Introduction

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 \textit{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 \textit{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
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A 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°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 *Spirodel polynriza* 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

*Spirodel polynriza* 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 *Spirodel polynriza* 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 EC50.
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 wastewate. 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 *Spirodea 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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