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PHYTOREMEDIATION OF WASTE WATER BY USING *AZOLLA-ANABAENA* CONSORTIUM AND ITS AQUATIC ASSOCIATES : A REVIEW

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Abstract

Water crisis in today's society is challenging not only for humans but also for all living creatures. Water being the basis of life has become a sparse resource. To circumvent this situation, reusability of water can be one of the suited alternatives. In order to achieve this goal, many techniques prevail, of which phytoremediation is considered to be more effective and significant. Essentially, the major water pollutant sources are generated from domestic, commercial and industrial usages were the pollutants are discarded without any sort of pre-treatments, resulting in an increased risk of environmental pollution. Primarily, phytoremediation process converts the waste water into usable water essentially by the help of plants. The breakthrough advantage of phytoremediation is attributed for its economical and environmental friendly processes. But, selection of plants to be used for the phytoremediation process is tricky. A number of plants are available which can be used for the detoxification process. Among several, *Azolla*, a potent biofertilizer is well known for its phytoremediation activity. Essentially, the water fern *Azolla* associates with an algal symbiont - *Anabaena azollae* and carries out the fixation of atmospheric nitrogen. Till date, the basis of phytoremedial activity displayed by *Azolla* is limited. It would be quite revealing to expose the role of *Azolla* in the phytoremediation process. This review article emphasizes on phytoremediation, plausible role of *Azolla* in this process and tried to arrive at strategies for developing better scientific models to handle abundant quantities of wastewater with the use of this water fern.

Key words: Phytoremediation, Waste water, *Azolla-Anabaena* consortium, Nitrogen fixation

Introduction

The environmental pollution is the major problem in the aquatic environment. Industrialization and rapid increase in human population have resulted in transformation of the natural environment. The environment became hostile, posing many threats to health and welfare because of pollutants released into the environment. The environment impacts of municipal waste water and industrial effluents discharge on receiving water are numerous and inputs of contaminants can affect the aquatic biota as well as the health of the coastal environment. With rapid industrialization across the globe, several remediation technologies have come out to deal with diverse categories of pollutants. Heavy metals are considered to be the chief and significant contaminants. Heavy metals continue to exert their effect for a prolonged period as compared to organic pollutants like pesticides or petroleum by-products. Heavy metals are exceedingly toxic for all biotic components present in the environment. Essentially direct water source or through biomagnifications, contamination of heavy metal results. More often in mining areas, elevated air concentrations too become a source of heavy metal contamination (Santona *et al*., 2006). Various conventional techniques are being used for obliteration of heavy metals, but evidently they require a huge capital cost along with several other negative effects. Additionally, chemical methods produce a huge amount of slurry and cost along with an increase in per capita (Hinchman *et al*., 1996). An efficient solution to deal with this current scenario has come up and referred to as phytoremediation, which utilizes plants for treatment of pollutants (Chaney *et al*., 1997). This review paper chiefly focuses on phytoremediation using *Azolla*. *Azolla*, a water fern represents the only example of pteridophyte harbouring symbiotic association with diazotrophic nitrogen-fixing cyanobacteria, and bacteria residing in leaf cavities (Sood

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and Ahluwalia, 2009; Satapathy and Chand, 2010). *Azolla* has high rate of multiplication (doubling time is around 2-3 days) coupled with high potential for N_2 fixation making its biomass rich in nitrogen and protein. Due to this it has been exploited as N-biofertilizer for rice as rice fields form an ideal environment for its growth. *Azolla* presents further potential uses, such as substrate for biogas production or to natural dyes extraction (anthocyanin-E-163, also with anti-oxidative action), as a biological control of submerged weeds, algal growth and insects, but essentially it can be utilized as feed supplements for aquatic and terrestrial animal and as a biofertilizer (Leterne *et al*., 2010). *Azolla* was studied as a biological regenerative life support system (BLSS) integrated in space missions (Soyus-Salyut 6) and Biosphere II project (Arizona) in order to promote food production, gas exchange, water reclamation and nutrient recycling (Carrapiço, 2002; Liu *et al*., 2008a, b). Due to increase in environmental awareness attracted scientific community to extend *Azolla* exploitation more vigorously in the area of phytoremediation because the fern can hyperaccumulate variety of pollutants such as heavy metals, radionuclides, dyes, and pesticides etc. from aquatic ecosystems along with other macrophytes (Padmesh *et al*., 2006; Rai and Tripathi, 2009; Mashkani and Ghazvini, 2009; Sood *et al*., 2011; Satapathy, 2000; Satapathy and Chand, 2009; Parida and Satapathy, 2013; Satapathy *et al*., 2013; Sunemia *et al*., 2013; Mishra *et al*., 2014; Pati and Satapathy, 2016; Mishra and Satapathy, 2017). *Azolla* possesses remarkable ability that proves it as a better plant system than many other macrophytes. These abilities include faster growth, efficient nitrogen fixing capacity and biomass disposal. *Azolla* has great possibility of use in bioremediation of waste waters and soils.

Phytoremediation

It is an innovative field meant for cleaning up of contaminated soil, water and air (Salt *et al*., 1998; Meagher, 2000; Pulford and Watson, 2003). This technology is an alternative or complementary one that could be applied along with or instead of mechanical cleaning methodologies which mostly require high capital investment, labour and intensive energy. Phytoremediation is the process of using plants (phyto) to clean up (remediate) polluted soil or water or air. Phytoremediation consists of mitigating pollutant concentrations in contaminated soils, water or air with naturally occurring or genetically engineered plants that have ability to accumulate, degrade or eliminate metals, pesticides, solvents, explosives, crude oil, and its derivatives etc. (Flathman and Lanza, 1998; Prasad and Freitas, 2003). Phytoremediation is an emerging technology that uses various plants to degrade, extract and immobilize contaminants from soil and water. This technology has been receiving attention lately as an innovative, cost-effective alternative to the more established treatment methods used at hazardous waste sites. Phytoremediation is one of the best solutions for removing pollutants from the sewage and making it suitable for reuse. Macrophytes-based wastewater treatment systems have several potential advantages compared with conventional treatment systems and can act as bio-filters in the wastewater treatment technologies. Aquatic macrophytes are able to remove a variety of nutrients from polluted water including the major agricultural pollutants N and phosphorus. Various aquatic plants have been used to remove nutrients and minerals from contaminated fresh water and also waste water; among them are *Eichhornia* (Cornwell *et al*., 1977), *Lemna* (Harvey and Fox, 1973), *Ipomoea* (Hashimoto, 1983), and *Azolla* (Kitoh *et al*., 1993; Mishra *et al*., 2007; Satapathy, 1995). It is an *in situ* process that utilizes the inbuilt characteristics of plants for environmental remediation. Development in this area can only be possible due to the collaboration and cooperation in the interdisciplinary research fields like plant biochemistry, molecular biology, soil chemistry, agronomy, environmental engineering and at the same time support at state and federal level.

Techniques of Phytoremediation

Different phytoremediation processes Fig. 1 were proposed to decontaminate the environmental toxicity (Vamerali *et al*., 2010).

(i) *Phytoextraction:*

In this process, plants uptake pollutants from soil and water, and translocate to and store in the harvestable biomass of the plants. Phytoextraction aims to remove pollutants from the contaminated sites. This process is usually observed in hyper-accumulating plants resistant to the pollutants.

