Paper from Proceeding of the National Conference "Science in Media 2012" Organized by YMCA University of Science and Technology, Faridabad, Haryana (India) December 3rd -4th 2012 Published by IJMRS's International Journal of Engineering Sciences, ISSN (Online): 2277-9698
www.ijmrs.com

Azolla: Role in Phytoremediation of Heavy Metals

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***Abstract*— The environmental pollution resulting from natural resources acquisition is one of the most severe problems nowadays. Phytoremediation is a new environmental friendly and economically attractive techniques using the plants for detoxification of their substrate. It is an integrated multidisciplinary approach to the cleanup of contaminated soils and water, which combines the disciplines of plant physiology, chemistry, and soil and water microbiology. Besides numerous application of *Azolla* in agriculture, e.g. as green manure, it was found that this plant possess a huge ability for phytoremediation. *Azolla*,an aquatic pteridophyte is an ideal choice because of its high multiplication rate, global distribution, high biomass production, high protein content, its growth habitat. It is not only used by farmers as biofertilizer but is also used as feed for livestock, as green manure, bioremediation of waste water and reclamation of saline soils. Both living as well as the non-viable biomass of *Azolla* can be used for heavy metal removal from wastewater. The growing concern about environmental degradation and shrinking natural resources necessitates the use to exploit the potential of this *Azolla –Anabaena* association more efficiently. This paper reviews the potential of *Azolla* in environmental management.**

Keywords: Green technology, Phytoremediation, Aquatic macrophyte, *Azolla,* Heavy metals.

I. INTRODUCTION

Global development, growing population and increasing urbanization has led to increase in pollution and overexploitation of the natural resources. Clean water and air are essential for life. The contamination of the environment by toxic compounds has become a worldwide problem. However the demand for a country's economic, agricultural and industrial development outweighs the demand for safe and pure environment. Accumulation of these toxic wastes into soil and water is a matter of serious concern as they enter the food chain and cause health hazards. The toxic substances include organic as well as inorganic compounds. Inorganic toxic substances that pollute environment are heavy metals, metalloids, other trace elements and radioactive materials that are added through mining processes and other geophysical modifications. Organic pollutants result partly from agricultural operations like application of herbicides, insecticides, fungicides, nematicides and numerous other applications of chemicals in everyday life.

Application of remediation approaches becomes imperative when the buildup of these toxic substances in water and soil is beyond permissible limits. Chemical processes are applied for treating the wastes, but they suffer from inherent limitations. Novel biotechnological approaches are being applied for treating wastes and these include bioremediation and phytoremediation. These are the terms used to describe those methodologies which employ living organisms to remove toxic compounds from the environment. While bioremediation refers to the use of lower organisms (algae, bacteria and fungi), phytoremediation refers to the using plants for treating polluted soil and water. Both these technologies share the advantage of in-situ application and environmental acceptability as compared to the other physico-chemical approaches [1].While phytoremediation using higher plants is best suited for treatment and reclamation of polluted soils, use of microorganisms and small water plants is more feasible for treating aquatic systems. Aquatic plants that have been used for treating wastewater includes *Lemna, Azolla, Sesbania* etc.

The fate of toxic substances in soil and aquatic environment depends on processes like non-specific absorption, specific absorption, precipitation, dissolution, oxidation, reduction, photochemical reaction, biotic processes etc [2]. Heavy metals exposure may cause neurobehavioral disorders such as fatigue, insomnia, decreased concentration, depression, irritability, gastric symptoms, sensory symptoms, and motor symptoms. Exposure to heavy metals has been linked with developmental retardation, various cancers, kidney disorders, autoimmunity, and even death in some instances of exposure to very high concentrations .At higher levels, mercury can damage vital organs such as lungs and kidneys. Another reason that toxic heavy metals are causing potential concern is that of bioaccumulation and biomagnifications. The metals may be transferred and accumulated in the bodies of animals or human beings through the food chain, which potentially causes DNA damage and carcinogenic effects caused by their mutagenic ability [3].Examples include Cd, Cr, and Cu, which have been associated with health effects ranging from dermatitis to various types of cancer. In addition, some metals occur in the environment as radioactive isotopes (*e.g.*, U238, Cs137, Pt239, and Sr90), which can greatly increase the health risk [4].

