Technical Bulletin No. 21

Management of Waste Water through Weed based Phytoremediation

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ICAR–DIRECTORATE OF WEED RESEARCH
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Dr. J.S. Mishra
Director
ICAR - Directorate of Weed Research, Jabalpur

Prepared and edited by

Mr. Dibakar Roy
Dr. P.J. Khankhane
Dr. K.K. Barman
Dr. C. Sarathambal
Dr. P.K. Singh
Dr. Jay G. Varshney
Dr. J.S. Mishra

Cover page design

Mr. Sandeep Dhagat

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Further information

ICAR - Directorate of Weed Research
Jabalpur (M.P.), India
Phone: 0761-2353934, 2353101
Fax: 0761-2353129
Email: dirdwsr@icar.org.in
Website: http://dwr.icar.gov.in

भारतीय कृषि अनुसंधान परिषद
कक्ष क्र. 101, कृषि अनुसंधान भवन-II, नई दिल्ली-110 012, भारत
INDIAN COUNCIL OF AGRICULTURAL RESEARCH
Room No. 101, Krishi Anusandhan Bhavan-II, Pusa, New Delhi- 110012, India

डॉ. सुरेश कुमार चौधरी
उप महानिदेशक (प्राकृतिक संसाधन प्रबंधन)
Dr. Suresh Kumar Chaudhari
Deputy Director General (Natural Resources Management)

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Foreword

In recent past Disposal of industrial effluents and sewage has become serious problem due to rapid industrial development and urbanization in India. Millions liters of untreated sewage and industrial effluents are being discharged in surface water bodies like rivers, ponds and lakes due to lack of adequate treatment facility in many cities. This has resulted in deterioration of water quality of surface water bodies in the form of eutrophication. Farmers of peri-urban areas have begun watering their crops, especially vegetables using city effluents resulting in accumulation of heavy metals in soils, crops, food-chain and environment. Hence, treatment of city and industrial effluents prior to recycling in agricultural fields is essential. The existing waste-water treatment plants are another couse of concern for environmentalists because of their high energy consumption, chemical use, emissions to the atmosphere, and sludge formation. In view of this, phytoremediation having low energy consumption and minimal byproduct footprint appears to be a feasible and environmental friendly approach for treating sewage and industrial effluents, and thereby reducing heavy metal loads in agricultural fields. In this context, some fast growing weedy plants with high biomass such as Water Hyacinth, Typha, Arundo, Phragmites, etc. have shown potential in removing contaminants from waste water.

The present publication entitled 'Management of waste water using weed-based phytoremediation' is a compilation of extensive research and demonstration carried out by the ICAR-Directorate of Weed Research, Jabalpur. I congratulate the authors for bringing out this publication in form of technical bulletin. I am sure that this bulletin would be helpful to researchers, policy makers and other stakeholders.

20/5/22

(S.K. Chaudhari)

Ph.: +91-11-25848364 Fax: +91-11-25848366 Email: ddg.nrm@icar.gov.in Website: www.icar.org.in

Preface

Clean water and sanitation is one of the sustainable development goals (SDGs) adopted by the United Nations. Water is inevitable necessity in human life apart from food and shelter. Surface and underground water are the major sources of clean water. However, with the rapid growth in population and increasing industrial development, many water sources have become polluted. Everyday huge quantities of waste water are generated from domestic uses or from industrial source. Presently in India an estimated volume of almost 72000 million liters waste water is generated per day. A major part of it remains untreated and is ultimately dumped to our natural water bodies like rivers, lakes and seas. Hence, wastewater must be adequately treated prior to discharge into the environment. Although the untreated sewage water generated from domestic use is rich in some plant essential nutrients like N, P and also organic matter. Farmers having access to this free of cost nutrient enriched water generally prefer it for irrigation. However, the use of industrial waste water for irrigation often causes entry of heavy metals in food chain. The running costs of the presently available sewage treatment facilities is gigantic, and thus to recycle this enormous volumes of waste water we need to have alternate low cost technologies.

Phytoremediation technique employs the application of plants for the remediation of wastewater. Weedy plants such as *Arundo donax, Typha latifolia, Vetivaria zizinoids, Phragmites karka, Eichhornia crassipes, Pistia stratiotes* etc., have the capacity to absorb excess contaminants such as organic and inorganic, heavy metals, and pharmaceutical pollutants present in agricultural, domestic and industrial wastewater. Growing such weeds in constructed wetlands for removing heavy metals and other contaminants from waste water can act as an alternate low cost sewage treatment technology. Extensive research work was carried out in this direction at ICAR-Directorate of Weed Research, Jabalpur. This bulletin contains some useful information generated on phytoremediation ability of a series of weedy plants and their usefulness for the purpose of wastewater treatment. We hope that this technical bulletin will help to understand weed based phytoremediation system, and will be useful to the researchers, students, planners, environmentalists and farmers concerned about sewage water recycling for agricultural purpose.

Authors

Content

1. Introduction

Rapid population bloom and unplanned urbanization in recent decades in India had tremendously increased fresh water demand from underground source, river and lakes for both domestic as well as industrial use. This fresh water utilization for various purposes ultimately leads to the generation of huge quantities of waste water. Nearly 80% of the fresh water supplied for domestic use passes out as wastewater. For example, in 1978 the total water supply in Class-I and Class-II cities were 8638 million litters per day (MLD) and 1533 MLD, which generated 7007 MLD and 1226 MLD waste water, respectively. By 2009, total waste water generation in Class-I cities increased roughly by 5 times, i.e. 35558 MLD. On the other hand, sewage treatment capacity increased from 2756 MLD in 1978 to 11554 MLD in 2009 (Fig. 1). This showed that the rate at which waste water generation increased, the creation of sewage water treatment facility did not increase proportionately. Since, our cities and urban areas restrained near river bodies, waste water generated through various sources are dumped directly into the rivers and lakes. Today most of the Indian rivers and lakes are polluted with this untreated sewage water and as well as with industrial waste water. Contamination of fresh water bodies with this untreated waste water or sewage water has become a serious issue.

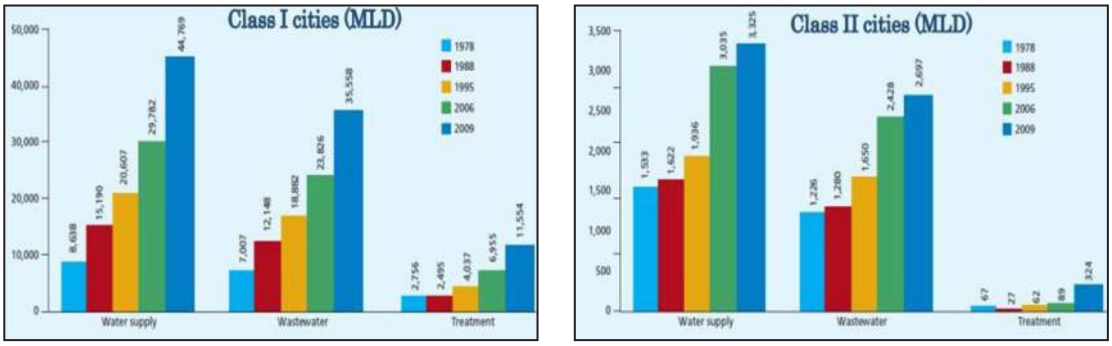


Fig.1. Status of sewage water or waste water generated in India (*Source*: CPCB technical report, 2016)

The farmers having fields adjoining to sewage canals prefer to use this waste water for irrigation purpose especially during summer and winter seasons. It is extremely valuable source for them as pumping cost from sewage drains is cheaper than from tube well. Further, sewage effluents from municipal origin are rich in organic matter and also contain appreciable amounts of major nutrients. As a result, the response of crops in terms of growth and yield is often better to sewage water irrigation than to tube well water irrigation. However this practice is also a major cause of heavy metal entry in food-chain; since, besides organic matter and nutrients, sewage water also contains several toxic heavy metals. During dry season, cultivated areas under peri-urban agriculture are worst affected by these contaminants/pollutants. Samra (2007) reported that continuous waste water irrigation enhances the available metal status in agricultural land by 2 to 100 times.

2. Current status of sewage water generation and sewage treatment plants

There are total 1631 sewage treatment plants (STPs) located in various parts of the country and out of them only 1093 STPs are operational, 102 are non-operational, 274 are under construction and 162 new STPs are proposed for construction (CPCB technical report, 2021). The total amount of sewage water generated in the country estimated nearly about 72368 Million liter per day (MLD) and major part of it is generated from Class-I cities (more than 10 lakh population). Presently out of these 72368 MLD sewage water, only 20236 MLD is being treated and remaining 52138 MLD waste water remaining untreated.

Presently, among the various states, maximum sewage water is generated by Maharashtra (9107 MLD) followed by Uttar Pradesh (8263 MLD), Tamil Nadu (6421 MLD), West Bengal (5457 MLD) and Gujarat (5013 MLD) (Table 1). Besides them, some other states like Andhra Pradesh, Madhya Pradesh, Karnataka, Kerala, Rajasthan, Delhi, Telengana and Bihar also generate significant volume (2000-5000 MLD) of waste water everyday. But in most of these states the installed sewage treatment capacity is far less compared to the volume of waste water generated. Only Punjab, Haryana, Delhi and Chandigarh have the capacity to treat about 70% of the total sewage water. However, about 40-70% of the total sewage water remains untreated in rest of the states. Again it is also noticed that almost in all the states the installed STPs hardly perform at their full potential. On an average, 64% of their full potential is being utilized.

Apart from domestic household activity, various industries also generate significant volume of waste water every day (Table 2). Pulp and paper industry is forerunner among them (generates 201.4 MLD waste water) followed by chemical industry (98 MLD), distillery (37 MLD), tannery (22.1 MLD) etc. This large volume of waste water is discharged into various drains causing entry of pollutants / heavy metals into drain water. The content of toxic heavy metals like Cd, Cr, Ni and Pb varies greatly in the drain water of different Indian cities (Table 3).

Table 1. State-wise sewage generation and sewage treatment capacity and actual treatment

S.No.	State	Total sewage generation (MLD)	Installed capacity		Actual quantity treated / capacity utilised		
			(MLD)	As % of sewage generated	(MLD)	As % of total sewage generated	As % of installed capacity
1.	Maharashtra	9107	6890	76	4242	47	62
2.	Uttar Pradesh	8263	3374	41	2510	30	74
3.	Tamil Nadu	6421	1492	23	995	15	67
4.	West Bengal	5457	897	16	213	4	24
5.	Gujarat	5013	3378	67	2687	54	80
6.	Karnataka	4458	2712	61	1786	40	66
7.	Kerala	4256	120	3	47	1	39
8.	MP	3646	1839	50	536	15	29
9.	NCT Delhi	3330	2896	87	2412	72	83
10.	Rajasthan	3185	1086	34	478	15	44
11.	Andhra Pradesh	2882	833	29	309	11	37
12.	Telangana	2660	901	34	706	27	78
13.	Bihar	2276	10	0	0	0	0
14.	Punjab	1889	1781	94	1360	72	76
15.	Haryana	1816	1880	104	1284	71	68
16.	Jharkhand	1510	22	1	15	1	68
17.	Odisha	1282	378	29	50	4	13
18.	Chhattisgarh	1203	73	6	6	0	8
19.	Assam	809	0	0	0	0	0
20.	J&K	665	218	33	49	7	22
21.	Uttarakhand	627	448	71	187	30	42
22.	Tripura	237	8	3	1.5	1	19
23.	Chandigarh	188	293	156	235	125	80
24.	Goa	176	66	38	25	14	38
25.	Manipur	168	0	0	0	0	0
26.	Puducherry	161	56	35	30	19	54
27.	Nagaland	135	0	0	0	0	0
28.	Himachal	116	136	117	51	44	38
29.	Meghalaya	112	0	0	0	0	0
30.	Mizoram	103	10	10	0	0	0
31.	Daman Diu	67	24	36	7	10	29
32.	Arunachal	62	0	0	0	0	0
33.	Sikkim	52	20	38	14	27	70
34.	A & N Islands	23	0	0	0	0	0
35.	Lakshadweep	13	0	0	0	0	0

