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Toxicity evaluation of lead and Chromium (VI) on Seed Germination and earlier seedling effect on *Ocimum* Seeds

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Abstract

The effect of heavy metals such as chromium (VI) and lead were studied on seed germination of two species viz. *Ocimum basilicum* L. and *Ocimum gratissimum* L. Seeds were examined at various concentrations of chromium (VI) and lead. Germination of seeds was inhibited by chromium (VI) even at a lesser concentration as compared to lead. The EC_{50}value for *Ocimum gratissimum* was found to be 90 ppm in case of chromium (VI) and 120 ppm in case of lead after 48 h of incubation. For *Ocimum basilicum* it was 45 ppm in case of chromium (VI) and 160 ppm for lead after 120 h of incubation. In addition to shortened length of plumule, a black spot in case of lead was clearly observed in *Ocimum basilicum* seeds. The samples were collected from Ranipet district in Tamil Nadu and observed concentration of heavy metals were more than toxic level.

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Key words:
Ocimum basilicum, Ocimum gratissimum, toxicity, germination, chromium (VI), lead.

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INTRODUCTION

Heavy metals are commonly defined as those whose density is more than 5 g/cm^{3}. They impose serious threats to mankind, animals as well as plants. Some of the important heavy metals which cause serious problems include lead, chromium, cadmium, mercury and arsenic.

Toxic heavy metals find their ways into environment through variety of sources such as metal smelters, industrial effluents including uses of fertilizers and pesticides, etc[1]. Among these industrial effluents the one discharged by tanneries cause serious problems.

Leather production is a major industry in India, which makes significant contribution to the country's foreign exchange earnings and provides employment opportunities to about three million people.

Lead is harmful for plants especially at high concentrations. It causes inhibition of growth inhibition by reducing the rate of photosynthesis, interference with cell division and with water absorption. Lead coats the surface of the leaf and reduces the amount of light reaching reducing the rate of photosynthesis, inhibiting respiration, encouraging an elongation of plant cells influencing root development by causing pre-mature aging.

Dissolved chromium reaches upto very high concentration due to polluted wastes discharged from tanneries, which is generally linked to the use of chromium (VI) compounds in several industries applications such as plating, metallurgy, pigments, and leather tanning[2]. Chromium (VI) is found be very toxic for plants. It interferes with several metabolic processes, causing toxicity to the plants as exhibited by reduced growth and phytomass, chlorosis, impaired photosynthesis, stunting and finally plant death[3]. *Ocimum basilicum* L. commonly known as 'Basil' belongs to the family *Laminaceae* and is an important medicinal and culinary herb[4]. It provides important essential oil which has insect repellent and antifungal activities[5]. It is a perennial herb, native to Asia, Africa, South America, and the Mediterranean but widely cultivated in many countries[6]. Basil is used as a medicinal herb in medical treatments such as for headaches, coughs, diarrhea, worms, and kidney malfunctions. Basil essential oil has been utilized extensively in the food industry as a flavoring agent, and in perfumery and medical industries[7].

Ocimum gratissimum too is widely distributed in various parts of Asia and Africa. Its leaves are used for abdominal pains, sore eyes, ear troubles, coughs and blocked noses. The flowers and leaves of the plant constitute a rich source of essential oils[8]. An infusion of the leaves is used as a disinfectant and as an insecticide[9].

Present study is done to examine the toxicity of lead and chromium (VI) on germination of *Ocimum basilicum L.* and *Ocimum gratissimum* seeds at varying concentrations. Sample water was collected from Ranipet District, Tamil Nadu, one of the major hubs for tannery industry.

MATERIAL AND METHODS

The plant seeds of *Ocimum basilicum* L. and *Ocimum gratissimum* L. were obtained from VIT University. Lead acetate and Potassium dichromate were obtained from Sisco research laboratories. The selected concentrations of lead acetate and potassium dichromate from 10 ppm to 200 ppm (20 different concentrations with 10 ppm increment) were prepared using deionized distilled water. The effect of metal concentration was studied between a range from 0 to 200 ppm.

Germination of Seeds and Metal Treatment

Germination of seeds was carried out in sterilized glass petri plates having double layer of whatman no. 1 filter paper wetted with 9ml solution of various concentrations of metals. Plates were covered and kept in dark at 25°C for germination to take place[10]. 20 sterilized seeds were used in each petri plates and deionized distilled water was used as control. Each step was done in triplicate. Small volume of each solution was added at particular intervals of time to prevent seeds. Seed were considered germinated only when length of plumule observed was more than 1mm long. Germination was observed after every 24 hours.

