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# Effect of Petroleum Polluted Soil on the Performance of *Phaseolus vulgaris* L.

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**Abstract:** A Greenhouse pot experiment was conducted at Department of Botany and Microbiology, King Saud University, Riyadh, KSA to study the effect of petroleum polluted soil (PPS) on the performance of *Phaseolus vulgaris* L.var. Strike. The effect of PPS (0-500g) on shoot length (SL) plant$^{-1}$, total leaf area (LA) plant$^{-1}$, shoot fresh weight (FW) and shoot dry weight DW) plant$^{-1}$, root fresh weight (R FW) and root dry weight (R DW) plant$^{-1}$ and content of heavy metals in shoot and root of plant was studied at 4 week (H1), 6 week (H2) and 8 week (H3) after planting. PPS treatments decreased all the growth characteristics at all stages. Heavy metal accumulation in plants was higher in root as compared to shoot at all stages. On the basis of the values of bioconcentration and translocation factor (1<). Plant of *Phaseolus vulgaris* was considered as the less efficient in hyper accumulation of heavy metals. These results suggest that *P. vulgaris* may be also less efficient in remediation of PPS.

**Key words:** Phytoremediation · *P. vulgaris* · Bioconcentration · Translocation · Heavy metals

## INTRODUCTION

Soil pollution is a major constraint for food production because it limits crop yield. Contamination of soil occurs due to the leakage of storage tanks and pipelines, land disposal of petroleum wastes and accidental spills. Petroleum polluted soil (PPS) is currently of much environmental concern. Petroleum contaminated soil is a harmful to both human and plant. Petroleum is a complex mixture of hydrocarbons with small amounts of organic compounds containing sulfur, oxygen and nitrogen, as well as compounds containing metallic constituents, particularly vanadium, nickel, iron and copper [1]. Petroleum hydrocarbons and heavy metals can affect soil characteristics sufficiently to result in significant losses in soil quality [2-4]. The phytoremediation for PPS is one of the promising environmental and cost effective approaches [5]. Phytoremediation refers to the use of plants to clean contaminated soil biologically through the ability to stimulate microbial degradation in a soil contaminated with crude oil [6, 7].

The common bean (*Phaseolus vulgaris* L.) is an herbaceous annual plant and widely cultivated in temperate and semitropical regions. *P. vulgaris* is leguminous plant that has ability to fix atmospheric nitrogen for the better performance of plants. The major objective of our study was to determine the effect of PPS on the performance of *P. vulgaris.*

## MATERIAL AND METHODS

A pot experiment was carried out in Green House, Department of Botany and Microbiology, King Saud University, Riyadh, KSA. Green been (*Phaseolus vulgaris* L. var. Strike) seeds were purchased from the local market (Riyadh). The petroleum polluted soil (PPS) was obtained from petrol station (Riyadh). PPS was sieved with a 5 mm stainless steel sieve after solar drying. The PPS was mixed fully with artificial soil (Perlite) at the rate of 0 ($T_1$), 100 ($T_2$), 200 ($T_3$), 300 ($T_4$), 400 ($T_5$) and 500 ($T_6$) g per kg PPS soil. The content of heavy metals in PPS was given in Table 1. Five seeds were planted in a pot containing mixed soil. Pots were arranged according to simple randomized design with single factor and five replicates of each treatment. After 2 weeks of planting, thinning was done and two healthy plants were maintained in each pot. Mineral nutrition was applied every 2 days with 200 ml Hoagland nutrient solution per pot. The plants were sampled at 4, 6 and 8 week after planting (H1, H2 and H3 respectively). The performance of the plant was assessed in terms of plant height, fresh and dry weight of root and shoot, total leaf area, content of heavy metals i.e. Cadmium (Cd), copper (Cu), lead (Pb),nickel (Ni) and Zinc (Zn). For the determination of heavy metals, plants samples were divided into roots and shoots and air-dried at 80°C for 24 h. The digestion approach was partly modified by Hseu [8] from that of Zheljazkov and Nielson [9]. One gram of sample was