(ii) *Phytostabilization:*

Plants reduce mobility and phytoavailability of contaminants in the environment. This process doesn't remove pollutants from contaminated sites but reduces mobility and excludes metals from plant uptake.

(iii) *Phytovolatilization:*

Hyper-accumulating plants uptake pollutants from soil and water, and translocates to the aerial parts of the plants, and volatilizes the pollutants in the air.

(iv) *Phytotransformation:*

This process is one kind of plant's defense mechanism to the environmental pollutants. The hyper-accumulating plants modify, inactivate, degrade (phytodegradation), or immobilize (phytostabilization) the pollutants through their metabolism.

(v) *Rhizofiltration:*

Usually aquatic plants perform this process. The hyper-accumulating aquatic plants adsorb and absorb pollutants from aquatic environments.

Role of Aquatic Macrophytes in Phytoremediation:

The limitations of Phytoremediation can be overcome by using plants having high biomass, faster growth rate, and ability to adapt with wide range of environmental conditions. Many terrestrial and aquatic plants have been screened for their ability to take up heavy metals from contaminated aquatic systems including ground water (Bennicelli *et al*., 2004; Miretzky *et al*., 2004; Khellaf and Zerdaoui, 2009; Narain *et al*., 2011; Veselý *et al*., 2011; Satapathy *et al*., 2012; Mishra *et al*., 2017). Aquatic macrophytes are more suitable for waste water treatment than terrestrial plants because of their faster growth and more biomass production, comparatively higher capability of contaminant uptake, and better purification effects due to direct contact with contaminated water. They also play a key role in the structural and functional aspects of aquatic ecosystems by various ways. Rapid urbanization and anthropogenic pressure is the main cause of nutrient accumulation in aquatic ecosystems, leads to eutrophication resulting into massive growth of the macrophytes and weeds. Eutrophication of a water body signifies the ageing of a lake. Various types of macrophytes including emergent, free floating, submerged are generally observed in an aquatic ecosystem play important role in removing nutrients. They have tremendous capacity of absorbing nutrients and other substances from the water (Satapathy, 2010) and hence bring the pollution load down. It is found to be most effective in removal of BOD, COD, nitrogen, phosphorus, organic carbon, suspended solids, phenols, pesticides, heavy metals etc. from waste water. The potential of aquatic marcophytes for heavy metal removal has been investigated and reviewed extensively (Brooks and Robinson, 1998; Cheng, 2003; Prasad and Freitas, 2003; Dhir *et al*., 2009; Dhote and Dixit, 2009; Marques *et al*., 2009; Rai, 2009; Satapathy and Chand, 2009, 2010; Sunemia *et al*., 2013; Mishra *et al*., 2016; Pati and Satapathy, 2016). Aquatic macrophyte can utilize large amounts of nitrogen and phosphorous and thus remove them from waste water. Many researchers have investigated the nitrogen and

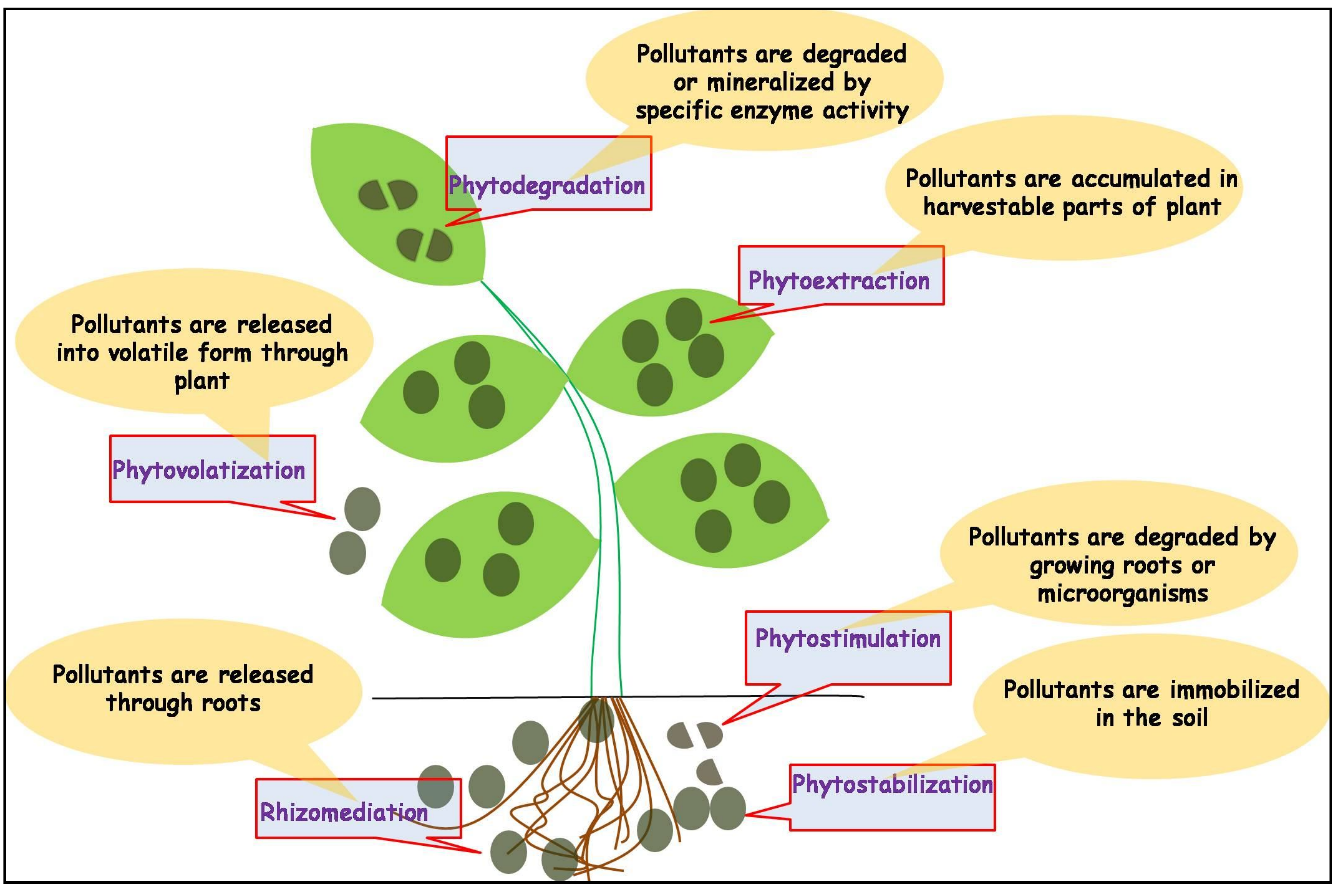

Fig. 1: Different kinds of phytoremediation processes include phytodegradation, phytoextraction, phytovolatization, phytostimulation, phytostabilization and rhizomediation.

phosphorous removal capacities of different aquatic plants (Satapathy and Chand, 1984; Satapathy, 2010). The heavy metals which are absorbed by aquatic macrophytes concentrate in their roots, shoots as well as leaves. However, the accumulation of heavy metals are much higher in roots than other parts of these plants (Mishra *et al*., 2009; Mufarrege *et al*., 2010; Mishra *et al*., 2016; Pati and Satapathy, 2016).

