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on

PHYTOREMEDIATION POTENTIAL OF VETIVER ZIZNIOIDES: A GREEN TECHNOLOGY TO REMOVE POLLUTANTS FROM PAMPA RIVER HIGHLIGHTING COLIFORM BACTERIA BY HYDROPONIC TECHNIQUE

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Phytoremediation potential of Vetiver ziznioides: A Green Technology to Remove Pollutants from Pampa River Highlighting Coliform bacteria by Hydroponic Technique

Introduction

Life began in water and no one live without water. Adequate supply of fresh and clean drinking water is a basic need for all human beings in the earth (Girija, 2013). Yet water pollution is one of the most serious ecological treats we face today. Therefore it is necessary to find a source of clean water. Pampa River, the third longest river in Kerala originates from Pulachimalai. The famous Sabarimala temple is located on bank of Pampa .The pollution of Pampa River is due to the Sabarimala pilgrimage, free flow of sewage, domestic waste and faecal matter into the river(Firozia et.al, 2013).

Phytoremediation is a bioremediation process that uses various types of plants to remove, transfer, stabilize or destroy contaminants in the water and soil. It is clear, simple, cost effective, eco-friendly process (Truong and Smeal, 2003). The Vetiver Zizanioids (V.Z) is most useful for phytoremedial application due to its unique morphological and physiological characteristics.

Coliform bacteria are a commonly used bacteria indicator of sanitary quality of food and water. Commonly it is found in faeces of warm-blooded animals. Coliform themselves are not normally cause serious illness but their presence is used to indicate other pathogenic organism(APHA, 1995).

The study focuses to remove the impurities and microbiological pathogens such as e-coli, coliform by Hydroponic technique using V.Z.

Objectives

The major objectives of the project are:

•To assess the water quality of Pampa River at different stations and to suggest remedial measures to alleviate the problem

•To evaluate the phytoremedial efficiency of V.Z in the Pampa River water in the laboratory

•To study and compare the extract of V.Z roots and leaves grown in the contaminated water and the control medium

•To study the antibacterial activity of dried root extract of V.Z against E.coli.

Materials and Methods

Water sample were collected from different 4 stations of River Pampa.

Station-I: Triveni, place near Sabarimala temple in the district of Pathnamthitta, Kerala. Pilgrims use the water in this region for sanitary purposes during pilgrimage season.

Station-II: Ranni is one of the largest taluk in Kerala. The renowed Hindu temple of Sabarimala is in this taluk situated approximately 60km from the main town, Ittiapara

Station-III: Parumala, a town in the district of Pathnamthitta, Kerala.

Station IV: Erumely , a holy place in Kottayam District . It is considered holy by both Hindus and Muslims and is on the way to Sabarimala. There are two temples in this town of Lord Ayyapapan and the famous Petta Thullal is done in November, December and January months. During Petta Thulal each devotees paints his body with green, black and kumkum colours. After Petta thullal devotees wash their bodies in Pampa River. Petta Thullal takes place almost every day from the first of the Malayalam month of Vrichikam till a couple of days prior to Sabarimala temple closing on January. This kumkum may contain Mercury, Lead and Synthetic dye.

About 2l of sample were collected for the comparison of Biological parameters like total coliform count and chemical parameter according to the standard procedure in APHA, 1995.

The sampling was carried out for a period of one year extends from between January 2015 to May2015. The work involved laboratory methods and field work to assess the pollution status and phytoremediation of waste in Pampa River using V.Z

The bottles were thoroughly cleaned with distilled water and rinsed with water samples before sampling. About 1.5 litres of water samples were collected monthly in sterile bottles of 2 litre capacity in the morning between 9.00 - 9.30 am without air bubbles from the five stations and were properly brought to the laboratory soon after the collection for analysis. The preservation of samples was done as per standard procedure of APHA (1995). Physical, chemical and biological parameters were measured by standard methods.

Plant material

Healthy V.Z clumps were selected from Vetiver nursery. Three week old V.Z slips of roughly same size were used. They were removed from the propagating field and the roots were cleaned carefully to remove any adhering soil. The shoots were cut back approximately in 10 cm height to reduce transpiration and root length 10 cm were used for phytoremediaton experiment.

Pot experiment

Pilot studies have been carried out in the laboratory, by using containers of 10 litre capacities to carry out the experiment. A preliminary trial was conducted over 6 months. Clumps of V.Z were grown in wastewater with floating technique in containers by the support of thermocol. Each container has one clumps and their axis was kept submerged in the wastewater and allowed to grow for 6 months. The pots were placed on a level concrete platform which was not shaded throughout the day to ensure good shoot growth. All the pots received the same amount of sunlight and heat during the trial. Control was also kept under the same conditions.