Source: CPCB technical report, 2021

Table 2. Sector specific industrial wastewater generation in India

Type of industry	Total units	Wastewater generation (MLD)
Pulp & Paper	67	201.4
Chemical	27	97.8
Sugar	67	96.0
Distillery	35	37.0
Tannery	442	22.1
Textile, Bleaching & Dyeing	63	11.4
Food, Dairy & Beverage	22	6.5
Others	41	28.6
Total	**764**	**500.8**

Source: CPCB technical report, 2021

Table 3. Sewage water quality in some Indian cities

Parameter	Vadodara[1]	Bharuch[1]	Ankleshwar[1]	Jabalpur[1]	Delhi[2]	Kolkata[3]	Varanasi[4]	FAO limit
pH	7.7	7.1	7.1	6.8-8.1	7.90	6.57	7.0	6.5-8.4
EC (dS/m)	1.3	1.3	2.5	0.71-1.20	1.2	2.60	2.28	<3.0
BOD(mg/L)	47.5	39.0	560	54.0	-	-	-	<100
Na (SAR)	5.1	2.0	5.3	NA	-	-	-	<10
F (mg/L)	6.85	6.34	25.55	NA		-	-	1.0
Zn	0.13	0.25	0.34	NA	134	1269	786	2.0
Cu	0.09	0.12	0.70	0.46-2.83	< 3.0	294	318	0.2
Mn	0.13	0.29	4.83	1.7-5.6	-	-	-	0.2
Cd	0.05	0.03	0.10	0.029-0.63	-	4.93	155	0.5
Co	0.24	0.15	0.56	NA	-	-	-	0.05
Cr	1.19	1.10	2.76	0.048-1.05	-	-	36	0.10
Ni	0.35	0.55	0.73	0.27-7.32	-	175	47	0.2
Pb	0.30	0.17	0.84	0.029-4.72	47.4	414	60	5.0

[1]Patel et al., 2004; [2]Annual Report 2015-16, NASF project, ICAR-DWR, [3]Roy et al., 2013; [4]Saha et al., 2017; [5]Singh and Agarwal, 2010

3. Impact of waste water irrigation on heavy metal built up in soil and metal entry in food chain

Soils under waste water irrigation act as a sink of heavy metals and other organic pollutants. Unlike organic pollutants, heavy metals, cannot be destroyed or changed to forms that are harmless. Recent reports suggest that more than 80% of toxic metals originally added 25 years back via sewage sludge application are still present in the top soil layers. Metals like cadmium are of special concern, due to its potential toxicity to biota at low concentrations. Lead contamination is increasing in soils, plant and animal food chains through addition and recycling of urban solid wastes, sewage/ sludge and industrial wastes. As compared to the light soils, black soils are more vulnerable to heavy metal accumulation; especially in surface layers. The soil samples collected from farmers field in Jabalpur and adjoining areas, where untreated sewage water is often used to irrigate the crops, showed accumulation of cadmium and lead above the critical limit of phyto-toxicity (Khankhane and Bisen, 2016). Compared to ground water irrigation, higher heavy metal content in soils receiving sewage water irrigation has also been reported by various other workers (Table 4).

Table 4. Heavy metal built-up in soil irrigated with waste water or sewage water in India

Heavy metal	Waste water irrigated soil	Ground water irrigated soil	Reference
Cadmium (Cd)	0.006	0.002	Gupta et al. (2009)
	0.6	BDL	Ghosh et al. (2011)
	2.63	-	Sharma et al. (2007)
Chromium (Cr)	0.055	0.034	Gupta et al. (2009)
	90.23	-	Sharma et al. (2007)
Lead (Pb)	0.896	0.613	Gupta et al. (2009)
	1.08	0.26	Ghosh et al. (2011)
	17.81	-	Sharma et al. (2007)
Nickel (Ni)	0.99	0.29	Ghosh et al. (2011)
	14.5	-	Sharma et al. (2007)

BDL – Below detectable limit

The sewage water and industrial waste are often drained to agricultural lands for growing crops, including vegetables. The sewage effluents are generally a rich source of organic matter and other nutrients, but sewage water irrigation is also known to contribute significantly to the heavy metal content of soils. The long-term application of untreated wastewater may result in a significant built-up of heavy metals in the soil and their subsequent transfer to the food chain through different plant parts, causing a potential health risk to consumers. The reports showed that the use of untreated wastewater for irrigation increased the contamination with Cd, Pb and Ni in the edible portions of vegetables (Khan et al. 2008 and Singh et al. 2010), and in some situations the bioaccumulation of Pb and Cd in vegetables was above the maximum permissible limit (Sharma et al., 2016). The heavy metal contamination in soil vis-à-vis their accumulation in edible parts of the vegetable crops like taro and raddish was studied by Gupta et al. (2009) in the wastewater irrigated fields in Durgapur, India. They found that metal accumulation was less in soil and higher in vegetables due to continuous bioaccumulation followed by biomagnifications. The gradient of metal accumulation followed in the order of Fe >Pb>Mn> Cr > Cd. The metal accumulation in roots of *Raphanus sativus* (radish) was greater than in *Colocasia esculentum* (taro); whereas, *Colocasia esculentum* shoots showed higher concentrations of metals compared to *Raphanus sativus* shoots (Fig.2). In the urban vegetable growing areas of Varanasi (UP) receiving long-term application of treated sewage water irrigation the highest total metal accumulation (Cd, Cr and Ni) was recorded in the edible parts of cabbage followed by radish, spinach, turnip, bean, tomato, coriander, carrot, potato, cauliflower and brinjal (Ghosh et al., 2011). Among the studied vegetables, the highest uptake of Cd and Cr was observed in radish; whereas highest Ni uptake was associated with cabbage. Similarly, the findings of All India Co-ordinated Research Project on Irrigation water management, Patna Bihar showed relatively higher levels of metal accumulation in vegetables like *Basella alba* (malabar spinach), *Amaranthus viridis* (red amaranthus), *Solanum melongena* (brinjal), *Momordica charantia* (bitter gourd), *Capsicum frutescens* (capsicum), *Brassica oleracea* var. *capitata* (cabbage), *Vigna unguiculata* (cowpea), *Luffa aegyptiaca* (sponge gourd), *Abelmoschus esculentus* (okra) and *Cucurbita maxima* (giant pumpkin) (Singh et al., 2016). Among them malabar spinach accumulated highest Ni (59 mg/kg), Cr (130 mg/kg) and Cd (11.7 mg/kg), and the Pb uptake was highest in *Amaranthus* sp.

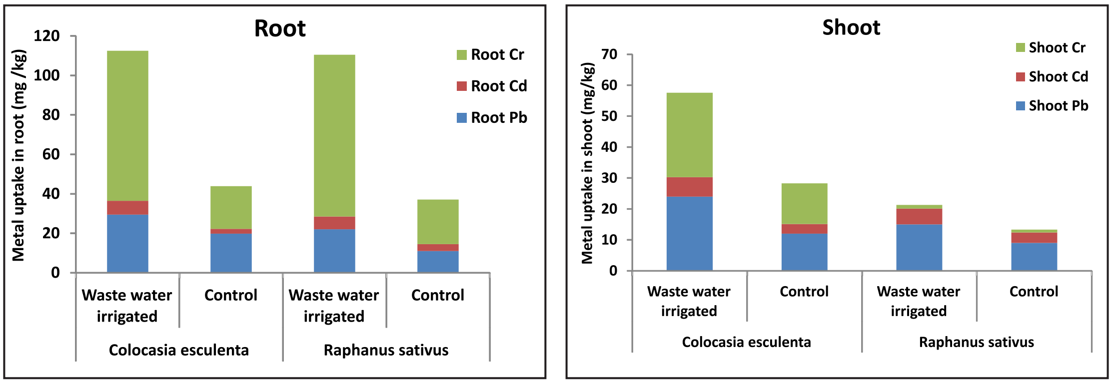


Fig.2. Influence of long term application of sewage water on metal uptake in Colocasia (taro) and Rahphanus (rasddish) in different parts. *Source*: Gupta et al., 2009

A study conducted in the drain water irrigated sites of Jabalpur to evaluate the heavy metal content in crops and associated weeds showed that the Cd content in the wheat and cauliflower was at the alarming level of 0.97 and 1.24 mg/kg, respectively (Khankhane and Bisen, 2016). The associated weeds differed in their capacities to remove and accumulate heavy metals. For example, Cd accumulation in *Sonchus arvensis* was 3.07 mg/kg, but it was negligible in case of *Vicia sativa*. Similarly, the associated weed *Chenopodium album*, which is commonly used as leafy vegetable, also showed fairly high level of Cd content (Table 5 & 6). This indicates that consumption of vegetables grown under such situation may cause serious risk to human health.

Table 5. Heavy metal accumulation in wheat and associated weeds irrigated with waste water

Plant species	Heavy metals (mg/kg)			
	Cu	Cd	Mn	Fe
Wheat grain	**19.1**	**0.97**	**40.7**	**11**
Avena ludoviciana	48.9	0.96	122.3	251
Chenopodium album	39.2	1.53	77.4	948
Medicago esculenta	38.8	1.10	52.0	864
Chicorium intybus	28.2	1.05	46.3	646
Vicia sativa	35.4	0.72	88.0	453
Parthenium hysterophorus	44.8	0.64	47.3	1065
Phalaris minor	BDL	1.35	BDL	BDL
Brassica. spp.	19.0	1.40	43.5	1352

Source: Khankhane and Bisen, 2016

Table 6. Heavy metal accumulation in cauliflower and associated weeds irrigated with waste water

Weed species	Heavy metals (mg/kg)			
	Cd	Cu	Mn	Fe
Avena ludoviciana	1.14	104	69.6	2041
Parthenium hysterophorus	1.12	49.4	BDL	2507
Vicia sativa	BDL	78.4	116	3485
Chenopodium album	1.11	31.8	66.4	547.5
Melilotus indica	1.30	BDL	19	BDL
Lathyrus sativa	0.77	BDL	132	BDL
Anagallis arvensis	1.55	20.5	30	1060
Alternanthera viridis	1.56	22.6	38	621
Sonchus arvensis	3.07	41.4	35.2	923
Eclipta alba	1.14	48.9	41.8	962
Cauliflower shoot	1.41	11.7	39.4	1887
Cauliflower head	1.24	13.6	25.3	521

Source: Khankhane and Bisen, 2016

Further, it was also noticed that the weed growth in the waste water treated fields was relatively higher than in tubewell water irrigated fields. As compared to tube well water, total weed density and weed dry matter under drain water irrigation were respectively 28 & 48 per cent higher in wheat, and 26 & 53 per cent higher in cauliflower (Khankhane and Bisen, 2016). This observation was further reaffirmed in a laboratory study conducted by using waste water from different localities of Jabalpur city. A significant increase in the germination, shoot and root length of wild oat (*Avena ludoviciana*) was recorded in the Petridishes treated with waste water as compared to the tube well water (Table 7, Fig. 3).

Table 7. Effect of waste water on germination, shoot and root length of wild oat at 6 DAS

Waste water sites	Germination (%)	Shoot length (cm)	Root length (cm)
Gohalpur	82.5	3.36	3.60
Baldeobagh	64.0	3.93	4.30
Ukhari	64.0	2.03	2.29
Uldena	78.6	3.48	3.74
Panagar	80.8	3.41	4.17
TW water (Control)	53.6	1.53	2.68
LSD (P= 0.05)	NA	0.40	0.68

Source: Khankhane and Varshney, 2010

Waste water — Tube well water

Fig.3. Germination of wild oat (*Avena ludoviciana*) in petri-dishes irrigated with waste water and fresh water

4. Waste water treatment technologies

The technologies being employed in India for treatment of domestic sewage /waste water are primarily Sequencing Batch Reactor (SBR), Activated Sludge Process (ASP), Up-flow Anaerobic Sludge Blanket (UASB), and Moving Bed Biofilm Reactor (MBBR). But the major drawback with these sewage treatment plants (STPs) is huge operational cost with no monetary benefits. The cost of activated sludge process (ASP) with a capacity of 1 MLD sewage is around 90 lakhs to 1 crore rupees, while the cost for new generation membrane bioreactor (MBR) is around 1.3-1.5 crore rupees (Kamyotra and Bharadwaj, 2011). Hence, there is a need of inexpensive sewage treatment technique, and phytoremediation could very well fit into this category. In phytoremediation technique green plants are used extensively or moderately for the removal of contaminants from water.

