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RESEARCH ARTICLE

PHYTOACCUMULATION OF PALAR RIVER WATER USING *Vetiveri azizanioides* and *Andrographis paniculata* AS PLANT REMEDIATORS

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ABSTRACT

River water is a major source for Agriculture and it is adequate for many villages and towns. Palar river in Vellore is the basin for the source of drinking water had been polluted by leather industry and deposition of hospital wastes. This available source of water is now being unable to use as the water parameters are very high and unfit for agriculture. To reduce these parameters, Phytoremediation technology had been practiced by employing *Vetiveri azizanioides* and *Andrographis paniculata* as plant phytoremediators using hydrophonic system. It was recorded that these plants were able to adapt itself in the Palar river water without altering its phytochemical and antioxidant properties. Plants were found to uptake the heavy metals from the water and continued growing.

INTRODUCTION

Water is a very important component for the survival and growth of all living organisms. Maintaining a good quality of water is important but nowadays water sources are getting polluted easily due to the emergence of various industries. The effluent of the industries when depleting the water makes the water unfit for the purposes as the physic-chemical parameters of the water is altered (Aksorn E and Chitsomboon B., 2013).

Palar River

The PalarRiver is a major river in southern India flowing through Vellore district. This river is used by many people in villages and towns on its basin as a source for drinking and agricultural use. But due to the disposal of wastes or chemicals from leather industries, PalarRiver has been polluted and now it is no longer used for drinking or cultivation purpose. The pollution of Palar river has led to many health problems and now it has to be treated to maintain it has good quality water.

Phytoremediation Method for Treatment of Water

Conventional wastewater treatment like biological nutrient removal technology or chemical method has been done for treating the wastewater. These technologies are not practised efficiently as it is very expensive, requires skilled personnel and dependent on electrical energy. Currently, phytoremediation has gained importance in wastewater treatment due to its low cost, energy efficient, environment friendly and minimal maintenance. Phytoremediation is a naturally occurring process which is carried out by plants in cleaning up and stabilizing the contaminated soils and ground water using the remarkable ability of plants to degrade environmental contaminants like chlorinated solvents, polycyclic aromatic hydrocarbons, pesticides/insecticides, explosives, surfactants and heavy metals. Plants are especially useful in the process of bioremediation because they prevent erosion and leaching that can spread the toxic substances to surrounding areas (Annie Melinda Paz-Alberto, Gilbert C. Sigua, 2013).

The application of phytoremediation is uptake of contaminants from wastewater effluents into their roots; binding the

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contaminant, into their root tissue, physically or chemically; and finally transport the contaminant into growing shoots which helps in the treatment of water and makes it fit for the agriculture and other purposes (C. Garbisu, 2002).

Hydrophinics Technology

Hydroponics is a hydroculture technique, which involves the growing of plants in a soil less medium, or an aquatic based environment. Hydroponic growing uses nutrients to feed the plants in water, without soil. This technique does not require pesticides, fertilizers and other chemicals, and there is no chance of soil-borne diseases. Water can be reused multiple times leading to water conservation practice. Plants grown through this technique are healthy and have shown better nutritional value. It has been proved that vitamin content is 50% more in hydroponically grown plants as compared to conventional ones (Gupta, R *et al*., 2013).

Plants as potential phytoremediators Vetiver (vetiveri azizanioidesl.)

Vetiver (*Vetiveri azizanioides* L.), a perennial grass, is a fast growing grass with a deep root system and high biomass production. As reported by the National Research Council, Vetiver has several unique characteristics (Dhanya, G. and D. Jaya, 2013). Due to its unique morphological characteristics, it has been used effectively in phytoremedial application for treating wastewater. Vetiver grass has a very high capacity for absorption of nutrients like nitrogen, phosphorus and heavy metals in wastewater and therefore, is a good plant for purifying water (Aksorn E and Chitsomboon B., 2013).

