Toxicity Experiment to Access the Impact of Petrochemical Organic and Inorganic Contaminants on the Seed Germination and Growth of *Pennisetum pedicellatum*

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***Abstract*—*Research in recent years has proved "phytoremediation" as a promising technology for clean-up of petroleum contaminated soils. The present study has been carried out to evaluate the effectiveness of grass Pennisetum pedicellatum as a potential plant for phytoremediation of organic and heavy metal contaminants. Seed germination and root/shoot biomass is one of the commonly used bioassay for eco-toxicity evaluation and selection of the plants for their prospective use in phytoremediation. The effect of PAH compounds (anthracene and phenanthrene); heavy metals (Zn,Cd and Pb) separately and in combination on the seed germination of Pennisetum pedicellatum was investigated using PAH and heavy metal spiked soil at the concentrations of 25,50,75 and 100 mg/kg, for a period of 30 days along with unspiked soil as control. It was observed that Pennisetum pedicellatum could efficiently tolerate and survive at a high concentration of 100mg/kg of PAH compounds, however a constant decline in the germination rate was observed if the same was compared to control. Higher concentration of contaminant especially heavy metals showed negative effect on root-shoot biomass of the plant, root length was more affected by metals than shoot length. Toxicity of heavy metals towards seed germination and elongation trial was Cd>Pb>Zn. The research findings suggest that heavy metal contamination proved to be more toxic to the plant than co-contamination (heavy metal and organic compounds) and organic contamination alone. Based on these results Pennisetum pedicellatum is recommended for follow-up investigations which could further develop the application of phytoremediation of petroleum contaminated soils.*

Keywords: *phytoremediation, toxicity, Pennisetum pedicellatum, PAH, heavy metal contamination.*

I. INTRODUCTION

Industrialization marks the extensive increase in the use of petroleum and its products. In the present era, contamination of petroleum hydrocarbons in soil-water ecosystem is a matter of immense concern. The release of petrochemical contaminants into the environment occurring due to accidental spills or leakage caused during transporting, storage, processing and use of petrochemical compounds include potentially toxic elements such as heavy metals and polycyclic aromatic hydrocarbons (PAH). Elevated levels of these compounds are known to affect soil toxicity [1].

Crude oil and petrochemical waste contains heavy metals like lead (Pb), cadmium (Cd), chromium (Cr), copper (Cu) and Zinc (Zn) etc. [1]. Although many metals (e.g Zn,Cu,Mn) are essential, but their higher concentrations causes oxidative stress by forming free radicals [2]. These inorganic ions are generally non-biodegradable [3] and have the ability to replace essential metals in pigments or enzymes hence impairing catalytic activity [2]. Cadmium and lead are the toxic elements of primary importance [4]. These metals are known to be toxic even at very low concentrations. Anthracene and Phenanthrene being tricyclic PAH compounds are listed in the 16 major priority pollutants [30] owing to their mutagenic and carcinogenic properties. Due to the potential environmental impact connected with the frequent release of these contaminants; there is a great need for removal of these organic chemicals from the soil to reduce health hazards.

Spillage of petroleum and petroleum-by products resulting in accumulation of mixture of organic and heavy metal contaminants has been reported at many places world over. Remediation of sites co-contaminated with organic and heavy metal contaminants may be a difficult task as most of the remediation technologies aim to degrade or remediate only a particular type of contaminant [5].

The conventional remediation techniques used for soil surface contaminated with petrochemical compounds are typically based on the removal of the contaminated zone through soil excavation, offsite treatments and other costly physical and chemical methods. To find more environment friendly and cost effective options, biological methods like "Phytoremediation" has been investigated [6] using the plant-microbe system for "clean-up" of the contaminated soils and ground water [7].

This emerging technology can be used for extensive treatment of sites with shallow contamination of organic or metal pollutants. Efficacy of phytoremediation strongly depends on the large and extensive penetrating root system and high transpiration rate of the plant used for this purpose [7] [11]. Plants not only enhance the microbial degradation of contaminant by facilitating oxygen required for substrate oxidation [8] in the root area, along root channels and loosed soil aggregates, but also they stimulate the degradation of organic chemicals in the rhizosphere by the release of root exudates, enzymes, and the build-up of organic carbon in the soil. For metal contaminants, plants depict the potential of phytoextraction, rhizofiltration or phytostabilization [9].