4.1 Phytoremediation

The term phytoremediation is used for a group of green eco-friendly technologies that fundamentally based on plants (aquatic, semi-aquatic and terrestrial) and related associated enzymes and microorganisms. Phytoremediation process in constructed wetlands has been used for the water purification in different parts of the world since 1950s. The plants used in these technologies are able to contain, degrade, or eliminate heavy metals, pesticides, solvents, crude oil and its derivatives, and various other contaminants from the media. The various processes by which phytoremediation takes place are phytoextraction, phytostabilization, rhizofiltration, rhizodegradation and phytovolatilization. These techniques are used depending upon the types of contaminants and ecological conditions.

***Rhizofiltration*:** Use of plant roots to absorb and adsorb pollutants, mainly metals from aquatic system. The plants are regularly harvested and incinerated. This system can be applied for treating sewage. For example *Arundo donax* plant can remove Cd from contaminated water by accumulating it mainly in its roots (Khankhane et al., 2017).

***Phytoextraction*:** It is moderately inexpensive and contaminants can be separated permanently. It involves growing plants to concentrate one or more heavy metals from contaminated soil and incorporate into plant biomass (from root to shoot). The plants are then harvested, incinerated and ash is placed in a confined area or heavy metals are extracted from it. For example, Chinese brake fern (*Pteris vittata*) was used for extraction of arsenic (As) from As contaminated soil (Yan et al., 2019).

***Phytostabilization*:** Use of plants to reduce the mobility/bioavailabilty of pollutants in the environment. Plant can help to stabilize pollutants by adsorbing them in outer root surface area or helps to precipitate within rhizosphere region. For example, Indian mustard (*Brassica juncea*) has been reported to stabilize mercury in soil (Raj et al., 2020). Similarly rapeseeds (*Brassica napus*), sunflowers (*helianthus annuus*), tomato (*Solanum lycopersicum*) and soapwarts (*Saponaria officinalis*) exhibit phytostabilization ability.

***Phytodegradation*:** Use of plants and associated microorganisms to degrade organic pollutants to less toxic forms to prevent their entry into the food chain or environment. The main phenomena are operated in plant rhizosphere region, where beneficial microbes reside and degrade organic pollutant. For example, *Typha latifolia* could degrade atrazine in hydroponic system (P´erez et al., 2021). Similarly reduction in atrazine, diazinon, and permethrin concentration in effluent of simulated agricultural runoff water by *Leersia oryzoides* (cutgrass), *Typha latifolia* (cattail), and *Sparganium americanum* (bur-reed) were reported when runoff effluents passed through these weeds in constructed wetland situation (Moore et al., 2013).

***Phytovolatilization*:** Plant take up toxic substances by their root system and translocate to their aerial parts especially in leaves, and finally release the toxic substances or a modified form thereof into the atmosphere as vapour. Rugh et al. (1996) reported that a mercury-resistant transgenic plant was developed by inserting an altered mercuric ion reductase gene (merA) into *Arabidopsis thaliana*, which volatilized mercury into the atmosphere.

4.2 Criteria of selecting plant for Phytoremediation

Several criteria are required to facilitate the choice of a suitable plant species for use in constructed wetlands under different climatic conditions. Among the numerous possible criteria that could affect the plant choice, some definite criteria were considered as the basis for the selection of plants in different climatic regions. The plant specific properties and adaptabilities criteria are: tolerance to flooded and water-saturated conditions (hydroperiod), adaptability to anaerobic and anoxic conditions (root zone oxygenation), propagation (vegetative by rhizomes and generative by seeds), life cycle (perennial and annual), growth characteristic (persistence and non-persistence), salinity tolerance (high, moderate and low), light demand (full sun, semi-shade and shade) and pH conditions (Acidity and Alkalinity).

A lot of wetland species cannot survive under permanently inundated conditions. These plant-specific hydro-period tolerance ranges should be considered when choosing suitable species for constructed wetlands. The individual tolerance ranges to flooding depend further on site-specific factors such as oxygen concentration in the water, presence of nutrients or toxic substances and the competitive ability with other wetland species. Weedy plants have advantages of producing high biomass, show greater tolerance to metals than crop plants, and require no fertilizers and plant protection measures.

Another important criterion is hyper accumulating ability of the plant. It is determined on the basis of bioconcentration factor (BCF) and translocation factor (TF).

BCF = toxic substance uptake by plant / toxic substance present in environment (soil or water)

TF = toxic substance present in shoot or stem / toxic substance present in roots; or, Toxic substance present in leaves / Toxic present in shoot or stem

For, terrestrial hyper accumulator plants the desired value of both BCF and TF is >1. But for aquatic weeds, as their dominant pathways is rhizofiltration, the desired value of BCF is >1, but TF for root to shoot, or shoot to leaves is < 1.

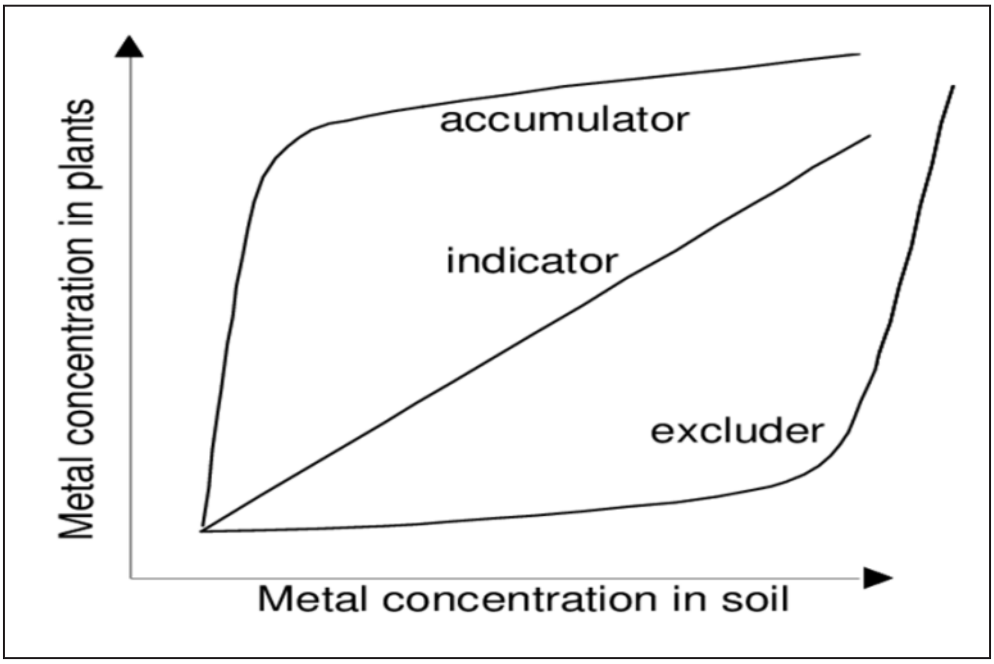


Fig.4. Difference in hyper accumulator, indicator and excluder plants (Baker, 1981)

5. Survey, identification and screening of potential plants for phytoremediation

5.1 Survey and identification of potential weedy plants

Weedy plants which naturally grow in waste water may be the ideal candidate for phytoremediation purpose. With this view, extensive survey work had been carried out for identification of various weedy plants growing in waste water carrying drains of Jabalpur (Khankhane and Vershney, 2008). The major weed species found in the drains were *Alternanthera philoxeroides, Polygonum persicaria, Commelina communis, Alternanthera sessilis, Ludwigia adgscendens, Mullugo verticillata* and *Amaranthus tricolor* (Fig. 5). The accumulation of heavy metals by different weed species varied depending upon the metal species. The order of heavy metal accumulation ability of the weeds was as follows:

Nickel: *Alternanthera philoxeoroides*> *Mullugo verticillata*> *Polygonum persicaria*> *Commelina communis*> *Ludwigia adgscendens*

Copper: *Mullugo verticillata*>*Ludwigia adgscendens*>*Amaranthus tricolor*>*Polygonum persicaria* > *Ageratum conyzoides*

Iron: *Convolvulus arvensis*> *Ludwigia adgscendens*> *Alternanthera philoxeoroides*> *Mullugo verticillata*> *Polygonum persicaria*

Zinc: *Alternanthera philoxeoroides*>*Amaranthus tricolor* > *Mullugo verticillata*> *Polygonum persicaria*> *Ipomoea aquatic*

Manganese: *Alternanthera philoxeoroides* >*Polygonum persicaria*> *Heliotropicum indicum*> *Commelina communis* >*Ludwigia adgscendens*

Mollugo verticillata

Commelina communis

Heliotropicum indicum

Convolvulus arvensis

Ipomea aquatica

Ludwigia adgscendens

Polygonum persicaria

Fig.5. Less explored weedy plants identified from west water containing drains near Jabalpur

In another survey, the heavy metal accumulation ability of the major *Terrestrial weed species* viz. *Calotropis procera, Argimone asteracantha, Sphaerantha indicus, Vetivaria zizinoides, Ipomoea carnea, Hyptis suaveolens, Cichorium intybus, Lantana camara, Parthenium hysterophorus, Xanthium strumarium* and *Arundo donax* growing along the road sides of Jabalpur were evaluated. The highest accumulation of lead was recorded in *Vetivaria zizinoides* (69 mg/kg) followed by *Arundo donax* (49 mg/kg), *Calotropis procera* (21.0 mg/kg) and *Sphaerantha indicus* (10.7 mg/kg). With relatively higher metal extraction ability (Table 8), the terrestrial weeds *Vetivaria zizinoides* and *Arundo donax* were found as potential phytoremediation agents for extraction of lead and manganese for contaminated sites.

Table 8. Heavy metal accumulation by road-side weed in nearby areas of Jabalpur (mg/kg)

Weed species	Lead	Accumulation ratio	Manganese	BCF
Calotropis procera	21.0	5.35	76.6	3.03
Argimone asteracantha	9.5	2.42	132.4	5.23
Sphaerantha indicus	10.7	2.73	118.7	4.69
Vetivaria zizinoides	69	17.6	161.5	6.38
Ipomoea carnea	4.0	1.02	136.1	5.38
Hyptis suaveolens	6.25	1.59	93.9	3.71
Chicorium intybus	3.0	0.76	68.8	2.71
Lantana camara	5.5	1.40	74.5	2.94
Parthenium hysterophorus	5.3	1.35	81.52	3.22
Crotalaria spectabilis	ND	-	44.0	1.74
Ageratum conyzoides	ND	-	34.1	1.35
Alternanthera sessilis	ND	-	124	4.90
Abutilon indicum	ND	-	27.8	1.09
Agaricus arvensis	ND	-	ND	-
Eleocharis geniculata	ND	-	101.5	4.01
Colcasia spp.	1.54	0.39	ND	-
Xanthium strumarium	2.15	0.55	65.4	2.58
Arundo donax	49	12.5	153.4	6.06

(Khankhane and Varshney, 2011)

Similarly a study was conducted to assess the water quality, as well as to identify dominant weed flora (aquatic macrophytes) present in different ponds of Jabalpur (Table 9). Both plant and water samples were collected from Ranital, Gullowa, Mansingh, Mahanadda and Adhartal ponds of Jabalpur during winter season. *Eichhornia crassipes*, *Alternanthera philoxeroides* and *Canna indica* were identified as the dominant weedy plants. The pond water was neutral in reaction with pH values ranging between 7.08 to 7.46 and the electrical conductivity (EC) ranged from 395 to 1678 uS/cm. The dissolved oxygen (DO) varied from 1.75 at Gullowa to 3.3 mg/L at Ranital Pond. The chloride content was above the permissible limit at Mahanadda, Ranital and Gullowa ponds. The heavy metal concentration in the pond water was in the order of Fe > Cd >Mn>Ni >Cu. The concentration of nickel, copper and manganese were far below their respective critical limits of 0.2, 1.5 and 0.5 mg L^{-1} for public uses. *Eichhornia crassipes* showed relatively greater accumulation of heavy metals in roots with average concentration of 20.9, 1.14, 59.5, 6171 and 352 mg/kg for nickel, cadmium, copper, iron and manganese, respectively. Similar metal accumulation pattern, except Fe, was shown by *Canna Indica*. Whereas, *Alternanthera philoxeroides* accumulated greater proportion Ni, Cd, Fe and Mn in shoots over roots (Table 10).