The term phytoremediation (phyto = plant and remediation = correct evil) was coined in 1991.

Phytoremediation is an emerging technology that uses various plants to degrade, extract, contain, or immobilize contaminants from soil and water. It is environment friendly, cost effective and solar driven process. It is an integrated multidisciplinary approach to the cleanup of contaminated soils and water, which combines the disciplines of plant physiology, chemistry, and soil and water microbiology. The harvested plant tissue, rich in accumulated contaminant is easily and safely processed by drying, ashing or composting. The volume of toxic waste produced as a result is just a fraction of that of many current, more invasive remediation technologies. Some metals can be reclaimed from ash or by desorption process in case of living biomass, thus reducing the generation of hazardous waste and generates recycling revenues.

A plant used for phytoremediation needs to be tolerant of the pollutants, grow rapidly with a high yield per hectare, accumulate the metal in harvestable parts, have a profuse root system and a high bioconversion factor. The bioconversion factor is the concentration of pollutant in the plant compared with that in environment. It needs to be more than 20 or above to be able to reduce contamination by 50%. The plants that accumulate high concentration of metals are called Hyperaccumulators and can accumulate 50-100 times more metal than normal plants[5].There are about 400 species that are hyperaccumulators. *Thalspi sp., Brassica sp., Alyssum sp. Salix, Populus, Alnus* are among the terrestrial plants and *Lemna, Azolla, Sesbania, Eichhornia. Pistia* etc.are the aquatic plants that have been used for treating wastewater .Hyperaccumulation involves adsorption, transport and translocation to areas where large quantities of metal can be stored.

MECHANISM OF PHYTOREMEDIATION

Phytoremediation can occur by the following mechanism:

1)Phytoextraction or phytoaccumulation: Phytoextraction is the removal of a contaminant from the soil, ground water or surface water by live plants. Phytoaccumulation occurs when the contaminant taken up by the plant is not degraded rapidly or completely, resulting in an accumulation in the plant.

2) Phytodegradation or phytotranformation: A contaminant can be eliminated by plant enzymes or enzyme co-factors[6;7].Dec and Bollag describe plants that can degrade aromatic rings in the absence of microorganisms. Polychlorinated biphenyls (PCBs) have been metabolized by sterile plant tissues. Poplar trees (*Populus* spp.) are capable of transforming trichloroethylene in soil and ground water [6,8]

3) Phytovolatilization:Phytovolatilization involves the use of plants to take up contaminants from the soil, transforming them into volatile form and transpiring them into the atmosphere. Phytovolatilization occurs as growing trees and other plants take up water and the organic and inorganic contaminants. [9].

4) Phytostabilization: Phytostabilization, also referred to as in-place inactivation, is primarily used for the remediation of soil, sediment, and sludges [10]. It is the use of plant roots to limit contaminant mobility and bioavailability in the soil. The plants primary purposes are to (1) decrease the amount of water percolating through the soil matrix, which may result in the formation of a hazardous leachate, (2) act as a barrier to prevent direct contact with the contaminated soil and prevent soil erosion and the distribution of the toxic metal to other areas . Phytostabilization can occur through the sorption, precipitation, complexation, or metal valence reduction.

5) Rhizodegradation: Rhizodegradation is a biological treatment of a contaminant by enhanced bacterial and fungal activity in the rhizosphere of certain vascular plants. The rhizosphere is a zone of increased microbial density and acitivity at the root/surface, and was described originally for legumes by Lorenz Hiltner in 1904 [11]. Plants and microorganisms often have symbiotic relationships making the root zone or rhizosphere an area of very active microbial activity [12,13,14,15]. Plants can moderate the geochemical environment in the rhizosphere, providing ideal conditions for bacteria and fungi to grow and degrade organic contaminants. Plant litter and root exudates provide nutrients such as nitrate and phosphate that reduce or eliminate the need for costly fertilizer additives.

6) Rhizofiltration: It is defined as the use of plants, both terrestrial and aquatic; to absorb, concentrate, and precipitate contaminants from polluted aqueous sources with low contaminant concentration in their roots.

