Advances in Environmental Research, *Vol. 3, No. 1 (2014) 1-9*
DOI: http://dx.doi.org/10.12989/aer.2014.3.1.001

Effect of automobile polluted soil on early seedling growth performance of Neem (*Azadirachta indica* A. Juss.)

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(*Received October 30, 2013, Revised December 10, 2013, Accepted January 01, 2014*)

Abstract. Effect of automobile polluted soil with five soil concentration (0 (Control), 25, 50, 75 and 100%) was observed on early seedling growth performance and biomass production of Neem (*Azadirachta indica* A. Juss). The treatment of 75% automobile polluted soil significantly ($p < 0.05$) decreased the seedling length (18.60 cm) of *A. indica*. The automobile polluted soil treatment with the concentration of 50% slightly increased the root length as compared to control. The automobile polluted soil treatment with the concentration of 25, 50, 75 and 100% negatively affected shoot length of *A. indica* as compared to control. The treatment of all concentration of automobile polluted soil progressively decreased the total leaf area *A. indica* as compared to control soil treatment. The automobile polluted soils also showed negative effects on biomass production of *A. indica*. The automobile polluted soil treatment at 25% concentration significantly ($p < 0.05$) affected shoot, leaves and seedling dry weight of *A. indica* as compared to control soil treatment. The order of relationship between production of *A. indica*'s seedling dry weight and automobile polluted soil treatment was observed as root > shoot > leaves > total seedling.

Keywords: automobiles; polluted soil; neem; seedling growth; biomass production

1. Introduction

The automobile activities usually involve changing of lubricating oil, servicing and greasing of motor parts and replacement of worn-out parts (Ajayi 2005) in the motor transport workshop. The disposal of spent lubricating oil other wastes used in cleansing during auto-mobile servicing pollute the soil and once these waste materials enter the soil they become part of the biological cycle that affects all forms of life (Mbah *et al*. 2009). Pollution caused by petroleum and its derivatives is the most prevalent problem in the environment (Millioli *et al*. 2009). Petroleum hydrocarbons (PHCs) are constituents of engine fuels, industrial solvents and many other products and are the most widespread among the organic contaminants due to extensive current use of oil

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http://www.techno-press.org/?journal=aer&subpage=7 ISSN: 2234-1722 (Print), 2234-1730 (Online)

and petroleum products throughout the world. They also have direct toxic influence on plants when they contact plant tissues. However, plants respond to PHCs differently (Sharonova and Breus 2012). Effect of automobile exhaust emissions on the two common avenue trees *Azadirachta indica* and *Polyalthia longifolia* has been studied with special reference to their cuticular and epidermal characteristics. In plants collected from heavy traffic density areas (HTDA) the epicuticular wax was reported severely damaged and its morphology altered with significant changes in cuticular and epidermal structures (Amit *et al*. 2000). The stress effects of motor vehicle exhaust gas (black carbon (BC), fine particles, VOCs and carbonyl compounds) resulted in an increase in concentrations of proline, glutamine, threonine, aspartic acid, glycine and phenylalanine and decreased concentration of arginine, serine, alanine and glycine in young needles of Norway spruce (*Picea abies*) seedlings (Viskari *et al*. 2000). The deposition of metal and the relocation of metals deposited on road surface by air and runoff water have led to contamination of soil (Nabuloa *et al*. 2006, Ogbonna and Okezie 2011, Turer and Maynard 2003, Viard *et al*. 2004). The effects of diesel exhaust emissions on 12 herbaceous species were studied with respect to growth, flower development, leaf senescence and leaf surface wax characteristics (Honour *et al*. 2009).