Toxicity due to the presence of contaminant on the normal growth and development of cultivated or native plant species is species specific as different plant species respond to contaminants differently [10].

In Phytoremediation studies, one of the preliminary steps involve screening and selection of plant species in determining its potential for survival/tolerance towards a particular contaminant. Thus the plant needs to be selected carefully with the aim to provide a maximum root surface area [6] [12]. Phytoremediation investigations by [14] [15] [16] have identified grasses as potential plants for their fibrous root system that provide extensive surface area for microbial colonization [7]. Such root systems form continuous dense rhizospheres providing ideal conditions for phytoremediation [6] [13].

Phytotoxicity tests are reported to be useful tools in assessing the risk of contaminated soil and to evaluate the efficacy of a remediation process. Kirk *et al* [10] used these Phytotoxicity assays for the selection of potential candidates for phytoremediation of petroleum contaminated soils [17]. Seed germination and root elongation, two critical stages in plant development, are sensitive to environmental contaminants. Reduced biomass in the presence of contaminant is another important factor studied during screening of suitable plant for phytoremediation. [18]

Pennisetum pedicellatum being a herbaceous perennial grass with a massive root system, ideal for phytoremediation studies for its rapid growth and capacity for producing high biomass, is widely used as a rehabilitation grass to overcome land degradation caused by overpopulation and unsustainable farming practices as it greatly improves ground cover which in turn controls runoff and soil loss. Moreover its fibrous root system strengthens the soil structure and improves water conservation capacities while effectively using deeper nutrients for growth. Phytoextraction ability of *Pennisetum pedicellatum* for remediation of metals like Cd,Zn,Pb,Cu,Ni, and Sc has already been studied [2] [19]. The plant has successfully been used for rhizospheric bioremediation of pesticides [20] indicating it's important to test its resistance towards soil contaminated with petrochemical hydrocarbons.

The main objective of the present study was to investigate and evaluate the effect of selected organic contaminants (Phenanthrene, Anthracene) and heavy metal (Pb,Cd,Zn) separately and in combination at different concentrations on the seed germination, root/shoot biomass of the grass *Pennisetum pedicellatum*.

II. Materials and Methods

A) Plant

The seeds of the studied plant species *Pennisetum pedicellatum* were procured from Indian Grass Land and Fodder Research Institute (IGFRI), Jhansi, Uttar Pradesh, India, and stored at cool dry place until used.

B) Soil Sampling and characterization

Soil for experiment was collected from a depth of about 0-15 cm along the banks of Surya River, Phalghar (located 100Km, north of Mumbai). Stones pieces, plant tissues and other unwanted materials were carefully removed from the soil prior to drying under laboratory conditions. The soil was screened through 2mm stainless steel sieve, and was characterized for its physico-chemical parameters. The moisture content of the soil was calculated by the weight difference before and after drying method to a constant weight. The pH and electrical conductivity (EC) were measured after 20 min of vigorous shaking of mixed samples at 1:2.5 (w/v) in deionized water using digital meters (Deluxe water & soil analysis Kit, Model 191E). Total nitrogen was determined according to APHA [21], organic carbon content was determined by using Warkley-Black method [6] [22].