Physico-chemical analysis of water

Analysis of water samples was carried out as per standard procedure (APHA, 1995) for Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), mercury and lead. Microbiological water quality checking was also carried out for the presence of coliforms using MPN technique.

Analysis of VZ planted in the wastewater

Plants were carefully removed from the experimental pot after 6 months. Root height, shoot height, number of clumps and number of leaves were noted. Plant samples were prepared by cutting and dividing into roots and leaves. Roots and leaves were cut into pieces and kept for shade dry at room temperature (28^0C) for about four days. The dried leaves and roots were powdered in the pulverizer separately. The dry weight of root and shoot were noted and Scanning Electron Microscopy (SEM) is conducted.

Soxhlet extractions were performed on plant samples (10g) using 200 ml of solvents. After putting the mixture into an extraction thimble, it was covered with glass wool and put in a soxhlet extraction apparatus. The soxhlet extracts were allowed to reflux for 24 hours and the solvent was separated from the extract in an evaporator at 45°C to about 5 ml and submitted to GCMS analysis after removing the wax content.

The dried leaves and roots were powdered in the pulverizer separately

Antimicrobial activity of VZ by disc diffusion method

VZ was used for investigating the antimicrobial activity against Escherichia coli Microbial Type Culture Collection (MTCC) 585. The shade dried roots were used for the present investigation. The organic solvent, chloroform was employed for the extraction of different bioactive principles. The various parts of the plants, were separated and subsequently washed to remove adhering dust particles. Then the roots were cut into small pieces, separately and used to extract the antimicrobial compounds. The plant extracts were prepared using soxhlet extraction (continuous extraction) method. About 10 g of dried roots of VZ was taken and placed in the soxhlet extractor. The extractor was allowed to run continuously for 24 hours and the samples were allowed to flush and the collected extracts were stored in a refrigerator at 4°C till usage.

RESULTS AND DISCUSSION

Hydrochemistry

Monthly intervals variation (Jan-May 2015) of chemical parameters of water sample in station I,II and III were noted

Chemical Oxygen Demand (COD).

COD determines the amount of organic pollutants found in waste water. COD is the measure of oxygen required to carry out the oxidation process of organic matter chemically. The permissible limit of COD as per WHO is 10mg/L (Sharma and Kaur, 1994).

The monthly intervals distributions of COD of different stations of the study area were reported graphically in Fig (I). All the study area showed high COD value. The high COD value could be due to added load of organic matter in wastewater (Neena et.al, 2007).

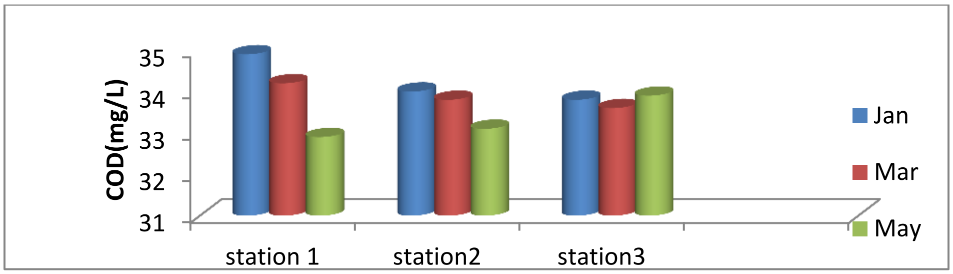


Fig (I) Monthly variation of COD

Biological Oxygen Demand (BOD)

It is amount of oxygen required for microbial oxidation of organic compounds in the water body. It is often expressed in mg of oxygen consumed per liter of sample during 5 days of incubation at 20°C. The permissible limit of BOD is 3.0mg/L (ISI, 1983). The monthly intervals distributions of BOD at different stations of the study area are reported graphically in Fig (II). The BOD value of water samples from all the three stations exceeded the permissible limit.

