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# Effect of organic contaminants on seed germination of *Lolium multiflorum* in soil

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**Abstract**
The seed germination trials have relevance in selection of the plants for their prospective use in phytoremediation. The effect of organic contaminants; anthracene and chlorpyrifos on the seed germination of *Lolium multiflorum* (ryegrass) has been investigated. The germination of ryegrass seeds was evaluated in the separately spiked soil with organic compounds at the concentrations 10, 25, 50, 75 and 100 mg/kg while un-spiked soil taken as control. There was no inhibitory effect of anthracene observed on germination as compared to the control whereas significant reduction and delay in seed germination was observed at the higher chlorpyrifos concentrations of 75 and 100 mg/kg. The results show the pesticide (chlorpyrifos) toxicity was not caused to the ryegrass seeds up to the concentration of 50 mg/kg. The level of seed germination was found to decrease with increasing concentrations of chlorpyrifos in the soil. The findings of this study assessed ability of ryegrass seeds to survive and tolerate the contaminants for phytoremediation studies.

**Keywords:** Ryegrass, Toxicity, Anthracene, Chlorpyrifos, Phytoremediation.



## Introduction

Among the broad rage of organic pollutants contaminating soil-water environment, polycyclic aromatic hydrocarbons and pesticides are of great environmental concern. Polycyclic aromatic hydrocarbons (PAHs) are composed of two or more fused aromatic rings and produced by combustion of organic matter. PAHs are hydrophobic organic contaminants and persist in the environment because of their recalcitrance to degradation and widespread occurrence in contaminated industrial wastelands. Thereby these compounds pose a health risk due to their toxic, mutagenic and carcinogenic properties (Corgie *et al.,* 2004; Maila and Cloete, 2002). Out of the 16 major toxic polycyclic aromatic hydrocarbons, anthracene is a tricyclic aromatic hydrocarbon that needs to be studied as signature PAH compound since its chemical structures are found in carcinogenic PAHs like benz(*a*)anthracene, dibenz(a,h) anthracene and benzo(a)pyrene (Moody et al., 2001).

While pesticides especially, insecticides like Chlorpyrifos, O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothioate, a broad spectrum organophosphorus insecticide, has been commercially used since the 1960s, particularly for the control of foliar insects on cotton, paddy fields, pasture and vegetable crops as well as applied directly to soil for the control of urban pests (e.g. root worms, borers, and termites) (Baskaran *et al.,* 2003). The repeated and indiscriminate uses in addition to the extreme stability of such pesticides have led to their accumulation in plants, animals, soils and sediments, thus effecting prevailing contamination of the environment (Dalvi and Salunke, 1975). Chlorpyrifos is toxicity class II - moderately toxic compound and has toxicological effects on humans, birds, wildlife, honeybees as well as marine organisms. Chlorpyrifos may also be toxic to some plants. Data also indicates that this insecticide and its soil metabolites can accumulate in certain crops (EXTOXNET PIP - CHLORPYRIFOS.mht, 1996). The potential environmental impact connected with the introduction and heavy use of these organic contaminants; there is a great need of removal of these organic chemicals to reduce its health hazards.

The conventional remediation techniques for these contaminants involve expensive physical and chemical treatments; Phytoremediation proves to be a promising technique that uses green plants and their associated microbes for environmental cleanup (Korade and Fulekar, 2008). This technology employs the naturally occurring processes by which plants and their microbial rhizosphere flora degrade and sequester organic or inorganic pollutants (Smits, 2005). The number of studies done show that the monocot species with fibrous rooting systems provide a high root surface area that interacts with soil microorganisms were mainly used in enhancing degradation rates of organic contaminants in planted soils (Banks *et al.*, 1999; Corgie *et al.*, 2004; Buyanovsky *et al.,* 1995; Fang *et al.,* 2001).