C) Soil Spiking

Selected Petrochemical Complex, Anthracene (Merk India 98%, Pure) and Phenanthrene (Sigma Aldrich; Germany 98% Pure), were weighed separately and dissolved in solvent di-chloromethane. In the treatment procedure, 25ml of the solvent solution containing contaminant was added to 25% (250g) of the soil sample. There after the solvent was allowed to get evaporated at room temperature (22-25°C) inside a fume-hood for 16 hours, after which the soil sample was mixed with the remaining 75% of the soil sample (750g). All samples were thoroughly mixed with a metal spatula. Soil was spiked to reach final concentration of contaminants at 25, 50, 75 and 100 mg/kg soil. The control soil samples received only the solvent. Similarly, the soil was amended with heavy metals: Cd as Cd $(NO_3)_2.H_2O$; Pb as Pb $(NO_3)_2$ and Zn as $ZnSO_4$, were mixed with deionized water and this water was mixed in the clean dry soil the varying concentrations of 25,50,75 and 100ppm applied for each heavy metal . For preparing the mixed contaminated soil, the soil was mixed with the organic contaminants in di-chloromethane at first, and it was dried in the fume hood until all the dichloromethane evaporated out. Then, this soil was mixed with heavy metal contaminants, dissolved in deionized water.

D) Seed Germination test and pot experiments

Pot culture experiments were conducted in the green house to study the stress tolerance of grass *Pennisetum pedicellatum* towards the organic and in-organic contaminants. The seeds were inspected and damaged seeds were removed. Before sowing, grass seeds were surface sterilized with 1% mercuric chloride solution for 1 minute followed by washing several times with distilled water to remove contaminants if any. Pots were sown with ten seeds each containing soil and sand mixture of 500gm in a ratio of 3:1 with 50gm of mycorrhizae inoculums in each pot. Three sets of contaminated pots were prepared for each contaminant condition. Uncontaminated soil was used for control. Plants were grown under natural light in greenhouse. After 7 days incubation, the number of germinated seeds was counted and germination rate was determined as (**Germination rate (%)** = number of germinated seeds/ number of sowed seeds x100).

In order to investigate the growth of plant species at varied concentrations of phenanthrene and anthracene respectively and in combination with heavy metals, germinated seeds were grown in contaminated soil in plastic pots for 30 days. After 30 days of plant growth period, roots of the plants were separated out from shoots and washed in deionized water. The roots, shoots and soil were dried in oven at 60°C (until it attained constant weight).The dry weights of roots and shoots were recorded as root biomass and shoot biomass.

E) Statistical Analysis

The data was analysed for mean and standard deviation (X±S.D) using standard statistical methods [31].

III. RESULTS and DISCUSSION

The advantage of germination trails to evaluate the stress tolerance capacity of plant is aimed at using it for phytoremediation of contaminated soils. The physico-chemical characteristics of soil play a critical role in contaminant degradation and mobility. The Physico-chemical analysis of soil collected for the experimentation is depicted in Table 1.

TABLE 1: Physico-chemical characteristics of experimental soil.

Soil Parameters	Alluvial Soil
pH	7.4
Moisture Content (%)	42.2
Electrical Conductivity (mMohs/100gm)	0.18
Organic Carbon (gm/kg)	72
Total Nitrogen (gm/kg)	2.8
Total Phosphorous (gm/kg)	0.24
Potassium (mg/kg)	23
Zn (ppm)	10.5
Cd,Pb (ppm)	Below Detection level

