ICAR ADHOC SCHEME

BIOREMEDIATION OF INORGANIC CONTAMINANTS OF RICE BASED WETLAND ECOSYSTEMS OF KUTTANAD, KERALA

157

FINAL REPORT

Principal Investigator

Dr. K.C. Manorama Thampatti

RALA AGRICULTURAL UNIVERSITY
RP (SOUTHERN REGION)
COLLEGE OF AGRICULTURE, VELLAYANI
THIRUVANANTHAPURAM - 695 522, KERALA

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Dr. K.C. Manorama Thampatti
Professor
Department of Soil Science & Agricultural Chemistry
College of Agriculture, Vellayani

KERALA AGRICULTURAL UNIVERSITY
NARP (SOUTHERN REGION)
VELLAYANI - 695 522
THIRUVANANTHAPURAM, KERALA

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- Staff, Department of Soil Science & Agricultural Chemistry, College of Agriculture, Vellayani
- Staff, Department of Plant Pathology, College of Agriculture, Vellayani
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K.C. Manorama Thampatti

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FINAL REPORT FOR ICAR - ADHOC SCHEME
(15.04.2005 to 14.04.2008)

DETAILS OF THE PROJECT

1. **Project Title**	:	**Bioremediation of Inorganic Contaminants of Rice Based Wetland Ecosystems of Kuttanad, Kerala**
2. **Sanction Order No.**	:	F.No/8-35/2003 – AFC dated 11.01.2003 of ADG, (AGRO), ICAR
3. **Report for the period**	:	15.04.2005 to 14.04.2008
4. **Date of Start**	:	15.04.2005
5. **Date of termination**	:	14.04.2008
6. **Institute's Name**	:	College of Agriculture Kerala Agricultural University
Place	:	Vellayani, Thiruvananthapuram
District	:	Thiruvananthapuram
State	:	Kerala
Dept./Division	:	Department of Soil Science & Agricultural Chemistry
Actual location	:	College of Agriculture, Vellayani
7. **Principal Investigator**		
Name	:	Dr. K.C. Manorama Thampatti
Designation	:	Professor
Division/Section	:	Department of Soil Science and Agricultural Chemistry
Experience	:	21 Years
Address	:	Department of Soil Science and Agricultural Chemistry, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala - 695 522

8.a. Co-Investgators

i)	**Name**	:	Dr. V.K. Girija
	Designation	:	Professor
	Division	:	Department of Plant Pathology
	Address	:	College of Agriculture, Vellayani Thiruvananthapuram, Kerala 695 522
ii)	**Name**	:	Dr. Sumam Susan Varghese
	Designation	:	Professor
	Division	:	Department of Soil Science and Agricultural Chemistry
	Address	:	College of Agriculture, Vellayani Thiruvananthapuram, Kerala 695 522
iii)	**Name**	:	Dr. Ushakumari, K.
	Designation	:	Professor
	Division	:	Department of Soil Science and Agricultural Chemistry
	Address	:	College of Agriculture, Vellayani Thiruvananthapuram, Kerala 695 522
iv)	**Name**	:	Dr. Usha, P.B.
	Designation	:	Professor
	Division	:	Department of Soil Science and Agricultural Chemistry
	Address	:	College of Agriculture, Vellayani Thiruvananthapuram, Kerala 695 522

8.b. Senior Research Fellow

1.	Mrs. Gouri Priya, Soil Science & Agrl. Chemistry	15.4.05 to 20.05.05
2.	Dr. Aparna, B., Soil Science & Agrl. Chemistry	21.05.05 to 03.10.05
3.	Mrs. Sheeba, P.S., Soil Science & Agrl. Chemistry	16.11.05 to 20.11.06
4.	Dr. Beena, V.I., Soil Science & Agrl. Chemistry	24.01.07 to 14.04.08

8.c. Skilled Assistant

1. Yamuna, R.L., Microbiology 13.09.06 to 31.01.08

9. Objectives (in brief)

- Identification and quantification of inorganic contaminants with special reference to heavy metals in soil, water and sediment of wetland rice ecosystem of Kuttanad
- Speciation of highly toxic contaminants and their pattern of accumulation in rice and other aquatic plants
- Develop bioremediation package for inactivation or de-toxification of contaminants in rice paddies
- Economic analysis of bioremediation

10. Relevance of the project

Kuttanad, *the rice bowl of Kerala* is a deltaic formation of four major river systems, Pampa, Achancoil, Manimala and Meenachil, confluencing into the Vembanad lake. The geographical area (1100 sq. km) extends from 9° $17'$ to 9° $40'$ N latitude and 76° $19'$ to 76° $33'$ E longitude, comprising vast stretches of backwaters, bordering mangrove formations and rice fields, the latter mostly reclaimed from the shallow stretches of the lake. The economy of the region is mainly dependent on rice though supported by coconut and fisheries. The region has been divided in to three distinct tracts viz., *kari, kayal* and *karappadam* based on soil characteristics, elevation, nearness to sea, extent of salinity etc. The rice cultivation in Kuttanad dates back more than a century. In the beginning of the 20^{th} century rice was cultivated here once in two or three years, which later achieved the status of double cropping during 1960's with the onset of green revolution. Rice cultivation in Kuttanad was always risky because of the annual flood submergence during monsoons and saline water intrusion during summer. To protect Kuttanad from floods, a spillway was constructed at Thottapally, which will drain off the excess floodwater during monsoons. A regulator was constructed across the Vembanad lake at Thanneermukkom to prevent saline water entry from the Arabian

Sea to the lake and from there to Kuttanad. The regulator will be kept closed during December to April, to prevent saline water entry to the rice fields. Though this was beneficial to rice cultivators, it had adversely affected the fishing community and had initiated several ecological disorders in the system. The regulator is considered as the major culprit for the ecological breakdown of Kuttanad (KSSP, 1992).

Being located at 0.5 to 2.2 m below mean sea level, the tract has been forced to serve as sink for many kinds of wastes ranging from industrial to agricultural sources generated within an area of 2000 sq. km. The heavy intensification pressure of cultivation enhances the release of large quantities of fertilizer, pesticide and herbicide residues to the system. The closure of the regulator aggravated the extent of pollution. The uptake of nutrients and pollutants are more complicated in a flooded system since various factors are operating simultaneously to shift the chemical equilibrium of each metal between solution phase and solid phase, which may affect their bioavailability and result nutrient imbalance within the system. Technogenic contamination of the system with toxic elements are reflected in the functioning of plants and biota which will have implication even on human health. Hence investigations to study the extent of toxic factors operating under such situations and how they can be corrected are highly essential in the tract.

The total rice production and productivity of Kuttanad showed a declining trend for the past few decades despite the use of high yielding varieties and modern farming techniques. The main reason attributed for the yield decline is the loss of soil health due to indiscriminative use of agrochemicals coupled with intensification of rice monoculture. In such situations self cleaning of soil does not take place or rather takes place very slowly warranting the need for new technologies for decontaminating the soils, restoring soil health and sustaining rice productivity. In the present scenario, the evolvement of methods for detoxification of pollutants occupies a prime place in our research needs. Bioremediation, a process of detoxification of contaminants through biological agents seems to be the best method for the restoration of soil health since it is eco-friendly and potentially cost effective. In view of serious degradation and pollution of resources, the present study attempts on quantification, speciation and bioremediation of major inorganic contaminants of Kuttanad.

11. (a) Technical programme approved for the scheme

Three watersheds, each representing the typical Kuttanad regions viz., *Kari, Kayal* and *Karappadom* will be selected for the study by an initial survey. The investigation comprises four parts viz.,

I. Identification and quantification of inorganic contaminants in the selected watersheds of Kuttanad rice ecosystem

II. Speciation of toxic contaminants in soil and sediment and their uptake by rice and other major aquatic plants

III. Identification of hyper accumulating plants and microbes with bioremediation capacity and quantification

IV. Pot culture experiments for detoxification of above contaminants through bioremediation and their field validation.

I. Identification and quantification of inorganic contaminants

Soil samples and field water samples will be collected during pre-monsoon, post-monsoon and summer seasons from the rice fields of the selected watersheds based on the field survey conducted for the estimation of inorganic contaminants. Simultaneously water and sediment from the canals surrounding the rice fields will also be drawn for the determination of inorganic contaminants. The samples will be analyzed for pH, EC, nitrate–N, ammoniacal-N, phosphorus, sulphur and heavy metals. Water soluble, exchangeable, organic form and residual form of contaminants in soil and sediment will be estimated. Based on their quantity present in the ecosystem the extent of contamination will be evaluated. The rating of toxicity will be done by comparing the existing levels with the permissible levels fixed by WHO or such other organizations.

Activity chart

Act. No.	Activity	I year			
		A	B	C	D
1	Survey and collection of soil and water samples from rice fields			*	*
2	Collection of sediment and water from canals			*	*
3	Estimation of inorganic contaminants - NH_4-N, NO_3-N, P, K, Ca, Mg, S, heavy metals (Fe, Mn, Zn, Cu and Cd) and Al of samples			*	*
4	Quantification of contaminants and rating of toxicity			*	*

A = April-June, B= July-September, C = Oct-Dec, D = Jan-March

II. Speciation of toxic contaminants and their pattern of accumulation in rice and other aquatic plants

Speciation of toxic contaminants identified from Part I will be attempted. For rice and aquatic plants, the pattern of accumulation of contaminants within the plant parts will be estimated.

Activity chart

Act. No	Activity	I year				II year			
		A	B	C	D	A	B	C	D
1	Collection of soil and sediment,				*				
2	Identification of plants having hyper accumulation capacity			*	*				
3	Collection of rice and aquatic flora					*	*	*	
4	Speciation of inorganic contaminants						*	*	
5	Uptake of contaminants by rice and aquatic flora							*	*
6	Collection and identification of microbes having bioremediation capacity					*	*	*	

A = April-June, B = July-September, C = Oct-Dec, D = Jan-March,

III. Experiments for identification of hyper accumulating plants and microbes with bioremediation capacity and quantification

This part of the experiment is envisaged to carry out in four stages.

1. Identification of microbes having bioremediation capacity

Microflora from native soil will be isolated by serial dilution plating. The microbes will be tentatively identified based on their cultural and morphological characters. The microbes belonging to already proven genera will also be selected for further study.

2. In vitro screening of microbes for rapid growth and bioremediation

Selected microbes will be artificially inoculated into sterilized broth containing graded doses of heavy metals. The ability of rapid growth of the microbes and the capacity for biosorption of heavy metals will be studied.

3. *In-vivo* screening of efficient microbes and VAM for biosorption of heavy metals in field condition

A pot culture experiment will be conducted in CRD with three replications using both the live and immobilsed forms of the microbes and appropriate control with graded doses of heavy metals. VAM will also be included in the study. Based on the colony and morphological characteristics and heavy metal absorption, bioremediation efficiency will be quantified.

4. Identification of hyper accumulators and evaluation of synergistic effect of hyperaccumulators and microbes

Experiments will be carried out to identify the hyper accumulators and the synergistic effect of microbes on heavy metal removal by hyperaccumulators with graded doses of heavy metals under this part. Vigorously growing plants in the polluted sites will be collected and chemically analyzed for the presence of heavy metals. The roots of these plants will be stained and observed for mycorhizal colonization, if any.

The native plants found to have phytoremediation capacity as well as known hyperaccumulators will be raised in pots by giving graded doses of contaminants. Based on the uptake of heavy metals, their bioaccumulation ability will be quantified to study the synergistic effect, treatments comprising hyperaccumulators with and without microbes and VAM will be included in the study. Heavy metals removed by different treatments will be estimated.

Activity chart

Act. No	Activity	II year			
		A	B	C	D
1	Soil collection and identification of microbes having bioremediation capacity	*	*		
2	In vitro screening of microbes for rapid growth and bioremediation	*	*		
3	*In-vivo* screening of efficient microbes and VAM for biosorption of heavy metals in field condition	*	*	*	
4	Collection of vigorously growing plants, rhyzosphere soil and setting up of pot culture experiments	*	*		
5	Analysis for the estimation of heavy metals and quantification of bioaccumulation capacity		*	*	*

A = April-June, B = July-September, C = Oct-Dec, D = Jan-March,

IV. Pot culture experiments for bioremediation/ detoxification of contaminants and field validation

Different materials including organic manures, composts, soil amendments, biological wastes, microbial inoculants etc. will be tested for their efficiency in inactivating or detoxifying the toxic pollutants by conducting pot culture experiments with soils collected from the above mentioned sites. Graded doses of toxic metals will be added to the rice soil along with the treatments and studies will be carried out to find out how effectively they can be inactivated. The treatments include the following.

1. Absolute control
2. Addition of organic amendments (FYM and coir-pith compost / vermicompost)
3. Addition of chemical amendment (lime)

4. Addition of biological waste (dairy waste)
5. Inoculation of microbes
6. Inoculation of VAM
7. Combined application of FYM and lime
8. Combined application of lime and dairy waste
9. Combined application of lime and microbial inoculants
10. Combined application of FYM, lime and microbial inoculants
11. Combined application of lime and dairy waste and microbial inoculants
12. Combined application of lime and VAM
13. Combined application of FYM, lime and VAM
14. Combined application of lime and dairy waste and VAM

The level of treatments will be fixed after studying the ability of above materials for sorption and desorption of toxic metals / pollutants by applying graded doses of toxic metals and treatment materials in an incubation study under laboratory conditions. The treatments will be replicated thrice. An experiment will be conducted in the main field for the field evaluation of the successful technology for bioremediation in one of the experimental site.

Activity chart

Act. No	Activity	II year				III year			
		A	B	C	D	A	B	C	D
1	Laboratory incubation study			*	*				
2	Setting up of different pot culture experiment & application of treatments			*	*	*			
3	Quantification and rating of bioremediation capacity					*	*		
4	Field validation of successful technology						*	*	*
5	Economic analysis, Statistical analysis and preparation of report							*	*

A = April-June, B= July-September, C = Oct-Dec, D = Jan-March

12. Technical persons employed

Sl. No.	Name of Post	Pay Scale	No. of Post	Total
1	Scientist	-		
2	Junior Research Fellow	-		
3	Senior Research Fellow	Rs. 8000/- + HRA	1	1
4	Research Associate	-		
5	Others – Skilled Assistant	Rs. 150/day	1	1

13. Total Outlay of the Scheme : Rs.15,02,500/-

14. Total amount sanctioned

Period	Pay and Allowance for SRF	Recurring contingencies	Non Recurring contingencies
I Year (15.04.2005 to 14.04.06)	96,000 + 4800 = 1,00,800	1,20,000	7,60,000
II Year (15.04.2006 to 14.04.07)	96,000 + 4800 = 1,00,800	1,20,000	Nil
III year (15.04.2007 to 14.04.08)	1,08,000 + 5400 = 1,13,400	1,20,000	Nil
Total	3,15,000	3,60,000	7,60,000

15. Total amount spent during the period

Period	Pay and Allowance for SRF	Recurring contingencies	Non Recurring contingencies
15.04.2005 to 14.04.06	61,917	92,987	7,62,007
15.04.2006 to 14.04.07	71,615	1,03,395	Nil
15.04.2007 to 14.04.08	91,135	1,13,640	Nil
Total	2,24,667	3,10,022	7,62,007

16. Detailed Progress Report

A. Description of the study area

Kerala state, lying between 8° 80′ to 12° 48′ N latitude and 74° 25′ to 77° 22′ E longitude, is a narrow strip of land in the southern west corner of Indian Penninsula. Kerala is divided in to five agro-climatic zones based on physiography, climate, soil characteristics, sea water intrusion, land use pattern, vegetation etc. The zones are "Southern zone, Central zone, Northern zone, High altitude zone and Special zone for problem soils". The study area "Kuttanad" has been classified under the special zone for problem soils. Kuttanad is a low-lying deltaic formation with backwaters, canals, stream networks and water ways encircling the rice fields, with more than 500 sq. km. of the tract lies 0.6 to 2.2 m below mean sea level. The garden lands of average elevation of 1 m above mean sea level covers an area of 304 sq. km.

It measures approximately 25 km east-west and 60 km north-south on the west coast of Kerala. The area encompasses 79 revenue villages lying in Karthikapally, Mavelikkara, Chengannur, Kuttanad, Thiruvalla, Chaganachery, Kottayam, Ambalapuzha, Cherthala and Vaikom thaluks, spread over the three districts of Alapuzha, Kottayam and Pathanamthitta.

In the geological past the region was a part of shallow coastal area of Arabian Sea. As a result of a geological uplift, a shallow bay was formed into which several rivers discharged. The silt carried out by these rivers got deposited at river mouths giving rise to present coast and converting the shallow bay in to a lake-lagoon backwater system extending from Alapuzha to Kochi and connecting the Arabian Sea by Kochi estuary. According to another theory the entire area was a dense forest, the legendary *Khandava vanam*, which caught fire and engulfed by the sea during the succeeding geological ages. Years later, sea receded exposing the land which forms a part of midland and coastal region of Kerala.

Geology and geographic setting

It is an alluvial belt formed of tertiary and quaternary sediments. The region appears as a saucer shaped basin flanked by sand dunes in the west and low lateritic

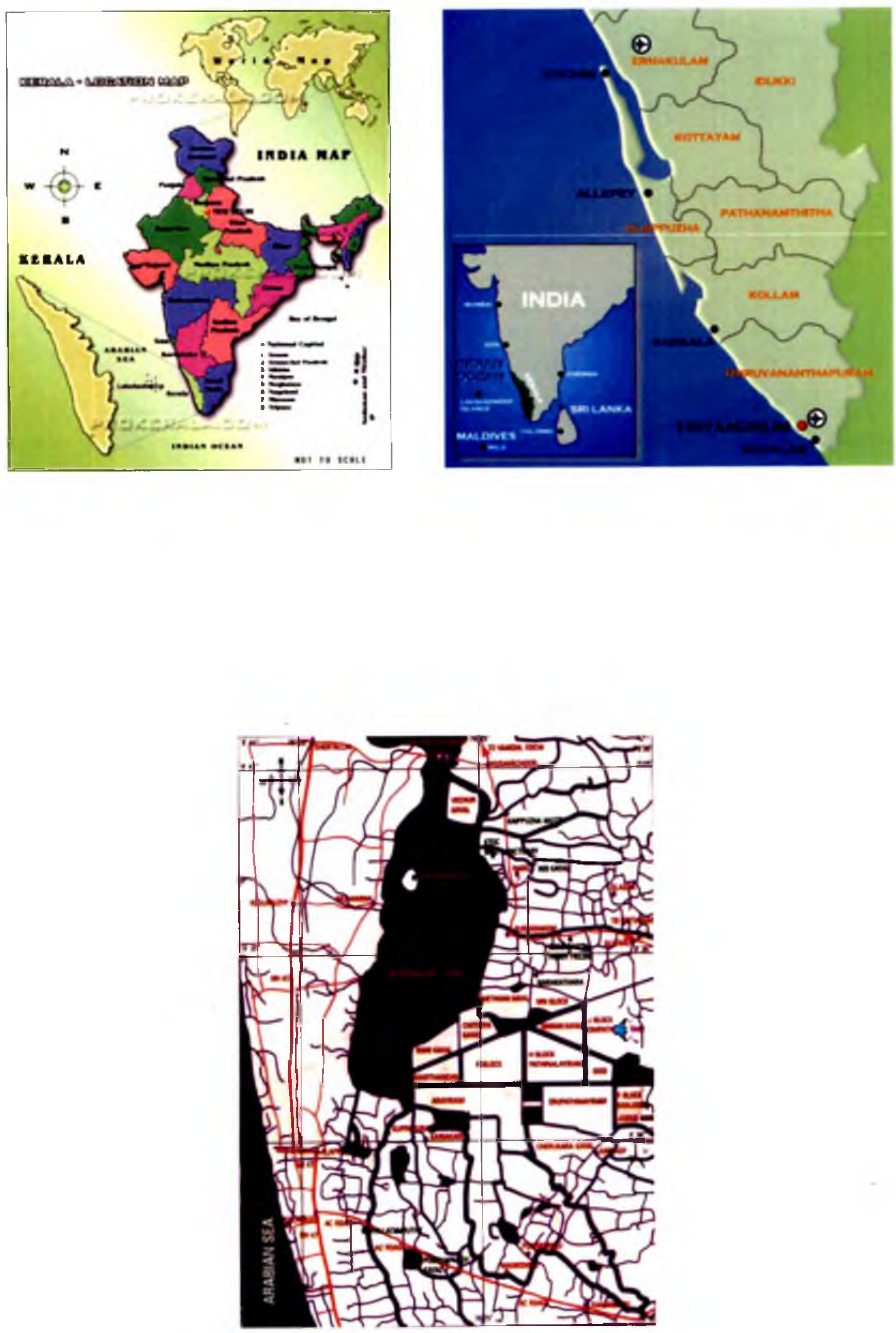


Plate 1. Location map of Kuttanad

hills in the east. The deltaic alluvium is alternating layers of sand and clay of varying sizes and variable percentage of organic matter.

Climate

The tract enjoys humid tropical climate with two spells of monsoons viz., South West monsoon during June-September and North East monsoon during October-November. Major part of the rain is received during the months of June, July and August. The cultivation season spreads from May to August (additional crop (kharif) rice) and from September to January (*Punja* (rabi) rice). Additional crop is practiced only in areas not affected by severe floods and *punja* is widely cultivated.

Normal climatic details of Kuttanad RRS, Moncompu, Alapuzha district

Months	Total Precipitation (mm)	Temperature (mean monthly)		Evaporation (mm)
		Max. °C	Min. °C	
January	80.2	32.5	22.4	61.8
February	63.6	32.8	23.0	70.0
March	13.0	33.7	24.8	82.2
April	177.6	34.5	25.4	79.1
May	293.0	32.4	26.0	76.4
June	612.6	31.6	23.2	33.5
July	452.1	30.9	24.4	55.4
August	248.0	29.5	24.4	73.7
September	516.4	30.6	24.8	91.3
October	340.8	30.7	24.7	78.8
November	122.0	31.8	23.6	78.1
December	14.2	33.0	22.4	104.2
Total	2993.5	--	--	--

Land use

Rice is the major crop of the area and is cultivated in large contiguous fields known as padasekharams or polders. These rice fields are surrounded by broad ring bunds (actually canals with broad bunds) that facilitated cultivation of miscellaneous tree crops, banana and vegetables on it and at the same time allow navigation through it, which are also the natural sites for capture fisheries. The entire area was covered with high yielding rice varieties and the farmers have profusely used the fertilsers and plant protection chemicals. In order to achieve food security, double cropping was introduced here with the onset of green revolution. As a result apart from normal punja season, an additional crop was also taken during April-May to August-September. During additional crop season, the area is affected by floods and during punja, severe salinity is the problem. Hydrological structures like Thottapally spill way to drain away the excess floodwater to Arabian Sea and Thanneermukkom regulator to prevent saltwater intrusion to Kuttanad were commissioned during 1955 and 1975 respectively. These developmental projects were not implemented as designed and the omissions that were made had adversely affected the tract's ecology, which was very well evidenced in yester years.

Soil characteristics

Soils of Kuttanad are generally acid saline in nature. In *kari* lands, acid sulphate soils are present. As per soil taxonomy the wetlands of Kuttanad has been classified into 15 soil series belonging to soil orders of Entisols and Inceptisols.

