

## THE EFFECTS OF AUTOMOBILE MOTOR WORKSHOP WASTE WATER ON SEEDLING GROWTH OF KIDNEY BEAN AND MUNG BEAN CROPS UNDER ABIOTIC STRESS

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**Abstract:** The waste water generation from the automobile motor workshop activities are responsible for environmental degradation in the form of water pollution and showed variable impact on plant growth. This paper gives information, assessment and screening about the effect of motor work shop waste water on seedling growth of two different types of legume bean crops namely, kidney bean (*Phaseolus vulgaris* L.) and mung bean (*Vigna radiata* (L.) R. Wilczek) in pot culture experiments. The results showed that the increase in treatment of 25%, 50%, 75%, and 100% of motor workshop waste water decreased the root, seedling length, number of leaflets, shoot, leaves dry weight and root/shoot ratio of common bean as compared to control. The waste water treatment of 25% significantly ( $p < 0.05$ ) decreased root growth performance and leaves dry weight of common bean as compared to control. The treatment of polluted water produced beneficial and harmful effects on root, shoot length, seedling size, number of leaves, leaf area and biomass production of mung bean. The treatment of waste water at 25% significantly ( $p < 0.05$ ) decreased shoot length, leaf area, shoot dry weight, specific leaf area and leaf area ratio as compared to control. Increase in concentrations of polluted water at 50% significantly effects seedling length, specific leaf area and leaf area ratio of polluted water on mung bean. The seedlings of *P. vulgaris* and *V. radiata* tested different percentage of tolerance to waste water treatment and found high in control treatment. The results showed that seedlings of *P. vulgaris* showed lowest (32.59%) percentage of tolerance to high concentration (100%) of polluted waste water treatment of workshop. The treatment of polluted water at 25, 50, 75 and 100% decreased the tolerance indices values in seedlings of *V. radiata* by 104.35, 83.37, 67.63 and 63.16 percent as compared to control. The decrease in seedling growth of growth parameter of *P. vulgaris* in this study revealed that it was might be due to abiotic stress produced by waste water. The chemical analysis of waste water showed gradually increase values of pH (7.61-9.32), electrical conductivity (0.56-2.62 mScm<sup>-1</sup>), chloride (13.60-50.96 mgL<sup>-1</sup>) and CaCO<sub>3</sub> (45.90-65.21 mgL<sup>-1</sup>) as compared to control.

**Keywords:** bean crops, growth, motor workshop, phytotoxicity assay, pH, soil.

### Introduction

Oil product residues from cars, motorbikes and automotive batteries contain heavy metals, cadmium, nickel, mercury, copper, and other chemical substances produce adverse impact on the plant growth and soil. The discharge of waste water thus pollute underground waters, rivers, and lakes. Insoluble, stable, and slowly degradable oil waste contain toxic chemicals and heavy metals, which enter human body. Sometimes “modern industrial blood” term used for Petroleum [HE & al. 2019]. Automobile service station, repair work shop release a large amount of wastewater which contain many toxic elements such as, fuel, dirt, detergent, solvent, hydrocarbons, heavy metals, total solids, engine oil and greases, chloride, sulfate, organic compounds and responsible for degradation of air, water and soil and effects on life, plant growth and productivity [POTTER & SIMMONS, 1998; ACHUBA, 2006; BONA & al.

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2011; ILEMOBAYO & KOLADE, 2008; KATHI & KHAN, 2011; MAZUMDER & MUKHERJEE, 2011; ASHA & al. 2016]. The effect of waste engine and crude oil pollution on number of plant species, crops, vegetation productivity and soil were observed [ATUANYA, 1987; EKUNDAYO & OBUEKWE, 1997; BENKA-COKER & EKUNDAYO, 1995; AMAKITRI & ONAFEGHARA, 1983; ANOLIEFO & VWIOKO, 1994; ISSOUFI & al. 2006]. Petroleum, heavy hydrocarbons, in contaminated soils showed a serious harm to the soil ecosystem, human health, biota, halophytes, seed germination percentage and biomass yield [KORADE & FULEKAR, 2009; EBADI & al. 2018; LIU & al. 2020; ZEB & al. 2020]. The impact of oil polluted water, petroleum and its products on plant growth required specific concern in ecological studies and can be studied by using bioassay techniques. The studies on plant behavior in petroleum contaminated soils for the identification and selection of oil pollution indicating species was carried out [MARANHO & al. 2009]. A deleterious effect of dielectric fluids in different oil contents 0.5, 1.0, 2.0, 2.5, 5.0, 7.5 and 10% in soil on soybean crop *Glycine max* (L.) Merr. growth was investigated [SANDERSON & al. 2018].

