TOXICITY OF Cr AND Pb DURING VEGETATIVE GROWTH OF SESAMUM INDICUM L.

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Date of online publication: 31st December 2019
DOI: 10.5958/2455-7218.2019.00027.5

In the present study, effect of Cr and Pb on seedlings growth of S. indicum has been studied under 10, 50 and 100 µM concentration of Cr and Pb. These heavy metal significantly affected all the growth parameters of seedling such as root and shoot length, leaf area, biomass production and root development at all concentrations when compared to control. PKDS-8 variety of Sesame was found to be more susceptible to both the heavy metals but maximum deleterious effect was observed at higher concentrations (50 and 100µM). The seedling growth of sesame was more sensitive to Cr in comparison to Pb. This preliminary information on the initial growth of seedling may help in the screening of the tolerance level of the target plant against two heavy metals for the proper growth and development of the sesame as now a day, this important oil yielding crop has emerged as a leading revenue generation resource of this region.

Keyword: Chromium, Heavy metals, Lead, Seedling growth, S. indicum

Heavy metal contamination is a major global problem. Soil having heavy metal is a great concern for agricultural production due to the disparaging effects on crop growth as well as on microorganism (Nagajyoti et al. 2010; Pourrut et al. 2011). Heavy metal contamination occurred through natural processes or by anthropogenic activities including rapid industrialization, inappropriate utilization and disposal of heavy metal containing wastes, extreme application of fertilizers and pesticides which is harmful to environmental as well as human health (Amel et al. 2016, Amin et al. 2018).

Lead (Pb) is one of the heavy metal which cause serious pollution to the soil generally due to industrial processes that affect growth of plant and soil microorganism (Pourrut et al. 2011, Amin et al. 2018). Lead contamination in soil causes reduction in seed germination seedling development, root elongation, chlorophyll production, transpiration, cell division, and lamellar organization in the chloroplast (Gupta et al. 2010). Anthropogenic source of lead are coal burning, lead batteries, lead arsenate pesticide application, lead based paints, gasoline, explosives, and the dump of municipal sewage sludge (Zheng et al. 2011).

Chromium (Cr) is a heavy metal that cannot be degraded. It occurs naturally as well as in rocks, soil and water (Amin et al. 2019) and causes soil pollution that is a major problem (Ashraf et al. 2017). Soils contaminated with chromium have an effect on the water and nutrient uptake and translocation, cell division, metabolic and biochemical processes of plant that is the main cause of reduction of seed germination and early seedling development and yield (Zeng et al. 2011, Singh et al. 2013, Singh et al. 2015). Cr also drops down the production of chlorophyll along with enzyme activities and plant biomass (Liu et al. 2008). The natural source of Cr is rocks but anthropogenic sources could be associated with dumping of Cr-containing solid wastes, liquids, alloying, ceramic glazes, leather tanning, electroplating, refractory bricks, metallurgy, wood preservation, paper production, textile dyes and paints (Butera et al. 2015, Rani et al. 2017).
S. indicum is a most ancient oil seed crop of Pedaliaceae family and cultivated in arid and semi-arid regions of Asia and Africa (Elleuch et al. 2007). The methyl ester is found in sesame seeds that can successfully be utilized as petro diesel hence it can be an alternative source for biodiesel (Saydut et al. 2008, Ahmad et al. 2011). It is used as a healthy food constituent (Osawa et al. 1985, Sankar et al. 2006). This oil has also recently been intended for many other productions (Ribeiro et al. 2016). The oil yield has been revealed to depend on ecological influences such as climate, type of soil and cultivars (Rahman et al. 2007).

Bearing in mind above facts, this study was set up to evaluate toxicity of Cr and Pb on plant growth parameters of Sesame by treatments in soil that could tolerate by this crop.