Aquatic macrophytes have been used during the last two decades for water metal removal competing with other secondary treatments, being the principal mechanism for metal uptake adsorption trough roots [18].The aquatic floating macrophytes Pistia stratiotes L.(water lettuce), Spirodela intermedia W. Koch (duckweed) and Lemna minor L. (duckweed) present a high growth rate and have been used for the removal of Cd, Cr and Pb from watercolumn [19, 20,21,22,23,24,25,26] S. intermedia and L. minor present the additional advantage of growing under varied climatic conditions.There are two general mechanisms associated with the separation of dissolved metals from water using aquatic plant biomass. The first is the fast metabolism(within minutes) independent surface reaction that has modeled as a diffusion processes and ends when the soluble metal ions bind or sorbs to the outer cell wall of the biomass. The second is a slow metabolism(within hours or days) dependent cellular uptake that has been modeled as a mass transfer process from the outer cell wall to the cell or cell wall interior[27].

AZOLLA IN PHYTOREMEDIATION

Azolla name is derived from Greek word *azo(* to dry) and allyo (to kill) . It means that plant dies when it dries. The genus *Azolla* belongs to the single genus family Azollaceae[28]. The six recognizable species within the genus are grouped under two subgenera: *Euazolla* and *Rhizosperma* [29].The four species under the sub-genus *Euazolla* are *A.filiculoides*, *A.caroliniana*, *A mexicana* and *A.microphylla*. It is thought that these four species originated from temperate, sub-tropical and tropical regions of North and South America.Although reproductive characters provide the most useful tool for taxonomic separation but in most of the samples sporocarps are usually absent hence identification is difficult. However, Zimmerman *et al*.[30] found three of these species (*A. caroliniana, A. mexicana* and *A. microphylla*) to have close taxonomic affinity and similar responses to phosphorus deficiency and recommended that they be considered as a single species.The two species under sub-genus *Rhizosperma* are *A. nilotica* and *A. pinnata*. *A.nilotica* is a native of East Africa and can be found from Sudan to Mozambique. *A.pinnata* has two different varieties that vary in their distribution patterns. *A.pinnata* var. *imbricata* originates from subtropical and tropical Asia while *A.pinnata* var. *pinnata* occurs in Africa and is known as African strain.

Azolla is a free floating water fern, which occurs in the symbiotic association with N_2 fixing blue green alga *Anabaena azollae.* It 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. For this attribute it has been exploited as N- biofertilizer for rice as rice fields form an ideal environment for its growth. *Azolla* is worldwide in distribution and grows in varied conditions from dilute water bodies to polluted waterbodies [31].It can uptake and accumulate nutrients directly from flood waters and has high affinity for P, Fe and K. It accumulates these nutrients several times more than its requirement then slowly releases these nutrients as it decomposes. Based on its capacity to concentrate nutrients like N, P and heavy metals it has also been used for treatment of waste waters [32]

Azolla has great possibility of use in bioremediation of wastewaters and soils. There are three main aspects of use of *Azolla* in bioremediation. These areas are:

a) For treatment of wastewaters rich in heavy metal pollutants.
b) Treatment of domestic sewage effluents which are rich in N and P wastes.
c) Bioremediation of saline soils.

Azolla possesses remarkable ability to absorb heavy metals such as chromium, zinc, nickel, cadmium copper and even uranium. The tolerance and concentration capacity of *Azolla* to different metal ions has been reported in earlier studies also. In 1985, Yong-Huang and Weizhen[33] from China studied tolerance of four *Azolla* species to Cu, Mn ,Fe, Zn, Mo, Co, Cd etc under laboratory conditions and found that concentration capacity of *Azolla* for metals affected its growth only slightly without any detrimental effect or not at all. This suggested possible exploitation of *Azolla* in concentrating heavy metals from polluted aquatic systems. Since then many processes using *Azolla* alone or in mixed culture with other aquatic plants for treating wastewaters have been studied and developed.