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Table 1: Heavy Metals Concentrations in Petrol Polluted Soil

| Element | Soil Samples (mg/kg) |
|---|---|
| Cu | 52 to 56 |
| Cd | 8 to 13 |
| Pb | 122 to 144 |
| Ni | 89 to 102 |
| Zn | 100 to 109 |

placed in a 250 mL digestion tube and 10 mL of concentrated $HNO_3$ was added. The sample was heated for 45 min at 90°C and then the temperature was increased to 150°C at which the sample was boiled for at least 8 h until a clear solution was obtained. Concentrated $HNO_3$ was added to the sample (5 mL as added at least three times) and digestion occurred until the volume was reduced to about 1 mL. The interior walls of the tube were washed down with a little distilled water and the tube was swirled throughout the digestion to keep the wall clean and prevent the loss of the sample. After cooling, 5 mL of 1% $HNO_3$ was added to the sample. The solution was filtered with Whatman No. 42 filter paper and <0.45 μm Millipore filter paper. It was then transferred quantitatively to a 25 mL volumetric flask by adding distilled water. The content of heavy metals i.e. Cd, Cu, Pb, Ni and Zn were determined by atomic absorption spectrometer (Perkin-Elmer model AAnalyst 300) [10].

Each pot was treated as one replicate and all the treatments were repeated five times. The data were analyzed statistically with SPSS-17 statistical software (SPSS Inc., Chicago, IL, USA). Mean was statistically compared by Duncan's multiple range test (DMRT) at P<0.05% level.

## RESULTS

Data revealed that *P. vulgaris* exhibited different nature at different levels of PPS for growth characteristics as well as accumulation of heavy metals (Figs. 1-3).

