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Germination of seeds in soil samples of heavy traffic zones of hyderabad telangana, India

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ABSTRACT

Plant toxicity bioassays through fast germinating agricultural crops can indicate the phytoremediation potential, effects on growth and survival and also assess extent of pollution. In the present study, the phytotoxic effect of heavy trafic/ petrol driven vehcile contaminated soil was studies on two agricultural crops namely Indian mustard (*Brassica juncea*) and *Eleusine coracana* (Ragi/ finger millet) in four different heavy trafic zone contamination. All the test plant species tolerated against contamination at 20-80% levels and the total percent seed germination was between 3 to 70 %. The change in physicochemical charecterstics of soil samples before and after seed germination suggesting cultivation of these crops in polluted areas helps in controlling of soil pollution.

KEYWORDS

Heavy traffic zone soil samples;
Phytoremediation;
Brassica junce;
Eleusine coracana;
% Germination;
Tolerance indices;
% Inhibition.

INTRODUCTION

The rapid advancement in Urbanization has not only brought progress to the present day world but it is also at a price too formidable to pay in terms of degradation of environment. Environmental contamination through anthropogenic activities is a wide spread and serious problem in many parts of the world. The occurrence and frequency of higher concentrations of pollutants in the atmosphere primarily depend on the magnitude and distribution of the sources of emissions, local topography, climate conditions and type of pollutant. The level of the pollution is mainly in correlation with the degree of the industrialization of the region and the traffic density[1-2].

In addition to the primary pollutants, carbon monoxide and nitrogen oxides, the third fraction in automobile exhaust is formed by incomplete combustion of hydrocarbons in oil and its derivations, which are probably directly less hazardous than the former two (they are weaker irritants), but they form very toxic and carcinogenic PAH (poly aromatic hydrocarbons) compounds. Automobile exhausts consist of benzopyrene, an already identified carcinogen[3]. The polluting of the roadside soil by heavy metals account for a constant increase in the number of automobiles became a considerable part of a common problem of the environmental aggravation. This lead to the death of roadside trees, erosion of roadside bends (as a result of root frame lesion), the decrease of birds and insect population etc,[4].

Heavy metals enter the roadside soil mainly as a result of transport work. When engine barrels and other inner parts are abraded, iron enters in the air; the combustion of ethylated fuel is the main source of lead. Aluminum, cobalt, copper, iron, manganese, lead, nickel, phosphorus, titanium, zinc and other elements, enter the roadside soil as a result of tire abrasion. Bearings, boxes, and brake oils are the source of copper and zinc[5].

When some of the above mentioned pollutants enter into the roadside soil they may remain there for many decades. Lead and cadmium are said to be the most dangerous pollutants among heavy metals[4-6]. The content of fly ash and particulate matter of soil that are rich in toxic pollutants can easily enter into human body and other living organisms through air and water and it leads to several serious diseases. To avoid the problem faced by global population the present study may help to control and utilization of polluted soil for seed cultivation and agricultural use, because plants can accumulate pollutants and minerals from the polluted soils. Soil pollution and its impacts and remedial measures have been studied with less focus compared to air and water. High-level metals are usually found in superficial soils and vegetation in areas is affected by mining activities and traffic emission. Generally, the total metal concentrations in polluted soils are required to assess the potential risk of these areas. However, there is usually a poor relationship between plant uptake and total metal content of heavy metals in soil. In the present study, the germination of seeds is carried out in the soil samples of heavy traffic areas of Hyderabad city and changes of the physical and chemical parameters of selected soil samples were examined with *Brassica juncea* and *Eleusine coracana* seeds.

MATERIAL AND METHODS

Careful soil sampling is essential for soil analysis. The sample must reflect the complete picture of the soil in that region. The samples were collected from the heavy traffic areas individually. The samples were collected in polyethylene bags and transported to the laboratory for analysis.

Selection of seeds

60 seeds were counted and selected for each set of germination. These seeds were selected based on floatation method. The seeds were dropped into water, seeds, which have floated, were discarded from the water, and the seeds, which sank in the water, were selected for germination.

Experimental set up for germination of seeds

Soil samples of four heavy traffic zone areas of Hyderabad city were taken in eight earthen pots. These pots were named according to their areas. In these pots, a 60 *Brassica juncea* seed is introduced in 4 earthen pots. As the same way 60 *Eleusine coracana* (Ragi) seeds are introduced in another 4 earthen pots. In another set of eight pots, 150 grams of polluted soil and 150 grams of normal soil are taken and 60 *Brassica juncea* (Indian Mustard) seed were planted in 4 pots and 60 *Eleusine coracana* seeds were introduced in another 4 pots. Each experiment was carried with triplicates. Germination and growth of germination are studied.