Azolla, a rewarding tool for phytoremediation:

Azolla is a free floating water fern that floats in the water and fixes atmospheric nitrogen because of its association with the nitrogen fixing heterocystous blue green alga *Anabaena azollae* in its dorsal leaves. *Azolla* is a small aquatic fern belonging to Phylum-Pteridophyta, Class-Polypodiopsida, Order-Salviniales, Family-Azollaceae with a monotypic genus (Wagnar, 1997; Pabby *et al*., 2003; Pabby *et al*., 2004; Sood and Ahluwalia, 2009). It is widely distributed in both tropical and temperate rice growing regions (Satapathy and Chand, 1984). The suitability of *Azolla* over other nitrogen fixers as a partial substitute for chemical nitrogen fertilizer is due to its high nitrogen fixing capacity, rapid growth in water logged rice soils, easy incorporation into the soil and the comparatively quicker availability of its nitrogen to the standing rice crop and non interference with the normal cultivation practices and crop development. The agronomic potential of *Azolla* - *Anabaena* association is related to its ability to grow rapidly and successfully in habitats lacking or having low levels of nitrogen, especially under waterlogged conditions. *Azolla* is one of the world's fastest growing aquatic macrophytes, with a doubling time of only 2-5 days (Taghi-Ganji *et al*., 2005; Zimmerman, 1985). The relatively quick decomposition of the biomass and rapid availability of its nitrogen to the standing crop makes it agronomically outstanding (Singh *et al*., 1981; Singh *et al*., 1982).

Potential utilities of *Azolla*

Azolla-Anabaena complex is eco-friendly as it never leads to contamination of the environment. It also does not compete with the rice plants for photosynthesis or nutrition. Such biological systems are able to provide 1.5-2.0 million tonnes of nitrogen for crop production in India whereas at least 3.3-4.4 million tonnes of urea will be required to give similar amounts of nitrogen. Besides, its use as a N-supplement in rice based ecosystems, it has also found limited use in crops such as taro, wheat, tomato, banana (Van Hove, 1989; Marwaha *et al*., 1992). *Azolla* presents further potential uses, such as substrate for biogas production or to natural dyes extraction (anthocyanin-E-163, also with anti-oxidative action), as a biological control of submerge weeds, algal growth and insects, but essentially it can be utilized as feed supplements for aquatic and terrestrial animal (Satapathy and Singh, 1985; Leterne *et al*., 2010). *Azolla* was studied as a biological regenerative life support system (BLSS) integrated in space missions (Soyus-Salyut 6) and Biosphere II project (Arizona) in order to promote food production, gas exchange, water reclamation and nutrient recycling (Carrapiço, 2002; Liu *et al*., 2008 a,b). The use of *Azolla* with high growth rate and productivity seems to be very promising to improve treated urban wastewater quality. The biosorption and bioadsorvent of NO_3^{-2}, PO_4^{-2} and SO_4^{-2} from sewage water using *Azolla* is well proven fact (Rakhshaee *et al.*, 2006; Parida and Satapathy, 2013). The fern can hyper-accumulate a variety of pollutants such as heavy metals, radionuclides, dyes, and pesticides etc. from aquatic ecosystems along with other macrophytes (Padmesh *et al*., 2006; Rai and Tripathi, 2009; Mashkani and Ghazvini, 2009; Sood *et al*., 2011; Satapathy and Chand, 2010). The free-floating habitat, ability to grow in N-deficit sites, known potential to tolerate wide range of pollutants, and accumulation of different heavy metals from contaminated sites reflect their exploitation a more promising candidate in future for phytoremediation (Arora *et al*., 2006; Umali *et al*., 2006). Interest in the use of this plant as a biological filter for the renovation of wastewater has increased now-a-days.

Environmental requirements of the *Azolla* - *Anabaena* consortium

Like other plants, *Azolla-Anabaena* symbiosis is also affected by environmental factors and this has been reviewed by Lumpkin and Plucknett (1980), Hamdi (1982), Watanabe (1982), and Lumpkin (1987a, b). Of the various environmental factors, the availability of water is most common factor limiting *Azolla* growth. The other important factors are nutrient availability, temperature, light and aspects of water quality such as pH, salinity and turbulence (Cary and Weerts, 1992; Lumpkin and Plucknett, 1982; Satapathy and Chand, 1984; Satapathy and Chand, 2004). In addition to abiotic factors, biotic factors like pests also affect the growth and cultivation of *Azolla* (Lumpkin and Plucknett, 1980; Lumpkin, 1987a; Van Hove, 1989; Satapathy and Singh, 1987).

Mineral nutrition

Azolla like other green plants requires all the macronutrients (except N) and micronutrients for its growth and nitrogen fixation by its symbiont (Becking, 1978; Kitoh and Shiomi, 1991; Yatazawa *et al*., 1980). The importance of some of micronutrients (Biswas *et* *al*., 2005) such as iron and trace elements like Mo has long been recognized for a successful and quick growth of *Azolla*, particularly in relation with its nitrogen fixation metabolism. Nitrogen fixation by *Azolla - Anabaena* requires cobalt and molybdenum (Zahran *et al*., 2007). Singh *et al*., (2010) studied the effect of micronutrients (e.g. Mo^{6+}, Mn^{2+}, Zn^{2+}, Cu^{2+} and Fe^{2+}) on cellular and extra-cellular activities of two *Azolla* species (*A. microphylla* and *A. filiculoides*) exposed to a p-deficient, saline (20 mM NaCl) medium. At lower concentrations (0 - 0.01 mM) of the micronutrients showed a significant enhancement in the given activity, whereas higher concentrations (10 Mm) played an inhibitory role. Macronutrients like potassium (K^{+}), calcium (Ca^{2+}) and Magnesium (Mg^{2+}) are also very important to yield a successful and rapid growth of the species (Biswas *et al*., 2005; Serag *et al*., 2000). Potassium and organic compounds of nitrogen and phosphorous in the biomass of *Azolla* can be good indicators for its use as a biofertilizer in domestic waste-waters or natural environments (Costa *et al*., 1999). According to Vidal and co-workers (1992) the optimum levels of P, K, Ca, Mg and Fe in the nutrient solution were 0.02 µg ml^{-1}, 5-12 µg ml^{-1},125 µg ml^{-1}, 15 µg ml^{-1} and 0.25 - 0.55 µg ml^{-1} respectively. Wagnar (1997) examined that the threshold levels of the micronutrients such as Fe, Mn, Mo and B for *Azolla* growth was 50, 20, 0.3 and 30 µg L^{-1} respectively. Jain *et al.* (1992) studied the toxic effect of some elements on *Azolla*. Their study showed that iron and manganese did not have any toxic effect on the anaerobic fermentation of *Azolla*, while copper, cobalt, lead and zinc showed toxicity. According to Yatazawa *et al*. (1980) the threshold levels of P, K, Mg and Ca required in the medium for *Azolla* growth were approximately 0.03, 0.4 and 0.5 m mol L^{-1} respectively; whereas full nitrogenase activity required 0.03, 0.6, 0 .5 and 0.5 m mol L^{-1} respectively. Olsen (1972) found that Na and Mn are also essential for the fern growth. The deficiency of Ca and P comparatively showed more pronounced effect on growth and N_2 fixation than deficiency of K and Mg as reported by Subudhi and Watanabe (1979) from their mineral nutrition study. Kitoh and Shiomi (1991) observed that *Azolla* required 0.3, 0.3, 0 .4, 0.08, 0.08 mM of K, Mg, Ca, P and S for growth and a concentration of 0.07, 0.06, 1.0, 0.05 Mm for nitrogen fixation.