Karappadam soil occur along the inland water ways and rivers, and spread over a larger part in upper Kuttanad covering an area of 40,000 ha. They are river borne alluvial soils lying 1.0-2.0 m below mean sea level. Soils are very deep, poorly drained, dark grey in colour with clay loam surface texture followed by silty clay subsoil. Subsoils also show presence of abundant prominent red and yellow mottles, gley horizons, streaks and concretions. The presence of sand pockets in the subsurface horizon is another feature (KAU, 1994). The soils are charactrised by high acidity, salt content and fair amount of organic matter.


Kuttanad - General view


Vembanad lake


Water way


Thanneermukkom regulator


Fe toxicity


Kuttanad fish


Cyperus pangorei


Eichhornia crassipes

Plate 2. Few characteristic features of Kuttanad

Kayal soils are found in reclaimed beds of Vembanad and kayamkulam lakes in Kottayam and Alapuzha districts covering an area of 8,000 ha. These soils are severely affected by salinity.

Kari lands are confined to a few pockets in non-contiguous manner along the coastal plains adjoining the backwaters in Alapuzha and Kottayam covering an area of 9000 ha. They exhibit characteristics of submerged forest area. The soils are deep, black in colour, heavy in texture, poorly aerated and ill drained with high organic matter content. Top soil is admixed with well decomposed organic matter. But very often this layer is under laid by partially decomposed fibrous plant residues and contains less than 50 per cent mineral matter. The kari soils are affected by severe acidity and periodic saline water inundation with consequent accumulation of soluble salts. In these soils free sulphuric acid is formed by the oxidation of sulphur compounds of organic residues or that accumulated from sea water by repeated inundation. The soils are of low fertility status and contained toxic quantities of Fe, Al and unidentified toxic organic compounds. The presence of large quantities of organic matter that resisted decomposition for a long time and high acidity, inspite of large accumulation of lime shells is generally observed.

Toxicity characteristics

Iron toxicity : The problem of iron toxicity is often encountered in Kuttanad soils and this is more serious in *kari* and *karappadam* soils rather than in *kayal* soils. The exchangeable Fe content in these soils increases during the first few weeks of flooding and then decreases. But the *kari* soils maintained the initial increase through out the crop period. The peak values of exchangeable Fe in *karappadam* and *kayal* soils are in the range of 30-270 mg kg^{-1} and in *kari* soils it is about 1508- 3411 mg kg^{-1}. The free Fe content of *karappadam* and *kari* soils varies from 7031 to 17187 and 7031 to 31250 mg kg^{-1} respectively (Thampatti and Jose, 2006, Thampatti *et al.*, 2006a).

Aluminum toxicity : Al toxicity in rice is lkely to occur at pH 4.5-5.0 for seedlings and 3.4-4.0 for older plants. The pH of Kuttanad soils especially that of *kari* soils falls under this range. The exchangeable Al content is very high in Kuttanad soils. The Al saturation of total and effectibve CEC is also very high in these soils. The exchangeable Al content of *karappadam, kayal and kari* soils ranges from 0.22 to 22.20, 2.40 to 22.30 and 4.42 to 41.20 cmol kg^{-1}, respectively. The water soluble Al content of these soils are in the range of 2-8, 1-5 and 5-16 mg kg^{-1} respectively (Thampatti and Jose 2006).

Apart from the above, the region is experiencing S toxicity in *kari* lands and toxicity due to the production of organic acids and that due to the residues of fertilizers, pesticides/herbicides and other effluents discharged to the system.

B. Work Report

The project was planned in four stages as furnished below.

I. Identification and quantification of inorganic contaminants in the selected watersheds of Kuttanad rice ecosystem

II. Speciation of toxic contaminants in soil and sediments and their uptake by rice and natural flora including aquatic plants

III. Identification of hyper accumulating plants and microbes with bioremediation capacity and quantification

IV. Pot culture experiments for detoxification of above contaminants through bioremediation and their field validation

The experiments as mentioned in the technical programme were carried out and the results are presented below.

I. Initiation of survey and collection of soil, sediment, water and plant samples and their chemical analysis

An initial survey was carried out in Kuttanad and based on that the following watersheds/villages viz., Ambalapuzha and Vechoor (*Kari* soils), Moncompu (*Karappadam* soils) and *kayal* lands of Kuttanad thaluk were selected for the study. Vechoor and Ambalapuzha villages representing potential acid sulphate soils (*kari*), Moncompu representing *karappadam* soils and *kayal* lands of Kuttanad were surveyed and farmers belonging to ten *Padasekhara Samithi's* (cluster of rice fields with an area of 125 to 200 ha) from each area were interviewed and samples were collected during Post-monsoon (October-November), Summer (February-March) and Pre-monsoon (April to June) seasons of 2005-06 and 2006-07.

The major constraint with regard to rice cultivation in the Vechoor area was the entry of saline water to the rice fields during *punja* season (Oct-Feb), though the area was protected from saline water entry by closing the Thanneermukkom regulator, which has been constructed for the prevention of saline water entry to Kuttanad. Every year the Thanneermukkom regulator will be closed before the highest tide that occurs during the mid week of December. The faulty practices behind the operation of the regulator (closure/opening) like improper closure of shutters, depositing big boulders beneath the shutters, partial lifting of shutters etc. were the major reasons for the entry of saline water to this area. Hence these farmers are taking only one crop of rice during May-June to September – October (Additional crop season). During this period they have to face the risk of flooding due to heavy South – West monsoon showers and the water brought down by the Meenachil river during its course towards Vemabanad lake. Many of the *Padasekhara Samithi's* have practiced aquaculture during the *punja* (rabi) season. Since the entire Kuttanad received very high monsoon showers during the year 2005-06, it was continuously flooded for long time, which had slightly delayed the initial sampling.

In Ambalapuzha area the major constraints are the extreme acidity and salt encrustation during the summer season. Most of the farmers prefer to take the additional crop of rice during rainy season and fish culture during summer season. Scarcity of seeds of suitable varieties of rice is also a problem in this area.

In *karappadam* and *kayal* lands the major havoc is due to the crop damage as the result of flood submergence during monsoon period. The decrease in soil fertility and acidity related problems were very common in this region. Though the Kuttanad region is surrounded by water the entire area faces severe water scarcity during summer. In both these areas the pollution due to intensive tourist activities and the diesel discharge from the motor boats are the newly emerging problems. Kainakari village of Kuttanad thulak occupying lowest position has been vulnerably affected by the fuel and oil discharge from the motor boats.

Soil, sediment, field water and canal water samples from the selected watersheds were collected during October-November (Post-monsoon), February-March (Summer) and April-June (Pre-monsoon). These samples were analyzed for major chemical characteristics and the results are presented below in tables 1 to 8.

The chemical characteristics of the study area showed variation during the different seasons. Though the trend in variation of different chemical characteristics was almost similar, their quantity varies with the site. The toxicity problems were much higher in *kari* soils compared to the *karappadam* and *kayal* soils. The studies on Kuttanad soils dates back to 1948 (Brito-Muthunayagam, 1948). Much change has occurred in chemical characteristics from that time onwards. The soil management in this tract is much cumbersome due to several toxic parameters like extreme acidity, salinity, toxic quantities of Fe, Al, S, organic acids, fertilizer residues etc. (Gopalaswamy, 1961; DEEM, 1987; KAU, 1994; Thampatti and Jose, 2005, Thampatti *et al.*, 2006b). In general *kari* soils are facing much problems for rice cultivation, often resulting patchy growth of rice plants (KAU, 2004).

The chemical analysis of the soils of the study area revealed that the *kari* soils (Tables 1 and 3) are extremely acidic and characterized by large quantities of available Fe and extractable Al. The acidity was highest during summer season and electrical conductivity was highest during pre-monsoon season. Lowering of water table during summer has initiated soil drying which caused an increase in acidity. During the pre-monsoon season , the sea water from the Arabian sea enters to Vembanad lake and from there to Kuttanad through the opened shutters of Thanneermukkom regulator. The presence of sea water had definitely enhanced the salinity level. Nair and Pillai (1990) and Thampatti and Jose (2002) also reported similar variations in the salinity levels of Kuttanad ecosystem in accordance with saline water entry.

The amount of different elements present in the soil showed variation with seasons. The NH_4-N was highest during post-monsoon season and lowest during pre-monsoon season. The oxidation status of soil, addition of nitrogenous fertilizers and saline water entry from Vembanad lake to Kuttanad had influenced the above parameters. One of the major reasons attributed for this reduction is the existence of a lean season / flood fallowing during the pre-monsoon period since, the dominant contributor towards the NH_4-N content being the chemical fertilizer Urea. Since the Thanneermukkom regulator is opened during the pre-monsoon period, the dilution effect due to the entry of saline water from Arabian Sea to Kuttanad had also contributed to the lower values for NH_4-N. NO_3-N content was highest during pre-monsoon season and lowest during post-monsoon season. The entry of nitrate salts through the saline water from the Sea is responsible for the increase.

The soils were generally deficient in P and lowest values were recorded during post-monsoon season. Elements like K, Ca, Mg and S were higher during the pre-monsoon period, definitely due to the influence of the brackish water entry to Kuttand. Fe and Al contents were highest during summer season. The enhanced acidity within the soil have maintained Fe and Al in soluble form. The other micronutrients viz., Mn, Zn and Cu showed very minute variation within these seasons.

Kari, karappadam and *kaya*l soils exhibited almost similar trend in variation of elemental concentration during different seasons, even though, the individual values showed wide variation among the soil types (Tables 1, 3, 5 and 7). Though the *karappadam* and *kayal* soils are also rich in above elements, their quantity remain lesser compared to the values of *kari* soils. The NH_4-N content was highest for Ambalapuzha *kari* soils while Vechoor *kari* showed highest NO_3-N content. The kayal soils recorded highest soil pH mainly because of their nearness to the Vembanad lake through which Arabian Sea is connected to Kuttanad tract. In case of electrical conductivity, the *kari* soil recorded the highest value and the same contained excess/toxic quantities of Fe, Al and S. While in *kayal* lands, even though the available S content was high the toxicity symptoms are rarely observed. The *karappadm* area (Moncompu) recorded very low values for available S. In general *kari* soils have more problem with the above elements. P deficiency is a common problem in Kuttanad and the excess amount of Fe and Al aggravate its deficiency further.

The sediment recorded still higher quantities for different elements compared to soil but it also followed the same trend as that of soil for elemental status in different seasons in *kari, karappadam* and *kayal* soils. The higher clay content in sediment might have helped in better retention of elements compared to soil.

Table 1. Chemical characteristics of soil and sediment of *kari* soils (Vechoor) during different seasons (mean values in mg kg^{-1})

Period	NH_4-N	NO_3-N	Available nutrients									Ex. Al	pH	EC dS cm^{-1}
			P	K	Ca	Mg	S	Fe	Mn	Zn	Cu			
SOIL														
Pre monsoon	92	17	2.5	232	1461	485	2834	325	5.2	3.1	2.1	46	5.1	2.4
Post monsoon	134	13	1.7	149	1000	491	2385	304	3.5	2.3	2.3	66	3.6	0.84
Summer	103	16	1.8	156	854	384	2662	342	5.0	2.5	2.0	88	3.8	1.08
SEDIMENT														
Pre monsoon	126	9	4.2	249	1675	506	2915	497	9.4	5.01	4.8	67.1	5.6	3.7
Post monsoon	167	5.1	2.5	172	1119	514	2149	437	4.0	3.4	3.0	66.9	4.0	0.73
Summer	156	7.2	3.8	191	1095	489	2158	584	8.6	4.4	4.0	102	3.8	0.92

Table 2. Chemical characteristics of field water and canal water of *kari* lands (Vechoor) during post-monsoon season (mg L^{-1})

Period	NH_4-N	NO_3-N	Available nutrients									Ex. Al	pH	EC dS cm^{-1}
			P	K	Ca	Mg	S	Fe	Mn	Zn	Cu			
FIELD WATER														
Pre monsoon	4.30	7.1	0.14	3.7	23.3	12.4	12.5	0.94	0.24	0.05	0.06	0.11	5.8	0.95
Post monsoon	5.9	5.1	0.04	2.4	15.4	9.06	8.3	0.82	0.17	-	-	0.098	6.5	0.34
Summer	6.0	6.3	0.07	2.9	20.2	10.3	10.2	1.20	0.23	0.05	0.07	0.16	4.9	0.80
CANAL WATER														
Pre monsoon	3.0	5.7	0.19	4.1	26.2	13.8	13.2	1.2	0.20	0.05	0.05	0.13	5.4	0.98
Post monsoon	3.2	4.2	0.06	2.6	17.9	8.9	8.2	0.76	0.13	0.03	0.02	0.11	6.8	0.45
Summer	3.6	4.71	0.09	3.2	19.2	9.7	8.2	1.40	0.18	0.05	0.05	0.14	4.8	0.85

Table 3. Chemical characteristics of soil and sediment of *kari* soils (Ambalapuzha) during different seasons (Mean values in mg kg^{-1})

Period	NH_4-N	NO_3-N	Available nutrients									Ex. Al	pH	EC dS cm^{-1}
			P	K	Ca	Mg	S	Fe	Mn	Zn	Cu			
SOIL														
Pre monsoon	94	16	5.4	237	1486	501	1898	314	11.1	3.9	3.8	49	5.1	2.3
Post monsoon	138	13	3.2	201	801	515	1336	311	6.9	2.8	2.0	72.3	4.1	0.67
Summer	107	13	5.1	188	776	485	1413	337	10.4	3.8	3.60	85.2	4.0	0.97
SEDIMENT														
Pre monsoon	121	11	5.1	254	1649	548	2906	401	17.0	4.6	4.5	64.2	5.5	3.6
Post monsoon	161	7.6	3.3	203	905	537	2310	421	13	3.2	2.3	85.2	4.2	0.70
Summer	137	9.2	4.8	209	848	512	2495	428	16.2	3.4	3.4	101	3.9	0.90

Table 4. Chemical characteristics of field water and canal water of *kari* lands (Ambalapuzha) during different season (Mean values in mg L^{-1})

Period	NH_4-N	NO_3-N	Available nutrients									Ex. Al	pH	EC dS cm^{-1}
			P	K	Ca	Mg	S	Fe	Mn	Zn	Cu			
FIELD WATER														
Pre monsoon	4.25	5.8	0.13	3.5	22.3	12.9	16.4	0.70	0.14	0.07	0.05	0.10	5.6	0.93
Post monsoon	5.2	4.8	0.04	2.2	14	7.8	11.7	0.66	0.10	0.04	0.02	0.09	5.2	0.76
Summer	6.3	5.7	0.07	2.5	16.7	8.3	15.3	0.9	0.13	0.07	0.05	0.15	4.3	0.80
CANAL WATER														
Pre monsoon	4.5	5.5	0.15	4.0	25.9	13.7	13.5	0.61	0.14	0.05	0.05	0.10	5.4	0.98
Post monsoon	4.6	4.8	0.05	2.5	14.9	7.0	10.9	0.64	0.11	0.01	0.01	0.09	5.9	0.68
Summer	5.3	5.0	0.07	2.6	16.0	9.0	12.1	0.77	0.13	0.04	0.05	0.12	4.6	0.79

Table 5. Chemical characteristics of soil and sediment of *kayal* lands of Kuttanad during different seasons (Mean values in mg kg^{-1})

Period	NH_4-N	NO_3-N	Available nutrients									Ex. Al	pH	EC dS cm^{-1}
			P	K	Ca	Mg	S	Fe	Mn	Zn	Cu			
SOIL														
Pre monsoon	82	14	4.82	185	1254	457	1621	228	12.6	3.0	2.9	25	5.8	1.6
Post monsoon	116	13	13	111	1342	495	1331	225	9.8	1.9	1.1	34.5	5.6	0.78
Summer	91	13	4.4	132	1176	426	1532	282	12.2	2.8	2.6	41.4	5.1	0.94
SEDIMENT														
Pre monsoon	91	10.6	5.0	198	1311	481	1675	239	12.8	3.1	2.3	31	5.7	1.7
Post monsoon	129	7.4	3.3	140	1135	499	1536	271	9.2	2.2	1.06	26.9	5.6	1.0
Summer	133	9.8	4.6	163	1030	415	1665	355	12.6	3.2	2.2	39.8	5.0	1.5

Table 6. Chemical characteristics of field water and canal water of *kayal* lands of Kuttanad during different seasons (Mean values in mg L^{-1})

Period	NH_4-N	NO_3-N	Available nutrients									Ex. Al	pH	EC dS cm^{-1}
			P	K	Ca	Mg	S	Fe	Mn	Zn	Cu			
FIELD WATER														
Pre monsoon	4.62	8.71	0.07	3.8	25.3	13.9	11.4	0.30	0.13	0.02	0.03	0.02	6.2	1.81
Post monsoon	5.12	5.71	0.03	1.13	15.2	5.34	4.06	0.46	0.09	0.02	0.01	0.02	6.2	0.61
Summer	5.52	7.76	0.06	1.53	15.8	5.44	4.26	0.56	0.12	0.03	0.03	0.04	6.0	1.02
CANAL WATER														
Pre monsoon	4.81	6.5	0.09	4.2	28.9	14.3	12.5	0.35	0.16	0.03	0.03	0.01	6.3	1.90
Post monsoon	4.86	5.08	0.05	1.38	18.9	9.46	6.82	0.66	0.11	0.03	0.02	0.04	6.4	0.64
Summer	5.34	6.10	0.07	1.79	16.4	9.90	7.20	0.85	0.16	0.04	0.03	0.05	6.1	1.21

Table 7. Chemical characteristics of soil and sediment of *karappadam* lands of Kuttanad (Moncompu) during different season (Mean values in mg kg^{-1})

Period	NH_4-N	NO_3-N	Available nutrients									Ex. Al	pH	EC dS cm^{-1}
			P	K	Ca	Mg	S	Fe	Mn	Zn	Cu			
SOIL														
Pre monsoon	103	15.8	2.9	185	815	432	155	87	22.5	2.9	2.7	21.7	5.5	0.94
Post monsoon	126	12	2.14	181	805	445	103	97	20	1.3	1.1	22	4.5	0.32
Summer	113	15.4	2.64	163	739	404	144	107	20.8	2.8	2.6	36.7	4.2	0.54
SEDIMENT														
Pre monsoon	124	16.5	3.0	194	820	470	162	92	20.4	2.9	1.5	25.3	5.5	1.05
Post monsoon	164	14	1.0	163	766	458	103	140	19	1.6	3.0	24	4.7	0.32
Summer	179	16	2.6	158	737	468	154	172	19.8	2.3	1.4	40.7	4.2	0.82

Table 8. Chemical characteristics of field water and canal water of *karappadam* lands of Kuttanad (Moncompu) during different season (Mean values in mg L^{-1})

Period	NH_4-N	NO_3-N	Available nutrients									Ex. Al	pH	EC dS cm^{-1}
			P	K	Ca	Mg	S	Fe	Mn	Zn	Cu			
FIELD WATER														
Pre monsoon	5.84	5.61	0.06	3.85	14.1	7.20	2.55	0.41	0.05	0.02	0.02	0.01	5.4	0.85
Post monsoon	7.7	3.9	0.04	2.3	10.8	5.9	1.5	0.58	0.03	0.02	0.01	0.07	5.1	0.32
Summer	8.72	4.71	0.06	2.50	12.1	6.74	2.35	0.68	0.05	0.02	0.02	0.08	4.8	0.47
CANAL WATER														
Pre monsoon	6.12	5.30	0.06	4.00	14.2	7.41	3.10	0.49	0.05	0.02	0.03	0.03	5.5	0.98
Post monsoon	6.4	3.8	0.02	2.32	8.62	5.02	2.22	0.60	0.05	0.02	0.02	0.05	5.9	0.40
Summer	7.26	4.12	0.06	2.44	9.22	5.41	2.89	0.69	0.05	0.02	0.03	0.07	5.3	0.45

On evaluating the changes in chemical characteristics of water in *kari* area of Vechoor (Table 2), it was observed that the NH_4-N content of field water gradually increased from 4.30 mg L^{-1} during pre monsoon season to 6.0 mg L^{-1} during summer season while NO_3-N showed higher content during pre monsoon season. The canal water recorded comparatively lesser values. This might be due to the mixing of sea water with lake water during the end of summer since the shutters of Thannermukkom regulator were kept opened during the month of May- June. The canal water and field water were well supplied with different elements indicating their vulnerability to eutrophication. The field water as well as canal water contained sizeable quantities of Ca, Mg and S. The nutrient load of both canal water and field water were lowest for kayal lands compared to to *kari* lands and *karappadam* except for NO_3- N (Tables 2, 4, 6 and 8).

Regarding the water quality parameters, the pH, EC, NO_3- N, P, K, Ca, Mg and S showed an increase during the pre-monsoon season compared to summer seasons for both field water as well as canal water, definitely due to the influence of saline water entry to the Kuttanad ecosystem. There was a reduction in NH_4-N, Fe and Al content during the same period. The three zones viz., *kari, kayal* and *karappadam* showed similar trends in water quality parameters. Comparing the water quality with that of ISI/WHO/EEPA standards fixed, the Fe, Mn, Cu and Al content of the study area exceeded the maximum permissible limits. In most of the cases, kari soils showed the highest values indicating that the extent of toxicity is more in these soils. The poor quality of water is an alarming problem in Kuttanad and improving the quality of water has to be addressed immediately and measures have to be undertaken for this with prime importance.

Table 9. Maximum permissible limit of elements in water for domestic purposes as per WHO/ISI/ EEPA standards (mg L^{-1})

Particulars	NO_3 -N	Ca	Mg	SO_4	Fe	Mn	Zn	Cu	Al	pH	EC dS m^{-1}
Domestic purposes	10	75	50	300 - 500	0.3	0.05 -0.10	5.0	0.01	0.1	6.5-7.5	0.75
Drinking water	10			400	0.3	0.05	5.0	1.0	0.05		
Toxic to aquatic organisms					0.3-1	0.05	0.01	0.001	0.005		

II. Speciation of toxic contaminants and their pattern of accumulation in rice and other aquatic plants

Different forms of elements in soils of Kuttanad (Fe, Al, S, Mn, Zn and Cu)

The soils collected from different parts of Kuttanad were analysed for different fractions of Fe, Al, S, Mn, Zn and Cu and the results are presented in Table 10 to 15.

Table 10. Different forms of iron in rice soils of Kuttanad (mean values)

Soil type	Different forms of iron (mg kg^{-1})				% of free Fe to total Fe
	Total	Free iron	DTPA extractable	Water soluble	
Kari soil - Vechoor	76450	67328	343	22.5	88.1
Kari soil - Ambalapuzha	75545	64270	337	20.6	85.1
Kayal soil	41325	30056	282	12.4	72.7
Karappadam soil	45924	35210	109	7.5	76.7

Table 11. Different forms of aluminium in rice soils of Kuttanad (mean values)

Soil type	Different forms of Al (mg kg^{-1})				% of free Al to total Al
	Total	Free Al	Extractable	Water soluble	
Kari soil - Vechoor	33915	21854	67.5	5.2	64.4
Kari soil - Ambalapuzha	34198	22110	72.9	5.4	64.6
Kayal soil	20750	10680	38.9	1.91	51.5
Karappadam soil	24455	14600	45.3	2.73	59.7

Table 12. Different forms of sulphur in rice soils of Kuttanad (mean values)

Soil type	Different forms of Sulphur (mg kg^{-1})			% of organic to total
	Total	Organic	Available	
Kari soil - Vechoor	11451	10649	2829	92.9
Kari soil - Ambalapuzha	10915	10041	2632	92.0
Kayal soil	6490	5516	1749	85.0
Karappadam soil	5140	3290	1238	64.0

Comparing different forms of Fe (Table 10), *kari* soils have a total Fe content of 7.5 to 7.6 per cent and 85 to 88 per cent of this remained in free or active form. This seems to be the main reason for the wide spread iron toxicity in these tracts. Wide spread iron toxicity has been reported from *kari* lands of Kuttanad (KAU, 1994; KAU, 2005; Thampatti *et al.*, 2006a). In *karappadam* and *kayal* lands, the total as well as the free Fe contents were much lower compared to *kari* soils. For aluminium (Table 11) also the trend is same as that of iron, but the contents of different forms of Al are much lower compared to Fe. But these amounts are sufficient to adversely affect the plant growth. In many areas root initiation has been adversely affected and number of healthy roots were also very few indicating the presence of excess Al in soil. For Sulphur (Table 12), the highest values are recorded by the *kari* soils (Vechoor) and the lowest by *karappadam* (Moncompu) soils.