Plant sampling and analysis

A seed was considered as germinated when root had emerged more than 2 mm. The number of germinated seeds per time is presented as seed germination rate. Germination percentage and tolerance indices[7] were determined by the following formula.

$$\text{Germination percentage} = \frac{\text{Number of germinated seeds}}{\text{Total number of planted seeds}} \times 100$$

$$\text{Tolerance indices} = \frac{\text{Mean root length of polluted area seeds}}{\text{Mean root length of control area seeds}} \times 100$$

The inhibition of seedling growth was expressed according to the formula[8].

$$\text{Percentage of inhibition} = \frac{\text{length of control} - \text{length of test}}{\text{Length of control}} \times 100$$

Characterization of soil samples

The soil samples were collected from selected heavy traffic zones of uppal, tarnaka, jeedimetla and shapoornagar Hyderabad and Secunderabad respectively. The soil samples collected from the traffic signals, junctions and from the sides of the roads of selected areas. The physical and chemical characteristics

TABLE 1 : Germination of *Brassica juncea* seeds in different polluted area soil samples

Days	control	Uppal soil	Tarnaka soil	Shapur nagar soil	Jeedimetla soil	Uppal + Normal soil	Tarnaka + normal soil	Jeedimetla + normal soil	Shapur + normal soil
1	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0
4	0.4 cm	0	0	0	0	0.6 cm	0.6 cm	0	0
5	0.7 cm	0	0.4 cm	0	0	0.9 cm	0.9 cm	0.4 cm	0
6	0.8 cm	0	0.6 cm	0	0	1.0 cm	1.6 cm	0.5 cm	0.6 cm
7	1.0 cm	0.4 cm	1.0 cm	0	0	1.7 cm	2.0 cm	0.6 cm	0.6 cm
8	1.2 cm	0.7 cm	1.7 cm	0	0	2.2 cm	2.7 cm	1.0 cm	0.9 cm
9	1.8 cm	0.7 cm	2.4 cm	0	0	3.6 cm	3.0 cm	1.2 cm	1.3 cm
10	2.6 cm	0.9 cm	3.0 cm	0	0	4.8 cm	3.4 cm	1.5 cm	1.7 cm
11	4.8 cm	1.2 cm	4.2 cm	0.4 cm	0	5.7 cm	4.1 cm	2.2 cm	2.4 cm
12	4.8 cm	1.6 cm	5.0 cm	0.7 cm	0	6.5 cm	4.7 cm	2.7 cm	2.9 cm
13	5.2 cm	2.0 cm	5.5 cm	0.8 cm	0	7.2 cm	5.2 cm	3.5 cm	3.1 cm
14	6.0 cm	3.0 cm	6.2 cm	1.0 cm	0	8.6 cm	5.6 cm	3.7 cm	3.4 cm
15	7.1 cm	3.7 cm	7.0 cm	1.0 cm	0	9.4 cm	6.0 cm	4.8 cm	3.6 cm
16	7.5 cm	4.0 cm	7.7 cm	1.2 cm	0.6 cm	10.8 cm	6.6 cm	5.5 cm	3.6 cm
17	8.2 cm	5.2 cm	8.5 cm	1.7 cm	1.0 cm	11.5 cm	7.5 cm	5.9 cm	4.9 cm
18	9.0 cm	6.0 cm	8.6 cm	2.2 cm	1.5 cm	12.1 cm	8.0 cm	6.7 cm	5.5 cm
19	9.6 cm	6.7 cm	9.4 cm	2.7 cm	1.5 cm	13.2 cm	8.4 cm	7.6 cm	6.2 cm
20	10.4 cm	7.3 cm	10.2 cm	3.5 cm	2.4 cm	13.9 cm	9.2 cm	8.0 cm	6.7 cm
21	10.8 cm	8.0 cm	11.6 cm	4.6 cm	2.7 cm	14.5 cm	9.8 cm	9.4 cm	7.0 cm

of collected soil samples has carried out by following standard procedures for all the soil samples before and after seed germination. The change in physical and chemical characteristics of soil samples before and after seed germination are represented in graphic mode.

RESULTS AND DISCUSSION

Germinated seeds were counted every other day until the time in which no germination was observed over three days ever after. Seeds were considered germinated when both the plumule and radical were extended to approximately more than 2mm from their junction[9]. The measurements of shoot length were carried out and mean shoot length of each soil sample with two different seeds were shown in TABLE 1 and 2.