Table 9. Assessment of water quality parameters in different ponds of Jabalpur

Water quality characteristics	Contaminated sites					
	Ranital	Gullowa	Mansing	Mahanadda	Adhartal	Mean
pH	7.15	7.26	7.16	7.46	7.08	7.22
EC (uS/cm)	938	1150	591	1678	395	950
DO	3.3	1.75	2.52	1.32	3.2	2.42
Chlorides (mg/L)	190	180	130	225	100	165
Phosphate (mg/L)	12	8	20	20	12	14.4
Heavy metals (mg/L)						
Nickel	0.158	0.092	0.12	0.03	0.126	0.105
Copper	0.06	0.002	0.02	0.09	0.005	0.035
Iron	1.27	0.108	0.14	1.10	0.168	0.557
Cadmium	0.043	0.077	0.62	0.17	0.020	0.186
Manganese	0.130	0.109	0.08	0.096	0.111	0.105

Source: Khankhane et al., 2014

Floating weed like water hyacinth with higher demand for nutrients and explosive growth rate has been put to use in cleaning up municipal and agricultural waste waters. It has been argued that water hyacinth's quest for contaminants can be turned in a more useful direction. Because of its fantastic ability of absorbing nutrients, rapid growth, low economic maintenance and many other benefits, this plant was recognized to be useful for waste water treatment for removing the heavy metals and other pollutants. The focus on water hyacinth as a key step in waste water recycling is due to the fact that it forms the central unit of recycling engine driven by photosynthesis, and therefore the process is sustainable, energy efficient and cost effective under a wide variety of rural and urban conditions.

On the basis of the above observation and similar other studies it was concluded that *Arundo donax, Typha latifolia, Vetivaria zizinoids, Ipomoea carnia* are the promising terrestrial weeds, and *Eichhornia crassipes* and *Alternanthera philoxeroides* are the most promising floating weeds for phytoremediation purpose.

Table 10. Heavy metal content (mg/kg) in plant parts of aquatic weeds

Name of Weeds	Plant part	Heavy metals (mg/kg)				
		Ni	Cd	Cu	Fe	Mn
Alternanthera Philoxeroides	Shoot	441	16.4	2448	3586	1666
	Root	149	12.0	2839	569	1196
Eichhornia Crassipes	Shoot	56.8	1.44	552	2645	1014
	Root	253	21.8	2868	6576	6624
Canna indica	Shoot	392	0.63	445	1414	951
	Root	465	4.24	719	959	2173

Source: Khankhane and Vershney, 2008

5.2 Screening of best of terrestrial weedy plant for lead removal from contaminated water in net house

Four plant species, namely, *Vetivaria zizinoides, Typha latifolia, Acorus calamus* and *Arundo donax* were exposed to different Pb levels, *viz.* 25, 100 and 400 mg/L. Significant differences among plant species were observed with regard to lead removal. These plants exhibit different types of Pb accumulation pattern; *Arundo donax, Acorus calamus* and *Typha latifolia* accumulated higher proportion of Pb in their roots (rhizofiltration mechanism), while *Vetivaria zizinoids* accumulated maximum portion of Pb in their shoots. At all the three tested levels of Pb in water, *Arundo donax* roots showed higher accumulating ability than the roots of *Typha latifolia, Acorus calamus* and *Vetivaria zizinoides*. *Arundo donax* accumulated 91, 120 and 671 mg/kg Pb in roots at the Pb concentration levels of 25, 100 and 400 mg/L, respectively (Fig.7A). In case of Pb accumulation by shoot, *Arundo donax* accumulated highest amount of Pb at higher concentration levels (100 and 400 mg/L); but at lower concentration level (25 mg/L), Pb accumulation was highest in *Vetivaria zizinoids* compared to other weeds (Fig.7B).

Fig.6. Nursery of *Arundo donax*, *Vetivaria zizinoids*, *Acorus calamus* and *Typha latifolia* at ICAR-DWR net house facility

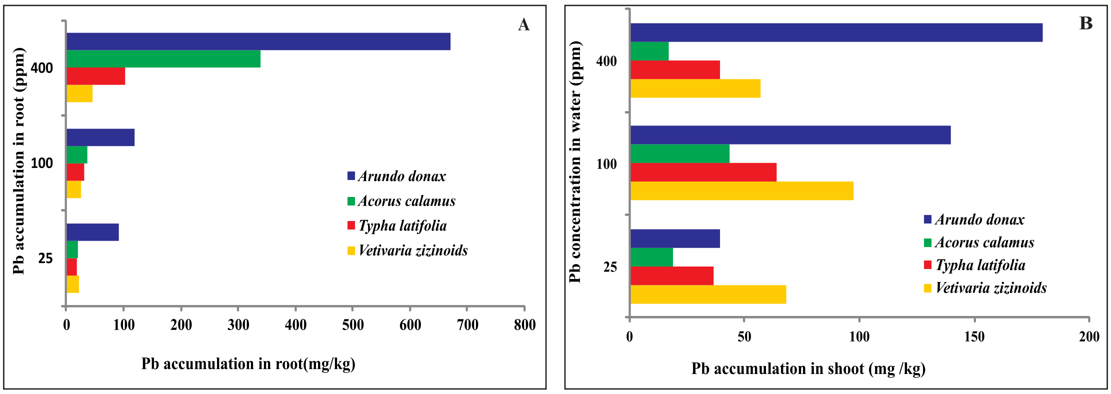


Fig.7. Lead (Pb) accumulation in roots (A) and shoot (B) under terrestrial weedy plants

6. Evaluation of small scale phytoremediation model for laboratory purpose

An investigation was carried out to study the performance of sub-surface wetland model using fast growing weed, *Arundo donax* for the treatment of drain water. A sub-surface rectangular wetland model made up of stainless steel of size 4'x 2'x 1' (Length, width and depth) was developed for the treatment of waste water. The three zones were maintained in the model including inlet, middle zone as a treatment bed and outlet zone

Treatment bed **Sedimentation zone**

Photo showing treatment of waste water in a model of constructed wetland

Fig.8. *Arundo donax* nurshary grown in hydroponics model, where stone chips are used to support root (*Source*: Khankhane and Versheney, 2015)

Waste water before treatment **Waste water after treatment**

Fig.9. Change in colour of waste water before and after phytoremediation (*Source*: Khankhane and Versheney, 2015)

Table 11. Performance of *Arundo donax* in a small scale wetland model

Parameter	Average concentration		% reduction of pollutants
	Drain water (before treatment)	Drain water (after treatment)	
pH	7.91	7.85	NA
EC (dS/m)	0.65	0.62	NA
Nitrates (mg/L)	50.0	15.0	70.0
Phosphates (mg/L)	3.50	2.0	42.8
Nickel (mg/L)	340	150	55.8
Copper (mg/L)	320	190	40.6

NA:Not applicable *Source*: Khankhane and Versheney, 2015

Arundo donax planted in the gravel medium (without soil) grew with a well spread entangling root system and no clogging occurred, as a result the treated water was easily discharged through the outlet of the filter bed. *Arundo donax* with a well spread entangling root system performed as a filter bed and withheld the suspended particles which reduced the colour of the waste water. As compared to the untreated waste water, the concentrations of nitrate, phosphate, nickel and copper in the treated water decreased to the extent of 70, 42.8, 55.8 and 40.6 per cent, respectively (Table 11). On the basis of the performance of *Aundo donax* in the sub-surface model, i.e. its ability to grow successfully in a gravel medium and phytoextraction potential for pollutants, it was decided to utilize this plant for treatment of drain water in field scale phytoremediation system for irrigation purposes.

7. Enhancement of Phytoremediation

7.1 Chemical enhancement

Various chemical substances are known to influence phytoremediation process. Chemical agents like EDTA, DTPA form chelate or metal-organic complexes with various heavy metals and consequently enhance metal uptake by plants.

An Investigation conducted at ICAR-DWR revealed that addition of EDTA enhanced the cadmium (Cd) accumulation by *Arundo donax* (Table 12). In absence of EDTA, Cd concentration in plant root increased with the increase in its concentration in water up to the highest tested level of 1200 mg/L. At all the tested levels of Cd, addition of 3 mg/L EDTA in the test solutions significantly increased Cd concentration in plant root over the corresponding no EDTA treatments. For example, at 100 mg/L level of Cd in test solution, Cd concentration in plant root increased from 105.9 mg/kg (in absence of EDTA) to 245.7 mg/kg when 3 mg/L EDTA was applied. In presence of EDTA, highest accumulation of Cd by plant roots was recorded at 800 mg/L level of Cd in test water, and root accumulation of Cd declined significantly beyond this test level. It may be noted that at higher level of Cd, *Arundo donax* showed toxic symptoms in terms of total biomass production, leaf area and root growth (**Table 13**).

Addition of EDTA increased bioaccumulation factor (BCF) of Cd in roots. The BCF of *Arundo donax* root increased from 1.059 (without EDTA) to 2.457 when 3 mg/L EDTA was applied at 100 mg/L Cd level; similarly the BCF increased from 1.96 to 4.176 when 6 mg/L EDTA was applied at 200 mg/L Cd level. In this study maximum BCF of 18.26 was obtained in case of the test solution containing 6 mg/L EDTA with 200 mg/L Cd. Subsequent increase in Cd concentration reduced BCF value, but EDTA effect was still there (**Fig. 10**).

Table 12. Interaction effect between cadmium levels and EDTA on Cd accumulation by shoot and root of *Arundo donax*

Cadmium nitrate (mg/L)	Cadmium accumulation in root		
	EDTA levels (mg/L)		
	0	3	6
Cd 100	106	246	669
Cd 200	392	835	3652
Cd 400	777	3096	3084
Cd 800	1977	3942	4225
Cd 1200	2227	2523	2707

LSD (P=0.05): Cd x EDTA = 996; *Source*: Khankhane et al. (2017)

Table 13. Effect of cadmium levels on leaf area, root length and dry weight of Arundo donax

Treatments	Leaf area (cm²)	Root length (cm)	Dry weight (g)
Cadmium conc.(mg/L)			
0	91	41.8	4.03
100	124	42.2	3.96
200	138	47.4	3.91
400	125	44.0	4.01
800	112	38.8	3.52
1200	90	30.1	3.19
LSD (P=0.05)	30	47.9	0.10
EDTA levels (mg/L)			
0	3.72	120	47.9
3	4.27	123	38.8
6	3.32	98	35.4
LSD (P=0.05)	0.73	21	7.1

Source: Khankhane et al. (2017)

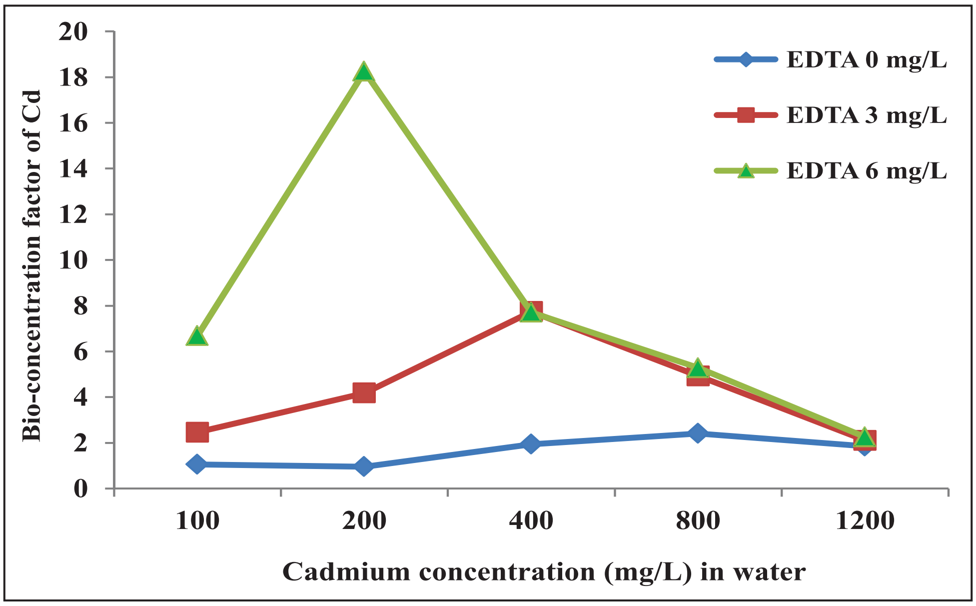


Fig.10. Influence of EDTA on bio-concentration factor of Cd by *Arundo donax* root
(*Source*: Khankhane et al. 2017)

7.2 Using microbes

Reports regarding enhancement of phytoremediation potential of various plants due to inoculation with plant growth promoting rhizobacteria (PGPR) are there in the literature. In view of this attempts are made to isolate Cd tolerant rhizobacteria for *Arundo donax*. Soil and plant samples were collected from different heavy metal contaminated areas of Jabalpur district. The rhizosphere soil of the dominant weed flora viz. *Typha latitfolia*, *Rumex crispus*, *Ageratum conyzoides*, *Chenopodium album*, *Eichhornia crassipes*, *Ammannia auriculala*, *Sidaa cuta* and *Cyperus rolundus* growing in the contaminated sites were sampled (**Table 14**). The PGPRs were isolated from the soil samples using serial dilution technique and King's B medium.

