samples (89.2% each).

Water sample collected during monsoon season revealed that phosphate content of WI was 1.44 mg/L before treatment. After two days of treatment it reduces to 1.27 mg/L with an efficiency of 11.5%. After 4 days it came down further to 1.04 mg/L (27.4% removal). After 8 days phosphate content was 0.92 mg/L with a removal efficiency of 36 % (*Table 39, Fig. 56a*). In W2, during the same season, water sample analysis shows initial phosphate concentration of 0.06 mg/L. After 2 days 12% of reduction took place and concentration came down to 0.05 mg/L. After 4 days it came down further to 0.04 mg/L (29%

removal). After 8 days phosphate measurement was 0.03 mg/L with a removal efficiency of 36% (*Table 40, Fig.56b*).

Water sample collected during post monsoon season revealed that phosphate content of WI was 3.41 mg/L before treatment. After two days of treatment it reduces to 3.01 mg/L with an efficiency of 11.5%. After 4 days it came down further to 2.43 mg/L (28.6 % removal). After 8 days phosphate content was 2.1 mg/L with a removal efficiency of 38.3% (*Table 41, Fig. 56a*). In W2, during the same season, water sample analysis shows initial phosphate concentration of 0.31 mg/L. After 2 days 12% of reduction took place and concentration came down to 0.27 mg/L. After 4 days it came down further to 0.22 mg/L (28.8% removal). After 8 days phosphate measurement was 0.19 mg/L with a removal efficiency of 36% (*Table 42, Fig. 56b*).

k) **BOD:** Tables 37 and 38 of pre monsoon sample treatment reveals the gradual reduction of BOD with time means that *Spirodela polyrhiza* mat effectively reduced BOD by 12% for WI sample and 13.3% for WII (reduced from 15 mg O2 L^{-1} at zero days reaching 13.2 mg O_2 L^{-1} for WI and reduced from 6 mg O_2 L^{-1} at zero days reaching 5.2 mg O2 L^{-1} 2days treatment). After 4 days it further reduced by 44% (reduced to 8.4 mg/L) and 33.3% (reduced to 4 O_2/L) for WI and WII respectively. After 8 days BOD stands at 3.5 mg/L (reduced by77%) for WI and stands at 2.8 mg/L (reduced by 53.3%) for WII (*Table 37 &38, Fig.57(a &b*).

Water sample collected during monsoon season revealed that BOD of WI was 13.52 O_2/L before treatment. After two days of treatment it reduces to 11.4 O_2/L with an efficiency of 15%. After 4 days it came

down further to 10.6 O_2/L (21% removal). After 8 days BOD was 6.28 O_2/L with a removal efficiency of 53.5%. In W2, during the same season, water sample analysis shows initial BOD of 1.83 O_2/L. After 2 days 16% of reduction took place and concentration came down to 1.53 O_2/L. After 4 days it came down further to 1.39 O_2/L (24% removal). After 8 days BOD measurement was 0.85 O_2/L with a removal efficiency of 54% (*Table 39 &40, Fig. 57(a & b)*).

Water sample collected during post monsoon season revealed that BOD of WI was 3.7 O_2/L before treatment. After two days of treatment it reduces to 3.1 O_2/L with an efficiency of 16.2%. After 4 days it came down further to 2.4 O_2/L (35% removal). After 8 days BOD was 2 O_2/L with a removal efficiency of 46%. In W2, during the same season, water sample analysis shows initial BOD of 2.0. O_2/L. After 2 days 16% of reduction took place and concentration came down to 1.69 O_2/L. After 4 days it came down further to 1.54O_2/L (24% removal). After 8 days BOD measurement was 0.93 O_2/L with a removal efficiency of 54 %. (*Table 41 & 42, Fig. 57 (a & b)*). Zimmo *et al*., (2005) found that BOD removal efficiency was higher in duckweed based ponds than in algae based ponds. Pandy, (2001) reported that in Delhi the duckweed ponds were operated at different flow rates giving hydraulic retention time from 5.4 to 22 days, a 30 - 50% reduction in phosphate, 56 - 80% reduction in ammoniac nitrogen and 66 - 80% reduction in BOD.

l) **COD:** During pre monsoon season, the sample analysis after treatment with *Spirodela* reveals that the COD has been reduced by 26% for WI and 24 % immediately after 2 days of phytoremediation (reduced from

the initial concentration of 178 mg/L to 132 mg/L and 261 mg/L to 198 mg/L). After 4 days it further reduced by 36% (reduced to 114 mg/L) for WI sample and reduced by 29% (reduced to 185 mg/L) and finally after 8 days, reduced to mere 56 mg/L (69%) for WI and reduced to 136 mg/L (48%) for WII (*Table 37&38, Fig. 58(a &b)).*

Water sample collected during monsoon season revealed that COD of WI was 197 mg/L before treatment. After two days of treatment it reduces to 134 mg/L with an efficiency of 32%. After 4 days it came down further to 126.1mg/L (36% removal). After 8 days COD was 63 mg/L with a removal efficiency of 68%. In W2, during the same season, water sample analysis shows initial COD of 186 mg/L. After 2 days 32% of reduction took place and concentration came down to 126 mg/L. After 4 days it came down further to 115.3 mg/L (38% removal). After 8 days COD measurement was 60 mg/L with a removal efficiency of 68% (*Table 39&40, Fig. 58a&b*)

Water sample collected during post monsoon season revealed that COD of WI was 228 mg/L before treatment. After two days of treatment it reduces to 155 mg/L with an efficiency of 32%. After 4 days it came down further to 150.4 mg/L (34% removal). After 8 days COD was 82 mg/L with a removal efficiency of 64%. In W2, during the same season, water sample analysis shows initial COD of 219 mg/L. After 2 days 26 of reduction took place and concentration came down to 162 mg/L. After 4 days it came down further to 140.1 mg/L (36% removal). After 8 days COD measurement was 74.4 mg/L with a removal efficiency of 66% (*Table 41 &42, Fig. 58 a&b*). Mandy's

experiment proved that duckweed treatment systems can tolerate maximum influent COD concentrations from 300 to 500 mg/l (Smith and Moelyowati, 2001). The results show that treatments in Pre monsoon, Monsoon and Post monsoon samples were capable of removing COD from water.

m) Heavy metals:

(a) Lead: During pre monsoon season Pb concentration was 4.3 mcg/L in the W1 sample solution. After 2 days of treatment it has been reduced by 35% to 2.79 mcg/L. After 4 days it further reduced by 65% to 1.52 mcg/L. Finally after 8 days Lead concentration is only .05 mcg/L, which means 99% removal. Pb concentration was 2.68 mcg/L in the W2 sample solution. After 2 days of treatment it has been reduced by 36% to 1.71 mcg/L. After 4 days it further reduced by 74% to 0.69 mcg/L. Finally after 8 days Lead concentration became 0 mcg/L, which means 100 removal (*Table 37&38, Fig. 59a &b*).

Water sample collected during monsoon season revealed that Pb content of WI was 1.82 mcg/L before treatment. After two days of treatment it reduces to 1.16 mcg/L with an efficiency of 36%. After 4 days it came down further to 0.62 mcg/L (66% removal). After 8 days Pb concentration was 0 mcg/L with a removal efficiency of 100%. In W2, during the same season, water sample analysis shows initial Pb content of 2.2 mcg/L. After 2 days 39% of reduction took place and concentration came down to 1.34 mcg/L. After 4 days it came down further to 0.41 mcg/L (81% removal). After 8 days Pb measurement was 0 mcg/L with a removal efficiency of 100% (*Table 39&40, Fig. 59a & b*).

During Post monsoon season, sample analysis revealed that Pb content of WI was 1.8mcg/L before treatment. After two days of treatment it reduces to 1.74 mcg/L with an efficiency of 3%. After 4 days it came down further to 1.51 mcg/L (16% removal). After 8 days Pb concentration was 0 mcg/L with a removal efficiency of 100%. In W2, during the same season, water sample analysis shows initial Pb content of 2.4 mcg/L. After 2 days 37% of reduction took place and concentration came down to 1.51 mcg/L. After 4 days it came down further to 0.53 mcg/L (78% removal). After 8 days Pb measurement was 0.03 mcg/L with a removal efficiency of 98.6% (*Table 41&42, Fig.59a & b*).

(b) Copper: After 8 days of treatment on sample collected during pre monsoon season Copper content in the water has been removed by 97% in W1 sample which means a reduction from initial concentration of 3.41 mcg/L to final concentration of 0.1 mcg/L . Copper shows removal by 62% after 2 days and removal by 65% after 4 days of treatment with *Spirodela polyrhiza* plant. Cu concentration was 2.2 mcg/L in the W2 sample solution. After 2 days of treatment it has been reduced by 62% to 0.83 mcg/L. After 4 days it further reduced by 71% to 0.63 mcg/L. Finally after 8 days Cu concentration became 0 mcg/L, which means 100 removal (*Table 37&38, Fig. 60a & b*).

Water sample collected during monsoon season revealed that Cu content of WI was 2.1 mcg/L before treatment. After two days of treatment it reduces to 0.79 mcg/L with an efficiency of 62%. After 4 days it came down further to 0.46 mcg/L (78% removal). After 8 days Cu

concentration was 0 mcg/L with a removal efficiency of 100%. In W2, during the same season, water sample analysis shows initial Cu content of 1.8 mcg/L. After 2 days 65% of reduction took place and concentration came down to 0.63 mcg/L. After 4 days it came down further to 0.39 mcg/L (78% removal). After 8 days Cu measurement was 0 mcg/L with a removal efficiency of 100% (*Table 39&40, Fig.60a &b*).

During Post monsoon season, sample analysis revealed that Cu content of WI was 2.43 mcg/L before treatment. After two days of treatment it reduces to 1.55 mcg/L with an efficiency of 36%. After 4 days it came down further to 0.77 mcg/L (69% removal). After 8 days Cu concentration was 0 mcg/L with a removal efficiency of 100%. In W2, during the same season, water sample analysis shows initial Cu content of 1.02 mcg/L. After 2 days 62% of reduction took place and concentration came down to 0.38 mcg/L. After 4 days it came down further to 0.24 mcg/L (76% removal). After 8 days Cu measurement was 0 mcg/L with a removal efficiency of 100% (*Table 41&42, Fig. 60a & b*).

(c) Zinc: Initial concentration of Zn was 60 mcg/L in W1 sample before treatment. Two days of treatment is only enough to remove 7.6% of Zn from the wetland water which means reduction to 55.4 mcg/L. Four days of treatment was enough to remove 57% of Zn (reduced to 48.5 mcg/L). Treatment with *Spirodela* was not successful since the concentration of Zn did not come down to the standard proposed by CPCB. Zn concentration was 23.2 mcg/L in the W2 sample solution. After 2 days of treatment it has been reduced by 22.2% to 18.04 mcg/L. After 4 days it further reduced by 61% to 9.09 mcg/L. Finally after 8 days Zn

concentration became just 5.2 mcg/L, which means 77.6% removal (*Table 37&38, Fig. 61a & b*). The final concentration was well within the range of CPCB standards.

Water sample collected during monsoon season revealed that Zn content of WI was 44.39 mcg/L before treatment. After two days of treatment it reduces to 35.8 mcg/L with an efficiency of 19%. After 4 days it came down further to 18.6 mcg/L (58% removal). After 8 days Zn concentration was 10.5 mcg/L with a removal efficiency of 76.3%. In W2, during the same season, water sample analysis shows initial Zn content of 11.13 mcg/L. After 2 days 22% of reduction took place and concentration came down to 8.65 mcg/L. After 4 days it came down further to 4.36 mcg/L (61% removal). After 8 days Zn measurement was 2.1 mcg/L with a removal efficiency of 81% (*Table 39&40, Fig. 61a & b*).