There was no more count of germination after 6th day incase of *Brassica juncea* seed and the number of germinated seeds were considered as final germinated seeds after 6 days. However the germination of seeds is observed after 6 days in case of *Eleusine coracana* seeds, there was no more count of germination of seeds after 11 days and the number of germinated seeds was considered as final germinated seeds after 11 days. From the data, % germination, % inhibition and Tolerance indices were calculated by using formulas, which described above for both *Eleusine coracana* and *Brassica juncea* seeds. The results are shown in Table 3 and 4. From the TABLE it was concluded that the % germination is higher in soil samples collected from the Uppal traffic area than normal soil sample. It indicates that the metal and minerals deposited in upaal soils are enhancing the seed germination process because of the presences of optimum levels of nutrients in the samples comparability to other polluted traffic area soil samples. Tolerance indices explain the healthy growth of roots and root length. The length of the root is an indirect measure of accumulation metals

TABLE 2 : Germination of *Eleusine coracana* seeds in different polluted area soil samples

Days	control	Uppal soil	Tarnaka soil	Shapur nagar soil	Jeedimetla soil	Uppal + Normal soil	Tarnaka + normal soil	Jeedimetla + normal soil	Shapur + normal soil
1	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0
7	0.3 cm	0.5 cm	0.3 cm	0	0	0.3 cm	0.3 cm	0	0
8	0.3 cm	0.7 cm	0.3 cm	0	0	0.5 cm	0.3 cm	0	0
9	0.5 cm	0.8 cm	0.5 cm	0	0	0.8 cm	0.5 cm	0	0
10	0.7 cm	0.8 cm	0.5 cm	0	0	0.8 cm	0.7 cm	0	0
11	1.0 cm	1.1 cm	0.7 cm	0	0	1.2 cm	1.0 cm	0	0
12	1.0 cm	1.4 cm	0.7 cm	0	0	1.4 cm	1.0 cm	0	0
13	1.0 cm	1.4 cm	0.9 cm	0	0	1.5 cm	1.0 cm	0	0
14	1.3 cm	1.8 cm	0.9 cm	0	0	1.8 cm	1.3 cm	0	0
15	1.9 cm	2.0 cm	1.4 cm	0	0	2.0 cm	1.9 cm	0	0
16	2.1 cm	2.3 cm	1.9 cm	0	0	2.3 cm	2.1 cm	0	0
17	2.5 cm	2.7 cm	2.2 cm	0	0	2.7 cm	2.3 cm	0	0
18	2.9 cm	3.3 cm	2.6 cm	0	0	3.3 cm	2.4 cm	0	0
19	3.2 cm	3.7 cm	3.1 cm	0	0	3.4 cm	2.4 cm	0	0
20	3.5 cm	4.0 cm	3.3 cm	0	0	3.5 cm	2.5 cm	0	0
21	3.8 cm	4.2 cm	3.3 cm	0	0	3.8 cm	2.6 cm	0	0

TABLE 3 : Analysis of germination percentage of *Brassica juncea* seeds

S.No	Name of the soil area	% of germination	Tolerance indices	% of inhibition
1	Normal	40	0	0
2	Uppal	46.6	80 %	2.63
3	Tarnaka	31.6	60 %	-7.4
4	Jeedimetla	3.3	50%	75
5	Shapur nagar	5	20%	57
6	Uppal + normal	41.6	90%	-34.2
7	Tarnaka + normal	28.3	85 %	9.25
8	Jeedimetla + normal	20	45 %	12.96
9	Shapur nagar + normal	33.3	20 %	35.15

and minerals from the soil samples. The highest root length and Tolerance indices was observed in soil samples of uppal + Normal soil and Uppal traffic area soil.

The Same experiment was performed with *Eleusine coracana* seeds and % germination, % Inhibition and Tolerance indices were calculated and presented in TABLE-4. From the TABLE it was concluded that the highest % germination was observed in soil samples of Tarnaka and Normal + Tarnaka comparatively control soil sample. Tolerance indices high in tarnaka + normal soil than the other soil sample but it is less than control soil sample. A 100% Tolerance indices was observed in normal soil in case of *Eleusine coracana* seeds compare with *Brassica juncea* seeds.

The growth response of two different seeds in same soil samples were calculated and shown in a graph mode and represented in Figure-3, 4 and 5. From Figure -3 it

