



## BIOCHEMICAL ALTERATION OF MUSTARD GROWN UNDER TIN CONTAMINATED SOIL

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### Abstract

Increasing demonstrative and man-induced behavior has made soils polluted all over the world leads to the health of the people. Nowadays, heavy metal stress influences a great decrease in the quality and quantity of the agricultural produce which ultimately leads to a great threat to the population health. An increase in bioavailable Tin (Sn) in the environment can contribute to its bioaccumulation in crops, which ultimately leads to the health of the people consuming the crops. The increasing concentration of heavy metals in soil affects the quality of the crops which can ultimately lead to the health of the people. Tin is one of these which affect the quality of the product but only when applied in higher concentration. Its concentration is toxic only when it is applied in a higher dose. The metal Tin (Sn) is produced using coal in a furnace to reduce the oxide ore and affect plant growth and quality to a greater extent. A pot experiment was conducted to check the effect of Tin (Sn) in a mustard plant. The main aim of this study is to mitigate the rhizosphere Tin (Sn) by mycorrhiza fungi with special reference to biochemical changes. In this study we perform the different biochemicals of plants viz; Total soluble sugar, Total soluble protein, Chlorophyll index, catalase activity to check the effects of Tin (Sn) on the plant growth and development. For this, Sn toxicity is created in the soil with stannous chloride (50ppm). 150 spores of Mycorrhiza fungi per kg of soil are also added to mitigate the effect of Sn in the plants. There is a significant increase in all the biochemical activities in treatment T1 (Mycorrhiza) as compared to the control (T0) and a slight decrease in treatment T2 in which metal (Sn) stress is there. Therefore, Mycorrhiza shows better results in the mitigation of Tin (Sn) stress.

**Keywords:** Agriculture, Biological, Chlorophyll, Environment, Growth, Injury, Membrane, Mitigation, Stability, Tin.

### Introduction

Tin is a chemical element with the symbol Sn and atomic no. 50 and atomic weight 118.710. The Discovery of Tin has been taking place around 3500 BC. Tin (Sn) is a naturally occurring heavy metal in the earth's crust. Its average concentration in the earth's crust is about 2 mg/kg. Due to the natural weathering of rocks, it becomes a part of many soils. Tin (Sn) is found in two oxidative states, stannous (+2) and stannic (+4) (Kumar and Dwivedi, 2018a; Kumar *et al.*, 2018b; Kumar *et al.*, 2018c; Kumar and Dwivedi, 2018d; Kumar *et al.*, 2018e; Kumar and Pathak, (2019f); Kumar *et al.*, 2019g; Siddique *et al.*, 2018h; Siddique *et al.*, 2018i; Pathak *et al.*, 2017j; Prakash *et al.*, 2017k; Kumar and Mandal, 2014L). The normal range of Tin (Sn) in unpolluted soils is less than or between 1 mg/kg to 200 mg/kg (Shacklette & Boerngen, 1984). Usually, Tin is used in canned foodstuffs or Tin cans are used for various energy drinks. But a limited concentration of Tin is required in the cans. The blind use of the metal concentration can lead to the hazardous effects in population and environment both. The main source of heavy metal is excessive use of fertilizers, mining industries, and smelting industries, sewage, and sludge prone areas, etc. At present heavy metals are used in rechargeable batteries which leads to the environmental pollution (Kumar, 2018vii; Kumar, 2018viii; Kumar and Pathak, 2018ix; Kumar and Pathak, 2018x; Kumar and Pathak, 2018xi; Kumar *et al.*, 2018xiii; Kumar and Pathak, 2018xiv). In the environment, the concentration of Tin is different in nature as well as man-induced conditions i.e. agriculture, mining and industrialization activities (Ashraf *et al.*, 2011). In some areas, Tin concentration exceeds higher reaching up to 1000 mg/kg due to these increase in the agricultural and mining practices (Kumar *et al.*, 2014m; Kumar *et al.*, 2014n; Kumar, 2013o; Kumar and Dwivedi, 2015p; Gogia *et al.*, 2014q; Kumar,

