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ORIGINAL ARTICLE

# Changes Germination, Growth and Anatomy *Vicia ervilia* in Response to Light Crude Oil Stress

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**KEYWORDS**

Oil pollution

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**ABSTRACT:** Thepetroleum concentrations are contaminant important sources in the environment. Hydrocarbon's contaminants depend on their type and concentration can cause variable toxicity in soils, on the other hand, different kinds of plants also response concentration of contaminant differently, because of the diversity of physiological and morphological characteristics. In this study the effect of different concentrations of light crude oil was investigated on stem length, germination and anatomical of *Vicia ervilia*. A factorial experiment was used with a randomized complete block design (RCBD) with three replications. The results showed that the germination and stem lengths decreased significantly ($p \leq 0.05$) with increasing concentrations of petroleum. Anatomical studies the changes of parenchyma, vascular bundles, epidermal and increase crack showed.

## INTRODUCTION

Increasing industrial productions are along with increasing pollutant in the environment [1]. Petroleum pollutant is an unavoidable consequence of population increasing and industrialization process. Soil pollution by petroleum concentrations is an important environmental problem [2]. Petroleum hydrocarbons are harmful for the alive and no alive components due to toxicity effects [3]. Pollutant in soil can insert into food chain [4] and this is dangerous for the health of human and other animals [5]. Petroleum concentrations can affects on vegetation growth through stimulate the soil bacteria, consume soil nutrients and reduce soil oxygen. Oil pollutions make disturbances in vegetations [6]. The signs of pollution in vegetation are numerous [6]. Petroleum cause chemically disturbances in metabolism and growth of vegetation [7], reduction of vegetation growth [8], autumn of areal organs (leaves), variations of pile, chlorophyll destruction, delay in flowering [9] and cell death [10]. In studies which were conducted by researchers, the performance of vegetation was studied in polluted soil by petroleum. The results showed that petroleum in some vegetations cause to decrease germination and growth (11, 3 and 12), delay or lack of germination [13, 14], increase root length and improve the vegetation growth in polluted soil [15, 16 and 17].

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In previous studies, the performance of vegetation was studied in polluted soil by petroleum. The obtained results showed that petroleum in some vegetations cause the following effects: decrease of germination and growth (11, 3 and 12), delay or lack of germination [13, 14], increase of root length and improve the vegetation growth in polluted soil [15, 16 and 17]. Results of several researches showed that the petroleum concentrations produce different degree of toxicity according to the type and their concentration. Mentioned items show that the petroleum pollution caused disturbances that are morphological, physiological and anatomy variations in vegetations. The anatomic properties of vegetations are very important, but a little attention has been paid to the effects of petroleum on vegetation anatomy which have grown in polluted soil. Therefore, in this study the effects of different concentrations of light crude oilon germination, stem length and anatomy of *Vicia ervilia* was investigated in polluted soil.

## MATERIALS AND METHODS

Soil was prepared from the adjacent agricultural lands. Soil was polluted by different concentration (1, 2, 3 and 4% W/W %) of petroleum and a soil without pollution was considered as control. Manual method was used to mix soil and petroleum [18]. Seeds with germination potential were planted in vessels contained by polluted and unpolluted soil at the depth of 1.5 cm. three replications were prepared from each concentration. All vessels were placed in free location with even temperature and environmental conditions. Control treatment was for the vegetation grown in unpolluted soil.

In this study the germination and stem height were measured. The germination rate in each vessel was calculated according to the number of planted seeds and following equation:

$$G = \frac{n}{N} \times 100$$

Where in this equation N is the number of planted seeds, n is the number of germinated seeds and G is the germination percentage [19]. The length of stem from the soil surface to top of the stem was measured using ruler in cm [20]. The lower part of stem was sampled for the anatomy experiments [10]. Vegetation texture was placed in solution of glycerin and ethanol 70%, because vegetation texture can be protected in these consolidators in long term [10]. The common blade was used to prepare sections from vegetation; the sample coloring was done using duplicated Carmen Stain**.** Prepared samples were assessed under the light Moticmicroscope and IXUS 220 HS canon camera was used to image samples. Excel was used to design diagrams and SPSS software was used to compare means by Duncan test at probability level of 0.05.

## RESULTS

The results of this study showed that the existence of light crude oil at the different concentrations had effects on germination of plants and the germination percentage decreased with increasing petroleum in soil. At concentration of 4% it was observed a delay in germination. 1005 germination was detected in control treatment. Germination at the concentrations of 1, 2, 3 and 4% were 77.7, 33.3, 22.2 and 72.2%, respectively (Figure 1).