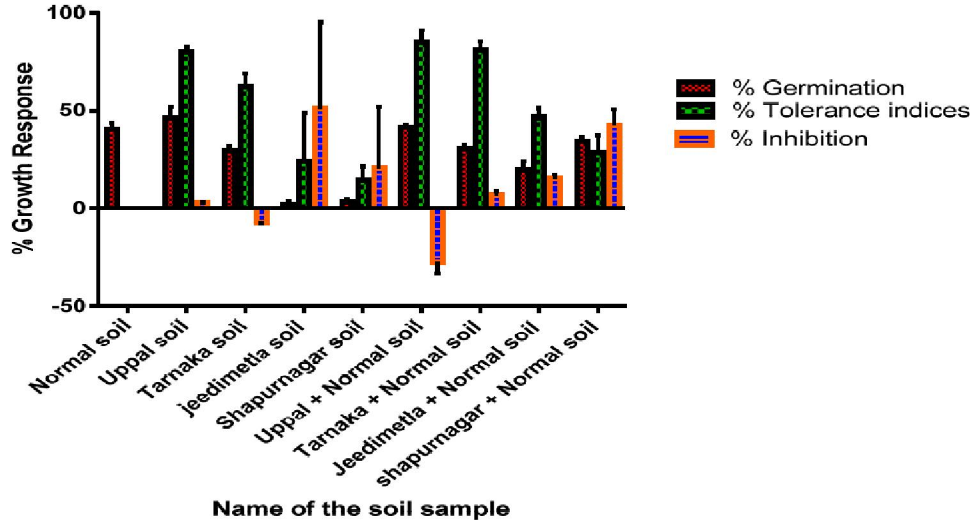

Figure 1 : Growth Response of *Brassica juncea* seeds in different soil samples

TABLE 4 : Analysis of germination percentage of eleusine coracana seeds

S.No	Name of the soil area	% of germination	Tolerance indices	% of inhibition
1	Normal	70	100	0
2	Uppal	43.3	38.89	25.93
3	Tarnaka	78.3	38.39	5.56
4	Jeedimetla	0	0	0
5	Shapur nagar	0	0	0
6	Uppal + normal	50	6	0
7	Tarnaka + normal	62	75	31.57
8	Jeedimetla + normal	0	0	0
9	Shapur nagar + normal	0	0	0

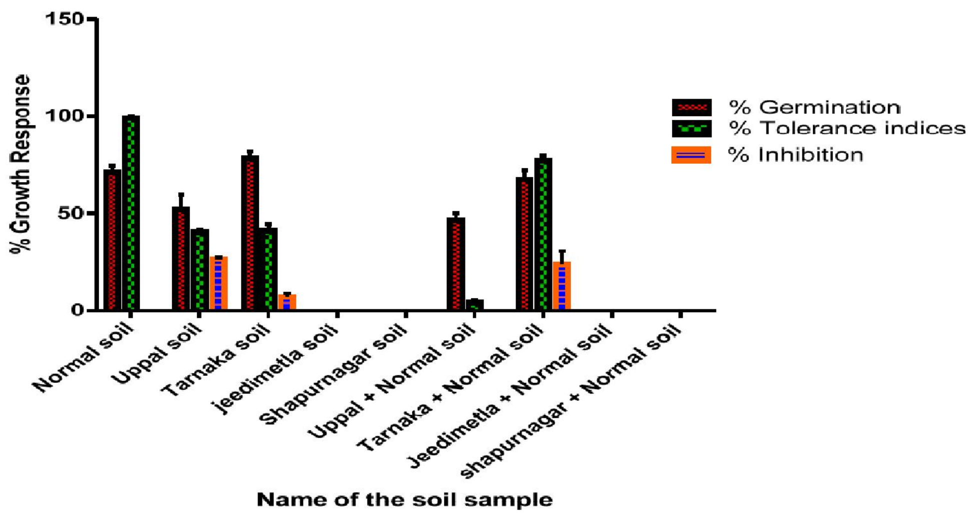

Figure 2 : Growth Response of *Eleusine coracana* seeds in different soil samples

can be concluded that the % germination of both the seeds in normal soil sample (control) is not similar it indicates that the soil sample which was used as control, red soil, enhances the seed germination of *Eleusine coracana* more compared to *Brassica juncea* seeds.

The % germination of both seeds in uppal traffic zone soil sample was almost similar comparatively. It indicates that the deposited minerals and metals on roadside sediments up to optimum level encourage seed germination process. In soil sample of Tarnaka traffic