Table 14. Selected PGPR isolates from rhizosphere of weed species collected from heavy metal contaminated sites

Botanical Name	Isolate code	No of isolates
Ageratum conyzoides	ACR	8
Ammannia auriculata	AAP	4
Chenopodium album	CAP	7
Cyperus rotundus	CRP	7
Eichhornia crassipes	ECU	6
Eryngium foetidum	EFP	2
Panicum repens	PRB	5
Rumex crispus	RCB	6
Sida acuta	SAU	6
Typha latifolia	TLP	7

(*Source*: Sarathambal et al., 2016)

Total 58 isolates having PGPR characteristics, i.e. ability to produce siderophores, plant hormone (IAA) and enzymes like 1- aminocyclopropane-1-carboxylate (ACC) deaminase, and P solubilisation were obtained (Table 15). Out of them 12 isolates were able to tolerate Cd up to 400 mg/L. The sequence analysis of the 16S rRNA genes indicates that these isolates belong to the phylum Firmicutes.

The isolates were screened based on maximum tolerance concentration (MTC) of various heavy metals such as Cd, Hg, Co, Ni and Zn. The 16S rRNA gene sequence analysis identified the isolates as *Bacillus* sp., *Bacillus subtilis* and *Bacillus licheniformis*. Among the isolates *Bacilllus* sp. (TLP2), *Bacillus subtilis* (RCB4), *Lysinibacillus* sp.(TLP7) and *Bacillus licheniformis* (RCP4) are the elite isolates.

Table 15. Plant growth promoting characteristics of bacteria associated with rhizosphere of weedy species (Sarathambal et al., 2016)

Isolate	[1]Siderophore	[2]ACC deaminase	[3]Available P	P-solubilisation efficiency (%)	[4]IAA
RCB1	22.4c	ND	0.34f	60e	17.7e
RCB4	54.6b	92.5a	0.67c	225b	42.4a
RCB5	38.4b	74.3b	0.55d	200bc	34.5b
AAP4	18.9cde	ND	0.68c	150cd	18.4e
TLP1	20.9cd	ND	0.46e	140d	12.3g
TLP2	66.3a	76.8b	0.96a	233a	30.8c
TLP7	13.5e	87.9ab	0.81b	50e	23.4d
SAU6	13.5e	ND	0.67c	250a	17.5e
RCP4	23.5cd	83.9ab	0.55d	125d	10.8h
ACR3	18.4de	ND	0.29g	150cd	ND
ECU6	20.4cde	ND	0.26g	33e	13.9f
CRP1	20.9cd	ND	0.32h	167cd	17.3e

[1]Catechol type, µg/mg protein; [2]nmoles of α-ketobutyrate/mg protein/h; [3]µg/ml; [4]µg/ml

Later the PGPR isolate *Bacillus* sp. (TLP2) had been tested on *Arundo donax* to study its role in cadmium uptake. It was noted that, the isolate *Bacillus* sp. significantly improves the growth, the activity of antioxidants enzymes, and the Cd uptake in *Arundo donax* (**Table 16**).

Table 16. Effect of plant growth promoting bacteria on plant biometric characters and Cd uptake in *Arundo donax*

Treatment	Plant height (cm)	No of tillers	Cadmium uptake (mg/kg)
Control (0 mg/L)	45.6e	11	0.5e
Cd 3 mg/L	56.8cde	13	0.6e
Cd 5 mg/L	53.6de	10	0.7e
Cd 3 mg/L + *Bacillus* sp.(TLP2)	60.5cde	15	0.9de
Cd 5 mg/L + *Bacillus* sp. (TLP2)	62.6bcde	14	1.5bc
Cd 3 mg/L + *Bacillus cereus*	69.8abcd	16	1.6bc
Cd 5 mg/L + *Bacillus cereus*	70.3abcd	14	1.5bc
Cd 3 mg/L + *Bacillus megaterium*	71.9abcd	13	1.4c
Cd 5 mg/L + *Bacillus megaterium*	72.9abcd	17	1.3cd
Cd 3 mg/L + *Bacillus* sp. (TLP2)+ AM	73.8abcd	15	1.4c
Cd 5 mg/L + *Bacillus* sp. (TLP2) + AM	75.9abcd	16	1.3cd
Cd 3 mg/L + *Bacillus cereus* + AM	84.9ab	19	2.3a
Cd 5 mg/L + *Bacillus cereus* + AM	89.4a	18	1.9ab
Cd 3 mg/L + *Bacillus megaterium*+ AM	72.5abcd	17	1.5bc
Cd 5 mg/L + *Bacillus megaterium* + AM	73.5abcd	16	1.4c
Cd 3 mg/L + AM	79.6abc	18.5	1.2cd
Cd 5 mg/L + AM	80.6abc	18.8	1.2cd

(*Source*: Sarathambal et al., 2016)

8. Developing constructed wetland model for rhizofiltration of heavy metals from sewage

Rhizofiltration involves use of plant roots to absorb, concentrate and/or precipitate hazardous contaminants from aqueous solutions. This method is often used for heavy metal contaminants viz., lead, chromium (III), nickel, lead, cadmium, iron, manganese, etc. which get strongly adsorbed to roots. Hydroponically cultivated plants rapidly remove heavy metals from water, and concentrate them in the roots and shoots. Rhizofiltration is effective when wetlands are created to pass the entire volume of contaminated water through the root zone of densely growing vegetation having capability to absorb large quantities of heavy metals from water. Shallow lagoons are commonly engineered as wetlands and maintained as facultative microbial systems. Wastewater is pumped through the system for the removal of contaminants by rhizofiltration.

Constructed wetlands provide ecological improvement through multipurpose waste water treatment systems. Wetland treatment systems fall into the following three major types:

i) Free water surface (FWS) systems: In a FWS, water flows horizontally at a low velocity over the top of the porous media and through wetland vegetation in shallow basins or channels.

ii) Subsurface flow systems (SFS): SFS is similar to the FWS system, but the water flows through the substrate. SFS require less land area and are performed better than FWS. In this wetlands the waste water flows through a highly permeable sediment and is collected in drain.

iii) Aquatic plant systems (APS): An APS is similar to FWS, but the water is in deeper ponds and the vegetation consists of floating or submerged plant systems.

Ideal wetland system consists with four main segments, i.e. (a) waste water inlet system followed by (b) sedimentation tank (pre treatment), (c) treatment tank (primary and secondary treatment tank) and (d) water outlet system (for irrigation or other purpose). Through inlet system the waste water enters in primary settling tank or sedimentation tank, in which waste water is allowed to stay for 24-48 hrs so that suspended solid particles get settled down at the bottom of the tank. After this, water is passed to treatment tank and is allowed to stay there for some days (7-10 days). In the treatment tank, base is filled with gravel and hyper accumulator plants are grown in water. Based on the contaminant load two or more treatment tanks may be used to treat waste water. Finally treated water is used for irrigation or other purposes.

Based on the performance of laboratory-scale constructed model and design, a hybrid constructed wetland system consisting of pre-treatment overhead settling zone and treatment zone having three pairs of sequential tanks (3m L x 2m B x 0.75m) was developed at ICAR-DWR farm. The selected weed species with phytoremediation potential was hydroponically grown in first pair of tanks. It was also grown in angular gravel media in second (surface) and third (sub-surface) pairs of tanks (Fig. 11 and Fig. 12). The polluted water from nearby drain was injected into pre-treatment overhead tanks for settling of solid particles at bottom, and then the water was directed through sequential treatment tanks. The water samples collected from the system were analyzed for nitrates, phosphates and different heavy metals at outlet point of hydroponic, surface and sub-surface tanks. The retention time of water in treatment zone was 2 to 3 days. After giving suitable detention time for the treatment, purified water is collected from outlet zone either for irrigation purpose or for its safe discharge in natural water bodies.

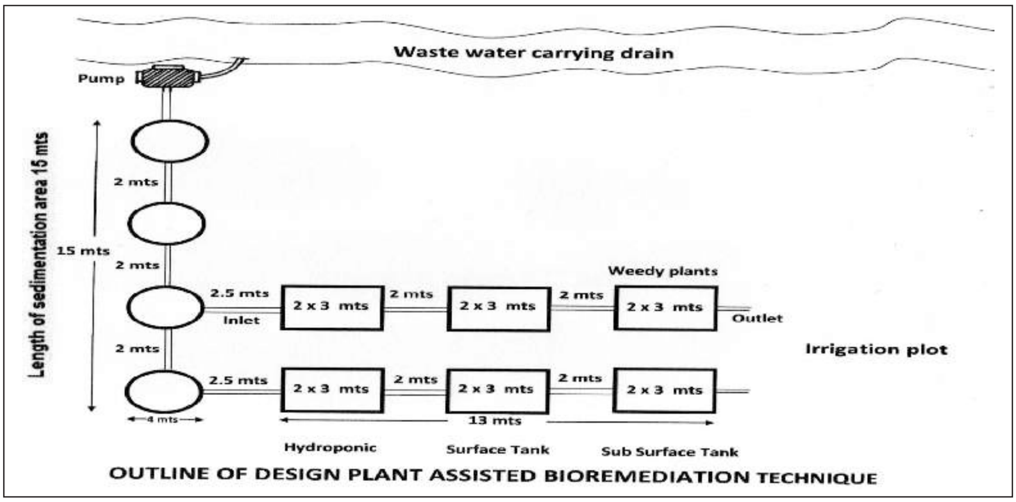


Fig.11. Layout of the constructed wetland

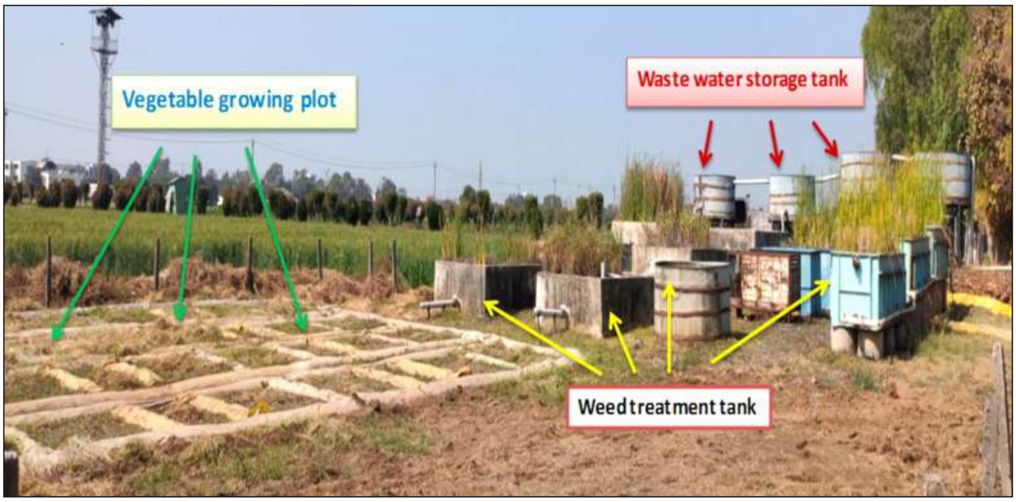


Fig.12. Wetland developed at ICAR-DWR, Jabalpur

9. Evaluation of Phytoremediation efficiency of weeds in large scale wetland system

9.1 Phytoremediation efficiency of Arundo donax

An investigation was carried out in a constructed wetland as described in section 8 to test the efficacy of weed-based phytoremediation system using *Arundo donax* for remediation of heavy metals in industrial drain water. The polluted water from waste water carrying drain was injected into pre-treatment overhead tanks. After settling of solid particles at bottom, the water from pre-treatment tanks was directed to treatment tanks. The fast growing *Arundo donax* was planted hydroponically in first pair of treatment tanks and in angular gravel media in surface and sub-surface tanks. It was observed that *Arundo donax* developed its extensive root system (110-134 cm in length) in hydroponically grown tanks (Table 17 and Fig 13). In surface and sub-surface tanks also its roots were highly entangled with angular gravels. The average density of plant grown in surface and subsurface tanks was 172.3/m^2. As far as water flow through porous media is concerned, no clogging occurred and there was free discharge of water from treatment tanks to irrigation plots through gravity flow.

