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Effect of Aluminum on Early Seedling Growth of Maize (*Zea Mays L.*) and *Flax* (*Linum usitatissimum*)

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Abstract:
Aluminum (Al) toxicity isa major constraint for crop production in acidic soils with pH is lower than 5 worldwide. Al is extremely toxic in terms of root elongation and is believed to be the primary factor in inhibiting plant growth. Al enters into the plant through root tip cells and stops root development process. It causes inhibition of cell elongation and cell division. further it reduces root length which results into poor intake of water and nutrients. And it also shows an impact on shoot and plant growth. In this paper the most remarkable symptoms of Al toxicity in plants and the latest findings in this area are addressed. In the present study different concentrations of Al 1.5mM,3.5mM,5.5mM,7.5mM,9.5mM were used to see the effect of Al toxicity on Maize (Zea mays L.) and Flax (Linum usitatissimum) seed germination, morphological changes - root length, shoot length, fresh weight ,dry weight, percent phytotoxicity, RGI(Relative growth index), chlorophyll content , carotenoidcontent. In both the plants (Maize and Flax) the growth of root and shoot were gradually inhibited from low concentrations of Al to the high concentrations of Al, the maximum inhibition of growth was observed at 9.5mM concentration of Al. Total chlorophyllcontent also decreased gradually with low to high concentrations of Al in the leaves of Flax and Maizeseedlings as well.

Keywords: *Acidic soil, phytotoxicity, relative growth index, chlorophyll, carotenoid*

1. Introduction

Concentration of Heavy Metals in the soils is considered to be toxic for the plant growth and development. There are about 50 heavy metals that are with toxicological impact on plant growth and healthof humans and animals. Generally, Pesticides and fertilizers are used to increase the production of the crops. They contain heavy metals, extensive use of these products can cause the contamination to the agricultural lands(Vlamic *et. al.,* 1985). Toxic heavy metals first accumulate in soil and reach the plant through roots or are taken up by the leaves of the plants from atmosphere .Heavy metals can cause various toxic effects on seed germination and plant growth and reduce the crop production.(Shankat *et. al.,* 1999). Further they cause alternation of normal metabolic pathways including respiration and photosynthesis by disrupting the cellular enzymes (Krupa *et. al.,* 1993).Al is the third most abundant element of earth crust behind that of Oxygen and Silicon.Al toxicity commonly occurs in oxisols and ultisols as well as heavily leached soils such as lateritic soils at the humid tropics. It is a major growth limiting factor on upland soils. With a pH of less than 5 i.e. it is estimated that 40% of arable soils of world are acidic and therefore Al toxicity hazardous to the plants (Vonuexkull & Muertos 1995). In the present study, Al toxicity and its tolerance on seed germination of Maize (*Zea mays*) and Flax (Linum *usitatissimum*) have been studied.
The inhibition of root growth by Al toxicity is the most recognized symptom and widely accepted measure of Al stress in plants. Al toxicity blocks the process of cell division. As a result, root becomes stunted and brittle, root hair development is poor and the root apices become swollen and damage. It causes extensive root injury leading to poor ion and water uptake,this is further inhibiting plant growth and development. Young seedlings are more susceptible than older plants. Plasma membrane of the root is the primary target of Al toxicity (Takabatake & Shimmen, 1997). The primary effect of Al on root membrane permeability appear in few minutes to hours after exposure to Al. It is likely that these effects are mediated by Al ability to bind to the carboxyl and phosphate groups of the cell wall and membrane respectively (Gunse *et al.,* 1997).Al toxicity also impact on other ions uptake-in Wheat roots grown in acid soils (pH < 5.5) For example Ca transport into root is more intensive at the root apex, which is also the primary site of Al accumulation and toxicity. The interactions between these two can cause decreased uptake and transport of Ca and with increased Al levels in Wheat plants (Tayler, 1988).

