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Phytoremediation potential of some abundantly growing indigenous herbs of crude oil contaminated sites

S. Akram and H. Deka*

Department of Botany, Gauhati University, Guwahati-781 014, India

***Corresponding Author Email :** hemendeka@gauhati.ac.in

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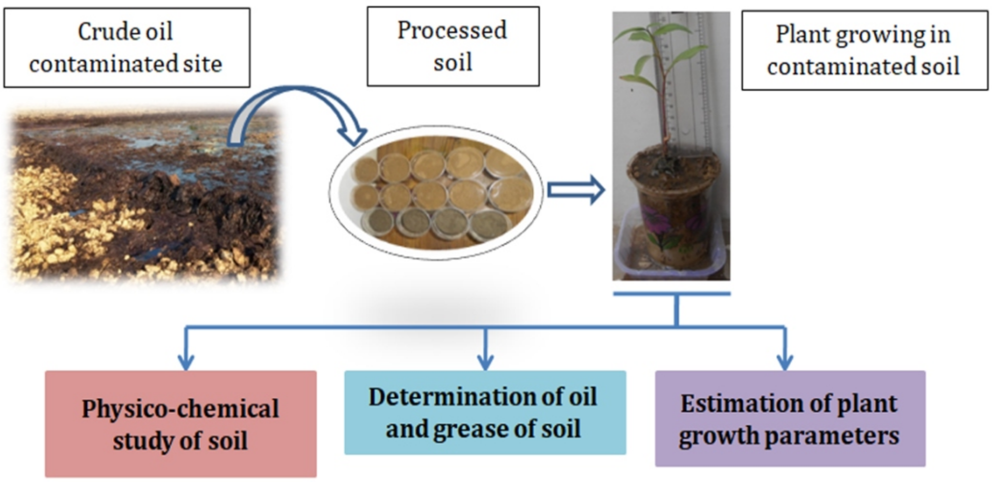
Abstract

Aim: To study the total oil and grease (TOGs) removal potential of three abundant herb species of crude oil contaminated sites, namely *Xanthium strumarium* (L.), *Ageratum conyzoides* (L.) and *Polygonum hydropiper* (L.) from oil contaminated soils.

Methodology: The pot experiment was carried out by taking soil samples of crude oil contaminated agricultural field. For each experimental plant, three replicas were maintained and a similar control setup was maintained without plants for comparing the results. Another control set up was maintained in non-contaminated soils to compare the plant growth parameters. Total oil and grease (TOG) contents, physico-chemical profiles of contaminated soil, plant growth/productivity parameters and functional groups were analyzed following the standard methods.



Results: The results showed that total oil and grease (TOG) contents decreased significantly after treatments by plants against the initial level and control. Plant height, number of leaves, chlorophyll contents and biomass were found to be lower in all the three plant species that were grown in contaminated soil as against the control treatment. The improved physico-chemical profiles of contaminated soil samples after treatment indicated the positive effect of plants in treated soil samples. FTIR data revealed difference in peak intensities and the presence of petroleum hydrocarbons in plants that were grown in oil-contaminated soils.

Interpretation: The herb species *Xanthium strumarium*, *Ageratum conyzoides* and *Polygonum hydropiper* showed the potential for removal of hydrocarbons from crude oil contaminated soil.

Key words: Crude oil, Hydrocarbons, Indigenous herbs, Phytoremediation

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Introduction

Crude oil pollution is a global problem from oil production industries (Markel *et al.,* 2004). In oil producing states of India, the crude oil associated burden is a perennial problem since the inception of oil industries. Again, in Assam most of the oil industries are situated near the agricultural field as well as in the periphery of human settlements. Contamination from crude oil takes place in various ways such as during oil extraction, storage, transportation, refining process of crude oil etc. All these activities ultimately pollute the nearby habitat (Urum *et al.,* 2006; Lai *et al.,* 2009). Continuous release of crude oil associated pollutants exert physico-chemical, biological and structural changes in soil systems and make them unfit for cultivation (Wei *et al.,* 2019). It has been reported that crude oil pollutants such as heavy metals (HMs) and polycyclic aromatic hydrocarbons (PAHs) are carcinogenic and mutagenic (Das and Chandran, 2011) which not only impose adverse affects on soil microbes but also other organisms, including human beings (Scott and Nelson, 2004; Anyika *et al.,* 2015). Considering the fact, it is necessary to remove the crude oil pollutants from soil.

Phytoremediation is a promising, cost effective and eco-friendly technique for remediation of crude oil polluted soils. It has been reported that plants are effective in degradation, transformation, assimilation, metabolism and detoxification of hazardous pollutants from soils (Cai *et al.,* 2010). The efficacy of plants for removal of crude oil pollutants has already been established by several workers around the globe (Han *et al.,* 2016; Cheng *et al.,* 2017). For example, some plants such as tall fescue, fire phoenix and maize have also been used for remediation of crude oil contaminated soil in recent past (Liu *et al.,* 2014a,b; Liao *et al.,* 2016). Nevertheless, the selection of plant species is crucial in phytoremediation, and even in most cases their success is site specific (Euliss *et al.,* 2007; Kirkpatrick *et al.,* 2006) and, therefore, use of indigenous plants of the polluted sites are always preferred.