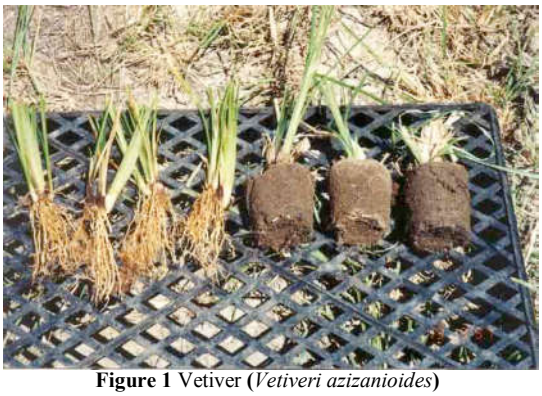


Figure 1 Vetiver (*Vetiveri azizanioides*)

Figure 2 Nilavembu (***Andrographis paniculata***)

Nilavembu (*Andrographis paniculata*) belongs to the family *Acanthaceae* which can be found all over southern India. Nilavembu consists of several active constituents in leaf and rhizome which is used to treat diabetes, anti-hepatitis-B virus and it also has anti-pyretic and Analgesic properties. Further the researches have revealed the nature of developing immuno protective chemicals from Nilavembu. It is proved that Nilavembu drinking water can relieve and prevent the effects of any kind of viral fever so Tamil Nadu Government has recommended this medicine for Dengue Fever.

MATERIALS AND METHODS

Sample Collection

The water sample was collected from the natural river source at the intake of the water supply plant located in river Palar province in Vellore district, Tamil Nadu. Soft water was collected from Genewin Biotech, Hosur and trialed as control. The samples were brought to the laboratory and analyzed for its chemical characteristics.

Plant Materials

The Vetiver (*Vetiveri azizanioides* L. Nash) and Nilavembu (*Andrographis paniculata*) plants were procured from Swamy Nursery, Hosur. The procured plants were removed from the soil and washed in the running water to ensure the elimination of adhering soil.

Panel Construction

The plants were placed in hydroponic system made up of floating rafts and plastic containers. Each floating panel consisted of 10 holes holding the crown of the plant. After all the plants were fixed in position, the panel was wrapped with the plastic mesh, thus sewing the edges. This will create a strong outer layer preventing the plants from falling into the water and to grab and raise the rafts from the water for monitoring purposes (Kanokporn Boonsong and Monchai Chansiri, 2008).

Phytochemical Analysis of the Selected Plants

The phytochemicals were screened in the selected plants before and after treatment to ensure the plants have not lost the presence of phytochemicals when kept in contact with polluted water (Harbone JB., 1998).

Qualitative Analysis Test for Tannins

To the extract, add strong potassium dichromate solution, yellow color precipitate indicates the presence of tannins and phenolic compounds.

Test for Saponins

10g of fresh sample dissolved in 100ml distilled water (1:10) to blend and filtered. The filtrate in the test tube and it was warmed in water bath, the stable persistent forth, was mixed

with 3drops of olive oil & shaken vigorously. Formation of emulsion indicates the presence of saponins.

Test for Flavonoids

1. The extract is treated with sodium hydroxide; formation of yellow color indicates the presence of flavones.
2. The extract is treated with concentrated H_2SO_4, formation of yellow color or orange color indicates the presence of flavones.

Test for Cardiac Glycosides

Borntragers Test

Add a few ml of dilute sulphuric acid to 1ml of the extract solution. Boil, filter with chloroform. The chloroform layer is treated with 1ml of ammonia. The formation of red color of the ammonical layer shows the presence of anthraquinones glycosides.

Test for Alkaloids Wagner's Test

Dissolve 2g of iodine and 6g of potassium iodide in 100ml of distilled water. Formation of brick red color or reddish brown color indicates the presence of alkaloids.

Test for Carbohydrates Molisch's Test

To 2ml of the extract, add 1ml of a-napthol solution, add concentrated sulphuric acid through the side of the test tube. Purple or reddish violet color at the junction of the two liquids reveals the presence.

Fehling's Test

To 1ml of the extract, add equal volume fehling solution A and B, upto heating formation of a brick red precipitate shows the presence of sugars.

Benedicts Test

To 5ml of the benedicts reagent, add 1ml of the extract solution and boil for 2 minutes and cool. Formation of red precipitate shows the presence of sugars.

Biurets Test

To 1ml of the extract, add 1ml of 10% NaOH and heated then add 1ml of 0.7% of cuso4. Formation of purple color solution shows the presence of protein.

Ninhydrin Test

To 1ml of the extract, add 0.25% of ninhydrin reagent and boil in water bath for 5minutes. Formation of blue color solution shows the presence of amino acids.

Test for Gums and Mucilage

Add 10ml of extract was slowly added to 25ml of absolute alcohol under constant stirring. The appearance of precipitation.

Test for Fixed Oils Spot Test

Small amount of extract was pressed between two filter paper. Formation of oil stain in the paper indicates the fixed oils.