**MATERIALS AND METHODS**

**Seeds procurement and sterilization:** Seeds of the black sesame variety PKDS-8 was procured from the Allahabad, Uttar Pradesh. In order to escape from microbial infection, seeds were surface sterilized with 0.1% HgCl₂ for 5 min and washed 7-8 times with sterilized distilled water (Pourakbar et al. 2007). The present investigation has been divided into two experiments, Petri dish and pot and both these were carried out simultaneously.

**Petri dish experiment:** The petri dishes were sterilized by keeping them in hot air oven at 60 °C for 48 hours. Sterilized petri dishes were lined with filter paper and to this 5 ml solution of 10, 50 and 100 µM concentration of CrO₃ and PbCl₂ were used. Twenty sterilized seeds were kept in each petri dish. Distilled water was used as control. The seeds were allowed to germinate at 35 to 40°C.

**Pot experiment:** Earthen pots were filled with 5 kg sieved soil and 10 seeds sterilized seed were sown in each pot. Pots were organized in a totally randomized design. Five plants in each pot were left, after one week of seed germination. When secondary leaf emerged out from the seedling then PbCl₂ & CrO₃ in increasing concentrations (10, 50 and 100 µM) to each pot were provided twice in a week followed by irrigation with distilled water. Nutrient solution was also supplied twice in a week. This experiment was performed in a greenhouse. Toxic symptoms of heavy metals were showed by plants during the experimental period. Plants were harvested 6 weeks.

**Germination percentage (%):** The percentage of seed germination was recorded till the 5th day after sowing. The visual emergence or protrusion of radicle was taken as the criteria for germination. The seed germination test was carried out according to rules laid down by International Seed Testing Association (1976). The germination percentage of seeds was calculated by the following equation. (Amin et al. 2019).

\[
\text{Germination percentage} = \left( \frac{\text{No of germinated seeds}}{\text{Total no of sowing seeds}} \right) \times 100
\]

**Measurement of morphological Parameters:** For the measurement of Root-shoot length of plants were taken in three replicate from pot. Root and shoot length were measured with the help of scale. Leaf area was determined by using standard graph papers methods. The leaves were outlined and the squares were measured under length and width. Averages of 5 leaves were taken and measured under length and width. For the studies of root development, plants were uprooted from the earthen pots and their roots were washed and counted. For the studies of root development, plants were uprooted with the help of water from the earthen pots and their roots were washed and counted manually.

**Determination of tolerance index:** Tolerance Index (TI) is the ratio among the growth
parameters (fresh weight of root & shoot dry of weight root & shoot, root & shoot length,) of the plants of CrO$_3$ and PbCl$_2$ treatment with the growth parameters of plants from control (untreated). TI was estimated by following method of Amin et al. (2019).

Tolerance index (%) = \frac{\text{Growth parameter in treatment}}{\text{Growth parameter in control}} \times 100

**Statistical analysis:** All experiments were conducted with three replicates and the data have been analyzed statistically using SPSS. To compare the means of the treatments, one way ANOVA was performed followed by Duncan's multiple range, Post Hoc tests at significance level of $p < 0.05$, to observe significance difference among means.

**RESULTS**

**Germination Percentage:** Cr and Pb had a very toxic effect on seed germination. In control, the seed germination was 88.9% but when seeds treated with Cr and Pb the germination decreased to 70% and 85%, respectively at 10 $\mu$M concentration. As the concentration of Cr and Pb increased 50 to 100 $\mu$M, it showed gradual decrement in the seed germination (Table 1).

**Measurement of morphological Parameters**

**Seedling Growth:** Seedling's length (root and shoot length) is also among primary determinants of plant growth. Treatment of Cr and Pb significantly ($p < 0.05$) inhibited growth in terms of root and shoots length in *S. indicum* (Table 1). Root and shoot length is directly related to the productivity. The deleterious effect of both heavy metal Cr and Pb was observed on root and shoot length of sesame plant.