Moreover, considering the better adaptability due to fibrous root systems; herbaceous and annual plants has been reported to be more successful in remediation of soils contaminated by crude oil (Hutchinson *et al.,* 2001; Kechavarzi *et al.,* 2007; Basumatary *et al.,* 2012a, b; Ikeura *et al.,* 2016). It has been reported that native herb species of crude oil polluted soils of Assam such as *Cyperus rotandus*; *Cyperus brevifolius; Axonopus compresus* are effective in removal of total oil grease contents from contaminated soils (Basumatary *et al.,* 2012a,b; Bordoloi *et al.,* 2012). Nonetheless, further investigation on phytoremediation potential of indigenous herbaceous community of crude oil polluted sites is needed in order to end up the lacuna of phytotechnology for remediation of polluted soils.

The herb species *Xanthium strumarium* L., *Ageratum conyzoides* L. and *Polygonum hydropiper* (L.) are found to grow abundantly in the crude oil polluted agro-ecosystems of Assam, India. Therefore, these herb species may possess some adaptive advantage to withstand with the adverse situations posed due to crude oil pollution. The aim of the present investigation is to study the total oil and grease (TOG) removal potential of Xanthium strumarium, Ageratum conyzoides and Polygonum hydropiper in crude oil polluted soils.

Materials and Methods

Collection of soil samples and physico-chemical analyses: The Lakowa oil field (25°01´NL, 94°50´EL) of Assam, India was selected as study site. The Lakwa oil field is under the operation of Oil and Natural Gas Commission (ONGC), India. The experimental soil samples were collected at a depth of 0-20 cm from the oil contaminated agricultural fields near the GGS4 (Group Gathering Station) where crude oil is stored before sending to the refineries. The collected soil samples were shade dried, debris were removed and finally grinded into fine particles for experimental use. The physico-chemical parameters of the collected samples were studied to understand the deterioration in soil conditions and included analysis of pH, conductivity, water holding capacity, total organic carbon, total Kjeldhal nitrogen, available phosphorus, total potassium and finally total oil and grease contents. The soil pH and conductivity were measured in 1:5 soil suspensions using digital pH (Universal 6331) and conductivity meter (Systronics 2485).

The water holding capacity of the soil samples were determined by the methods of Piper (Singh *et al.,* 1991). Total organic carbon content level was measured by Walkey and Black titration method (Walkey and Black, 1974). Total Kjeldhal nitrogen content of the soil was estimated by microkjeldhal method (Jackson, 1973). Available phosphorus was estimated spectrophotometrically (Shimadzu UV1601) following the stannous chloride method (APHA, 1998). Total potassium content was determined by the acid digestion method using a flame photometer with standard solution (APHA, 2017). Total oil and grease content of the soil samples were extracted with dichloromethane in soxhlet extractor (Martin Jr *et al.*1991) and measured gravimetrically (Villalobos *et al.*, 2008).

Experimental plants: *Xanthium strumarium* L., *Ageratum conyzoides* L. and *Polygonum hydropiper* L. were selected as experimental plants.These three species were found abundantly in the crude oil contaminated sites of Assam, India. However, for the experimental purpose plant seeds were collected locally and grown in pots separately. The same aged seedlings were selected for experimental trials.

Experimental setup: The experiment was carried out in plastic pots in net house. The duration of the experiment was fixed for 60 days. In each pot, equal amount of contaminated soil (200 gm) was taken, mixed properly after watering and left for three days to settle down. Then a single plant seedling was introduced to the respective pots. Separate set up was maintained for each plant species. A similar control set up was also maintained without plants for comparison of the results. Another control set up was also maintained in non-contaminated soil to compare the plant growth parameters. In each case, three replicas were taken for statistical analysis of the results. The physico-chemical properties along with TOGs of the contaminated soil were analyzed before introduction of the plants as well as after completion of the experimental trial following the methods as mentioned in collection of soil samples and physico-chemical analysis section. Plant growth parameters that includes morphological characters such as shoot length, root length and number of leaves, leaf area index (LAI), biomass values and chlorophyll contents were analyzed at the beginning and by end of the experimental trials following the standard methods.

FTIR Analysis: FTIR Spectroscopy was conducted by following the KBr palate method. Shoot portion of each plant species were dried and grind separated in mortar and pestle. Powder of the stems was then mixed with KBr in 1:80 ratio and pellets were prepared by establishing pressure of 10 kg cm^2 for about 1 min. Spectra ranging between wave numbers 4500 and 400 cm^{-1} were recorded with Fourier transform infrared spectroscopy (IR Affinity1). Functional groups were identified and interpreted (Pavia *et al.,* 2008).

Statistical Analyses: SPSS software (2018 version) was used for statistical analysis. The significant difference in values of physico-chemical properties of the soil samples, plant growth parameters were determined by pair-sample t-test, One-way ANOVA and LSD test.