2. Materials and Methods

The seeds of Maize and Flax were collected randomly from the research field of Agricultural University, Karimnagar, Telangana, India. Then healthy seeds were selected and they were soaked for 12hrs in distilled water and surface sterilized with 0.01M Mercuric chloride for 2 minutes and thoroughly washed with distilled water several times. The seeds of uniform size were spread in large sized

Petri plates lined with three layers of *Wattman* No1 filter paper containing 10 ml of varying concentration of Aluminum. 10 seeds of Maize & Flax were placed in each Petri plate. The different concentrations of Al selected for the present experiment was 1.5 mM, 3.5mM, 5.5mM, 7.5mM, 9.5mM given in the form of Aluminum Sulphate. For control the seeds were kept in Distilled water for germination. For each treatment five replicates were maintained. The experiment was carried out under normal laboratory conditions with a photo period of 8 hours per day and a temperature of approximately 30 ± 2^0c during the day and 22 ± 2^0 c during the dark period. The seeds were allowed to germinate for 10 days. They were then removed and separated into individual parts for further analysis. In the present study, different parameters were analyzed to see the Al toxicity, they are morphological changes, root and shoot length, percent phytotoxicity, dry weight, relative growth index (RGI), chlorophyll a, chlorophyll b, total chlorophylls and carotenoids.

2.1. Growth Parameters

- → *Morphological Changes:* -Seedlings of Maize and Flax observed for morphological changes. The visual symptoms of toxicity if any were noted on the 10^{th} day.
- → *Root and Shoot length:* - The seedlings were detached into roots and shoots and length of each part was measured using a graph paper.
- → *Percent phytotoxicity:* - It was calculated as follows:
  Percent phytotoxicity = $\frac{\text{root length of control—root length of test}}{\text{Root length of control}} \times 100$
- → *Dry weight:* - The seedlings were separated into roots and shoots, gently blotted and their fresh weight was recorded, the same were dried in a hot air oven at 90^0c for 48 hours to obtain constant dry weights.

2.2. Chlorophyll Estimation

The total Chlorophyll content was estimated according to the method of Arnon1949. 0.2 grams of leaf material was cut into small pieces and homogenized with 10 ml of 80% acetone in a clean mortar. The green slurry was centrifuged at 3000rpm for 12 minutes. The supernatant was transferred into a clean test tube and the residual pigment in the pellet is re-extracted with 10 ml acetone. The process is repeated till a complete white pellet is obtained. The total volume is made upto 25 ml with 80% acetone. The optical density was determined at 663&645 using 80% acetone solvent as blank in a spectrophotometer.

Total Chlorophylls = (0.D 645 × 20.2) + (0.D 663 × 8.02) V/1000× W
Chlorophyll a = (0.D 663 × 12.7) -- (0.D 645 × 2.69) V/1000× W
Chlorophyll b = (0.D 645 × 22.9) -- (0.D 663 × 4.68) V/1000× W

2.3. Estimation of Carotenoids

Carotenoids were estimated according to the method of Zakaria *et al*.,(1979).0.2 grams of leaf was cut into small pieces , homogenized and saponified with 1.5 ml of 12% alcoholic KOH in a water both at 60^0c for 30 minutes. The saponified extract was transferred to a separating funnel containing 5 to 12 ml of petroleum ether and mixed well. The lower aqueous solution was then relocated to another separating funnel and the upper petroleum ether layer containing the carotenoids was collected the extraction was repeated until the aqueous layer became color less. A small pinch of anhydrous sodium sulphate was added to the petroleumether extract to remove excess moisture. The final volume of the petroleumether extract was noted .The observance of the yellow color was read in a spectro photometer at 450 nano meter,

Amount of total carotenoids = $\frac{A_{450} \times \text{volume of sample} \times 100 \times 4}{\text{Weight of the sample}}$

3. Results & Discussion

Inhibition of root and shoot growth of Maize and Flax was observed in all Al concentration after 3-4 days of germination. At higher concentrations of Al treatment death of leaf tips observed at higher concentration of Al treatment in both Maize and Flax. Decreased Photosynthetic activity caused chlorosis and necrosis in the leaves. Reduced leafnumber, leaf size, and total biomass were observed by Thomas., 1986. Overall stunting and purpling of stems observed in Maize and Flax. The roots are stubby - brittle and root tips became thick and turn brown in Maize and Flax. According to Mossor –Pietra-Snewska *et. al.,* 1997. Another toxic effect of Al observed was that the roots were curled in Maize and Flax. Aluminum also resulted in the formation of smaller young leaves that are curled along the margin with yellow tips and having necrotic spots. Older leaves show marginal chlorosis with subsequent lethality in Maize. (Pavan & Bingham 1982 & Foy 1984).