Scientists around the world are working for conserving of water [HUSSIN & al. 2002]. Water pollution is an important problem in many parts of the world. The discharge of waste water from motor workshop into the immediate environment is an important environmental issue and affecting on agronomic crop. Many researchers have drawn their attention on the effects of polluted waste water on growth of bean crops. Therefore, the present study was carried out with the aim to study the effects of waste water on the growth of two commercially an important agronomic legume bean crop, *Phaseolus vulgaris* L. and *Vigna radiata* (L.) R. Wilczek of Pakistan.

### **Material and methods**

This experimental study was conducted in green house of the Department of Botany, University of Karachi, Pakistan in the month of October having temperature in range of 33/27 °C. Seeds of *Phaseolus vulgaris* L. (white kidneys beans) and *Vigna radiata* (L.) R. Wilczek (mung bean) were bought from the local seed store of Lahore, city and used for experiments. The waste water from motor transport workshop located at Orangi town was obtained and considered as standard solution of 100%. From the standard solutions further different concentrations as 0, 25, 50, 75 and 100% in distilled water were prepared. Distilled water was used as control for experiment. The seeds were imbibed in distilled water for half an hour to break seed dormancy. The eight seeds were sown in garden loam soil at 1 cm depth in plastic pots of 7.3 cm in diameter and 9.6 cm depth. The pot was kept moist by adding tap water when necessary. Seedlings were allowed to grow for two weeks to reach at a reasonable height. Three best seedlings of equal height were selected and treated with different 0, 25, 50, 75 and 100% concentrations of waste water. This experiment was replicated three times with the design of completely randomized and lasted for four weeks. The height of the plants was measured using a steel scale. The numbers of leaves were counted. The plants were carefully uprooted and the seedlings part rinsed with clean water. The root, stem and leaves were kept in marked paper envelope and finally place in an oven at 80 degree centigrade for 24 hours to obtained dry weight. Oven dried weights for roots, shoot, leaves and total plant weight was recorded. Leaf area were determined as follows:

**For leaf area:**

Leaf area = Length  $\times$  Breadth  $\times$  2/3

Leaf weight ratio was determined according to the Eq. 1.

$$\text{Leaf weight ratio} = \text{Leaf dry weight} / \text{Total plant dry weight. (Eq. 1)}$$

Tolerance indices of seedlings were determined with the help of following formula [IQBAL & REHMATI, 1992].

$$\text{Tolerance indices (T.I.)} = \frac{\text{Mean root length of waste water treated seedlings}}{\text{Mean root length of control seedlings}} \times 100$$

**Waste water analysis**

The waste water sample was collected from motor transport workshop located at Orangi town motor workshop from Karachi for analysis. Calcium carbonate was determined by acid neutralization as described by ANONYMOUS (1954). pH of waste water was determined by direct pH reading meter (MP-220, Mettler, Toledo). Chlorides were found through titration by Mohr's Method [ALLEN & al. 1974]. Electrical Conductivity (E.C.) were determined by direct AGB 1000 electrical conductivity meter.

**Statistical analysis**

The means as well as standard errors were calculated. Data collected were subject to one-way analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT) using personal computer software packages COSTAT version 3.00 statistically analyzed. Level of significance for these tests was at  $P < 0.05$ .