The test of results

The number of germinated seeds in petri plates was recorded for analysis. Statistical analysis is done using Graph Pad Prism (version 5.0). Each data point represents the average of three replicas and error bars indicates ± SEs.

Result and Discussion

Effect of chromium (VI) and lead on germination of *Ocimum basilicum* and *Ocimum gratissimum* are summarized in graphs depicted in figure 1 and 2. Readings were taken with reference to control from 1st germination to 100% germination. Germination was affected by incubation time and concentration of metals. Rate of germination increased with increase in incubation time. The metal concentration at which germination of seeds inhibited by 10% (EC_{10}) and 50% (EC_{50}) are tabulated in table 1.

Effect of Chromium (VI)

Chromium (VI) is more toxic for *Ocimum basilicum* than *Ocimum gratissimum*. The EC_{50} value for this metal on *Ocimum basilicum* was found to be 45 ppm after 48 h and for *Ocimum gratissimum* it was found to be 90 ppm after 120 h incubation. At later incubations *Ocimum gratissimum* showed same EC_{50} value but it increased in the case of *Ocimum basilicum*.

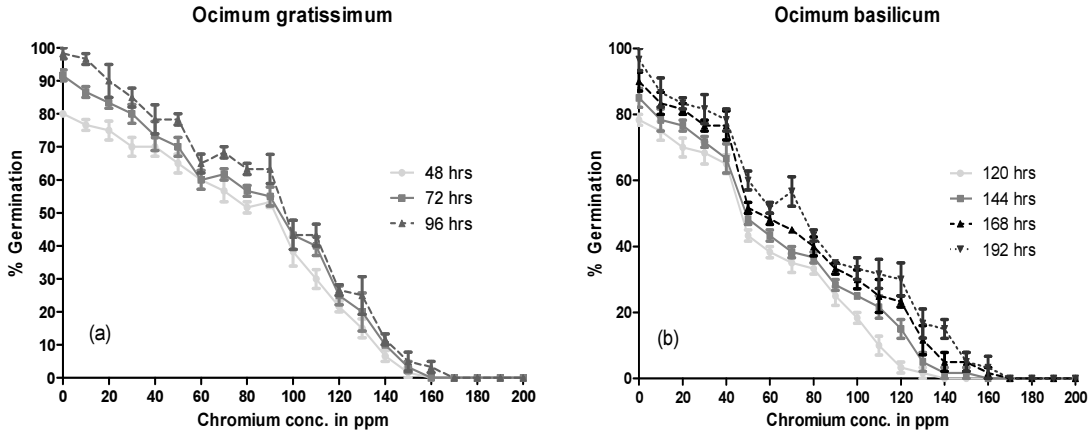

Figure 1: The effect of varying concentrations of chromium (VI) on percentage germination of seeds of (a) *Ocimum gratissium* and *(b) Ocimum basilicum.*

Effect of Lead

Lead is less toxic than chromium (VI) in same concentrations. The EC_{50} value of lead for *Ocimum gratissium* was 120 ppm at 48h and for *Ocimum basilicum* it was 160 ppm at 120h. These EC_{50} value increased with incubation time.