Test for Steroids Salkowski Test

Dissolve the extract in chloroform and add equal volume of conc. H2SO4. Formation of bluish red to cherry color in chloroform layer and green fluorescence in the acid layer represent the steroidal components in the tested extract (A. Jeyasankar *et al*., 2013).

Quantitative Analysis Extraction of Plant Materials

The powdered plant material was sequentially extracted with ethanol solvent and then filtered. The filtered content was then subjected to rotary vacuum evaporator until solvents were completely evaporated to get the solidified crude extracts (Memnune Sengul *et al*., 2009). The crude extracts were stored and maintained at 4^{o}C (S. Arivoliand Samuel Tennyson, 2013).

Antioxidant Activity Determination

The total antioxidant capacity of the extract was determined with phosphomolybdenum, using –ascorbic acid as standard. An aliquot of 0.2 mL (containing 1.0 mg) of the extract was combined with 2.0 mL of the reagent (0.6 M sulfuric acid, 28.0 mM sodium phosphate and 4.0 mM ammonium molybdate). The blank solution was made by mixing 2.0 mL of the reagent solution with the appropriate volume of the same solvent used to dissolve the sample. The tubes were capped and incubated in water bath at 95 °C for a period of 90 minutes. The sample should be allowed to cool down to room temperature. The absorbance of the sample was measured against blank solution at 695nm (S. P., Kangade Y. P *et al*., 2012).

Total Flavonoid Content Determination

Quercet in was used as standard to determine the total flavonoid content of the plant extract. The dry extract (10 mg) was dissolved in 1.0ml of 80% ethanol. An aliquot of 0.5 mL was taken out of it and added to a test tube containing 4.3ml of 10% aluminium nitrate. The mixture was incubated at room temperature for 40 minutes and then the absorbance measured at 415 nm (D. Marinova *et al*., 2005).

Total Phenolic Content Determination

Total soluble phenolic content of the plant extract was determined with Folin-Ciocalteu reagent using pyrocatechol as standard. The amount of 25 mg of the dry extract was dissolved in 20 mL of distilled water and the total where, volume was transferred to an erlenmeyer flask. It was diluted to 46 mL by adding distilled water. One mL of Folin-Ciocaulteu reagent was added to the extract solution in the flask and the mixture was shaken vigorously for 3 minutes, after which 3 mL of 2% sodium carbonate solution was added. The flask was covered with aluminium foil in order to protect the formed complex from light. The mixture was shaken

occasionally at room temperature for 2 hours and then the absorbance was measured at 760 nm (Syed Majid Bukhari *et al*., 2013).

Water Quality Analysis

Before the Vetiver and Nilavembu are transplanted in the polluted water, initial analysis of water for pH, Electrical conductivity (EC), Total dissolved solids (TDS), Total Hardness, Biological Oxygen Demand (BOD),Chemical Oxygen Demand (COD), Sodium (Na), Potassium (K), Calcium (Ca), Magnesium (Mg), Iron, Chloride, Sulphates, Nitrates were done (Tiwari T.N. and Mishra M. A., 1985).

Ph

The pH is determined by digital pH meter which gives direct values of pH (Gupta, 1984).

Electrical Conductivity (Ec) and Total Dissolved Solids (Tds)

EC and TDS are determined by using digital conductivity meter (Gupta, 1984).

Total Hardness

The hardness in the water sample is determined by titrating against 0.02M EDTA disodium salt solution using Eriochrome Black T as an indicator (APHA, 1998).

BOD

BOD (Biological Oxygen Demand) is the amount of dissolved oxygen required for the biochemical decomposition of organic compounds and the oxidation of certain inorganic materials The BOD test was performed by incubating a sealed water sample for a standard five-day period for determining the change in dissolved oxygen (DO) content (B.B. Singh, 1995).

Chemical Oxygen Demand (Cod)

COD is the amount of dissolved oxygen required to cause chemical oxidation of the organic material in water. Both BOD and COD are key indicators of the environmental health of a surface water supply (APHA, 1998).

Sodium and Potassium

Flame photometer was used for the determination of sodium and potassium (APHA, 1998).

Calcium and Magnesium

Calcium and Magnesium is determined by titrating standard solution of EDTA solution using Ammonium purpurate and Eriochrome Black T as an indicator respectively (APHA, 1998).

Iron

Iron content in the water sample was determined by Atomic \ absorption spectrophotometer (WHO, 1984).

Chloride

The chloride content is determined by titrating thewater sample against silver nitrate solution using potassium chromate as an indicator (WHO, 1984).