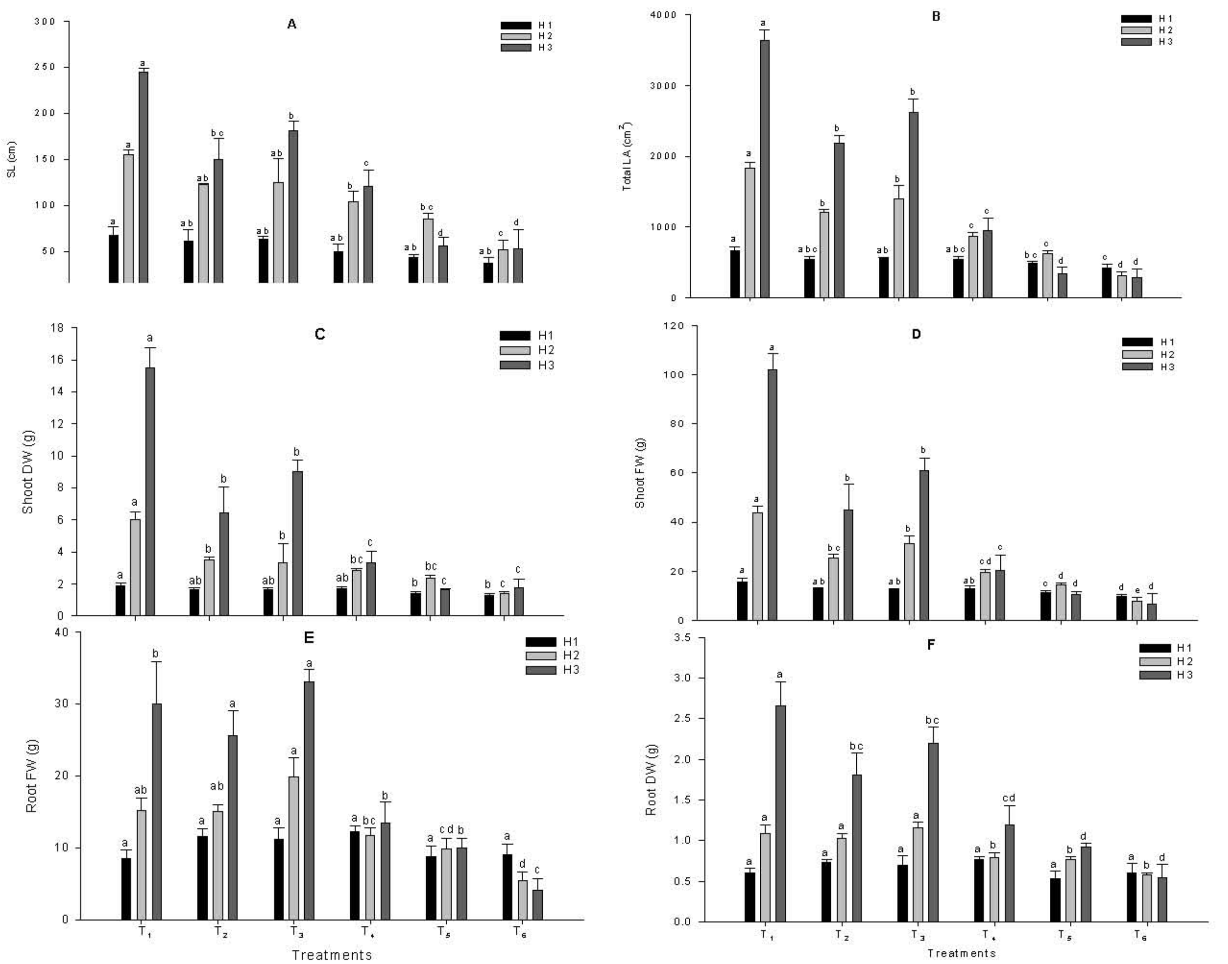


Fig. 1: Effect of petroleum polluted soil on (A) SL, (B) total LA, (C) shoot FW, (D) shoot DW, (E) root FW and (F) root DW of Phaseolus vulgaris L. Bars followed by the same letter do not differ statistically at P < 0.05 (Duncan Multiple Range Test). Average of five determinations are presented with bars indicating S.E.

