

## Screening of potential plants for Phytoremediation in a Hydrocarbon exploration site of Tripura, India

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### Abstract

Phytoremediation is the use of plants to remediate an area of any types of contaminants. This technique is becoming popular in recent years due to its cost effective and environment friendly nature. The present study was conducted in a hydrocarbon exploration site of Tripura to determine the types of plants present in the area and to check the concentration of elements like iron, chromium, and sulphur in the plants that are found during the study. The adjacent site was also explored for comparative analysis and structural diversity. It was observed that only herb species was found in the hydrocarbon exploration area. There was no shrub species in the area. However, in the adjacent area both herb and shrub species were found. The element content in the plants shows that all the plants absorbed some amount of the mentioned elements in their root and shoot part. The study is significant as no earlier study of such kind has been conducted in Tripura.

**Key words :** Phytoremediation, remediate, contaminants, natural gas exploration site, metals.

In order to develop a healthy and useful ecosystem, key role is played by the biodiversity of animal and plant species which in turn play an important part in profitable values to man and environment<sup>20</sup>. Therefore, identifying the plants and animal species present in a site plays a key role in maintaining the ecosystem of that site. Factors such as human disturbance, widespread foraging, trampling, arrival of invasive species and soil erosion majorly influenced vegetation structure<sup>19</sup>. We depend on soil for a variety of reasons. As one of the fundamental components of an ecosystem, soil is a crucial component for human survival and growth<sup>24</sup>. With increasing human population coupled with the increasing pressure on land due to urbanization, industrialization etc., the area of land under contamination also increases. Irrespective of the potential expenses, it is desirable from an

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environmental standpoint to rehabilitate contaminated sites to the highest degree<sup>7</sup>. There are many techniques available for cleaning up the contaminated soil. Traditional methods like landfilling and incineration which are used to wipe out crude oil contaminated soils can be labor demanding and expensive<sup>9</sup>. Phytoremediation may be an effective alternative for contaminants cleanup as it can be conducted on the site itself, is cost effective when compared with other current technologies and is relatively environmentally friendly<sup>16</sup>. Since, it has the desirable advantages; it is becoming popular in recent years as far as restoration ecology is concerned. When plants are used to eliminate contaminated mediums such as soil, sediment, surface, and ground water, it is known as phytoremediation<sup>8</sup>. Plants have been performing this process since time memorial. It is just that we came to know about the process just few decades ago. Phytoremediation is fast becoming accepted as an economical method for contaminated site clean-up toxic metals, radionuclides, and dangerous organics<sup>13</sup>. It can be used in any site which is contaminated. Finding a plant species which tolerates or is resistant to a specific contaminant is the first goal in phytoremediation with an aim to maximizing its capacity<sup>22</sup>. While selecting plant species, it is also important to select native plants which are already present in the area in order to save the time and expenditure of plant acclimatization<sup>5</sup>. The most effective remedial native plant species that are well adjusted to the ecological conditions of a given place must be identified in order to successfully perform phytoremediation studies<sup>19</sup>. Normally, essential elements like Cu, Fe, Zn, Ca, K, Mg and Na are accumulated by plants for growth and

development from soil solutions but some non-essential elements like Pb, Al, Cr, As, Cd are also taken up which have no biological activity<sup>1</sup>. Organic pollutants like polychlorinated biphenyls, nitroaromatics, polychlorinated biphenyls and linear halogenated hydrocarbons can be mineralized entirely by using plants such as poplar trees, alfalfa, willow, and various grass varieties<sup>10</sup>. Plants are the favourable means for enhancing the elimination of stored persistent organic pollutants like PAHs for those soils which are contaminated for a long time by assuming that plants can survive on the site through supporting the spread of microbes which can degrade those contaminants<sup>12</sup>. Aged heavy mineral oil can be remediated by adding amendments such as mineral nutrients or organic amendments by a process called bioremediation<sup>11</sup>. The present study was conducted in a hydrocarbon exploration site in Tripura to find out the vegetation types in the site. The metal content in the soil and plant samples collected were also determined in order to have an idea about the level of contamination in the site. In order to have a precise valuation of site contamination and remedial substitutions, we need to have a clear idea about the physical attributes of the site and the level and type of contamination<sup>23</sup>. The data so obtained can be utilized in future remediation experiment.