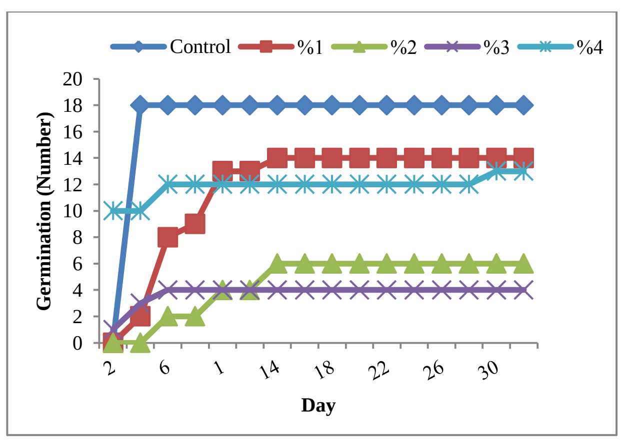


Figure 1. Germination of *Vicia ervilia* (Based on the counting of the number of germination per day)

Statistical analysis of data showed the significant difference among treatments. In comparison to control and contaminant treatments, it was not observed a significant difference between the control and treatments of 1 and 4% (Figure 2).

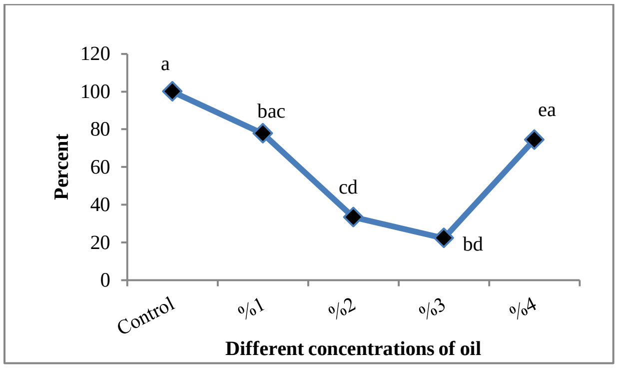


Figure 2. Germination rate of *Vicia ervilia* (At least a common subscript shows that there isno significant difference at probability level of 5%)

The height of stem was measured every 5 days to determine the difference in growth variations. The plant in initial days had more height growth at concentration of 3% as compared to other contaminant concentrations. But at the end of growth period it showed significant reduction compared to treatment of 1 and 2% (Figure 3).

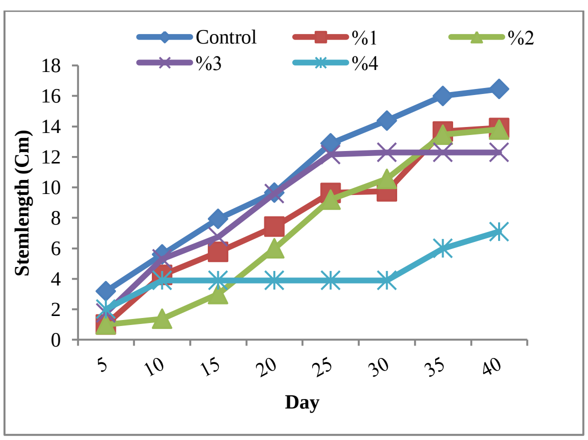


Figure 3. measuring stem height of *Vicia ervilia*
(Every 5 day in growth period)

The results of the statistical analysis of the mean of stem height and investigation of the effects of concentration on stem height showed that there was significant difference among the treatments at probability level of 5%, but there was no significant difference among the control and treatments of 1 and 2% (Figure 4).

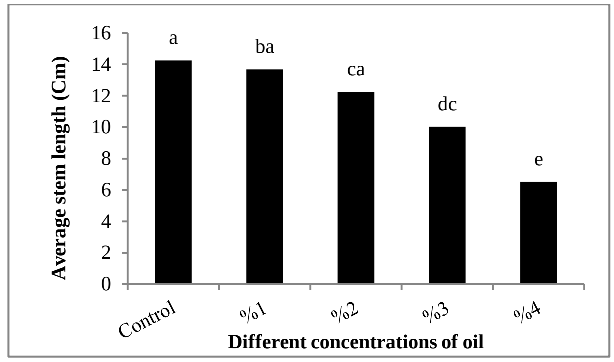


Figure 4.mean height of *Vicia ervilia*stem in cm(Columns with same subscript shows that there isno significant difference at probability level of 5%)

The stem of the vegetation grown in contaminant soil was different with the stem of vegetation grown in non-contaminant soil. These variations are shown in Figures 5 and 6.