Water

As it is clear from the etymology of *Azolla*, derived from a Greek origin, namely '*azo'* and '*ollyo'* which means "killed by drought" (Carrapiço *et al*., 2000); this fern cannot survive without water. In other words, water is a vital and important factor for the survival of *Azolla*. This small aquatic fern should float on the water surface to stay alive. Though, it is able to grow on a wet mud surface or wetted peat litter, this fern prefers to grow in free-floating conditions (Serag *et al*., 2000) on calm water surfaces, and may thus be found on the surface of ponds, canals, and lakes as well as on some slow-moving rivers (Ghorbanzadeh and Tajer Mohammad, 2009; Satapathy and Chand, 1984). Water is a fundamental requirement of *Azolla*. It can survive only a few days in a paddy field once the field is drained. Maximum nitrogenase activity has been observed in *A. caroliniana* at 88-95% moisture levels of the fresh mass, but when moisture contents drop to 80% nitrogenase activity decrease to less than one-fifth of the maximum (Helcher and Dawson, 1995). A shallow water depth of 5 cm or less is best, although *Azolla* can grow satisfactorily in greater depth.

pH

The response of *Azolla* to pH depends on many factors such as temperature, light intensity, nutrients (nitrogen and phosphorus), and the presence of soil and iron (Wagnar, 1997). Usually, *Azolla* prefers a medium near to neutrality or to some extent, acidic conditions. The optimum pH range for *Azolla* growth is 4.5-7, although it can survive within a range of 3.5-10 (Watanabe *et al*., 1977; Lumpkin and Plucknett, 1980; Lumpkin, 1987b; Satapathy, 2010). At high light intensity (60,000 lux), optimum pH is 9-10, whereas at low light intensity (15,000 lux), optimum pH is 5-6 for maximum relative growth rates of *Azolla*. Growth was not supported in acidic soil of pH of 3.0-3.8 or at an alkaline pH 8.4 (Singh, 1977). Nitrogen fixation was found to be optimal at a pH of 6.0 with a temperature of 20°C (Ashton, 1974; Lumpkin and Plucknett, 1980). In green house experiments, Cary and Weerts (1992) found that, at a water temperature of 25°C, both *A. pinnata* and *A. filiculoides* showed maximum growth at pH values of 5-7. *Azolla pinnata* showed greater tolerance to a wide pH range than did *A. filiculoides*, the latter growing much more poorly at pH values of 4 and 8. Preferably, optimum growth in *Azolla* is dependent not only on pH but also on other environmental conditions.

Phytoremediation and Environmental Management

Global industrialization and rise in population over the past few decades have added huge loads of pollutants in the water resources (CPBC, 2008). Discharge of untreated or partially treated industrial and domestic wastewater, leaching of pesticides and residues of fertilizers and transportation activities are the most important factors that affect the quality of ground and nearby surface water bodies (Ezzat *et al*., 2002;

Satapathy, 2000). It is evident that anthropogenic sources are responsible for pollution and environmental degradation in order to exploit nature for means of livelihood. All components of the biosphere are facing threats of pollution by a variety of organic/inorganic pollution because of manmade activities that alter the normal biogeochemical cycle (Prasad and Freitas, 2003). Due to acute scarcity of fresh water resources and ever increasing volumes of wastewaters, it has become imperative for both developed and developing nations to conserve water and address problems of water scarcity and wastewater disposal. The best way to conserve water is to recycle it. Use of treated waste water for irrigation and other non-residential purposes is being encouraged. More than 70% of the surface water is polluted by industrial and sewage effluents generated in cities. A large number of industries including textile, paper and pulp, printing, iron and steel, electroplating, coke, petroleum, pesticide, paint, solvent and pharmaceutical etc. consume large volumes of water and organic chemicals which differ in their composition and toxicity. With the increase in world population, the water consumption has increased manifold which caused increase in sewage effluents. For developing countries like India, the discharge of effluents from these industrial units and domestic waste water from municipalities to various water bodies leading to water pollution is a matter of great concern. The purification and removal of nutrients, organic pollutants with conventional method is not cost effective and sustainable (Deval *et al*., 2012). Several conventional physico-chemical methods, such as membrane filtration (Yoon *et al*., 2009), chemical precipitation (Matlock *et al*., 2002; Ramos *et al*., 2009), ion exchange (Inglezakis and Loizidou, 2007), chemical oxidation or reduction (Mitra *et al*., 2011), electrochemical treatment (Rana *et al*., 2004), solvent extraction (Miretzky *et al*., 2006) and activated carbon adsorption (Malik, 2003) have been used to remove heavy metals and other contaminants from effluents. Each of the remediation technology has specific benefits and limitations (EPA, 1997) but in general none of them is cost-effective (Volesky, 2001; Rai, 2009). Many studies have been conducted to improve the water quality through natural means to overcome this problem. Stewart (1970), Wooten and Dodd (1976), and Conwell *et al*., (1977) were among the pioneers to demonstrate the nutrient removal potential of aquatic plants. Seidal (1976) and Wolverton and McDonald (1976) experimentally proved the importance of aquatic plants in removing organic contaminants from aquatic environments. Therefore, this plant-based remediation technology, a concept called phytoremediation offers a cost-effective, non-intrusive and safe alternative to conventional clean up technologies.

Advantages and limitations of Phytoremediation

The primary motivation behind the development of phytoremediation technologies is their eco-friendly and cost effective nature. Phytoremediation takes advantage of the unique, selective and naturally occurring uptake capabilities of plant root systems, together with the translocation, bioaccumulation and pollutant storage/ degradation abilities of the entire plant body. Besides being aesthetically pleasing, phytoremediation is on average tenfold cheaper than other physical, chemical or thermal remediation methods since it is performed *in situ*, is solar driven and can function with minimal maintenance once established. Phytoremediation of soil metals has been successfully carried out at military sites, agricultural fields, industrial sites and mine tailings (Bañuelos, 2000; Winter Sydnor and Redente, 2002). Inorganic pollutants that can be phytoremediated include plant macronutrients such as nitrate and phosphate (Horne, 2000), plant trace elements such as Cr, Cu, Fe, Mn, Mo and Zn, nonessential elements such as Cd, Co, Fe, Hg, Se, Pb, V and W (Horne, 2000; Blaylock and Huang, 2000) and radioactive isotopes such as 238U, 137Cs and 90Sr (Dushenkov, 2003; Dushenkov and Kapulnik, 2000). After phytoremediation the hyper-accumulating plants can be used for retrieval of the precious heavy metals as bio-ores.

The use of phytoremediation is also limited by the climatic and geologic conditions of the site to be cleaned, the temperature, soil type and the accessibility for agriculture equipment (Salt and Kramer, 2000; Schmoger *et al*., 2000). The other limitations of this technology include long clean up times required, the potential for introducing the contaminant into food chain, bioavailability of contaminant and toxicity encountered in establishing and maintaining vegetation at waste sites.