Table 13. Different forms of manganese in rice soils of Kuttanad (mean values)

Soil type	Different forms of mangenese (mg kg^{-1})				% of free Mn to total Mn
	Total	Free Mn	DTPA extractable	Water soluble	
Kari soil - Vechoor	102	78	16	6	76.4
Kari soil - Ambalapuzha	112	81	17	8	72.3
Kayal soil	71	47	11	3	66.2
Karappadam soil	65	45	10	3	69.2

Table 14. Different forms of Zn in rice soils of Kuttanad (mean values in mg kg^{-1})

Soil type	Total	DTPA Extractable	Double acid extractable 0.05 N HCl + .025 N H_2SO_4	Water soluble
Kari soil - Vechoor	86	7	8	0.6
Kari soil - Ambalapuzha	76	5	7	0.4
Kayal soil	62	3	4	0.3
Karappadam soil	25	3	3	0.3

Table 15. Different forms of copper in rice soils of Kuttanad (mean values in mg kg^{-1})

Soil type	Total	DTPA Extractable	Double acid extractable 0.05 N HCl + .025 N H_2SO_4	Water soluble
Kari soil - Vechoor	54	1.2	1.5	0.08
Kari soil - Ambalapuzha	50	1.3	1.4	0.09
Kayal soil	36	0.65	0.8	0.03
Karappadam soil	28	0.58	0.7	0.03

Comparing different forms of Mn (Table 13), *kari* soils have a total Mn content of 102 to 112 mg kg^{-1} and 72 to 76 per cent of this remained in free or active form. But Mn toxicity was not reported from Kuttanad yet. In *karappadam* and *kayal* lands, the total as well as the free Mn contents were much lower compared to *kari* soils. For zinc (Table 14) also the *kari* soils showed higher contents but were much lower compared to Fe. Copper also followed the same trend as that of zinc, but the water soluble forms were present in very negligible amounts (Table 15).

III. Analysis of natural flora for chemical composition and hyper-accumulation ability

III.a. Analysis of natural flora for chemical composition

Kuttanad is blessed with a large variety of plants and is really a hot spot of biodiversity. The green plants are most relevant component in decontamination of negative effects of pollution. These plants could act as sentinels for monitoring trace element pollution and hence they can be successfully employed for phytoremediation (Bashmakov, 2005). The profusely growing natural flora collected from the study area were collected and analysed for their chemical composition. The analytical results of some of the promising species are presented below.

Table 16. Chemical composition of natural flora (shoot) of Kuttanad wetland ecosystem

Sl. No	Species	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	Al
		mg kg^{-1}		%			mg kg^{-1}				
1	*Eclipta alba*	2325	8750	1.68	0.33	0.34	455	50	15	11	315
2	*Hydrocotyle asiatica*	2625	7250	3.76	0.76	0.13	1295	25	80	17	1867
3	*Bacopa monnieria*	3200	18500	3.44	2.20	0.17	2375	20	34	19	1798
4	*Boreria hispida.*	2500	11250	2.16	1.39	0.22	1415	14	135	12	35
5	*Commolina bengalensis*	3287	18500	2.64	0.52	0.170	2185	5	66	20	1982
6	Fern	1512	7250	2.16	0.43	0.11	1225	20	24	21	512
7	*Eichhornia crassipes*	0.41	2.15	3.75	1.68	0.45	22865	120	125	95	1728
8	*Cynadon dactylon*	3666	43000	4.96	1.62	0.22	7480	175	112	41	1420
9	*Cyperus pangorei*	361	31000	4.46	2.30	0.28	5780	205	76	28	969
10	*Eliocaris fimbrinalis*	6102	45000	3.05	0.70	0.31	10051	178	64	22	1529
11	*Chenapodium sp*	2132	8000	3.1	0.93	0.43	429	71	83	105	509
12	*Altrnathera sissiles*	5328	2500	2.84	1.29	0.79	804	33	125	37	1010
13	*Hydrophila auriculata*	1078	23250	4.5	1.53	2.19	1067	96	90	23	1236
14	*Salvinia molesta*	4105	21500	3.75	1.68	0.45	22865	120	125	95	1115
15	*Panicum sp*	3310	31000	3.12	0.09	0.99	3820	45	34	22	845
16	*Hydrilla*	2000	5500	4.00	0.60	0.32	24251	53	211	198	2526

Table 17. Chemical composition of natural flora (root) of Kuttanad wetland ecosystem

Sl. No	Species	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	Al
		%					mg kg^{-1}				
1	*Eclipta alba*	0.18	0.80	1.92	0.72	0.27	1455	95	39	59	125
2	*Hydrocotyle asiatica*	0.21	0.90	1.6	1.2	0.22	1640	20	58	46	759
3	*Bacopa monnieria*	0.19	1.72	4.5	1.2	0.21	3735	25	26	15	1967
4	*Boreria hispida.*	0.13	0.53	3.4	0.76	0.10	405	10	31	8	-
5	*Commolina bengalensis*	0.27	1.95	0.96	0.57	0.26	2750	10	46	15	1669
6	Fern	0.13	0.77	2.08	0.62	0.11	310	10	27	17	459
7	*Eichhornia crassipes*	0.30	1.2	3.75	1.20	0.32	8405	74	105	90	2300
8	*Cynadon dactylon*	0.15	2.60	4.52	2.68	0.55	4200	100	40	22	1039
9	*Cyperus pangorei*	0.48	1.55	3.92	1.39	0.19	6140	125	56	38	1563
10	*Eliocaris fimbrinalis*	0.51	2.25	2.95	1.10	0.27	8427	94	59	43	1425
11	*Chenapodium sp*	0.25	0.95	3.00	0.52	0.19	1031	79	86	85	569
12	*Altrnathera sissiles*	0.13	0.87	3.45	0.98	0.12	2116	88	131	40	933
13	*Hydrophila auriculata*	0.11	0.70	3.15	0.52	0.24	456	65	45	24	740
14	*Salvinia molesta*	0.31	1.36	2.74	0.89	0.44	8245	96	105	21	1250
15	*Panicum sp*	0.33	2.60	0.96	0.52	0.13	7800	49	27	22	600
16	*Hydrilla*	0.20	0.60	2.8	0.61	0.16	10620	58	196	49	1238

Hydrocotyl asiatica, Bacopa monnieria, Commolina bengalensis, Cynadon dactylon, Cyperus pangorei, Salvinia molesta, Eichhornia crassipes, Panicum sp., *Hydrilla* etc. contained substantial quantities of Fe and Al and *Altrnathera sissiles, Hydrophila auriculata, Salvinia molesta, Eichhornia crassipes, Panicum* sp. and Hydrilla contained substantial quantities of sulphur. Phytotechnologies using such plants that can accumulate toxic metals are quite hopeful in decontaminating the soil and water. Phytoextraction and phytostabilization are the two processes commonly employed for ecosystem restoration (Prasad *et al*., 2005). The experiments using these selected plants were carried out and the results are presented in the following sections.

III.b. Phytoextraction of elements

1. Experiment for phytoextraction of Fe

The following plants viz., *Hydrocotyle asiatica, Bacopa monnieria, Commolina bengalensis, Cynadon dactylon, Eichhornia crassipes, Monochoria vaginalis, Pistia stratiotes* and *Cyperus pangorei* were selected to study their phytoextraction ability of iron. Pot culture experiments were conducted with graded levels of iron (0 to 3000 mg kg^{-1}). The experiment was started with during October 2005. Ferrous sulphate was used to provide the graded doses of iron. The treatments include

1. Absolute control
2. 600 mg Fe per kg of soil
3. 1200 mg Fe per kg of soil
4. 1800 mg Fe per kg of soil
5. 2400 mg Fe per kg of soil
6. 3000 mg Fe per kg of soil

The plants have established well under these treatments. On 45th day the plants were harvested and biometric observations were taken before harvest. Fresh weight and dry weight of shoot and root were measured. The samples were separated in to root and shoot, dried, powdered and subjected to chemical analysis for Fe. The Fe accumulation factors for the above plants were worked out and the results of promising plants are presented in tables 18 to 21.

An increase in shoot and root dry matter production with graded doses of Fe was observed up to 1800 mg kg^{-1} for *Hydrocotyle asiatica* and 2400 mg kg^{-1} for *Bacopa monnieria,* while *Eichhornia crassipes* and *Cyperus pangorei* maintained positive correlation up to the highest level tried, ie., 3000 mg kg^{-1}. Among the tested plants, the biomass production was highest for *Eichhornia crassipes.*

Table 18. Drymatter production by different plants (g $plant^{-1}$) on 45^{th} day

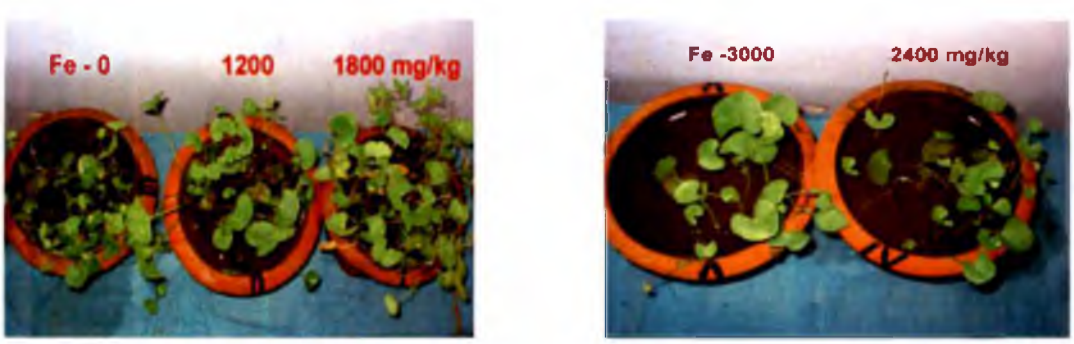
Treatments, Rates of Fe (mg kg^{-1})	*Hydrocotyle asiatica*		*Bacopa monnieria*		*Eichhornia crassipes*		*Cyperus pangorei*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero Fe	26.50	1.98	11.48	5.24	25.1	15.2	12.71	14.71
600	30.10	2.30	12.04	6.52	30.2	19.8	24.45	20.3
1200	33.29	2.34	12.22	7.00	36.0	24.2	15.39	22.69
1800	36.89	2.41	12.66	7.13	41.1	27.3	16.39	23.99
2400	26.71	2.46	12.96	7.50	42.5	28.1	17.28	24.55
3000	18.97	1.79	9.73	4.17	44.2	30.7	19.05	25.35
CD (0.05)	2.11	NS	NS	NS	1.28	1.17	1.51	2.14

Table 19. Iron content of different plants (mg kg^{-1}) on 45^{th} day

Treatments, Rates of Fe (mg kg^{-1})	*Hydrocotyle asiatica*		*Bacopa monnieria*		*Eichhornia crassipes*		*Cyperus pangorei*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero Fe	1624	4616	2135	4653	10210	6400	1441	10510
600	1989	12062	3211	9434	18200	8630	1616	15710
1200	4563	16345	3709	12290	26410	10500	1836	16415
1800	6418	19527	4364	7582	29540	12250	3412	16820
2400	6927	22036	2691	7497	32840	14400	3945	17359
3000	7218	25636	2618	7425	36700	15940	4090	17440
CD (0.05)	32.8	48.5	21.4	17.6	26.7	22.5	20.3	22.9

Bacopa monnieria - Fe @ 0 - 3000 mg / kg

Cyperus pangorei - Fe @ 0 - 3000 mg / kg



Hydrocotyle asiatica - Fe @ 0 - 3000 mg / kg

Plate 3. Phytoextraction of iron by different plants

Application of Fe had increased Fe content of both shoot and root of all tested plants. At the highest dose of Fe, *Bacopa monnieria* showed a decrease in shoot and root Fe content. Most of the tested plants were found to be good accumulators of Fe up to an iron content of 2400 mg kg^{-1}. *Eichhornia crassipes* and *Cyperus pangorei* exhibited phytoremediation capacity up to highest tested dose ie., 3000 mg Fe kg^{-1}. *Eichhornia crassipes* recorded the highest content of Fe in its shoot. Highest value for root Fe content was recoded by *Hydrocotyle Asiatica.*

Comparing the Fe removal by these plants, *Hydrocotyle asiatica* and *Bacopa monnieria,* removed more Fe with graded doses of Fe up to 1800 mg kg^{-1}only, while *Eichhornia crassipes* and *Cyperus pangorei* responded positively up to 3000 mg Fe kg^{-1} and the highest quantity was removed by *Eichhornia crassipes.*

Table 20. Iron removal by plants under graded doses of Fe (mg $plant^{-1}$)

Treatments, Rates of Fe (mg kg^{-1})	*Hydrocotyle asiatica*		*Bacopa monnieria*		*Eichhornia crassipes*		*Cyperus pangorei*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero Fe	43.0	9.13	24.5	24.3	256.3	97.3	18.31	154.0
600	59.9	27.7	38.7	61.5	549.6	170.9	23.7	318.9
1200	151.9	38.2	45.3	86.3	950.7	254.1	28.2	372.5
1800	236.7	47.1	55.2	54.1	1214.1	334.4	55.9	488.4
2400	185	54.2	34.8	56.2	1395.4	404.6	68.2	426.1
3000	136.9	45.8	25.5	30.9	1622.2	489.3	77.9	442.1
CD (0.05)	7.80	4.52	3.26	4.88	12.10	8.12	5.21	6.86

Accumulation factor for iron was calculated by the following formula to evaluate the hyper accumulating capacity of tested plants.

$$\text{Accumulation factor} = \frac{\text{Fe content in treated plant} - \text{Fe content in absolute control}}{\text{Amount of added iron}}$$

Table 21. Accumulation factor for iron under graded doses of Fe

Treatments Rates of Fe (mg kg^{-1})	*Hydrocotyl asiatica*		*Bacopa monnieri*		*Eichhornia crassipes*		*Cyperus pangorei*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
600	0.60	12.41	1.79	7.91	13.31	3.71	0.29	8.66
1200	2.45	9.77	1.31	6.36	13.52	3.41	0.32	4.92
1800	2.66	8.28	1.23	1.63	10.73	3.25	1.09	3.50
2400	2.20	7.25	0.23	1.18	9.43	3.33	1.04	2.85
3000	1.86	7.00	0.16	0.92	8.83	3.18	0.88	2.31

When the accumulation factor was calculated it was found to be higher for root compared to shoot for all plants except *Eichhornia crassipes*. *Eichhornia crassipes* recoded the highest accumulation factor for its shoot and this clearly proved its suitability for phytoremediation studies. *Hydrocotyl asiatica* and *Bacopa monnieria* showed comparatively higher values for the factor in their roots. In both the above cases the value for the "accumulation factor" decreased with increasing doses of Fe. *Cyperus pangorei* showed an increase in accumulation factor with graded doses of Fe for its shoot while root behaved reversely.

The results of the study clearly indicated the suitability of the above plants as phytoextractors for Fe. *Eichhornia crassipes* was found to be the ideal one since it has removed more Fe by the shoot itself. Soltan and Rashed (2003) reported the ability of *Eichhornia crassipes* to survive under heavy metal contaminated environment and its extraction ability of these metals. Though both *Eichhornia crassipes* and *Cyperus pangorei* are notorious aquatic weeds, the study revealed their possibility for decontaminating the water bodies. The study also indicated the possibility of utilizing these plants for removing Fe from the effluents discharged to the constructed lagoons.

2. Experiment for Phytoextraction of Al and S

The following plants viz., *Hydrocotyle asiatica, Bacopa monnieria, Cyperus pangorei* and *Pistia stratiotes* were subjected to study their hyper accumulation capacity of aluminium and sulphur by giving graded of Al and S. The pot culture experiment was started during October 2006. Aluminium sulphate was used to provide the graded doses of Al. The treatments include

1. Absolute control
2. 500 mg Al per kg of soil
3. 1000 mg Al per kg of soil
4. 1500 mg Al per kg of soil
5. 2000 mg Al per kg of soil

The above treatments provide sulphur @ zero, 890, 1780, 2690 and 3480 mg S respectively per kg of soil.

The plants have established well under these treatments. On 45th day the plants were harvested and biometric observations were taken before harvest. Fresh weight and dry weight of shoot and root were measured. Total biomass production was also measured. The samples were separated in to root and shoot, dried, powdered and subjected to chemical analysis for Al and S. The results are presented in Tables 22 to 27.

Table 22. Drymatter production (shoot) by different plants (g $plant^{-1}$)

Treatments, Rates of Al (mg kg^{-1})	*Hydrocotyle asiatica*	*Bacopa monnieria*	*Cyperus pangorei*	*Pistia stratiotes*
Zero Al	10.0	7.2	12.0	5.1
500	11.2	9.3	13.1	5.3
1000	12.3	10.1	14.2	5.4
1500	9.8	8.2	13.6	5.0
2000	8.7	7.0	12.4	4.6
CD (0.05)	0.18	0.32	0.59	0.14

Table 23. Drymatter production (root) by different plants (g plant^{-1})

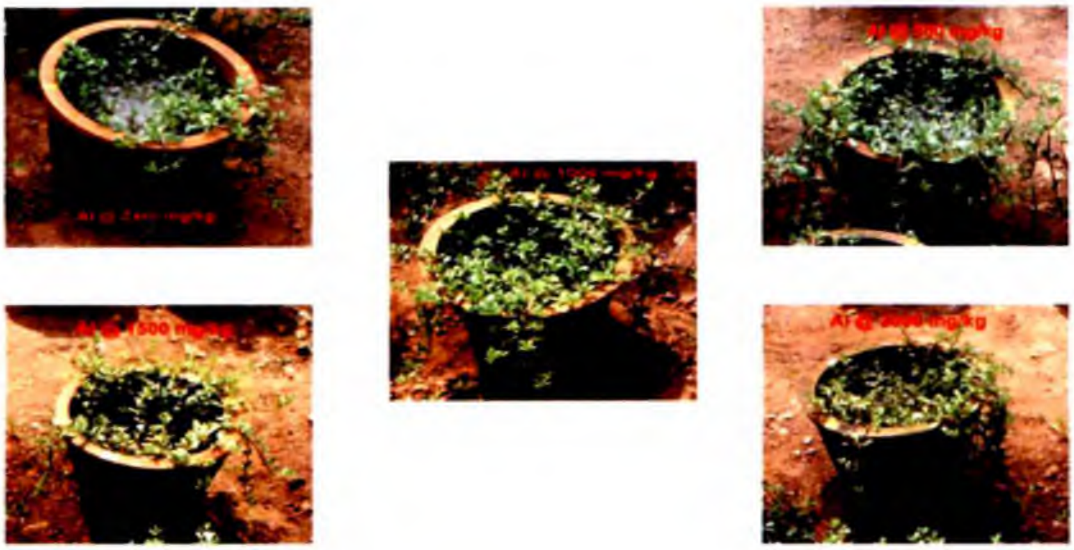
Treatments, Rates of Al (mg kg^{-1})	*Hydrocotyle asiatica*	*Bacopa monnieria*	*Cyperus pangorei*	*Pistia stratiotes*
Zero Al	1.5	4.2	11.7	4.8
500	2.1	5.2	14.6	6.2
1000	2.2	5.4	15.2	6.4
1500	2.1	4.8	15.0	5.1
2000	1.8	4.0	12.1	4.2
CD (0.05)	0.17	0.20	1.78	0.58

An increase in shoot dry matter production with graded doses of Al and S was observed for all plants up to a level of 1000 mg Al kg^{-1} of soil. *Cyperus pangorei* recorded the highest dry matter production for both shoot and root. Though, *Hydrocotyle asiatica* and *Bacopa monnieria* also produced sizeable quantity of shoot biomass, their root biomass production was in small quantities only. Above 1000 mg Al kg^{-1} of soil, the growth of plants was poor.