The *Azolla* processes are superior to traditional methods of metal removal from effluents when:

a) Environmental and ecological constraints exist
b) Concentrations of metals in wastewaters are extremely low, but more than permissible limits (i.e. 1-20 ppm). At such low concentrations no chemical means of metal ion are removal are effective.
c) *Azolla* being free floating , its biomass is very easy to harvest.

Azolla processes for heavy metal removal occur in two forms-

1. Active
2. Passive

In active process, metal removal occurs as *Azolla* plants grow in containers such as tanks or ponds containing wastewater, while in passive (biosorption) process the effluents are passed through dry *Azolla* biomass packed into filters. Both types of processes have been proved to be efficient in treating fresh, brackish and polluted water. The possibility of using *Azolla* by active processes for purifying wastewaters has been explored earlier also where *Azolla* species in combination with other aquatic plants have been used. *Azolla* being free floating aquatic plant is best suited for growth in wastewaters than rooted aquatic plants.

In the recent past, exciting developments have occurred in the use of *Azolla* biomass for removal of heavy metals by passive processes using Azolla biofilters or biomatrix. The *Azolla* biomatrix can be used, not only for removal of toxic heavy metals, but also for concentration of precious metals. The distinct advantages of these processes using biomatrix are:

1. High binding capacity of *Azolla* biofilters for metals where binding to the extent of Pb-10%, Cd-3.7%, U-3.5%, Cr3+-9%, Cr6+-2% and Ni-3% has been achieved.
2. Single step polishing of effluents containing toxic heavy metals to extremely low concentration (like Pb down to 5ppb, Cr, Cd and Ni down to 20ppb).
3. *Azolla* biofilters retain heavy metal binding capacity at pH between 3.0-11.0.
4. The binding capacity is retained upon acid wash.
5. *Azolla* biomass for preparing biofilters can be easily obtained and *Azolla* biomass is produced in outdoor farms and polyhouses. *Azolla* farming is cheap, reproductive and large scale biomass production is being developed further.
6. Also metal laden *Azolla* biomass is easily decomposable and combustible
7. *Azolla* processes have been proved to be superior to active charcoal, zeolite, active earth, etc., and they are comparable to cubolite.
8. *Azolla* forms proper matrix at has very high amount of pectin less of cellulose and thus allow the flow of solution.
9. These processes can also be used for detoxification of the radioactive wastes.

Effect of *Azolla* on physiochemical properties of the soil was studied by Awoden M.A.[34]Soil pH ,organic matter,N,P,K, Ca, Mg and Na increased with reduction in soil bulk density but increased soil porosity. *Azolla* is able to grow in the saline conditions ,if adapted to it in a stepwise manner. Growth behaviour of *Azolla pinnata* at various salinity levels and induction of high salt tolerance studies showed adaptation involved the development of a capability in the plants to regulate ion concentration [35].

Azolla sp. can be exploited for treatment of tannery and other Cr contaminated wastewaters [36]. It was found that *A. pinnata* and *Lamna minor* removed the heavy metals iron and copper from polluted water [37].The pollutants at low concentration could be treated by passing it through ponds and can be reused for agriculture purpose. Results found by Cohen-Shoel [38] shows biofiltration of toxic elements by *Azolla* biomass. *Azolla* exhibits a remarkable ability to concentrate metals cu, Cd, Cr, Ni, Pb and nutrients directly from pollutants [39] or sewage water [40]. It was found [41] that intact and treated biomass can remove heavy metals from water and wastewater.

In a review on Azolla Wagner [32] calls Azolla "a green gold mine ". The mankind is threatened by drastic global environmental changes triggered by his own activities, we need to investigate and develop alternative strategies for conducting our affairs. The application of *Azolla* as biofertilizer , as a green manure, as mosquito repellant, in weed control as feed for livestocks , in production of biogas[42] and bioenergy, play a significant role in maintaining or improving the state of global environment. There is a definite need to exploit the potential of the aquatic pteridophyte in a more efficient manner in the future, through biotechnological interventions. Therefore a combination of approaches involving basic and applied research should be taken towards making *Azolla* more resistant to environmental fluctuation and also less labour-intensive, so that its actual utilization is diversified and enhanced in agriculture, industry and environmental management.

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