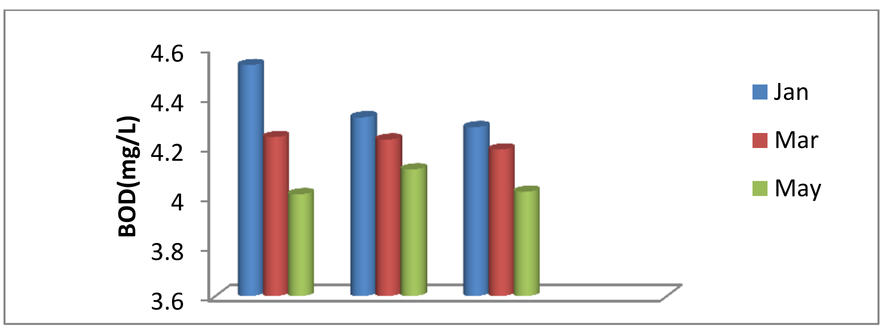
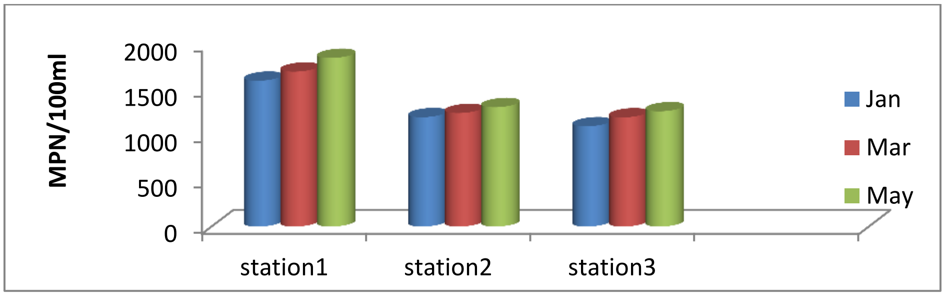


Fig (II) The monthly variation of BOD

MPN of Coliform

The most basic test for bacterial contamination of a water supply is the test for total coliform bacteria (Girija, 2013). Total coliform counts give a general indication of the sanitary condition of a water supply. The MPN method is based on the ability of coliform group of organisms to ferment lactose and produce CO_2. The organic matter is the food of coliform bacteria. The acceptable limit of MPN/100ml proposed by Indian standard limits>10 and 0 for faecal coliform.

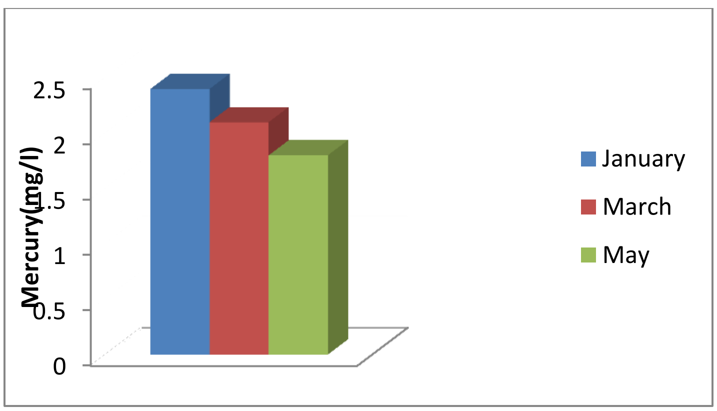
The monthly intervals distributions of coliform of different stations were recorded graphically in Fig(III). The highest MPN value was seen in station (I), this might be due to the highest amount of organic matter.



Fig(III)The monthly variation of coliform

Mercury

Mercury is considered as most dangerous pollutant because Mercury that settles and remain in sediments. The permissible limit of Mercury in drinking water is 0.002mg/l (Kapoor et al,1999). The monthly distribution of Mercury of the study area(Station IV) were represented graphically represented in Fig (IV)



Fig(IV) The monthly variation of Mercury

Lead

Lead accumulates in the body of water organism. Body of phytoplankton can be disturbed when lead interfere and finally it enters in human body. The permissible limit of Lead is 0.015mg/l (Kapoor et al,1999). The monthly distribution of Lead in the study area were graphically represented in Fig (V)