Figure 1a: Seed Germination under different Concentrations of Aluminum

Figure 1a: Effect of Al on early Seedling growth of Maize

Figure 1b: Maize seedling without Al concentration

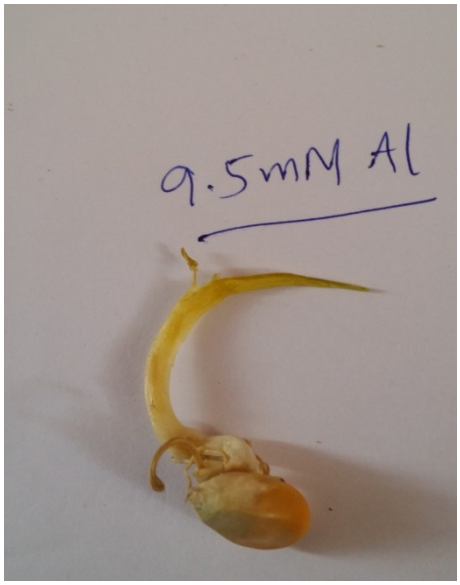

Figure 1c: Effect of Al on Maize seedlings

Figure 1

Figure 2: Effect of Al on Flax Seedlings

3.1. Root and Shoot Length

Data pertaining to root and shoot length of both Maize and Flax is represented in Table 1. Increasing concentration of Aluminum caused linear decrease in the root and shoot length in both Maize and Flax seedlings. At 9.5mM the % decrease in the root length was 21.8% in Maize and 12.06% in Flax. According to Muguira & Elgawhary 1979; Blamey et. al., 1990; Taylor, 1991; Kochian, 1995 the main symptom of Al toxicity is rapid inhibition of root growth. Al was found to induce abnormalities in the root system which include dwarfing of roots (Kerridge *et al.,* 1971). Reduction and inhibition of the growth of the main axis of root with thickening and mottling (Eleftheriou *et al.,*1993; Barcelo and poschenrieder.2002; Jorge and Menossi 2005; Jemo *et. al.,* 2006.) Root thickening and browning was observed by (Foy 1984) root curling was observed in Maize (Eleftheriou *et al.,* 1993; Barcelo and poschenrieder.2002).

Metal conc (in mM)	**Maize**		**Flax**	
	shoot length in cm	root length in cm	shoot length in cm	root length in cm
Control	5.2 ± 0.24	9.6 ± 0.08	6.5 ± 0.29	5.8 ± 0.42
1.5 mM	5.0 ± 0.21	5.5 ± 0.16	4.0 ± 0.23	3.9 ± 0.27
3.5 mM	3.7 ± 0.26	4.8 ± 0.18	3.7 ± 0.19	2.4 ± 0.23
5.5 mM	3.6 ± 0.24	2.4 ± 0.14	3.9 ± 0.14	2.0 ± 0.17
7.5 mM	3.1 ± 0.27	2.5 ± 0.12	3.8 ± 0.10	1.4 ± 0.12
9.5 mM	2.5 ± 0.14	2.1 ±0.10	3.1 ± 0.07	0.7 ± 0.08

Table 1: Effect of Al on shoot and root length of Maize and Flax.

3.2. Percent Phytotoxicity

It was calculated on the basis of root length and is shown in Table no 2.

A linear relation was observed between the concentration of Al and % of the phtotoxicity in both the plants (Maize and Flax). At 9.5mM % phtotoxicity of Al was maximum (i.e. 78.125 in Maize and 87.93 % in Flax). It was more in Flax than Maize similar trend in % phtotoxicity as a result of Al was observed in wheat, barley, rice. A marked correlation was observed between % phtotoxicity and RGI in both the plants. Increased in % phtotoxicity resulted in the decrease of RGI(Figure3and Figure3a)

Metal conc in mM	**Maize**	**Flax**
Control	0%	0%
1.5mM	42.70%	32.75%
3.5mM	50.00%	58.62%
5.5mM	75.00%	65.51%
7.5mM	73.95%	75.86%
9.5mM	78.12%	87.93%

Table 2: Percent Phytotoxity of Aluminum on Maize and Flax.