A hydrocarbon exploration site (23°43' 40.65" N, 91°18'34.23" E) of Tripura was selected for the reconnaissance survey. The suitable quadrat sampling techniques were used to collect soil and plant samples from the location as well as from the nearby site. The quadrat size used was 5m x 5m for shrubs and 1m x 1m for herbs. The collected plants were identified using the "The Flora of Tripura

State<sup>2</sup>. The Important Value Index (IVI) was calculated as sum of relative frequency (RF), relative density (RD) and relative abundance (RA) of each species<sup>21</sup>. The biodiversity index was determined using package PAST software 1.89<sup>6</sup>. The statistical data was analyzed using

SPSS software Java 22. The Translocation factor was calculated by using Deng, Ye & Wong 2004 method<sup>3</sup>.  $TF = \frac{Metal_{shoot}}{Metal_{root}}$ . The Bioconcentration factor was calculated using the same process by Zayed, Gowthaman & Terry, 1998<sup>25</sup>.  $BCF$

$$= \frac{\text{Trace element concentration in plant tissues} \left(\frac{mg}{kg} \text{ at harvest}\right)}{\text{Initial concentration of the element in the external nutrient solution} \left(\frac{mg}{L}\right)}$$

Table-1. Herb species found in Hydrocarbon exploration site

Plant name	Common name	Family	Habit	Biomass (g)	IVI
<i>Ageratum conyzoides</i> (L.)	Goatweed	Asteraceae	Annual	0.85	18.81
<i>Cynodon dactylon</i> (L.) Pers.	Bermuda grass	Poaceae	Perennial grass	7.74	134.24
<i>Centipeda minima</i> (L.)	Sneeze wort	Asteraceae	Perennial	3.51	81.91
<i>Digitaria sanguinalis</i> (L.) Scop.	Crab finger grass	Poaceae	Annual	3.01	65.02

Table-2. Herb species found in adjacent site

Plant name	Common name	Family	Habit	Biomass (g)	IVI
<i>Axonopus compressus</i> (Sw.) P.Beauv	Carpet grass	Poaceae	Perennial	6.86	127.03
<i>Cynodon dactylon</i> (L.) Pers.	Bermuda grass	Poaceae	Perennial grass	9.87	87.52
<i>Centipeda minima</i> Linn.	Sneeze wort	Asteraceae	Perennial	3.30	18.13
<i>Chrysopogon aciculatus</i> (Retz.) Trin.	Love grass	Poaceae	Perennial	2.60	21.88
<i>Desmodium triflorum</i> (L.) DC.	Creeping tick trefoil	Fabaceae	Annual or perennial	4.42	56.43
<i>Ageratum conyzoides</i> (L.)	Goatweed	Asteraceae	Annual	2.47	37.10
<i>Centella asiatica</i> (L.) Urban	Indian pennywort	Apiaceae	Perennial	1.15	94.16
<i>Vernonia cinerea</i> (L.) Less.	Ash colored fleabane	Asteraceae	Annual	1.90	16.41
<i>Mimosa pudica</i> (L.)	Touch-me-not	Fabaceae	Annual or perennial	5.52	83.90
<i>Ipomoea alba</i> (L.)	Moon vine	Convolvulaceae	Perennial	12.71	35.97

Table-3. Shrub species found in adjacent site

Plant name	Common name	Family	Habit	Biomass (g)	IVI
<i>Chromolaena odorata</i> L.	Christmas bush	Asteraceae	Perennial	78	91.29
<i>Melastoma malabathricum</i> L.	Indian rhododendron	Melastomataceae	Annual or perennial	17	131.15
<i>Urena lobata</i> L.	Caesarweed	Malvaceae	Perennial	33.94	46.08
<i>Lantana camara</i> L.	Lantana	Verbenaceae	Perennial	29	31.48

Table-4. The biodiversity index of the herb and shrub species found in the Hydrocarbon exploration site and adjacent site are given in the table below-

Site	Type	Shannon index (H) (Mean± SEM)	Simpson index (D) (Mean± SEM)	Evenness (Mean± SEM)
Hydrocarbon exploration site	Herb	0.70±0.25	0.55±0.15	0.98±0.01
Adjacent site	Herb	0.76±0.29	0.63±0.14	0.66±0.14
	Shrub	1.10±0.15	0.40±0.05	0.74±0.10

It was observed that Shannon index and Simpson index for herb was higher in adjacent site.

Evenness was found to be higher in Hydrocarbon exploration site.

Table-5. Significant means differences between hydrocarbon exploration site (herb) and adjacent site (Herb).