Results and Discussion

The results of TOGs dissipation is presented in Fig. 1. The results revealed that there was significant reduction in TOGs level after treatment by each plant species. The initial TOG contents of contaminated soil was 197 gm kg^{-1} which reduced significantly to 54 gm kg^{-1}, 78 gm kg^{-1} and 69 gm kg^{-1} after the treatment with Ageratum conyzoides, *Polygonum hydropiper* and *Xanthium strumarium* respectively. Thus, reduction in TOG levels over the initial value was 72%, 60%, and 64% for *Ageratum conyzoides, Polygonum hydropiper* and *Xanthium strumarium,* respectively. The present findings corroborates with the results of Peng *et al.* (2009), who reported about 63% reduction in oil content by *Mirabilis jalapa* in the crude oil contaminated soil. Similarly, Ikhajiagbe *et al.* (2017) reported upto 46% reduction in TOG contents from oil contaminated soil employing *Eleusine indica*. Degradation of petroleum hydrocarbon was found higher by fescue, sorghum, cowpea, alfalfa and black rush plants (Merkl *et al.,* 2004).

On the other hand in the control treatment, a 16% dissipation of TOG content was recorded which may be due to oil evaporation along with other natural and microbiological process (Chukwuma *et al.,* 2019). The TOG dissipation percentage increased 4.5, 3.8 and 4 fold for *Ageratum conyzoides, Polygonum hydropiper* and *Xanthium strumarium,* respectively over control treatment may be attributed to the synergistic action of plants and microbes in the soil (Asemoloye *et al.,* 2019). Although, correlation of rhizosphere microbial population with the studied herbs has not been presented in this investigation. It has already been established that of biodegradation significantly accelerates when there is rise in microbial population in the rhizosphere zone (Basumatary *et al.,* 2012a). The results of the physico-chemical analysis are presented in Table 1. The results revealed that there were changes in the physico-chemical conditions of soil after treatment by the experimental plant species.

Soil pH indicates the soil health and availability of other nutrients for the plant. The crude oil contaminated soil sample was found to be acidic with a pH value of 4.27 at the beginning of experimental trials. The pH level in soil samples changes to 6.07, 6.10 and 6.59, respectively, post treatment with *Ageratum conyzoides, Polygonum hydropiper* and *Xanthium strumarium*. This indicates that there was shifting of acidic condition of soil towards the near-neutral range. Oil contaminated soil are generally acidic in nature is due to the presence of toxic acids and pollutants (Barua *et al.,* 2011; Oyem and Oyem, 2013; Basumatary *et al.,* 2012b). The change in the acidic nature of oil contaminated soil after treatment by plants may be due to microbial action upon toxic acids/pollutants and uptake of pollutants by the treated plant species (Egharevba *et al.,* 2017), although further planned study is required to prove this hypothesis.

The initial value of conductivity in the contaminated soil sample was found to be 0.08 mS ds^{-1}. The conductivity values of the soil changed to 0.32, 0.54 and 0.69 mS ds^{-1} respectively, post treatment with *Ageratum conyzoides, Polygonum hydropiper* and *Xanthium strumarium.* The lower conductivity values of oil contaminated soil indicate the adverse effect of crude oil pollution. Non-polar nature of crude oil reduces ionic movement in the soil (Akpoveta *et al.,* 2011; Osuji and Nwoye, 2007), there by reducing the conductivity. Increasing trends in soil conductivity of contaminated soil after plant treatment may be due to plant-microbe interaction which ultimately degrades the oily layer, besides releasing some ions in soil system (Baruah *et al.,* 2014).

The crude oil contaminated soil samples showed reduced level of water holding capacity (9.86) during the start of the experiment. Lower values of water holding capacity may be due to the hydrophobic nature of crude oil and the formation of oil coating over the soil surface. Similar findings were reported by Vara *et al.* (2012). After harvesting the plants, water holding capacity was found to be 29.3%, 26.6% and 25.43% for *Ageratum conyzoides, Polygonum hydropiper* and *Xanthium strumarium,* respectively. The enhanced value of water holding capacity may be due to reduction of total oil grease contents from the soil (Baruah *et al.,* 2014). The water holding capacity of 25.04% or more is suitable for agricultural purpose (Baruah *et al.,* 2013).

Initial level of total organic carbon in the oil contaminated soil sample was 18.75% whereas post plant treatment, the values decreased by 8.15%, 12.8% and 9.58%, respectively, *Polygonum hydropiper* and *Xanthium strumarium*. Since crude oil contains

Table 1: Physico-chemical analysis of crude oil contaminated soils at the beginning and post plant treatment by *Ageratum conyzoides*, *Polygonum hydropiper* and *Xanthium strumarium*

Parameters	Post plant treatments				
	Initial	Control	*A. conyzoides*	*P. hydropiper*	*X. strumarium*
pH	4.27±0.1^{a}	4.69±0.24^{a}	6.07±0.9^{c}	6.10±0.04^{d}	6.59±0.15^{k}
Conductivity (mS ds^{-1})	0.08±0.007^{a}	0.09±0.003^{a}	0.32±0.009^{d}	0.54±0.02^{b}	0.69±0.12^{p}
Water holding capacity (%)	10.78±1.1^{a}	9.86± 0.7^{a}	29.3±1.2^{k}	26.6±0.9^{b}	25.43±0.9^{p}
Total organic carbon (%)	17.11±1.1^{a}	18.75±1.2^{a}	8.15±0.5^{c}	12.8±0.5^{b}	9.58±0.87^{d}
Total nitrogen (%)	890±40^{a}	884.3±34^{a}	503±10^{b}	420±70^{c}	390±30^{d}
Available phosphorus (mg kg^{-1})	30±1.7^{a}	28.7±2.3^{a}	23.4±3^{b}	11±0.51^{c}	8.6±2.1^{d}
Total potassium (mg kg^{-1})	272.56±8^{a}	266.9±8.6^{a}	210.8±9^{b}	220.03±5.9^{c}	223.53±6.1^{d}