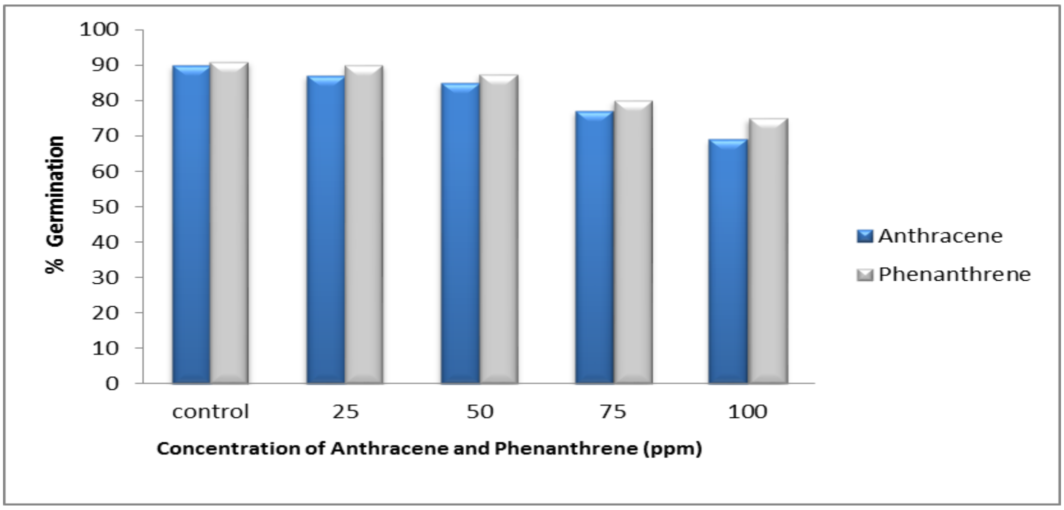

Fig 1: Effect of organic compound (Anthracene & Phenanthrene) on seed germination of *Pennisetum pedicellatum* in soil

Seed germination percentage was determined at various concentrations of Phenanthrene, anthracene and the heavy metals to investigate and compare the stress tolerance of *Pennisetum pedicellatum* towards organic and metal contaminants (Figs 1-3).

These results illustrate that *Pennisetum pedicellatum* can efficiently survive and tolerate anthracene and phenanthrene concentration even at high concentration of 100mg/kg. The results however indicate that the germination rates of grass seeds showed a tendency to decline (from 91% to 69%) as the concentration of contaminants is increased in the soil. The findings of the present seed germination study are in accordance with the observations made by [23] where they examined that there was no adverse effect of PAHs on germination and subsequent growth of seven grasses and legumes in fresh and aged contaminated soils. Seed germination trials done by [24] also reported that the percentage seed germination did not vary significantly between anthracene contaminated soils and control soil using rye grass.

While assessing the seed germination percentage of *Pennisetum pedicellatum* in heavy metal (Fig 2) contaminated soil at 25,50,75 and 100 ppm, the seeds were found to grow at all concentration of metal contamination. However, the percentage of seed germination decreased with increasing concentration of the heavy metals. The percentage of seed germination significantly decreased in Cd, Pb and Zn at 50 and 75ppm as compared to control. The seed germination percentage was arrested from 90% in control to 33 % at 100ppm Cd, followed by 42% at 100ppm Pb and 50% at 100ppm Zn.

Fig. 3 clearly depicts that there are significant negative effects on seed germination of *Pennisetum pedicellatum* in combined contamination of PAH and heavy metal. There was a sharp decline from 90% to 42% in seed germination with the increase in the combined contaminants of anthracene and heavy metals (Zn,Pb,Cd) or phenanthrene and heavy metals.

Based on the growth characteristics it can be observed that the germination rate and root/shoot biomass were greatly influenced by the contamination conditions. In general, higher seed germination and root/shoot biomass was observed in organic contaminated soil (Fig 4), followed by mixed contaminated soil and heavy metal contaminated soil. The root shoot biomass and seed germination of heavy metal and mixed contaminated soil were considerably less compared to the control. These observations are found in accordance with the earlier research findings [5], where the phytotoxicity of organic contaminants and heavy metals were studied for four plant species viz: oat plant, rye grass, tall fescue and sunflower. It was observed that plants in the soil with organic contamination alone had growth characteristics similar to that in uncontaminated soil.

All metals tested had more hampering effect on root dry weight than shoot. This can be attributed to the fact that root growth is known to be more sensitive than shoot growth to metal toxicity [25]. Zn is an essential micronutrient, which is required only in small amounts for plants. But higher concentration of Zn is known to inhibit plant growth [26]. Pb and Cd are non-essential heavy metals, which are not known to have any metabolic function in plants, and are toxic to plants at high concentration [27]. A high percentage of tolerance was found in *Pennisetum pedicellatum* at 25 ppm of Cd, Pb and Zn. Increase in the concentration of heavy metal treatment at 50ppm reduced the percentage of tolerance and the lowest percentage of tolerance was found at 100ppm. It was however observed that the tolerance in *Pennisetum pedicellatum* at 100ppm of Cd was lower than that of Pb. The plant found to be more tolerant to Zn- toxicity as compared to Pb and Cd. There can be a special mechanism of Zn uptake existing in *Pennisetum pedicellatum* and that it may be classified as a new Zn hyper-accumulator, when exposed or grown on much higher Zn contaminated sites [2].