Diversity indices	Variable	Mean ± SD	MD	SED	t-value
Simpson index (D)	Hydrocarbon exploration site (Herb)	0.71 ± 0.51	0.05	0.39	0.13
	Adjacent site (Herb)	0.76 ± 0.59			
Shannon index (H)	Hydrocarbon exploration site (Herb)	0.55 ± 0.31	0.08	0.21	0.38
	Adjacent site (Herb)	0.63 ± 0.29			
Evenness	Hydrocarbon exploration site (Herb)	0.98 ± 0.03	0.32	0.14	2.35
	Adjacent site (Herb)	0.66 ± 0.27			

Significant at 0.05 level of confidence;  $t_{(0.05)}(6) = 2.45$ .

Significant at 0.01 level of confidence;  $t_{(0.01)}(6) = 3.71$ .

Table-5 reveals that there was insignificant difference between Hydrocarbon exploration Site (Herb) and Adjacent site (Herb) on Simpson, Shannon & Evenness, as the calculated value  $t = 0.13, 0.38 \& 2.35$  were lesser than the tabulated value  $t = 2.45$  ( $p > 0.05$ ).

Further, it was also found that there was insignificant difference between Hydrocarbon exploration site (Herb) and Adjacent site (Herb) as the calculated value of  $t$  were lesser than the tabulated value  $t = 3.71$  ( $p > 0.01$ ).

Table-6. Iron, Chromium and Sulphur content in soil sample collected from the sites.

Site	pH	Moisture content (%)	Organic Carbon content (%)	Iron ( $\text{mgkg}^{-1}$ )*	Chromium ( $\text{mgkg}^{-1}$ )*	Sulphur ( $\text{mgkg}^{-1}$ )*
Hydrocarbon exploration site	6.1	11.28	0.6	23.67 $\pm$ 0.01	29.64 $\pm$ 0.24	155.99 $\pm$ 3.25
Adjacent site	6.2	7.49	1.7	17.19 $\pm$ 0.00	27.31 $\pm$ 1.01	116.68 $\pm$ 1.51

(\* Mean $\pm$  SEM; N=3)

Table-7. Iron, Chromium and Sulphur content in plant sample collected from Hydrocarbon exploration site.

Plant name	Plant tissue	Iron ( $\text{mgkg}^{-1}$ )*	TF	BCF <sub>root</sub>	Chromium ( $\text{mgkg}^{-1}$ )*	TF	BCF <sub>root</sub>	Sulphur ( $\text{mgkg}^{-1}$ )*	TF	BCF <sub>root</sub>
<i>Ageratum conyzoides</i> L.	Root	0.21 $\pm$ 0.04	2.67	0.01	2.74 $\pm$ 0.68	0.73	0.09	52.97 $\pm$ 0.33	1.93	0.34
	Shoot	0.56 $\pm$ 0.03			2.00 $\pm$ 0.65			101.97 $\pm$ 0.57		
<i>Cynodon dactylon</i> L. Pers.	Root	1.29 $\pm$ 0.07	0.37	0.05	4.13 $\pm$ 0.80	0.65	0.14	156.56 $\pm$ 0.87	1.70	1.00
	Shoot	0.48 $\pm$ 0.03			2.70 $\pm$ 0.46			266.42 $\pm$ 0.66		
<i>Centipeda minima</i> L.	Root	0.50 $\pm$ 0.02	0.38	0.02	4.87 $\pm$ 0.61	0.67	0.16	145.06 $\pm$ 0.33	1.76	0.93
	Shoot	0.19 $\pm$ 0.05			3.11 $\pm$ 0.71			255.90 $\pm$ 0.57		
<i>Digitaria sanguinalis</i> (L.) Scop.	Root	2.11 $\pm$ 0.06	0.63	0.09	4.75 $\pm$ 0.67	0.61	0.16	140.12 $\pm$ 0.87	1.08	0.90
	Shoot	1.33 $\pm$ 0.02			2.90 $\pm$ 0.78			151.31 $\pm$ 0.57		

(\* Mean $\pm$  SEM; N=3)

The highest iron, chromium and sulphur content in both root and shoot parts were observed in *Paspalum notatum*, *Centipeda minima* and *Cynodon dactylon* respectively. Only *A. conyzoides* have TF greater than 1

for iron. The remaining plants have both TF and BCF values of iron and chromium less than 1. In case of sulphur, TF values in all the plants were greater than 1 whereas only *C. dactylon* have BCF value greater than 1.

Table-8. Iron, chromium and sulphur content in plant sample collected from adjacent site.