**Leaf area:** The productivity of crop is indirectly dependent on leaf area. When the

<table>
<thead>
<tr>
<th>Meshes</th>
<th>Control</th>
<th>Cr</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germination (%)</td>
<td>88.9%</td>
<td>70%</td>
<td>85%</td>
</tr>
<tr>
<td>Leaf Area</td>
<td>36.21 cm$^2$</td>
<td>20.65 cm$^2$</td>
<td>25.96 cm$^2$</td>
</tr>
<tr>
<td>Root Fresh weight</td>
<td>3.14 g</td>
<td>2.34 g</td>
<td>2.24 g</td>
</tr>
<tr>
<td>Root Dry weight</td>
<td>1.31 g</td>
<td>1.54 g</td>
<td>1.45 g</td>
</tr>
<tr>
<td>Shoot Fresh weight</td>
<td>8.35 g</td>
<td>7.68 g</td>
<td>7.28 g</td>
</tr>
<tr>
<td>Shoot Dry weight</td>
<td>3.24 g</td>
<td>2.94 g</td>
<td>2.74 g</td>
</tr>
<tr>
<td>No. of primary</td>
<td>8.74</td>
<td>7.84</td>
<td>7.34</td>
</tr>
<tr>
<td>No. of secondary</td>
<td>11.41</td>
<td>9.61</td>
<td>8.71</td>
</tr>
</tbody>
</table>

Data are presented as means of three replicates. Mean ± SE sharing the same letter non-significant according to Duncan's Multiple Range Test ($p < 0.05$) in the same column.
surface area is large then productivity will be higher. Table 1 depicted the effect of these heavy metals on leaf area in *S. indicum*. The treatment of Cr showed high inhibitory effect at 100 µM concentration compared to Pb over control.

**Root Development:** Data of table 1 indicated the effect of Cr and lead on root development. The inhibitory effect of heavy metal Cr is high in comparison to Pb. However, lower concentration of these heavy metals, proved beneficial for the development of secondary and tertiary roots. At 10 µM concentration heavy metal Cr and Pb showed toxic effect in secondary roots by reduction 11.49 % and 18.42 %. While at 50 µM concentration it was 27.53 % and 32.29 % respectively but at higher 100 µM concentration it was reduced more 45.96 % and 49.48 % over control. The inhibitory effect of heavy metal Cr and Pb on tertiary root development was also found at lower concentration 10 µM where it was 10.14 % and 21.55% over control. The reduction was 25.31 % and 34.20% over control at 50 µM concentration and at higher concentration of both metals Cr and Pb, the reduction was 38.42 % and 40.47 % over control.

**Biomass:** Data present in Table 1 showed the inhibitory effect of these two heavy metals on biomass production. This effect was showed by more effectively by Cr rather than Pb. In case of higher concentration (50 µM) of Cr and Pb, root dry weight decreased up to 32.55 % and 26.74 % over control but at 100 µM concentration upto 43.02 % and 37.20 % over control. In case of shoot dry weight, it decreased 20 % and 15 % at 10 µM concentration of Cr and Pb. However higher concentration (50 µM), its reduction over

![Figure 1: Effect of heavy metals (Cr and Pb) interaction on Tolerance indices showing in different growth parameters in *S. indicum*. Bars and error bars showing means of three replicate and SE respectively.](image)
control is 30% and 25% but at 100 µM concentration shoot dry weight decreased upto 40% and 35% over control.

**Tolerance indices:** Tolerance indices (TIs) for *S. indicum* were found different under Cr and Pb treatment (Figure 1). *S. indicum* had the TIs for leaf area (44.35% and 52.72%), root lengths (48.21% and 77.98%) and shoot lengths (58.36% and 82.30%), root fresh weights (72.50% and 75%), and shoot fresh weights (47.50% and 52.50%), root dry weights (56.98% and 62.79%), shoot dry weights (60% and 65%), secondary root no (54.04% and 50.52%) and tertiary root no (59.52% and 59.52%) at 100 µM Cr and Pb treatment respectively.