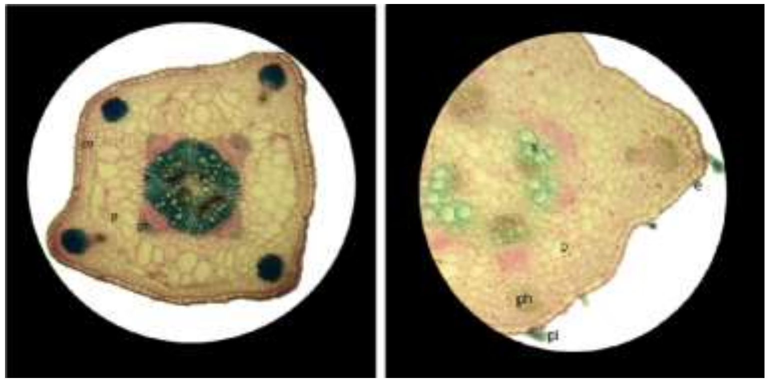

Figure 5. Increasing of pile in contaminant sample compared to control (Left sample is control and right sample is contaminant sample)
e: Epidermal, ph: Phloem, X: Xylem, co: Collenchyma, p: Parenchyma, Pi: pile

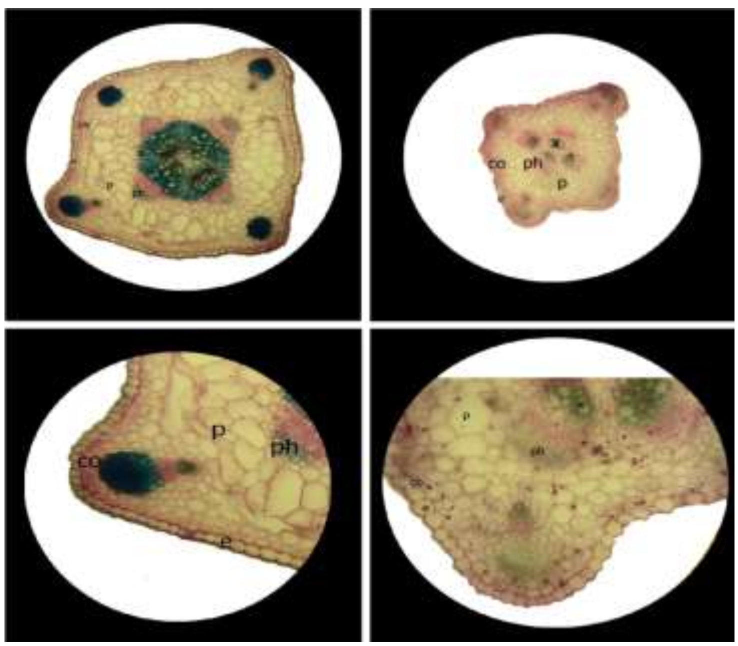

Figure 6. comparison of the cross section of Vicia ervilia and Variation in contaminant sample (right) and control (left)
e: Epidermal, ph: Phloem, X: Xylem, co: Collenchyma, p: Parenchyma

Anatomical studies showed the changes of parenchyma, vascular bundles, epidermal and increase crack. As it was shown in Figure 5, control sample of *Vicia ervilia* has determined section and shape, in contaminant samples, it can be observed and an increase in swell and variations in cross section shape. In Figure 6 (down figure) control sample had large parenchyma cells and regular epidermal but in contaminant samples the size of Parenchyma cells decreased but its frequency increased. Moreover the irregularity in epidermal size can be observed. Phloem vascular of contaminant samples was irregular and compacted as compared to control.