**Results and discussion**

Water pollution due to automobile repair workshop is a major global environmental concern both in developing and underdeveloped countries. Water pollution by petroleum products are an important ecological problem likewise air and soil pollution problems. The impact of crude oil into the environment was received worldwide attention [MILLIOLI & al. 2009]. In present study the variable effects of different levels 25%, 50%, 75% and 100% of polluted waste water on the seedling growth performances of legume bean crops, *Phaseolus vulgaris* and *Vigna radiata* and waste water properties were recorded (Table 1). The adverse effect of the various treatments level of polluted water on common and mung bean was measured by assessing through bioassay test. The polluted water affected root, shoot length, seedling size, number of leaves and leaf area as compared to control of both legume crops. These findings are in close conformity with the findings of another researcher's for eggplant, *Capsicum annum* L. and *Lycopersicon esculentum* Mill. and *Chromolaena odorata* (L.) R. M. King & H. Rob. species [ANOLIEFO & EDEGBAI, 2001; ANOLIEFO & al. 2003; VWIOKO & FASHEMI, 2005; RAHBAR & al. 2012]. The mean root, shoot, leaves, total seedling dry weight, root/shoot ratio, leaf weight ratio, specific leaf area and leaf area ratio of *P. vulgaris* and *V. radiata* were also highly affected as compared to control treatment. The negative impact of waste water application at 100% on mung bean plant growth as compared to control (without waste water).

The negative effects of engine oil on germination of perennial rye grass and maize growth performance reported [ISIRIMAH & al. 1989; ODJEGBA & SADIQ, 2002; SIDDIQUE

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& ADAMS, 2002]. This study demonstrated that treatment of waste water at high concentration in soil has significant effect on the seedling growth performance of *P. vulgaris*. The polluted water treatment at 100% significantly decreased shoot (2.23 cm) and leaves dry weight (0.10 g) of *P. vulgaris* as compared to control. The increase in polluted water level at 75-100% gradually decreased root, seedling height, number of leaflets, root, shoot dry weight of *P. vulgaris* as compared to control (0%) and produced abiotic stress. Abiotic stresses, such as drought, salinity, and heavy metals limit crop productivity and disturb plant growth worldwide [CANTER, 2018; WAQAS & al. 2019].

**Table 1.** Seedling growth parameter and biomass production of *P. vulgaris* under different concentration (0, 25, 50, 75, 100%) of polluted waste water.

Seedling growth parameters	Waste water concentration (%)				
	0	25	50	75	100
Root length (cm)	PV7.18±3.20a VR7.20±0.96a	5.33±2.20a 5.88±0.69a	4.17±2.08a 7.20±1.07a	5.40±2.88a 8.17±1.77a	2.34±0.69a 8.12±1.02a
Shoot length (cm)	14.15±2.30b 22.85±1.06c	8.82±2.11ab 18.10±2.44b	7.74±3.87ab 19.30±0.68bc	8.66±4.33ab 15.24±0.96ab	2.23±0.99a 13.27±0.12a
Seedling length (cm)	21.34±5.50a 30.06±0.18b	14.14±4.31a 23.99±2.96a	11.91±5.95a 26.51±1.75ab	14.07±7.09a 23.41±1.12a	4.57±1.45a 21.39±0.89a
Number of leaflets	15.00±3.05a 16.00±1.00a	9.66±1.66a 13.66±1.85a	6.66±3.33a 13.66±1.66a	10.00±5.00a 12.33±2.66a	4.66±1.45a 13.33±1.66a
Leaf area (cm <sup>2</sup> )	4.88±2.02a 38.53±5.10c	8.14±0.45a 24.60±4.00b	5.30±2.69a 23.56±2.38ab	2.86±1.44a 16.40±1.45ab	3.53±0.84a 12.95±2.95a
Root dry weight (g)	0.04±0.00a 0.02±0.00ab	0.02±0.00ab 0.02±0.00ab	0.02±0.01ab 0.04±0.01b	0.02±0.01ab 0.01±0.00a	0.01±0.00a 0.03±0.00ab
Shoot dry weight (g)	0.04±0.00a 0.04±0.00b	0.03±0.00a 0.03±0.00ab	0.02±0.01a 0.02±0.01a	0.02±0.01a 0.02±0.00ab	0.01±0.00a 0.01±0.00a
Leaves dry weight (g)	0.05±0.00b 0.04±0.00a	0.04±0.00b 0.04±0.00a	0.03±0.01b 0.04±0.00a	0.02±0.01ab 0.03±0.00a	0.01±0.00a 0.15±0.10a
Total plant dry weight (g)	0.13±0.00a 0.10±0.01a	0.09±0.02a 0.10±0.00a	0.04±0.03a 0.23±0.02a	0.07±0.03a 0.07±0.00a	2.24±0.02a 0.20±0.11a
Root / shoot ratio	1.04±0.15a 0.47±0.07a	0.63±0.18a 0.72±0.03a	0.88±0.48a 2.08±0.50c	0.77±0.39a 0.83±0.33a	0.43±0.29a 1.66±0.16bc
Leaf weight ratio	0.37±0.03a 0.44±0.03a	0.45±0.02a 0.40±0.02a	1.67±0.17a 1.70±1.38a	0.23±0.11a 0.46±0.07a	0.60±0.19a 0.61±0.12a
Specific leaf area (cm <sup>2</sup> g <sup>-1</sup> )	104.85±43.2a 820.00±63.1c	199.38±27.9a 560.65±45.92b	117.96±58.9a 611.99±92.8bc	71.58±36.13a 494.44±30.9b	262.46±150a 194.16±150a
Leaf area ratio (cm <sup>2</sup> g <sup>-1</sup> )	39.35±17.27a3 65.78±37.5b	90.83±15.78a 229.46±25.6ab	312.16±282a 124.44±63.54a	24.85±12.45a 233.01±40.2ab	176.52±76.36a 97.60±41.16a