Soil is a precious natural resource, but its quality is deteriorated due to several anthropogenic activities (Ramakrishnaiah and Somashekar 2002). Since contamination of soil with refinery products deteriorates its biochemical and physiochemical properties, it also limits the growth and development of plants, whose nutritive and technological value can be low and often questionable (Wyszkowski *et al*. 2004). Auto mobile workshops play an important role in socio_economic dynamics (Enabulele and Obayagbona 2013). In other parts of the developing world, oil spills at auto-mechanic workshops left uncared for over the years and its continuous accumulation may cause serious environmental problems because of its hazardous nature where used motor oil disposed of improperly contains potentially toxic substances, such as lead, arsenic, zinc and cadmium (Abdulsalam and Omale 2009). The increasing utilization of petroleum divided products such as gasoline, diesel and motor oils, has led to a marked increase in soil contamination (Houshmandfar and Asli 2011). The effects of petroleum products on plants have been studied by some researchers around the world (Anon 2003, Shahid 2007, Njoku *et al*. 2009). Trapp *et al*. (2001) studied the phytotoxicity of fresh and weathered diesel and gasoline to willow and poplar trees. Merkl and Schultze-Kraft (2005) reported that legumes died within 6 to 8 weeks in heavily crude oil contaminated soil, whilst the grasses showed reduced biomass production. A reduction in primary root length of peanut, cowpea, sorghum and corn affected with the treatment of different concentrations of diesel oil into the soil recorded (Ogbo 2009). The toxic effect of five levels (1, 2, 3, 4 and 5%) of spent and unspent diesel fuel on two agricultural crop plants *Zea mays* and *Arachis hypogaea* was investigated where the growth of plumule and radicle were reported negatively affected in all levels of contamination (Ehiagbonare *et al*. 2011). The effects of metal-polluted soil on growth, biomass and uptake of trace metals, nutrients in *Alnus incana* and *A. glutinosa* seedlings and rate of nitrogen fixation, counts of microbial groups, and activities of phosphomonoesterases and phenolic compounds in soil observed (Lorenc-Plucińska *et al*. 2013).

Neem (*Azadirachta indica*. A. Juss) tree has been known to be useful in soil enrichment and for insect, pest and disease control (Xuan *et al*. 2004). *A. indica* (Family Meliaceae) commonly called as neem is an important tropical and sub tropical tree that has found varied use in ecological, medicinal and agricultural sectors (Atawodi and Atawodi 2009). Neem tree is the official tree of the Sindh Province (Pakistan) and is very common in all cities of Sindh. There are projects underway for planting this tree in all over Sindh Province. The information on the effects of polluted soil due to automobile activities on early seedling growth of local trees species are scanty in the country. Therefore, the present study was performed with the aim to find out the relationship between *A. indica* seedling growths performances using different polluted soil concentration.

2. Materials and methods

Seeds of *A. indica* were randomly collected from the Karachi University Campus. Seeds were separated from the pulp, washed with distilled water and air dried at room temperature. The seeds having any sign of insect or infection were discarded. Garden soil was collected from the Department of Botany for mixing with polluted soil to make various treatments. Polluted soil samples were collected from the premise of Motor Transport Workshop located at University of Karachi campus. The composite soil was taken in plastic bags from both sites and brought in laboratory for further studies. The soil samples were air dried and removed any large size boulder or stone. Different soil concentration of automobile polluted soil such as 0%, 25%, 50%, 75% and 100% were prepared. Pure garden soil served as control and 100% was considered as totally polluted soil. A sufficient quantity of *A. indica* seeds were grown in large pots under natural environmental condition. The seed germination was examined on daily basis and provided with seed normal water. A seed was considered to be germinated when the protruding radical reached a length of 2 mm. The seedlings of *A. indica* having the same size were transferred in other pots for soil treatment. In each pot single seedlings was added. The seedlings were checked daily and provided tap water daily for irrigation. These pots are kept in natural environmental conditions at the Department of Botany, University of Karachi. The experiments were laid out in completely randomized design (CRD) in five replicates. Data were recorded for vegetative characteristics such as seedling height (cm), circumference of plants (cm), root length (cm) and shoot length (cm).

Leaf area was calculated using following formula.

Leaf length × leaf breadth × 2 / 3

Seedlings were harvested and kept in oven at 80°C for 24 hours to get constant weight for the determination of root, shoot, leaves and total seedling dry weight (g). The experiment lasted for six week. Data obtained from this study were subjected to one-way Analysis of Variance (ANOVA) using COSTAT Statistical Package v. 03 and mean separation done according to Duncan Multiple Range Test at $p < 0.05$ on personnel computer.

3. Results

The application of automobile polluted soil on seedling length (cm), root, shoot length (cm) and total leaf area of *A. indica* showed negative responses of effects as compared to control. The treatment of polluted soil with different concentration also produced inhibitory effects on root, shoot and seedling dry weight (g) of *A. indica* as compared to control soil treatments (Figs. 1-8; Table 1). The automobile polluted soil concentration of 25 and 50% showed slight decrease in seedling length of *A. indica* as compared to control (Fig. 1). The increase in automobile polluted soil concentration treatment at 75% significantly ($p < 0.05$) affected seedling length of *A. indica* as compared to control. Automobile polluted soil concentration at 25% slightly increases in root growth as compared to control soil treatment (Table 1). An increase in automobile polluted soil treatments at 50% concentration further decreased the root growth of *A. indica* as compared to