During Post monsoon season, sample analysis revealed that Zn content of WI was 3.71 mg/L before treatment. After two days of treatment it reduces to 2.96 mcg/L with an efficiency of 20%. After 4 days it came down further to 1.55 mcg/L (58% removal). After 8 days Zn concentration was 0.96 mcg/L with a removal efficiency of 74%. In W2, during the same season, water sample analysis shows initial Zn content of 58.2 mcg/L. After 2 days 22% of reduction took place and concentration came down to 45.2 mcg/L. After 4 days it came down further to 22.81 mcg/L (61% removal). After 8 days Zn measurement was 13.2 mcg/L with a removal efficiency of 77.30% (*Table 41 &42, Fig.61a &b*).

(p) Cadmium: Cd concentration was 2.33 mcg/L in the W1sample collected during pre monsoon period. After 2 days of treatment it has

been reduced by 3% to 2.26 mcg/L. After 4 days it further reduced by 15% to 1.98 mcg/L. Finally after 8 days Cd concentration became 0 mcg/L, which means 100% removal. Cd concentration was 0.47 mcg/L in the W2 sample solution. After 2 days of treatment it has been reduced by 4.5% to 0.44 mcg/L. After 4 days it further reduced by 58% to 0.19 mcg/L. Finally after 8 days Cd concentration became 0 mcg/L that means 100 % removal (*Table 37&38, Fig.62a &b*).

Water sample collected during monsoon season revealed that Cd content of WI was 0.48 mcg/L before treatment. After two days of treatment it reduces to 0.46 mcg/L with an efficiency of 3%. After 4 days it came down further to 0.4 mcg/L (16% removal). After 8 days Cd concentration was 0 mcg/L with a removal efficiency of 100%. In W2, during the same season, water sample analysis shows initial Cd content of 0.2 mcg/L. After 2 days 15% of reduction took place and concentration came down to 0.17 mg/L. After 4 days it came down further to 0.08 mcg/L (58% removal). After 8 days Cd measurement was 0 mcg/L with a removal efficiency of 100% (*Table 39&11, Fig.62a & b*).

During Post monsoon season, sample analysis revealed that Cd content of WI was 2.33 mcg/L before treatment. After two days of treatment it reduces to 2.26 mcg/L with an efficiency of 3%. After 4 days it came down further to 1.98 mcg/L (15% removal). After 8 days Cd concentration was 0 mcg/L with a removal efficiency of 100%. In W2, during the same season, water sample analysis shows initial Cd content of 0.25 mcg/L. After 2 days 4.5% of reduction took place and concentration came down to 0.24 mcg/L. After 4 days it came down further to

0.1 mcg/L (58% removal). After 8 days Cd measurement was completely removed from the solution (*Table 41&42, Fig. 62a &b*).

4.3.2.2 Bioconcentration (BCF) and accumulation of metals

The bioconcentration factor (BCF) provides an index of the ability of the plants to accumulate metal element with respect to the element concentration in water. The bioconcentration factor is more significant than the amount accumulated in plants since it indicates the plant's ability to accumulate trace elements relative to their concentration in the external nutrient solution (Del-Campo Marin & Oron, 2007).

***Table 43**: The values indicate the BCF values after 8 days of treatment period. Values are mean of three replicates of experiments and expressed with significance P<0.05 by one-way ANOVA with Tukey-Kramer multiple comparisons test.*

Sample	Cu	Pb	Cd	Zn
W1 pre monsoon	971	987	1000	486
W2 Pre monsoon	998	1000	1000	776
W1 monsoon	1000	1000	1000	763
W2 Monsoon	1000	1000	1000	811
W1 Post monsoon	1000	1000	1000	741
W2 post monsoon	1000	988	1000	773

In Pre monsoon season W1 water treatment results in highest accumulation of Pb (BCF=950). The remaining metals shows accumulation in order Cu>Zn>Cr>Co>Hg>Mn>Ni. Nickel shows least accumulation hence lowest BCF value. In W2 the BCF value decreases in order Cd>Fe>Pb>Cu>Zn>Cr>Hg>Co>Mn>Ni. Cd has accumulated completely (BCF=1000). Ni again least absorbed in this station also.

In Monsoon season W1, the BCF value decreases in order Pb>Hg>Cu>Co>Zn>Cr>Mn>Ni Cd has accumulated completely (BCF=1000). Ni again least absorbed in this season too (BCF=342.34). In W2 Fe and Cd were absorbed completely (BCF=1000) It is followed by Pb>Cu>Hg>Zn> Cr> Co>Mn>Ni.

In Post monsoon season, W1 water treatment shows BCF in following order. Pb> Hg>Cu> CO>Zn> Cr> Mn>Ni. Pb has 1000 as BCF. Nickel is poor accumulator (BCF=312). In W2 Cd and Fe were completely removed (BCF=1000). It is followed by Pb> Cu> Zn> Cr> Co>Hg> Co> Ni.

In all seasons, for Cu, Pb, Hg, Cd, and Zn , the BCF values were approximately 1000 and the removal percentage was high; however the BCF values were very low for Ni. Based on these results, we can conclude that *Spirodela* could be a good candidate for the phytoremediation of low concentrations of these metals from polluted water (*Table 43*).

4.3.3. Discussion

Culley *et* al., (1981) found that the upper temperature tolerance limit for duckweed growth was around 34°C. Duckweed tolerance allows it to be used for year-round wastewater treatment in areas where tropical macrophyte, such as water hyacinths, can only grow in summer (Cheng *et* al., 2002). In the current study the temperature never became the limiting factor for duckweed growth. The reduction in pH is due to absorption of nutrients or by simultaneous release of H+ ions with the uptake of metal ions by the hydrophytes (Mahmood., *et* al., 2005). The range of pH for optimum growth of *S. polyrhiza* reported in India was 6.8-8.5 (Gopal and Rizvi, 2008).

The reduction observed in sample total alkalinity could be explained by the interpretation of Filbin and Hough (1985), who reported that the plant may use up to 86% aqueous inorganic carbon and bicarbonate (HCO_3) from water column instead of atmospheric CO2, which may result in carbon limitation in some hydrophytes. In pre monsoon season the death of a large number of plants and hence microbial decomposition of organic matter increased, producing excess CO_2 in water column, leading to increased total alkalinity (Peavy *et* al., 1986).

Ammonification followed by biological nitrification/de-nitrification is the main cause of reduction in nitrate content of the solution. Plants can directly absorb nitrates. Numerous studies have proven that the major removal mechanism for nitrates in most of the constructed wetlands is microbial nitrification/ denitrification (Vymazal *et* al., 2007). In addition, the metals (Pb, Cd etc.) seem exhibit some inhibitory effect on nitrogen uptake by cattail plants (Lim *et* al., 2003). Besides plant uptake, denitrification may also contribute to the decreased NO3-N concentration in the treatment plots as a more anaerobic condition at water surface was created by the growing plants. Other anaerobic micro-sites may also contribute to NO3-N removal through denitrification (Gumbricht, 1993; Reddy *et* al., (1983). According to Ayyasamy *et* al., (2009), the sharp reduction in nitrate removal rate is due to osmotic pressure at higher concentrations not supporting the uptake of nitrate (Eaton, 1941).

Duckweeds prefer ammonia nitrogen (NH4-N) as a source of nitrogen and will remove ammonia preferentially, even in the presence of relatively high nitrate concentrations. According to the results of laboratory experiments, duckweed tolerate**s** concentrations of elemental N as high as 375 mg/l

(Rejmánková *et* al., (1979). Macrophyte plants can volatilize ammonia from the solution. Ammonia can also oxidized by the nitrifying bacteria in aerobic zones, and nitrate is converted to free nitrogen or nitrous oxide in the anoxic zones by denitrifying bacteria(Vymazal, 2007).The crude protein content of duckweed however, seems to increase over the range from trace ammonia concentrations to 7-12 mg N/L (Leng *et* al., (1995).

The reason for reduction in EC could be due to the fact that besides receiving storm water from the land, the W1 also received effluents from the nearby industries in Eloor and seafood processing factories in Kannamaly and other runoff factory compound may be enriched with salts including heavy metals. The varying levels of EC in water samples from both sites over different seasons might be due the external additions of salts and also might be due to different binding capacities of the roots, root excretions and competition by the roots to get nutrients from the liquid medium. Similar increase in electrical conductivity in rooting medium has been reported earlier (Mane *et* al., 2011). Mahmood *et* al., (2005) also observed the reduction in conductivity due to absorption of pollutants by plants. Electrolyte conductivity appears to have some effect on the growth of different species of duckweed. Gopal and Rizvi (2008), reported the maximum biomass of *L. perpusilla* and *S. polyrhiza* from roadside pools and ditches in India within a electrolyte conductivity range of 650-1 000 µS/cm. Khondker *et* al., (1993) recorded the complete disappearance of *S. polyrhiza* by the end of May when a sharp fall in conductivity and alkalinity was observed.

The reduction in turbidity may be attributed to decrease the concentration of suspended material because of settlement on the bottom and adsorption on

aquarium glass and this was shown in statistical analysis, as it recorded significant correlation between suspended solids and turbidity ($r=0.94$; $p<0.05$). The estimation of TSS before and after the treatment corroborates the findings of Pandy, (2001) regarding discharged duckweed treatment system in Halisahar, Likewise Huang, Han and Lin (2007), record a clear reduction in resuspension of sediment in Taiho lake during 41 days which covered by floating aquatic plants , and this result agreed with the study of Al-Sabunji and AlMarashi (2002). The mass of plant in the surface minimizes wind-induced turbulence and mixing and the removal of suspended solids occurs through gravity sedimentation in the zone under the surface layer of the hydrophyte (Rai and Munshi, 1979; Brix, 1997). The ability of the plant for sedimentation, filtration/adsorption and biological degradation (hydrolysis) helps to remove TSS from the samples. In addition, the presence of plants decreased water disturbance by wind, thus reducing sediment resuspension. The plant growth blocks available sunlight for algae and phytoplankton growth, which, together with sedimentation, contributes to clearer water. The much larger decrease in turbidity than TS indicated that algae and phytoplankton contributed a high proportion to water turbidity while minimal to TSS due to their negligible biomass weight.

This decrease in TDS was due to the plant capacity to take some organic and inorganic ions. The increase in these parameters during the rainy season was likely due to the input of storm water, which carried soil particles and solutes, including nutrients. The growth of *Spirodela polyrhiza* improved water quality by significantly decreasing TS and turbidity in water of the treatment plots . Khosravi *et* al. (2005) reported the importance of TDS uptake by *Azolla filiculoides* for their growth in wetlands. Groudev *et* al. (2001)

observed reduction of total dissolved solids from 2620 ppm to 1230 ppm in treatment of acid mine drainage from an uranium deposit by means of a natural wetland. A good reduction (90 %) of total suspended solids by constructed wetland plants with a retention time of 7 days was reported (Amelia, 2001).

Phosphate can be precipitate with cations of metals present in the medium. It may be getting adsorbed on clay or organic substances present in the medium or it may be directly absorbed by the plants (Reddy and Sacco, 1981) Watson *et* al., (1989) states that main mechanisms for phosphorus removal in constructed wetlands are adsorption, complex formation and precipitation, plant absorption (plant uptake), and biotic assimilation. The roots of hydrophytes worked as a giant biological absorber that removed organic matter of all kinds including phosphorus. At the same time, microorganisms residing in the submerged roots in the wastewater were degrading other pollutants which then were absorbed by the plants. The latter may contribute less but is an essential process and accelerate the former. High percentage of phosphate removal were reported by Koner and Vermaat (1998; Al-Sabunji (2002) and Jassim (2008). Perniel *et* al. (1998) also found that Lemna minor monoculture consistently removed the largest amount of ammonia and phosphorus from storm water. But the results in this experiment displayed a relatively low efficiency of TP removal. During the present experiment, *Spirodela* used phosphate for growth and reduced its concentration until reaching 30% in the end of experiment.