These organic contaminants may be degraded in the root zone of plants or taken up, followed by degradation, sequestration, or volatilization depending on their physico-chemical properties. The properties of the contaminants like hydrophobicity and volatility which are proposed to have influence over their fate and behavior in the soil-water environment may also attribute to the toxicity of contaminants to plant (Smits 2005). The plant species - specific differences observed in seed germination and tolerance were very wide. Therefore it is often becomes necessary to asses the plant toxicity of the compounds for phytoremediation studies. Seed germination is commonly used method for measuring soil toxicity. The assay, also serves as bioindicator response endpoint due to its simple methodology, moderate sensitivity to toxicants and its potential use *in situ* and *ex situ*, both. Thus, the aim of the present study is to estimate the effect of anthracene and chlorpyrifos on the seed germination of ryegrass for its potential use in phytoremediation for environmental clean-up.

## Materials and Methods

Anthracene (98% pure) was purchased from Merck, India, and technical grade Chlorpyrifos (O, O-diethyl-O-(3, 5, 6-trichloro-2-pyridyl) phosphorothioate) 94% purity, was obtained from AIMCO Pesticides, Maharashtra, India. The physical and chemical properties of organic contaminants are listed in Table 1. Ryegrass was chosen for present seed germination studies to evaluate its use in phytoremediation experiments. Ryegrass was found to grow in a broad range of soil textures, adapts well to poorly drained soils and has a high level of tolerance to climatic variations. This plant also develops highly branched fibrous roots (large surface area and deep root penetration) and has quick seedling emergence (4-6 days) which is advantageous for removal of organics from soil (Parrish *et al.,* 2004). Seeds of annual ryegrass (*Lolium multiflorum*) were obtained from National Seeds Corporation limited (NSC), Beej Bhawan, New Delhi, India. Soil used for the pot culture experiments was collected from a depth of about 0-15 cm along the banks of Surya River, Palghar (located 100 km away from Mumbai). The soil was screened through 2 mm stainless steel sieve, and stored in a plastic bag at room temperature until use. The soil collected was characterized for its properties. The physico-chemical characteristics of soil and the standard methods applied are given in Table 2.

The dried and sieved soil used for fortification showed no background contamination of PAHs and pesticides. Soil was then mixed with sand at 3:1 ratio and 20% of laboratory developed mycorrhiza inoculum for the experiment (as the same composition was used in phytoremediation studies). Soil was spiked with anthracene and chlorpyrifos separately according to the procedure used by Brinch et al. (2002) to obtain final concentrations of both the organic contaminants to 10, 25, 50, 75 and 100 mg/ kg. The same protocol was used without contaminants as the control. Seeds of ryegrass were surface sterilized by soaking in 30% (v/v) $H_2O_2$ for 20 min and washed several times with distilled water (Binet et al. 2001). The spiked and un-spiked mixtures were taken in the Petri plates (9cm diameter) and made moist enough to sow the seeds. Ten seeds per Petri plate were sown and moisture was maintained during germination. Experiments were conducted at room temperature (26-27°C at day and 23-24°C at night) in dark. The experiment was run for 7 days and done in three sets. The seeds were considered germinated with the emergence of radicles. The statistical significance of the treatment effects on the percent seed germination were compared with control by unpaired t- test ($p < 0.05$) (Mahajan 1991).

**Results and Discussion**

The effect of contaminant on microorganisms and plants was reported to be depending on the concentration and the kind of contamination. In the current research study, the effect of anthracene and chlorpyrifos on ryegrass seed germination was assessed. Anthracene was selected as a compound representing PAH group and chlorpyrifos was chosen as representative of Pesticide group of organic contaminants. The effect of these compounds on seed germination was observed at varying concentrations and the comparison was made.

**Table1. Physical and chemical properties of organic contaminants.**

| Properties | Anthracene[a] | Chlorpyrifos[b] |
|---|---|---|
| Molecular weight | 178 | 350.5 |
| Melting Point | 216°C | 41-42°C |
| Boiling Point | 340°C | Decomposes at 160°C (approx) |
| Water Solubility | 0.07mg/L | 0.7mg/L |
| Log $K^{\dagger}_{ow}$ | 4.45 | 4.82 |
| Henry's law constant | $1.8X10^{-6}$ atm-$m^3$/mol | $1.23X10^{-5}$atm-$m^3$/mol |
| Vapor pressure | 1.96X10-4 mm Hg | 1.87X10-5 mm Hg |