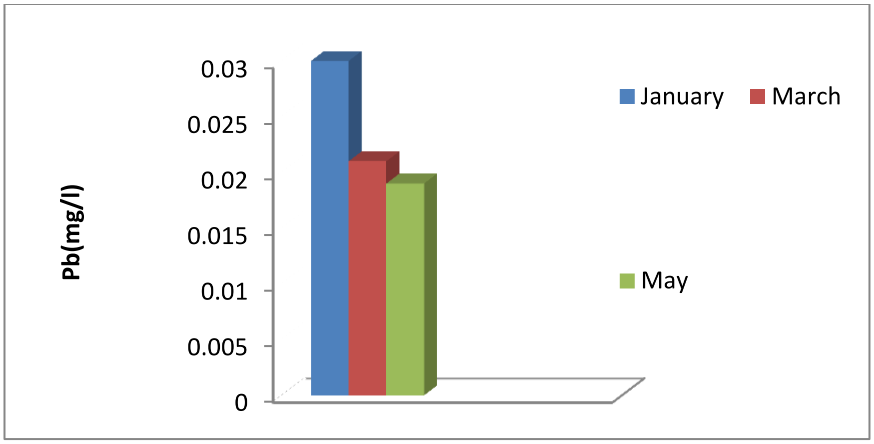


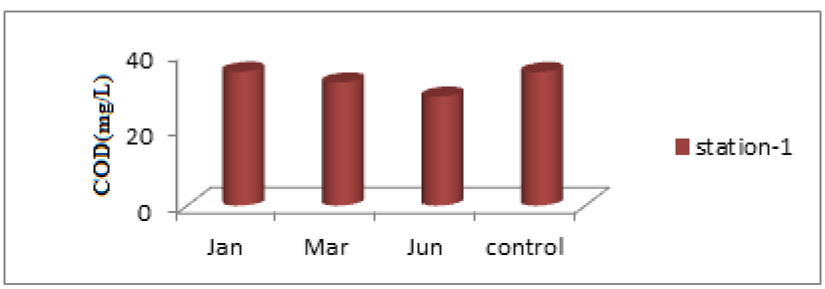
Fig (V) The monthly variation of lead

Phytoremediation using V.Z

Phytoremediation is a new area of biotechnology that take advantage of act that certain species of plants and fungi flourish by accumulating waste material in wastewater. Contaminants such as metals, pesticides, solvents, crude oil and its derivatives have been mitigated in phytoremediation. Plants absorb contaminants through root systems and transforms through shoot systems. The current study on the treatment performs of V.Z in water collected by floating platform technique. Water collected from station (I) and station IV were used for phytoremediation using V.Z because these station were found to be more contaminated than station (II) and (III).

COD

COD in the water sample were decreased by an average of 95% in the treatment pots as compared with that in the control pot. So many works has been done showing the efficiency of V.Z in removing COD. Liao et.al (2003) showed that within 4 days of planting V.Z in pig farm wastewater the COD level reduced to 64% of the initial value. Nyakango and Van Bruggen (1999) also showed a satisfactory removal of COD by planting V.Z in wastewater.

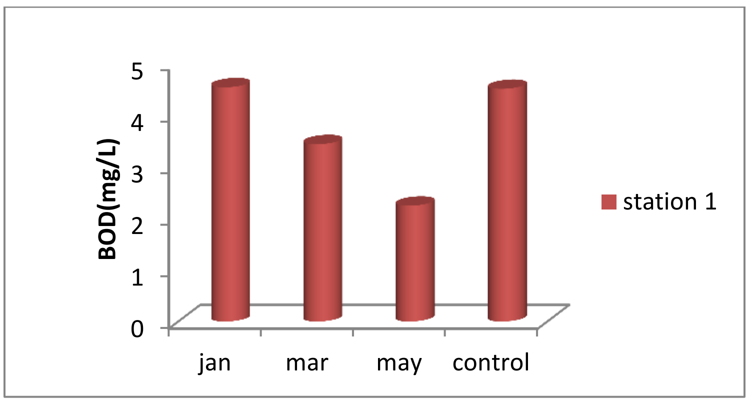


Fig(VI) Variation of COD value after planting V.Z and control of medium

BOD

BOD in the water samples were decreased by an average 95% in the treatment pots as compared with that in the control pots. The decrease in BOD as a result of remediation can be directly attributed to a reduction in the concentration of organic matter in the wastewater as a result of plant growth. (Girija, 2013). So many works has been done showing the efficiency of V.Z in removing BOD from wastewater.

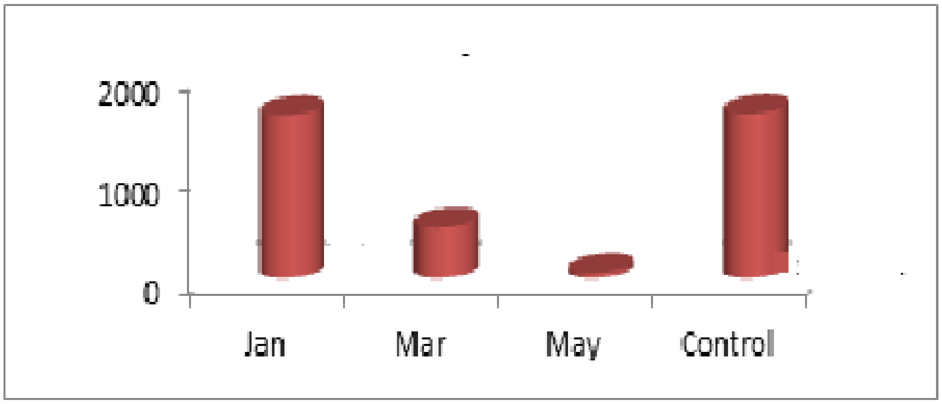
Liao et.al(2003) found that when V.Z were grown in pig farm wastewater with BOD at 500 mg/L could reduce to 68% within four days. 90% removal efficiency of BOD by V.Z was reported by Lakshmanperumalsamy et.al (2008) on the 60th day of planting.