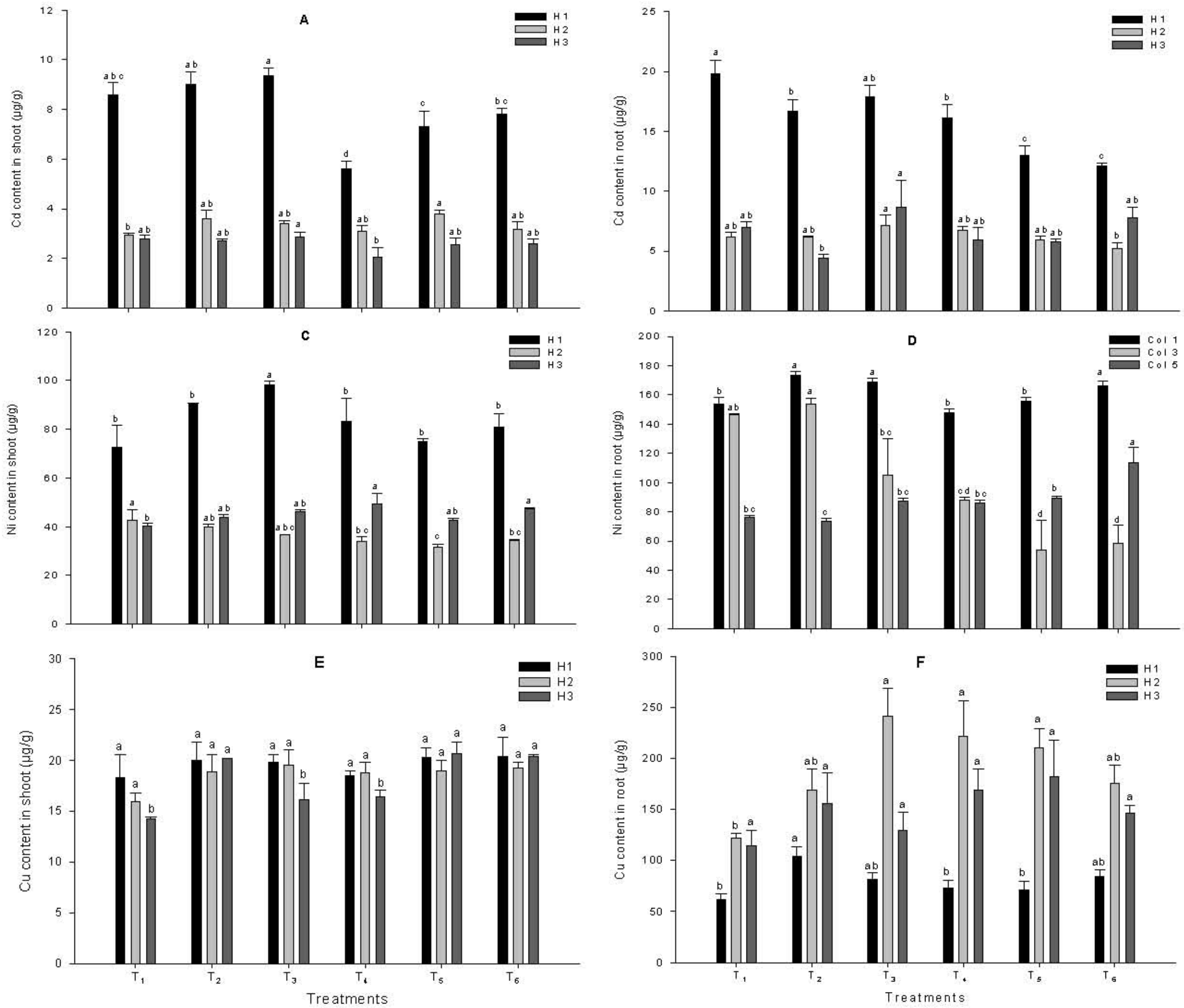


Fig. 2: Effect of petroleum polluted soil on (A) Cd content in shoot, (B) Cd content in root, (C) Ni content in shoot, (D) Ni content in root, (E) Cu content in shoot, (F) Cu content in root of Phaseolus vulgaris L. Bars followed by the same letter do not differ statistically at $P < 0.05$ (Duncan Multiple Range Test). Average of five determinations are presented with bars indicating S.E.

PPS stress significantly inhibited SL, total leaf area, Shoot FW and DW, root FW and DW. Maximum reduction of SL, Shoot FW and DW, root FW and DW and total leaf area was recorded with $T_6$ at all sampling dates. Treatment $T_6$ resulted in the shortest phaseolus plants compared with the control ($T_1$) at all stages. However, treatment $T_6$ showed parity with $T_2$, $T_3$, $T_4$, $T_5$ at H1 and $T_5$ also at H2 and H3 for SL (Fig. 1A). The lowest LA was recorded with $T_6$ treatment at all stages (Fig. 1B). However, $T_5$ and $T_6$ being at par with each other for total LA at H1 and H3. Data (Figs. 1C and 1D) illustrate that maximum reduction for shoot FW and DW was observed with treatment $T_6$ of PPS at all stages. However, treatment $T_4$ being at par with each other at H1 and H3 and treatments $T_4$ and $T_5$ showed parity with $T_6$ for shoot DW at H3. Data recorded in figs 1E and 1 F revealed that root FW and DW decreased with increasing levels of PPS stress. Treatments $T_6$ exhibited maximum reduction for root FW and DW at H2 and H3 as compared with $T_1$. However, there was not a significant difference among the treatments for root FW and DW at H1. Data revealed that accumulation of Cd was greater in root compared with shoot of *P. vulgaris* (Fig. 2A). Treatments $T_5$ and $T_6$ at H1, treatments $T_1$, $T_2$, $T_4$, $T_5$ and $T_6$ (except $T_3$) at H2 and H3 for Cd content in root showed parity with each other. Similarly the accumulation of Ni was greater in root as compared to shoot at all stages (Figs. 2C and 2D). Treatment $T_1$ gave the lowest value for Ni content in shoot as well as root at H1 and H3 (except H2). However, treatments $T_4$ and $T_6$, being at par, gave lowest value for Ni content in shoot while $T_5$ and $T_6$, gave