Zimmo et al. (2005) found that BOD removal efficiency was higher in duckweed based ponds than in algae based ponds. Pandy (2001) reported that

in Delhi the duckweed ponds were operated at different flow rates giving hydraulic retention time from 5.4 to 22 days, a 30 - 50% reduction in phosphate, 56 - 80% reduction in ammoniacal nitrogen and 66 - 80% reduction in BOD. In concurrence with the present findings, Oron *et* al. (1988) mentioned that the duckweed contribution for the removal of organic material is due to their ability to direct use of simple organic compounds.

Korner and Vermaat (1998) mentioned that duckweed significantly enhanced COD removal in shallow batch systems. Pandy (2001) reported that COD removal was in the range of 70% - 80% in the discharged duckweed treatment system at Halisahar, West Bengal. However in the present study, the COD and BOD removal by the macrophyte were not up to the capacity of *Lemna minor*. The ability of *Spirodela* for sedimentation, filtration/adsorption of organic substances and biological degradation (aerobics and anaerobic) with the help of microbes associated with their fronds results in bringing down BOD and COD. Organic compounds are degraded both aerobically and anaerobically by the heterotrophic microorganisms in the wetland systems depending on the oxygen concentration in the bed (IWA, 2000). And the removal was not referred to the metals added to sewage according to Lim et al. (2003). Hydrophytes can supply required oxygen by oxygen leakage from the roots into the rhizosphere to accelerate aerobic degradation of organic compounds in wetlands. This assumption was confirmed in our study, since the COD or BOD removal in aquariums with *Spirodela* plants was significantly higher than those without the plant. The reduction in pH favors microbial action to degrade biochemical oxygen demand (BOD) and chemical oxygen demand (COD) in the wastewater. According to Reddy(1981), the presence of plants in wastewater depletes dissolved CO2 during the period of

photosynthetic activity and an increase in DO of water, thus creates aerobic conditions in wastewater, which favors the aerobic bacterial activity to reduce the BOD and COD (Fonkou *et* al., 2002).

Members of Lemnaceae have been shown to possess a great ability to accumulate tolerate high concentrations of heavy metals (Landolt & Kandeler, 1987). These characteristics of Lemnaceae suggest a possible application for the efficient removal of metals from wastewater. The present study revealed that duckweed system induced effluent reduction in all heavy metals during both monsoon and post monsoon seasons (Table 3-6). Metal availability and bioaccumulation is governed by several environmental factors, viz. chemical speciation of the metal, pH, organic chelators, humic substances, presence of other metals and anions, ionic strength, temperature, salinity, light intensity, oxygen level and other prevailing electrochemical functions (Greger, 1999). Viet *et* al. (1988) reported that duckweed plants proved to be an excellent bioaccumulator of various heavy metals, which allowed it to treat a variety of wastewaters including industrial and highly polluted wastes. Hammouda *et* al., (1995) evaluated the efficiency of duckweed aquatic treatment in heavy metals removal in various water systems data obtained suggested a maximum reliability of systems with mixtures containing high ratios of wastewater. Mane *et* al., (2011) indicated that at lower concentrations of heavy metals, the plant growth was normal and removal efficiency was greater. The metals studied in Eloor samples showed Cd>Pb>Cu>Zn pattern of absorbance in both wetland water samples. The removal mechanisms include ionic adsorption/ precipitation/exchange in the medium or direct absorption by the plant. In Kannamaly metals like Copper, Lead, Zinc and Cadmium were found in the wetland water and removed by the plant by greater extend. The eight metals

studied showed Cd>Pb>Cu>Zn pattern of absorbance in both wetland water samples. The removal mechanisms include ionic adsorption/precipitation/ exchange in the medium or direct absorption by the plant. Phytoremediation can be classified as phytoextraction, phytodegradation, phytostabilization, phytostimulation, phytovolatilization and rhziofiltration (Susarla *et* al., 2002). Rhziofiltration, also referred to as phytofiltration, is based on hydroponically grown plants that have shown to be most efficient in removing heavy metals from water (Raskin *et* al., 1997). Phytoextraction was considered to have taken less part relatively in metal removal but it should have been promoted by nutrients.

The bioconcentration factor (BCF) is more significant than the amount accumulated in plants since it indicates the plant's ability to accumulate trace elements relative to their concentration in the external nutrient solution (Del-Campo Marin & Oron, 2007).Several cases of accumulation of heavy metals such as Zn, Cu, Pb, Cd, Ni and Cr, have been thoroughly studied in several wetland plant species, such as *E. crassipes*, *Typha latifolia, Spartina alterniflora* and *Phragmites australis* (Li *et* al., 2008). Bioconcentration factor (BCF) is a useful parameter to evaluate potential of the plants in accumulating metals and this value is calculated on a dry weight basis. The change in BCF of *Spirodela polyrhiza* .with respect to different metals were studied in present investigation to know capacity of the plant to concentrate metals from varied water samples at the end of treatment. As a fact larger BCF implies better phytoaccumulation capability and tissues with BCF greater than 1,000 are considered high, and less than 250 low, with those between classified as moderate (Zayed *et* al., 1998 b).

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Chapter 5

Summary and Conclusions

The results obtained from the current study suggest that the test material *S. polyrrhiza* should be used in the biomonitoring and phytoremediation of municipal, agricultural and industrial effluents because of their simplicity, sensitivity and cost-effectiveness. The study throws light on the potential of this plant which can be used as an assessment tool in two diverse wetland in Ernakulam district.

Higher plant tests are relatively underdeveloped and seldom used. In the assessment study, two wetlands each from Eloor and Kannamaly were selected. ASGR, Td, DW/FW ratio, Protein and Carbohydrate content, Estimation of Chloroplast pigments and morphological parameters were studied. Even though numerous studies have been made in Eloor in last three decades, not a single study has been made till date on the use of macrophytes as a tool. The study shows water sample from W2 is affecting plant health than W1 in Eloor. Parameters affected most during Pre monsoon period and less during monsoon period. There was a high growth rate occurs during monsoon followed by post monsoon and pre monsoon season. The less dilution of toxicants during pre monsoon may affect the growth rate in pre monsoon period. Plants can tolerate mild pollution evident from increase in chlorophyll content, ASGR,

carbohydrate, protein and biomass may be due to hormesis. Presences of heavy metals are clear due to the nature of toxicity. It is clear that W2 sample is much polluted than W1 sample may be due to the proximity to Kuzhikkandam creek. Kannamaly village is not an ecological hot spot like Eloor. The study area is bordered by seafood processing and chitin extracting factories which discharge the effluents directly to the wetland bodies. The results of the physico-chemical parameters revealed that, most of the parameters fall within CPCB standards. From W1 to W2 samples there was a gradual decrease in the pollution level. This shows that, even though all the parameters are within the permissible limits of water standards there is a need to take appropriate measures of pollution control by the concerned authorities to keep the water quality parameters within the permissible limits as the population and industrial activities in the area are increasing.

The results show the usefulness of combining physicochemical analysis with bioassays as such approach ensures better understanding of the toxicity of chemical pollutants and their influence on plant health. The results shows the suitability of *Spirodela* plant for surface water quality assessment as all selected parameters showed consistency with respect to water samples collected over a 3-monitoring periods. Similarly the relationship between the change in exposure period (2, 4 and 8 days) with the parameters were also studied in detail. *Spirodela* are consistent test material as they are homogeneous plant material; due to predominantly vegetative reproduction. New fronds are formed by clonal propagation thus, producing a population of genetically homogeneous plants. The result is small variability between treated individuals.

Ernakulam district is witnessing rapid development of industries in the area. Lots of new metallic and non metallic compounds with little knowledge of their toxicity may enter into the water bodies, causing the uncertain harmful effects on non-target aquatic organisms and human beings. A biomonitoring network may provide important information on the aquatic pollution level, bioaccumulative factor and potential ecological effects and thus exhibiting the precaution function for occurrence of the dangerous poisoning accidents. Lack of the accordant criteria of biomonitoring limits its wide use and the possible comparison in the actual evaluation of aquatic metal pollution. It is the need of the hour to enact the legislative standards for more biomonitoring approaches.

Metal toxicity issues in plants and soils are a significant problem throughout the wetlands in Ernakulam district. It only by understands the relationships between bioavailable metal fractions in the water and plant responses to metals that we can make decisions regarding metal toxicity in plants. At present, researches on the toxicity and bio accumulation potential of two well known and widely present heavy metals Copper (Cu) and Lead (Pb) were studied as the plant was subjected to the 8 days of exposure with various concentration (1 mg/L to 80 mg/L) of the heavy metals. For biomonitoring, same parameters were selected. The study proves that Copper is enhancing the parameters while Pb is inhibitory at lower concentrations. At higher concentrations Cu is more toxic than Lead. Both the metals are eliminated more in the lower concentrations. From the present study, It is clear that *Spirodela* plants can play an important role in the toxicity and accumulation of metals like Copper and Lead. The metals are thereby made available to heterotrophs and, thus, reintroduced into the food web via fish to birds and humans. Additionally, these vascular aquatic macrophytes are involved in the

biogeochemical cycles of nutrient and non-essential elements in many aquatic ecosystems. These plants often take up elements in excess of need and can accumulate essential as well as non-essential elements to concentration many times higher than those of the surrounding waters. In view of the increasing aquatic pollution, the study was aimed at understanding the importance of these macrophytes in accumulation of toxic metals and suggesting the remedial measures, if any for the preservation and restoration of the wetland ecosystems. This will enable the rehabilitation of metal contaminated areas with appropriate wetland species, allows identification of metal toxicities when they occur and allow for the effective regulation of metal emissions.

It has been observed that phytoremediation of water samples collected from Eloor and Kannamaly using the floating plant system is a predominant method which is economic to construct, requires little maintenance and eco friendly. Phytoremediation with *Spirodela* is quite promising. These plants bring water quality within the standards after the exposure with 8 days by absorption and accumulation. A large number of heavy metals can be removed and BCF determination shows the plants have great affinity towards toxic metals like Pb, Cu, Cd, Hg etc. Many researchers have used these plants for the removal of water contaminants including heavy metals. Since their treatment capabilities depend on different factors like climate, contaminants of different concentrations, temperature, retention time etc, a standardization of procedure is required for phytoremediation Therefore, an available knowledge and techniques for removal of water contaminants and advances in waste water treatment can be integrated to assess and control water pollution. However, application of the treatment with *Spirodela polyrhiza* can substantially reduce the pollutant loads. High metal removal rates have been

reported in both wetland sites studied were quite promising. Constructed wetlands with *Spirodela* mat may help to prevent the spread of contamination from source to the aquatic environment.

An unfortunate question that some developing countries have had to face as food crops such as wheat and corn have been used to create bio fuels. While some of these 'first generation' bio fuels have been produced with some success over the last few decades, it has also led to food shortages and has had a major economic impact on food prices. Therefore, there has been growing interest in finding 'second generation' alternatives to food crops that "don't grow on arable land and instead can be used specifically for bio fuels. One promising candidate is duckweed called *Spirodela polyrhiza* that can convert high nitrogen and high phosphorus water into much cleaner water and at the same time massively increase in biomass and "it can kill two birds with one stone".