Fig(VII) Variation of BOD value after planting V.Z and control of medium

MPN of Coliform

In the present study MPN of Coliform was found to decrease by planting V.Z by an average of 94% in the treatment pots as compared with that in the control pots. This may be due to the increase in Dissolved Oxygen (DO), which oxidize the organic matter resulting in the depletion of coliforms. The main factor influenced the rising of DO was due to Oxygen translocation through the root system of V.Z. The increase in DO in the solution may also be due to photosynthesis (Girija,2013). In the control sample which was kept idle for 6 months there has been a steady increase of coliform bacteria. This may be due to the favorable condition for the growth of coliform in stagnant water. Similar work has been carried out by Truong and Hart (2001) and found that V.Z successfully removed the waste products from the septic tank effluent in Australia.

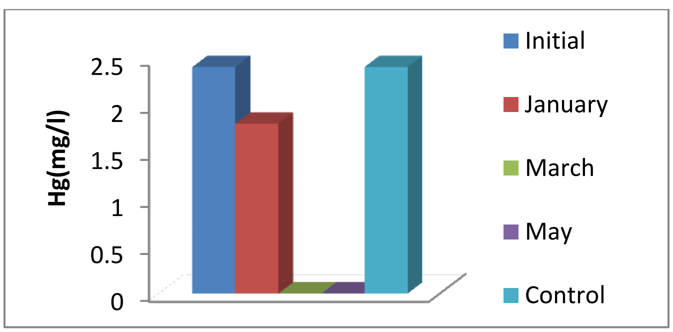


Fig(VIII) Variation of MPN value after planting V.Z and control of medium

4.5.3 Mercury

In the present study mecury concentration decreased in the water samples by an average of 99% in the treatment pots, as compared with that in the control pots after 6 months. Variation of mercury concentration after planting V.Z for 2, 4, and 6, months and the control were depicted in Fig (IX).

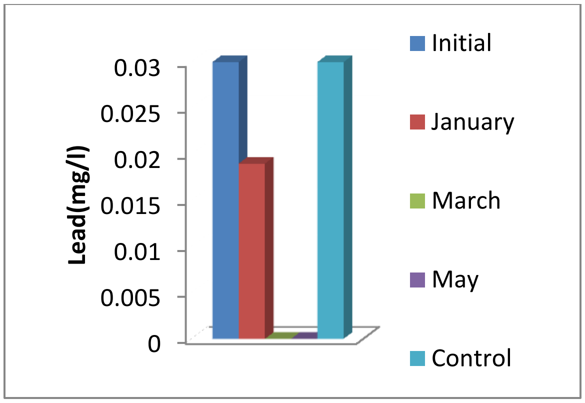
Few work has been done showing the effect of V.Z in removing Mercury. Paul Truong (2000) showed that the Mercury pollution in the Lake and Yolo countries of Northern California can be decreased by planting V.Z.



Fig(IX) Variation of Hg after planting V.Z and control of medium

4 Lead

Concentration of lead decreasedby 99% after planting V.Z for every 2 months . Fig (X) shows the variation of lead concentration after planting VZ for 2, 4, and 6 months of planting V.Z and the control were depicted in Fig (X). Few work has been done showing the effect of V.Z in removing Lead. Veralika Singh et al (2014) showed that 80-94% of lead can be removed by using V.Z from synthetic wastewater.



Fig(X) Variation of Pb after planting V.Z and control of medium

Chemical Analysis of Sample after Treatment with V.Z

UV-Vis Spectral Analysis

UV-Vis spectral analysis of water sample taken after every 2 months of treatment with V.Z. The dye content in the water sample is measured using UV-Vis spectra. Fig (XI) shows the UV-Vis spectra of dye content in the water sample measured in January, March and May. The absorbance in the graph clearly indicates that V.Z is highly beneficial for wastewater treatment.