Values are mean of three replicates ±SD. Different letters in the same rows indicate significant differences in values among different treatments

Table 2: Morphological changes in *Ageratum conyzoides*, *Polygonum hydropiper* and *Xanthium strumarium* after 60 days of experimental trials in control and crude oil contaminated soils

Morphological parameters	Plant species											
	Ageratum conyzoides				*Polygonum hydropiper*				*Xanthium strumarium*			
	Control		Contaminated		Control		Contaminated		Control		Contaminated	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Shoot length (cm)	5±0.4^{a}	18.7±1.1^{b}	5.3±0.3^{a}	10.3±0.5^{d}	9.1±1.6^{e}	21.5±2.1^{f}	9.1±1.7^{g}	14.2±1.9^{h}	10.6±1.6^{i}	19.1±4.4^{j}	10.7±1.7^{p}	12±3.1^{m}
Root length (cm)	3.5±0.3^{a}	4.9±0.4^{p}	3.4±0.4^{a}	3.9± 0.6^{k}	3.2±0.7^{b}	5.9±0.4^{q}	3.2±0.7^{b}	4.5±0.6^{d}	3.2±0.7^{b}	5.0±0.45^{x}	3.3±0.8^{b}	4.2±0.6^{z}
Number of leaves	7.6±0.57^{a}	26.6±2.8^{b}	8±0.3^{d}	20±2^{k}	5±1^{p}	14±2.6^{q}	4.9±1.2^{p}	9.3±0.58^{c}	5±1^{p}	10±3^{x}	5±1^{p}	8.1±2^{y}

Values are mean of three replicates ± SD, Different letters in the same rows indicate significant differences in values among different treatments

Table 3: IR spectra along with their assignments in the shoot samples of experimental plants grown on control and crude oil contaminated soils

Bands and Peaks (cm^{-1})	Assignments	Samples
3700-3100	O-H stretching of flavonoids/phenolic compounds	Present in all samples, More intense in the plant samples of contaminated soils except Xanthium
2920.22	C-H stretching indicating presence of alkane	Present in all samples, more intense in control plant samples
2360.76	O-H stretching of carboxylic acid compounds such as benzoic acid	Present in all samples, highly intense in Xanthium of contaminated soils
2065.76	C-C compounds representing alkenes	Present dominantly in Polygonum samples
1736.87	C=O stretching of aldehydes	Clear and intense in Ageratum samples of contaminated soils
1620.20	C= C stretching of alkenes such as 1-hexadecane, cyclohexane etc.	Present in all samples, More intense in the plant samples of contaminated soils except Xanthium
1381.03	C-F stretching of fluoride/monoflourohexane	Present in all samples, More sharp and intense in the plant samples of contaminated soils
1062.87	C-O stretching of alcohols/ethers/esters/carboxylic acids	Present in all samples, sharp and intense in control plant samples

organic carbon, the contaminated soil samples showed elevated level of organic carbon (Shaker *et al.,* 2009; Chukwuma *et al.,* 2010; Nwazue, 2011). The reduction in TOCs may be attributed to the microbial utilization of organic carbon from the soil. The present findings is agree with the previous study of Mrayyan and Battikhi (2005) who have reported up to 28% reduction in TOC level in the oil contaminated soil. It has been found that plant's exudate enhances the microbial population which in turn act on organic carbon and reduces the TOC levels during phytoremediation trials.

The initial values of total Kjeldhal nitrogen (TKN), available phosphorus (AP) and total potassium (TK) in the contaminated soil were recorded as 890 mg kg^{-1}, 30 $mgkg^{-1}$ and 272.56 $mgkg^{-1}$, respectively. The results showed that TKN, AP and TK values in soil samples decreased significantly by end of the experimental trials irrespective of the experimental plant species used in the experiment. The decrease in TKN values in the treated soil was found within 1.7-2.2 folds for *Ageratum conyzoides, Polygonum hydropiper* and *Xanthium strumarium*. Similarly, decrease in AP was 1.2, 2.7 and 3.5 folds in the soil samples treated with *Ageratum conyzoides, Polygonum hydropiper* and *Xanthium strumarium,* respectively. Whereas, average TK decrease was recorded 1.2 fold in the soil samples after 60 days of treatment in all the plant treated trials. It has been suggested that microbial population in the contaminated soil utilize a significant portion of the macro as well as micronutrients besides using hydrocarbons as the sole carbon source and this process become more accelerated in presence of plants. Moreover, it is also true that plants utilize major portion of the soil nutrients for their growth and metabolic activities which ultimately decreases NPK concentrations in soil (Verma *et al.,* 2012).