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APPENDICES

Appendix A

Environmental & Analytical
Toxicology

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Research Article Open Access

Aquatic Macrophyte Spirodela *Polyrrhiza* as a Phytoremediation Tool in Polluted Wetland Water from Eloor, Ernakulam District, Kerala

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Abstract

This study involved a laboratory experiment on the efficiency of the plant duckweed Spirodela polyrrhiza in improving the quality of two polluted wetlands of Eloor industrial area, Ernakulam, Kerala. The efficiency was tested by measuring some of physicochemical characteristics of the control and plant treatments after each eight days. All the parameters show considerable rate of reduction. In wetland I, The highest rates of reduction after 8 days of treatment were for heavy metals, accounting 95%, 79%, and 66% for Lead, Copper and Zinc, respectively, followed by 53% for Chromium, 45% for Mercury, 26% for Cobalt, 20% for manganese and 7% for Nickel. Other factors like pH, BOD, COD, Nitrate, Phosphate, sulphate, TDS, TSS and Turbidity reduced by 12%, 37%, 49%, 100%, 36%, 16%, 53%,85% and 52% respectively. In wetland II also heavy metals were removed with Cd (100%), Fe (98%), Pb (91%), Cu (74%) Zn (62%) and Hg (53%) removed more efficiently. The results showed that this aquatic plant can be successfully used for wastewater pollutants removal. Other physiochemical parameters like pH, BOD, COD, Nitrate, Phosphate, sulphate, TDS, TSS and Turbidity reduced by 14%, 40%, 60%, 100%, 38%, 65%, 73%, 85%, and 51% after 8 days of treatment.

Keywords: Phytoremediation; Spirodela polyrrhiza; Lemnaceae; Wetland; Heavy metals

Introduction

Wetlands support a wide array of flora and fauna and deliver many ecological, climatic and societal functions. Scientists often refer to wetlands as the "kidneys" of the earth. Kerala is well known for its wetlands. Eloor, an island of 11.21sq/km, on the Periyar River is home to more than 247 chemical industries and large number of wetlands. The soil, water bodies and the wetlands in and around Eloor have been contaminated with heavy metals. Duckweed based wastewater systems are promising to be used in effluent treatment considering organic matter, pathogen and nutrient removal [1]. Besides, duckweeds are floater plants, which reduce suspended solids by blocking light penetration. Thus, light availability causes algae die off, which settle or disintegrate. Ran et al. [2] points the advantages of using duckweeds due to its high production rate, easy manual harvest from the surface, high protein and low fiber content The aim of the present investigation was to evaluate the effectiveness of duckweed *Spirodela polyrrhiza to* remove all impurities as well as heavy metals from the water samples taken from two sites of polluted welands in Eloor. Among macrophytes, duckweeds are very small floating aquatic macrophytes belonging to the Lemnaceae family which grow on the nutrient rich surface and in fresh waters and they are known for their efficiency in nutrient uptake [3]. Likewise, Lemnaceae have the greatest capacity in organic matter removal and in absorbing the micro-elements such as potassium, calcium, sodium and magnesium among others. However, duckweed plants grow only in the upper water surface layer where mainly pollutant removal takes place [4]. In India phytoremediation techniques are on initial scale and detail investigations are necessary for further research.

Materials and Methods

Sample collection

One sample (Wetland 1) was collected approximately 8 metres north of the Kuzhikundam Thodu creek, at a location approximately 10 m northwest of the HIL site boundary (Latitude 10° 04´51.7´´N and Longitude 76° 17´32.5´´E). (Table 1) The second sample (Wetland 2) was collected from the wetlands southwest of the "Amanthuruthu" wetland area, approximately 150 metres west of the HIL (Hindustan Insecticides Limited) site, and approximately 80 metres south of the Kuzhikundam Thodu creek (Latitude 10° 04´48.1´´N Longitude 76° 17´22.7´´E). The samples taken from both sites were analyzed for physiochemical parameters.

Duckweed treatment system

Spirodela polyrhiza is a floating aquatic macrophyte belonging to the family Lemnaceae and can be found worldwide on the surface of fresh and brackish waters [5]. The Lemna and Spirodela are among the most standardized test organisms in aquatic ecotoxicology [6-9]. The wetland water collected had undergone preliminary sieving step to get rid of large suspended solids. The transferred water was immediately collected into the aquariums in laboratory conditions (as replicates). The treatment system with growing duckweed in three small glass aquariums (length18 inches) was constructed in laboratory set up.Each aquarium was 10 inches deep and 9 inches wide. These aquariums were arranged in such a way that light availability is maximum. The sides were covered to prevent light entering except at the top [10], Duckweed (*Spirodela polyrhiza.*) plants were collected from an unpolluted natural pond near Fort Kochi, Kerala. The stocks were cleaned by tap water then washed by distilled water. Approximately 50 g of fresh, wet *Spirodela polyrrhiza* plants were stocked into each of the three aquariums. Each aquarium was supplied sequentially with wetland water diluted with distilled water in 1:4 ratios. Each of the three aquariums was filled with

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Physiochemical parametrs	Before treatment	After 2 days of treatment	Removal efficiency	After 4 days of treatment	Removal efficiency	After 8 days of treatment	Removal efficiency
PH	8.2	7.42	10	7.34	10.4	7.2	12.1
BOD (mg/L)	110	96	13	87	21	69	37.2
COD(mg/L)	320	196	39	178	44.3	162	49.3
Nitrate(mcg/L)	27	15	46	4.3	84.0	0	100
Phosphate(mcg/L)	11	9.2	16.36	8.2	25.4	7	36.3
Sulphate(mg/L)	500.12	469	6.30	444.2	11.1	421	16
TDS (mg/L)	3210.3	3111	3.09	2928.11	9	1522	53
TSS (mg/L)	218.41	99	55	65.12	70.1	32	85.4
Turbidity(NTU)	29	22.4	23	19.7	32.06	14	52
Copper(mcg/L)	65	28.22	57	21.56	67	14	79
Lead(mcg/L)	26	17.31	33.4	9.43	64	1.3	95
Zinc(mcg/L)	212	177.21	16.4	92.4	56.4	72.3	66
Chromium(mcg/L)	118	101.23	14.2	82	31	56	53
Cobalt(mcg/L)	7.2	6.4	11.1	5.2	28	5.2	28
Manganese(mcg/L)	8	8	5	7.2	10	6.4	20
Mercury(mcg/L)	2	2	0	1.8	10	1.1	45
Nickel(mcg/L)	19.3	19.1	1.03	19	4	18	9

Table 1: Physiochemical parameters of wetland I measured after 2,4 and 8 days of treatment using duckweed Spirodela polyrrhiza.

same dilutions of wetland water. An aquarium is kept with same dilution but without macrophyte is considered as control. The experiment was kept under laboratory conditions of temperature (25+2) and lighting (8 light: 16 dark). Detention time of duckweed was 8 days in the first reactor, 4 days and 2 days in the second and third one. After harvesting, new and prewashed duckweed was inserted. Water volume reduction by volatilization was compensated by addition of pure water.

Analytical methods

A single sample collection has been done from the study area. The water collected from the site was analysed for physio chemical characteristics. The parameters of study are pH, BOD, COD, Nitrate, Phosphate, Sulphate, TDS, TSS and Turbidity before and three weeks after the experiment. Analysis revealed that wetland water is a cocktail of variety of metals including heavy metals. Metals like Copper, Lead, Zinc, Chromium, Cobalt, Manganese, Mercury and Nickel were present.

During the treatment process subsurface (under duckweed mat) water samples for physico-chemical, were collected in polyethylene bottles from all sides of each tank and then mixed. This procedure carried out every week. Initial and final measurements after three weeks of exposure were made. The percentage of removal or removal efficiency was also calculated. Physico-chemical analysis was carried out according to standard methods for examination of water and wastewater [11]. Field parameters were measured in situ. The statistical analysis was done using STATISTICA software.

Results and Discussion

The results of efficiency of *Spirodela* in scavenging contaminants indicate that the presence of this macrophyte was an important element for contaminant removal in wastewater. Hydrophytes can supply required oxygen by oxygen leakage from the roots into the rhizosphere to accelerate aerobic degradation of organic compounds in wetlands. This assumption was confirmed in the present study, since the accumulation of heavy metals was higher in plants than water. Rhziofiltration, also referred to as phytofiltration, is based on hydroponically grown plants that have shown to be most efficient in removing heavy metals from water [12].

In physio-chemical analysis different parameters (colour, pH, BOD, COD, Nitrate, Phosphate, Sulphate total dissolved solids, TSS and turbidity of wetland I and II were studied. During sample collection colour of the wastewater samples was turbid or slightly yellowish. The level of colour in the wastewater may be due to the presence of total dissolved solids.

The pH of water from wetland I was alkaline 8.2 and for wetland II was 8.4 were found to be in the optimum range for duckweed growth [4]. After 2 days of treatment it has reduce to 7.2 and 7.9 for WI and WII. In the remaining two treatment chambers the pH remains 7.2 and 7.4 respectively after 4days and 7.2 for both samples after 8 days of treatment (Tables 1 and 2) In the present experiment temperature ranged between 21.7°C and 23°C which was within temperature tolerance limit for duckweed growth as mentioned by [13] who found that the upper temperature tolerance limit for duckweed growth was around 34°C. Duckweed tolerance allows it to be used for year-round wastewater treatment in areas where tropical macrophytes, such as water hyacinths, can only grow in summer [14].

Turbidity was reduced by 23% from 29 NTU to 22.4 NTU after 2 days for WI. It further reduced to 19.7(32%) after 4 days of treatment. After 8 days it was 13.8 NTU which means almost half of the turbidity has been removed. In WII turbidity was reduced by 25.13% from 382 NTU to 286 NTU after 2 days. It further reduced to 262 (31.4) and to 189 NTU (50.5%) after 4 days and 8 days of treatment respectively (Figure 1). This may be attributed to decrease the concentration of suspended material because of settlement on the bottom and adsorption on aquarium glass and this was shown in statistical analysis, as it recorded significant correlation between suspended solids and turbidity ($r=0.94$; $p<0.05$).

Figure 2 shows total suspended solids (TSS) values decreased by increasing treatment periods, showing maximum concentration of 218.4 and 359 for WI and WII respectively before treatment. The concentration sides down to 98.66 and 181 $mg.L^{-1}$ after 2 days (55% and 49.5% respectively) and further reduced to 65.12 mg/L (70.1%) and 125.6 (65.01%) after 4 days and finally decreased by 85.4% and 85.2% (31.7 mg/L and 52.8 mg/L respectively) which corroborates the findings of Pandy [15] regarding discharged duckweed treatment system in Halisahar, Likewise Huang et al. [16] recorded a clear reduction in

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Physiochemical parameters	Before treatment	After 2 days of treatment	Removal efficiency	After 4 days of treatment	Removal efficiency	After 8 days of treatment	Removal efficiency
PH	8.4	8	6.0	7.4	12	7.2	14.2
BOD (mg/L)	341	292.1	14.3	271.2	20.4	205.1	40.0
COD(mg/L)	679	511.2	25.0	464.4	32.0	268.3	60.4
Nitrate(mcg/L)	12.0	6.1	48.0	1.1	91.0	0	100
Phosphate(mcg/L)	13.1	12.0	10.0	10.4	21.0	8.1	38.0
Sulphate(mg/L)	133.0	15.0	89.04	27.4	79.3	46.2	65.1
TDS (mg/L)	593.1	477.0	20.0	266.3	55.1	158	73.3
TSS (mg/L)	359	181	50.0	126.0	65.0	53.0	85.2
Turbidity(NTU)	382	286	25.1	262	31.4	189	51.0
Copper(mcg/L)	63.3	36.0	44.0	22.0	66.0	16.4	74
Lead(mcg/L)	34.4	23.0	34.0	13.0	63.0	3.0	91
Zinc(mcg/L)	301	258	14.2	144.2	52.0	113.1	62.4
Chromium(mcg/L)	121	105.3	13.0	85.0	30	62.3	49.0
Cobalt(mcg/L)	8	7.2	10	6.3	21.2	5.0	40
Manganese(mcg/L)	7.3	7.1	3.0	6.4	12.3	5.1	30.1
Mercury(mcg/L)	3.4	3.1	9.0	3.0	18.0	2.0	53.0
Nickel(mcg/L)	22.3	21	6.0	18.1	19.0	18.0	22.0
Iron(mg/L)	5.3	5.1	4.0	4.2	21.0	0.1	98.1
Cadmium(mg/L)	3	3	0	3.0	7.0	0	100

Table 2: Physiochemical parameters of wetland II measured after 2, 4 and 8 days of treatment using duckweed Spirodela polyrrhiza.

resuspension of sediment in Taiho lake during 41 days which covered by floating aquatic plants, and this result agreed with the study of Al-Sabunji et al. [17].