Conclusion and Future perspectives

Domestic waste water includes household waste liquid from toilets, baths, showers, kitchens, sinks etc. that is disposed by means of sewers. Usually the major source of water pollution is sewage especially in and around large urban centres. The composition of sewage water is quite variable depending upon the contributing source, mode of collection and treatment provided. Although a large proportions of these sewage waters is organic in nature and contains essential plant nutrients but sometimes toxic metals are also present in appreciable amounts. Organic substances present in sewage are carbohydrates, lignin, fats, protein and their decomposed products, soaps as well as various natural and synthetic organic chemicals from the process industries. Sewage also contains the inorganic substances from domestic and industrial sources, including a number of potentially toxic elements such as cadmium, chromium, copper, lead, zinc and iron (Dipak and Arti, 2011). Besides this, domestic waste water also contains a good number of pathogenic microbes. Sewage pollution of the urban areas has become a growing concern throughout the globe, and over populated towns and cities of Odisha are no exceptions. Effective and economic control of sewage is one of the primary responsibilities of any industrialists, urban authorities and governments. Conventional physico-chemical methods of treatment are invariably cost-intensive and cannot be employed in all industries especially in small and medium scale industries. In this situation biological treatment systems may serve as one of the alternatives. It may be critical to search for new photo-autotrophic organisms with high growth rates and high utilization potential, which could be mass cultured in waste water and play a dual role of cleansing the water and serving as a source of feed and fertilizer. Free living blue-green algae, *Azolla-Anabaena* consortium may ideally be suited to perform these functions by virtue of their high growth rates and their known nutritional and fertilizer value.

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References

Arora, A., S. Saxena and D.K. Sharma (2006). Tolerance and phytoaccumulation of chromium by three *Azolla* species. *World Journal of Microbiology and Biotechnology,* **22:** 97-100.

Ashton P.J. (1974). The effect of some environmental factors on the growth of *Azolla filiculodies.* In: The Orange River Progress Report, E.M.V. Zinderen (ed.), South Africa: Baker Bloemfontein, 123-138.

Banuelos, G.S. (2000). Factors influencing field phytoremediation of selenium laden soils. In: Phytoremediation of contaminated soil and water, N. Terry and G. Banuelos (eds.), Boca Raton, Lewis, 41-61.

Becking, J.H. (1978). Ecology and physiological adaptations of *Anabanea* in the *Azolla – Anabaena azollae* symbiosis. In: Environmental role of nitrogen fixing blue green algae and a symbiotic bacterium, U. Granhall (ed.), Stockholm. *Ecol. Bull.*, **26:** 266-281.

Bennicelli, R.Z., Stezpniewska, A. Banach, K. Szajnocha and J. Ostrowski (2004). The ability of *Azolla caroliniana* to remove heavy metals (Hg(II), Cr(III), Cr(VI)) from municipal waste water. *Chemosphere,* **55:** 141-146.

Biswas, M., S. Parveen, H. Shimozawa and N. Nakagoshi (2005). Effects of *Azolla* species on weed emergence in a rice paddy ecosystem. *Weed Biology Management,* **5:** 176-183.

Biswasi, S.P., M. Mohanty, C. Pradhan and K.B. Satapathy (2013). Phytoremediation of Aqueous solutions contaminated with Nickel (Ni) by exploitation of *Azolla microphylla* & *Salvinia molesta*: a novel Bioseparation process for waste water treatment. In: Industrial and Environmental Biotechnology, K. Pramanik (ed.), Department of Biotechnology & Medical Engineering, National Institute of Technology, Rourkela - 769 008, India, 283-292.

Blaylock, M.J. and J.W. Huang (2000). Phytoextraction of metals. In: Phytoremediation of toxic metals using plants to clean up the environment I. Raskin and B.D. Ensley (eds:), Wiley, New York 53-70.

Brook, R.R. and B.H. Robinson (1998). Aquatic phytoremediation by accumulator plants. In: Plants that hyperaccumulate heavy metals: Their role in phytoremediation, microbiology, archaeology, mineral exploration and phytomining, Brook, R.R (ed.), Wallingford, UK: CABI International 203–226.

Carrapiço, F. (2002). *Azolla-Anabaena*-Bacteria as a Natural icrocosm. In: SPIE Astrobiology Conference "Instruments, Methods and Missions for Astrobiology IV". San Diego, 261-265. ISBN 0-8194-4209-7.

Carrapiço, F., G. Teixeria and M.A. Diniz (2002). *Azolla* as Biofertilizer in Africa. A challenge for the future. *Revista de Ciencias Agrarias*, **23:** 120-138.

Cary, P.R. and P.G.J. Weerts (1992). Growth and nutrient composition of *A. pinnata* R.Br. and *A. filiculoides* Lam. as affected by water temperature, nitrogen and phosphorus supply, light intensity and pH. *Aquatic Botany,* **43:** 163-180.

Chaney, R.L., M. Malik and Y.M. Li (1997). Phytoremediation of soil metals. *Current Opinion in Biotechnology,* **8(3):** 279-284.

Cheng, S. (2003). Heavy metals in plants and Phytoremediation. *Environmental Science and Pollution Research,* **10:** 335-340.

Cornwell, D.A., J. Jr. Zoltek and C.D. Patrinely (1977). Nutrient removal by water hyacinths. *J. Water Pollut. Control Fed*., **49:** 57–65.

Costa, M.L., M.C.R. Santos and F. Carrapico (1999). Biomass characterization of *A. filiculoides* grown in natural ecosystems and wastewater. H*ydrobiologia*., **415:** 323-327.

Central Pollution Control Board (2008). Status of water quality in India 2007, New Delhi, India: CPCB.

Deval, C.G., A.V. Mane, N.P. Joshi and G.D. Saratale (2012).

Phytoremediation potential of aquatic macrophyte *Azolla caroliniana* with references to zinc plating effluent. *Emir Journal of Food Agriculture,* **24:** 208-223.

Dhir, B., P. Sharmila and P.P. Saradhi (2009). Potential of aquatic macrophytes for removing contaminants from the environment. *Critical Reviews in Environmental Science and Technology,* **39:** 754–781.

Dhote, S. and S. Dixit (2009). Water quality improvement through macrophytes - a review. *Environmental Monitoring and Assessment,* **152:** 149-153.

Dipak, S. and D. Arti (2011). Assessment and treatment of municipal wastewater of Indore city of India. *Archives of Applied Science Research,* **3(1):** 450-461.

Dushenkov, S. and Y. Kapulnik (2000). Phytofiltration of metals. In: Phytoremediation of toxic metals using plants to clean up the environment I. Raskin and B.D. Ensley (eds.). Wiley, New York. 89-106.

Dushenkov, S. (2003). Trends in phytoremediation of radionuclides. *Plant Soil,* **249:** 167-75.

EPA (Environmental Protection Agency) (1997). Electrokinetic laboratory and field processes applicable to radioactive and hazardous mixed waste in soil and ground water. EPA 402/R-97/006.Washington, DC.

Ezzat, M.N., H. Shehab, A.A. Hassan, M. El Sharkawy, A. El Diasty, I. El Assiouty, F. El-Gohary and A. Tczap (2002). Survey of Nile system pollution sources*:* Report No. 64. APRP.

Flathman, P.E. and G.R. Lanza (1998). Phytoremediation: current views on an emerging green technology. *Journal of Soil Contamination,* **7:** 415-432.

Fogg, G.E., W.D.P. Stewart, P. Fay and A.E. Walsby (1973). The blue-green algae. Academic Press, London, England.