[a] Data from Sims and Overcash (1983)
[b] Data from ATSDR (1997)
† Octanol-water partition coefficient

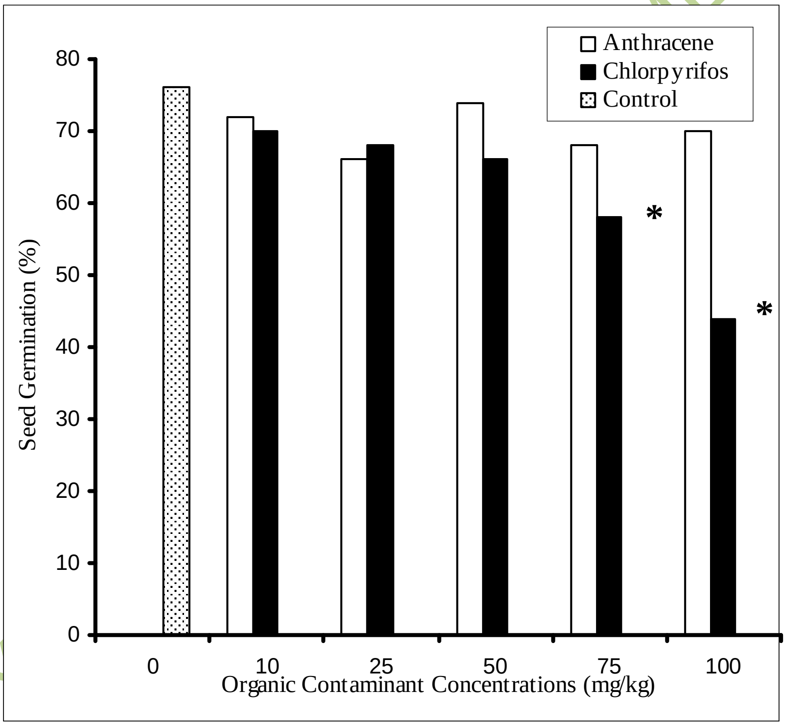
**Table 2. Soil characterization***.

| Parameters | Method Applied | Value |
|---|---|---|
| pH | APHA 1998 | 7.4 |
| Electrical Conductivity (mMohs) | APHA 1998 | 0.46 |
| Cation exchange capacity (meq/100gm) (Jackson 1973) | Ammonium acetate extraction at pH 7.0 | 7.2 |
| Organic carbon (%) | Walkley–Black method (Jackson 1973) | 1.3 |
| Total Nitrogen (%) | APHA 1998 | 0.24 |
| Phosphorous (%) | APHA 1998 | 0.039 |
| Potassium (mg/kg) | APHA 1998 | 25 |

*The values are the average of three replicates.
APHA- American Public Health Association

The percentage germination of the ryegrass seeds in organic contaminant amended soil is presented in Figure 1. These results illustrate that ryegrass can efficiently survive and tolerate the anthracene concentration even at high concentration of 100mg/kg. The percentage seed germination did not vary significantly between anthracene contaminated soils and control soil. However the percentage seed germination reduced and delayed along the increasing chlorpyrifos concentrations in soil. The differences in seed germination rate observed to be non significant between 10 to 50 mg/kg of chlorpyrifos contaminated soil compared to the control one ($p< 0.05$). This indicated that chlorpyrifos would not be toxic to ryegrass up to the concentrations 50mg/kg. At higher concentrations of the chlorpyrifos (75 and 100 mg/kg); seed germination resulted to be 58% and 44% compared to the 76% of the control where fewer seeds could tolerate and germinate with delay in the contaminated soil.

**Figure 1. Effect of organic contaminants on seed germination of ryegrass in soil.**




***** indicates significant difference ($p < 0.05$, n=3) of percentage seed germination grown in control and contaminated soil.