It was also revealed that total dissolved solids (TDS) of WI and WII recorded their minimum values of 1522 mg/L (52.5%) and 158 mg/L (73.3%) after 8 days treatment. It was 3111 mg/L (3% reduction) and 476.5 mg/L (19.6% reduction) after 2 days of treatment. It was 2928.11 mg/L (8.7% reduction) and 266.3 mg/L (55.10%) after 4 days. Majority of TDS was reduced between 4 days to 8 days of treatment. This decrease was due to the plant capacity to take some organic and inorganic ions (Figure 3).

Results in (Tables 1 and 2) shows that sulfate concentration in WI and WII recorded by 6.3% and 89.04% as reduction percentage during 2 days, 11.18% and 79.33% during 4 days and 15.8% and 65.15% after 8 days of Phytoremediation (Figure 4). The cause of reduction may be due to plant ability to absorb different types of pollutants and accumulated in their tissues [18]. But the sulphate reduction percentage after 8 days of treatment is found to be insufficient. It may be assumed that Spirodela polyrhiza is a poor tool for phytoremediation of sulphate from waste water. The phosphate content from the wetland water I and II were 11 mcg/L and 11.71 mcg/L respectively. After 2 days of treatment, it has been reduced by 16.3% and 47.9% and after 4 days it has been reduced by 25.4% and 20.61% respectively. After 8 days it has been reduced by 36.36% to 7.0 mcg/L for WI and reduced by 37.6% to 8.17 after 8 days of growth (Figure 4). The removal of phosphate is comparatively better than sulphate. Similarly Nitrate content was 27 mcg/L for WI and 11.7 mcg/L for WII. It has been reduced to 14.7 mcg/L (45.55%) and 6.1 mcg/L (47.9%) respectively with 2 days of treatment. It was further down to mere 4.3 mcg/L and 1.1 mcg/L after 4 days (84% and 90.6% respectively). Eight days of treatment was enough to remove nitrate from the water completely from both samples (100%) (Figure 5).

Tables 1 and 2 also reveal the gradual reduction of factor like BOD, COD, Phosphate, Nitrate etc. with time. Data revealed that Spirodela polyrhiza mat effectively reduced BOD by 12.7% for WI and 14.34% for WII (reduced from 110 mg O2 L-1 at zero days reaching 96 mg O2 L-1 for WI and reduced from 341 mg O2 L-1 at zero days reaching 292.1 mg O2 L-1 2days treatment). After 4 days it further reduced by 20.9% (reduced to 87 mg/L) and 20.46% (reduced to 271.2 mg/L) for WI and WII respectively. After 8 days BOD stands at 69 mg/L (reduced by37%) for WI and stands at 205.1 mg/L (reduced by 39.85%) for WII. Zimmo et al. [5] found that BOD removal efficiency was higher in duckweed based ponds than in algae based ponds. Pandey [15] reported that in Delhi the duckweed ponds were operated at different flow rates giving hydraulic retention time from 5.4 to 22 days, a 30 - 50% reduction in phosphate, 56 - 80% reduction in ammoniacal nitrogen and 66 - 80% reduction in BOD (Figure 6). In concurrence with the present findings, Oron et al. [19] mentioned that the duckweed contribution for the removal of organic material is due to their ability to direct use of simple organic compounds. The COD has been reduced by 38.75% for WI and 24.71% immediately after 2 days of phytoremediation (reduced from the initial concentration of 320mg/L to 196 mg/L and 679 mg/L to 511.2 mg/L). After 4 days it further reduced by 44.37% (reduced to 178mg/L) for WI and reduced by 31.60% (reduced to 464.4 mg/L) and finally after 8 days, reduced to mere 162 mg/L (49.375%) for WI and reduced to 268.3 mg/L (60.4%) for WII (Figure 7). Korner et al. [20] mentioned that duckweed significantly enhanced COD removal in shallow batch systems. Pandey [15] reported that COD removal was in the range of 70% - 80% in the discharged duckweed treatment system at Halisahar. However in the present study, the COD and BOD removal by the macrophyte were not up to the capacity of Lemna minor.

Ferrara et al. [21] indicated the reliability of wastewater treatment by some aquatic plants including duckweed in adsorption of the heavy metals cadmium and zirconium. Viet et al. [22] reported that duckweed plants proved to be an excellent bioaccumulator of various heavy metals, which allowed it to treat a variety of wastewaters including industrial and highly polluted wastes. Hammouda et al. [23] evaluated the efficiency of duckweed aquatic treatment in heavy metals removal in various water systems data obtained suggested a maximum reliability of systems with mixtures containing high ratios of wastewater. In the present study metals like Copper, Lead, Zinc, Chromium, Cobalt, Manganese, Mercury and Nickel were found in the wetland water and removed by the plant by greater extend. The eight metals studied, showed Pb>Cu>Zn>Cr>Hg>Co>Mn>Ni pattern of absorbance. Pb

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concentration was 26 mcg/L in the control solution. After 2 days of treatment it has been reduced by 33.4% to 17.3 mcg/L. After 4 days it further reduced by 63.7% to 9.43 mcg/L. Finally after 8 days Lead concentration is only 1.3 mcg/L, which means 95% removal (Figure 8). After 8 days of treatment Copper content in the water has been removed by 78.76% which means a reduction from initial concentration of 65 mcg/L to final concentration of 13.8 mcg/L (Figure 9). Copper shows

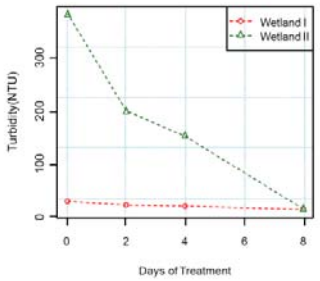

Figure 1: This may be attributed to decrease the concentration of suspended material.

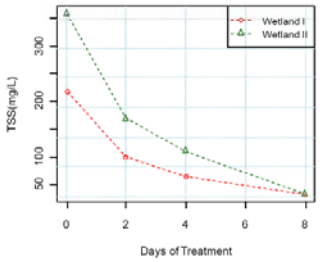

Figure 2: Total suspended solids (TSS) values decreased by increasing treatment periods.

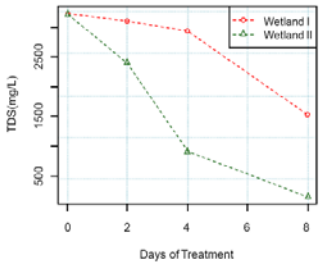

Figure 3: Majority of TDS was reduced between 4 days to 8 days of treatment.

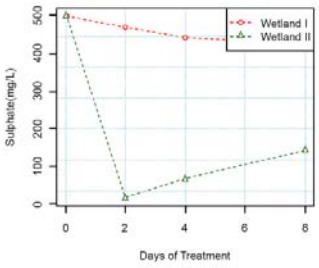

Figure 4: Sulfate concentration in WI and WII recorded as reduction percentage during 2 days.

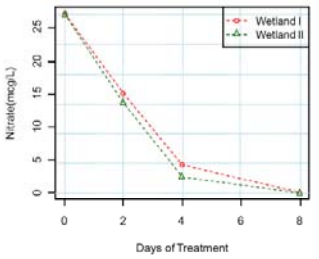

Figure 5: Eight days of treatment to remove nitrate from the water completely from both samples.

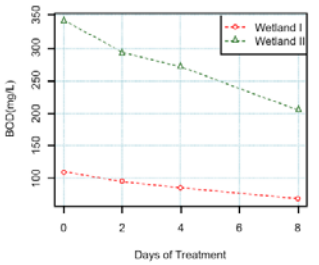

Figure 6: Different flow rates giving hydraulic retention time.

removal by 56% after 2 days and removal by 66.83% after 4 days of treatment with Spirodela polyrhiza plant. Initial concentration of Zn was 212 mcg/L. Two days of treatment is only enough to remove 16.4% of Zn from the wetland water which means reduction to 177.21 mcg/L. Four days of treatment was enough to remove 56.4% of Zn (reduced to 92.4 mcg/L) See (Figure 10). Chromium concentration was 118 mcg/L before treatment. It has been reduced by 14.21%, 30.71% and 52.5% after 2, 4 and 8 days of treatment (Figure 11). Cobalt concentration

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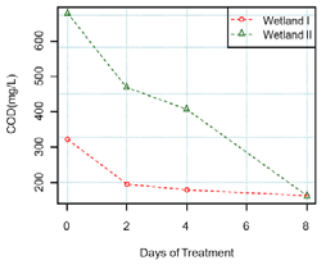

Figure 7: After 4 days it further reduced for WI.

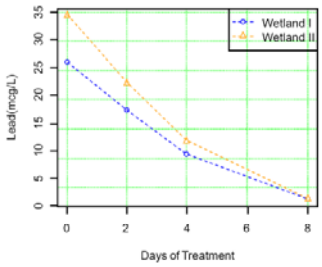

Figure 8: Lead concentration removal.

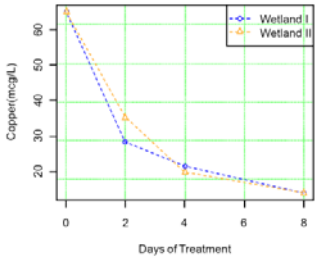

Figure 9: After 8 days of treatment Copper content in the water reduction from initial concentration to final concentration.

was very low initially i.e.7.2 mcg/L. After 2 days it has been reduced by mere 11.11%. After 4 days the concentration was measured as 5.2 mcg/L i.e. 27.77%. Interestingly even after 8 days of treatment the cobalt concentration did not came down any further i.e. it remains at 27.77% removal (Figure 12). The Manganese concentration has been reduced by 20% from 8 mcg/L to just 6.4 mcg/L even after 8 days of treatment. This reveals that Spirodela polyrhiza is a poor accumulator of Manganese (Figure 13). Mercury concentration remains the same even after 2 days of treatment i.e. 2.0 mcg/L. The concentration reduced to 1.1 mcg/L after 8 days of treatment with the removal efficiency of 45% (Figure 14). Nickel shows the least removal after the treatment regime of 8 days. It has been reduced from 19.3 mcg/L to 17.6 mcg/L with removal efficiency of just 8.80% that shows that Spirodela is a poor accumulator of Nickel (Figure 15). Iron and Cadmium were present in Wetland II but were absent in wetland I. Fe were removed 98.1% and Cd removed completely (100%) after 8 days of treatment (Figure 16 and 17).

Conclusion

In the current study macrophyte duckweed Spirodela polyrhiza was

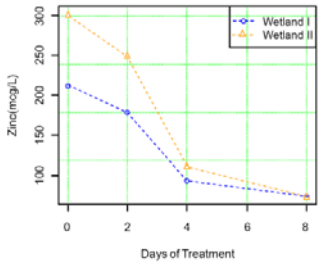

Figure 10: Four days of treatment for removal of Zn.

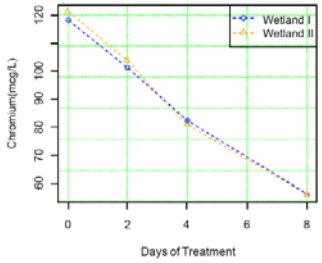

Figure 11: Chromium concentration before treatment.