Hamdi, Y.A. (1982). Application of nitrogen-fixing systems in soil improvement and management. FAO Soils Bull. 49. Food and Agriculture Organization, Rome.

Harvey, R.M. and J.L. Fox (1973). Nutrient removal using *Lemna minor*. *J. Water Pollut. Control Fed.,* **45:**.

Hashimoto, S. (1983). Wastewater treatment by channel flow system and food production. *Water Purif. Liq. Wastes Treat.,* **24:** 17-24 (in Japanese).

Hechler, W.D. and J.O. Dawson (1995). Factors affecting nitrogen fixation in *Azolla caroliniana*. *Trans. Illinois State Acad. Sci.,* **88:** 97-107.

Hinchman, R.R., M.C. Negri and E.G. Gatliff (1996). Phytoremediation: using green plants to clean up contaminated soil, groundwater and wastewater. In: Proceedings of the International Topical Meeting on Nuclear and Hazardous Waste Management- *Spectrum,* **96:** 1-13.

Horne, A.J. (2000). Phytoremediation by constructed wetlands. In: Phytoremediation of contaminated soil and water, N. Terry and G. Bañuelos (eds.) Boca Raton, Lewis. 13-40.

Inglezakis, V.J. and M.D. Loizidou (2007). Ion exchange of some heavy metal ions from polar organic solvents into zeolite. *Desalination,* **211:** 238-248.

Jain, S.K., G.S. Gujral, N.K. Jha and P. Vasudevan (1992). Production of biogas from *Azolla pinnata* R. Br and *Lemna minor* L.: Effect of heavy metal contamination. *Bioresource Technology,* **41:** 273-277.

Kannaiyan, S. (1981). *Azolla* biofertilizer for rice. INSFFR Training Seminar. Int. Rice Res. Inst., Philippines, 1-11.

Khellaf, N. and M. Zerdaoui (2009). Phytoaccumulation of zinc by the aquatic plant, *Lemna gibba* L. *Bioresource technology,* **100:** 6137-6140.

Kitoh, S. and N. Shiomi (1984). Nutrient removal by *Azolla* from the mineral nutrient solution and wastewater. *Water Purif. Liq. Wastes Treat.,* **25:** 561-567.

Kitoh, S. and N. Shiomi (1991). Disappearance of symbiotic algae in the *Azolla-Anabaena* association subjected to transitory exposure to ammonium. *Soil Sci. Plant Nut.,* **37:** 323-329.

Kitoh, S., N. Shomi and E. Ljheda (1993). The growth and nitrogen fixation of *Azolla filiculoides* Lam. in polluted water. *Aquatic Bot.,* **46:** 129-139.

Leterne, P., A.M. Londoño, D.C. Ordoñez, A. Rosales, F. Estrada, J. Bindelle and A. Bulgen (2010). Nutritional value and intake of aquatic ferns (*Azolla filiculoides* Lam. and *Salvinia molesta* Mitchell.) in cows. *Animal Feed Science and Technology,* **155:** 55-64.

Liu, X., M. Chen, Z. Bian and C. Liu (2008a). Studies on urine treatment by biological purification using *Azolla* and UV photocatalytic oxidation. *Advances in Space Research,* **41(5):** 783-786.

Liu, X., M. Chen, Z. Bian and C. Liu (2008b). Research on some functions *Azolla* in CELSS systems. *Acta Astronautica,* **63(7-10):** 1061-1066.

Lumpkin, T.A. (1987a). Collection, maintenance and cultivation of *Azolla*. In: Symbiotic Nitrogen Fixation Technology. G.H. Elkan (ed.), Marcel Dekker, Inc., New York: 55-193.

Lumpkin, T.A. (1987b). Environmental requirements for successful *Azolla* growth. In: International Rice Research Institute, *Azolla* utilization : Proceedings of the Workshop on *Azolla* Use. International Rice Research Institute, Los Banos, Philippines, 89-97.

Lumpkin T.A. and D.L. Plucknett (1980). *Azolla*: botany, physiology and use as a green manure. *Econ. Bot.,* **34:** 111-153.

Malik, P.K. (2003). Use of activated carbons prepared from sawdust and rice husk for adsorption of acid dyes: a case study of Acid Yellow 36. *Dyes and pigments,* **56:** 239-249.

Marques, A.P.G.C., A.O.S.S. Rangel and P.M.L. Castro (2009). Remediation of heavy metal contaminated soils: Phytoremediation as a potentially promising clean-up technology. *Critical Reviews in Environmental Science and Technology,* **39:** 622-654.

Marwaha T.S., B.V. Singh and S.K. Goyal (1992). Effect of incorporation of *Azolla* on wheat (*Triticum aestivum var.* HD-2329). *Acta Bot. Indica,* **20:** 218-220.

Mashkani, S.G. and P.T.M. Ghazvini (2009). Biotechnological potential of *Azolla filiculoides* for biosorption of Cs and Sr: Application of micro-PIXE for measurement of Biosorption. *Bioresource Technology,* **100:** 1915-1921.

Matlock, M.M., B.S. Howerton and D.A. Atwood (2002). Chemical precipitation of heavy metals from acid mine drainage. *Water Research,* **36:** 4757-4764.

Meagher, R.B. (2000). Phytoremediation of toxic elemental and organic pollutants. *Current opinion in plant Biology*, **3:** 153-162.

Miretzky, P., A. Saralegui and A.F. Cirelli (2004). Aquatic macrophytes potential for the simultaneous removal of heavy metals (Buenos Aires, Argentina). *Chemosphere*, **57(8):** 997-1005.

Miretzky, P., A. Saralegui and A.F. Cirelli (2006). Simultaneous heavy metal removal mechanism by dead macrophytes. *Chemosphere*, **62:** 247-254.

Mishra, M., C. Pradhan and K.B. Satapathy (2014). Decontamination of Lead from aquatic environment by exploitation of floating macrophyte *Azolla microphylla* Kauf. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, **8(12):** Ver. III: 17-23.

Mishra, M., A. Mohapatra and K.B. Satapathy (2016). Effect of lead on plant growth, lead accumulation and biochemical changes of *Pistia stratiotes* L. *International Journal of Biosciences*, **9(6):** 66-78.

Mishra, M. and K.B. Satapathy (2017). Phytoremediation of Lead Contaminated Wastewater through Aquatic Weeds. LAP LAMBERT Academic Publishing, 17 Meldrum Street, Beau Bassin 71504, Mauritius, 193 p.

Mishra, M., A. Mohapatra and K.B. Satapathy (2017). Potential of *Salvinia cucullata* Seg. and *Salvinia molesta* D.Mitch in phytoremediation of lead contaminated waste water. *The Journal of Bioprocess Technology*, **103:** 514-522.

Mishra S., S. Srivastava, R.D. Tripathi and P.K. Trivedi (2007). Thiol metabolism and antioxidant systems complement each other during arsenate detoxification in *Ceratophyllum demersum* L. *Aquatic Toxicology,* **86:** 205-215.

Mishra, V.K., B.D. Tripathi and K.H. Kim (2009). Removal and accumulation of mercury by aquatic macrophytes from an open cast coal mine effluent. *Journal of Hazardous Materials,* **172:** 749-754.