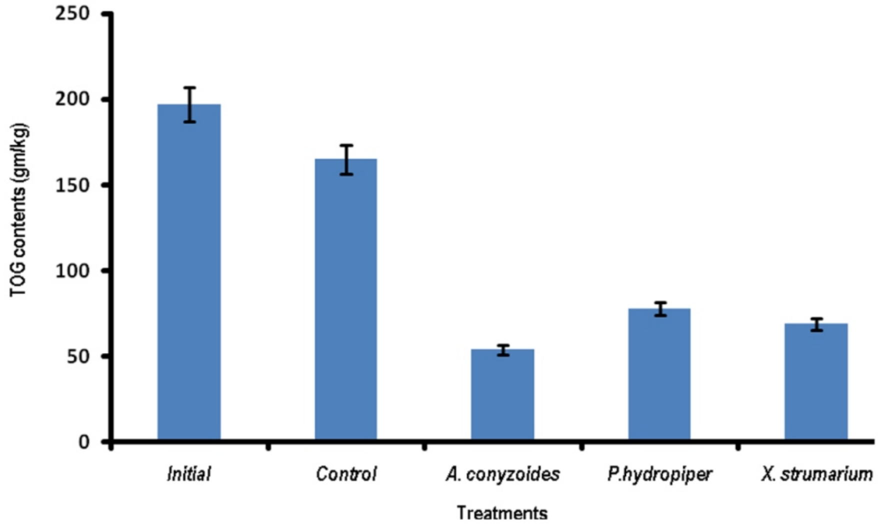

Fig. 1: Total oil and grease (TOGs) content in the crude oil contaminated soil after 60 days of treatment by *Ageratum conyzoides, Polygonum hydropiper* and *Xanthium stromerium*. Values are means of three replicates, bars indicates SD.

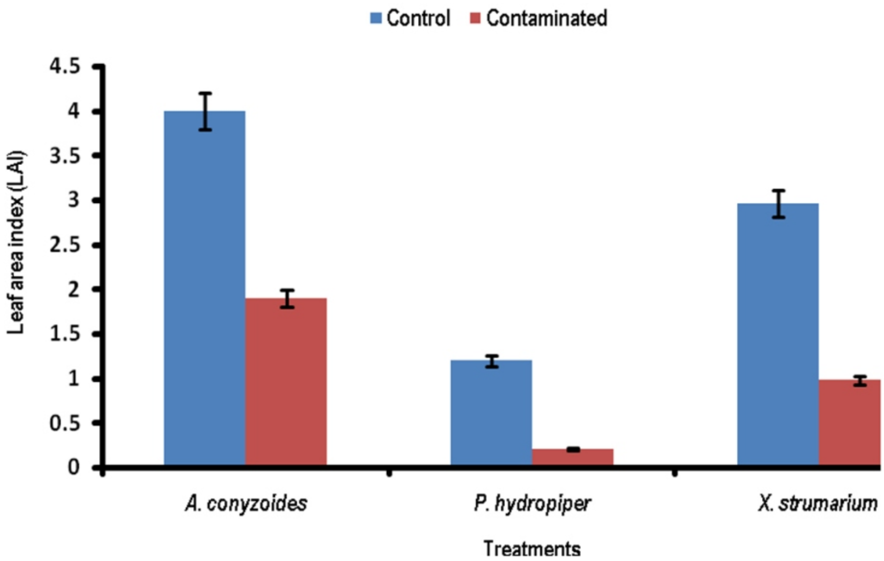

Fig. 2: Leaf area index (LAI) of *Ageratum conyzoides, Polygonum hydropiper* and *Xanthium strumarium* grown on control and oil contaminated soils. Values are means of three replicates, bars indicates SD.

Table 2 shows variations in plant growth as indicated by morphological parameters such as leaf number, shoot and root length among the studied plants grown on control and contaminated soil. The results revealed that there were significant differences in the number of leaves, shoot and root lengths in all test plants grown in control and contaminated soils. Similarly, results of the plant productivity parameters *i.e.,* leaf area index (LAI), biomass and leaf chlorophyll contents are presented in Fig. 2, Fig. 3 and Fig. 4, respectively. The results revealed that LAI, plant biomass and chlorophyll a, chlorophyll b, total chlorophyll content of *Ageratum conyzoides, Polygonum hydropiper* and *Xanthium strumarium* grown in the control soil were significantly higher as against the same plants grown on contaminated soil. As a whole, crude oil contamination caused negative effects on both growth and productivity of the test plants, which is obvious as crude oil associated pollutants impose abiotic stress on the plants (VJ and Okunnu, 2012; Samal and Santra, 2002; Anthony, 2001;

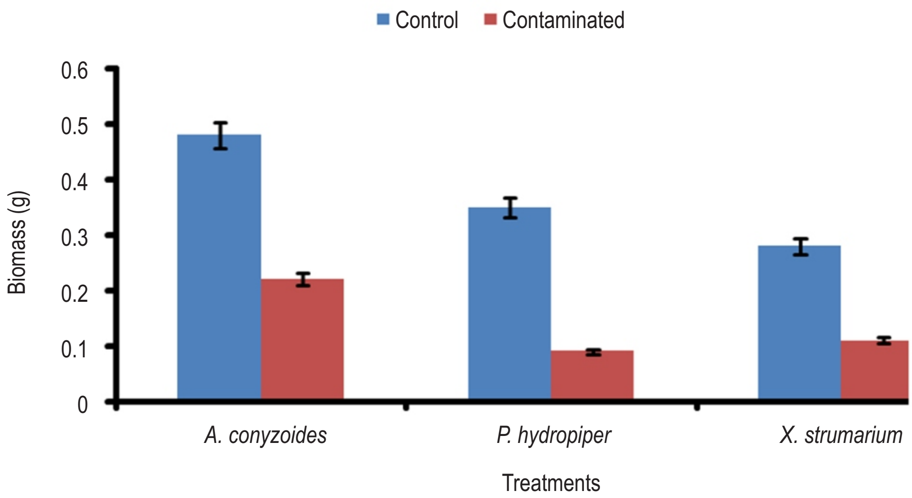

Fig. 3: Shoot and root biomass values *Ageratum conyzoides, Polygonum hydropiper* and *Xanthium strumarium* grown on control and oil contaminated soils. Values are means of three replicates, bars indicates SD.