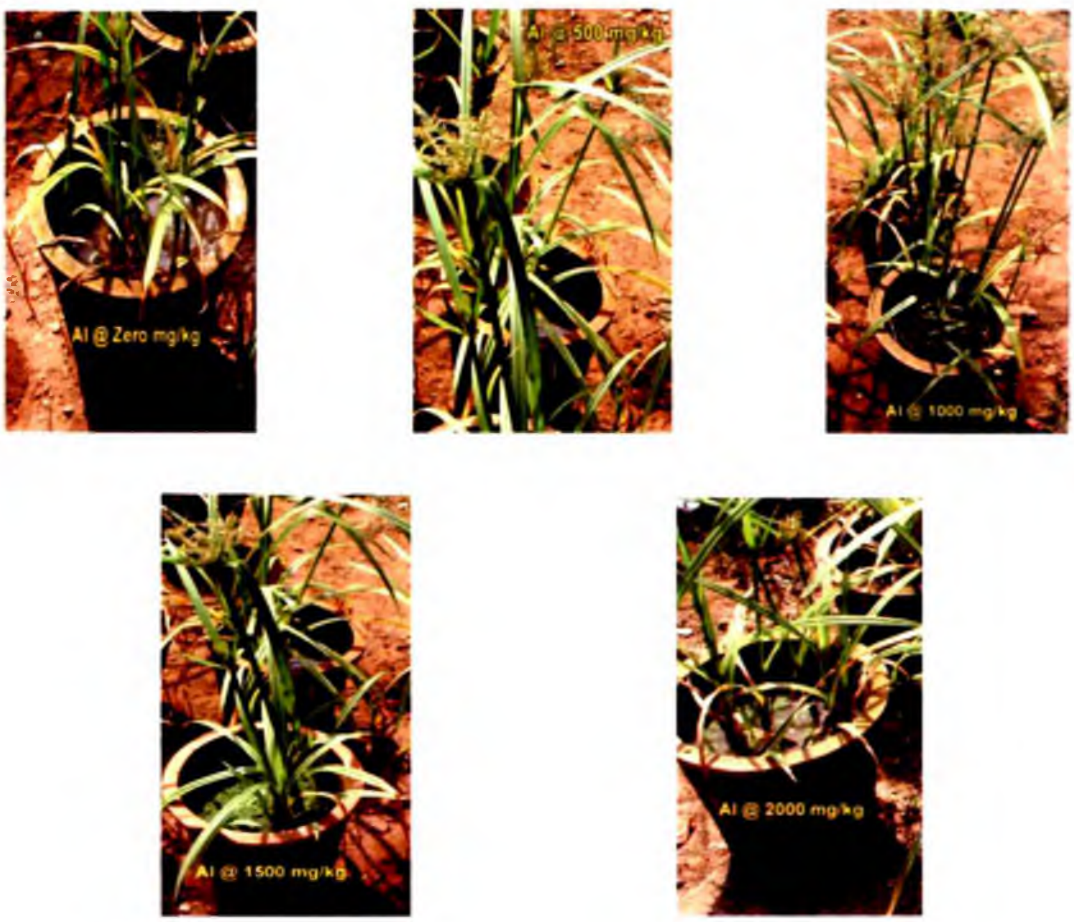
Application of Al had increased Al content of shoot for most of the tested plants to a level of 500 mg Al kg^{-1} of soil except *Hydrocotyle asiatica,* which showed an increase up to 1000 mg Al kg^{-1} of soil. For root, most of the tested plants showed an increase in Al content with its graded doses up to 1000 mg kg^{-1} except *Cyperus pangorei.*

Table 24. Al content (mg kg^{-1}) of plants under graded doses of Al and S

Treatments, Rates of Al (mg kg^{-1})	*Hydrocotyle asiatica*		*Bacopa monnieria*		*Cyperus pangorei*		*Pistia stratiotes*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero Al	460	540	429	440	426	426	816	975
500	686	610	734	520	555	759	880	1060
1000	914	920	649	458	420	828	745	1156
1500	820	396	537	357	358	885	678	980
2000	744	358	512	348	306	540	601	800
CD (0.05)	17.06	7.19	5.57	8.23	3.60	8.41	8.78	6.45



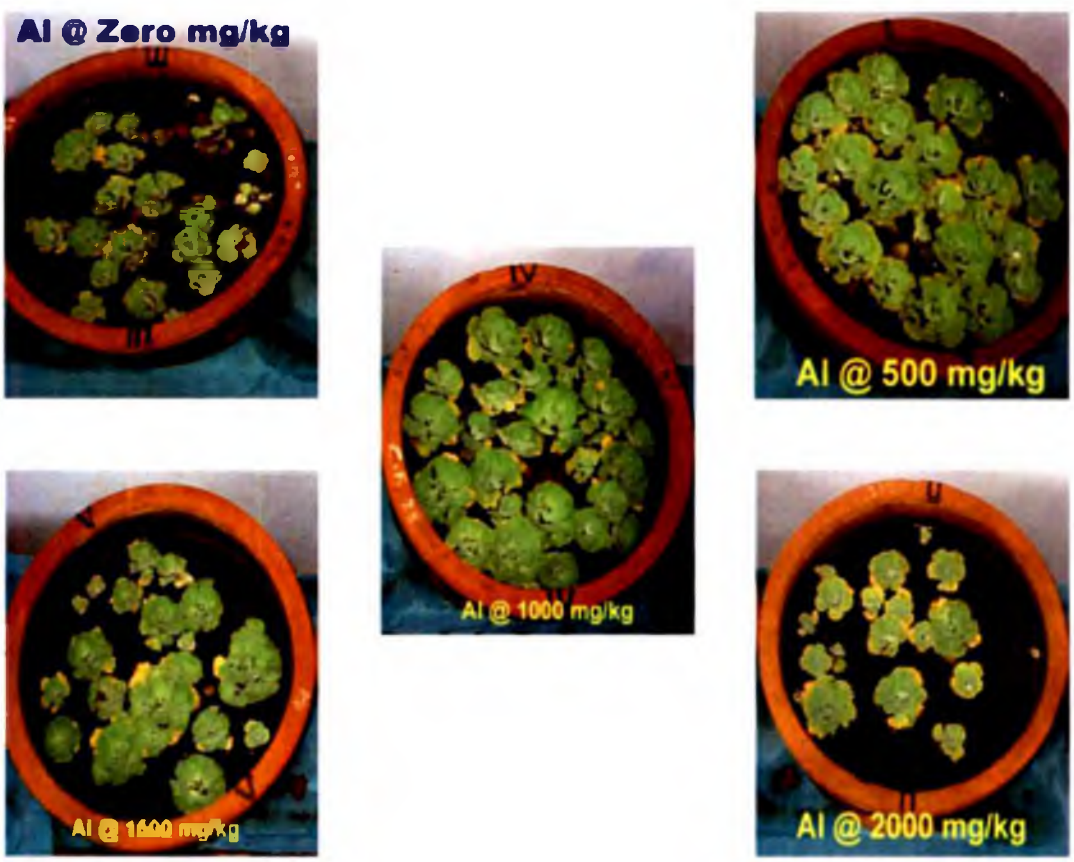
Bacopa monnieria - Al @ 0 - 2000 mg / kg



Cyperus pangorei - Al @ 0 - 2000 mg / kg

Plate 4. Phytoextraction of Al by different plants

Hydrocotyle asiatica - Al @ 0 - 2000 mg / kg



Pistia stratiotes - Al @ 0 - 2000 mg / kg

Plate 4 (Contd....)

Table 25. S content (mg kg-1) of shoot under graded doses of S

Treatments Rates of S (mg kg^{-1})	*Hydrocotyle asiatica*		*Bacopa monnieria*		*Cyperus pangorei*		*Pistia stratiotes*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero S	925	2091	1096	720	705	564	376	600
890	1120	3080	1304	936	739	836	584	856
1780	1584	3211	1309	1021	824	843	504	912
2690	2092	3464	800	980	896	910	478	924
3480	2178	3722	744	845	984	936	456	955
CD (0.05)	5.63	4.60	5.15	2.64	3.09	3.99	6.83	6.61

Table 26. Al removal (mg $plant^{-1}$) by plants under graded doses of Al

Treatments Rates of Al (mg kg^{-1})	*Hydrocotyle asiatica*		*Bacopa monnieria*		*Cyperus pangorei*		*Pistia stratiotes*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero Al	4.60	0.81	3.08	1.85	5.11	4.98	3.92	4.69
500	7.68	1.28	3.81	2.70	7.27	11.08	5.46	6.57
1000	11.24	2.06	6.55	2.47	.5.96	12.59	4.77	7.39
1500	8.03	0.85	4.40	1.71	4.87	13.27	3.46	4.99
2000	6.47	0.65	3.58	1.39	3.79	6.53	2.52	3.36
CD (0.05)	3.81	1.56	2.09	2.86	1.71	1.40	2.27	8.74

Application of graded doses of Al and S had resulted variable response in different plants. *Hydrocotyle asiatica* and *Cyperus pangorei* showed positive response up to the highest level of Al and S tested (2000 mg Al and 3480 mg S kg^{-1} of soil) in shoot. *Bacopa monnieria* showed an increase in shoot S up to 1780 mg S kg^{-1} of soil and *Pistia* *stratiotes* up to 890 mg S kg^{-1} of soil. For root S content, all the tested plants except *Bacopa monnieria* responded positively up to the highest levels of Al and S (2000 mg Al and 3480 mg S kg^{-1} of soil). *Bacopa monnieria* responded positively only up to 1500 mg Al and 2690 mg S kg^{-1} of soil.

Table 27. S removal (mg/plant) by plants under graded doses of S

Treatments Rates of S (mg kg^{-1})	*Hydrocotyle asiatica*		*Bacopa monnieria*		*Cyperus pangorei*		*Pistia stratiotes*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero S	9.25	3.13	7.89	3.02	3.59	2.71	4.51	7.02
890	12.54	6.46	12.12	4.86	3.92	5.18	7.65	12.49
1780	19.48	7.19	13.22	5.51	4.44	5.40	7.15	13.86
2690	20.50	7.44	6.56	3.84	4.48	4.64	6.50	13.87
3480	18.95	6.79	5.20	3.38	4.52	3.93	5.65	11.55
CD (0.05)	8.53	9.61	5.61	0.39	4.65	7.01	8.86	7.41

Comparing the plants, *Hydrocotyle asiatica* and *Bacopa monnieria* showed positive responses up to a level of 1000 mg Al kg^{-1}. *Cyperus pangorei* and *Pistia stratiotes* showed variable response for Al removal by shoot and root. Root showed positive response up to 1000 mg Al kg^{-1} and shoot to a level of 500 mg kg^{-1}. For S removal, *Hydrocotyle asiatica* showed positive response to the highest level of S applied while most of the remaining plants showed positive response to 1780 mg S kg^{-1}.

The accumulation factors for Al and S were also calculated and positive values were recorded only by *Hydrocotyle asiatica* for Al and S. *Hydrocotyle asiatica* can be considered as a phytoextractor for Al and S. Barley and horse bean (Grauer and Horst, 2003) were reported to be the accumulators for Al and their accumulation rates are 1000 and 100 μg g^{-1} respectively.

3. Experiment for Phytoextraction of Zn, Cu, Cd and Pb

The following plants viz., *Bacopa monnieria, Hydrocotyle asiatica, Cyperus pangorei* and *Eichhornia crassipes* were tested for their accumulation ability of Zn, Cu, Cd and Pb. Pot culture experiments were conducted with the above plants by giving graded doses of Zn, Cu, Cd and Pb. The treatments are as follows.

Element	Levels
Zn	0, 10, 20 and 40 mg Zn as zinc nitrate kg^{-1} of soil
Cu	0, 10, 20 and 40 mg Cu as copper nitrate kg^{-1} of soil
Cd	0, 5, 10 and 20 mg Cd as cadmium nitrate kg^{-1} of soil
Pb	0, 5, 10 and 20 mg Pb as lead nitrate kg^{-1} of soil

The plants have established well under these treatments. On 45th day the plants were harvested and biometric observations were taken before harvest. Fresh weight and dry weight of shoot and root were measured. The samples were separated in to root and shoot, dried, powdered and subjected to chemical analysis for Zn, Cu, Cd and Pb.

1. Zinc

Table 28. Drymatter production by different plants (g $plant^{-1}$) on 45th day

Treatments, Rates of Zn ($mg\ kg^{-1}$)	*Hydrocotyle asiatica*		*Bacopa monnieria*		*Cyperus pangorei*		*Eichhornia crassipes*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero Zn	4.7	1.6	4.5	1.39	13.2	12.1	20.9	15.2
10	6.8	2.0	16.3	2.63	15.1	7.7	50.6	23.0
20	9.0	5.9	18.6	4.76	20.1	14.2	32.3	24.5
40	6.7	2.3	21.3	5.27	22.3	22.7	30.1	25.7
CD (0.05)	0.25	0.31	0.39	2.42	0.48	0.20	0.34	0.4

The plants *Bacopa monnieria* and *Cyperus pangorei* showed an increase in plant dry weight with levels of Zn upto 40 mg kg^{-1} while *Hydrocotyle asiatica* and *Eichhornia crassipes* showed positive response up to 20 and 10 mg Zn kg^{-1} of soil only.

Table 29. Zinc content of different plants (mg kg^{-1}) on 45^{th} day

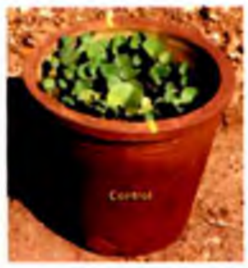
Treatments, Rates of Zn (mg kg^{-1})	*Hydrocotyle asiatica*		*Bacopa monnieria*		*Cyperus pangorei*		*Eichhornia crassipes*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero	80	59	34	28	70	303	315	722
10	115	72	114	51	120	367	427	831
20	143	86	86	63	146	415	510	867
40	165	90	95	70	178	482	534	952
CD(0.05)	5.21	3.06	4.32	3.54	4.75	5.28	4.52	6.94

Among the plants tested, *Eichhornia crassipes* showed the highest content of Zn and the root portion contained more of the element compared to shoot. Bashmakov (2005) reported the better removal of Zn by the roots of *Chenopodium album* compared to shoot. All the tested plants showed an increase in Zn content with the graded levels of Zn. The highest quantity of Zn was removed from the soil by *Eichhornia crassipes* followed by *Cyperus pangorei* (Fig. 1) indicating its accumulation ability. The accumulation factor was also highest for *Eichhornia crassipes*. Soltan and Rashed (2003) reported the ability of *Eichhornia crassipes* to phytoextract Zn, Cu, Cd and Pb from natural ponds.

Table 30. Zn removal by plants under graded doses of Zn (µg $plant^{-1}$)

Treatments, Rates of Zn (mg kg^{-1})	*Hydrocotyle asiatica*			*Bacopa monnieria*			*Cyperus pangorei*			*Eichhornia crassipes*		
	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total
Zero	376	94	470	153	39	192	924	3739	4663	8583	10974	19557
10	782	144	926	1174	134	1308	1812	2825	4637	21606	19113	40719
20	1287	507	1794	1600	300	1900	2935	5893	8828	16473	21241	37714
40	1106	207	1313	2024	369	2393	3969	10941	14910	16073	24466	40539

E. crassipes

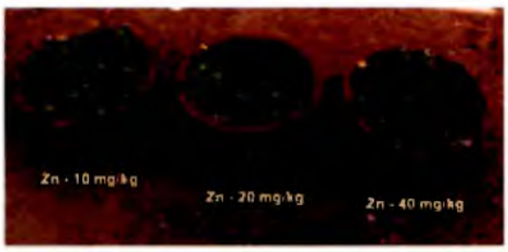


H. asiatica

B. monnieria

C. pangorei

Control



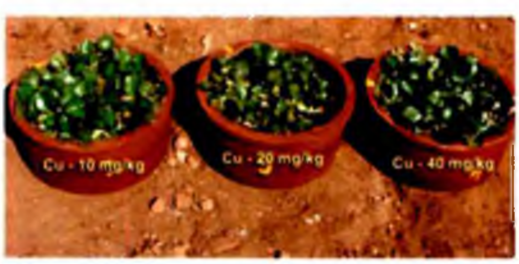
E. crassipes

C. pangorei

H. asiatica

B. monnieria

Zinc @ 10 - 40 mg / kg



E. crassipes

C. pangorei

H. asiatica

B. monnieria

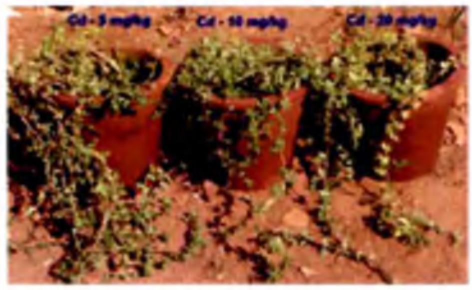
Copper @ 10 - 40 mg / kg

Plate 5. Phytoextraction of Zn, Cu, Cd and Pb by different plants

E. crassipes

C. pangorei

H. asiatica



B. monnieria

Cadmium @ 5 - 20 mg / kg

E. crassipes

C. pangorei

H. asiatica

B. monnieria

Lead @ 5 - 20 mg / kg

Plate 5 (Contd....)

Table 31. Accumulation factor for Zinc

Treatments, Rates of Zn (mg kg^{-1})	*Hydrocotyle asiatica*		*Bacopa monnieria*		*Cyperus pangorei*		*Eichhornia crassipes*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
10	3.50	1.30	8.00	2.30	5.00	7.00	11.2	10.9
20	3.15	1.35	2.60	1.75	3.80	5.61	9.75	7.25
40	2.12	0.78	1.50	1.05	2.70	4.48	5.40	5.75

2. Copper

Table 32. Drymatter production by different plants (g plant^{-1}) on 45th day

Treatments, Rates of Cu (mg kg^{-1})	*Hydrocotyle asiatica*		*Bacopa monnieria*		*Cyperus pangorei*		*Eichhornia crassipes*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero Cu	4.5	1.7	4.6	1.42	13.0	12.2	20.6	15.3
10	5.8	3.6	10.4	3.19	12.1	22.0	30.5	22.7
20	5.9	2.1	7.7	2.36	19.3	17.4	28.8	19.2
40	8.5	1.9	6.0	1.19	17.6	9.6	22.0	14.7
CD (0.05)	0.15	0.17	0.19	0.07	0.25	0.30	0.22	0.28

The plants *Bacopa monnieria* and *Cyperus pangorei* showed an increase in plant dry weight with levels of Cu up to 20 mg Cu kg^{-1} of soil, while *Hydrocotyle asiatica* and *Eichhornia crassipes* showed positive response up to 10 mg Cu kg^{-1} of soil only.

Table 33. Copper content of different plants (mg kg^{-1}) on 45th day

Treatments, Rates of Cu (mg kg^{-1})	*Hydrocotyle asiatica*		*Bacopa monnieria*		*Cyperus pangorei*		*Eichhornia crassipes*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero	2.4	6.5	19.3	2.5	2.7	7.2	2.6	2.7
10	2.6	9.2	32.6	15.0	12.8	15.3	42.2	16.8
20	8.9	14.6	46.9	34.3	25.3	27.6	70.1	20.3
40	10.7	16.1	58.2	50.8	56.5	61.2	92.5	26.6
CD (0.05)	1.25	1.60	2.58	2.87	2.15	3.21	2.58	1.27

Bacopa monnieria, Cyperus pangorei and *Eichhornia crassipes* were found to contained appreciable amounts of Cu in their shoot and root. Copper content was more in shoot for *Bacopa monnieria,* and *Eichhornia crassipes* while *Cyperus pangorei* showed a higher concentration in root. The highest quantity was removed by *Eichhornia crassipes* followed by *Cyperus pangorei* (Fig. 2).

Table 34. Copper removal by plants under graded doses of Cu (μg $plant^{-1}$)

Treatments, Rates of Cu (mg kg^{-1})	*Hydrocotyle asiatica*			*Bacopa monnieria*			*Cyperus pangorei*			*Eichhornia crassipes*		
	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total
Zero	10.8	11.0	21.8	88.7	3.6	92.3	35.1	87.8	123	54.0	41.3	95.3
10	15.1	34.0	49.1	339	47.9	387	155	337	492	1295	385	1680
20	52.5	30.7	133	356	80.9	437	488	478	968	2018	386	2404
40	64.2	30.5	94.7	349	60.5	410	994	587	1581	2035	393	2436

3. Cadmium

Table 35. Drymatter production by different plants (g $plant^{-1}$) on 45^{th} day

Treatments, Rates of Cd (mg kg^{-1})	*Hydrocotyle asiatica*		*Bacopa monnieria*		*Cyperus pangorei*		*Eichhornia crassipes*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero Cd	4.7	1.4	4.5	1.4	13.1	12.0	21.4	15.1
5	6.3	1.8	21.4	3.96	13.6	13.4	54.5	14.9
10	9.3	2.2	17.9	3.33	13.2	13.5	53.3	13.0
20	11.8	3.7	10.5	2.31	16.5	13.8	30.5	8.0
CD (0.05)	0.13	0.16	0.35	0.12	0.13	0.38	0.33	0.32

The plants *Hydrocotyle asiatica* and *Cyperus pangorei* showed an increase in plant dry weight with levels of Cd while *Bacopa monnieria* and *Eichhornia crassipes* showed positive response up to 10 mg Cd kg^{-1} of soil only.

Table 36. Cadmium content of different plants (mg kg^{-1}) under graded doses of Cd

Treatments, Rates of Cd (mg kg^{-1})	*Hydrocotyle asiatica*		*Bacopa monnieria*		*Cyperus pangorei*		*Eichhornia crassipes*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero	12	0.4	9.7	0.6	6.2	2.1	12.1	1.4
5	14	1.6	12.8	8.6	13.3	9.6	53.2	11.9
10	18	3.2	13.4	9.7	8.7	10.0	68.2	13.5
20	21	6.1	13.1	11.2	7.7	9.2	76.8	17.2
CD (0.05)	0.56	0.51	1.02	0.84	0.45	0.62	1.23	0.78

Hydrocotyle asiatica and *Eichhornia crassipes* contained higher quantities of Cd compared to other plants. The highest quantity was removed by *Eichhornia crassipes.* Both these plants contained higher quantity in shoot which is considered as a quality to be possesed by a good phytoextractor. All the tested plants showed positive response to graded levels of Cd. The highest quantity of Cd was removed by *Eichhornia crassipes* when Cd was applied @ 10 mg kg^{-1} (Fig. 3).

Table 37. Cadmium removal by shoot under graded doses of Cd (μg $plant^{-1}$)

Treatments, Rates of Cd (mg kg^{-1})	*Hydrocotyle asiatica*			*Bacopa monnieria*			*Cyperus pangorei*			*Eichhornia crassipes*		
	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total
Zero	58	0.56	58.6	44	0.84	44.8	81	25	112	259	18.3	277
5	90	2.88	92.9	273	34	30.7	180	128	308	2902	178	3080
10	170	7.04	177	240	32	272	115	135	270	3637	183	3020
20	248	22.5	271	138	26	164	127	127	259	2342	138	2480

4. Lead

Table 38. Drymatter production by different plants (g plant^{-1}) on 45th day

Treatments, Rates of Pb (mg kg^{-1})	*Hydrocotyle asiatica*		*Bacopa monnieria*		*Cyperus pangorei*		*Eichhornia crassipes*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero Pb	4.5	1.6	4.4	1.45	13.4	12.0	21.7	15.2
5	6.0	1.7	27.6	10.7	22.1	12.2	24.9	18.4
10	9.7	1.7	12.9	7.2	19.2	10.7	35.0	24.5
20	3.9	0.6	7.53	3.28	18.9	6.8	48.6	35.0
CD (0.05)	0.34	0.20	0.36	0.19	0.53	0.42	0.39	0.13

The plants *Hydrocotyle asiatica* and *Bacopa monnieria Cyperus pangorei* showed an increase in plant dry weight with levels of Pb up to 10 mg kg^{-1} while *Eichhornia crassipes* showed positive response up to 20 mg Pb kg^{-1} of soil.