Mitra, P., D. Sarkar, S. Chakrabarti and B.K. Dutta (2011). Reduction of hexa-valent chromium with zero-valent iron: Batch kinetic studies and rate model, *Chemical Engineering Journal (Amsterdam, Netherlands),* **171:** 54-60.

Mufarrege, M.M., H.A. Hadad and M.A. Maine (2010). Response of Pistia stratiotes to heavy metals (Cr, Ni and Zn) and phosphorous. **Archives of Environmental Contamination and Toxicology, 58:** 53-61.

Narain, S., C.S.P. Ojha, S.K. Mishra, U.C. Chaube and P.K. Sharma (2011). Cadmium and Chromium removal by aquatic plant. *International Journal of Environmental Sciences,* **1(6):** 1297-1304.

Newton, J.W. (1976). Photo-production of molecular hydrogen by a plant algal symbiotic system. *Science,* **169:** 559-560.

Olsen, C. (1972). On biological nitrogen fixation in nature, particularly in blue-green algae. *Compt. Rend. Trav. Carlsberg Lab*., **37(12):** 269-283.

Pabby, A., R. Prasanna and P.K. Singh (2004). Morphological characterization of cultured and freshly separated cyanobionts (Nostocales, Cyanophyta) from *Azolla* species. *Acta Botanica Hungarica*., **46:** 211–223.

Pabby, A., R. Prasanna, S. Nayak and P.K. Singh (2003). Physiological characterization of the cultured and freshly isolated endosymbionts from different species of *Azolla*. *Plant Physiology and Biochemistry*, **41:** 73–79.

Padmesh, T.V.N., K. Vijayraghavan, G. Sekaran and M. Velan (2006). Application of *Azolla rongpong* on biosorption of acid red 88, acid green 3, acid orange 7 and acid blue 15 from synthetic solutions. *Chemical Engineering Journal*, **122:** 55-63.

Parida, P. and K.B. Satapathy (2013). Phytoremediation of urban waste water using *Azolla pinnata* R. Br. and *Oryza sativa* L. - a case study of Vyasanagar municipality in the district of Jajpur, Odisha. *Biohelica*, **3(1&2):** 65-7.

Pati, S. and K.B. Satapathy (2016). Phytoremediation potential of aquatic macrophyte *Azolla pinnata* R.Br. and *Salvinia molesta* Mitchell for removal of Chromium from waste water. *International Journal of Science, Environment and Technology*, **5(4):** 2146 -2160.

Prasad, M.N.V. and H. Freitas (2003). Metal hyperaccumulation in plants-Biodiversity prospecting for phytoremediation technology. *Electronic Journal of Biotechnology,* **6:** 285-321.

Pulford, I.D. and C. Waston (2003). Phytoremediation of heavy metal - contaminated land by tree: a review. *Environment International,* **29:** 592-540.

Rai, P.K. (2009). Heavy metal phytoremediation from aquatic ecosystems with special reference to macrophytes. *Critical Reviews in Environmental Science and Technology,* **39:** 697-753.

Rai, P.K. and B.D. Tripathi (2009). Comparative assessment of *Azolla pinnata* and *Vallisneria spiralis* in Hg removal from G.B. Pant Sagar of Singrauli Industrial region, India. *Environmental Monitoring and Assessment,* **148:** 75-84.

Rakhshaee, R., M. Khosravi, Taghi Masoud and M.T. Ganji (2006). Kinetic modeling and thermodynamic study to remove Pb(II), Cd(II), Ni(II) and Zn(II) from aqueous solution using dead and living *Azolla filiculoides*. *Journal of Hazardous Materials*, **134:**120–129.

Ramos, W.D.S., T. Poznyak, I. Chairez and I. Cürdova (2009). Remediation of lignin and its derivatives from pulp and paper industry wastewater by the combination of chemical precipitation and ozonation. *Journal of Hazardous Materials*, **169:** 428-434.

Rana, P., N. Mohan and C. Rajagopal (2004). Electrochemical removal of chromium from wastewater by using carbon aerogel electrodes. *Water Research*, **38:** 2811-2820.

Salt, D.E. and V. Kramer (2000). Mechanism of metal hyperaccumulation in plants. In: Phytoremediation of toxic metals: Using plants to clean up the environment I. Raskin and B.D. Ensley(eds.). Wiley, New York. 231-246.

Salt, D.E., R.D. Smith and I. Raskin (1998). Phytoremediation. *Annual review of Plant Physiology - Plant Molecular Biology*, **49:** 643 –688.

Santona, L., P. Castaldi and P. Melis (2006). Evaluation of the interaction mechanisms between red muds and heavy metals," *Journal of Hazardous Materials*, **136:** 324-329.

Satapathy, K.B. (1995). Growth performance of *Azolla* and rice in domestic sewage and its impact on pollution control. Environment Change and Management R.C. Mohanty (ed.) Kamal Raj Enterprises, New Delhi. 124-128.

Satapathy, K.B. (2000). Decontamination of hazardous chemicals of municipal sewage and industrial effluents by exploitation of hydrophytes. In: Proc. Natl. Sem. Management of hazardous chemicals, G.D. Rath (ed.) Multi-disciplinary Centre on Safety, Health and Environment, Bhubaneswar, 70-80.

Satapathy, K.B. (2010a). Effect of various doses of super phosphate, cow dung and cattle-slurry on the growth of *Azolla*. *Biohelica*, **1(1):** 41- 44.

Satapathy, K.B. (2010b). Effect of soils of varied pH on the growth and N_2 fixation of the water fern *Azolla*. *Plant Science Research*, **32:** 114-118.

Satapathy, K.B. and P.K. Chand (1984). Studies on the ecology of *Azolla pinnata* R. Br. of Orissa. *J. Indian Bot. Soc*., **63:** 44-52.

Satapathy, K.B. and P.K. Chand (2004). Environmental influence on the growth and N_2 fixation of free-living and symbiotic Cyanobacteria from low land rice fields of Orissa. Science for Sustenance J.K. Panigrahi *et al*., (eds.), ISCA (Bhubaneswar chapter), Bhubaneswar, 29-51.

Satapathy, K.B. and P.K. Chand (2009). Phytoremediation of chromium toxicity in water resources of 'Sukinda chrome zone' using *Azolla* and its floating associates. In: Proc. of Natl. Sem. on Recent Trends in Monitoring and Bioremediation of Mine and Industrial Environment. P.G. Department of Botany, North Orissa University, Baripada, 54-63.

Satapathy, K.B. and P.K. Chand (2010). *Azolla* : A Biofertilizer and Waste disposer. VDM Verlag Dr. Muller Aktiengesellschaft & Co. KG Dudweiler Landstr. 99, 66123 Saarbrucken, Germany, 191 p., 2010 (ISBN: 978-3-639-19138-7).

Satapathy, K.B. and P.K. Singh (1985). Control of weeds by *Azolla* in Rice. *J. Aquat. Plant Manage*, **23:** 40-42.

Satapathy, K.B. and P.K. Singh (1987). Effect of pesticides carbofuran and phorate on the growth of water fern *Azolla pinnata* R. Br. varieties and their pests. *Beiterage Trop. Landwirtsch Veterinarmed*, **25:** 411-417.