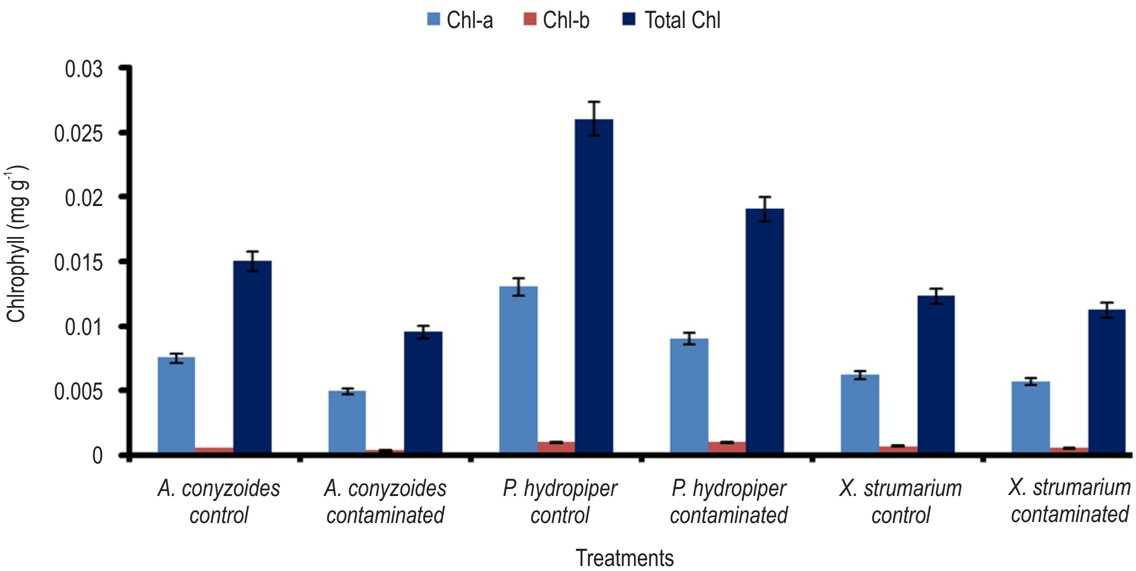

Fig. 4: Chlorophyll-a, Chlorophyll-b and total Chlorophyll contents of leaves of *Ageratum conyzoides, Polygonum hydropiper* and *Xanthium strumarium* grown on control and oil contaminated soils. Values are means of three replicates, bars indicates SD.

Ekundayo *et al.,* 2001), thus resulting differences in growth and productivity in two contrasting habitat conditions. It has been suggested that presence of aliphatic, aromatic and high molecular weight organic compounds of crude oil inhibits the chlorophyll synthesizing enzyme and as a result chlorophyll production of the test plants was reduced (Akapo *et al.,* 2011). This chlorophyll deficiency of plants growing in oil contaminated soil is further related with retardation in growth of plants. Moreover, water deficient condition in oil contaminated soils lead to slower photosynthesis and transpiration, and finally reduce the biomass values (Chaineau *et al.,* 1997).

FTIR spectroscopy was conducted to understand the changes in the functional groups in the experimental plants under abiotic stress conditions induced by crude oil contaminations. The FTIR spectra was measured within the range of 500-4500 cm^{-1}. The main absorbance bands of the IR spectra for different samples assigned for different functional groups are presented in Table 3.

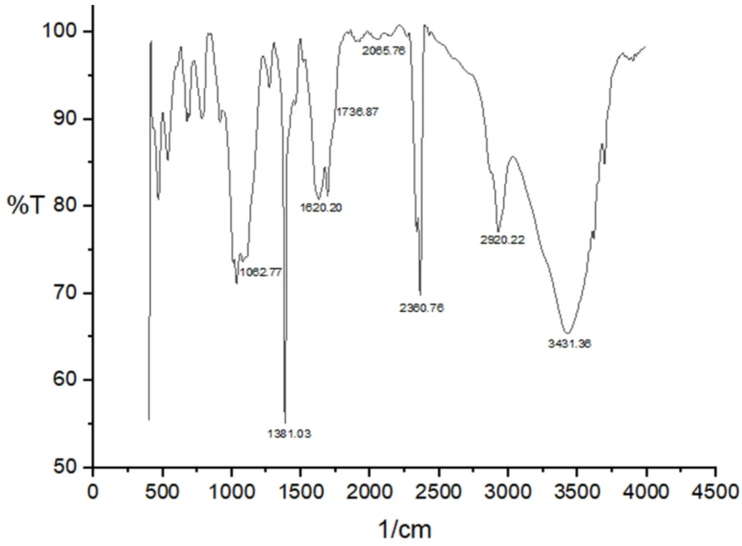

Fig. 5a: FTIR spectra of *Ageratum conyzoides* grown on control soil.