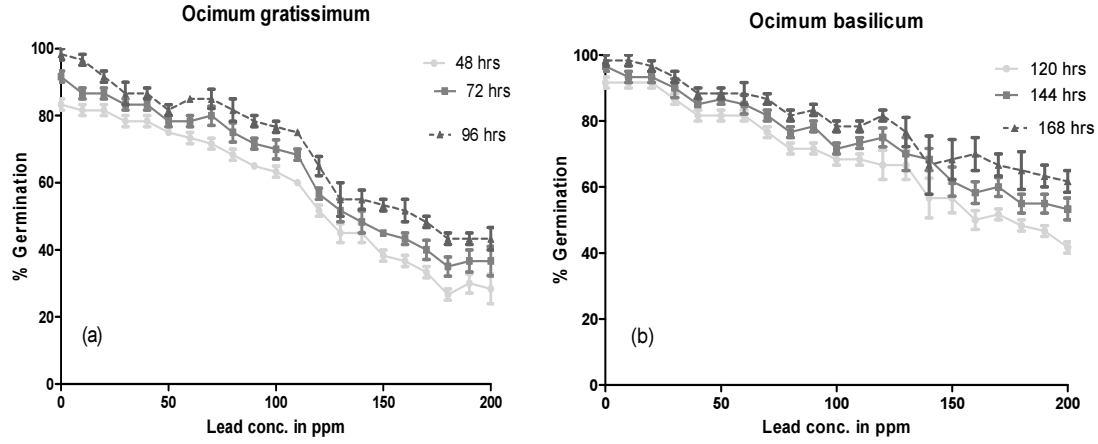

Figure 2: The effect of varying concentrations of lead on percentage germination of seeds of (a) *Ocimum gratissium* and (b) *Ocimum basilicum.*

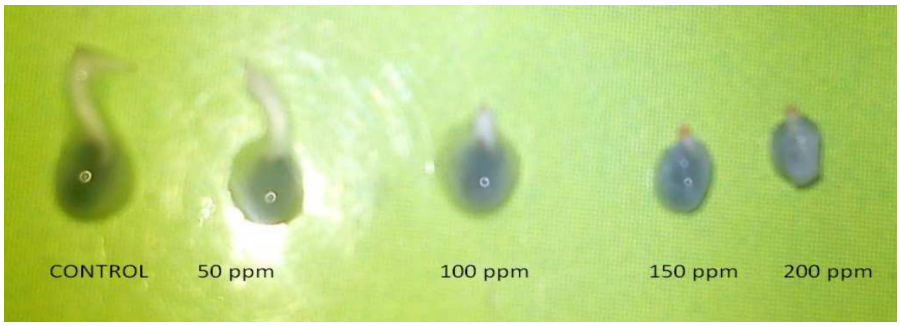

Figure 3: Effect of lead on germination time and plumule length on *Ocimum basilicum* L. seeds (after 48 hours). Black spot on tip of seeds clearly can see in last two seeds.

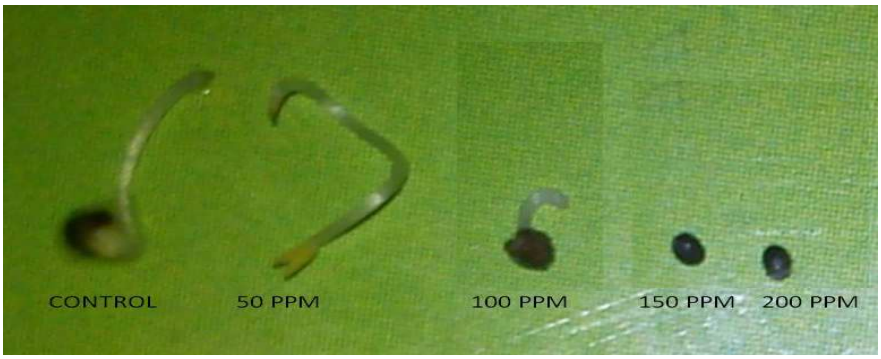

Figure 4: Effect of lead on Ocimum *gratissimum* L. seeds germination and plumule length (after 120 hours).

Table 1: Effective concentrations of Cr (VI) and Pb on seed germination of *Ocimum gratissimum* and *Ocimum basilicum* at different incubation time.

Plant	Duration of time	Effective Concentration of metal (ppm)			
		Cr (VI)		Pb	
		EC_{10}	EC_{50}	EC_{10}	EC_{50}
Ocimum gratissimum	48	135	90	>200	120
	72	140	90	>200	130
	96	145	95	>200	160
Ocimum basilicum	120	110	45	>200	160
	144	125	50	>200	200
	168	130	50	>200	>200
	192	145	60	-	-

Conclusion:

Thus toxic levels of chromium (VI) and lead for germination of *Ocimum* seeds were found out. Chromium (VI) was more toxic on *Ocimum basilicum* than *Ocimum gratissimum* but it was just opposite in case of lead. By AAS[11], it was found out that concentration of lead in the samples collected from Ranipet was 112 ppm and chromium(VI) was 48 ppm which is above toxic levels according to the experiment and thus measures needs to be taken to curtail the persistent pollution from tannery industries. The effects were observed in *Osmium* seeds but similar results can be expected with other plants also. If continued could seriously affect the surrounding flora of the place.

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