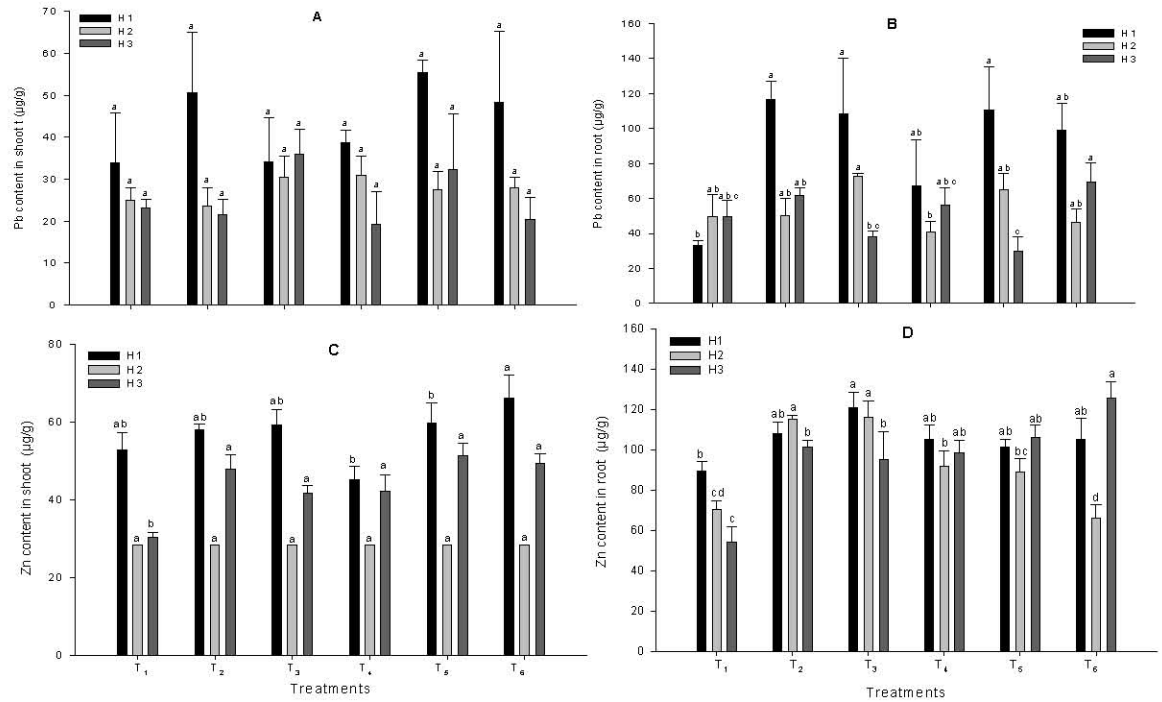


Fig. 3: Effect of petroleum polluted soil on (A) Pb content in shoot, (B) Pb content in root, (C) Zn content in shoot and (D) Zn content in root of Phaseolus vulgaris L. Bars followed by the same letter do not differ statistically at P < 0.05 (Duncan Multiple Range Test). Average of five determinations are presented with bars indicating S.E.

the lowest value for Ni content in root at H2 (Fig. 2D). Accumulation of Cu in shoot at H1 and H2 and in root at H1 and H3 was statistically insignificant (Fig. 2E and 2F). Treatments $T_1$, $T_3$ and $T_4$, gave the lowest value for Cu content in shoot. However, $T_5$ and $T_6$, being at par, gave highest value for Cu content in shoot (Fig. 2E). Accumulation of Pb in shoot was found non-significant statistically at all stages (Fig 3A). However, treatments $T_1$, $T_4$ and $T_5$ gave the lowest value for Pb content in root at H1, H2 and H3 respectively (Fig. 3B). Accumulation of Zn was found maximum in root as compared to shoot at all stages (Fig. 3C and 3D). Zn content in shoot was found non-significant at H2. Treatments $T_4$ and $T_5$, being at par with $T_1$, $T_2$ and $T_3$, gave the lowest value for Zn content in shoot at H1. At H3, treatment $T_1$ exhibited lowest value at all stages. However, treatment $T_1$, being at par with the rest of the treatments at H2 (Fig. 3D).

## DISCUSSION

The *P. vulgaris* responded widely in terms of growth characteristics and accumulation of heavy metals at different levels of PPS stress. PPS-exposed plants exhibited stunted growth (Fig. 1). The observed decrease in SL, total LA, shoot FW and DW and root FW and DW, with increasing levels of PPS over the controls (Figs. 1A, 1B, 1C, 1D, 1E and 1F) may be ascribed to the maximum accumulation of hydrocarbon that can create unsatisfactory environment for plant growth through a number of process; (i) oil could displace air from soil pore space, (ii) an increase in the demand for oxygen brought about by activity of oil-decomposing microorganisms [11] and (iii) hydrophobic environment due to hydrocarbon, which limits water absorption to roots [12]. These results coincide with earlier studies of Nwoko *et al.* [13]; Diab [14]. Therefor, it could be possible reason to suggest that *P. vulgaris* is sensitive to PPS and unsuitable for remediation.

In the present study, metal concentrations in plants growing in PPS were not very high and none of the PPS-exposed plant showed metals concentration 1000 mg $kg^{-1}$ in shoot as well as root (Figs. 2 and 3). i.e. *P. vulgaris* is not hyperaccumulator. Jung and Thornton [15]; Rosselli *et al.* [16] reported that elevated soil pH and high organic matter limited plant availability of heavy metals in the soil, resulting in low plant uptake of these metals. Moreover, the ability of this plant to withstand and accumulate heavy metals may be not useful for phytostabilization. Bioconcentration factor (BCF) and translocation factor (TF) is very important parameters to

Table 2: Accumulation and translocation of Cd, Ni, Cu, Pb and Zn in *P. vulgaris* L.