Phytotoxicity seems to be lesser when organic contaminants are present along with heavy metals compared to the situations when heavy metals were present alone. Fig 4, 5 & 6 indicate that the plant *Pennisetum pedicellatum* has a better root/shoot biomass and germination rate in organic contaminated soil and mixed contaminated soil as compared to heavy metal contamination, this can be explained due to the lower bioavailability of heavy metals in the presence of organic contaminants. The presence of organic contaminants can influence the metal mobility in soils [5] [28]. The difference in sorption of heavy metal contaminants in presence of organic contaminants may be the reason of lower bioavailability of heavy metals in combined contaminated soils [29].

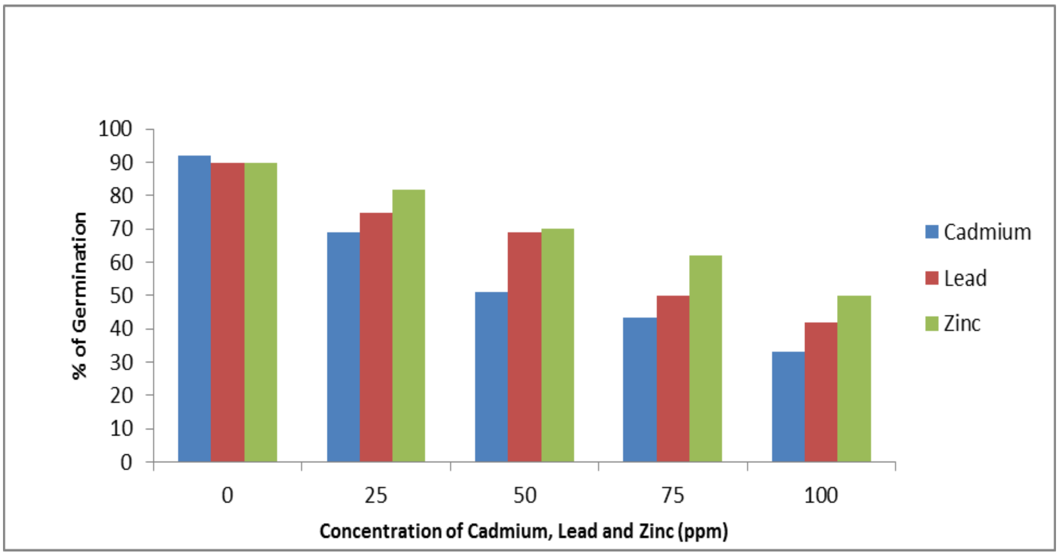

Fig 2: Germination of Grass seeds in heavy metal (Zn, Pb and Cd) spiked soil.