3.3. Dry Weight

The data on dry weight of root and shoot is shown in table no 3. The dry weight of both roots and shoots decreased with increase in Al treatment .At 9.5mM the root and shoot dry weight in Maize showed a reduction of 89.3% and 69.8% respectively, where as the decrease in Flax was 79.4% and 67.8%. Inhibition of growth at toxic levels of Al was shown by (Jemo *et al.,* 2006, Pereira*et al.,* 2006) and similar result shown in roots of rice (Fagria 1982, in Maize (Sierra *et al* .,2006)

3.4. RGI

The RGI was calculated from the dry weight of roots and shoots of Maize and Flax and is depicted in Table no 3. The RGI of roots and shoots of both Maize and Flax decreased with increased with increasing the concentration of metal treatment. Similar results were obtained for Zn and Ni treated pigeon pea cultivars. Ata concentration of 9.5mM of Al the RGI was 10% in the maize Roots where as it was 20% in the roots of Flax.

Metal conc (in mM)	**Maize**				**Flax**			
	Root	RGI	Shoot	RGI	Root	RGI	Shoot	RGI
Control	5.54 mg	100%	9.86mg	100%	2.52mg	100%	2.98mg	100%
1.5mM	3.24mg	58.48%	7.45mg	75.55%	2.14mg	84.92%	2.56mg	85.90%
3.5mM	1.63mg	29.42%	4.56mg	46.24%	2.13mg	84.52%	2.32mg	77.85%
5.5mM	1.57mg	28.33%	4.22mg	42.79%	1.82mg	72.22%	1.54mg	51.67%
7.5mM	1.24mg	22.38%	3.58mg	36.30%	1.64mg	65.07%	1.32mg	44.29%
9.5mM	0.59mg	10.64%	2.98mg	30.22%	0.52mg	20.63%	0.96mg	32.21%

Table 3: Effect of Al on dry matter accumulation (in mg) and relative growth index (RGI in %) of Maize and Flax seedlings