The findings of the present seed germination study are in accordance with the observations made by Smith *et al.* (2006) where they have examined the effect of PAHs on germination and subsequent growth of seven grasses and legumes in freshly and aged contaminated soil. They found that none of the treatments adversely affected germination of the plants where there was no reduced growth of ryegrass observed over the period of 12 weeks in the treated soil as compared to the other six species. In the work done on phytotoxicity of ancient gaswork soils, Henner et al. (1999) explained that volatile, water-soluble low molecular-weight hydrocarbons (<3 rings) such as benzene, toluene, xylene (BTX), styrene, indene, naphthalene and other toxic substances strongly inhibited plant germination and growth in contrast to the high molecular weight PAHs (3-5 rings) which did not show any phytotoxicity under the conditions studied. The research carried out by Maila and Cloete (2002) assessed that germination of *Lepidium sativum* decreased with increase in the PAH concentrations in artificially contaminated soil; while the percentage seed germination at 50 ppm was found to be 75% which reduced to 16% at the concentration of 1000 ppm. However the highest concentration of PAH considered in the said study was much higher (1000 ppm) than the maximum one used in the research work presented in this paper (100 mg/kg or ppm).

Cabrera et al. (1994) have validated the use of plants for evaluation of environmental pollutants such as pesticides because plants are direct recipients of agrotoxics, so they are important material for environmental monitoring of places affected by such pollutants. Effects of pesticides on the germination of weed seeds were explored by Gange et al. (1992) where the effects of three pesticides, chlorpyrifos (a contact insecticide), dimethoate (a systemic insecticide) and iprodione (a contact fungicide) on seed germination of 20 weed species were examined in the laboratory. Chlorpyrifos reduced germination in the annual grass and one annual forb and iprodione in one perennial forb. In 2007, Wang et al. examined the fate of chlorpyrifos in soil-crop system where concentrations of chlorpyrifos in wheat seedlings at harvest correlated positively with the initial concentrations spiked in the soil. Wheat and oilseed rape seedlings were found to absorb 0.257-4.50µg/g and 0.249-2.02 µg/g compound irrigated at the concentrations 1-10µg/g. The fresh weight of oilseed rape seedlings were also reported to be significantly reduced at 10µg/g compared to 1µg/g and control. In experiments performed by Asita and Makhalemele, 2008, the pesticides, chlorpyrifos, alpha-thrin, efekto virikop and springbok were assessed for their cytotoxicity and genotoxicity in the onion plant. Cytotoxicity was determined by comparing the mitotic index (MI) of treated cells with that of the negative control. Chlorpyrifos induced all the different types of damages to the cell division apparatus at 0.068% and 0.034% (680 and 340 ppm) concentrations but not at the lower concentration of 0.017% (170 ppm). The wide range of genotixic effects induced by chlorpyrifos is indicative of the wide range of targets in the cell that are susceptible to its effects including, spindle fibres, chromosomes, kinetochore, centriols and enzymes. The study thus reasons out that chromosome lagging and bridges, pulverized and stick chromosomes, multipolar anaphase and telophase may be the possible mechanism of causing chlorpyrifos toxicity to the plant.

The technical grade hexachlorocyclohexane (tech-HCH) was also showed to have effect on radish and green gram seeds; marked reduction in germination percentage and seeding vigor index as well as increased abnormalities with increasing concentration of tech-HCH was observed. The germination of these seeds was found almost completely inhibited at 100 µg HCH level on moist filter paper and soil (Bidlan et al. 2004). The results obtained in the present study for chlorpyrifos toxicity are in agreement with the findings of the other studies done on seed germination and toxicity with the other plants. The potency of seed germination of ryegrass indicates the significance of the plant and its beneficial use for phytoremediation of specific contaminants.

## Conclusion

The seed germination of *Lolium multiflorum* was not affected by the anthracene amended in the soil. The seed germination declined with increase in chlorpyrifos concentrations showing significant reduction and delay in seed germination at 75 and 100 mg/kg concentrations. Ryegrass indicates higher potential of survival and tolerance at the high concentration of anthracene than chlorpyrifos in the respective contaminated soil. Therefore the nature and concentrations of the organic contaminants found to contribute in setting up the toxicity of the compounds to the plant.

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