Plant name	Plant tissue	Iron (mgkg <sup>-1</sup> )*	TF	BCF <sub>root</sub>	Chromium (mgkg <sup>-1</sup> )*	TF	BCF <sub>root</sub>	Sulphur (mgkg <sup>-1</sup> )*	TF	BCF <sub>root</sub>
<i>Axonopus compressus</i> (Sw.) P.Beauv	Root	0.01±0.02	3.27	0.00	20.18±0.26	0.31	0.74	286.49±0.57	1.31	2.46
	Shoot	0.36±0.04			6.22±0.69			375.29±1.51		
<i>Cynodon dactylon</i> (L.) Pers.	Root	0.09±0.02	0.44	0.01	4.13±0.32	2.06	0.15	205.25±0.87	2.34	1.76
	Shoot	0.04±0.03			8.51±0.47			480.87±0.57		
<i>Centipeda minima</i> (L.)	Root	0.24±0.05	0.25	0.01	14.12±0.54	0.70	0.52	176.96±0.57	1.63	1.52
	Shoot	0.06±0.00			9.91±0.82			288.13±0.87		
<i>Chrysopogon aciculatus</i> (Retz.) Trin.	Root	0.22±0.04	0.45	0.01	13.92±0.71	0.23	0.51	264.45±1.43	0.19	2.27
	Shoot	0.10±0.03			3.27±0.27			51.32±0.87		
<i>Desmodium triflorum</i> (L.) DC.	Root	0.09±0.00	4.33	0.01	9.66±0.67	0.32	0.35	218.40±0.57	1.35	1.87
	Shoot	0.39±0.03			3.07±0.25			294.05±0.87		
<i>Ageratum conyzoides</i> (L.)	Root	0.19±0.03	4.05	0.01	1.58±0.55	3.55	0.06	46.22±0.22	4.26	0.40
	Shoot	0.77±0.03			5.61±0.62			196.70±1.14		
<i>Centella asiatica</i> (L.) Urban	Root	0.11±0.02	4.55	0.01	5.89±0.71	3.69	0.22	48.69±0.57	2.51	0.42
	Shoot	0.50±0.05			21.74±0.82			122.36±1.19		
<i>Vernonia cinerea</i> (L.) Less.	Root	0.14±0.04	0	0.01	6.18±0.72	1.21	0.23	105.59±0.87	0.98	0.90
	Shoot	0.00±0.01			7.98±1.27			103.95±0.57		
<i>Mimosa pudica</i> (L.)	Root	0.08±0.08	1.38	0.01	14.93±0.14	0.88	0.55	66.45±2.05	3.17	0.57
	Shoot	0.11±0.03			13.18±0.39			210.51±0.57		
<i>Ipomoea alba</i> (L.)	Root	0.03±0.01	1.33	0.00	7.27±3.11	1.19	0.27	104.93±0.57	3.49	0.90
	Shoot	0.04±0.02			8.64±0.75			366.08±0.87		
<i>Chromolaena odorata</i> (L.)	Root	0.08±0.01	4.88	0.01	9.05±0.94	1.47	0.33	125.00±0.87	3.06	1.07
	Shoot	0.39±0.05			13.26±0.60			382.20±1.14		
<i>Melastoma malabathricum</i> (L.)	Root	0.10±0.02	4.9	0.01	4.01±0.43	2.58	0.15	60.20±0.87	4.60	0.52
	Shoot	0.49±0.03			10.36±0.28			276.62±0.57		
<i>Urena lobata</i> (L.)	Root	0.54±0.03	0.43	0.03	4.62±0.60	1.05	0.17	569.34±0.33	0.15	4.88
	Shoot	0.23±0.03			4.83±0.44			86.84±0.33		
<i>Lantana camara</i> (L.)	Root	0.28±0.05	0.75	0.02	7.98±0.86	0.90	0.29	58.89±1.43	5.81	0.50
	Shoot	0.21±0.03			7.20±0.60			342.07±0.87		
<i>Hevea brasiliensis</i> Muell. Arg.	Leaves	0.94±0.01	-	-	2.70±0.27	-	-	281.55±0.57	-	-
	Stem	0.39±0.03			9.13±0.26			682.16±0.57		

(\* Mean± SEM; N=3)

The highest concentration of iron, chromium and sulphur in shoot was observed in *Ageratum conyzoides*, *Centella asiatica* and *Cynodon dactylon* respectively while in root part, iron and sulphur was highest in *Urena lobata* and chromium content was highest in *Axonopus compressus*. More than half of the plants have TF values greater than 1 for iron, chromium, and sulphur.  $BCF_{root}$  values of iron and chromium was found to be very less.  $BCF_{root}$  value of sulphur was found to be greater than 1 in some plants.