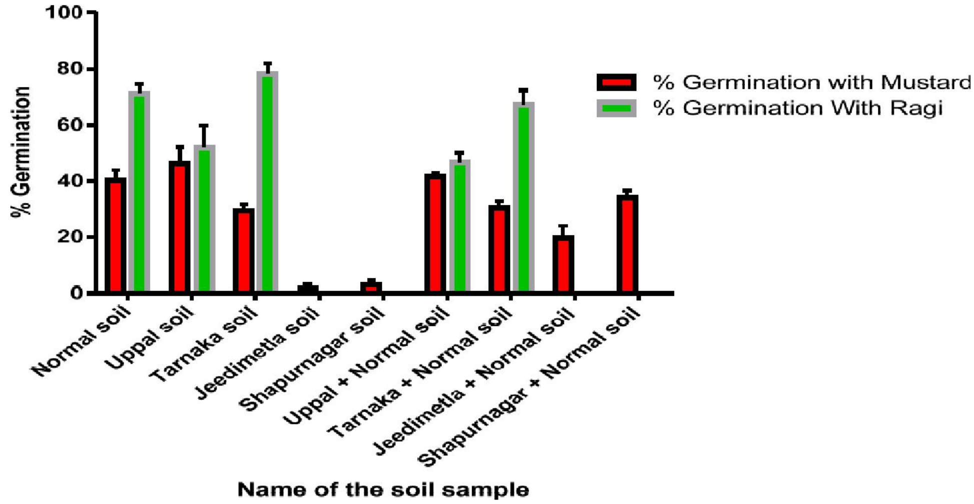

Figure 3 : % germination of *Brassica juncea* and *Eleusine coracana* in different soil samples

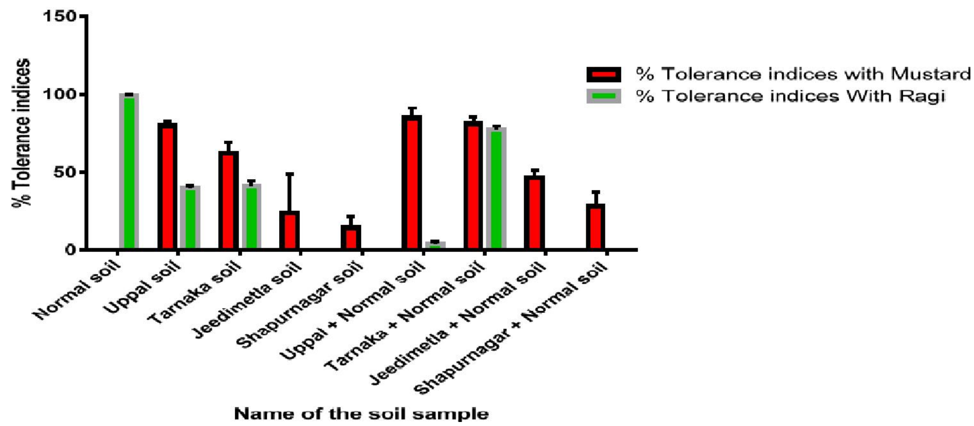

Figure 4 : Tolerance indices of *Brassica juncea* and *Eleusine coracana* in different soil samples

zone the % germination of *Eleusine coracana* is higher than *Brassica juncea* seeds. It indicates that the pH, Ec and Lead deposition of Tarnaka soil is less than the Uppal soil. The low pH, EC and metal concentration favors the seed germination of *Eleusine coracana* more than the *Brassica juncea*. No % germination was observed with *Eleusine coracana* in soil samples of shapurnagar and jeedimetla where as less % germination was observed in case of *Brassica juncea* seeds. It indicates that the soil samples collected from these two traffic zones were highly polluted and seeds cannot germinate in these conditions. Another set of seed germination experiments performed by taking equal quantities of traffic zone soil and control soil samples. The highest % germination was observed in uppal + Normal soil and Tarnaka + Normal soil when compared to other combinations for *Brassica juncea* and *Eleusine coracana* seeds respectively. No seed % germination was observed in soil samples of Jeedimetla + Normal soil and Shapurnagar + Normal soil for *Eleusine coracana* seeds but less % germination were observed with *Brassica juncea* seed in same soils. It indicates that pollutant levels in these two area soil samples did not decrease even when mixed with control soil sample.

According to the tolerance test, tolerance to pollutants released from petrol, diesel and fuel driven vehicles was lower in both the seeds when compared to control. This information can be considered a contributing step in exploring and finding of the tolerance limit of *Brassica juncea* and *Eleusine coracana* seeds in different traffic zone soil samples. Jeedimetla and shapurnagar soil samples were found highly toxic to seedling growth of *Eleusine coracana* when compared to *Brassica juncea* seeds. These findings can be an useful indicator of pollutant/metal (Pb) tolerance to some extent for plantation of this species in metal contaminated area. How fairly low amounts of lead absorbed over many years could lead to extinction of such an important plants species is unknown. In the metal contaminated areas, further research is needed to determine different levels of metals in the environment and various parts of the plants.

Statistical analysis

The experimental data was tested with two way ANOVA (Analysis of Varience) using Graphpad prism version 6.04 software and the report concludes that no significant change in % germination was observed in all polluted traffic zone soil samples with control soil sample except in Jeedimetla and Shapurnagar traffic zone soil samples produced ($p < 0.008$ and 0.0135) significant effect when germinated with *Brassica juncea* seeds. From the statistical report with multiple comparisons within polluted soils wherein we observed a significant change in % germination was observed in soil samples of jeedimetla vs uppal ($p < 0.0014$), shapurnagar vs uppal ($p < 0.002$), uppal+ Normal vs jeedimetla ($p < 0.006$) and uppal+ normal vs shapurnagar ($p < 0.0095$).