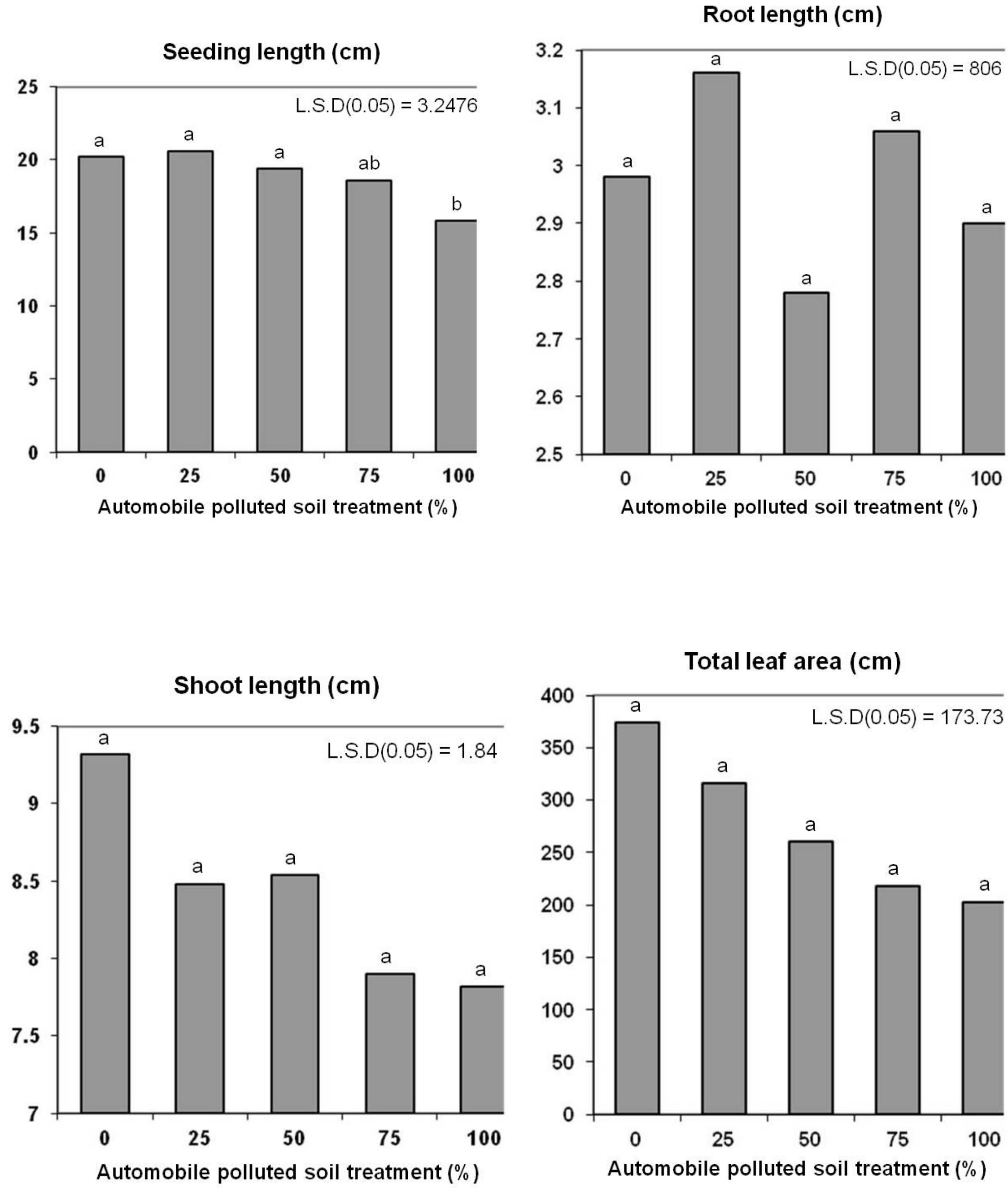

Fig. 1-4 Effects of different concentration of automobile polluted soil (0, 25, 50, 75 and 100%) treatment on seedling length (cm), root length (cm), shoot length (cm) and total leaf area. Values followed by the same letters are not significantly different ($p < 0.05$) according to Duncan's Multiple Range Test