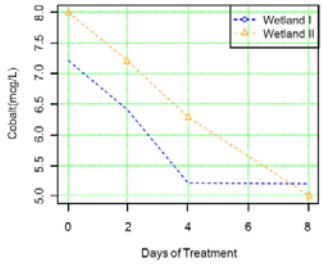

Figure 12: Stability after 8 days of treatment with cobalt concentration.

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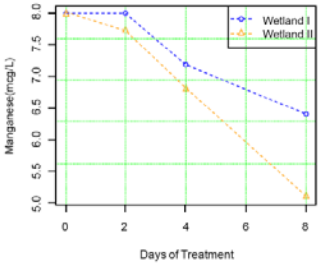

Figure 13: *Spirodela polyrhiza* is a poor accumulator of Manganese.

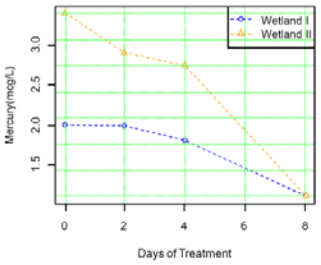

Figure 14: The concentration reduced after 8 days of treatment with the removal efficiency.

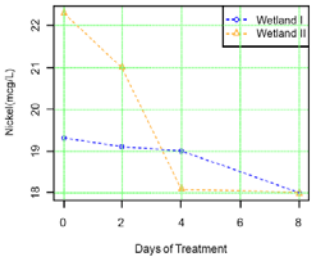

Figure 15: Nickel shows the least removal after the treatment regime of 8 days.

employed as effective phytoremediation agent in the polluted wetland water from Eloor. Constructed wetlands with Spirodela mat may help to prevent the spread of heavy metal contamination from land to the aquatic environment. High metal removal rates of close to 100% have been reported in both wetlands is quite promising. The advantage of lagoon treatment systems that use aquatic plants as productive 'sinks' for wastewater nutrients from a wide range of sources. It is easy and cheap to construct and operate suggests they are a suitable alternative for wastewater purification.

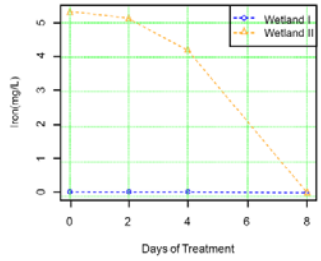

Figure 16: Iron and Cadmium were present in Wetland II.

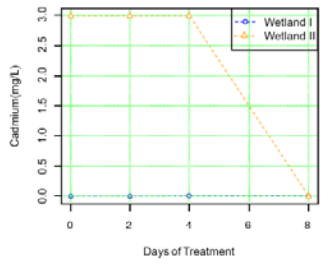

Figure 17: Fe and Cd removal completely after 8 days of treatment.

Acknowledgement

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Appendix B

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Phytotoxicological Assessment of Two Wetlands in Eloor, Kochi Using Aquatic Macrophyte Spirodela *Polyrhiza*.

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Abstract: *In the current study, the duckweed aquatic macrophyte Spirodela polyrrhiza was employed for assessing the toxicity of two wetlands in the Eloor industrial estate, Ernakulam district, Kerala, South India. The assessments were made according to OECD guidelines for testing (2006). The studies involve study of growth parameters, Growth Index, Biomass and changes in productivity. The water samples were collected from two different wetland sites at the same time. The spirodela plants were introduced into several dilutions of wetland water samples. The parameters were measured after 7 days of exposure. All samples except control affected all parameters. The results of this study emphasize the significance of duckweeds as standard and reliable testing material for biological parameters in polluted aquatic ecosystem.*

Keywords: *Growth inhibition, Spirodela polyrrhiza, Growth Index, macrophyte, duckweed*

I. Introduction:

Wetlands support a wide array of flora and fauna and deliver many ecological, climatic and societal functions. Scientists often refer to wetlands as the" kidneys" of the earth. . Kerala is well known for its wetlands. Eloor, an island of 11.21 sq/km, on the Periyar River is home to more than 247 chemical industries and large number of wetlands. The soil, water bodies and the wetlands in and around Eloor have been contaminated with heavy metals. Standardised ecotoxicity test methods frequently uses duckweed species Spirodela polyrrhiza due to their advantages such as rapid vegetative propagation , sensitivity to toxicants, easy culturing under axenic conditions (Lakatos et al .1993).

II. Materials and methods:

Duckweed Spirodela polyrrhiza were obtained from an unpolluted natural pond near Fort Kochi, Kerala, India. It is a floating aquatic macrophyte belonging to the family Lemnaceae and can be found world wide on the surface of fresh and brackish waters (Zimmo, 2003). The Lemna spp.are among the most standardized test organisms in aquatic ecotoxicology(EPA 1996;DIN2000,2001;Eberius 2001;OECD 2002). One sample (Wetland 1) was collected approximately 8 metres north of the Kuzhikundam Thodu creek, at a location approximately 10 m northwest of the HIL site boundary (Lat 10^0 04'51.76"N and Long 76^0 17'32.55"E) . (See table 1)

The second sample (Wetland 2) was collected from the wetlands southwest of the "Amanthuruthu" wetland area, approximately 150 metres west of the HIL (Hindustan Insecticides Limited) site, and approximately 80 metres south of the Kuzhikundam Thodu creek (Lat 10^0 04'48.13"N Long 76^0 17'22.75"E) (see Table 2).

Test solutions were prepared by diluting water samples of wetland 1 and 2 with distilled water. The solutions were prepared in 100%, 50%, 25%, 10%, 5% and 0.5% concentrations of wetland water plus a control and undergo seven days of exposure, plants were harvested , washed with double distilled water, blotted and used for the study of various parameters. The parameters include study of vegetative characters, growth parameters and study photosynthetic pigments. All the tests were conducted in six replicates.

III. Analysis of parameters:

1. Study of growth parameters

1. A.Dry weight*:*

All colonies are collected from each of the test vessels and rinsed with distilled or deionised water. They are blotted to remove excess water and then dried at 60 °C to a constant weight. Any root fragments should be included. The dry weight should be expressed to an accuracy of at least 0.1 mg.

***1. B. Fresh weight**:*

All colonies are transferred to pre-weighed plastic tubes with small (1 mm) holes in the rounded bottoms. The tubes are then centrifuged at 3000 rpm for 10 minutes at room temperature. Tubes, containing the now dried colonies, are re-weighed and the fresh weight is calculated by subtracting the weight of the empty tube.

1. c Dry weight-Fresh weight

ratio can be determined from above estimations. The plant growth index was calculated as follows

Growth Index = Biomass (t = 7 days)
Biomass (t = 0)

1. D. .Doubling time:

To determine the doubling time *(Td)* of frond number and adherence to this validity criterion by the study, the following formula is used with data obtained from the control vessels:

Td = ln 2 /μ Where μ is the average specific growth rate .

1. e. Average specific growth rate:

This response variable is calculated on the basis of changes in the logarithms of frond numbers, and in addition, on the basis of changes in the logarithms of another measurement parameter (total frond area, dry weight or fresh weight) over time (expressed per day) in the controls and each treatment group. It is sometimes referred to as relative growth rate . The average specific growth rate for a specific period is calculated as the logarithmic increase in the growth variables -frond numbers and one other measurement variable (total frond area, dry weight or fresh weight) - using the formula below for each replicate of control and treatments:

$\mu_{i-j} = \frac{\ln(N_j) - \ln(N_i)}{t}$

where:

- μi-j : average specific growth rate from time i to j
- Ni : measurement variable in the test or control vessel at time i
- Nj : measurement variable in the test or control vessel at time j
- t : time period from i to j.

1.f. Percentage of growth inhibition:

Percent inhibition of growth rate (Ir) may then be calculated for each test concentration according to the following formula

$\% Ir = \frac{(\mu C - \mu T) \times 100}{\mu C}$

where:

- % Ir : percent inhibition in average specific growth rate
- μC : mean value for μ in the control
- μT : mean value for μ in the treatment group

2. .Estimation of photosynthetic pigments.

The chlorophyll estimation is an important study parameter for the estimation of impact of pollution on photosynthetic activity. About 200mg of treated plants were weighed. This is taken in a mortar with 5mlof 90% acetone and 1ml of Magnesium carbonate. It is then ground thoroughly with pestle. This is then kept at 4^{o}C for 4 hours for the pigments to elute. The solution is then centrifuged at 2500 rpm for 15 minutes. The extract is then decanted to a volumetric flask and the volume is made up to 50 ml with 90% acetone. The absorbance at 750, 663, 645,510 and 480 were measured in the spectrophotometric analysis using Hitachi-U-2000 spectrophotometer.

Statistical Analysis

Analysis of variance for each test were conducted using STATISTICA software (One way Anova). The significant difference between treatments were determined by Duncan's multiple range test ($P<0.05$). Each test was conducted in six replicates.

IV. Results and conclusions:

The concept of average specific growth rate is based on the general exponential growth pattern of duckweed in non-limited cultures, where toxicity is estimated on the basis of the effects on the growth rate, without being dependent on the absolute level of the specific growth rate of the control; slope of the

concentration-response curve or on test duration. The use of average specific growth rate for estimating toxicity is scientifically preferred. In the current study ASGR and frond doubling time (Td) of the control and treatment with 0.5% concentration yield the same result. The inhibition of growth in this concentration is negligible. As the concentration of effluent increases, all the parameters vary. When the frond doubling time exceeds 2.5, the test solution is considered toxic. In the study 50% and 100% concentration treatments in wetland 1 and 2 shows Td values more than 2.5, thus found to be toxic. The values are given in table 3.

Plant growth index were measured after 7 days of exposure with different dilutions of water from both wetlands. At 0.5 % and 5% dilution GI is greater than control values in both waters. At 10% W2 treatment shows less GI than control but W1 still has values above control which shows the water quality of W1 is better than W2. From 25% GI values shows sharp decline in both treatments. The finding is given in table 4.

The growth indexes of both wetland water are similar in lowest concentration (0.5%). From 5% dilution onwards it is quite clear that growth of Spirodela is affected more in wetland II. But at 25% dilution GI is surprisingly similar. At extreme a concentration again GI varies. The differences in GI under different concentrations are illustrated in Table 3.

Changes in Dry weight fresh weight ratio indicate that in exposed Spirodela plants, growth retardation takes place in comparison to the control. At 0.5 % concentration, the biomass yield is same as that in control. In 5% concentration of wetland 1and 2 water, slight increase in DW/FW ratio recorded in wetland 2 effluents than control. At 10% and 25% solutions, the DW/FW ratio is higher in weland water 1 in comparison with Wet 2. But in 50% and 100% the ratio shows sharp decline. It was noted that at 50% concentration, spirodela growing in highly polluted weland 2 water yield higher FW/DW ratio than those growing in wetland 1 (Table 5). The decline in biomass ratio may be due the presence of excess heavy metals present in wetland. It has been shown that accumulation of heavymetals disturb the plant water status which eventually results in osmotic stress and growth reduction (Perfus- Barbeoch et al.2002; Poschen-reider and Barcelo 2004). Water especially in wetland 2. In wetland 1 the biomass (DW/FW ratio) percentage were 100, 100.7,103.9, 100, 78.5, 64.27,and 57.1 for dilutions 0.5, 5, 10, 25, 50 and 100 respectively. Similarly for wetland 2 it was 100, 99.9, 100.07, 78.4, 74.9, 64.2, and 57.04 for dilutions 0.5, 5, 10, 25, 50 and 100 respectively.