The experimental data of % germination of *Eleusine coracana* (ragi) seeds was tested with two way ANOVA and from the report it was observed a significant change ($p < 0.0001$) in % seed germination in all the soil samples compared with control soil sample except in Tarnaka ($p > 0.05$) and tarnaka+ normal soil ($p > 0.67$). From the statistical report with multiple comparison with in polluted soil samples when germinated with ragi seeds a significant change in % germination was observed in all soil samples except in Uppal, Jeedimetla, Shapurnagar and their combination with control soil sample. There is no significant difference ($p > 0.9999$) was observed within polluted soil samples.

According to the tolerance test, tolerance to different soil treatments in mustard and ragi was lower when compared to control. This is due to deposition of high content of toxic compounds (Lead) in soil. The excessive amount of toxic elements usually cause a reduction in plant growth[10-11]. Many other studies[12-16] have also reported seed germination and seedling growth inhibition by heavy metal. When we performed the multiple comparison within polluted soils for the tolerance test, we observed that, Uppal and Tarnaka traffic zone soil samples showed a more significant difference in tolerance compared with other soils. Surprisingly, the tolerance indices of Tarnaka + Normal soil and Uppal + Normal soil were observed to be high when compared to individual samples of tarnaka and uppal traffic zone area soil samples with regards to both *Brassica juncea* and *Eleusine coracana* seeds. This information can be considered a contributing step in analysis of tolerance indices of *Brassica juncea* and *Eleusine coracana* in different roadside soil sediments. From the report we can conclude that the disposal of these roadside soil samples (Uppal and Tarnaka heavy traffic zone soil) in agricultural land which specially cultivating *Brassica juncea* and *Eleusine coracana* may be give a better remediation solution of soil pollution and we would observe high growth rate of crops.

Physicochemical analysis of soil samples before and after seed germination

To find out the remediation efficiency of pollutant removal from soil with *Brassica juncea* and *Eleusine coracana* the physicochemical analysis of soil samples were carried out before and after seed germination (21 day from seed germination). The change in pH, Electro conductivity, Salinity, Nitrate Nitrogen, Nitrite Nitrogen, Phosphorus and lead concentration with both *Eleusine coracana* and *Brassica juncea* seeds in 4 different traffic zone soil samples (Tarnaka, Uppal, Jeedimetla and Shapurnagar) are shown in Figure-5 to Figure-9. From the graphs, it was found that all the soil samples are had a normal range of pH (6.5 to 7.5) before seed germination, except in Shapurnagar and Uppal traffic zones soils, which may be due to higher usage of vehicles and heavy industrial activities by using organic and inorganic salts in these areas. However, the clay amount in uppal soil is very high when compared to shapurnagar soil. pH of the soil is an important parameter as it will increase the mobility/ solubility of metals in the soil environment. The loading and retaining capacity of metals increases with increase in pH levels of the soils. The excess amount of minerals, toxic compounds and heavy metals in roadside sediments may biomagnify, they increase toxicity and physical, chemical and biological properties of the soil, and it leads to soil pollution problems. The nature of the soil can be changed by adding organic garbage and compost. Taking that factor into consideration germination of seeds has been carried out in the soils to evaluate the capacity

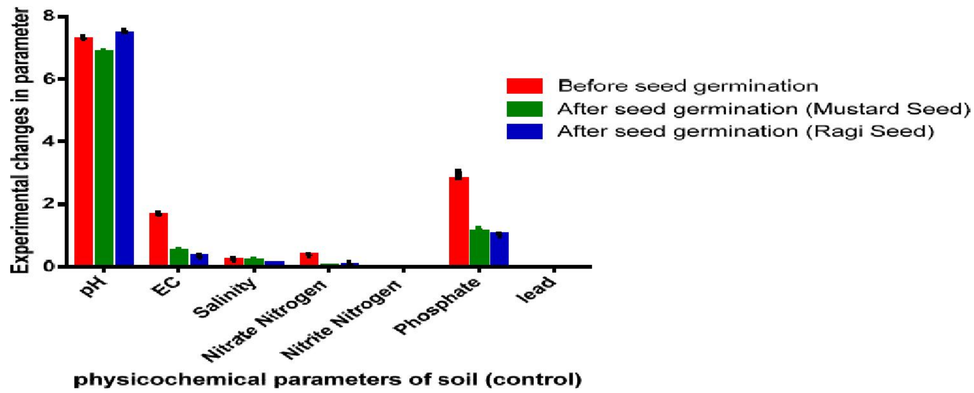

Figure 5 : Physicochemical parameters of control soil sample before and after seed germination