Table 17. Growth parameters of *Arundo donax* shown in wetland phytoremediation system

Treatments tanks	Plant height (cm)				Density of plants (No/m^2)	
	Row-I		Row-II		Row-I	Row-II
	Shoot	Root	Shoot	Root		
Hydroponics	93.9	134	73.1	110	149	141
Surface	103	-	116	-	172	136
Sub-surface	107	-	109	-	224	212
Mean	101.3	-	99.36	-	181.6	163

Source: Annual Report 2011-12, ICAR-DWR

Water samples collected from the outlet points of sedimentation, hydroponic, surface and subsurface tanks after treatment period were analysed for nitrates, phosphates and different heavy metals. As compared to untreated drain water, the concentrations of total soluble salts (TSS), nitrate, copper, nickel, zinc and manganese have reduced to the extent of 64.0, 88.4, 69.3, 62.4, 78.0 and 61.7 per cent, respectively in the treated water (Table 18). Unlike nitrate removal, which was reduced by 88.4%, phosphate removal was relatively lower i.e. 46.3% .

Table 18. Contaminants in drain water after treatment in constructed wetland system using *Arundo donax*

Treatments	TSS (mg/L)	NO_3 (mg/L)	PO_4 (mg/L)	Cu (mg/L)	Zn (mg/L)	Mn (mg/L)	Fe (mg/L)	Bacteria (cfu/ml)
Sedimentation Tank	238	19.5	2.72	0.388	0.43	1.78	1.66	6.5×10^4
Tank I (Hydroponics)	152	9.40	1.90	0.340	0.41	1.17	1.25	24×10^3
Tank II (Surface)	134	8.79	1.71	0.187	0.35	0.93	0.92	14.7×10^2
Tank III (Sub-surface)	89	2.29	1.56	0.141	0.12	0.69	0.55	8.1×10^2
Waste water (Untreated)	249	20.3	2.91	0.468	0.54	1.83	1.73	18.7×10^4
Efficiency (%)	64.0	88.4	46.3	69.36	78.0	61.7	68.2	-

Source: Annual Report 2011-12, ICAR-DWR

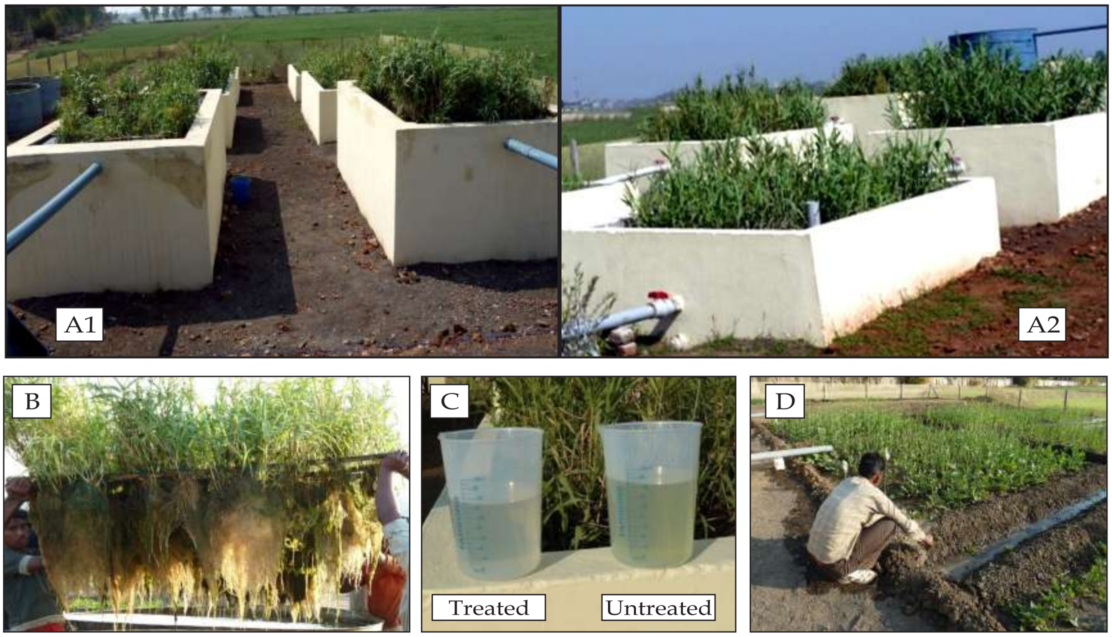


Fig.13. Large-scale demonstration of weed (*Arundo donax*) assisted phytoremediation of waste water and subsequent re-use as irrigation in mustard growing plots. (Fig.A depicted *Arundo donax* growing treatment tank, Fig.B. *Arundo donax* roots, Fig.C *Arundo donax* treated clear water vs waste water, Fig.D *Arundo donax* treated waste used as irrigation water in mustard growing plots)

Reduction in microbial population in the waste water and treated water is depicted in Fig.14. Reduction of Pb, Cd and Ni content in the waste water after treatment in hydroponic (Tank 1), surface (Tank 2) and subsurface tanks (Tank 3) are depicted in Fig.15. The Data revealed that in the final treated water the content of Ni, Cd and Pb decreased by 63, 48 and 77%, compared to untreated water. The result showed that among the tested heavy metals *Arundo donax* was most efficient for removal of Pb.

Microbes colony in waste water

Microbes colony in waste water of sedimentation tank

Microbes colony in water treatment by sub-surface tank

Fig.14. Sequential reduction of bacterial population in waste water after treatment with *Arundo donax* in constructed wetland system

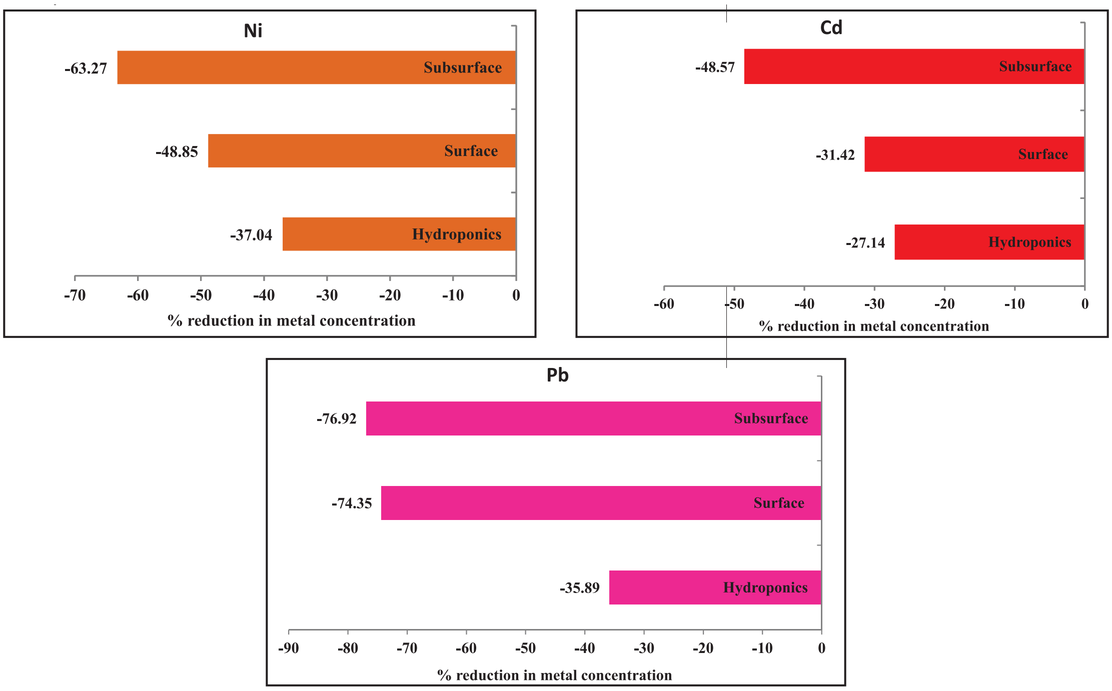


Fig.15. Reduction in Ni, Pb and Cd by *Arundo donax* in large-scale wetland system

9.2 Phytoremediation efficiency of Typha latifolia

Phytoremediation potential of *Typha latifolia* was evaluated in a constructed wetland system as described in section 8. It was grown hydroponically in the first treatment tank, while in second (Surface) and third (Sub-surface) treatment tanks it was grown on angular gravel media (Fig.17A. Development of extensive root system of *Typha latifolia* was noticed in the hydroponic tank (Fig. 18). Contaminated waste water from drains pumped into first sedimentation tank and which was then passed through hydroponic, surface and subsurface tank in sequential manner. The concentration of nitrate, phosphate iron, copper and cadmium in drain water was 2.82, 1.84, 0.3282, 0.0363 and 0.0126 mg/L respectively. After treatment period, water samples were collected from the outlet points of sedimentation, hydroponic, surface and subsurface tanks, and were analysed for nitrates, phosphates and different heavy metals like Fe, Cu and Cd.

According to Environmental protection agency (EPA, 1986) the phosphorus content in the stream was not more than 0.1 mg/L as, indicating that P content in the drain water used in this study was beyond the permissible limit. Similarly according Food and Agriculture Organization (FAO, 2005), maximum permissible limit for Cd in irrigation water is 0.01 mg/L. Since the Cd content in the drain water used in this study was slightly higher than maximum permissible limit, it should not be used directly for irrigation purpose.

It was noted that, turbidity (Fig.19A), metal and nutrient content in waste water reduced gradually as the water sequentially passed from sedimentation tank to final outlet of the subsurface tank. For example, Fe content of water collected from the outlets of sedimentation, hydroponic, surface and sub-surface tanks decreased by 31.65%, 52.83, 80.19 and 89.15% respectively from its initial value. Similar trend was also noticed in case NO_3, PO_4, Cu and Cd. Reduction in metal and nutrient content of the water after full sequence of treatment by *Typha latifolia* is given in Fig.16. It showed that, highest reduction was observed for Fe (89.15%) followed by NO_3 (80.39%), Cu (56.9%), PO_4 (42.6) and Cd (26.24%).