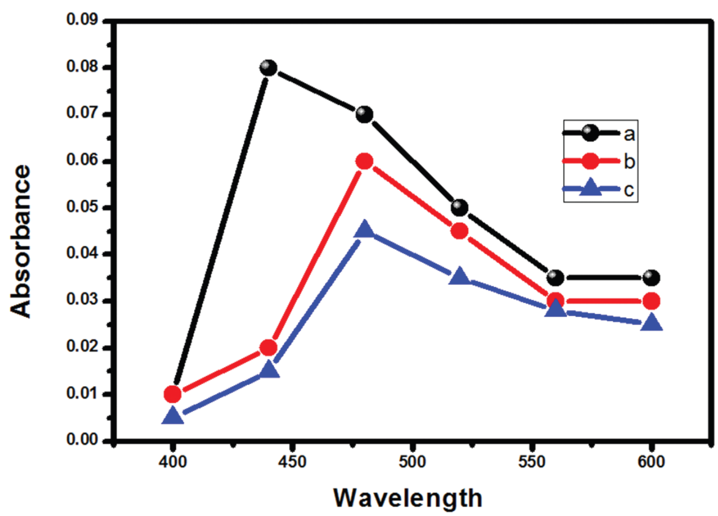
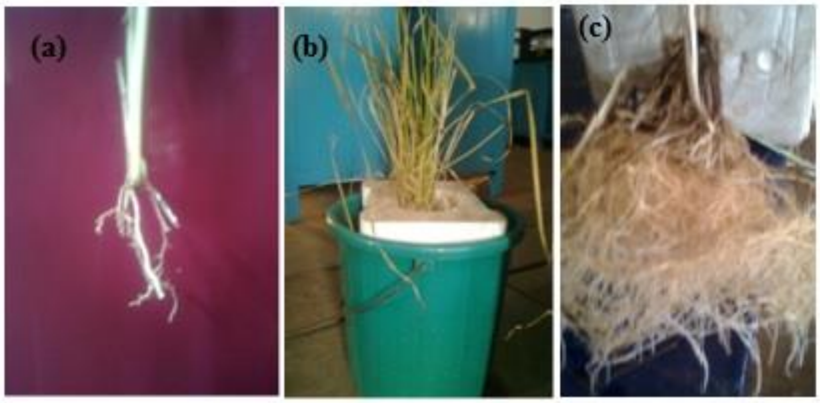


Figure (XI): UV-vis spectral analysis analysis of dye content after, (a) 2 months, (b) 4 months, (c) 6 months of planting Vetiver zizanioides.

Figure(XII) shows the growth performance of VZ after 6 months of planting. Variation of the color of wastewater after planting VZ with an interval of 2 months is shown in Figure (XIII). The reduction in the color intensity was due to the high dye absorption efficiency of VZ.



Figure(XII) Growth performance of VZ shoot and root in the wastewater

(a)at starting period(b)Shoot after 6months (c) root after6months

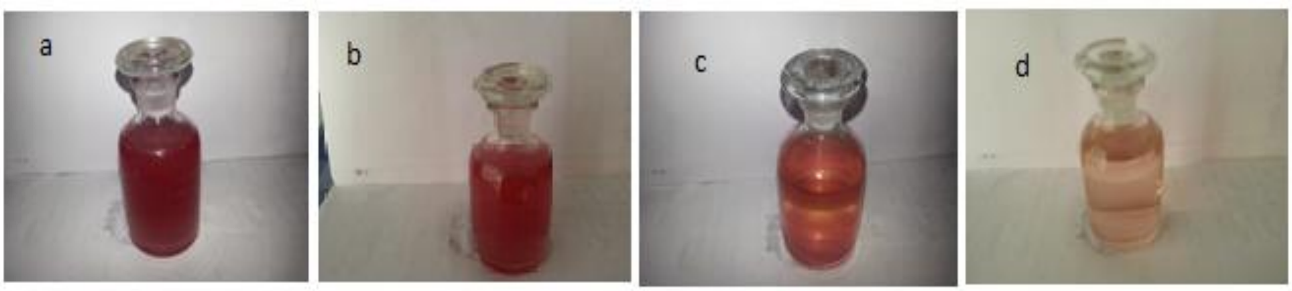


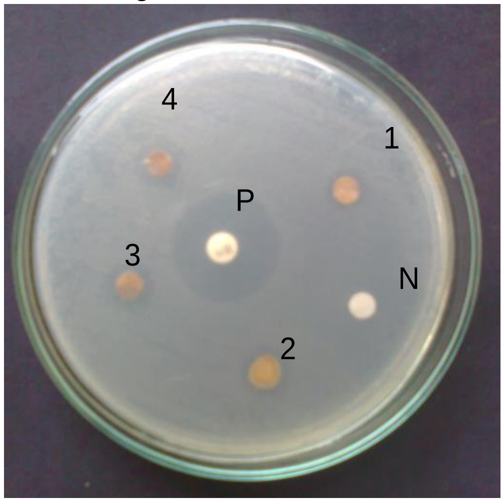
Figure (XIII) Water samples from the control pot and treatment pot