Symbol used: PV = *Phaseolus vulgaris* L.; VR = *Vigna radiata* (L.) R. Wilczek;

Number followed by the same letters in the same row are not significantly different according to Duncan Multiple Range Test at <0.05 level. ± Standard Error

Seedling growth is sensitive to environmental stresses [SULEIMAN & al. 2009]. The variation in the seedling growth parameter in different level of waste water were recorded. An increase in the degradation of oil hydrocarbons occurs in the soils inhabited by the plants [MURATOVA & al. 2003]. SUMATHI & al. (2008) provided an evidence in a study about the adverse effect of the refinery waste on the growth of the *Lens culinaris* Medik. at higher

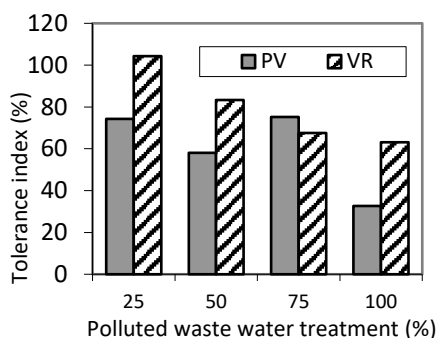
concentrations. The polluted water treatment significantly decreased root length (5.66 cm), shoot length (8.78 cm), seedling length (14.45 cm), number of leaflets (9.00) and leaf area ( $12.29 \text{ cm}^2$ ) as compared to control. Similar trend of decrease in root, shoot dry weight for mung bean was observed. The polluted water treatment at higher concentrations gradually decreased total seedling dry weight (0.09 g), leaf weight ratio (0.31), specific leaf area ( $447.86 \text{ cm}^2 \text{ g}^{-1}$ ) and leaf area ratio ( $142.51 \text{ cm}^2 \text{ g}^{-1}$ ) as compared to control. Polluted contaminated water at higher level (25-50%) affected root growth performance of *V. radiata* due to development of unsuitable growth condition by oil pollution. The contamination with petroleum affects the development of plants due to different physical effects. According to BONA & SANTOS (2003) oil diminishes the soil capacity for retaining water, thus interfering with plant growth.

The effects of waste water on biomass production were also observed. The negative effects of oil contamination on the total biomass of *Avena sativa* L., *Secale cereale* L. and *Hordeum vulgare* L. was observed. MARANHO & al. (2006) investigated the effect of petroleum pollution on the leaf structure of *Podocarpus lambertii* Klotzsch ex Endl. (Podocarpaceae). Our data also showed the influence of oil polluted soil on leaf area of *P. vulgaris* as compared to control. Seedlings biomass of both crops were less productive in oil contaminated water sample and most productive than control soil sample treatment. The results showed that crude oil level (control, 1, 2, 3, 4% W/W), affected fresh and dry weights of the root and the shoot, root volume, stem diameter, number of leaves, leaf area, and stem height affected by ( $p < 0.05$ ) for Eucalyptus [TAHERI & al. 2018]. PETUKHOV & al. (2000) used plant as biotests of soil and water pollution with petroleum and petroleum product. The continuous decrease in seedling growth of growth parameter of *P. vulgaris* in this study revealed that it is due to abiotic stress.