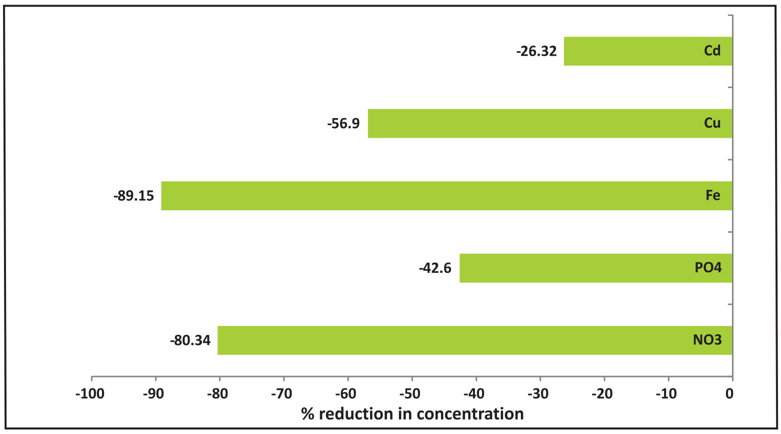


Fig.16. Reduction in Cd, Cu, Fe, NO_3 and PO_4 content by Typha latifolia in large-scale wetland system

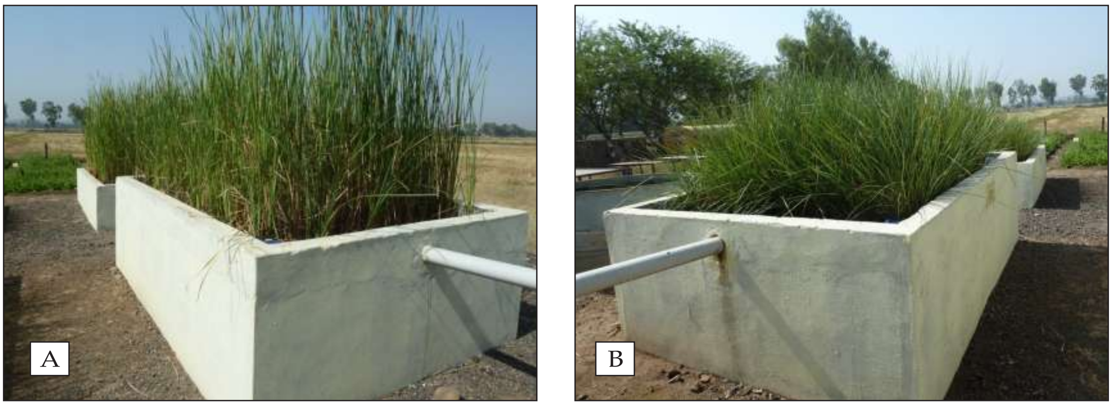
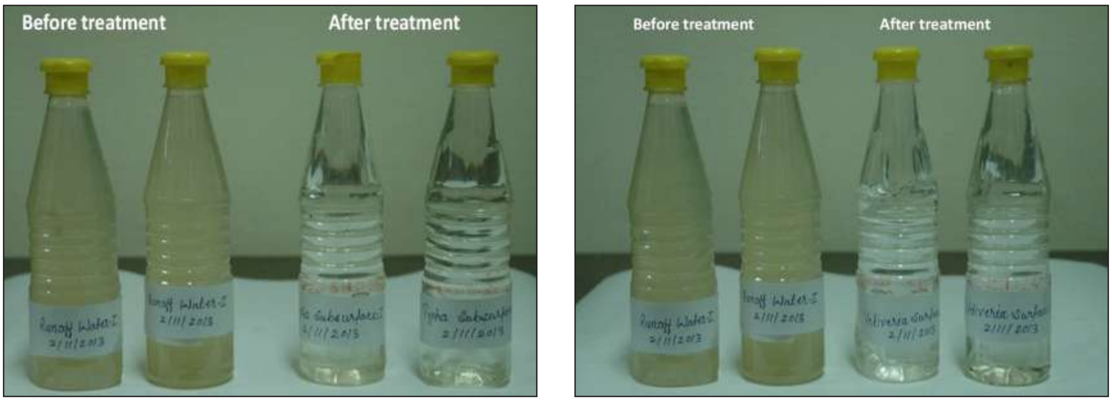


Fig.17. Typha and Vetivria growth in constructed wetland

Fig.18. Root growth of *Tyhpa latifolia* in wetland



Typha treated **Vetivaria treated**

Fig.19. Comparison of waste water before and after treatment

9.3 Phytoremediation efficiency of Vetivaria zizinoides

Phytoremediation potential of *Vetivaria zizinoids* was evaluated in a constructed wetland system as described in section 8. It was grown hydroponically in the first treatment tank, while in second (Surface) and third (Sub-surface) treatment tanks it was grown on angular gravel media (Fig.17B). Contaminated waste water from drains pumped into first sedimentation tank and which was then passed through hydroponic, surface and subsurface tank in sequential manner. The concentration of nitrate, phosphate iron, copper and cadmium in drain water was 2.82, 1.84, 0.3282, 0.0363 and 0.0126 mg/L respectively. After treatment period, water samples were collected from the outlet points of sedimentation, hydroponic, surface and subsurface tanks, and were analysed for nitrates, phosphates and different heavy metals like Fe, Cu and Cd.

It was noted that, turbidity (Fig.19B) metal and nutrient content in waste water reduced gradually as the water sequentially passed from sedimentation tank to final outlet of the subsurface tank. For example, NO_3 content of water collected from the outlets of hydroponic, surface and sub-surface tanks decreased by 58.8, 70.45 and 80.34% respectively from its initial value. Similar trend was also noticed in case PO_4, Fe, Cu and Cd. Reduction in metal and nutrient content of the water after full sequence of treatment by *Vetivaria zizinoids* is given in Fig. 20. It showed that, highest reduction was observed for NO_3 (79.34%) followed by Fe (68.52%), Cu (45.18%), PO_4 (38.34) and Cd (30.95%).

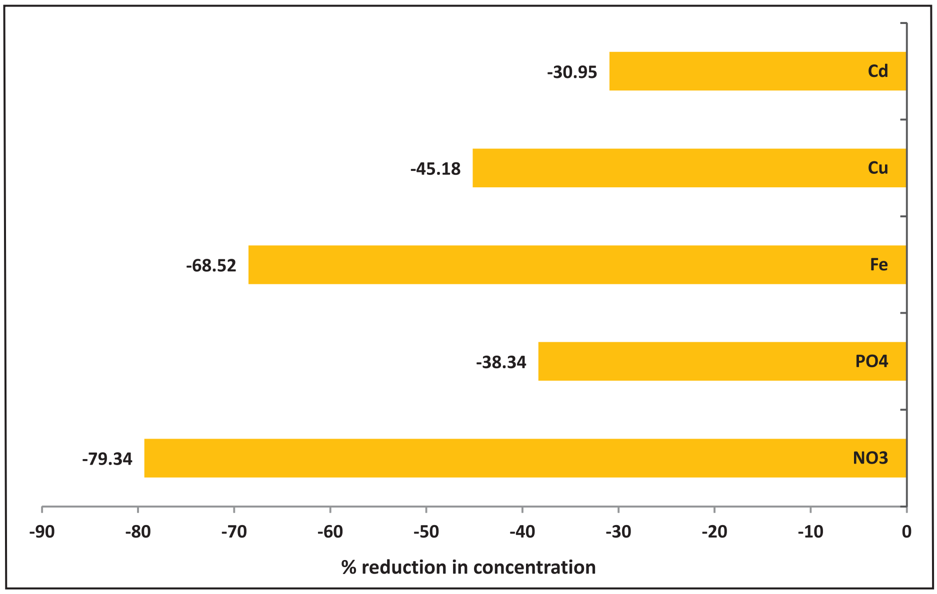


Fig. 20. Reduction in Cd, Cu, Fe, NO_3 and PO_4 content by *Vetivaria zizinoids* in large-scale wetland system

10. Evaluation of treated water as irrigation source for vegetables

Waste water obtained after treatment in *Typha* and *Vetivaria* based wetland system was used for irrigation in fenugreek (Fig.22) and tomato (Fig.24) to assess the impact of phytoremediation measure on reduction in heavy metal accumulation in edible parts such as leaf and fruits of these crops. Plant samples collected at their full maturity for laboratory analysis.

In fenugreek, matured leaves were used for Cd. Relatively higher content of Cd was detected in fenugreek leaves irrigated with waste water, whereas lower proportion Cd was observed when irrigated with *Typha latifolia* and *Vetivaria zizinoides* treated water. The leaf samples collected from the plots receiving irrigation with Typha treated water showed about 74% less Cd content compared to those receiving untreated waste water irrigation. Similarly *Vertivaria ziznoides* treatment showed 45% less Cd content in fenugreek leaves (**Fig. 21.**).

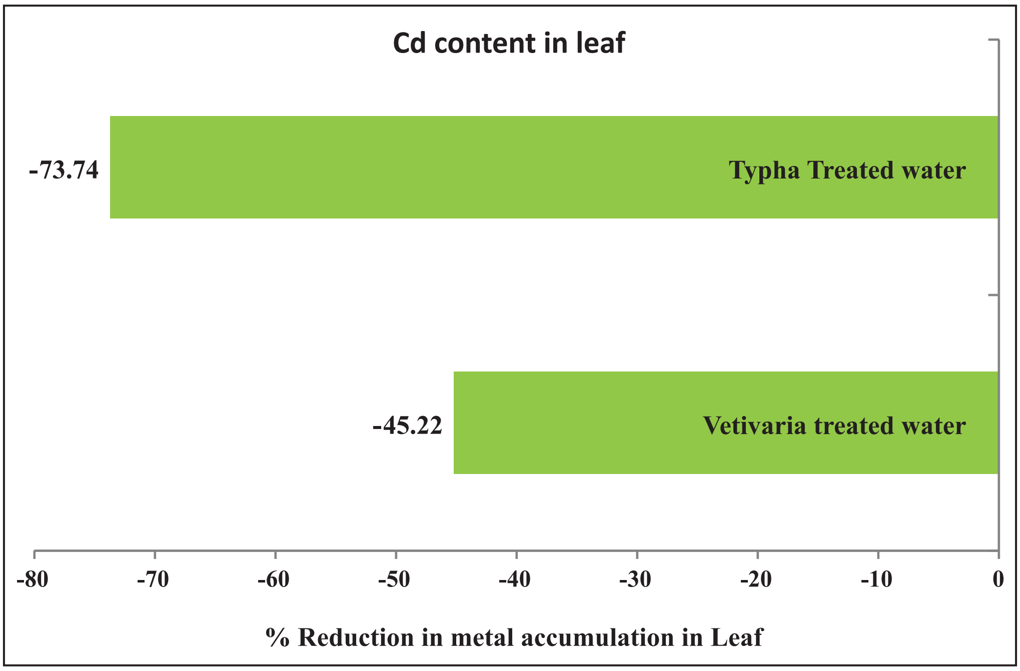


Fig. 21. Reduction in Cd content in leaf of fenugreek irrigated with Typha and Vetivaria treated water