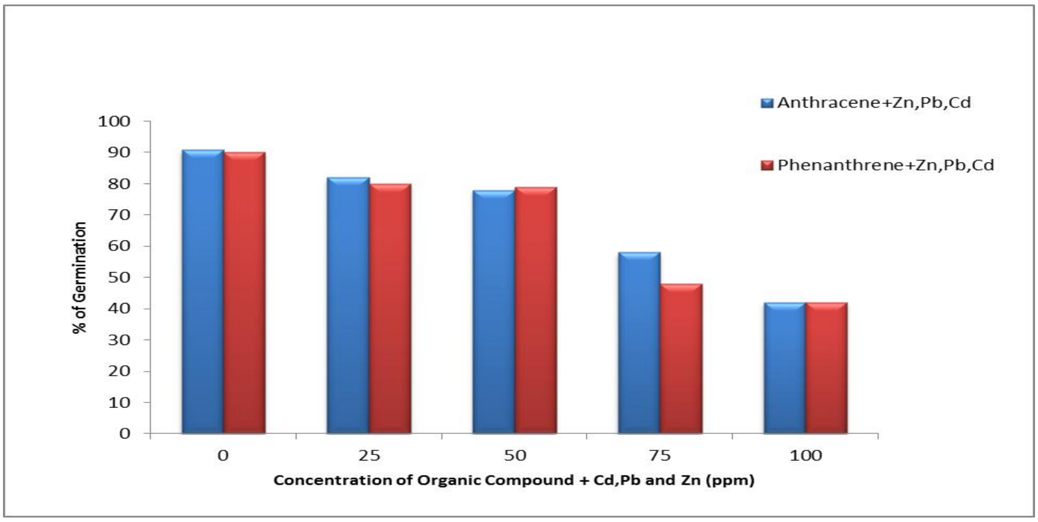

Fig 3: Germination of Grass seeds in soil spiked with both Organic Contaminant (Anthracene, Phenanthrene) and heavy metal (Zn, Pb, Cd).

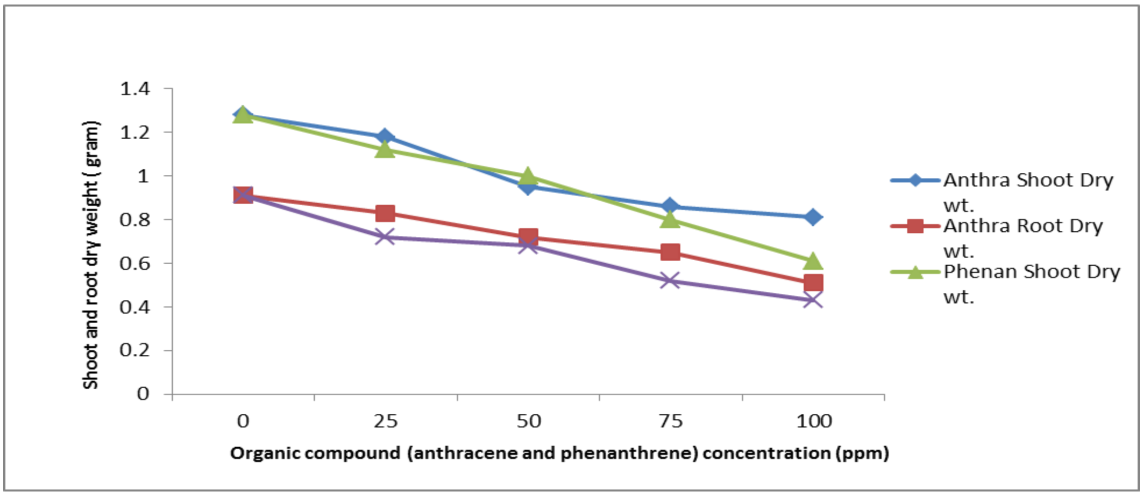

Fig 4: Shoot and root dry biomass of *Pennisetum pedicellatum* plant grown in varying concentration of Phenanthrene and Anthracene spiked soil.