Chlorophyll and carotenoids are the central part of the energy manifestation of every green plant system and therefore, any significant alteration in their levels is likely to cause a marked effect on the entire metabolism of the plant. The productivity of plants is directly related with changes in the content of photosynthetic pigments chlorophyll a , chlorophyll b and Carotenoids. Industrial wastewater not only affects the chlorophyll content but the chlorophyll activity also(song and Huang,2001;Baron et al., 1995; Lewis, 1995). In the study photosynthetic pigments were inhibited due to metal toxicity. Duckweed leaves started to show signs of chlorosis (pigment loss) following 7d exposure to surface water samples. At 50% and 100% dilutions necrosis could also seen. The Carotenoids contents are found to be less affected. At the end of 7 days of exposure 0.5% dilution of wetland 1 and 2 shows slight increase in chlorophyll a content (Hormesis) while at the same dilution and Carotenoids content remain unchanged. From 5% to 100% all pigments shows gradual decrease in concentration. Chl *b* degraded at a much slower rate than chl *a* which indicates that greater damage of pollutants present in water samples on chl *a*.The loss of photosynthetic pigment content has been reported in duckweed plants following exposure to Cu, Pb and Ni (Axtell et al.2003; Hou et al.2007; Kanoun- boule et al.2009).The destruction of photosynthetic pigments by heavy metals could be due to : impairment of ETC , replacement of Magnesium ions associated with chlorophyll ring, inhibition of important enzymes (Van Assche and Clijsters 1990) associated with chlorophyll synthesis or peroxidation processes in chloroplast membrane lipids by reactive oxygen species (Sandalio et al.2001).

Acknowledgement:

The authors are grateful to the School of Environment studies, CUSAT, for providing technical assistance to carry out the work.

V. Conclusion:

The study revealed that both welands are highly polluted. Wetland 2 has more pollutants compared with wetland 1 which is evident from the assessment of vegetative , growth and photosynthetic pigment parameters. The study also points towards the importance of conservation of wetlands in the area.

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Table 1-Wetland I

Table 2-Wetland II.

Table 3: *ASGR, Td and Ir% of S. polyrrhiza afer 7 days of treatment with various dilutions of wetland I and 2. Mean N(i)=5, Mean T(j)=7 and T(i)=0. Standard deviations were presented by error bars. Each values are means of sixreplicates. The* ***significant difference between treatmentsis P<0.05.***

Site	Medium	mean Nj	mean ASG (μ)	Td	%Ir
	control	16	4.258	1.272	0
	0.5	16	4.258	1.272	0
	5	15	3.871	1.4	9.08
	10	14	3.484	1.555	18.177
	25	12	2.71	2	36.35
	50	10	1.935	2.801	34.21
Wet I	100	9	1.548	3.501	63.64
	0.5	16	4.258	1.272	0
	5	15	3.871	1.4	9.08
	10	14	3.484	1.555	18.177
	25	13	3.097	1.75	27.26
	50	9	1.548	3.501	63.64
Wet 2	100	8	1.161	4.668	72.73

Table 4: *Growth Index of Spirodela plant in different dilutions of Wetland 1 and 2 after 7 days of exposure.Standard deviations were presented by error bars. Each values are means of sixreplicates. The significant difference between treatmentsis P<0.05.*

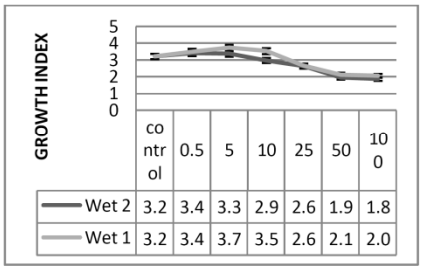


Table 5: *Biomass DW/FW ratio of S. polyrrhiza after 7 days of exposure in water from Wetland 1 and 2. Standard deviations were presented by error bars. Each value is means of sixreplicates. The significant difference between treatmentsis P<0.05*

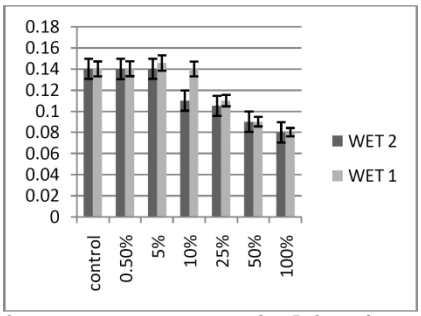


Table 6: *Relative photosynthetic pigment concentrations after 7 days of exposure in different dilutions of wetland 1. Standard deviations were presented by error bars. Each values are means of sixreplicates. The significant difference between treatmentsis P<0.05.*

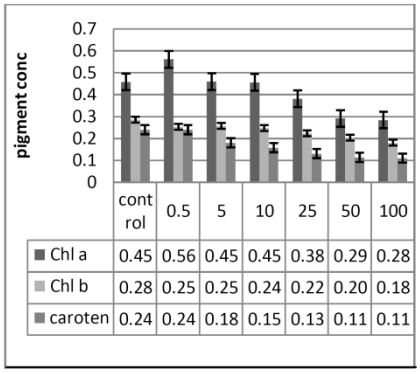
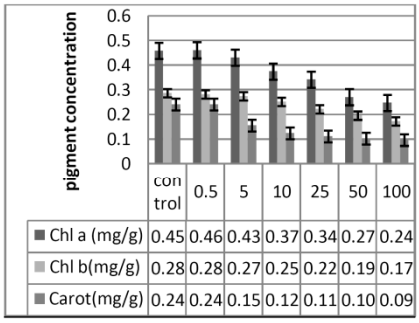


Table 7: *Relative photosynthetic pigment concentrations after 7 days of exposure in different dilutions of wetland 2. Standard deviations were presented by error bars. Each values are means of sixreplicates. The significant difference between treatmentsis P<0.05.*



Appendix C

Environmental & Analytical Toxicology

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Research Article Open Access

Phytotoxicological Assessment of Two Backwater Wetlands in Kannamaly, Ernakulam Using Aquatic Macrophyte - *Spirodela Polyrhiza.*

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School of Environmental Studies, Cochin University of Science and Technology, Kerala, India

Abstract

In the current study, the duckweed aquatic macrophyte Spirodela polyrrhiza was employed for assessing the toxicity of two backwater wetlands in the Kannamaly, Chellanam panchayath, Ernakulam district, Kerala, South India. The assessments were made according to OECD guidelines for testing (2006). The studies involve study of growth parameters, Growth Index, Biomass and changes in productivity. The water samples were collected from two different wetland sites at the same time. The spirodela plants were introduced into several dilutions of wetland water samples. The parameters were measured after 7 days of exposure. All samples except control affected all parameters. The results of this study emphasize the significance of duckweeds as standard and reliable testing material for biological parameters in polluted aquatic ecosystem.

Keywords: Growth inhibition; Spirodela polyrrhiza; Growth Index; Macrophyte; Duckweed

Introduction

Wetlands support a wide array of flora and fauna and deliver many ecological, climatic and societal functions. Scientists often refer to wetlands as the" kidneys" of the earth. Kerala is well known for its wetlands. The Kerala coast is bordered by 29-backwaters running parallel to the shoreline. The water quality of these backwaters is deteriorating due to population explosion, rapid industrialization, silting, tourism and agricultural activities. Effluents from industries are major cause of pollution in coastal area. The waste water/effluents from seafood processing plant have high organic content. The effluents from Shrimp processing plants locates at Kannamaly, Chellanam panchayath, Ernakulam district directly discharge the waste water to the neighboring water bodies. Apart from raising the BOD at immediate vicinity, limited effluents do not cause any severe damage to the system. But at high levels, often cause severe pollution and adversely affect the aquatic flora and fauna. Standardised ecotoxicity test methods frequently uses duckweed species Spirodela polyrrhiza due to their advantages such as rapid vegetative propagation , sensitivity to toxicants, easy culturing under axenic conditions [1]. Spirodela polyrrhiza has tolerance to moderate saline conditions. Duckweeds are salinity tolerant, adapt with time to high salinity, remove salinity, and have a potential for desalination in agricultural detention ponds [2].

Objectives

The objective of current study to assess the toxicity of water from two wetlands by standard testing procedure that includes growth analysis and photosynthetic pigment analysis.

Materials and Methods

Duckweed Spirodela polyrrhiza were obtained from an unpolluted natural pond near Fort Kochi, Kerala, India. It is a floating aquatic macrophyte belonging to the family Lemnaceae and can be found worldwide on the surface of fresh and brackish waters [3]. The duckweeds .are among the most standardized test organisms in aquatic ecotoxicology [4-7]. One sample (Wetland 1) was collected approximately 50 meters south of the Kannamaly pilgrim centre and close to India Seafoods Factory, at a location approximately Lat 9.8704°N and Long 76.2665°E) (Figure 1).

The second sample (Wetland 2) was collected from the wetlands south to the " wetland I area, approximately 1.8 km away and located at Lat 9.8612°N Long 76.2642°E) (Figure 2).

Wetland water samples collected from two sites were analysed according to APHA standards [8]. Metals were analysed using AAS. Test solutions were prepared by diluting water samples of wetland 1 and 2 with distilled water. The solutions were prepared in 100%, 50%, 25%, 10%, 5% and 0.5% concentrations of wetland water plus a control (water taken from an unpolluted site near Kumbalangi, 3 km away from Kannamaly. After seven days of exposure, plants were harvested, washed with double distilled water, blotted and used for the study of various parameters. The parameters include study of vegetative characters, growth parameters and study photosynthetic pigments. All the tests were conducted in six replicates.

Figure 1: One sample (Wetland 1) was collected approximately 50 metres south of the Kannamaly pilgrim centre and close to India Seafoods Factory.

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Citation: Loveson A, Sivalingam R (2013) Phytotoxicological Assessment of Two Backwater Wetlands in Kannamaly, Ernakulam Using Aquatic Macrophyte - *Spirodela Polyrhiza*. J Environ Anal Toxicol 3: 180. doi:10.4172/2161-0525.1000180

Figure 2: The second sample (Wetland 2) was collected from the wetlands south to the wetland I area, approximately 1.8 km away.

Analysis of Parameters

Physio chemical analysis of wetland water

Study of growth parameters:

1.a. Dry weight: All colonies are collected from each of the test vessels and rinsed with distilled or deionised water. They are blotted to remove excess water and then dried at 60°C to a constant weight. Any root fragments should be included. The dry weight should be expressed to an accuracy of at least 0.1 mg.

1.b. Fresh weight: All colonies are transferred to pre-weighed plastic tubes with small (1 mm) holes in the rounded bottoms. The tubes are then centrifuged at 3000 rpm for 10 minutes at room temperature. Tubes, containing the now dried colonies, are re-weighed and the fresh weight is calculated by subtracting the weight of the empty tube.

1.c. Dry weight-Fresh weight ratio can be determined from above estimations. The plant growth index was calculated as follows

$$\text{Growth Index} = \frac{Biomass\ (t = 7\ days)}{Biomass\ (t = 0)}$$

1.d. Doubling time: To determine the doubling time *(Td)* of frond number and adherence to this validity criterion by the study, the following formula is used with data obtained from the control vessels:

$$Td = \ln 2 / \mu$$

Where μ is the average specific growth rate.

1. e. Average specific growth rate: this response variable is calculated on the basis of changes in the logarithms of frond numbers, and in addition, on the basis of changes in the logarithms of another measurement parameter (total frond area, dry weight or fresh weight) over time (expressed per day) in the controls and each treatment group. It is sometimes referred to as relative growth rate. The average specific growth rate for a specific period is calculated as the logarithmic increase in the growth variables -frond numbers and one other measurement variable (total frond area, dry weight or fresh weight) - using the formula below for each replicate of control and treatments:

$$\mu_{i-j} = \frac{\ln(N_j) - \ln(N_i)}{t}$$

where:

μ_{i-j}: average specific growth rate from time i to j

N_i: measurement variable in the test or control vessel at time i

N_j: measurement variable in the test or control vessel at time j

t: time period from i to j

1.f. Percentage of growth inhibition: Percent inhibition of growth rate (Ir) may then be calculated for each test concentration according to the following formula

$$\%Ir = \frac{(\mu C - \mu T)}{\mu C} \times 100$$

where:

% Ir: percent inhibition in average specific growth rate

μC: mean value for μ in the control

μT: mean value for μ in the treatment group

Estimation of photosynthetic pigments

The chlorophyll estimation is an important study parameter for the estimation of impact of pollution on photosynthetic activity. About 200mg of treated plants were weighed. This is taken in a mortar with 5mlof 90% acetone and 1ml of Magnesium carbonate. It is then ground thoroughly with pestle. This is then kept at 4°C for 4 hours for the pigments to elute. The solution is then centrifuged at 2500 rpm for 15 minutes. The extract is then decanted to a volumetric flask and the volume is made up to 50 ml with 90% acetone. The absorbance at 750, 663, 645,510 and 480 were measured in the spectrophotometric analysis using Hitachi-U-2000 spectrophotometer.