Sulphate and Nitrate

UV-Visible Spectrophotometer was used to determine the sulphate and nitrate content (WHO, 1984).

Nutrient Analysis

Total N in effluent was measured using the Kjeldhal method in which the sample was digested using concentrated H2SO4 followed by steam distillation after alkalination with NaOH. The amount of N in the sample was determined by titrating with H_2SO_4. Total P was determined colorimetrically using UV-Visible spectrophotometer and potassium using flame photometer. Total Zn, Ni and Mn were determined using the atomic absorption sprectrophotometric method (AAS). At the end of the experiment the treated water were collected and analyzed for physico-chemical parameters. The entire study group plants were uprooted and the morphological changes like plant height, number of leaves, leaf area, and root length were determined. Then the oven dried plant materials were used for the analysis of nutrients such as total nitrogen, phosphorous, sodium, and potassium in the root and leaves (APHA, 1998).

RESULTS AND DISCUSSION

Table 1 Physico-Chemical Parameters of Soft and Palarwater before treatment

Parameters	LIMITS	Soft Water	Palar Water
pH	6.5-8.5	7.13	7.38
Electrical conductivity (µS)	<1000	44	1944
Total dissolved solids (ppm)	<500	28	1254
Total Hardness (mg/l)	<60	18	732
BOD (mg/l)	<30	2	64.23
COD (mg/l)	<250	4	1326.11
Sodium (mg/l)	<15	29	46.54
Potassium (mg/l)	<28	19	23.75
Calcium (mg/l)	<75	7	621.46
Magnesium (mg/l)	<30	2	39.05
Chloride (mg/l)	<250	38	791.70
Sulphate (mg/l)	<200	21	261.61
Nitrate (mg/l)	<45	26	36.13

Table 2 Physico-Chemicl Parameters of Soft and Palarwater after treatment

Parameters	LIMITS AS PER IS 10500:9001	Soft Water	Palar Water
pH	6.5-8.5	7.09	7.02
Electrical conductivity (µS)	<1000	50	185
Total dissolved solids (ppm)	<500	32	119
Total Hardness (mg/l)	<60	15	126
BOD (mg/l)	<30	2	6
COD (mg/l)	<250	2	12
Sodium (mg/l)	<15	8	16
Potassium (mg/l)	<28	19	11
Calcium (mg/l)	<75	6	30
Magnesium (mg/l)	<30	1	15
Chlorides (mg/l)	<250	31	115
Sulphates (mg/l)	<200	21	103
Nitrates (mg/l)	<45	23	37

The results showed that the physico-chemical parameters of soft water used as control are within the prescribed limit of WHO, ICMR and BIS. In palar water EC, TDS, BOD, COD, Calcium, sulphate are above the standard limits.

After 10 days of experimental study using the selected plants, it was found that all the parameters of Palar River water were getting reduced and lie within the standard limits of IS 10500:9001. Thus Vetiver and Nilavembu has the ability to treat the palar water effectively by hydrophonics technology and these plants found to uptake the heavy metals for its growth and reduced the water parameters.

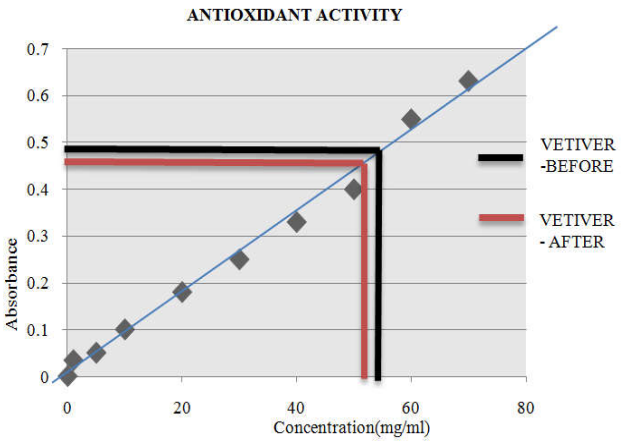
The phytochemical analysis was done before and after the treatment with polluted water which showed almost same results of the presence of phytochemicals and did not alter any of the phytochemicals under the hydrophonic treatment using polluted water.