**DISSCISION**

The present observations indicate that the deleterious effect of Cr and Pb on seed germination was noted at various concentrations (10, 50 and 100 µM). The reduction of seed germination may also resulting from the interference of amylase and protease enzymes with lead and chromium and other stress (Sengar *et al.* 2009, Agnihotri *et al.* 2007), which are necessary for hydrolysis of starch and also make available sugar to developing embryos. The toxicity of these heavy metal affects amylase activity resulting availability of sugar decreases for developing embryos causing reduction in seed germination (Amin *et al.* 2019) Similar results of seed germination inhibitions were observed by many other workers in various concentrations of heavy metals (Khan and Khan 2010, Amin *et al.* 2018, 2019). According to Kopittke *et al.* (2007), Pb is strongly inhibited to Germination even at micro molar levels.

Heavy metals have strong affinity with cell division, including inducement of chromosomal aberrations and abnormal mitosis resulting plant growth inhibition (Jiang *et al.* 2001, Radha *et al.* 2010). The decrease in root and shoot length is primarily due to the interference of Cr with water and mineral nutrient uptake which in turns, induced mineral deficiency in plants, resulted in reduced root cell division and cell elongation along with extension of cell cycle leading to reduced root length under Cr toxicity. Uptake of Cr by seedling can directly interact with sensitive plant a tissue as leaves and disturbed the processes of photosynthesis that affect cellular metabolism of shoots, so reducing height of plant (Sundaramoorthy *et al.* 2010, Shaikh *et al.* 2013, Amin *et al.* 2019).

Many researchers found that the effect of heavy metal like Cr and Pb may reduce the leaf area markedly as the concentration increased (Dar 2010). Heavy metal reduced the leaf area through perturbation in metabolic process. This adverse effect of heavy metals can be reversed following calcium application on leaf area in *S. indicum* (Abd-Allah *et al.*, 2017).

It has been found that lower concentration of Cr and Pb are beneficial but as concentration increase the reduction rate also increase and harmful for both secondary and tertiary roots. Our results are in agreement with the reports that steady increase concentration of heavy metals in soil and showed that could significantly affect the root growth and development (Agnihotri *et al.* 2006, Sundaramoorthy *et al.* 2010, Amin *et al.* 2019).

The shoot dry weight was more affected than root dry weight with higher concentration of both these metals but Cr causes more reduction in shoot dry weight. Similar results on decrement in biomass production in several other crops have also been observed (Singh *et al.* 2013, Kamel 2008). The decreased biomass mainly due to the inhibition of water and mineral uptake might also be due to the disturbed metabolic activities and low photosynthetic reactions under high Cr stress (Miao and Yan 2013, Amin *et al.* 2018, 2019). High plant biomass (fresh weight and dry weight) is the first prerequisite for high
yielding plant and mainly based on growth performance of particular species (Sundaramoorthy et al. 2010). Under Cr treated soil, the TIs for root and shoot length, root and shoot fresh weight, root dry weight, shoot length, shoot dry weight, root length and shoots fresh weight of the plant by Cr treatment while in the case of Pb the shoot length presented the highest TI, followed by leaf area, root length, root fresh weight, root dry weight and shoot dry weight, secondary root, tertiary root and shoots fresh weight of the plant by Pb treatment. It was concluded that S. indicum had higher tolerance to Pb than Cr heavy metal. Similar results were reported by Amin et al. (2018, 2019). Srinivasan et al. (2014) observed that inhibition in growth parameters is a common response to heavy metal stress.

**CONCLUSION**

This preliminary study can be concluded that the screening of heavy metal Cr and Pb may lead to the toxic level. Following the optimum level, the farmers may grow this oil yielding crop in the region whose soil had the limited value of these two heavy metals and can increase the productivity level. Cr and Pb treatment in soil had a significant effect on the growth characteristics of plant. S. indicum could be suitable crop plant to be grown in Cr and Pb contaminated soil. Further work is requisite to understand the mechanisms of metal tolerance in plants.

We are thankful to The Head, Department of Botany, School of Life Sciences, Khandari Campus, Dr. Bhimrao Ambedkar University, Agra for providing necessary facilities to carry out the experiments.

**REFERENCE**