The screening of plant species with their ability to grow on contaminated soil is considered an important step in the planning for phytoremediation program. The seedling growth of *Impatiens balsamina* L. and *Crotalaria retusa* L. was observed in areas adjacent to automobile service stations in Sri Lanka for their tolerance to used lubricating oil (ULO) contaminated soil [GAMAGE & al. 2020]. The seedlings of *P. vulgaris* and *V. radiata* were tested for tolerance in different level to waste water treatment. The presence of heavy metal likewise cadmium might reason for reduction in seedling growth. The use of cadmium (Cd) in agricultural soils transfer to crop plants which can pose a potential health risk to consumers. The concentrations of cadmium in spinach leaves, potato tubers, onion bulbs and wheat grain grown in commercial horticultural operations across New Zealand (NZ) showed that certain soil and environmental factors can be a key influence for determining Cd accumulation in the edible parts of some plant [ZICHENG & al. 2020].

The seedlings of *P. vulgaris* and *V. radiata* showed high percentage of tolerance to waste water treatment in control treatment (Figure 1).

The results showed that seedlings of *P. vulgaris* showed lowest (32.59%) percentage of tolerance to high concentration (100%) of polluted waste water treatment of workshop as compared to control. The seedlings of *V. radiata* showed similar trend of decrease in tolerance with increasing 25, 50 and 100% levels of pollution of motor workshop polluted water as compared to control. The tolerance in seedlings of *P. vulgaris* to polluted water were reduced with the values 58.07% percent when treated with 50% as compared to control. The treatment of polluted water at 25, 50, 75 and 100% decreased the tolerance indices values in seedlings of *V. radiata* by 104.35, 83.37, 67.63 and 63.16 percent as compared to control. The variation in the seedling growth parameter in different level of waste water were recorded. The decrease in seedling growth of growth parameter of *P. vulgaris* in this study revealed that it was might be due to abiotic stress produced by waste water. The analysis of waste water from 0, 25, 50, 75 and 100% showed variation in chemical properties (Table 2).



**Figure 1.** Percentage of tolerance in *Phaseolus vulgaris* L. (PV) and *Vigna radiata* (L.) R. Wilczek (VR) in different concentration of polluted waste water (25, 50, 75 and 100%) as compared to control.

**Table 2.** Analysis of motor workshop waste water

Soil parameter	Motor workshop waste water concentration (%)				
	0	25	50	75	100
pH	7.61a±0.30	8.33b±0.27	8.99ab±0.14	8.57b±0.21	9.32c ±0.10
EC (mScm <sup>-1</sup> )	0.56a±0.23	1.66b±0.00	1.77bc±0.17	2.28bc±0.22	2.62d±0.22
Chloride (mg L <sup>-1</sup> )	13.60a±4.19	29.53b±2.90	45.28c±0.98	50.96c±3.40	49.30c±2.50
Calcium carbonate (%)	45.90a±0.50	47.21a±1.74	52.21a±4.76	62.42b±2.36	65.21b±2.65

Number followed by the same letters in the same column are not significantly different according to Duncan Multiple Range Test at <0.05 level. ± Standard Error. Electrical conductivity (EC)

Similar, effect of crude oil pollution from an accidental blowout of an oil well on soil pH, temperature, crude oil content and its flora was studied [DEBOJIT, 2006]. The automobile work shop waste water values gradually increase values of pH (7.61-9.32), electrical conductivity (0.56-2.62 mScm<sup>-1</sup>), chloride (13.60-50.96 mgL<sup>-1</sup>) and CaCO<sub>3</sub> (45.90-65.21 mgL<sup>-1</sup>) as compared to control (Table 2).

### Conclusion

The present study concludes that the significant changes in the seedling growth performances of *P. vulgaris* and *V. radiata* in terms of seedling growth and biomass production was due to treatment of different concentration (25, 50, 75 and 100%) of waste water as compared to control. The reduction was directly proportional to the stress of waste water concentrations. A clear reduction in the shoot, seedling length, number of leaves, leaf area, shoot and leaves productivity of *P. vulgaris* was observed at highest 100% oil polluted water treatment. The significant changes of decrease in shoot length, seedling growth, leaf growth and total seedling dry weight of *V. radiata* grown in the waste water treated soil was also recorded. The waste water bioassay studies can be served as good pollutant indicator of water pollution monitoring.

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