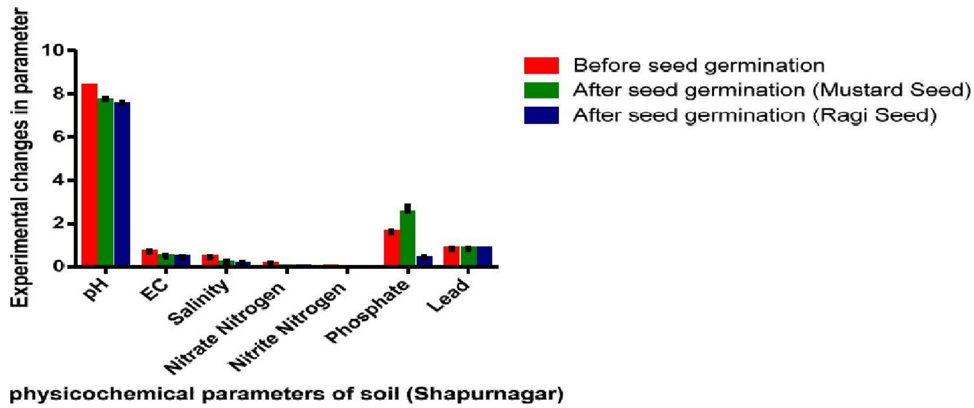

Figure 6 : Physicochemical parameters of shapurnagar soil sample before and after seed germination

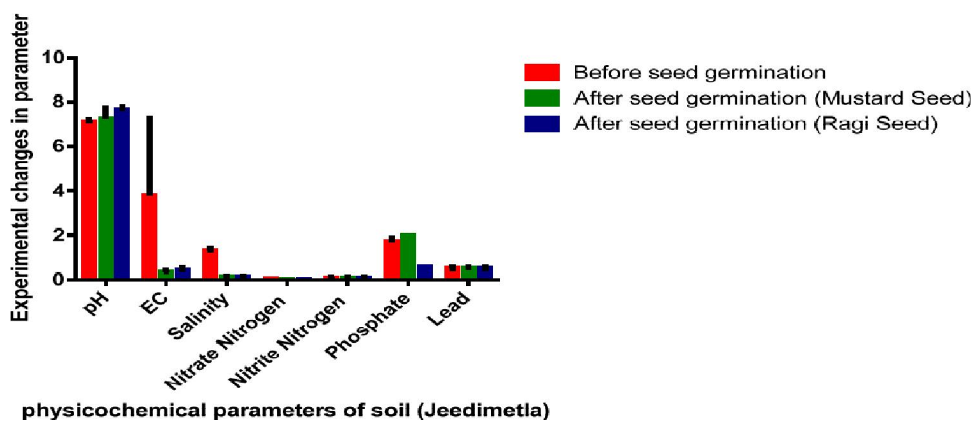

Figure 7 : Physicochemical parameters of jeedimetla soil sample before and after seed germination

of seeds to remediate the soils. High alkalinity interferes with seed germination and plant growth. It will reduce the uptake capacity of nutrients. After germination of seeds the pH of the soils has decreased to a very small extent. This may be due to constant soil moisture and it will result in dilution effect of soil pH due to presence of hydroxyl ions.

Electrical conductivity (EC) is defined as the ability of the soil to conduct electricity. It is indirect measurement and can be correlated to many physicochemical characteristics of soils. The Electrical conductivity of the different traffic zone of Hyderabad city before seed germination and after seed germination (*Brassica juncea* and *Eleusine coracana*) was shown in Figure 5 to 9. In the present study, highest EC was observed in jeedimetla and shapurnagar soil samples and lowest EC was observed in Uppal and Tarnaka soil samples. The difference in the electrical conductivity is due to differences in type of industrial activities and high usage of petrol driven vehicles in these areas. The ideal electri-

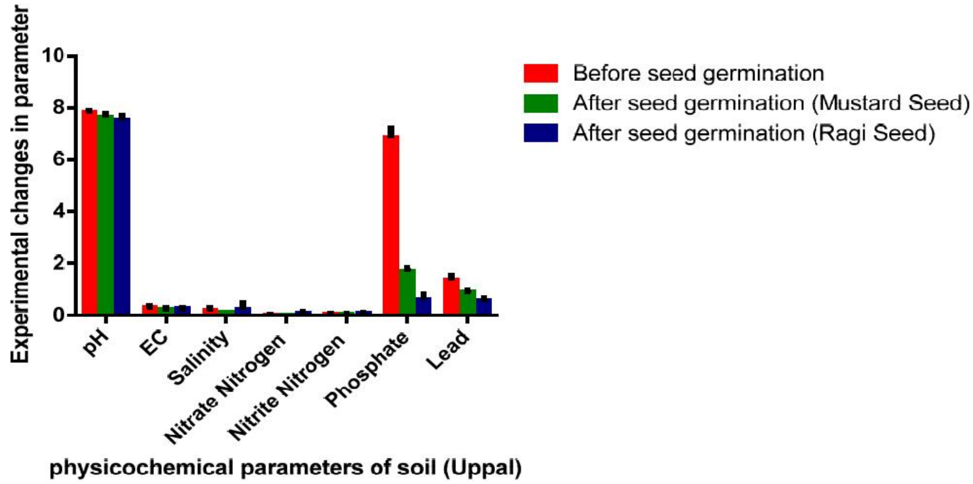

Figure 8 : Physicochemical parameters of uppal soil sample before and after seed germination