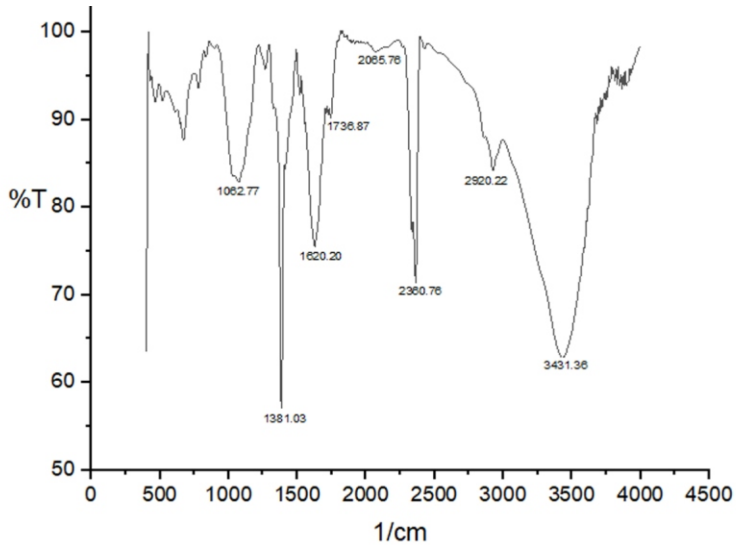

Fig. 5b: FTIR spectra of *Ageratum conyzoides* grown on oil contaminated soil.

The results revealed that most of the peaks and bands that represent various functional groups are common and found in the plant samples obtained from both control and contaminated soils.

However, there were variations in intensities of peak and bands between the plant samples grown in control and contaminated soils. The broad band between 3700 3100 cm^{-1} due to O-H stretching indicated the presence of flavonoids/ phenolic compounds in all samples. The intensity of this band was higher in the plant samples of contaminated soils baring *Xanthium* (Table 3). On the other hand, the peak at 2920.2 cm^{-1} due to C-H stretching representing aliphatic alkane was found to be highly intense in all control plant samples. Although peak at 2360.76 cm^{-1} (O-H stretching) for carboxylic acid compounds such as benzoic acid was found in all samples, however, but the highest intensity of peak was observed in *Xanthium* grown in contaminated soil. The peak at 2065.76 cm^{-1} representing alkenes was dominant by only in the shoot samples of *Polygonum*. The peak at 1736.87 cm^{-1} due to C=O stretching for aldehydes was very distinct in the *Ageratum* shoot samples of contaminated soils whereas the peak

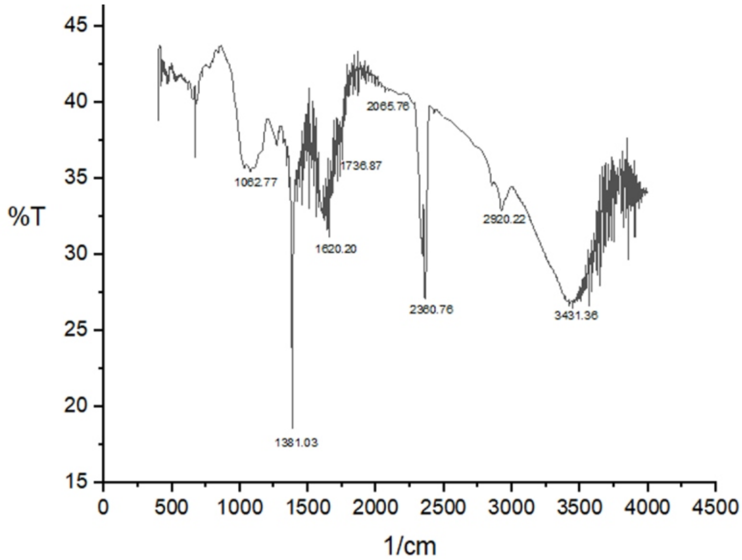

Fig. 5c: FTIR spectra of *Polygonum hydropiper* grown on control soil.

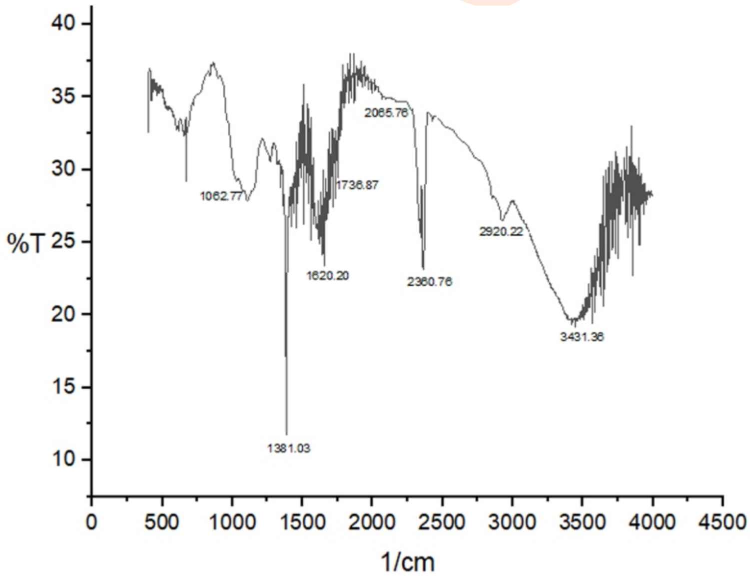

Fig. 5d: FTIR spectra of *Polygonum hydropiper* grown on oil contaminated soil.

at 1062.87 cm^{-1} due to C-O stretching for alcohols was more prominent in all control samples. The peak at 1620.2 cm^{-1} due to C=C stretching for alkenes such as 1-hexadecane and cyclohexane was found in all samples, however, peak intensity was higher in the plant samples of contaminated soils. The peak at 1381.03 cm^{-1} for C-F stretching of fluoride/monoflourohexane was found to be more intense and sharp in the plant samples of contaminated soils.