Table 39. Lead content of different plants (mg kg^{-1}) under graded doses of Pb

Treatments, Rates of Pb (mg kg^{-1})	*Hydrocotyle asiatica*		*Bacopa monnieria*		*Cyperus pangorei*		*Eichhornia crassipes*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero	2.7	1.2	2.9	2.1	11.2	9.4	13.1	14.2
5	4.4	2.8	6.8	6.0	18.8	16.1	19.5	18.9
10	8.3	7.5	16.2	14.2	29.1	20.4	27.4	29.3
20	13.2	10.2	30.2	23.0	54.4	38.3	61.6	63.7
CD (0.05)	0.61	0.53	0.24	0.21	0.42	0.38	0.95	0.63

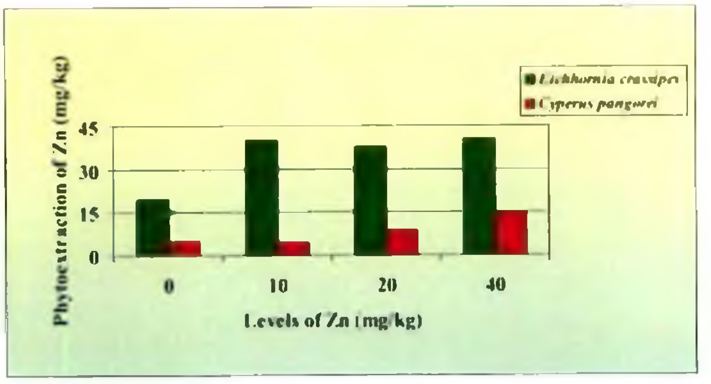


Fig. 1. Phytoextraction of zinc

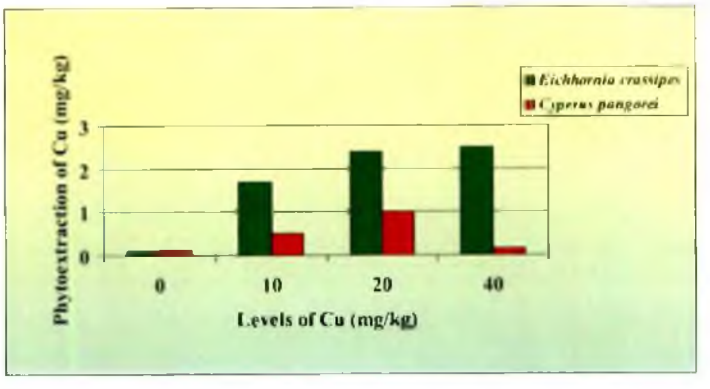


Fig. 2. Phytoextraction of copper

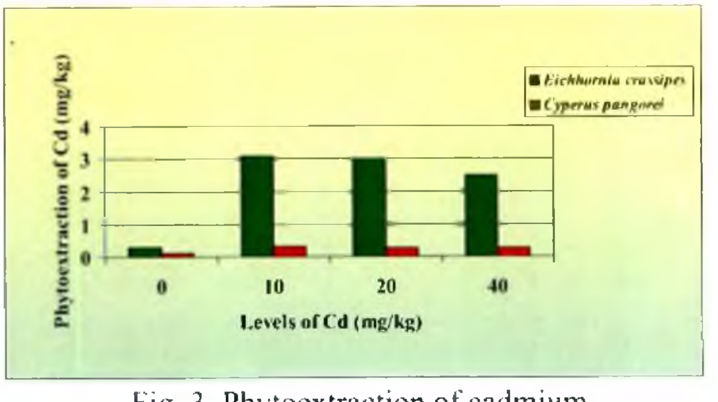


Fig. 3. Phytoextraction of cadmium

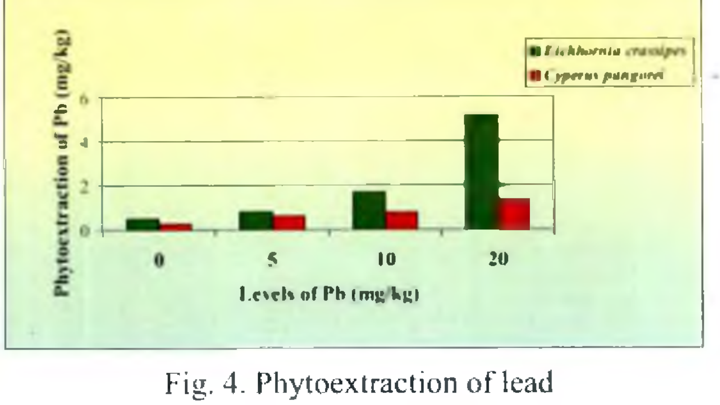


Fig. 4. Phytoextraction of lead

Eichhornia crassipes and *Cyperus pangorei* showed higher concentration of Pb. *Cyperus pangorei* was found to accumulate Pb in shoot while *Eichhornia crassipes* accumulated Pb in root. All the tested plants showed an increase in their Pb content with graded levels of Pb. The highest quantity was removed by *Eichhornia crassipes* followed by *Cyperus pangorei.*

Table 40. Lead removal by shoot under graded doses of Pb (µg $plant^{-1}$)

Treatments, Rates of Pb (mg kg^{-1})	*Hydrocotyle asiatica*			*Bacopa monnieria*			*Cyperus pangorei*			*Eichhornia crassipes*		
	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total
Zero	12.2	1.92	14.4	12.8	3.0	15.8	150	108	258	284	216	500
5	26.4	4.76	31.2	120	43.2	163	416	196	612	485	352	837
10	80.5	12.8	93.3	212	72.4	285	559	218	777	959	718	1677
20	51.5	12.2	63.7	227	75.4	303	1028	310	1338	2993	2230	5223

Comparing the hyperaccumulation /phytoextraction ability of different plants for various elements, *Eichhornia crassipes* was found to be the best extractor followed by *Cyperus pangorei.* The accumulation factors for different elements were also highest for *Eichhornia crassipes.* These weeds could be used for environmental clean up of aquatic systems including removal of heavy metals.

IV. Experiments for identification of hyper accumulating plants and microbes with bioremediation capacity and quantification

1. Mycological studies

Mycological studies with the collected soil from the rice fields and that from the rhizosphere of natural flora were initiated. Rhizosphere soil from vigorously growing plants collected from rice fields in the selected watersheds were subjected to serial dilution and plating technique for isolation of the fungi associated. 10^{-4} dilution were transferred to petridishes containing Rose Bengal Agar and incubated. Observations on the colony forming units (cfu) per gram of soil were taken and the results are furnished below.

Table 41. Population of fungi in the rhizosphere of plants and soil

Name of plant	cfu/g soil	Predominant fungi
Cyprus pangorei	268 x 10^4	*Pencillium, Trichoderma, Aspergillus*
Ludwijia perinis	30 x 10^4	*Pencillium and Trichoderma*
Alternathera sissiles	45 x 10^4	*Aspergillus, Pencillium*
Hydrocotyl asiatica	25 x 10^4	*Pencillium, Trichoderma, Fusarium*
Kari Soil - Vechoor	30 x 10^4 to 120 x 10^4	*Pencillium, Trichoderma, Fusarium*

Common saprophytic fungi were present in the rhizosphere soil of all the selected plants. All these fungi were conidia forming and belonged to sub division *Deuteromycotina*. Most of these are fast growing and can establish well under polluted environments (Romero *et al.*, 2002).

2. Mycorrhizal colonization in hyperaccumulators

Mycorrhizal colonization was noticed in all the treatments for all the plants tested. The highest colonization was noticed for *Cyperus pangorei* followed by *Bacopa monnieria* and *Hydrocotyle asiatica.*

In general, there was increase in mycorrhizal colonization as the concentration of iron increased. The percentage of colonization observed in treatment plants were statistically superior compared to that of absolute control. Accordingly *Bacopa monnieria* recorded the maximum colonization in treatment receiving 2400 mg Fe kg^{-1} followed by that receiving 3000 mg Fe kg^{-1}. These treatments were superior to absolute control. Similarly *Cyperus pangorei* showed more mycorrhizal colonization in 3000 mg Fe kg^{-1} and showed a gradual but significant decrease as concentration of Fe decreased. *Hydrocotyle asiatica* also showed a similar trend. However, the mycorrhizal colonization in 1800, 2400 and 3000 mg Fe kg^{-1} were on par but significantly higher to absolute control.

These observations point to the fact that mycorrhizal colonization was more pronounced in plants grown under high Fe concentration. This indicated the favourable influence of mycorrhizae on metal tolerance/ stress tolerance of such plants. The presence of mycorrhizae in these plants may help to make immune under high levels of Fe and to absorb it in more efficient manner. However, in these plants the hyphal growth of mycorrhizal fungi was efficient but showed decreased number of vesicles and arbuscules. Even in plants with lesser mycorrhizal colonization, stress tolerance will be induced by the fungal partners.

Table 42. Mycorrhizal colonization (%) under graded doses of iron and sulphur

Treatments Rates of Fe (mg kg^{-1})	*Hydrocotyle asiatica*	*Bacopa monnieria*	*Pistia stratiotes*	*Monochoria vaginalis*	*Cyperus pangorei*
Zero Fe	78.28	75.34	84.14	77.48	79.83
1200	85.48	84.78	84.22	81.52	74.67
1800	78.56	84.29	83.22	79.74	82.84
2400	84.85	89.20	84.76	80.48	86.36
3000	85.76	85.30	84.88	81.62	92.74

CD (0.05) for crops- 2.66 CD (0.05) for iron – 1.19

3. Growth of fungi on soil extract of heavy metal contaminated wetland soil

The ability of fungal isolates to grow on the soil extract broth prepared from heavy metal contaminated soils of Kuttanad was studied. For this, 1 kg wetland soil was boiled with 1 litre of water for 30 minutes and allowed to settle for 1 hour. The supernatant liquid was sieved through muslin cloth. The soil extract broth was prepared by adding 100 ml of the prepared extract to 1litre of the medium. Aliquots of 50 ml were dispensed in 100 ml conical flask and sterilized in an autoclave.

The fungi isolated from the polluted soils were grown on PDA in sterile petridishes. Culture discs (1 cm diameter) were cut from the respective fungal culture and aseptically transferred to the soil extract broth and incubated 28 ± 1°C for 7 days. The mycelial mats were then filtered through Whatman No.1 filter paper and dried in a drying oven to constant weight. Based on the biomass produced, *Trichoderma virens* and *Trichoderma pseudokoningii* were selected as the fungal candidate for toxicity studies. A strain of *Trichoderma atroviride* isolated from sludge obtained from water treatment plant has been found capable of multi metal removal (Errasquin and Vazquez, 2003). Moreover, Trichoderma being widely exploited for plant disease biocontrol and growth enhancement capability, their scope for addition in rice ecosystems for bioremediation is more.

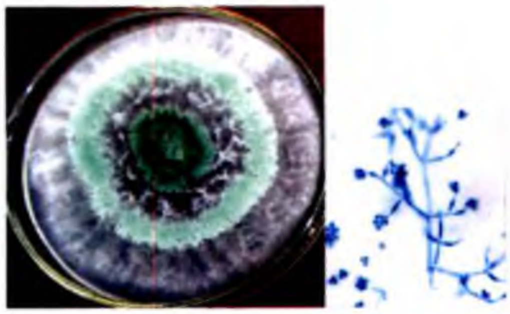
Table 43. Dry weight of fungal mycelium in soil extract of wetlands

Name of fungus	Extent of growth in medium	Mycelial dry weight (g)
Trichoderma virens	++++	1.1
Trichoderma pseudokoningii	++++	0.74
Fusarium sp.	++	0.53
Aspergillus	+++	0.63
Penicillium	+	0.29

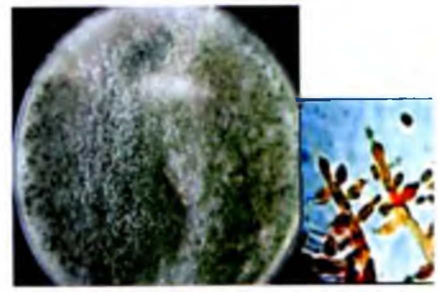
++++ - Profuse growth   +++ - sufficient growth

++ - Scanty growth   + - poor growth

Removal of Fe by *Trichoderma* spp. was studied through growing these fungi in media supplemented with graded doses of $FeSO_4 . 7 H_2O$. The rapidly growing *Trichoderma* spp. were found capable of removing appreciable quantity of heavy metals from the medium as indicated by the heavy metals detected in their mycelial samples. *T.virens* was found to have high potential and could take up 15 to 25 % of the iron in the medium.The concentration of Fe absorbed in the vegetative body was proportional to the quantity of Fe supplied in the medium.



Trichoderma virens



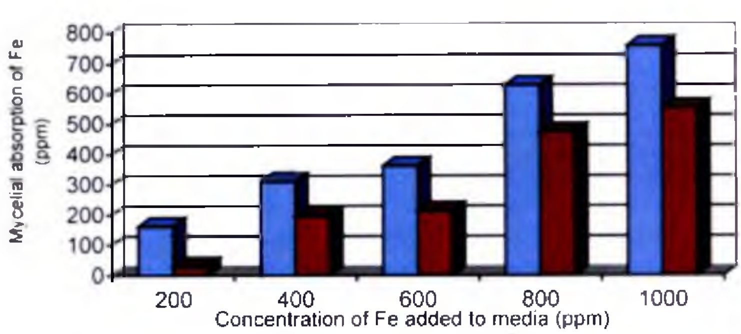
Trichoderma pseudokoningii

4. Biosorption studies

Growth inhibition was achieved by inoculating actively growing mycelial discs of two actively growing fungi to solutions containing graded doses of aqueous Fe *ie*., 200, 400, 600, 800 and 1000 mg L^{-1}. The volume of each solution was 100 ml. The mycelial growth rate was measured after 7 days.

Graded doses of aqueous Fe *ie*., 200, 400, 600, 800 and 1000 mg L^{-1}. were prepared by diluting standard $FeSO_4$ solution to the desired concentrations. The freshly diluted solutions were used for each biosorption study. The sorption experiments were conducted in 100 ml flasks containing 50 ml of Fe solutions with initial concentrations ranging from 200 to 1000 mg L^{-1}. Actively growing mycelial discs of fungi were inoculated to solutions containing graded doses of aqueous Fe. During the adsorption process, the flasks were agitated on a shaker for 48 hours under ambient temperature (28±1°C). To determine the concentration of the adsorbed metal ions, the fungal biomass in the form of mycelial mat in the sample solutions was removed by filtration and routine analysis was carried out to measure the Fe concentration spectrophotometrically.

Fig. 5. Uptake and removal of iron by *Trichoderma* sp.



T. virens was found to have high potential and could take up 50 to 75 % of the iron in the medium. The concentration of Fe absorbed in the vegetative body was proportional to the quantity of Fe supplied in the medium.

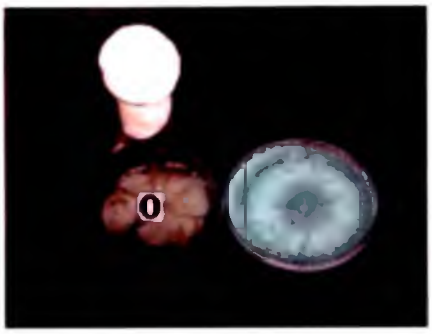
5. Metal training of fungus for enhanced bioremediation

1. Iron

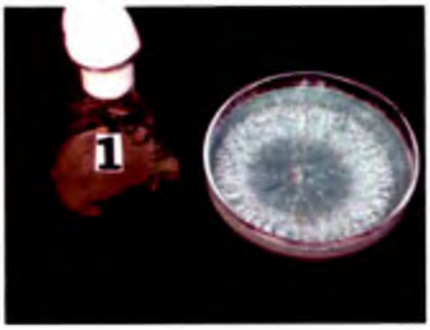
Within a particular species, differences in metal sensitivity and tolerance have been demonstrated for different strains and ecotypes. Tolerance can be increased by training the fungi on media containing graded levels of metal. Some fungi retain the acquired tolerance while others revert to original state. Training the selected fungi to low to high concentration of metal incorporated media will help to identify metal tolerant ones which will take up the metal in a better manner and adapt to polluted habitats. Such an attempt was initiated in this experiment.

T. virens and *T. pseudokoningii* were grown in Richards medium and were trained to increasing levels of metals by serial transfer every 3 days to medium amended with increasing concentration of the respective metal. The media were adjusted to pH 5.0 as metals occur as divalent cation at this pH. Control was maintained on the same medium without addition of metals. Accordingly, the fungus *T. virens* was serially transferred to 200 -1000 mg L^{-1} Fe containing medium and the growth, colour and sporulation at different concentrations of Fe was recorded (Table 44 and 45). The experimental observations revealed that the fungus produced abundant mycelial growth and sporulation during the serial transfers to higher metal concentrations up to 800 mg L^{-1}. At 1000 mg L^{-1}, the fungus showed scanty and delayed growth without sporulation.

General view

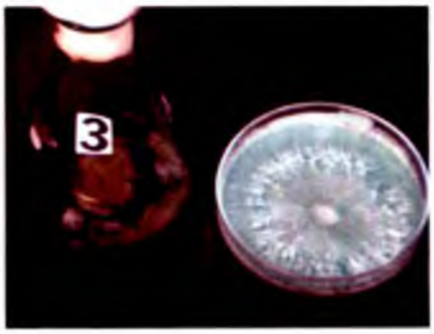


Non-trained (wild)

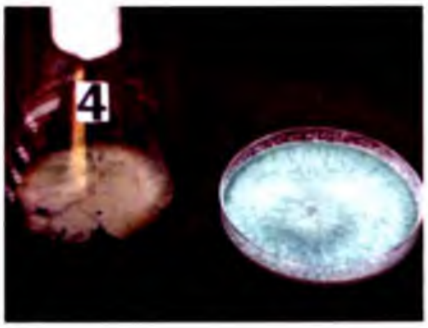


Trained at 200 mg/l Fe

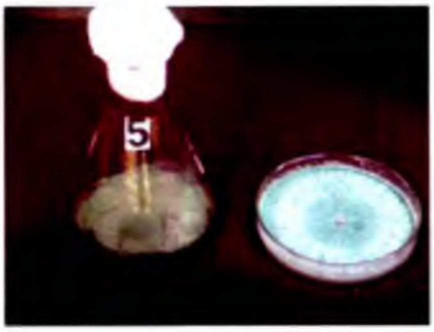
Trained at 400 mg/l Fe



Trained at 600 mg/l Fe



Trained at 800 mg/l Fe



Trained at 1000 mg/l Fe

Plate 6. Growth of metal (Fe) trained *T. virens* at various concentration of Fe in solid and liquid medium

Table 44. Growth and colour of *T. virens* on metal (Fe) training

Fe cocentration mg L^{-1}	Nature of growth (Days after inoculation)						Colour (Days after inoculation)					
	2	3	4	5	6	7	2	3	4	5	6	7
200	++	++++	++++	++++	++++	++++	-	G	G	G	G	G
400	++	++++	++++	++++	++++	++++	Sp.g	G	G	G	G	G
600	++	++++	++++	++++	++++	++++	-	G	G	G	G	G
800	+	++	++++	++++	++++	++++	-	-	-	-	SP.g*	Sp.g*
1000	-	-	-	-	+	++	-	-	-	-	-	Sp.g *

G- Dark green Sp.g – Sparse green Sp.g* - Sparse green at edges

Table 45. Sporulation of *Trichoderma virens* on metal (Fe) training

Fe mg L^{-1}	Sporulation (Days after inoculation)					
	2	3	4	5	6	7
200	-	++	+++	++++	++++	++++
400	+	+++	++++	++++	++++	++++
600	-	++	++++	++++	++++	++++
800	-	-	-	-	+	+
1000	-	-	-	-	-	+

G- Dark green, Sp.g – Sparse green, Sp.g* - Sparse green at edges

The biomass from 200-800 mg L^{-1} was collected by filtration and dried to constant weight. The dried mycelial mat of the treated and untreated fungus were acid digested and are being analyzed for metal biosorption. The Fe trained fungus was transferred serially to 20, 40, 60, 80 and 100 mg L^{-1} of Cu after keeping respective control. The nature of growth of the non-trained and the trained *T. virens* at various concentrations from 200- 1000 mg L^{-1} were plated on Richards medium and the extent of mycelial growth was measured from 24-96 hours of inoculation (Table 46). The trained *Trichoderma virens* from 200, 400 and 600 mg L^{-1} produced profuse mycelial growth right from 24 hours of inoculation and achieved full growth (9 cm) in petridishes within 3 days. Trained isolates performed in a better way than in the non-trained counter part. The trained fungus transferred from 800 mg L^{-1} showed delay in initiation of mycelial growth in the first two days. However, this further gained momentum and achieved full growth in 96 hours. The isolate from 1000 mg L^{-1} showed very slow and retarded growth. Although high Cu concentration (80 and 100 mg Cu L^{-1}) inhibited the growth of the fungus, training the fungus by successive sub-culturing in media cultures progressively increasing the concentration of the metal and giving sufficient time for adaptability helped the fungus to withstand and tolerate higher metal concentration. The result shows the adaptive mechanism and reflects upon the tolerance of the trained strain to increasing metal concentration. Poor tolerance of strains to heavy metals that hamper metal cleaning process can be addressed by developing training strategies. Valix and Loon, (2003) suggested metal training of *Aspergillus* spp. and *Penicillium simplicissinium* for heavy metal tolerance. Based on theses results the fungus trained on 800 mg L^{-1} Fe was considered for continuing the training on Cu at different concentrations.

Table 46. Comparison of mycelial growth of metal (Fe) trained and non- trained *Trichoderma virens*

Fungus	Mycelial growth (cm)			
	24 hour	48 hour	72 hour	96 hour.
Non-trained *T. virens*	3.0	6.9	7.8	9.0
Trained *T. virens*				
200 mg L^{-1}	4.1	7.0	9.0	9.0
400 mg L^{-1}	4.2	7.5	9.0	9.0
600 mg L^{-1}	4.0	7.2	9.0	9.0
800 mg L^{-1}	-	1.2	8.0	9.0
1000 mg L^{-1}	-	-	3.2	4.0

T. pseudokoningii

T. virens

T. virens

Sporulated culture of *T. virens*

Plate 7. Inoculum production of *Trichoderma* spp.

2. Copper

Trichoderma virens was grown in Richards medium amended with increasing concentration of the respective metal. Thus the fungi were initially serially transferred to 200- 1000 mg L^{-1}. The fungal growth from the highest level of metal tolerated was used to study the tolerance to Cu.

In the first set of trial, *T. virens* was initially plated at the centre of petridishes containing PDA medium. After three days growth, 1cm diameter mycelial discs were cut and inoculated on Richards broth containing 200 mg of Cu. The growth of fungus in this amended broth was watched and subsequently transferred loopful of fungal biomass to the subsequent dilution *ie*., 400 mg. This was repeated with the concentrations 600, 800 and 1000 mg. The growth, colour and sporulation during serial transfers were studied (Tables 47 and 48).

The biomass from 200-800 mg was collected by filtration and dried to constant weight. The dried mycelial mat of the treated and untreated fungus was digested and analyzed for metal biosorption. Based on these observations the fungal culture grown on 800 mg Cu kg^{-1} was considered for continuing the training on Cu.

A loopful of *T. virens* trained on 800 mg Cu L^{-1} of Fe was taken and inoculated into 100 ml. Richard's broth containing 20 mg Cu L^{-1}. After the fungus was fully grown, one loopful of the fungal growth was further inoculated to medium containing 40 mg Cu L^{-1}. This was repeated by serial transfer from the lower concentration to the other higher concentrations. Mycelial mats were harvested after 7 days growth and dried to constant weight. The sporulation in the various concentrations was also studied. The wild or non-trained *T. virens* was also inoculated at various concentrations for comparison.

It was observed that the trained *T. virens* showed better tolerance and growth in Cu amended media as compared to the wild. Both the trained and the wild showed increase in mycelial weight with increase in the metal concentration up to 80 mg L^{-1}. The training allowed the fungus to grow profusely at 20-80 mg Cu L^{-1}, whereas the non-trained/wild isolate produced only very less growth in all the concentrations tested.

Table 47. Mycelial growth of *Trichoderma virens* (trained and non-trained) under different concentrations of Cu

Metal concentration mg Cu L^{-1}	Mycelial weight (g)	
	Trained *T. virens*	Non-trained *T. virens*
0	0.98	0.68
20	1.06	0.65
40	0.98	0.75
60	0.98	0.69
80	1.16	0.73
100	0.90	0.67

Table 48. Sporulation of *Trichoderma virens* (trained and non-trained) under different concentrations of Cu.

Treatments	Metal concentration (ppm)				
	20	40	60	80	100
Trained *T.virens*	++++	++++	+++	+++	+++
Non-trained *T.virens* (Wild)	++++	+++	+++	++	+

++++ - Profuse +++ - sufficient ++ - Scanty + - Poor

Both the isolates sporulated profusely in the lowest concentration (20 mg kg^{-1}) of Cu. The trained fungus showed the peak of sporulation at 80 mg L^{-1} and showed sufficient sporulation for all the concentrations tested. The non-trained produced lesser sporulation for the concentrations of 80 to 100 mg L^{-1}. Based on the mycelial growth and sporulation, single spore isolate of the trained fungus from 80 mg $L^{-1}$was maintained as the metal trained isolate of *T. virens* for use in further studies.