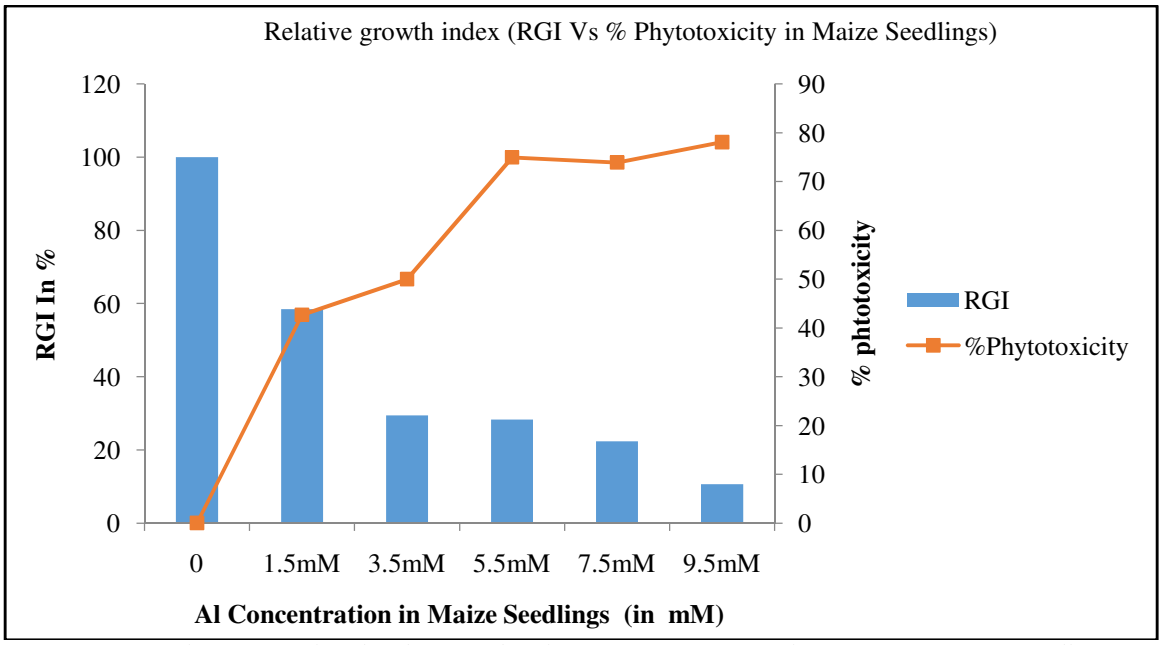

Figure 3: Relative growth index decreased with increase in percent phytotoxicity in Maize seedlings

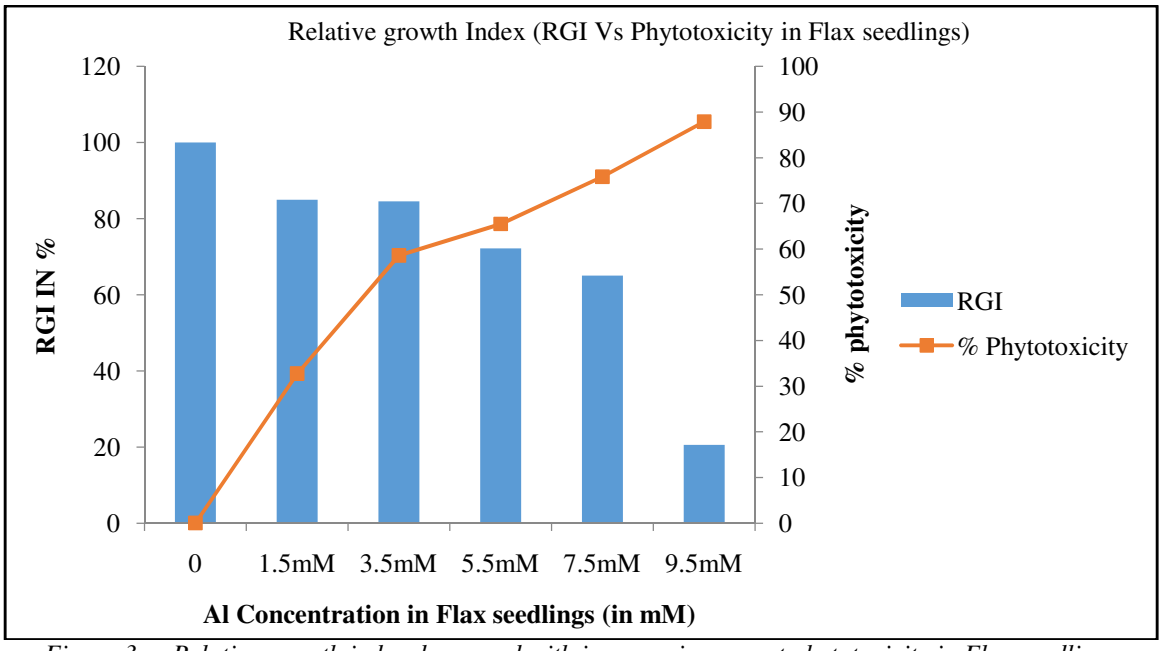

Figure 3a.: Relative growth index decreased with increase in percent phytotoxicity in Flax seedlings

3.5. Chlorophylls and Carotenoids

The contents of Carotenoids were increased progressively with the increase in the concentration of Al applied. Chl a, Chl b and total Chlorophyllconcentrations declined with elevated concentrations of Al. The present data was confirmed by D.B. Milivojevic, D.D. Stojanovic, S.D. Drinic. Dec-2000 Vol 43.Who realized remarkable Al induced reductions in the quantity of Chlorophyll pigments. (Sarkunan *et al.,*1984) Chlorophyll a and Chlorophyll b which was accompanied by degradation of thylakoids in the chloroplast (Pettersson *et al.,* 1985). Mihailovic *et. al.,* 2008 found that Al decreased chlorophyll content in Al sensitive Maize in bred line (B-73). Pereira *et al.,* 2006 demonstrated that Al effects Chlorophyll synthesis by inhibiting the activity of aminolevulinic acid dehydratase enzyme (ALA-D) responsible for the formation of monopyrole porphoblonogen is a part of the Chlorophyll molecule as well as the cytochromes and also greatly impairs plant growth. According to Parviz, Malekzadeh, P., R. Sheikhakbari Mehr and A. A. Hatamnia. 2015, the concept of photo synthetic pigments i.e.chla,chl b were decreased with increased Al concentrations in Maize. (*Zea mays*).