## DISCUSSION

Germination is the most important vegetative stage of plants [21]. Germination rate was observed in all the concentrations of petroleum but the germination rate decreased with increasing petroleum concentration in soil which this was in agreement with the findings of Njoku and colleagues, and Kirk and colleagues [22, 23]. Decreasing of germination was happened due to reducing oxygen and moisture [24], poor accessibility nutrient for plant [25] and increasing toxicity materials of petroleum compositions in soil [26]. Delay in germination at 4% concentration was in agreement with the findings of Kisic and colleagues, which reported that petroleum compositions provided a soft layer around the seed and prevented from access to oxygen and water [27]. This caused delaying or lacking germination in polluted soil by oil. The mean of stem length of *Vicia ervilia*in three replications from control to oil concentrations of 4% were respectively equal to 14.26, 13.69, 12.26, 10.03 and 6.53. The stem length was significantly decreased with increasing the concentrations of light crude oil ($P<0.05$). Reduction of vegetation growth by contaminant soil was proved by several researchers [28, 29 and 30]. Daryabeigizand and colleagues found that the light crude oil compounds hadn't significant negative effects on germination but it had significant effects during the vegetative stages [31]. This result showed that the oil by its toxicity effects prevented the natural growth of plant. Mendelssohn and Hester reported that the decreasing of stem length was due to physical, chemical and biological variations by petroleum compounds in soil [32]. Moreover reduction of stem length can be due to decreasing accessible nutrients for plants [33] or poor ventilation of soil which caused that the growth prevented and its length decreased [34]. According to the reports and results of statistical investigations of different concentrations of oil as compared to control showed that the toxicity compounds of light crude oil had negative effect on germination and growth of *Vicia ervilia* and the severity of effects increased with increasing the concentration of petroleum in soil. Species had degrees of sensitivity based on their morphological and physiological properties; therefore it is important to investigate the anatomical properties of plants. The observed variations were due to the effects of petroleum compounds in soil grown plants. *Vicia ervilia*is dicotyledonous species which have 3 epidermal layers, parenchyma and vascular bundles in the structure of stem. In contaminant treatment the pile increased. Increasing pile can be a tool of sourcing more petroleum compounds. Moreover an irregularity was observed in vascular bundles. Xylem and phloem vascular were compacted [35] and decreased by increasing the concentration of petroleum which this was not in agreement with the finding of Omosun and colleagues, [10]. The response of vegetation to decrease nutrients and nitrogen of soil reduced xylem and phloem vascular. Thus, reduction of vascular bundles led to decrease transporting petroleum into plant. Decreasing of the vegetation growth showed the disturbances in performance of xylem and phloem vascular in providing water and nutrient under the stress of soil contaminant by petroleum. This result was in agreement with the finding of Agbogidi and Eshegbeyi, and Agbogidi and Ofuoku [34, 36]. Increasing of parenchyma was in agreement with the findings of Gill and colleagues, [6]. This change has been occurred to produce stability in response to stress and/or preventing from the entering petroleum compounds in to cells. The cross section *Vicia ervilia*has changed. Nogueira and colleagues, in their research showed the changing of cross section [37]. Irregularity in epidermal was due to the disturbances in plant morphology. Epidermal thickness increased slowly which this was in agreement with the finding of Sharma and colleagues, and Gill and colleagues [38, 6]. They found the epidermal variations in vegetation grown in contaminant soil. Epidermal variations was occurred due to the preventing the reduction of inter tissue water [38]. In Table 1 observations of the present study are given compared to other studies.

Table 1. Observations of the present study comparison with other studies

| The parameters Studied | Observations in this study | Correspondence | Nonconcurrence |
|---|---|---|---|
| **Germination** | Germination rate of was observed in all the concentrations of petroleum but the germination rate decreased with increasing petroleum concentration in soil. | Njoku*et al*., 2011; Kirk *et al*., 2002 | — |
| **Delaygermination** | Delay in germination was observed at a concentration of 4 percent. | Kisic*et al*., 2009 | — |
| **Averagestem length** | The stem length was significantly decreased with increasing the concentrations of light crude oil ($P<0.05$). | Okonokhua*at el*., 2007; Shahriari*et al*., 2007; Ogboghodo*et al*., 2003 | — |
| **Anatomical** | The pile was increased in contaminant treatments. | — | — |
| | Irregularity was observed in vascular bundles. | — | — |
| | Xylem and phloem vascular were compacted by increasing the concentration of petroleum. | Kofidis*et al*., 2008 | — |
| | Xylem and phloem vascular were decreased by increasing the concentration of petroleum. | — | Omosun*et al*., 2008 |
| | Decreasing of the vegetation growth shows the disturbances in performance of xylem and phloem vascular in providing water and nutrient under the stress of soil contaminant by petroleum. | Gill *et al*., 1992 | — |
| | Increasedparenchymaltissue. | Nogueira*et al*., 2011 | — |
| | The cross section Vicia ervilia has changed. | Sharma *et al*.,1980; Gill *et al*., 1992 | — |

## CONCLUSIONS

In this study the effect of different concentrations of light crude oil was investigated on stem length, germination and anatomical of *Vicia ervilia*. The results showed that the oil had negative effect on germination and growth of *Vicia ervilia*. Germination and stem lengths decreased significantly with increasing concentrations of petroleum. The results of anatomical studies showed that the species changed its structure in response to petroleum contaminant which these variations were adaption mechanisms in response to contaminant location. *Vicia ervilia* can be generated in all petroleum concentrations and the growth continued in period, so it can be described that the plant had relative resistance against soil with low pollution. In recent years many countries which produce petroleum suggested Phytoremediation practices. In this process some plans can be generated in contaminant soil to petroleum.

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