6. Single spore cultures

To determine whether the metal resistance is transferred to the spores, loopful of spores from the non and trained fungus were suspended independently in sterile distilled water and transferred to Richard's agar medium amended with different concentrations of the metal keeping non- amended medium inoculated with the wild (non-trained) fungus as check. The mycelial growth and sporulation for various treatments were measured.

Table 49. Mycelial growth of *T. virens* under different treatments of iron

Fungus	Mycelial growth (cm)			
	24 h.	48 h.	72 h.	98 h.
Non-trained *T.virens* (Wild)	3.0	6.9	7.8	9.0
Trained *T.virens*		-		
200 mg	4.1	7.0	9.0	9.0
400 mg	4.2	7.5	9.0	9.0
600 mg	4.0	7.2	9.0	9.0
800 mg	1.5	6.0	8.0	9.0
1000 mg	-		3.2	4.0

7. Comparison of sporulation of *T. virens* on metal training

The sporulation of *T.virens* (trained and non-trained) wild isolates were compared in the medium amended with graded doses of Fe. Both the isolates produced sporulation at 200 to 1000 mg L^{-1}. In media containg 800 mg L^{-1} Fe, there was medium sporulation. Trained *T. virens* sporulated more compared to non-trained.

Table 50. Details of sporulation by *Trichoderma virens*

	Metal concentration (mg Fe kg^{-1})				
	200	400	600	800	1000
Trained *T. virens*	++++	++++	++++	++	+
Non-trained *T. virens* (Wild)	++++	++++	+++	-	-

++++ - Profuse +++ - Sufficient ++ - Scanty + - Poor

V. Synergistic effect of hyperaccumulators and microbes

Six separate experiments were carried out for studying the synergistic effect of hyperaccumulators and microbes on heavy metal absorption, with *Eichhornia crassipes* as the test crop since it produce more biomass within a short span of time and accumulate most of the heavy metals without any toxicity symptoms as evidenced from the previous experiments. These are the general criteria to be followed for selection of a phytoextractor (Kramer, 2005). The accumulation factors for various elements were also highest for *Eichhornia crassipes*. The elements tested include heavy metals like Fe, Cu, Zn, Cd and Pb, and Al. *Eichhornia crassipes* were grown in pots containing graded doses of Fe (0, 400, 800, 1200 mg kg^{-1}), Zn (0, 10, 20, 40 mg kg^{-1}), Cu (0, 10, 20, 40 mg kg^{-1}), Cd and Pb (0, 5, 10, 20 mg kg^{-1}) and Al (0, 100, 200, 400 mg kg^{-1}). The microbial treatments include *Pseudomonas fluorescens* (Root dip in 2% slurry for 30 min), *Trichoderma virens* and *Trichoderma pseudokoningii* (Root dip for 30 minutes in 20 % slurry) along with control treatment. The treatments were replicated thrice.

The plants have established well under these treatments. The plants receiving higher doses of Fe (800 and 1200 mg kg^{-1}) showed typical toxicity symptoms initially, but subsequently recovered and showed satisfactory growth. On 45^{th} day the plants were harvested. Fresh weight and dry weight of shoot and root were taken. The samples were separated in to root and shoot, dried, powdered and subjected to chemical analysis for Zn, Cu, Cd, Pb, Fe and Al. The results on dry matter production and phytoextraction ability of the tested plants for Zn, Cu, Cd, Pb, Fe and Al are presented in Tables 51 to 68.

1. Zinc

The dry matter production, Zn content and Zn removal by *Eichhornia crassipes* were significantly influenced the treatments. The treatment without microbial inoculation receiving Zn @10 mg kg^{-1} of soil recorded the highest shoot weight and root weight but at higher levels of Zn the dry matter production decreased. The treatment receiving microbial inoculants showed an increase in shoot and root dry matter production up to 20 mg Zn kg^{-1} of soil. Among the microbial cultures, *Trichoderma virens* receiving Zn @ of 20 mg kg^{-1} showed the highest dry weight.

Table 51. Dry matter production by *Eichhornia crassipes* at different levels of Zn (mg plant^{-1})

Treatments, Rates of Zn (mg kg^{-1})	Control		*Pseudomonas fluorescens*		*Trichoderma virens*		*Trichoderma pseudokoningii*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero Zn	20.3	14.6	24.9	17.3	20.9	16.3	20.8	14.8
10	34.9	21.5	25.2	18.0	26.8	18.8	30.2	20.9
20	30.6	20.5	32.8	22.9	34.1	23.3	31.6	22.2
40	29.8	19.8	30.0	20.2	30.3	20.4	28.1	22.5
CD (0.05)	0.49	0.24	0.49	0.24	0.49	0.24	0.49	0.24

Table 52. Zn content in *Eichhornia crassipes* at different levels of Zn (mg kg^{-1})

Treatments, Rates of Zn (mg kg^{-1})	Control		*Pseudomonas fluorescens*		*Trichoderma virens*		*Trichoderma pseudokoningii*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero Zn	65	65	70	70	65	65	70	70
10	120	315	250	750	120	700	300	500
20	210	550	700	800	210	850	600	750
40	250	750	900	1250	500	1050	701	801
CD (0.05)	5.86	4.51	5.86	4.51	5.86	4.51	5.86	4.51

The Zn content of both shoot and root showed positive response to the graded doses of Zn by showing an increase in Zn content with levels of Zn for all microbial treatments and control. The application of microbial cultures has increased the Zn content of both shoot and root. *Pseudomonas fluorescens* recorded the highest value for Zn in both shoot and root at 40 mg Zn kg^{-1} of soil.

Table 53. Zn removal by *Eichhornia crassipes* at different levels of Zn (mg $plant^{-1}$)

Treatments, Rates of Zn (mg kg^{-1})	Control		*Pseudomonas fluorescens*		*Trichoderma virens*		*Trichoderma pseudokoningii*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero Zn	1.3	0.9	1.7	1.2	1.4	10.6	1.5	1.0
10	4.1	7.5	6.3	13.5	3.2	13.1	9.1	10.5
20	6.4	11.3	22.9	18.3	7.2	19.8	18.9	16.7
40	7.4	14.9	27.0	25.2	15.1	21.4	19.6	18.0
CD (0.05)	0.06	0.11	0.06	0.11	0.06	0.11	0.06	0.11

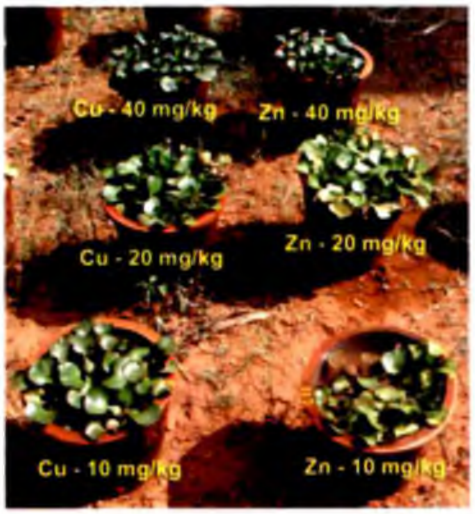
The microbial application had showed a synergistic effect on Zn removal from soil and this was more evident on Zn removal by shoot. *Pseudomonas fluorescens* had removed the highest quantity of Zn (Fig. 6) followed by *Trichoderma pseudokoningii.* The Zn removal by shoot was more compared to root for all treatments except control. This might be due to better mobilization of the element in presence of microbes.

2. Copper

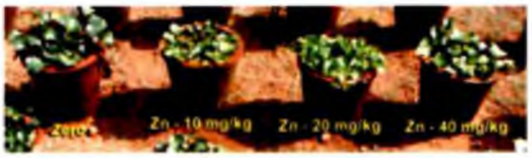
Table 54. Dry matter production by *Eichhornia crassipes* at varying levels of Cu (mg $plant^{-1}$)

Treatments, Rates of Cu (mg kg^{-1})	Control		*Pseudomonas fluorescens*		*Trichoderma virens*		*Trichoderma pseudokoningii*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero Cu	21.5	14.6	21.0	14.0	20.0	14.6	20.6	14.2
10	29.7	22.5	28.9	19.8	29.4	20.2	34.8	23.2
20	26.9	18.7	32.9	23.2	30.6	21.3	26.9	18.6
40	21.9	15.0	22.0	15.7	34.8	24.0	24.6	17.2
CD (0.05)	0.39	0.27	0.39	0.27	0.39	0.27	0.39	0.27

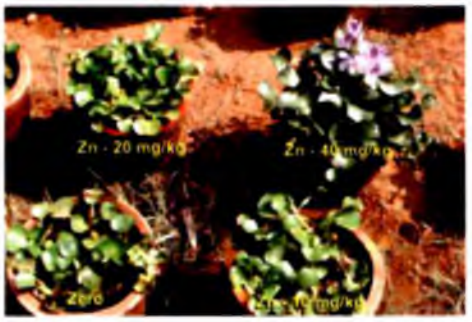
Absolute control



Without microbial treatments

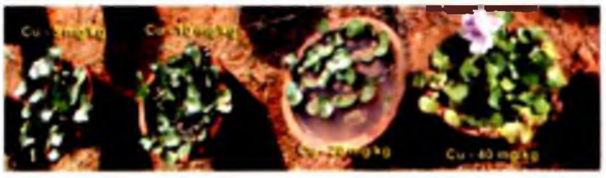


Zn + *P. fluorescens*

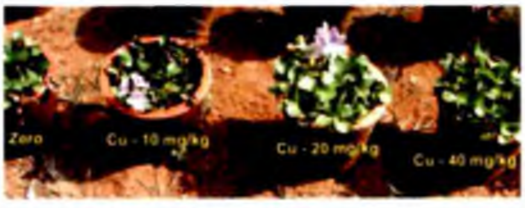


Zn + *Trichoderma virens*

Zn + *Trichoderma pseudokoningii*



Cu + *P. fluorescens*



Cu + *Trichoderma virens*

Cu + *Trichoderma pseudokoningii*

Plate 8. Effect of microbes on phytoextraction of Zn and Cu by *E. crassipes*

The treatments had significantly influenced the dry matter production, shoot Cu content and Cu removal by *Eichhornia crassipes*. The microbial application had showed differential performance towards dry matter production under graded levels of Cu. The treatment without microbial application and that received *Trichoderma pseudokoningii* showed enhanced dry matter production up to 10 mg Cu $kg^{-1}$of soil only, while *Pseudomonas fluorescens* responded positively up to 20 and *Trichoderma virens* up to 40 mg Cu kg^{-1} soil respectively. The total Dry matter production was also highest for *Trichoderma virens*.

Table 55. Cu content in *Eichhornia crassipes* at different levels of Cu (mg kgt^{-1})

Treatments, Rates of Cu (mg kg^{-1})	Control		*Pseudomonas fluorescens*		*Trichoderma virens*		*Trichoderma pseudokoningii*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero Cu	11.0	9.0	1.4	8.0	1.7	70	1.8	52
10	14.0	20	3.2	88	5.1	155	5.2	161
20	42.5	52	7.1	132	7.6	210	7.5	216
40	67.5	63	8.4	260	8.9	311	8.8	346
CD (0.05)	NS	1.69	NS	1.69	NS	1.69	NS	1.69

The application of microbial treatments have significantly reduced the Cu content of shoot and had greatly increased the Cu content of root in *Eichhornia crassipes*. In all the cases it showed positive response to the graded levels of Cu. The highest Cu content was recorded by the *Trichoderma pseudokoningii* followed by *Trichoderma virens*. However, the Cu removal by both shoot and root was highest for *Trichoderma virens*, (Fig. 7) definitely under the influence of higher dry matter production. In general, Cu is the most difficultly phytoextracted element from soil due to its high affinity for bonding with organic molecules.

Table 56. Cu removal by *Eichhornia crassipes* at different levels of Cu (mg plant^{-1})

Treatments, Rates of Cu (mg kg^{-1})	Control		*Pseudomonas fluorescens*		*Trichoderma virens*		*Trichoderma pseudokoningii*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero Cu	0.24	0.13	0.03	0.11	0.03	1.02	0.04	0.74
10	0.42	0.45	0.09	1.74	0.15	3.10	0.18	3.80
20	1.14	0.97	0.23	3.06	0.24	4.47	0.20	4.05
40	1.48	0.95	0.18	4.08	0.31	7.46	0.22	6.02
CD (0.05)	0.93	0.11	0.93	0.11	0.93	0.11	0.93	0.11

3. Iron

Application of microbial treatments had significantly reduced the dry matter production by *Eichhornia crassipes* at higher levels of Fe compared to the control treatment. The control treatment alone showed positive response to levels of Fe while the microbial treatments showed better performance in the absence of Fe towards dry matter production. Both shoot and root Fe content showed positive relation with graded levels of Fe. The concentration was much higher in shoot compared to root. Though the highest Fe content was recorded by the treatment receiving "*Trichoderma pseudokoningii*" + 1200 mg Fe kg^{-1} of soil" the highest quantity was removed by "control treatment receiving 1200 mg Fe kg^{-1} of soil" (Fig. 8). It can be concluded that the tested microbes have no synergistic effect on Fe removal and at the same time expressed an antagonistic effect. The microbes have assisted the plants to perform better under zero levels of Fe by producing more biomass and Fe removal. Reports are there that at insufficient/deficient levels of Fe, *Trichoderma virens* helped the plants to extract more Fe with the help of siderophores.

Table 57. Dry matter production by *Eichhornia crassipes* at graded levels of Fe (mg plant^{-1})

Treatments, Rates of Fe (mg kg^{-1})	Control		*Pseudomonas fluorescens*		*Trichoderma virens*		*Trichoderma pseudokoningii*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero Fe	21.5	15.1	33.2	23.6	34.8	24.0	33.4	23.6
400	28.2	19.8	28.8	20.7	30.4	20.6	30.2	21.3
800	39.7	27.6	28.0	19.8	28.2	19.7	29.1	20.5
1200	42.3	36.2	24.9	20.2	22.7	16.4	29.0	20.0
CD (0.05)	0.24	0.19	0.24	0.19	0.24	0.19	0.24	0.19

Table 58. Fe content in *Eichhornia crassipes* at graded levels of Fe (mg kg^{-1})

Treatments, Rates of Fe (mg kg^{-1})	Control		*Pseudomonas fluorescens*		*Trichoderma virens*		*Trichoderma pseudokoningii*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero Fe	10600	6420	13115	6835	14878	7124	11405	6712
400	22800	8405	24810	8738	28139	8916	23205	8629
800	28300	9834	31718	10325	34408	11424	30710	9930
1200	32600	11528	33570	12970	35388	13407	33480	12225
CD (0.05)	9.43	59.99	9.43	59.99	9.43	59.99	9.43	59.99

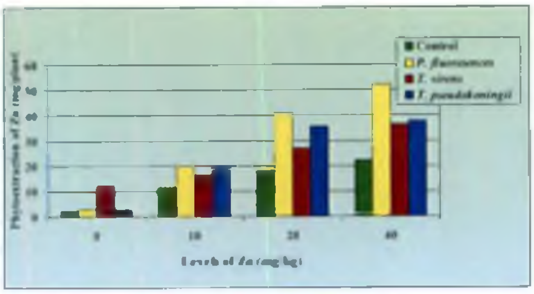


Fig. 6. Phytoextraction of zinc in presence of microbes

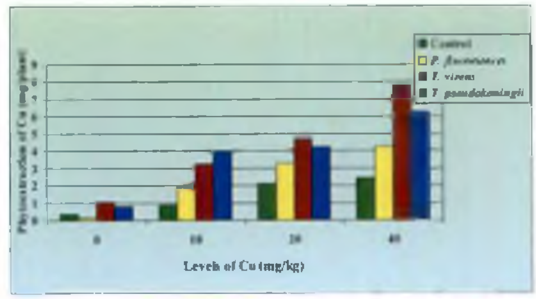


Fig.7. Phytoextraction of Cu in presence of microbes

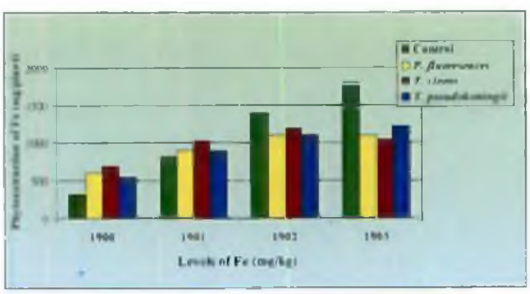


Fig.8. Phytoextraction of Fe in presence of microbes

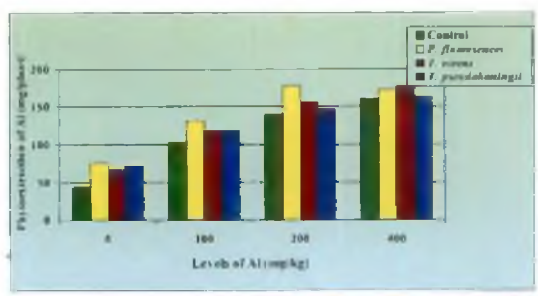


Fig.9. Phytoextraction of Al in presence of microbes

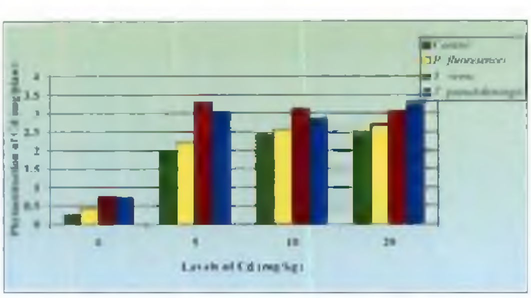


Fig.10. Phytoextraction of Cd in presence of microbes

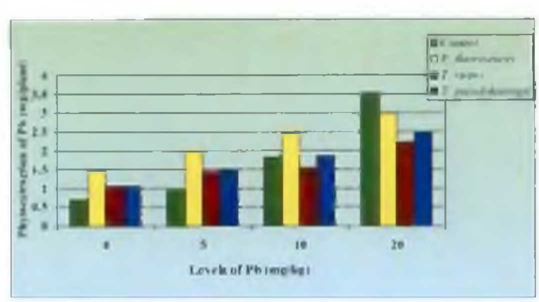


Fig.11. Phytoextraction of Pb in presence of microbes

Table 59 . Fe removal by *Eichhornia crassipes* at different levels of Fe (mg plant^{-1})

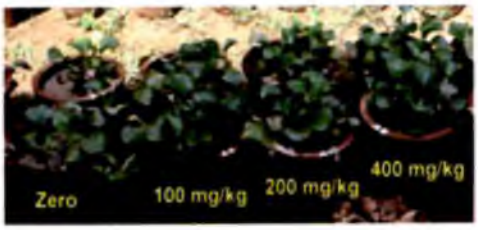
Treatments, Rates of Fe (mg kg^{-1})	Control		*Pseudomonas fluorescens*		*Trichoderma virens*		*Trichoderma pseudokoningii*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero Fe	227	96	435	161	517	170	381	158
400	642	166	714	180	855	183	700	184
800	1123	271	888	204	970	225	894	204
1200	1378	417	835	261	803	219	971	244
CD (0.05)	3.04	1.20	3.04	1.20	3.04	1.20	3.00	1.10

4. Aluminium

Dry matter production by *Eichhornia crassipes* showed an increase up to the level of 100 mg Al kg^{-1} of soil for most of the treatments and the highest value was recorded by *Pseudomonas fluorescens*. Al concentration within the plant parts maintained positive relation with garded levels of Al. Highest values were recorded by the treatment receiving *Trichoderma virens* + Al @ 400 mg kg^{-1}.

Table 60. Dry matter production by *Eichhornia crassipes* at different levels of Al (mg plant^{-1})

Treatments, Rates of Al (mg kg^{-1})	Control		*Pseudomonas fluorescens*		*Trichoderma virens*		*Trichoderma pseudokoningii*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero Al	21.7	15.3	24.8	28.5	29.3	20.6	33.0	23.2
100	30.3	21.3	34.7	24.6	30.9	21.0	31.5	21.8
200	26.9	22.9	36.0	24.0	28.8	20.4	28.6	20.1
400	22.4	24.6	26.7	22.1	28.8	20.3	27.2	19.2
CD (0.05)	0.78	0.19	0.78	0.19	0.78	0.19	0.78	0.19



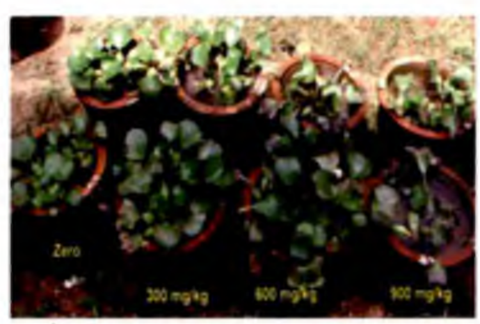
P. fluorescens + Al

T.pseudokoningii + Al

Trichoderma virens + Al

Experimental site

P. fluorescens + Fe



Trichoderma pseudokoningii + Fe

Tricoderma virens + Fe

Fe toxicity symptoms
in Eichornia

Fe toxicity symptoms

Plate 9. Effect of microbes on phytoextraction of Fe and Al by *E. crassipes*

Table 61. Al content of *Eichhornia crassipes* at graded levels of Al (mg kgt^{-1})

Treatments, Rates of Al (mg kg^{-1})	Control		*Pseudomonas fluorescens*		*Trichoderma virens*		*Trichoderma pseudokoningii*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero Al	736	1810	839	1933	874	1959	848	1930
100	1728	2300	1911	2644	1959	2784	1926	2635
200	2414	3244	2631	3460	2770	3734	2693	3476
400	2925	3830	2986	4205	3047	4388	2990	4247
CD (0.05)	59.90	12.13	59.90	12.13	59.90	12.13	59.90	12.13

Comparing the amount of Al accumulated, it was observed that root had accumulated more of Al compared to shoot. The highest quantity of Al was removed by *Pseudomonas fluorescens* receiving Al @ 200 mg kg^{-1} followed by *Trichoderma virens* receiving Al @ 400 mg kg^{-1} (Fig. 9).

Table 62. Al removal by *Eichhornia crassipes* at graded levels of Al (mg plant^{-1})

Treatments, Rates of Al (mg kg^{-1})	Control		*Pseudomonas fluorescens*		*Trichoderma virens*		*Trichoderma pseudokoningii*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero Al	15.9	27.7	20.8	55.1	25.6	40.3	27.9	44.7
100	52.3	49.0	66.3	65.0	60.5	58.4	60.8	57.4
200	64.9	74.2	94.7	83.0	79.7	76.1	76.4	69.8
400	65.5	94.2	79.7	92.9	87.7	89.0	81.3	81.5
CD (0.05)	6.49	3.21	6.49	3.21	6.49	3.21	6.49	3.21

5. Cadmium

Application of microbial cultures had favourably influenced the dry matter production by *Eichhornia crassipes* only up to a level of 10 mg Cd kg^{-1} of soil. At higher levels of Cd there was a decrease in dry matter production, might be due to the toxic effect of excess Cd in soil. The dry matter production was highest for *Trichoderma virens* at 5 mg Cd kg^{-1} of soil. Cd content of both shoot and root increased with levels of Cd and the highest value was obtained for *Trichoderma virens* at 20 mg Cd kg^{-1} in shoot. The Cd content of the shoot was very high compared to that of root. The Cd removal from soil was also highest for *Trichoderma virens* at 10 mg Cd kg^{-1} (Fig. 10). The shoot had removed more Cd from soil compared to root.