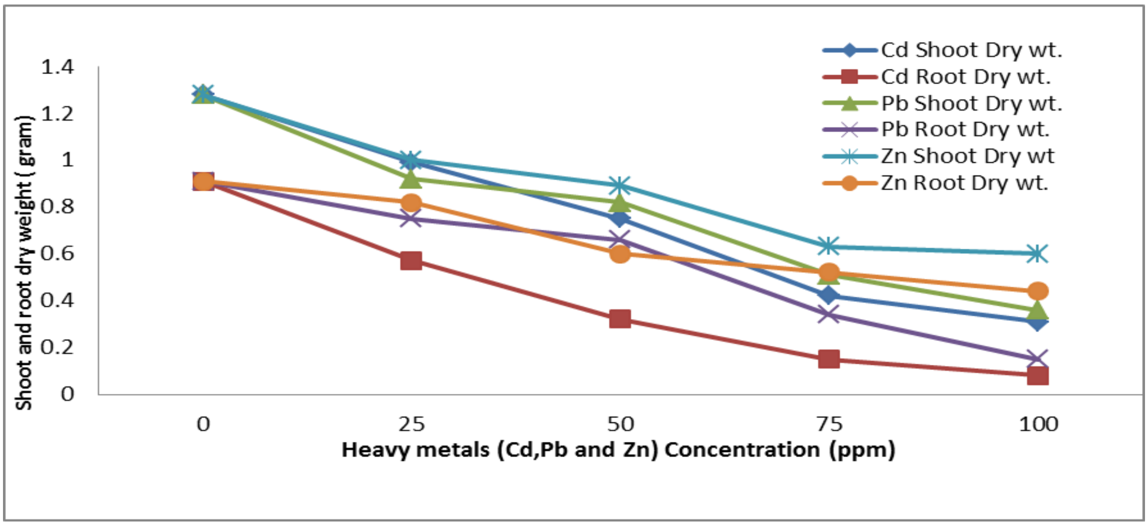

Fig 5: Shoot and root dry biomass of *Pennisetum pedicellatum* plant grown in varying concentrations of Cd, Pb and Zn spiked soil.

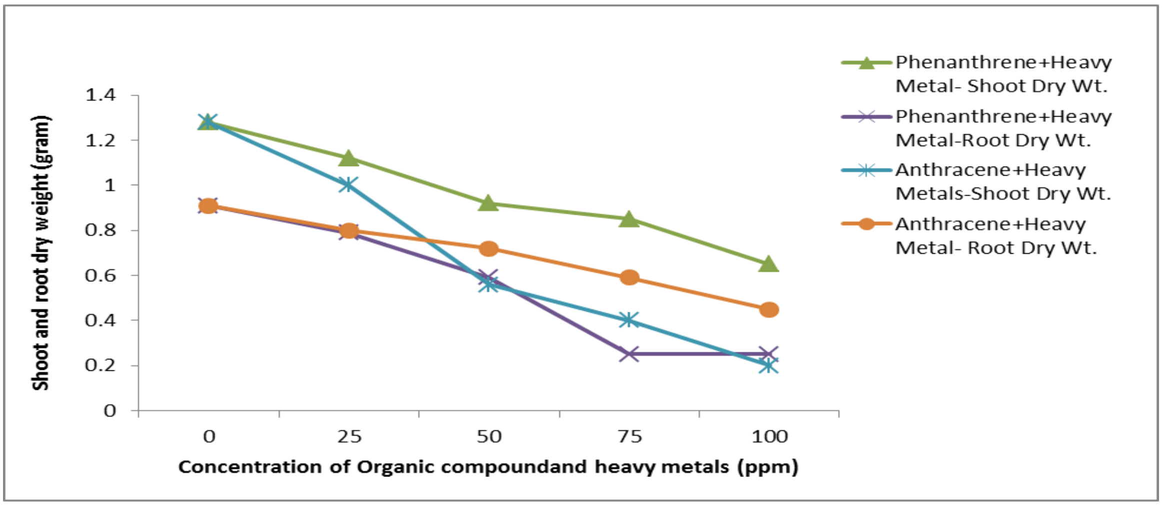

Fig 6: Shoot and root dry biomass of *Pennisetum pedicellatum* plant grown in varying concentration of Selected organic compound + heavy metal (Zn,Cd,Pb) spiked soil.

IV. Conclusion

Seed germination and cumulative shoot/ root dry matter of *Pennisetum pedicellatum* reflects the ability of the grass species to germinate, grow and develop in the presence of petrochemical organic compound and hence it can establish and survive in organic compound contaminated soil and does not show significant outward sign of phytotoxicity at the highest concentration. There was significant reduction and delay in seed germination of grass seeds at higher concentrations of heavy metals and mixed contaminations (PAH and heavy metal).

Pennisetum pedicellatum can prove to be a good phytoremediation plant considering its growth characteristics in both the alternates, where the sites are contaminated with only one group of contaminants or in combined contamination conditions. Plant Toxicity trials showed that *Pennisetum pedicellatum* was notably tolerant to the petrochemical contamination tested, demonstrating it's potential to effectively remediate the contaminated soil surfaces. The potency of phytotoxicity test of *Pennisetum pedicellatum* indicates the significance of the plant and its beneficial use in future rhizospheric bioremediation studies.

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