(a)initial (b) after 2 months (c) after 4 months (d) after 6 months of planting VZ

Analysis of the Antimicrobial activity

Escherichia coli (E.coli) is a major coliform bacteria present in water, as a first pointer, we have screened the antibacterial activity of dried root extract against bacteria E.coli. The antibacterial activity of dried roots extract of V.zizanioides using chloroform was investigated and the results are given in Table 1. The crude chloroform extract of roots showed the maximum inhibitory activity against E.coli MTCC 585 (18 mm) at 200 µg concentration/disc. The other concentrations viz., 10,50, 100 µg/disc also exhibited antimicrobial activity against E. coli at the level of 8 mm, 10 mm and 12 mm respectively Fig.(XIV). The positive control Chloramphenicol (20 µg/disc) (w/v) reported the antimicrobial activity of 22 mm and the negative control (Chloroform) showed no activity against the pathogenic bacteria, E. coli MTCC 585.

Antimicrobial activity of *Vetiveria zizanoides*(L.)
against *Escherichia coli* MTCC 585



1= 10 µg/disc
2= 50 µg/disc
3= 100 µg/disc
4= 200 µg/disc
P= Positive control
Chloramphenicol
(20 µg/disc)
N= Negative control
Chloroform

Fig. (XIV) Antimicrobial activity of V.zizanioides

Concentration of the extract (µg/disc)	Zone of Inhibition(dia. in mm) using the solvents
10	8
50	10
100	12
200	18
Positive control Chloramphenicol (20µg/disc)	22
Negative control Chloroform	No activity

Table I. Antibacterial activity of dried roots of Z against E. coli MTCC 585

The antibacterial activity of dried roots of VZ using the organic solvent, chloroform was investigated and from the results, it was found that the chloroform extract of roots showed the maximum inhibitory activity against E.coli MTCC 585 (18 mm) at 200 µg concentration/disc. The other concentrations reported a moderate activity against E.coli MTCC 585. Perhaps the variations may be due to the polarity of solvents which determines the type of reaction and solubility of compounds. Jayashree et al. (2011) reported that the crude chloroform extract of VZ root and shoot fractions controlled more number of pathogenic bacteria and fungi. Similarly, Goun et al. (2003) also reported the antimicrobial activity of VZ oil. The plant also contains active ingredients used in traditional medicine and as botanical pesticide. The chemical composition of the oil is extremely complex and consists of sesquiterpenes and their derivatives, belonging to 11 structural classes (Khalil and Ayoub, 2011). These may be the possible reasons for the antimicrobial property of crude extracts of the dried VZ roots. The antimicrobial action may be due to the physiological variation of the gram negative bacterial cell wall of E.coli. However, to explain the exact mode of action, the active compounds were screened and molecular mechanism of the bacteria also has to be determined by additional experimental studies.

Scanning electron microscopy (SEM)

SEM result of VZ root and shoot with reference shows a modification of the surface. This is because of the absorption of heavy metals such as mercury and lead. The sample root surface shows greater variation than reference root. This indicates that the pollutant was largely absorbed in the root biomass. There is a little variation in shoot surface as compared to the reference shoot. This may be due to the accumulation of heavy metals in root biomass with little transportation to shoot (Figure XV).

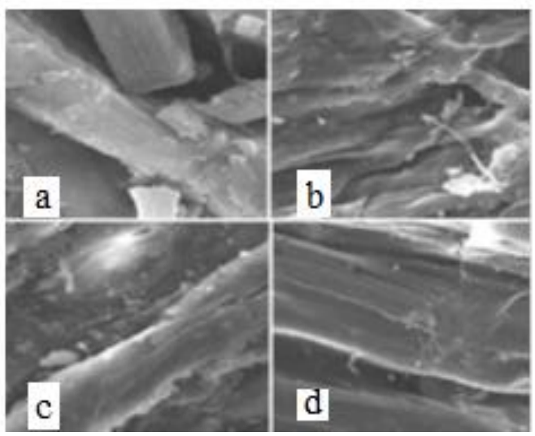


Figure (XV) Scanning electron microscopy analysis of Vetiver zizanioides root and shoot.

(a) Sample root, (b) reference root, (c) sample shoot (d) reference shoot.