Table 63. Drymatter production by *Eichhornia crassipes* at graded levels of Cd (mg plant^{-1})

Treatments, Rates of Cd (mg kg^{-1})	Control		*Pseudomonas fluorescens*		*Trichoderma virens*		*Trichoderma pseudokoningii*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero Cd	21.4	15.1	27.2	25.6	34.1	23.8	33.9	23.9
5	34.5	14.9	32.4	22.9	35.2	24.3	39.2	27.5
10	33.3	13.0	30.4	21.4	33.7	23.8	32.7	23.2
20	30.5	8.0	28.1	19.8	27.1	19.1	31.3	22.2
CD (0.05)	0.20	0.27	0.20	0.27	0.20	0.27	0.20	0.27

Table 64. Cd content in *Eichhornia crassipes* at graded levels of Cd (mg kg^{-1})

Treatments, Rates of Cd (mg kg^{-1})	Control		*Pseudomonas fluorescens*		*Trichoderma virens*		*Trichoderma pseudokoningii*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero Cd	12	1.4	14	1.9	21	2.2	20	2.0
5	53	12	59	13	76	15	68	14
10	68	14	73	16	81	19	75	18
20	76	17	81	21	94	27	90	22
CD (0.05)	0.73	0.18	0.73	0.18	0.73	0.18	0.73	0.18

Table 65 . Cd removal by *Eichhornia crassipes* at graded levels of Cd (mg plant^{-1})

Treatments, Rates of Cd (mg kg^{-1})	Control		*Pseudomonas fluorescens*		*Trichoderma virens*		*Trichoderma pseudokoningii*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero Cd	0.25	0.02	0.38	0.05	0.72	0.05	0.68	0.05
5	1.82	0.18	1.91	0.30	2.67	0.37	2.66	0.38
10	2.26	0.17	2.21	0.35	2.67	0.45	2.45	0.42
20	2.31	0.13	2.27	0.42	2.54	0.51	2.81	0.49
CD (0.05)	1.82	NS	1.82	NS	1.82	NS	1.82	NS

6. Lead

The inoculation of microbial cultures had negatively influenced the dry matter production by *Eichhornia crassipes* under graded doses of Pb since the control treatment showed higher values for dry matter production and Pb removal. The control treatment receiving Pb @ 20 mg kg^{-1} produced highest quantity of dry matter. Among the microbial treatments, *Pseudomonas fluorescens* showed an increase in dry matter production up to 10 mg Cd kg^{-1}of soil only, while others have responded positively to 20 mg Pb kg^{-1}of soil. Pb content of all the treatments showed an increase with levels of Pb. The highest content was recorded by *Pseudomonas fluorescens* receiving Pb @ 20 mg kg^{-1}. The highest quantity of Pb was removed by control treatment receiving Pb @ 20 mg kg^{-1} (Fig. 11).

Table 66. Dry matter production by *Eichhornia crassipes* at graded levels of Pb (mg plant^{-1})

Treatments, Rates of Pb (mg kg^{-1})	Control		*Pseudomonas fluorescens*		*Trichoderma virens*		*Trichoderma pseudokoningii*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero Pb	21.7	15.2	32.9	23.3	25.5	18.0	26.6	18.9
5	24.9	18.4	33.7	23.7	31.2	21.7	30.1	21.2
10	35.0	24.5	34.7	24.5	26.8	21.9	33.1	22.6
20	48.6	35.1	30.3	24.3	33.0	23.2	35.2	24.8
CD (0.05)	0.40	NS	0.40	NS	0.40	NS	0.40	NS

Table 67. Pb content in *Eichhornia crassipes* at graded levels of Pb (mg kg^{-1})

Treatments, Rates of Pb (mg kg^{-1})	Control		*Pseudomonas fluorescens*		*Trichoderma virens*		*Trichoderma pseudokoningii*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero Pb	22	15	30	21	24	20	24	21
5	25	18	39	28	30	22	32	24
10	35	25	48	33	37	25	38	26
20	48	35	64	44	41	37	44	39
CD (0.05)	0.35	0.36	0.35	0.36	0.35	0.36	0.35	0.36

Table 68. Pb removal by *Eichhornia crassipes* at different levels of Pb (mg plant^{-1})

Treatments, Rates of Pb (mg kg^{-1})	Control		*Pseudomonas fluorescens*		*Trichoderma virens*		*Trichoderma pseudokoningii*	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Zero Pb	0.48	0.22	0.98	0.49	0.61	0.43	0.65	0.39
5	0.62	0.33	1.32	0.65	0.95	0.48	0.95	0.51
10	1.22	0.61	1.67	0.80	0.98	0.55	1.26	0.59
20	2.33	1.20	1.92	1.06	1.36	0.85	1.54	0.95
CD (0.05)	0.27	NS	0.27	NS	0.27	NS	0.27	NS

Microbial inoculation has a favourable influence on the extraction of Zn Cu, Al and Cd. The synergistic effect due to microbial treatments was very much evident in case of Zn for both shoot and root while for Cu, it was evident for root only. The effect was more for *Pseudomonas fluorescens* in case of Zn and *Trichordma virens* for Cu. Instead of synergistic effect, Fe showed an antagonistic effect due to microbial treatments. In case of Al, the synergistic effect was more evident on shoot and among the microbes, *Pseudomonas fluorescens* showed profound effect.. The microbial effect was synergistic for Cd for both shoot and root, but it was more evident at lower levels of Cd. The effect was more prominent for *Trichordma virens*. The microbial effect was not synergistic for Pb.

VI. Pot culture experiments for bioremediation/ detoxification of contaminants

Different materials including organic manures, soil amendments, composted wastes and microbial inoculants were tested for their efficiency in inactivating metal present in toxic quantities (Fe and Al). The materials were tested by growing rice under graded doses of iron and aluminium with the following treatments. The treatments were replicated thrice.

Levels of Fe

Zero, 300, 600 and 900 mg kg^{-1}

Levels of Al

Zero, 100 and 200 mg kg^{-1}

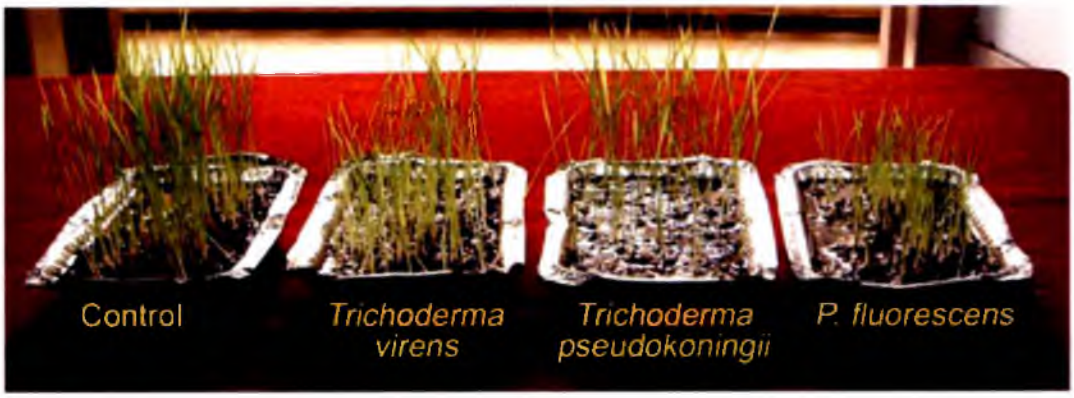
Treatments

1. Absolute control
2. Application of FYM @ 5 t ha^{-1}
3. Application of vermicompost @ 2.5 t ha^{-1}
4. Application of microbial inoculants

   a) *Pseudomonas fluorescens* @ 2% - Root dip for 30 min

   b) *Trichoderma virens*- @ Seed treatment and seedling root dip for 30 min in 20% slurry

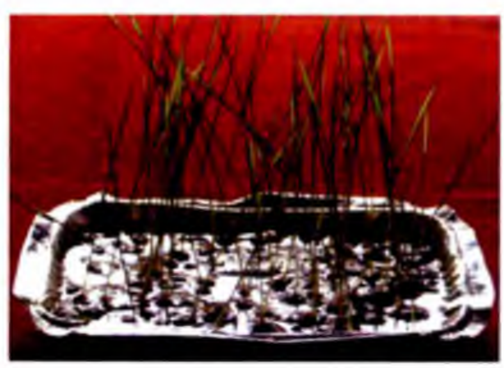
   c) *Trichoderma pseudokoningii*- @ Seed treatment and seedling root dip for 30 min in 20% slurry



Control

P. fluorescens

Trichoderma virens



Trichoderma pseudokoningii

Plate 10. Rice seedlings inoculated with different microbial cultures

For treating the seedling with *Pseudomonas fluorescens*, two per cent (2%) slurry of the same is prepared by mixing 20 g *Pseudomonas fluorescens* with one liter of water and the seedling roots were dipped in the slurry for 30 minutes before transplanting.

For *Trichoderma virens* and *Trichoderma pseudokoningii*, 20% slurry was prepared by mixing 200 g of microbial inoculant with one liter of water. Seed treatment was done with the above slurry for both *Trichoderma virens* and *Trichoderma pseudokoningii* before sowing the seeds in the nursery. Seedling roots were also dipped in the slurry for 30 minutes before transplanting.

Lime @ 600 kg ha^{-1} was applied to all treatments since the soils are highly acidic and the crop could not sustain with out the addition of lime. Rice variety "Uma" was planted and observations were taken. The crop was harvested and biometric observations, dry weight of grain, straw and root were recorded. The dried samples were analysed for their Fe and Al content.

VI.a. Bioremediation of iron

The results of the experiment for bioremediation of iron are presented in tables 69 to 77. The grain yield and straw yield were significantly influenced by the treatments and the highest values were recorded by the treatment receiving "*Trichoderma virens* + Fe @ 600 mg kg^{-1}". In general, yield increase for grain and straw was observed up to the Fe level of 600 mg kg^{-1}. This shows that with good management practices, higher levels of Fe can be made use for increasing the yield. Comparing the effect of manures on grain yield under graded levels of Fe, vermicompost was significantly superior to FYM, while their effect was non significant for straw yield and root weight. In all the cases root weight was found to increase with levels of Fe and the highest root weight was recorded by the treatment receiving *Trichoderma virens* with FYM + 900 mg Fe kg^{-1} of soil.

Table 69. Grain yield of rice at different levels of Fe (g $plant^{-1}$) at harvest

Treatments, Rates of Fe (mg kg^{-1})	Control		*Trichoderma virens*		*Trichoderma pseudokoningii*		*Pseudomonas fluorescens*	
	FYM	VC	FYM	VC	FYM	VC	FYM	VC
Zero Fe	12.0	12.4	13.1	13.0	12.5	12.6	13.0	12.9
300	12.5	13.2	13.8	13.7	13.1	14.0	13.2	13.3
600	13.9	15.7	14.7	16.7	14.2	16.6	14.4	15.2
900	13.4	15.1	14.3	15.9	13.5	15.3	13.8	15.0
CD (0.05)	Organic manure : 0.0791 Microbial inoculants/iron : 0.1119							

Table 70. Straw yield of rice at different levels of Fe (g $plant^{-1}$) at harvest

Treatments, Rates of Fe (mg kg^{-1})	Control		*Trichoderma virens*		*Trichoderma pseudokoningii*		*Pseudomonas fluorescens*	
	FYM	VC	FYM	VC	FYM	VC	FYM	VC
Zero Fe	20.4	20.6	22.1	22.0	20.8	20.8	20.9	20.9
300	22.1	22.5	23.4	23.7	21.6	21.5	22.8	22.9
600	23.8	23.7	24.9	24.7	24.1	24.0	24.3	24.4
900	23.6	23.4	24.5	23.9	23.2	24.4	24.1	24.1
CD (0.05)	Organic manure : NS Microbial inoculants/iron : 0.0959							

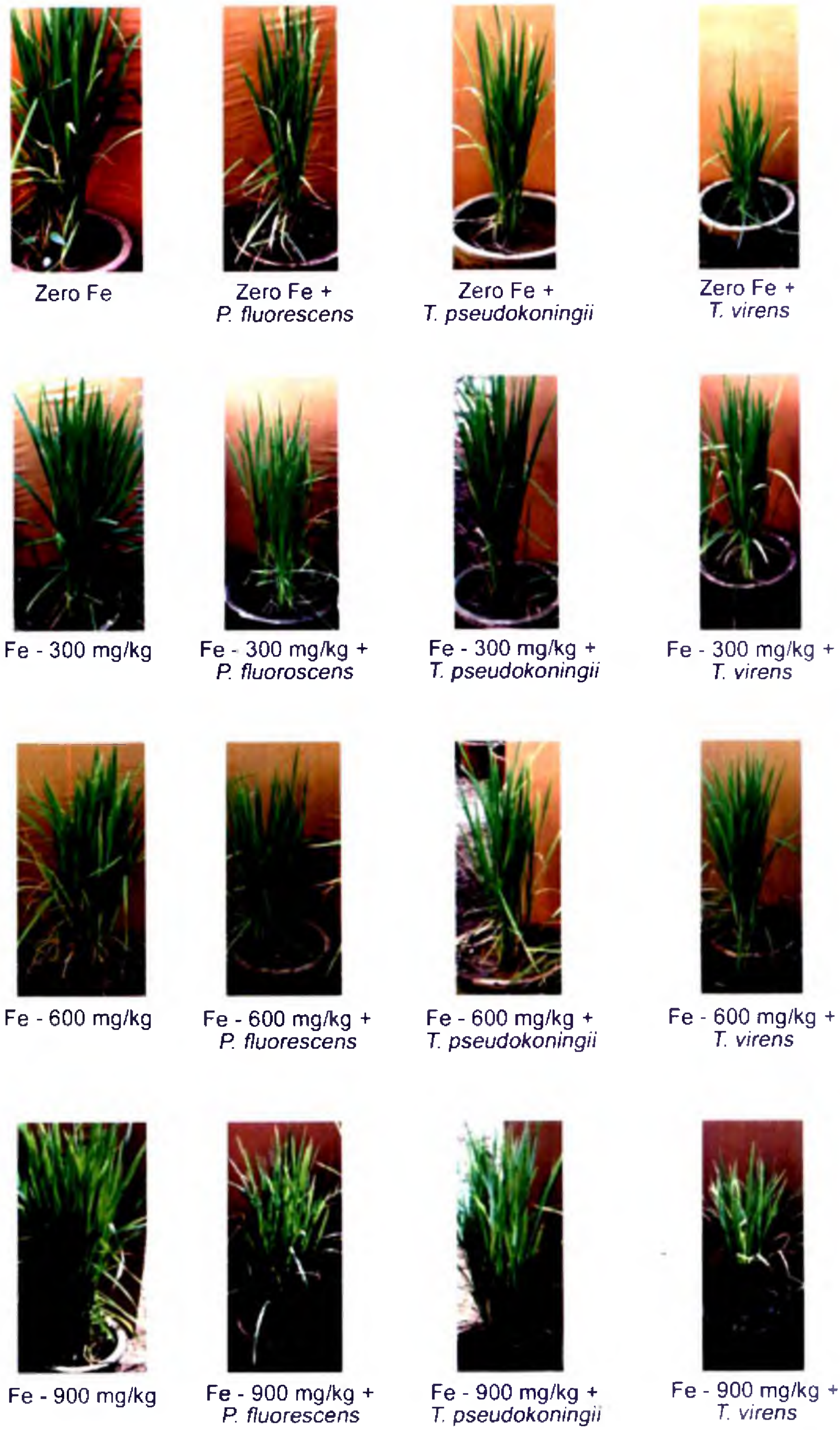


Plate 11. Rice under different levels of Fe in microbial inoculated treatments

Table71. Root weight (g plant^{-1}) of rice at different levels of Fe at harvest

Treatments, Rates of Fe (mg kg^{-1})	Control		*Trichoderma virens*		*Trichoderma pseudokoningii*		*Pseudomonas fluorescens*	
	FYM	VC	FYM	VC	FYM	VC	FYM	VC
Zero Fe	3.2	3.3	3.6	3.5	3.3	3.3	3.3	3.4
300	3.7	3.6	4.1	4.2	3.7	3.7	3.8	3.9
600	3.9	3.8	4.4	4.4	3.9	3.9	4.0	4.0
900	4.0	3.9	4.6	4.5	4.1	3.9	4.2	4.2
CD (0.05)	Organic manure : NS Microbial inoculants/iron : 0.0328							

Table 72. Fe content of rice grain (mg kg^{-1}) at different levels of Fe at harvest

Treatments, Rates of Fe (mg kg^{-1})	Control		*Trichoderma virens*		*Trichoderma pseudokoningii*		*Pseudomonas fluorescens*	
	FYM	VC	FYM	VC	FYM	VC	FYM	VC
Zero Fe	315	420	380	375	380	388	388	463
300	388	425	375	425	405	400	422	500
600	396	430	388	435	420	408	425	525
900	415	438	393	488	463	463	445	605
CD (0.05)	Organic manure : 14.19 Microbial inoculants/iron : 20.08							

The highest Fe content was recorded by *Pseudomonas fluorescens* with vermicompost and Fe @ 900 mg kg^{-1} in grain. Control treatment with microbial application reduced the Fe removal by grain. All the treatments showed positive response to graded doses of Fe. Application of vermicompost has enhanced the Fe removal by rice grains.

Table 73. Fe content of rice straw (mg kg^{-1}) at different levels of Fe at harvest

Treatments, Rates of Fe (mg kg^{-1})	Control		*Trichoderma virens*		*Trichoderma pseudokoningii*		*Pseudomonas fluorescens*	
	FYM	VC	FYM	VC	FYM	VC	FYM	VC
Zero Fe	348	337	300	320	301	293	275	275
300	350	358	308	331	318	300	320	305
600	368	390	316	342	323	323	350	308
900	374	500	333	345	338	395	355	324
CD (0.05)	Organic manure : 9.27 Microbial inoculants/iron : 13.11							

Fe content of straw also showed positive response to graded doses of Fe for all treatments. In contrast to the grain Fe content, microbial application had reduced the Fe content of straw and higher values were recoded by the control treatment. The microbial inoculation had facilitated the Fe movement to the grains. Application of vermicompost has enhanced the Fe removal by rice straw compared to FYM.

Table 74. Fe content (mg kg^{-1}) of rice root at different levels of Fe at harvest

Treatments, Rates of Fe (mg kg^{-1})	Control		*Trichoderma virens*		*Trichoderma pseudokoningii*		*Pseudomonas fluorescens*	
	FYM	VC	FYM	VC	FYM	VC	FYM	VC
Zero Fe	3735	2300	3000	2950	3500	2800	3000	2850
300	3850	3525	3250	3250	3800	2000	3125	3575
600	4500	4350	3625	4025	4350	2200	3525	3750
900	6000	4450	4600	4275	5250	2850	4500	4000
CD (0.05)	Organic manure : 9.27 Microbial inoculants/iron : 13.11							

Fe content of the root was very high for all treatments compared to straw and grain. The highest value was recorded by control receiving FYM and Fe @ 900 mg kg^{-1}.

Table 75. Fe removal by rice grain at different levels of Fe (mg $plant^{-1}$) at harvest

Treatments, Rates of Fe (mg kg^{-1})	Control		*Trichoderma virens*		*Trichoderma pseudokoningii*		*Pseudomonas fluorescens*	
	FYM	VC	FYM	VC	FYM	VC	FYM	VC
Zero Fe	3.78	5.20	4.98	5.52	4.75	4.88	5.04	5.97
300	4.85	5.61	5.17	5.82	5.30	5.60	5.57	6.65
600	5.50	6.75	5.70	7.26	5.96	6.77	6.12	7.90
900	5.56	6.61	5.61	7.75	8.25	7.08	6.14	9.07
CD (0.05)	Organic manure : 0.018 Microbial inoculants/iron : 0.025							

Table 76 . Fe removal by straw of rice at different levels of Fe (mg $plant^{-1}$) at harvest

Treatments, Rates of Fe (mg kg^{-1})	Control		*Trichoderma virens*		*Trichoderma pseudokoningii*		*Pseudomonas fluorescens*	
	FYM	VC	FYM	VC	FYM	VC	FYM	VC
Zero Fe	7.09	6.94	6.63	7.04	6.26	6.09	5.74	5.74
300	7.73	8.05	7.20	7.84	6.86	6.45	7.29	6.98
600	8.75	9.20	7.87	8.44	7.78	7.75	8.50	7.5
900	8.82	11.7	8.16	8.24	7.84	9.36	8.55	8.04
CD (0.05)	Organic manure : 0.027 Microbial inoculants/iron : 0.019							

Table 77. Fe removal (mg plant^{-1}) by rice root at different levels of Fe at harvest

Treatments, Rates of Fe (mg kg^{-1})	Control		*Trichoderma virens*		*Trichoderma pseudokoningii*		*Pseudomonas fluorescens*	
	FYM	VC	FYM	VC	FYM	VC	FYM	VC
Zero Fe	11.9	7.5	10.8	10.3	11.5	9.2	9.9	9.7
300	14.2	12.6	13.3	13.6	14.0	7.4	12.5	13.9
600	17.5	16.5	15.9	17.7	16.9	8.5	14.1	15.0
900	24.0	17.3	21.1	12.3	20.5	11.1	18.9	16.8
CD (0.05)	Organic manure : 0.131 Microbial inoculants/iron : 0.186							

The Fe removal by grain was also highest for *Pseudomonas fluorescens* with vermicompost and Fe @ 900 mg kg^{-1} in grain. Control treatment with microbial application reduced the Fe removal by grain. Application of vermicompost has enhanced the Fe removal by rice grains. In contrast to the Fe removal by grain, microbial inoculation had reduced the Fe removal by straw and higher values were recoded by the control treatment. The highest value was recorded by the control treatment receiving vemicompost and Fe @ 900 mg kg^{-1}. The root also followed the same trend but, the removal was highest for treatment receiving FYM and Fe @ 900 mg kg^{-1}. The ability of microorganisms in inactivating and there by reducing the uptake of potentially toxic elements was confirmed through this study.

VI.b. Bioremediation of aluminium

The results of the experiment for bioremediation of aluminium are presented in tables 78 to 86. The treatment had significantly influenced yield parameters and Al content of the crop.