Figure 4: Chlorophyll Estimation by Arnon Method of Maize Seedlings.

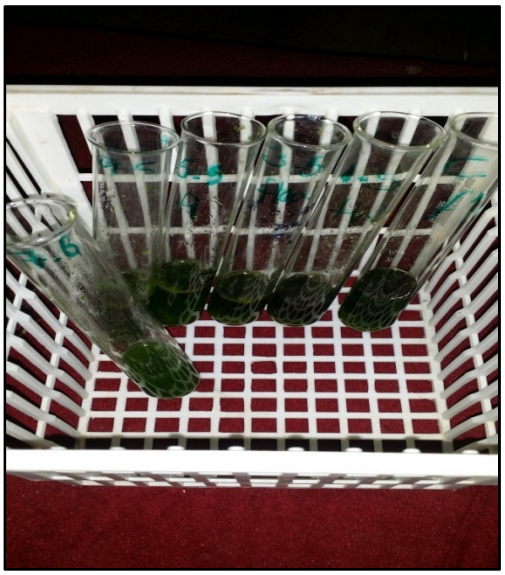

Figure 4a: Chlorophyll Estimation by Arnon Method of Flax Seedlings.

	Maize				Flax			
Al conc (in mM)	**Chl a**	**Chl b**	**Chl a+b**	**Carotenoids**	**Chl a**	**Chl b**	**Chl a+b**	**Carotenoids**
Control	3.59	1.79	5.38	1.42	3.21	1.99	5.20	1.57
1.5mM	2.82	1.68	4.5	1.98	2.67	1.63	4.30	1.74
3.5mM	2.57	1.34	3.91	2.03	2.48	1.37	3.85	1.82
5.5mM	2.12	1.29	3.41	2.07	1.65	0.93	2.58	1.91
7.5mM	1.32	0.59	1.91	2.16	1.29	0.78	2.07	2.08
9.5mM	0.72	0.36	1.08	2.48	0.58	0.42	1.00	2.72

Table 4: Chl a, Chl b, total Chlorophyll (µg/gm fw) and Carotenoids of Al treated Maize and Flax Seedlings.