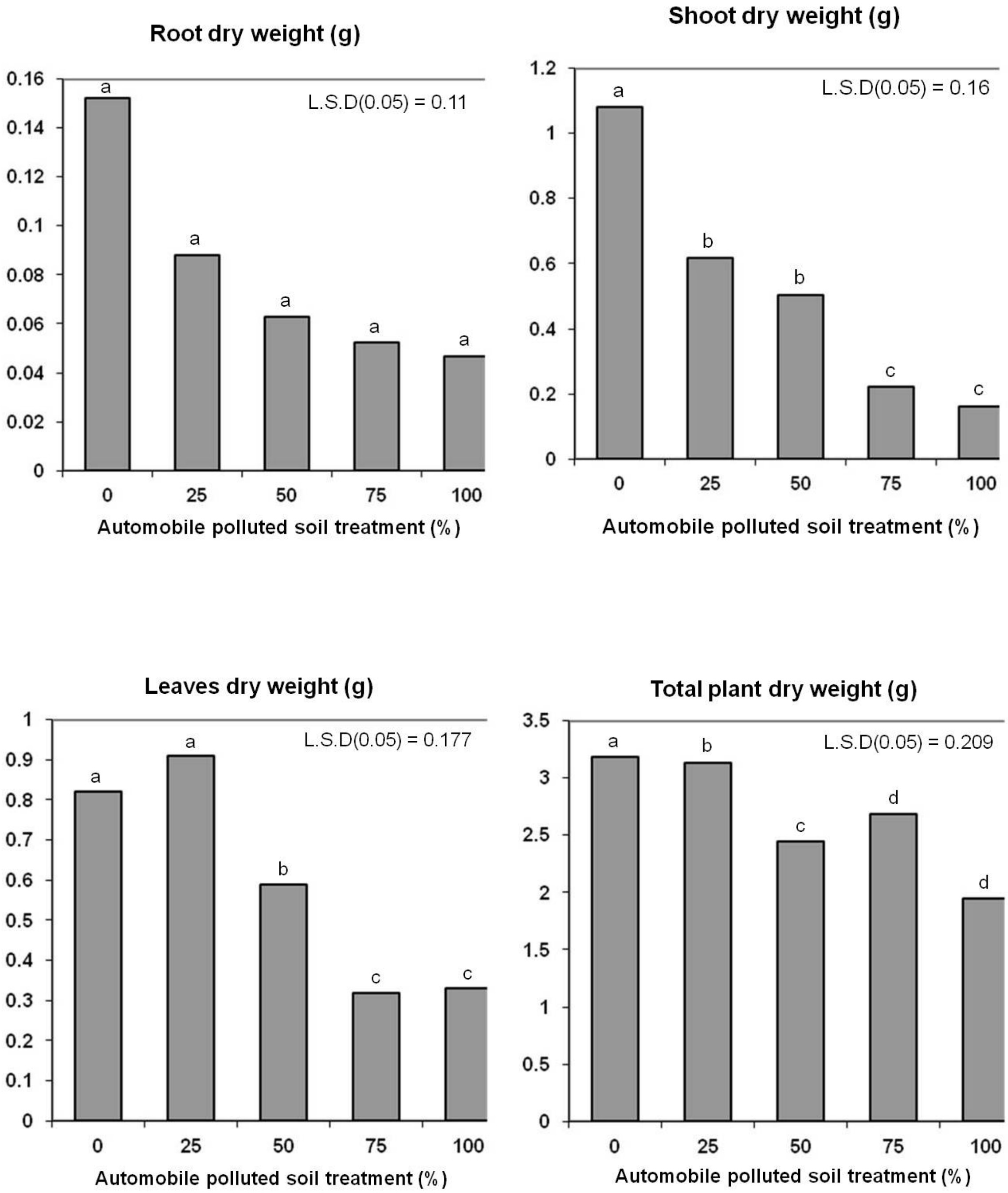

Fig. 5-8 Effects of different concentration of automobile polluted soil (0, 25, 50, 75 and 100%) treatment on root, shoot, leaves and total plant dry weight (g). Values followed by the same letters are not significantly different ($p < 0.05$) according to Duncan's Multiple Range Test

Table 1 Percentage decrease or increase in seedling growth parameter and biomass production of *Azadirachta indica* A. Juss. under different concentration (25, 50, 75, 100%) of automobile polluted soil as compared with control soil (0%)