Table 78 . Grain yield of rice at different levels of Al (g plant^{-1}) at harvest

Treatments, Rates of Al (mg kg^{-1})	Control		*Trichoderma virens*		*Trichoderma pseudokoningii*		*Pseudomonas fluorescens*	
	FYM	VC	FYM	VC	FYM	VC	FYM	VC
Zero Al	12.0	13.0	13.1	12.4	12.5	13.0	12.6	12.9
100	10.6	12.1	11.3	12.1	9.6	12.0	9.8	12.2
200	10.2	11.6	10.8	11.5	9.3	11.4	9.2	11.5
CD (0.05)	Organic manure : 0.042 Aluminium : 0.052 Microbial inoculants : 0.059							

Table 79. Straw yield of rice at different levels of Al (g plant^{-1}) at harvest

Treatments, Rates of Al (mg kg^{-1})	Control		*Trichoderma virens*		*Trichoderma pseudokoningii*		*Pseudomonas fluorescens*	
	FYM	VC	FYM	VC	FYM	VC	FYM	VC
Zero Al	20.4	20.6	22.1	22.0	20.8	20.8	20.9	20.9
100	20.3	20.6	20.7	20.9	20.6	20.8	20.6	20.7
200	18.4	18.8	19.5	19.1	19.0	18.9	19.0	19.2
CD (0.05)	Organic manure : 0.054 Aluminium : 0.066 Microbial inoculants : 0.077							

Zero Al +
T. pseudokoningii

Zero Al +
T. virens

Zero Al
without microbial
treatment

Zero Al +
P. fluorescens

Al - 100 mg / kg +
T. pseudokoningii

Al - 100 mg / kg +
T. virens

Al - 100 mg / kg +
without microbial
treatment

Al - 100 mg / kg +
P. fluorescens

Al - 200 mg / kg +
T. pseudokoningii

Al - 200 mg / kg +
T. virens

Al - 200 mg / kg +
without microbial
treatment

Al - 200 mg / kg +
P. fluorescens

Plate 12. Rice under different levels of Al in microbial inoculated treatments

Table 80. Root weight (g plant^{-1}) of rice at different levels of Al at harvest

Treatments, Rates of Al (mg kg^{-1})	Control		*Trichoderma virens*		*Trichoderma pseudokoningii*		*Pseudomonas fluorescens*	
	FYM	VC	FYM	VC	FYM	VC	FYM	VC
Zero Al	3.2	3.3	3.6	3.5	3.3	3.3	3.8	3.4
100	2.8	2.6	3.1	3.0	2.9	2.9	2.9	3.0
200	2.4	2.1	3.0	2.8	2.7	2.8	2.8	2.8
CD (0.05)	Organic manure : 0.037 Aluminium : 0.045 Microbial inoculants : 0.052							

Grain yield was highest for the treatment receiving *Trichoderma virens* along with FYM at zero level of Al, clearly indicating the adverse effect of Al on rice yield. The lowest yield was recorded by *Pseudomonas fluorescens* with 200 mg Al kg^{-1} of soil. Straw yield also followed the same trend as that of grain. The root weight was also affected by the treatments and control treatment recorded lowest weight, clearly expressing the favourable influence of microbes on crop growth by reducing the uptake of toxic elements.

Table 81. Al content of rice grain (mg kg^{-1}) at graded levels of Al at harvest

Treatments, Rates of Al (mg kg^{-1})	Control		*Trichoderma virens*		*Trichoderma pseudokoningii*		*Pseudomonas fluorescens*	
	FYM	VC	FYM	VC	FYM	VC	FYM	VC
Zero Al	60	61	66	65	71	70	70	68
100	85	86	80	79	78	78	78	75
200	78	78	75	78	74	76	75	74
CD (0.05)	Organic manure : 0.394 Aluminium : 0.483 Microbial inoculants : 0.558							

Table 82. Al content of rice straw (mg kg^{-1}) at graded levels of Al at harvest

Treatments, Rates of Al (mg kg^{-1})	Control		*Trichoderma virens*		*Trichoderma pseudokoningii*		*Pseudomonas fluorescens*	
	FYM	VC	FYM	VC	FYM	VC	FYM	VC
Zero Al	110	115	108	109	107	110	101	114
100	126	131	128	130	125	128	128	130
200	121	119	111	118	119	115	118	124
CD (0.05)	Organic manure : 1.32 Aluminium : 1.62 Microbial inoculants : 1.88							

Table 83. Al content (mg kg^{-1}) of rice root at graded levels of Al at harvest

Treatments, Rates of Al (mg kg^{-1})	Control		*Trichoderma virens*		*Trichoderma pseudokoningii*		*Pseudomonas fluorescens*	
	FYM	VC	FYM	VC	FYM	VC	FYM	VC
Zero Al	500	500	500	480	490	625	400	450
100	2000	1718	2005	2375	3937	812	2100	937
200	1625	1650	1100	1415	1100	812	1062	612
CD (0.05)	Organic manure : 35.45 Aluminium : 43.31 Microbial inoculants : 50.13							

Table 84. Al removal by rice grain (mg plant^{-1}) at graded levels of Al at harvest

Treatments, Rates of Al (mg kg^{-1})	Control		*Trichoderma virens*		*Trichoderma pseudokoningii*		*Pseudomonas fluorescens*	
	FYM	VC	FYM	VC	FYM	VC	FYM	VC
Zero Al	0.72	0.76	0.86	0.85	0.89	0.98	0.91	0.88
100	0.90	1.04	0.90	0.95	0.75	0.76	0.94	0.91
200	0.80	0.90	0.81	0.89	0.85	0.71	0.87	0.85
CD (0.05)	Organic manure : 0.007 Aluminium : 0.009 Microbial inoculants : 0.011							

Table 85. Al removal by rice straw (mg plant^{-1}) at different levels of Al at harvest

Treatments, Rates of Al (mg kg^{-1})	Control		*Trichoderma virens*		*Trichoderma pseudokoningii*		*Pseudomonas fluorescens*	
	FYM	VC	FYM	VC	FYM	VC	FYM	VC
Zero Al	2.24	2.37	2.38	2.40	2.20	2.29	2.11	2.38
100	2.56	2.73	2.71	2.72	2.58	2.66	2.64	2.68
200	2.23	2.27	2.16	2.25	2.26	2.18	2.27	2.39
CD (0.05)	Organic manure : 0.020 Aluminium : 0.033 Microbial inoculants : 0.038							

Table 86. Al removal (mg $plant^{-1}$) by rice root at different levels of Al at harvest

Treatments, Rates of Al (mg kg^{-1})	Control		*Trichoderma virens*		*Trichoderma pseudokoningii*		*Pseudomonas fluorescens*	
	FYM	VC	FYM	VC	FYM	VC	FYM	VC
Zero Al	1.60	1.6	1.80	1.68	1.62	2.06	1.52	1.53
100	5.60	4.5	6.22	7.13	11.41	2.35	6.09	2.81
200	3.90	3.5	3.30	2.35	2.97	2.27	2.97	1.71
CD (0.05)	Organic manure : 0.017 Aluminium : 0.021 Microbial inoculants : 0.024							

All the treatments showed a positive response to Al up to the level of 100 mg kg^{-1} for Al removal. The highest value was recorded by control treatment receiving vermicompost along with Al @ 100 mg kg^{-1} for grain and straw. Al removal by the grain and straw followed the same trend. Al content of the rice root did not show a definite trend in Al content and removal. However, root showed a positive response to a level of Al @ 100 mg kg^{-1}only.

Among the microbes, *Trichoderma virens* was found to have more favourable influence on the grain and straw yield with minimum uptake of Al under the graded doses of Al. At zero level of Al, the difference in grain yield for various microorganisms were low compared to higher levels.

There are also reports that rice can accumulate high amounts of different elements like, Cd, Co, Cu, Cr, Ni, Pb and Zn (Kashem and Singh, 2001; Kim *et al.*, 2002). Cereal crops in combination with certain soil/plant treatments were shown as perspective tools to improve metal phytoextraction and removal of metals from contaminated soils (Kim *et al.*, 2002).

VII. Field experiment

Field experiments were laid out in farmer's field at two sites in Kuttanad viz., Moncompu and Viyyapuram, for validation of results obtained from the pot culture experiments. The experiment with 10 treatments was laid out during the *punja* (November to March) season. Due to the continuous monsoon rains, the planting was slightly delayed in both the sites selected. The sowing was done during the last week of November and harvesting was done during April. The rice variety "Uma" was sown in the experiment area. The crop growth was good and all cultivation practices as mentioned in the Package of Practices Recommendations of Kerala Agricultural University were followed. The details regarding the experimental site and treatments are given below.

Table 87. The details of experimental site

Name and address of Farmer	Name and location of rice field	Experimental area (m^2)	Plot size (m^2)
Sri. Mohanan	Moolae Pongabra padasekharam Thekkekara, Moncompu	1000	100
Sri. Raghavan	Potta Kalakkad padasekharam Viyyapuram	1000	100

Treatments

1. FYM @ 5 t ha^{-1} + Control
2. FYM @ 5 t ha^{-1} + *Pseudomonas fluorescens* @ 2% - Seed dip for 30 minutes
3. FYM @ 5 t ha^{-1} + *Trichoderma virens* @ 2 kg mixed with 100 kg neemcake–cowdung mixture ha^{-1}
4. FYM @ 5 t ha^{-1} + *Trichoderma pseudokoningii* @ 2 kg mixed with 100 kg neemcake–cowdung mixture ha^{-1}
5. Vermicompost @ 2.5 t ha^{-1} + Control
6. Vermicompost @ 2.5 t ha^{-1} + *Pseudomonas fluorescens* @ 2% - Seed dip for 30 minutes
7. Vermicompost @ 2.5 t ha^{-1} + *Trichoderma virens* @ 2 kg mixed with 100 kg neemcake-vermicompost mixture ha^{-1}
8. Vermicompost @ 2.5 t ha^{-1} + *Trichoderma pseudokoningii* @ 2 kg mixed with 100 kg n eemcake-vermicompost mixture ha^{-1}
9. POP recommendation
10. Farmer's Practice

Plate 13. Field experiment at Moolae Pongabra Padasekharam, Moncompu

For treating the seeds with *Pseudomonas fluorescens,* two per cent (2%) slurry of the same was prepared by mixing 20 g *Pseudomonas fluorescens* with one liter of water and the seeds were soaked in the slurry for 30 minutes before sowing. Apart from the seed treatment, soil application of the talc based formulation of the same at 2% level was also done before sowing the seeds.

For *Trichoderma virens* and *Trichoderma pseudokoningii,* the talc based preparations of the same were mixed neemcake–cowdung mixture/neemcake-vermicompost mixture, covered it with perforated polythene sheet and incubated for 5 days for multiplication of the fungi, in shade. Again it was mixed well and kept for three more days for further multiplication. It was incorporated in to the soil before sowing.

All the treatments except Farmer's Practice received fertilizers @ 90:45:45 kg ha^{-1}. Lime @ 600 kg ha^{-1} was applied to all treatments except farmer's practice. Rice variety "Uma" was planted and observations were taken. The crop growth was good. It was visually observed that the treatments receiving microbial inoculants were less susceptible to pest and diseases. The crop was harvested during the first week of April 2008. Biometric observations and dry weight of grain, straw and root were recorded. The dried samples were analysed for their Fe and Al content. The results of the experiment are furnished below in tables 88 to 93.

Table 88. Grain yield of rice (t ha^{-1}) under different treatments

Location	FYM				Vermicompost				POP	Farmers Practice
	Control	Pseudomonas	*T. virens*	*T. Pseudo-koningii*	Control	Pseudomonas	*T. virens*	*T. Pseudo-koningii*		
Viyyapuram	6.35	6.75	6.70	6.60	6.15	6.60	6.30	6.40	6.50	6.22
Moncompu	6.25	6.72	6.70	6.55	6.10	6.20	6.20	6.35	6.50	6.25
CD (0.05)	Organic manure : 0.056 Microbial inoculants : 0.082 Organic manure X Microbial inoculants : 0.115									

The treatments had significantly influenced the grain yield and straw yield of rice. In both the locations, treatment receiving FYM and pseudomonas recorded the highest grain yield, followed by *Trichoderma virens.* In pot culture studies with graded levels of Fe and Al (Tables 69,78), *Trichoderma virens* was found to perform better compared to pseudomonas. But at zero levels of Fe and Al, there was not much difference in grain yield for these two treatments and as the levels of Fe and Al increased, *Trichoderma virens* performed better. Similarly at natural condition, pseudomonas might have performed better with regard to grain yield and metal uptake. The field experiments in soils differing in their elemental status with microbial treatments alone could reveal the bioremediation behaviuor of pseudomonas and *Trichoderma virens* in actual field conditions.

The straw yield was highest for POP recommendation. On comparing the effect of manures on grain yield and straw yield, FYM was found to give higher yields than vermicompost. However, the vermicompst was applied at half the rate of FYM and this indicated the need for modifying the dose of vermicompost in field situations.

Table 89. Straw yield of rice (t ha^{-1}) under different treatments

Location	FYM				Vermicompost				POP	Farmers Practice
	Control	Pseudomonas	*T. virens*	*T. Pseudo-koningii*	Control	Pseudomonas	*T. virens*	*T. Pseudo-koningii*		
Viyyapuram	13.6	15.0	15.0	14.0	13.5	13.0	14.0	14.0	15.5	14.5
Moncompu	13.8	14.0	14.0	13.9	13.0	12.9	13.5	14.3	16.0	15.0
CD (0.05)	Organic manure : 0.309 Microbial inoculants : 0.438 Organic manure x Microbial inoculants : 0.619									

The treatment receiving pseudomonas with FYM recorded the lowest Fe content in both grain and straw. For the root Fe content, reverse was the trend.

Plate 14. Field experiment at Potta Kalakkod Padasekharam, Viyyapuram

Table 90. Fe content of rice grain (mg kg^{-1}) under different treatments

Location	FYM				Vermicompost				POP	Farmers Practice
	Control	Pseudomonas	*T. virens*	*T. Pseudo-koningii*	Control	Pseudomonas	*T. virens*	*T. Pseudo-koningii*		
Viyyapuram	288	278	280	290	280	324	306	290	281	320
Moncompu	276	275	278	290	275	325	300	275	277	322
CD (0.05)	Organic manure : 2.97 Microbial inoculants : 4.21 Organic manure x Microbial inoculants : 5.95									

Table 91 Fe content of rice straw (mg kg^{-1}) under different treatments

Location	FYM				Vermicompost				POP	Farmers Practice
	Control	Pseudomonas	*T. virens*	*T. Pseudo-koningii*	Control	Pseudomonas	*T. virens*	*T. Pseudo-koningii*		
Viyyapuram	353	340	418	409	360	369	460	412	351	358
Moncompu	355	333	415	400	365	370	462	410	349	358
CD (0.05)	Organic manure : 2.48 Microbial inoculants : 3.51 Organic manure x Microbial inoculants : 4.97									

The treatment receiving pseudomonas with FYM recorded the lowest Al content in grain, straw and root. Application of FYM and vermicompost had differently influenced the Fe content of root. Root Al content was lowest for the control treatment receiving FYM.

Table 92. Al content of rice grain (mg kg^{-1}) under different treatments

Location	FYM				Vermicompost				POP	Farmers Practice
	Control	Pseudomonas	*T. virens*	*T. Pseudo-koningii*	Control	Pseudomonas	*T. virens*	*T. Pseudo-koningii*		
Viyyapuram	80	60	64	61	71	70	62	131	81	64
Moncompu	81	61	62	60	73	71	62	131	83	62
CD (0.05)	Organic manure : 0.823 Microbial inoculants : 1.160 Organic manure x Microbial inoculants : 1.647									

Table 93. Al content of rice straw (mg kg^{-1}) under different treatments

Location	FYM				Vermicompost				POP	Farmers Practice
	Control	Pseudomonas	*T. virens*	*T. Pseudo-koningii*	Control	Pseudomonas	*T. virens*	*T. Pseudo-koningii*		
Viyyapuram	113	93	116	161	105	175	106	188	137	115
Moncompu	110	94	114	155	100	170	104	180	131	118
CD (0.05)	Organic manure : 2.19 Microbial inoculants : 3.10 Organic manure x Microbial inoculants : 4.39									

On evaluating the different sources for their bioremediation capacity based on rice yield, Fe and Al removal, it was found that FYM performed better compared to vermicompost. Comparing the microbial inoculants, for their bioremediation capacity, pseudomonas was found to be the best followed by *Trichoderma virens* in terms of yield and reducing the uptake of potentially toxic elements. These bacteria might have been able to reduce the metal uptake of rice by biosorption process. Biosorption is particularly suited as a polishing step in wetland systems with low to medium metal concentrations ranging from few to 100 mg kg^{-1} (Volesky, 2001). Therefore application of. pseudomonas to rice in Kuttanad could not only reduce the pest and disease incidence and there by increase yield, but also could produce quality rice with least uptake of potentially toxic elements.

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Plate 15. Project evaluation by ICAR Team

17. Summary

- *Kari* soils contained higher quantity of available Fe, S and extractable Al compared to *karappadam* and *kayal* soils

- Sediment contained still more quantities of almost all the elements tested compared to soil samples except nitrate nitrogen, and Ca and Mg in certain cases

- Nutrient load of water is also high posing the threat of eutrofication, in all selected watersheds of Kuttanad

- All the chemical characteristics showed an increase during pre-monsoon period compared to summer and post-monsoon season, clearly indicating the chances of metal accumulation during pre-monsoon period except NH_4- N.

- The chemical characteristics of sediment were also very similar to that of soil, the values being slightly higher for certain parameters.

- Regarding the water quality parameters, the pH, EC, NO_3- N, P, K, Ca, Mg and S showed an increase during the pre-monsoon season compared to summer season for both field water as well as canal water, definitely due to the influence of saline water entry to the Kuttanad ecosystem. There was a reduction in NH_4-N, Fe and Al content during the same period.

- Comparing the water quality with that of ISI/WHO/EEPA standards fixed, the Fe, Mn, Cu and Al content of the study area exceeded the maximum permissible limits.

- *Kari* soils showed the highest values for total Mn and 72 to 76 per cent of this remained in free or active form. In *karappadam* and *kayal* lands, the total as well as the free Mn contents were much lower compared to *kari* soils.

- For Zn also, *kari* soils showed highest value compared to *kayal* and *karapadam* soils. Cu also followed the same trend as that of Zn, but the water soluble forms were present in very negligible amounts.

- Among the plants tested for hyper-accumulation capacity for Fe and S, viz., *Hydrocotyl asiatica, Bacopa monnieria, Commolina bengalensis, Cynadon dactylon* and *Cyperus pangorei*, the *Cyperus pangori* was found to extract largest quantity of Fe and S at 900 mg Fe as ferrous sulphate per kg of soil.

- Accumulation factor for Fe was found to be higher for root than shoot and the same was negative for *Commolina bengalensis*

- Comparing the plants, *Hydrocotyle asiatica* and *Bacopa monnieria* removed more Fe with its graded doses up to 1800 mg kg^{-1} only, while *Cyperus pangorei* and *Eichhornia crassipes* removed Fe up to 3000 mg kg^{-1}.

- All the tested plants showed positive response to graded doses of Al and S up to a level of 1000 mg Al kg^{-1} of soil for dry matter production. *Cyperus pangorei* recorded the highest dry matter production for both shoot and root.

- *Hydrocotyle asiatica* and *Cyperus pangorei* showed positive response up to the highest level of Al and S tested. The highest quantity of Al was extracted by *Cyperus pangorei* and S by *Hydrocotyle asiatica.*

- All the tested plants viz., *Eichhornia crassipes, Hydrocotyle asiatica, Bacopa monnieria* and *Cyperus pangorei* showed a positive response Zn, Cu, Cd and Pb application

- *Eichhornia crassipes* showed the highest content of Zn and the root portion contained more of the element compared to shoot. The highest quantity of Zn was removed from the soil by *Eichhornia crassipes* followed by *Cyperus pangorei.*

- The highest quantity of Cu was also removed by *Eichhornia crassipes* followed by *Cyperus pangorei*

- *Hydrocotyle asiatica* and *Eichhornia crassipes* contained higher quantities of Cd compared to other plants. The highest quantity was removed by *Eichhornia crassipes.* Both these plants contained higher quantity in shoot. The highest quantity of Cd was removed from the soil by *Eichhornia crassipes* when Cd was applied @ 10 mg kg^{-1}.

- *Eichhornia crassipes* and *Cyperus pangorei* showed higher concentration of Pb. The highest quantity was removed by *Eichhornia crassipes* followed by *Cyperus pangorei.*

- *Bacopa monnieria* recorded the maximum mycorrhizal colonization in treatment receiving 2400 mg Fe kg^{-1} followed by that receiving 3000 mg Fe kg^{-1}.

- The rapidly growing *Trichoderma* spp. were found capable of removing appreciable quantity of Fe from the medium as indicated by the Fe detected in their mycelial samples. *T. virens* was found to have high potential and could take up 50 to 75 % of the iron in the medium.

- Trained isolates of *Trichoderma* spp. Showed profused growth and sporulation compared to non-trained counter parts.

- *Trichoderma virens* and *Pseudomonas fluorescens* showed a synergistic effect on dry matter production and Zn removal by *Eichhornia crassipes* and was more evident on shoot.

- The microbial treatments have significantly reduced the Cu content of shoot and had greatly increased the Cu content of root in *Eichhornia crassipes* with a positive response to the graded levels of Cu. The highest Cu content was recorded by the *Trichoderma pseudokoningii* followed by *Trichoderma virens*.

- The plants showed a positive response to graded levels of Fe in presence of microbial treatments, but the synergistic effect was not observed on dry matter production.

- *Pseudomonas fluorescens* showed highest synergistic effect on Al removal by *Eichhornia crassipes.*

- *Eichhornia crassipes* showed positive response to graded levels of Cd and the highest value was obtained for treatment receiving *Trichoderma virens* at 20 mg Cd kg^{-1}.

- The grain yield and straw yield were significantly influenced by the treatments and the highest yield was recorded by the treatment receiving *Trichoderma virens* along with Fe @ 600 mg kg^{-1}. The Fe content and Fe removal were highest for *Pseudomonas fluorescens* with vermicompost and Fe @ 900 mg kg^{-1} in grain.

- Grain yield and straw yield were highest for the treatment receiving *Trichoderma virens* along with FYM at zero level of Al, clearly indicating the adverse effect of Al on rice yield. All the treatments showed a positive response to Al up to the level of 100 mg kg^{-1}.

- Validating the experimental results, it was observed that application of FYM @5 t ha^{-1} + Pseudomonas @ 0.20% recorded the highest grain yield with lowest Fe and Al content. But the straw yield was highest for POP recommendation

18. Results which can be exploited on pilot or field scale

- *Eichhornia crassipes* and *Cyperus pangorei* can be used as good phytoextractors for Al and heavy metals viz., Fe, Zn, Cu, Cd and Pb. The fungai, *Trichoderma virens* and *Pseudomonas fluorescens* could synergize the heavy metal extraction by plants from soil. Thise technology can be successfully utilized for the purifying heavy metal loaded effluents discharged from factories and can be effectively used in constructed lagoons/wetland for purification.

- Use of *Pseudomonas fluorescens* and *Trichoderma virens* along with FYM increased the grain yield and straw yield of rice in acid sulphate soils of Kuttanad, with least removal of persistent toxic elements of the area viz., iron and aluminium

19. Publication

1. Girija, V. K., K.C. Manorama Thampatti., Lulu Das. and Arthur Jacob. 2006. Exploitation of fungi for cleaning up the environment. Paper presented at Indian Environmental Congress 2006 held at Amrithapuri, Kollam, Kerala.

2. Manorama Thampatti, K.C., P.B. Usha and V.I. Beena. 2007. Aquatic macrophytes for biomonitoring and phytoremediation of toxic metals in wetlands of Kuttanad. Paper presented at Kerala Environmental Congress 2007 held at Thiruvananthapuram, Kerala.

3. Girija, V. K., K.C. Manorama Thampatti and R.L. Yamuna. 2007. Diversity of filamentous fungi associated with heavy metal contaminated soils of Kuttanad. Paper presented at Kerala Environmental Congress 2007 held at Thiruvananthapuram, Kerala.

20. Contribution made by Co-operators

Assisted in selection of fields in Kuttanad region, experimental work and in documentation of the project results. The work on mycological studies was contributed solely by the co-investigator from the Department of Plant Pathology.

21. Others - Nil

K. C. Manoram
Signature of Principal Investigator

K.C. Manorama Thampatti
Professor

Date: 23.01.09

Signature 23/01/09
Head of Institution/Station