Statistical analysis

Analysis of variance for each test was conducted using STATISTICA software (One way Anova). The significant difference between treatments were determined by Duncan's multiple range test ($P<0.05$). Each test was conducted in six replicates.

Results and Discussions

Physiochemical properties of water from both wetlands are given in Table 1. The values of BOD, Nitrate, phosphate, Ammonia, TDS, TSS, Turbidity and heavy metals Cu, Pb, Zn and Cd were significantly high in wetland 1 in comparison to the control.

Sl.No	Parameter (Unit)	Control wetland	Wetland 1	Wetland 2
1	Temp(°C)	28.8	29.1	28.8
2	Conductivity(mS)	1.67	4.1	2.3
2	pH	7.9	8.2	7.8
3	Salinity (ppt)	1.8	1.8	1.8
4	DO(mg O2/L)	5	2.65	3.2
5	BOD(mg O_2/L)	8	112	12.24
6	Nitrate(mg/L)	45	12.28	0.91
7	Phosphate(mg/L)	6.2	14.42	1.57
8	Ammonia	1.2	28.09	0.75
9	TDS	2100	2811	2220
10	TSS	112	55.42	24.89
11	Turbidity	11	413.2	13.8
12	Cu(mg/L)	2.4	2.43	2.2
13	Pb(mg/L)	1	3.71	2.68
14	Zn(mg/L)	3.2	112.21	23.2
15	Cd(mg/L)	not detected	2.33	0.47

Table 1: Results of physio chemical analysis of Wetland 1 and 2 water before exposure. Each values are means of triplicates. The significant difference between treatments is $P<0.05$

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Duckweeds show great tolerance to changes in physiochemical parameters of water. The growth rate of duckweed is favoured by organic pollutants as well as inorganic nutrients. Gopal and Chamanlal [9] reported the maximum biomass of L. perpusilla and S. polyrrhiza from roadside pools and ditches in India within an electrolyte conductivity range of 650-1 000 µS/cm.

The concept of average specific growth rate is based on the general exponential growth pattern of duckweed in non-limited cultures, where toxicity is estimated on the basis of the effects on the growth rate, without being dependent on the absolute level of the specific growth rate of the control; slope of the concentration-response curve or on test duration. The use of average specific growth rate for estimating toxicity is scientifically preferred. In the current study ASGR and frond doubling time (Td) of the control and treatment with 0.5% and 5% concentration I WI yield the same result.WII water shows different result ASGR and Td remains same as control up to 25% concentration. The inhibition of growth in this concentration is negligible. As the concentration of effluent increases, all the parameters vary. For the test to be valid, the doubling time of frond number in the control must be less than 2.5 days (60 h) (OECD guidelines) [7]. When the frond doubling time exceeds 2.5, the test solution is considered toxic. In the study only un dilutes 100% concentration water from wetland 1 shows Td values more than 2.5, thus found to be toxic. The values are given in Table 2.

Plant growth index were measured after 7 days of exposure with different dilutions of water from both wetlands. At 0.5 % and 5% dilution GI is greater than control values in both waters. At 10% W2 treatment shows less GI than control but W1 still has values above control which shows the water quality of W1 is better than W2. From 25% GI values shows sharp decline in both treatments. The finding is given in Table 3.

The growth index of both wetland water is similar in lowest concentration (0.5%). From 5% dilution onwards it is quite clear that growth of Spirodela is enhanced more in wetland I. This may be due to the increase in crude protein content of duckweed however, seems to increase to a maximum of ~40 percent DM over the range from trace ammonia concentrations to 7-12 mg N/L [10]. Khondker, Islam and

Site	Medium	Mean Nj	Mean ASG (µ)	Td	%Ir
	control	16	4.258	1.272	0
	0.5	16	4.258	1.272	0
	5	16	4.258	1.272	0
	10	15	3.871	1.4	9.08
	25	14	3.484	1.555	18.177
	50	13	3.097	1.75	27.26
Wet I	100	10	1.935	2.801	34.21
	0.5	16	4.258	1.272	0
	5	16	4.258	1.272	0
	10	16	4.258	1.272	0
	25	16	4.258	1.272	0
	50	15	3.871	1.4	9.08
Wet 2	100	15	3.871	1.4	9.08

Table 2: ASGR, Td and Ir% of S. polyrrhiza after 7 days of treatment with various dilutions of wetland I and 2. Mean N(i)=5, Mean T(j)=7 and T(i)=0. Standard deviations were presented by error bars. Each value is means of six replicates. The significant difference between treatments is P<0.05.

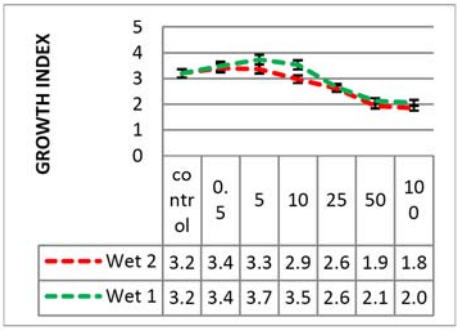

Table 3: Growth Index of Spirodela plant in different dilutions of Wetland 1 and 2 after 7 days of exposure. Standard deviations were presented by error bars. Each values are means of six replicates. The significant difference between treatments is P<0.05.

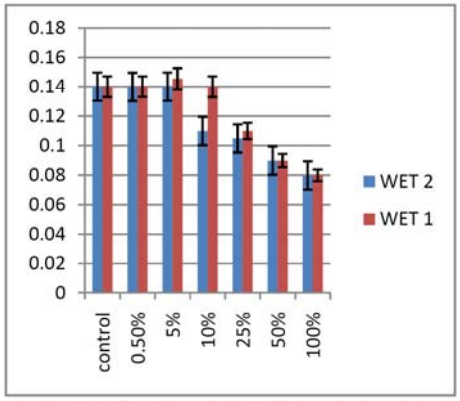

Table 4: Biomass DW/FW ratio of S. polyrrhiza after 7 days of exposure in water from Wetland 1 and 2. Standard deviations were presented by error bars. Each values are means of six replicates. The significant difference between treatments is P<0.05.

Makhnun [11] observed that both phosphate and silicate concentrations had significant positive correlation with the biomass of L. perpusilla in Bangladesh. But at 25% dilution GI is surprisingly similar. At extreme concentrations again GI vary. The differences in GI under different concentrations are illustrated in Table 4.

Changes in Dry weight fresh weight ratio indicate that in exposed Spirodela plants, growth retardation takes place in comparison to the control. At 0.5 % concentration, the biomass yield is same as that in control. In 5% concentration of wetland 1and 2 water, slight increase in DW/FW ratio recorded in wetland 2 effluents than control. At 10% and 25% solutions, the DW/FW ratio is higher in weland water 1 in comparison with Wet 2. But in 50% and 100% the ratio shows sharp decline. It was noted that at 50% concentration, spirodela growing in highly polluted weland 2 water yield higher FW/DW ratio than those growing in wetland 1 (Table 5).

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The decline in biomass ratio may be due the presence of excess heavy metals present in wetland. It has been shown that accumulation of heavy metals disturb the plant water status which eventually results in osmotic stress and growth reduction [12,13]. Water especially in wetland 2. In wetland 1 the biomass (DW/FW ratio) percentage was 100, 100.7, 103.9, 100, 78.5, 64.27, and 57.1 for dilutions 0.5, 5, 10, 25, 50 and 100 respectively. Similarly for wetland 2 it was 100, 99.9, 100.07, 78.4, 74.9, 64.2, and 57.04 for dilutions 0.5, 5, 10, 25, 50 and 100 respectively.

Chlorophyll and carotenoids are the central part of the energy manifestation of every green plant system and therefore, any significant alteration in their levels is likely to cause a marked effect on the entire metabolism of the plant. The productivity of plants is directly related with changes in the content of photosynthetic pigments chlorophyll a, chlorophyll b and Carotenoids. Industrial wastewater not only affects the chlorophyll content but the chlorophyll activity also [14-16]. In the study photosynthetic pigments may be inhibited due to metal toxicity. Industrial wastewater not only affects the chlorophyll content but the chloroplast activity also [14-16].

Duckweed leaves started to show signs of chlorosis (pigment loss) following 7d exposure to surface water samples. At 50% and 100% dilutions necrosis could also seen. The Carotenoids contents are found to be less affected. At the end of 7 days of exposure 0.5% dilution of wetland 1 and 2 shows slight increase in chlorophyll a content (Hormesis) while at the same dilution and Carotenoids content remain unchanged. From 5% to 100% all pigments shows gradual decrease in concentration. Chl *b* degraded at a much slower rate than chl *a* which indicates that greater damage of pollutants present in water samples on chl *a*. The loss of photosynthetic pigment content has been reported in duckweed plants following exposure to Cu, Pb and Ni [17-19]. The destruction of photosynthetic pigments by heavy metals could be due to: impairment of ETC, replacement of Magnesium ions associated with chlorophyll ring, inhibition of important enzymes [20] associated with chlorophyll synthesis or peroxidation processes in chloroplast membrane lipids by reactive oxygen species [21] (Table 6).

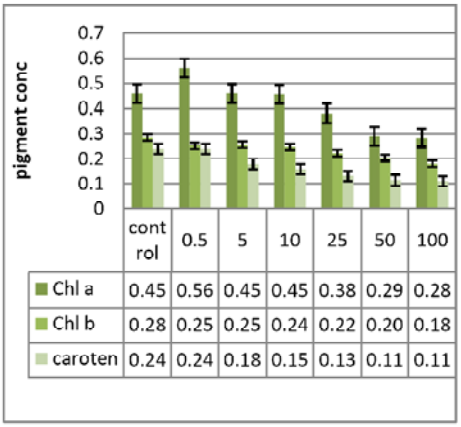

Table 5: Relative photosynthetic pigment concentrations after 7 days of exposure in different dilutions of wetland 1. Standard deviations were presented by error bars. Each values are means of six replicates. The significant difference between treatments is P<0.05.

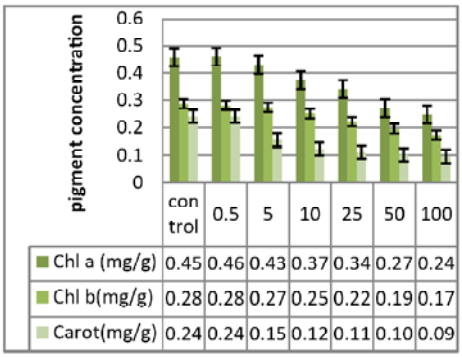

Table 6: Relative photosynthetic pigment concentrations after 7 days of exposure in different dilutions of wetland 2. Standard deviations were presented by error bars. Each values are means of six replicates. The significant difference between treatments is P<0.05.

Conclusion

The study revealed that both welands are highly polluted. Wetland 2 has more pollutants compared with wetland 1 which is evident from the assessment of vegetative, growth and photosynthetic pigment parameters. The study also points towards the importance of conservation of wetlands in the area.

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