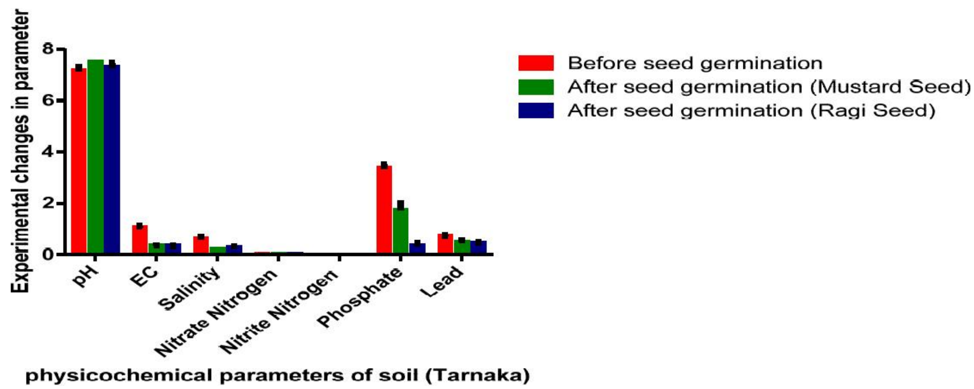

Figure 9 : Physicochemical parameters of tarnaka soil sample before and after seed germination

cal conductivity of the soils should be in the range of 0.2 to 1.2 ms/cm. The electrical conductivity decreased in all soil samples after seed germination in case of both the crops. The increased in organic matter in seedlings and the germination of seeds reacted with the soluble salts present in the soils and decreased electrical conductivity thus favoring the pre treatment of the soils by seed germination.

Nitrate Nitrogen (NO_3^-) should be present in the soils and it causes denitrification, when NO_3^- levels are high, then the soils are said to possess the high denitrification capacity. In the present study, the NO_3^- levels were observed to decrease after germination. This may be due to uptake of NH_3^- or NO_3^- by germinated seeds or may have been leached and transported to the root zone. Roots of mustard consist of taproot system and occupy 1 to 2% of the topsoil and absorption of nutrients is high in upper zone of soil, which showed a decrease in NO_3^- level in our analysis. Ragi root system is fibrous in nature and it will distribute completely and take nutrients. We observed reduced NO_3^- content in soil samples which germinated with ragi seeds. Major differences were not observed in % decrease as both germinated seeds have capacity to uptake nutrients equally.

Nitrite Nitrogen (NO_2^-), is toxic to plants and microorganisms. It will not accumulate in soils but detectable amounts occur in only in calcareous soils. In all types of soils is present in traceable amounts, which is favorable for plantation. Presence of trace amount of was observed in all collected soil samples and it is utilized by seeds while germination process.

Phosphorous is an essential element and it is involved in vital plant growth processes. The function of phosphorous is to store energy and energy transfer. It is an important component of several biochemical processes, especially ATP synthesis, which in turn is utilized by other bio pathways. A plant seed itself contains a store of nutrients to keeps its embryo alive and to provide all essential nutrients. It is highly essential in the earliest stages of germination. Seed phosphorous levels effects seed growth and root growth at early stages of seed germination. After the root system develops, it takes up phosphorous from the soil. Phosphorous content in mustard seed is 4% and in ragi seed, it is 0.25-0.30%, which indicates that mustard seed has more phosphorous content compared to ragi seeds. Due to high content of phosphorous in mustard seed the seed germination has taken less days (6 days) when compared to ragi seeds (11 days). The uptake of phosphorous after germination was significantly observed to increase which may be attributed to the measured decrease in phosphorous level in soil.

Soil pH, other factors such as the presence of competing ligands, the ionic strength of the soil solution and the simultaneous presence of competing metals are known to significantly affect the sorption processes of particular elements through a soil profile[17]. In the present study, exchangeable lead concentrations werefound to decrease in both seed germination process in all soil samples, similar results were observed in case of mustard seed germination process in Małgorzata Poniedziałek et al., 2010; jae-min lim et al 2003[18-21].

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