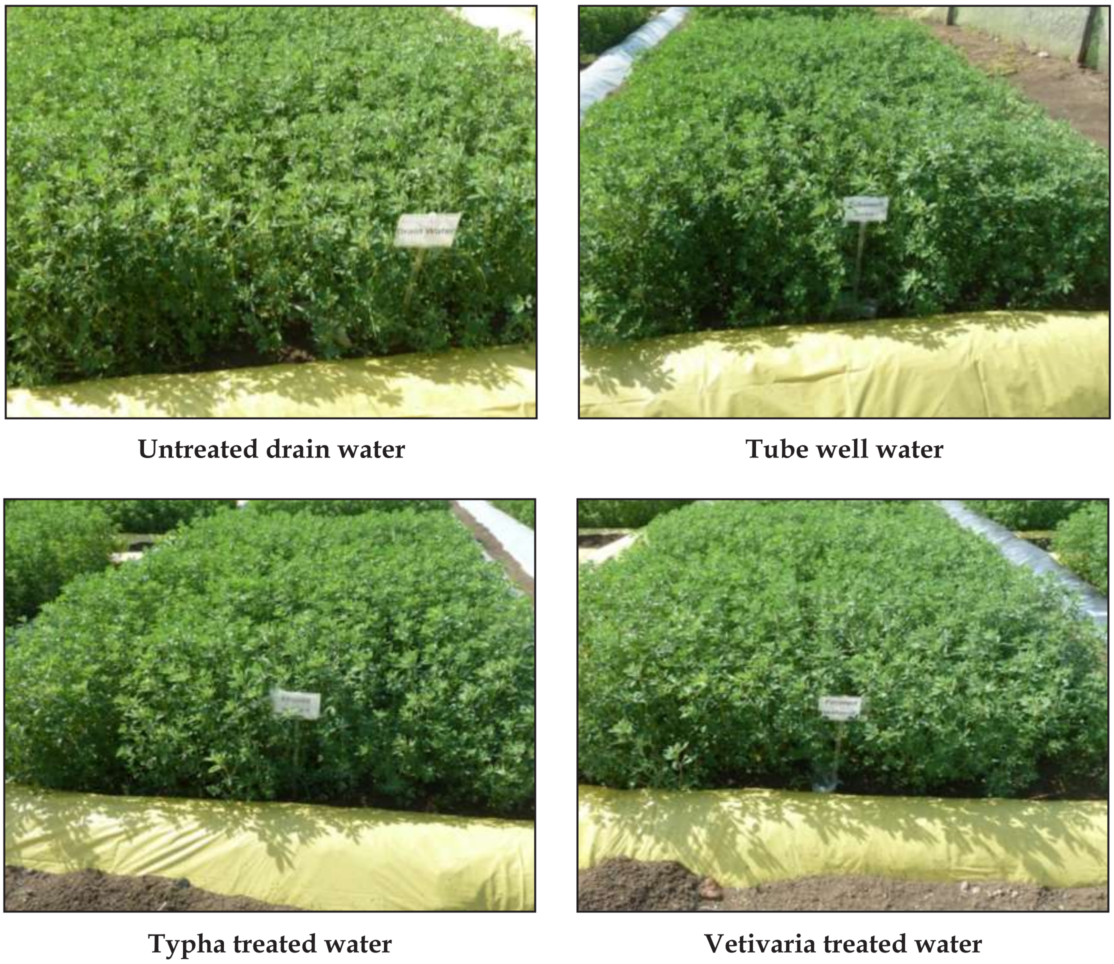


Fig. 22. Growth of fenugreek in the experimental plots receiving irrigation with untreated drain water, tube well water, Typha treated water and Vetivaria treated water (*Source*: Annual Report 2016-17, ICAR-DWR

Similar observations were also recorded in case of tomato fruit samples. Irrigation with *Typha latifolia* and *Vetivaria zizinoides* treated water reduced Cd content in tomato fruits by 47 and 43% compared to untreated waste water irrigation (Fig. 23). These indicates that Typha based phytoremediation system is more efficient than Vetivaria based phytoremediation system in reducing Cd load in food chain.

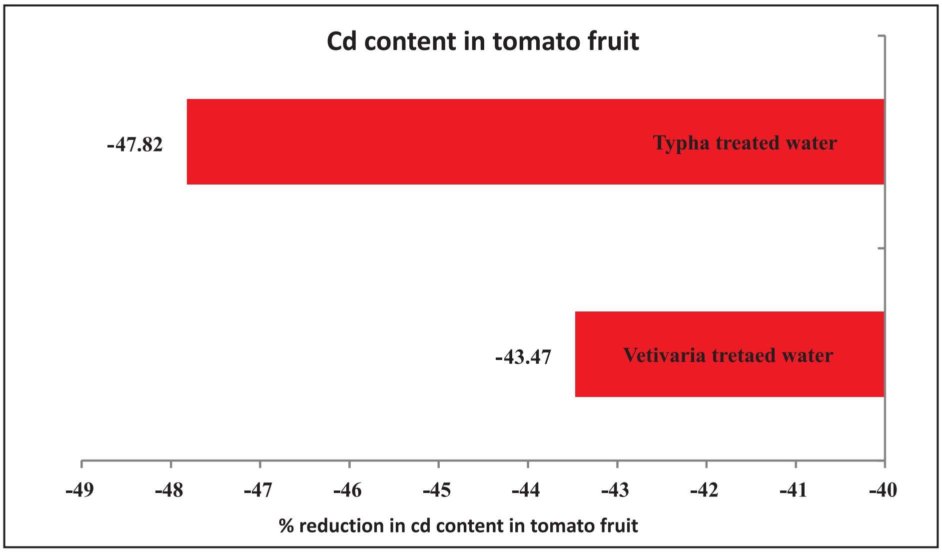


Fig. 23. Reduction in Cd content in tomato (fruit) receiving Typha and Vetivaria treated water over untreted drain water

Untreated drain water

Tube well water

Typha treated water

Vetivaria treated water

Fig.24. Growth of tomato in the experimental plots receiving irrigation with untreated drain water, tube well water, Typha treated water and Vetivaria treated water

(*Source*: Annual Report 2014-15, ICAR-DWR)

11. Management of post-harvest biomass

While selecting the local weedy plants for phytoremediation, their further uses are to be assessed for economic considerations. Several known weeds have been put to certain economic use since ages. Composting can significantly reduce the volume of harvested biomass. The economic utilization of aquatic weeds, such as water hyacinth, for composting depends on the cost of harvesting and transportation. Using dry weed biomass as mulch in the adjoining areas of its production site could be an economically more viable means of utilization. Use of weed biomass mulch in crop fields was found to help in increasing crop production. For example, Barman et al (2009) observed that the water hyacinth mulch was superior over rice straw mulch in increasing potato yield in black cotton soils of Jabalpur. However, the plants biomass generated from the phytoremediation sites containing heavy metals should not be used for composting and other agricultural uses such as mulching. Recent reports suggested that some weed species such as Vetiveria zizinoides and Typha may be used for making cooling mats, brooms, etc. Similarly, water hyacinth can be used for making paper and production of ethanol. At ICAR-DWR, the terrestrial weed *Arundo donax*, having semi-hard stem, was exploited for making baskets (**Fig. 25**).

Fig.25. Busket made of *Arundo donax* and Bamboo at ICAR-DWR

12. Demonstration of weed based phytoremediation techniques at farmer's field

The *Eichhornia crassipes* (water hyacinth) and *Pistia stratiotes* (water lettuce) based phytoremediation systems were constructed and their performance were demonstrated in Urdua village (Fig.27). In both systems waste water coming from Jabalpur city was used for treatment purpose. Both the systems significantly reduced turbidity, sodium, chloride, sulphate, chromium and phenol content within 7 days. Compared to untreated waste water, water hyacinth treated water showed 97, 70, 40, 55 and 76 % lesser turbidity, Na, Cl, SO4, Cr and phenol respectively. The result showed that (Fig.26) water hyacinth was a better phytoremediating agent than *Pistia stratiotes*.

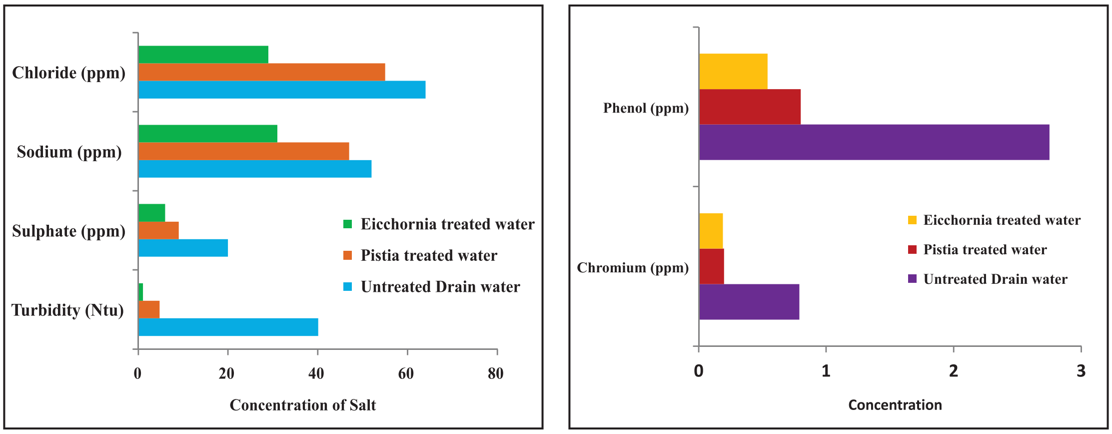


Fig. 26. Comparative assessment of water hyacinth and water lettuce based phytoremediation system for removal of heavy metals and other impurities from contaminated drain water

Fig.27. Floating aquatic weed based phytoremediation model constructed in urdua village, Jabalpur

13. Conclusion

India currently generates about 72400 million litres of waste water every day. But, the existing sewage treatment plants can actually treat only 20350 MLD. The utilization of this huge volume of untreated waste water as irrigation source is leading to entry of heavy metals in food chain. Another problem often encountered due to sewage water irrigation is increased weed menace in field. Huge operational cost is associated with modern sewage treatment facilities; hence, it is need of the hour to develop low-cost technology for creating economically and environmentally sustainable waste water recycling system. Our study showed that phytoremediation system with selected weedy plants can be utilized for recycling sewage water for agricultural use. Weed based phytoremediation system can significantly reduce turbidity and contents of nitrate, sulphate, phosphate and heavy metals, viz. cadmium, chromium, nickel and lead, in treated water. Various weedy plants were collected from sewage water containing drains, ponds and associated polluted areas in Jabalpur, and they were evaluated for effective phytoremediation ability. *Arundo donax*, *Typha latifolia*, *Vetivaria ziznoides*, *Acorus calamus*, *Phragmites karka* among the terrestrial weeds, and *Eichhornia crassipes* and *Pistia stratiotes* among floating weeds were found suitable for phytoremediation purpose. The effectiveness of the weed based phytoremediation systems for various pollutants depended on the choice of plants employed. In general, it was observed that terrestrial weeds like *Arundo donax*, *Typha latifolia* and *Vetivaria zizinoids* performed better than the free floating aquatic weeds like *Eichhornia crassipes* and *Pistia stratiotes*. The *Arundo donax* was the best agent for phytoremediation of cadmium and lead, as it can tolerate very high levels of these metal species in water and perform phytoremediation work efficiently. It accumulated these heavy metals more in roots compared to shoots. The treatment of metal contaminated drain water in a large scale constructed wetland with *Arudno donax* as phytoremediation agent had significantly lowered cadmium and lead content in it. Similarly, *Typha latifolia* and *Vetivaria zizinoids* have also showed excellent phytoremediation potential of Cd containing drain water. Treated water from *Typha latifolia* and *Vetivaria zizinoids* based constructed wetlands had been evaluated as irrigation source in fenugreek and tomato. It was observed that compared to untreated drain-water; accumulation of cadmium in the edible parts of these crops was significantly lower when treated water was used for irrigation. These findings showed that the weed based phytoremediation systems have potential to serve as low cost solution for waste water treatment and can be successfully implemented in large scale

14. Way Forward

These aquatic weeds are easily available in nearby contaminated water bodies. These weed based water treatment facility may be constructed easily for personal as well as societal benefits. Policy makers should re-think in this direction, as this municipal waste water can become potential source of irrigation water only after small treatment. Same time it is not possible to construct costly modern sewage treatment plants (STP) each and every municipality area. Here the weed based phytoremediation system can perform efficiently. The government incentives may be given to those farmers who are willing to construct weed based waste water treatment system for purchasing / constructing items like storage tanks, motors for pumping waste water from drain to settling tank and sequential treatment tanks near agricultural field. Further social awareness programmes / campaigns are needs to be organized from municipal Corporation or village panchayat regular basis so that common peoples come forward.

I. One of the key aspects to the acceptance of phytoremediation pertains to the measurement of its performance, ultimate utilization of by-products and its overall economic viability.

II. To date, commercial phytoremediation has been constrained by the expectation that site remediation should be achieved by using locally available plant species. So far, most of the phytoremediation experiments have taken place using limited plant species exposed to single metal or lower concentration of heavy metals in large volume of water such as sewage water.

III. Plant consortia is required to be developed for the remediation of multi-pollutant real contaminated sites. Optimization of the process, proper understanding of plant heavy metal uptake, enhancement of phytoremediation process and proper disposal of biomass produced is still needed.

IV. New Methods like production of biogas, vermin-composting required to be explored for the management of post harvested plant biomass.

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