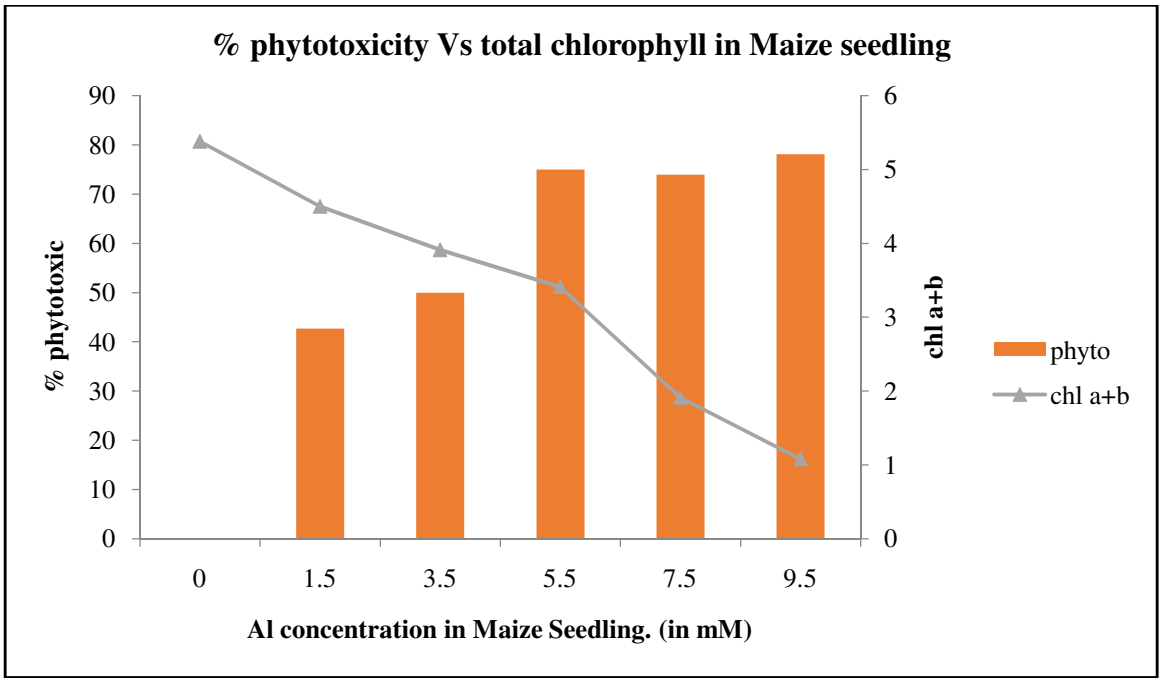

Figure 5: Total chlorophyll decreased with increase in % Phytotoxicity in Maize Seedlings

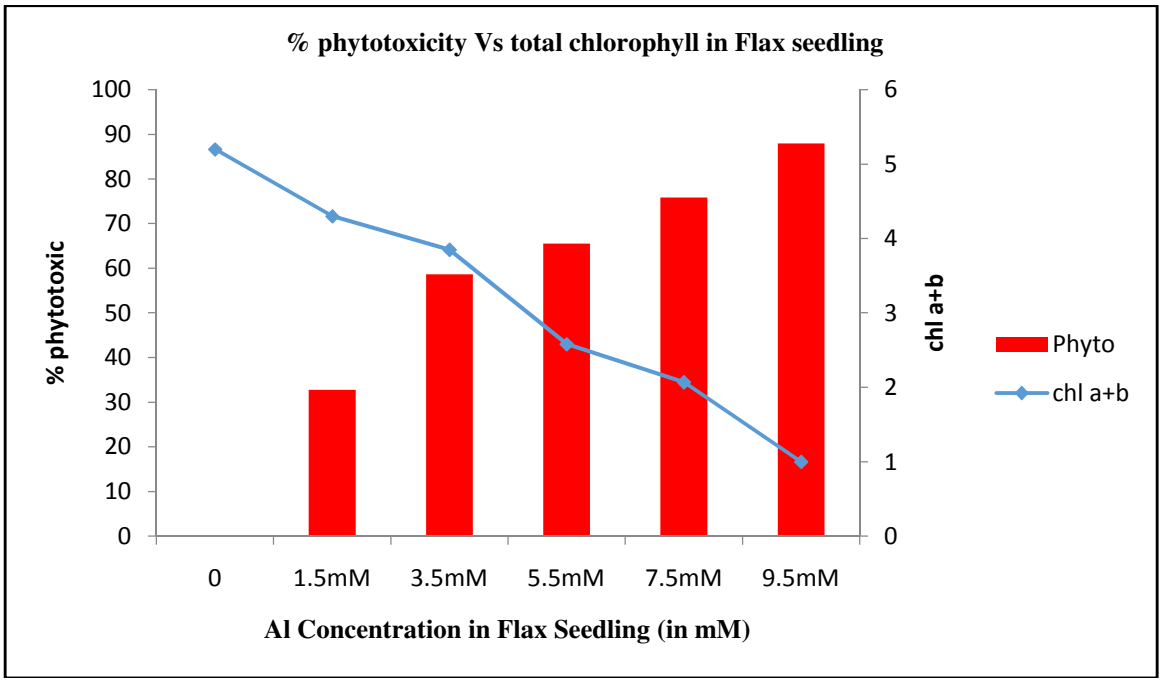

Figure 5a: Total chlorophyll decreased with increase in % Phytotoxicity in Flax Seedlings

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