Table 3 Phytochemical Analysis of the Selected Plants

Phytochemical tests	Vetiver		Nilavembu	
	Before	After	Before	After
Alkaloids(wagnerstest)	+	+	+	+
Carbohydrates Molisch test	+	-	+	+
Fehlings test	+	-	+	+
Benedicts test	+	+	-	-
Tannins	-	-	-	-
Flavonoids Shinoda test	+	+	+	+
Protiens Biuret test	-	-	-	-
Amino acids Ninhydrin test	+	+	+	+
Saponins	-	-	-	-
Phytosterols Salkowski test	-	-	-	-
Fixed oils Spot test	+	+	-	-
Gums & mucilage	-	-	-	-
Glycosides Borntragers test	+	+	+	+

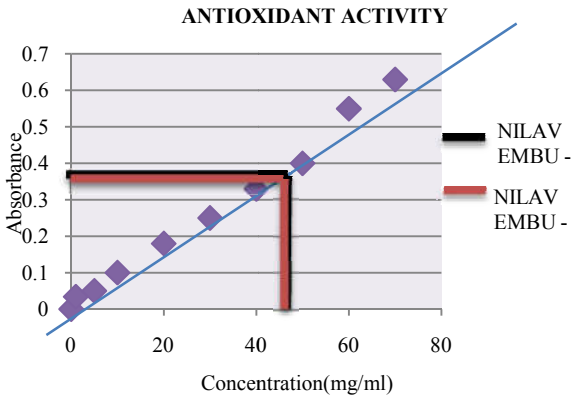
Antioxidant Activity in Selected Plants

The antioxidant activity of the selected plants was measured before and after treatment and the results were recorded.



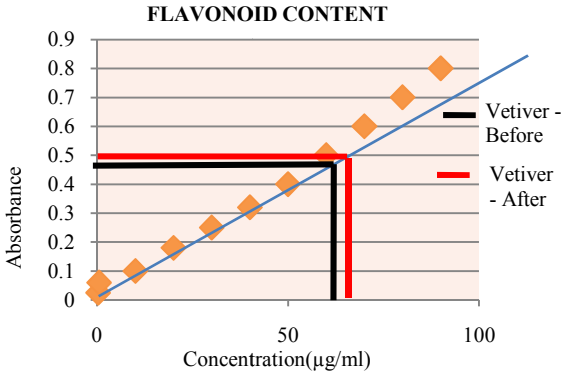
Graph 1 Graph showing the Antioxidant activity in Vetiver plants

The antioxidant activity in Vetiver and Nilavembu was measured and found that the Vetiver had 56 mg/ml before treatment and 54 mg/ml after treatment which did not vary so much after contact with polluted water. Nilavembu showed 42 mg/ml before treatment and 41.5 mg/ml after treatment which was almost the same. This proved the efficiency of the selected plants towards Hydrophonic technology. The Flavonoid content in Vetiver and Nilavembu was measured and found that the Vetiver had 61 mg/ml before treatment and 63 mg/ml after treatment which showed