Gas chromatography with mass spectrum

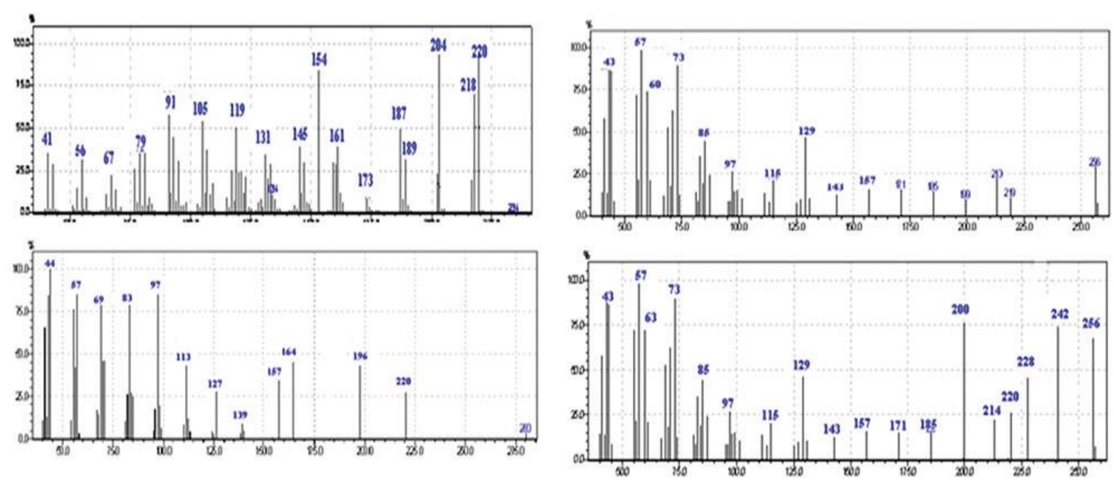
(GC-MS) analysis

The chloroform extract after removing the wax content, of powdered VZ root and shoot planted in the medium and control were used for the GC-MS analysis. The wax content was found to be more in the plants grown in the polluted medium. The reason was due to the fact that the plant has to produce more wax to face the stress condition in the polluted water. GC-MS study of VZ revealed qualitative analysis of different constituent before and after planting the polluted medium. The chemical samples chloroform identities of a number of compounds from root and leaf extract were determined by matching their recorded mass spectra with data bank spectra (NIST and WILLEY libraries) provided by the instrument. The compounds identified in the root extract before planting in the contaminated water at retention time 14.8 were terpinen-4-ol (mol.wt.154), vetivone (mol.wt.218), khusimone (mol.wt.204), and khusimol (mol.wt.220). Similar components were reported by the GC-MS analysis of VZ roots grown under different cultivation conditions (Pripdeevech etal 2006). The above components were found to be absent in the root extract after planting VZ in the polluted water. This may be due to the fact that metabolism plays a significant role in phytodegradation of contaminants as well.

The components identified in the leaf extract of VZ at retention time 14.8 before planting in the contaminated water were 2,4-Pyrimidinedione (mol.wt.157), 2-Piperidinone (mol.wt.113), 7-Methylcaprolactam (mol.wt. 127), 2-Methyl-2-(3-oxobutyl)-1,3-cyclohexanedione (mol.wt.196), and 4,7-decadiynoicacid (mol.wt.164). In the extract of leaf after planting in the polluted medium, several new components such as pentadecanoic acid (mol.wt.242), hexadecanoic acid (mol.wt.256),tetradecanoic acid (mol.wt.228), and dodecanoic acid (mol.wt.200) were observed Figure (XVI).

Phytodegradation can result in the formation of toxic intermediate chemicals from the original contaminant or result in the creation of fewer toxic compounds, thus having a beneficial effect. Plants absorb contaminants through root systems and store them in the root biomass and transport them to the stem or leaves.

a in control b in wastewater



c in control d in wastewater

Figure (XVI) Gas chromatography with mass spectrum of root extract. (a and b)

And shoot extract (c and d) of VZ.

CONCLUSION

The phytoremediation efficiency of VZ was evaluated by planting it in wastewater of Pampa river by a floating platform technique in the lab. It has been shown that VZ is highly capable of reducing coliform bacterial counts by 94% for 6 months of planting. Mercury and lead in station IV was found to be decreased by 99% by planting VZ for 6 months. From the UV-vis spectral analysis, a reduction in dye removal was also observed by planting VZ for 6 months in the wastewater.

The ability of VZ to absorb harmful heavy metals, bacteria, and dye should be of great concern to all because fresh water and effective sanitation are basic necessities of all human beings. The fact that VZ reduces disease causing bacteria should be a reason enough to consider it as a solution for environmental remediation of pollution. In addition, no harm can be caused by testing it in the environment, as greater harm is occurring by doing nothing.

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