Growth parameters	Soil concentration (%)			
	25	50	75	100
Seedling length	1.98+	3.96	7.92	21.78
Root length	6.04+	6.71	2.68+	2.68
Shoot length	9.10	8.36	15.32	16.09
Total leaf area	15.411	30.28	41.52	45.81
Root dry weight	41.97	58.55	65.39	69.34
Shoot dry weight	42.98	53.69	79.70	85.23
Leaves dry weight	10.70+	28.22	61.07	59.36
Total plant dry weight	21.45	44.38	70.74	73.76

control. There concentration of automobile polluted soil at 25% recorded total leaf area (315.99) as compared to control (373.56) in *A. iundica*. Further increase in automobile polluted soil concentration at 50% and 75% decreased the leaf area (260.44 and 218.43) of *A. indica* as compared to control concentration (Fig. 4). Biomass production of *A. indica* showed similar response of negative effects of automobile polluted soil stress as compared to control soil treatment. The root dry weight was decreased with the treatment of automobile polluted soil at 25%, 50%, 75% and 100% concentration as compared to control soil (Fig. 5). The lowest root dry weight (0.0466 g) was recorded with the automobile polluted soil treatment at 100% concentrations as compared to control soil (0.1520 g). Automobile polluted soil treatment at 25% concentration showed a significant ($p < 0.05$) reduction in shoot growth of *A. indica* as compared to control soil treatment (Fig. 6). The automobile polluted soil treatment with 50% showed significant ($p < 0.05$) effects on leaves dry weight of *A. indica* as compared to control soil. An increase in automobile polluted soil treatment at 75% recorded further decrease in leaves dry weight of *A. indica*. The automobile polluted soil treatment also showed significant ($p < 0.05$) effects on total seedling dry weight of *A. indica* as compared to control soil. Automobile polluted soil treatment at 25% significantly decreased in total seedling dry weight of *A. indica* as compared to control (Fig. 8).

4. Discussion

In present investigation the seedling growth performance and biomass production of *A. indica* were found dependent with the increase in concentration of automobile polluted soil treatment as compared to control soil. The treatment of contaminated soil produced negative effects on seedling length of *A. indica* due to the fact that the soil of the motor transport workshop area is polluted. Germination and seedling establishment are vulnerable stages in the plant life cycle (Vange *et al.* 2004). The accumulation of pollutant usually makes the soil polluted with toxic substances and in results reduction in germination and growth performances of plants observed. In another studies the properties of soils within the vicinity of automobile workshops another investigation the effects of soil polluted due to addition of crude oil, automotive gasoline oil, and spent engine oil negatively affected the growth of cowpea (*Vigna unguiculata*) (Adedokun and Ataga 2007). Reduction in seedling growth of *A. indica* due to automobile polluted soil treatment is in agreement of other researchers. A gradual reduction in shoot length, chlorophyll and protein levels of *Amaranthus hybridus* with spent oil was observed (Elaigwu *et al*. 2009, Odjegba and Sadiq 2002). The effect of automobile polluted soil on leaf area of *A. indica* was also recorded which might be due to imbalances in the availability of nutrient from the polluted soil and disturbances in biological equilibrium. Petrol and diesel oil normally effect on mineral and biological components in soil (Wyszkowski and Ziolkowska 2008). Studies on biomass estimation are important from the point of view of forecasting productivity, nutrient budgeting and for maintaining proper felling-cycles in tree species (Banik and Bhosale 1999). The automobile polluted soil concentration treatment also significantly ($p < 0.05$) affected on total seedling dry weight of *A. indica*. The biomass production of *A. indica* plant was found significantly decreased with the increase in concentration of automobile polluted soil as compared to control soil treatment. The presence of toxic substance such as petrol, gasoline, diesel fuel, metals and particulate matter in the substrate and availability to seedlings of *A. indica* is an important cause of reduction in seedling dry weight production. The negative impact of petroleum based products can be attributed to the fact that as these substances continue to penetrate soil deeper and deeper, they block air spaces that allow air and water to enter soil layers and as a result, the soil becomes more compact, its physical, chemical and biological properties deteriorate (Wsyzkowski *et al*. 2004). Metal contamination in the environment is a global concern and metal contamination of top soil and dispersion in the vicinities of auto repair workshops in Iwo, Nigeria has been observed (Ipeaiyeda *et al*. 2007, Leung *et al*. 2013). Uptake of heavy metals into seedlings of *A. indica* from the automobile polluted soil can happen via roots from the soil and transported to the leaves resulting in damaging effects on seedling performance of *A. indica*.

5. Conclusions

Contamination of soil by petroleum product is an important environmental issue. In this study it was concluded that the treatment of automobile polluted soil with the ratio of 25, 50, 75 and 100% concentration negatively affected the seedling growth performance of neem. The seedling of *A. indica* treated with automobile polluted soil at 100% concentration was found highly toxic. Results indicated that the treatment of automobile polluted soil responded differently to seedling growth performance of *A. indica*. An increase in the concentration of polluted soil from 25 to 100% concentration thus decreased the seedling growth of *A. indica* possibly due to bioavailability and presence of toxic elements present in the substrate.

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