Satapathy, K.B., A. Mohapatra and P. Parida (2012). Phytoremediation of Steel plant effluents by using native macrophytes – A case study of Neelachal Ispat Nigam Limited (Odisha). Plant and Stress Management, H.K. Patra (ed.), P.G. Department of Botany, Utkal University, Bhubaneswar - 751 004 (Odisha), 31-37.

Satapathy, K.B., A. Mohapatra and P. Parida (2013). Exploitation of Selected Aquatic and Marshy Macrophytes in Detoxifying Waste Water containing Steel Plant Effluents - A Case Study of Neelachal Ispat Nigam Limited, Odisha (India). Industrial and Environmental Biotechnology K. Pramanik (ed.), Department of Biotechnology & Medical Engineering, National Institute of Technology, Rourkela - 769 008, India, 363-370.

Schmoger, M.E., M. Oven and E. Grill (2000). Detoxification of arsenic by phytochelatins in plants. *Plant Physiol*., **122:** 793-801.

Seidal, K. (1976). Macrophytes and water purification. In Biological control for water pollution, J. Tourbier and R.W. Pierson (ed.), Pennsylvania: Pennsylvania University Press. 109-121.

Serag, M.S., A. El-Hakeem, M. Badway and M.A. Mousa (2000). On the ecology of *A. filiculoides* Lam. in Damietta District, Egypt. *Limnologica*., **30:** 73-81.

Shome, G., D.P. Kushari and G. Shome (1996). Effect of different phosphorus concentrations on the growth of some *Azolla* species. *Environ. Ecol*., **14:** 546-549.

Singh, A.L., P.K. Singh and P.L. Singh (1988). Comparative studies on the use of green manuring, organic manuring and *Azolla* and blue green algal biofertilizers to rice. *J. Agri. Sci. Camb*., **110:** 337-343.

Singh, P.K. (1977). Multiplication and utilization of fern *Azolla* containing nitrogen-fixing algal symbiont a green manure in rice cultivation. *RISO*, **26:** 125-137**.**

Singh, P.K., B.C. Panigrahi and K.B. Satapathy (1981). Comparative efficiency of *Azolla*, blue-green algae and other organic manures in relation to N and P availability in a flooded rice soil. *Plant and Soil*, **62:** 35-44.

Singh, P.K., K.B. Satapathy, S.P. Mishra, S.K. Nayak and R.N. Patra (1982). Application of *Azolla* in rice cultivation. Proc. Natl. Symp. on Biol. Nitrogen fixation. IARI, New Delhi. 423-450.

Singh, S.S., A.K. Mishra and R.S. Upadhyay (1981). Potentiality of *Azolla* as a suitable P-biofertilizer under salinity through acid phosphatase activity. *Ecological Engineering*., **36:** 1076-1082.

Singh, S.S., A.K. Mishra and R.S. Upadhyay (2010). Potentiality of *Azolla* as a suitable P-biofertilizer under salinity through acid phosphatase activity. *Ecological Engineering*., **36:** 1076-1082.

Sood, A. and A.S. Ahluwalia (2009). Cyanobacterial–plant symbioses with emphasis on *Azolla-Anabaena* symbiotic system. *Indian Fern Journal*, **26:** 166–178.

Sood, A., S. Pabbi and P.L. Uniyal (2011). Effect of paraquat on lipid peroxidation and antioxidant enzymes in aquatic fern *Azolla microphylla* Kual. *Russian Journal of Plant Physiology*, **58:** 667-673.

Sood A., P.L. Uniyal, Radha Prasanna and A.S. Ahluwalia (2012). Phytoremediation Potential of Aquatic Macrophyte, *Azolla*. *Ambio*., **41(2):** 122-137.

Stewart, K.K. (1970). Nutrient removal potential of various aquatic plants. *Hyacinth Control Journal*, **8:** 34–35.

Subudhi, B.P.R. and I. Watanabe (1979). Minimal level of phosphate in water for growth of *Azolla* determined by continous flow culture. *Curr. Sci*., **48:** 1065-1066.

Taghi-Ganji, M., M. Khosravi and R. Rakhshaee (2005). Biosorption of Pb (II), Cd (II), Cu (II) and Zn (II) from the wastewater by treated *A. filiculoides* with H2O2/MgCl2.

Umali, L.J., J.R. Duncan and J.E. Burgess (2006). Performance of dead *Azolla filiculoides* biomass in biosorption of Au from wastewater. *Biotechnology Letters*, **28:** 45-49.

Vamerali, T., M. Bandiera and G. Mosca (2010). Field crops for phytoremediation of metal-contaminated land- A review. *Environ. Chem. Lett*., **8:** 1-17.

Van Hove, C. (1989). *Azolla* and its multiple uses with emphasis on Africa. *Food and Agriculture Organization*, Rome.

Veselý, T., P. Tlustoš and J. Száková (2011). The use of lettuce (*Pistia stratiotes* L.) for rhizofiltration of a highly polluted solution by cadmium and lead. *International Journal of Phytoremediation*, **13:** 859-872.

Vidal, I., L. Longeri and M. Leal (1992). Effect of nutritional composition on the development and nitrogen fixation of *Azolla filiculoides*. *Turrialba*, **42:** 509-519.

Volesky, B. (2001). Detoxification of metal-bearing effluents: Biosorption for the next century. *Hydrometallurgy*, **59:** 203-216.

Wagnar, G.M. (1997). *Azolla*: a review of its biology and utilization. *Botanical Review*, **63:** 1-26.

Watanabe, I. (1982). *Azolla-Anabaena* symbiosis – Its physiology and use in tropical agriculture. Y.R. Dommergues and H.G. Diem (eds.), Microbiology of tropical soils and plant Productivity. Martinus Nighoff/W. Junk, The Hague, 169-185.

Watanabe, I. C.R., N.S. Espianas Berja and B.V. Alimagno (1977). Utilization of the *Azolla-Anabaena* complex as a nitrogen fertilizer for rice, *IRRI Res Paper Ser.*, **11:** 1-15.

Winter Sydnor, M.E. and E.F. Redente (2002). Reclamation of high elevation, acidic mine waste with organic amendments and topsoil. *J. Environ. Qual*., **31:** 1528-1537.

Wolverton, B.C. and R.C. McDonald (1976). Don't waste water weeds. *New Scientist*, **71:** 318-320.

Wooten, J.W. and D.J. Dodd (1976). Growth of water hyacinth in treated sewage effluent. *Economic Botany*, **30:** 29–37.

Yatazawa, M., N. Tomomatsu, N. Hosoda and K. Nunome (1980). Nitrogen fixation in *Azolla-Anabaena* symbiosis as affected by mineral nutrient status. *Soil Sci. Pl. Nutr*., **26:** 415-426.

Yoon, J., G. Amy, J. Chung, J. Sohn and Y. Yoon (2009). Removal of toxic ions (chromate, arsenate and perchlorate) using reverse osmosis, nanofiltration and ultrafiltration membranes. *Chemosphere*, **77:** 228-235.

Zahran, H.H., A.H. Abo–Ellil and E.A. Al-Sherif (2007). Propagation, taxonomy and ecophysiological characteristics of the *Azolla-Anabaena* symbiosis in fresh water habitats of Beni-Suef Governorate (Egypt). *Egyptian Journal of Biology*, **9:** 1-12.

Zimmerman, W.J. (1985). Biomass and pigment production in three isolates of *Azolla* II. response to light and temperature stress. *Annals of Botany*, **56:** 701-709.