As a whole the results showed both increase as well as decrease in intensities of various bands and peaks among the studied plant samples. This clearly indicates the adverse effects of crude oil on the test plants. It has been suggested that increase and/or decrease as well as shifting in bands/peaks occurs when plants are exposed to abiotic stress such as crude oil contaminations (Liu *et al.,* 2012), which usually vary from species to species. Moreover, the increase in the intensities of peaks/bands in the plant samples of contaminated soils may be due to uptake and metabolism of crude oil components by the experimental plants (Devatha *et al.,* 2019). Further, various compounds such as alcohols, phenols, aliphatic hydrocarbons, carboxylic acids etc., are component of plants (Bobby *et al.,*

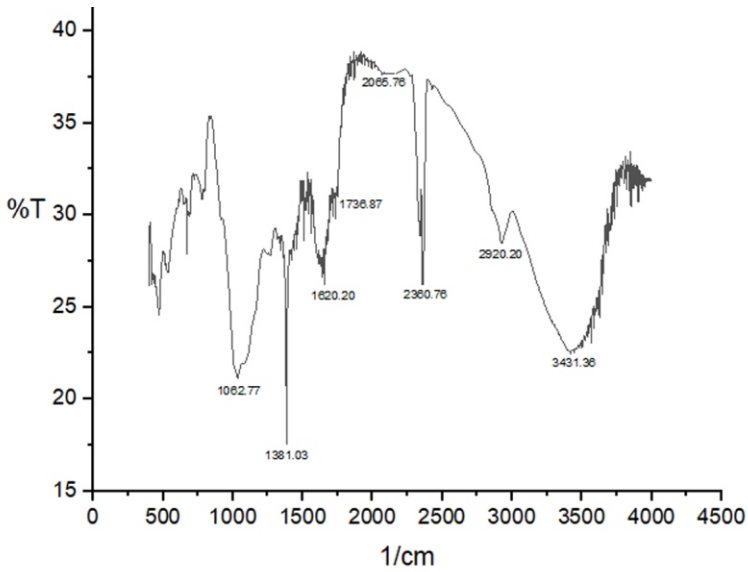

Fig. 5e: FTIR spectra of *Xanthium strumarium* grown on control soil.

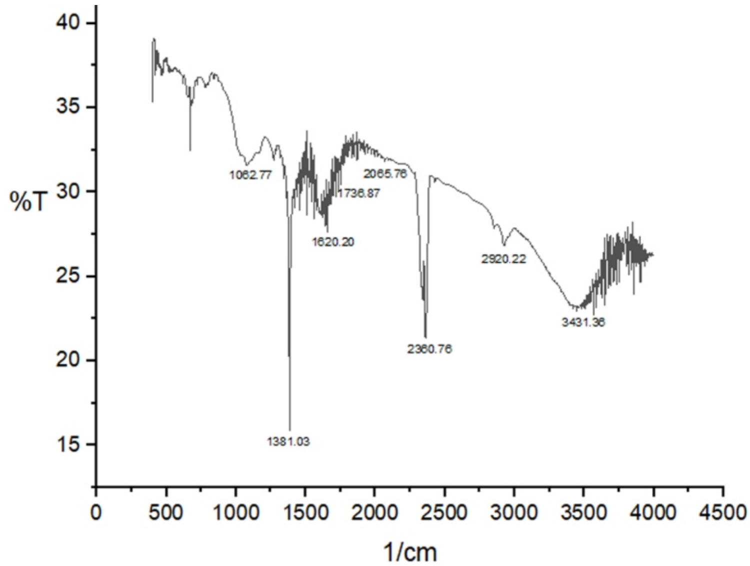

Fig. 5f: FTIR spectra of *Xanthium strumarium* grown on oil contaminated soil.

2012) which justify the presence of intense bands/peaks indicating the presence of these compounds in control plant samples.

In the present study, it was found that *Ageratum conyzoides* was the most potent herb, followed by *Xanthium strumarium* and *Polygonum hydropiper* in removal of total oil and grease from the crude oil contaminated soils. FTIR analysis clearly showed the uptake as well as metabolism of oil and grease by the experimental plants from the contaminated soil. Nevertheless, further planned study is needed on plant- microbe synergy to understand the mechanism of oil and grease or hydrocarbons dissipation from contaminated soils.

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Add-on Information

Authors' contribution: S. Akram: Carried out the experimental works and wrote the manuscript with Hemen Deka; **H. Deka:** Developed the concept of this experimental works, provided laboratory facilities and guided Shaleh Akram and wrote the manuscript.

Research content: The research contents is original and has not been published elsewhere

Ethical approval: Not Applicable.

Conflict of interest: The author declares that there is no conflict of interest.

Data from other sources: Not Applicable

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