| | | Bioconcentration factor (BCF) | | | | | Translocation factor (TF) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatments | DAS | Cd | Ni | Cu | Pb | Zn | Cd | Ni | Cu | Pb | Zn |
| PPS 0 | H1 | 2.48 | 1.72 | 1.18 | 0.27 | 0.90 | 0.43 | 0.47 | 0.30 | 1.03 | 0.59 |
| | H2 | 0.78 | 1.65 | 2.35 | 0.41 | 0.70 | 0.48 | 0.29 | 0.13 | 0.50 | 0.40 |
| | H3 | 0.87 | 0.86 | 2.21 | 0.40 | 0.54 | 0.45 | 0.53 | 0.12 | 0.47 | 0.56 |
| PPS 100 | H1 | 2.09 | 1.95 | 2.01 | 0.96 | 1.08 | 0.54 | 0.52 | 0.19 | 0.43 | 0.54 |
| | H2 | 0.77 | 1.72 | 3.25 | 0.41 | 1.15 | 0.59 | 0.26 | 0.11 | 0.47 | 0.25 |
| | H3 | 0.55 | 0.83 | 2.99 | 0.51 | 1.01 | 0.44 | 0.59 | 0.13 | 0.35 | 0.47 |
| PPS 200 | H1 | 2.24 | 1.90 | 1.56 | 0.89 | 1.21 | 0.52 | 0.58 | 0.25 | 0.31 | 0.49 |
| | H2 | 0.89 | 1.18 | 4.65 | 0.59 | 1.16 | 0.47 | 0.35 | 0.08 | 0.42 | 0.24 |
| | H3 | 1.08 | 0.98 | 2.49 | 0.31 | 0.95 | 0.40 | 0.53 | 0.12 | 0.94 | 0.44 |
| PPS 300 | H1 | 2.01 | 1.66 | 1.40 | 0.55 | 1.05 | 0.35 | 0.56 | 0.25 | 0.58 | 0.43 |
| | H2 | 0.84 | 0.99 | 4.26 | 0.34 | 0.92 | 0.46 | 0.39 | 0.08 | 0.75 | 0.31 |
| | H3 | 0.74 | 0.96 | 3.24 | 0.46 | 0.99 | 0.30 | 0.57 | 0.10 | 0.34 | 0.43 |
| PPS 400 | H1 | 1.63 | 1.75 | 1.37 | 0.91 | 1.01 | 0.56 | 0.48 | 0.28 | 0.50 | 0.59 |
| | H2 | 0.74 | 0.60 | 4.04 | 0.53 | 0.89 | 0.64 | 0.59 | 0.09 | 0.42 | 0.32 |
| | H3 | 0.72 | 1.01 | 3.50 | 0.24 | 1.06 | 0.43 | 0.48 | 0.11 | 1.09 | 0.48 |
| PPS 500 | H1 | 1.51 | 1.87 | 1.61 | 0.81 | 1.05 | 0.64 | 0.49 | 0.24 | 0.49 | 0.63 |
| | H2 | 0.65 | 0.65 | 3.38 | 0.38 | 0.66 | 0.61 | 0.59 | 0.11 | 0.61 | 0.43 |
| | H3 | 0.97 | 1.27 | 2.81 | 0.57 | 1.26 | 0.33 | 0.42 | 0.14 | 0.29 | 0.39 |

check the ability of plants for pytoremediation. A plant's ability to accumulate metals from soil can be estimated using BCF, which is defined as the ratio of metal concentration in the root to that in soil. A plant's ability to translocate metals from the roots to the shoots is measured using the TF, which is defined as the ratio of metal concentration in the shoots to the roots [17]. According to the Fitz and Wenzel [18], plants exhibiting TF and BCF values less than one are unsuitable for phytoextraction. In the present study, all the treatments gave almost highest values of BCF (higher than one) for Cd, Ni and Zn (except at $T_1$) at H1. However, all the treatments gave higher values than one for Cd content at all stages. It may be due to Cu high concentration in PPS. However, at all stages, TF values were found less than one at all treatments (Table 2). However, no significant co-relations were found between concentration and translocation of heavy metals (data not shown). Based on the TFs values of all plants fed with PPS, the plants were less efficient in translocating the heavy metals. These results postulated that there were other factors that caused plant growth inhibition. Polycyclic aromatic hydrocarbons that are present in petroleum might be accumulated in root as well as in shoot and caused the inhibition of plant growth performance [19].

From the results of our study, it can be concluded that PPS at all levels decreased SL, total LA, shoot FW and DW, root FW and DW. Heavy metals contents have no effect on the performance of *P. vulgaris*. Based on the values of BCFs and TFs (Table 2) of all PPS-fed plant samples, plant was considered as the less efficient for phytoremediation. Phytoremediation potential of genotypes of *P. vulagris*, particularly for use in PPS areas, need to be investigated.

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