The experiment demonstrated the types of plants found in a hydrocarbon exploration site as well as their adjacent site. In the hydrocarbon exploration site, there was absence of shrub species. The main reason for this is that there are generally little or no space for plants to grow in the site as the site is exclusively used for the exploration purpose. The soil analysis data shows that all the studied metals *i.e.*, iron, chromium, and sulphur content were found to be higher in the Hydrocarbon exploration site when compared with the adjacent site. Shannon-Weiner and Simpson diversity increases within creasing richness of a particular homogeneity pattern, but it happens otherwise sometimes. The Simpson diversity is less sensitive to richness and more susceptible to homogeneity than the Shannon index, which is more prone to homogeneity<sup>18</sup>. In the experiment, it was found that both the Shannon index and Simpson index for herb was higher in adjacent site. If the value of evenness approaches zero, it means that there is larger change in abundance of species while a higher value depicts that all the species are abundant

equally and evenly distributed within the sample quadrat<sup>14</sup>. Evenness was found to be higher in Hydrocarbon exploration site than the adjacent site. The BCF specifies the ability of the plant to absorb the trace element in the plant with concern to the trace element concentration in the media<sup>25</sup>. Values above 1 are high for the translocation factor, which is a measure of the concentration of metal in aerial portals to that in roots<sup>15</sup>. It was also observed that all the plants found in both the sites have some level of the metals in either their root or shoot parts. This ignites our hope to conduct future phytoremediation studies in the site by using plants that are found in the site. Some of the plants found in the study site were used in phytoremediation experiment by different researchers.

In case of Hydrocarbon exploration site, *D. sanguinalis* was found to contain high iron in both the root and shoot part but the TF value was highest in *A. conyzoides* which means that although *D. sanguinalis* absorbed more iron the translocation of iron from root to shoot part was more efficient in case of *A. conyzoides*. However, the BCF value was less than 1 which means that plants are not able to take up iron effectively from the soil. *C. minima* have the highest chromium content but both TF and BCF values of all the plants were found to be less than 1. *C. dactylon* has the highest sulphur content among all the plants and both the TF and BCF values were greater than 1. This means that the mentioned plants have ability to take up sulphur from the soil and efficiently transfers it to other parts. Earlier study by Devi & Dasgupta<sup>4</sup> also reported the highest phosphorus content in this plant. The TF values of sulphur for all the plants were

greater than 1 while BCF values were less than 1 except for one plant. Nevertheless, it should be noted that the plants collected from this site was recently established and hence this may account for the low metal content or less TF and BCF value.

For adjacent site, iron content in shoot and root was observed in *A. conyzoides* and *U. lobata* respectively. *A. conyzoides* also have a very high TF value of 4.05 which means that the metal is efficiently transfer from the root and stored it in the shoot.  $BCF_{root}$  of iron of all the plants were found to be very less in all the plants. *C. asiatica* and *A. compressus* recorded the highest chromium content in shoot and root part with TF values of 3.69 and 0.31 respectively. The  $BCF_{root}$  value of chromium in all the plants was less than 1. The highest sulphur content in shoot and root was observed in *C. dactylon* and *U. lobata*, respectively. The  $BCF_{root}$  values of both the plants were also found to be greater than 1. Out of 14 plants found in the site, 10 plants have TF values more than 1 which means that most of the plants found can effectively translocate the absorbed metals from the root to the shoot. 7 plants were also found to have  $BCF_{root}$  values greater than 1 demonstrating that the plants can efficiently absorbed the metal from the soil.

The main aim of the study was to identify plant species which are found in the hydrocarbon exploration site and to determine the element content of the different plants found in the site. The data so obtained can be used in future phytoremediation study. There is no previous study on the element content in a hydrocarbon exploration site in Tripura and hence this study can be used as a baseline

data for future study. From the experiment, it is found that there are many plants species which can effectively accumulate metals from the soil and transfer it to other plant parts. While selecting plants, those which have high TF and BCF values can be selected as high values means that they are able to accumulate the metals from the site. More future studies are needed as phytoremediation is in very initial stage as technology is concerned. Studies can be also being focused on increasing the efficiency using various methods.

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