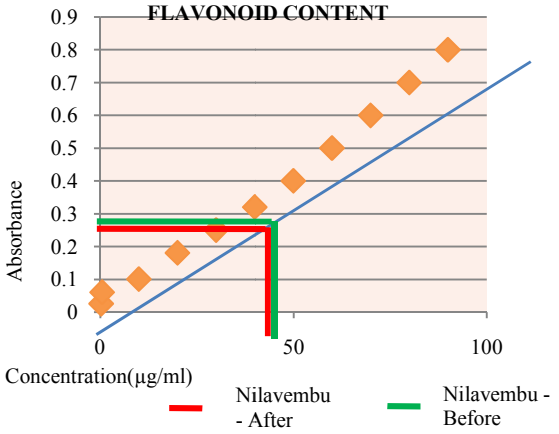


Graph 2 Graph showing the Antioxidant activity in Nilavembu plants

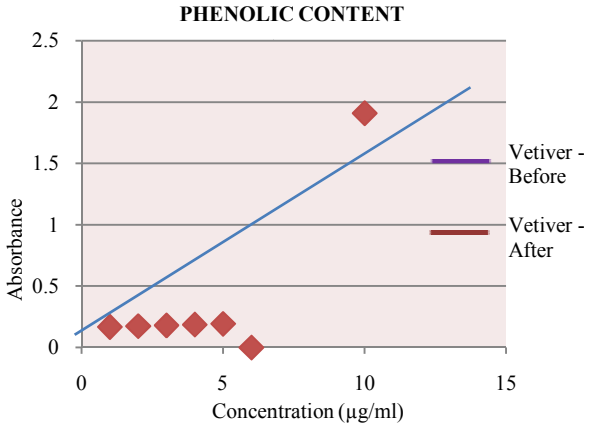
Total Flavonoids



Graph 3 Graph showing the Total Flavonoid content in Vetiver plants

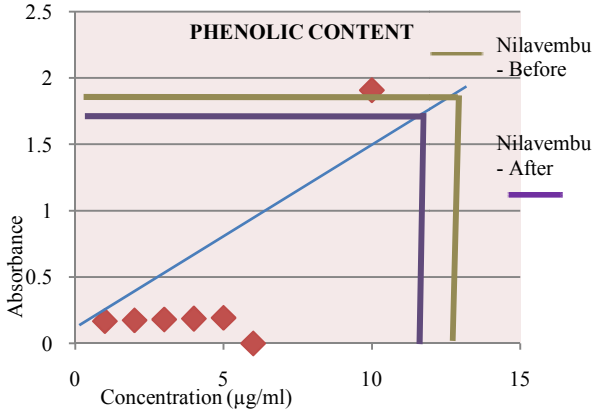


Graph 4 Graph showing the Total Flavonoid content in Nilavembu plants



Graph 5 Graph showing the Phenolic content in Vetiver plants

increased Flavoid content after contact with polluted water. Nilavembu showed 43 mg/ml before treatment and 42.5 mg/ml after treatment which was almost the same.



Graph 6 Graph showing the Antioxidant activity in Nilavembu plants

The Phenolic content in Vetiver and Nilavembu was measured and found that the Vetiver had 56 mg/ml before treatment and 54 mg/ml after treatment which did not vary so much after contact with polluted water. Nilavembu showed 42 mg/ml before treatment and 41.5 mg/ml after treatment which was almost the same. This proved the efficiency of the selected plants towards Hydrophonic technology.

Changes In The Plant Morphology And Biomass

Table 4 Changes in the plant morphology and biomass of Vetiverand Nilavembuplants

	Soft Water		Palar Water	
	Vetiver	Nilavembu	Vetiver	Nilavembu
Plant Height (cm)	84.3	86.4	83.1	87.5
Root Length (cm)	96.2	94.5	98.7	92.1
Total wet biomass (cm)	172.4	172.3	173.8	171.7

The results obtained shows that vetiver plants treated with palar water showed higher root length and biomass production when compared with control. Nilavembu plants showed higher growth and moderate root length and biomass production when compared with soft water. Bothe the plants did not change its plant morphology and biomass after the tretmern in polluted water.

Uptake of Heavy Metals by Vetiver and Nilavembu Roots From Water

The uptake of heavy metals like copper, iron and molybdenum in vetiver roots was found to be greater in palar water than in soft water. In nilavembu roots uptake of Zinc, copper, iron, manganese, boron and molybdenum was higher when compared with control.

Table 5 Uptake of heavy metals by Vetiver and Nilavembu roots

	Soft water		Palar Water	
	Vetiver	Nilavembu	Vetiver	Nilavembu
Zinc (ppm)	25	24	24	25
Copper (ppm)	9	5	11	10
Iron (ppm)	39	37	42	40
Manganese (ppm)	44	46	39	49
Boron (ppm)	16	12	16	15
Molybdenum (ppm)	5	3	8	7

Uptake of Heavy Metals by Vetiver and Nilavembu Leaves

Table 6 Uptake of Heavy metals by Vetiver and Nilavembu leaves

	Soft water		Palar Water	
	Vetiver	Nilavembu	Vetiver	Nilavembu
Zinc (ppm)	19	13	26	11
Copper (ppm)	11	28	17	21
Iron (ppm)	29	27	53	38
Manganese (ppm)	58	41	49	42
Boron (ppm)	29	20	23	32
Molybdenum (ppm)	2	7	9	5

Plants showed high uptake of heavy metals such as zinc, copper, iron, molybdenum in Vetiver leaves treated with Palar water. Nilavembu leaves were also able to uptake the heavy metals but lesser than the Vetiver leaves.

CONCLUSION

The study showed a positive reduction in EC, TDS, Nitrogen, phosphorus, potassium and certain heavy metals found in palar river water using hydroponic technique by potential phytoremediators such as Vetiver and Nilavembu plants. The phytochemical compounds and the antioxidants present in the plants did not get altered as the plants were able to uptake the heavy metals from water and could sustain its phytochemicals and antioxidant property which is found to be a natural remedy for treating the river water and make it useful for agricultural purposes and other purposes.

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