2014r; Kumar *et al.*, 2012s; Mishra *et al.*, 2012t; Kumar *et al.*, 2011u; Kumar *et al.*, 2011v; Kumar and Pathak, 2016w; Pathak *et al.*, 2016x; Kumar *et al.*, 2018y; Kumar *et al.*, 2018z). Tin as lower concentration usually not affect plant growth but its higher concentration affects the plant and human health. Its toxicity causes the disease like fatigue, headaches, diarrhea, vomiting, muscular weakness and paralysis, anemia, excessive damage to the liver and kidneys, and affects the various levels of neurotransmitters in the brain (Kumar *et al.*, 2018aa; Kumar *et al.*, 2018bb; Kumar *et al.*, 2018cc; Kumar and Dwivedi, 2018gg; Kumar *et al.*, 2018ff; Kumar *et al.*, 2018cd; Kumar and Pathak, 2018kk; Kumar and Pathak, 2018pq; Singh *et al.*, 2020a; Singh *et al.*, 2020b; Sood *et al.*, 2020; Bhadrecha *et al.*, 2020; Singh *et al.*, 2020c; Sharma *et al.*, 2020; Singh *et al.*, 2020d; Bhati *et al.*, 2020; Singh *et al.*, 2019 and Sharma *et al.*, 2019). Nowadays, Tin toxicity is going to be found in the crops which are incorporated in the plant tissues and affect the people who consume these crops. Therefore, it is important to understand the uptake of Tin (Sn) by different crops and its accumulation in different crops to save our food products. 40-700 mg/kg Tin (Sn) concentration in sewage and sludge is used for the production of crops in some areas which are toxic to human health (Kumar, 2018i; Kumar, 2018ii; Kumar, 2018iii; Kumar, 2018iv; Kumar, 2018v; Kumar, 2018vi; Kumar, 2018vii; Kumar, 2018viii; Kumar and Pathak, 2018ix; Kumar and Pathak, 2018x; Kumar and Pathak, 2018xi; Kumar *et al.*, 2018xiii; Kumar and Pathak, 2018xiv; Kumar and Pathak, 2018xv; Kumar and Pathak, 2018xvi; Kumar and Pathak, 2018xvii; Kumar and Pathak, 2018xviii).

## Materials and Methods

### Growing conditions and sample preparation:

A pot experiment was conducted at agriculture farm, School of agriculture, Lovely Professional University, Phagwara region, Punjab which is a North-Eastern state and comes under central plain zones. In Phagwara, the average annual temperature is 24.1 °C / 75.3 °F and the rainfall is 686 mm / 27.0 inch. The soil of central Punjab ranges from sandy loam to clayey with pH value ranges from 7.8 to 8.5. The temperature falls to 5°C in winters and maximum up to 40-45°C in summers. Mustard (*Brassica juncea*, L.) seeds variety PBR-357 were grown in 12 pots having dimensions 30×25 diameter and height respectively, in a completely randomized design. The seeds are collected from the Punjab Agriculture University Ludhiana. Four treatments (T, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>) and three replications (R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>) were taken to check and analyze the results. Before 2 days of sowing, Tin (Sn) toxicity is created in the soil of the pots by the application of Stannous chloride (SnCl<sub>2</sub>.2H<sub>2</sub>O) 50 ppm per 10 kg of soil and Mycorrhiza 150 spores per kg of soil was also added as a treatment. Leaf samples of 15 days after sowing were taken for the estimation of different biochemical in the plant (Table 1).

**Table 1:** Treatment details

Treatments	Details of the Treatments
T	Control
T <sub>1</sub>	Mycorrhiza (150 spores per kg of soil)
T <sub>2</sub>	Tin (Sn) 50ppm conc. per 10 kg of soil
T <sub>3</sub>	Tin (Sn) (50 ppm) + Mycorrhiza (150 spores)

### Overall detail and layout of the experiment

**Table 2:** Experimental Details

S. No.	Particulars	Details
1.	Design	CRD
2.	Genotype	PBR-357
3.	No. of Treatments	4
4.	No. of Replications	3
5.	Soil per pot	10 Kg
6.	Total number of pots	4*3= 12

### Observations to be recorded are:

The leaf sample of 15 days after sowing (DAS) was taken for the estimation of biochemical changes in the plant. The standard procedure adopted for the estimation of biochemicals for different treatment are given below:

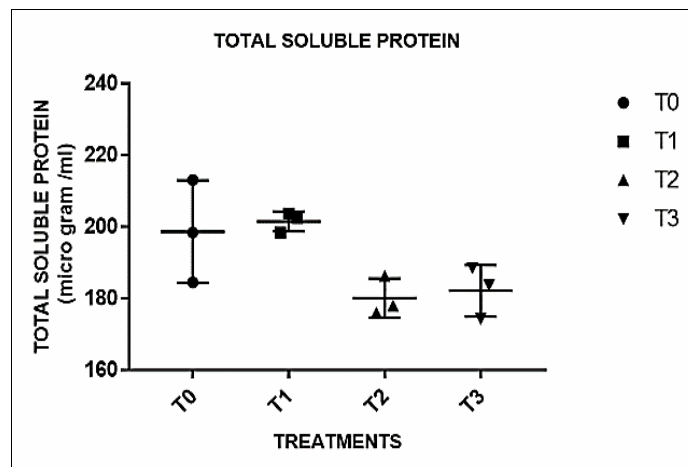
- Total Soluble Sugar:** It is estimated by the method proposed by Sadasuvam and Manickam (1992).
- Total Soluble Protein:** It is estimated by the method proposed by Bradford, (1976).
- Total Phenols:** For the estimation of total phenol the protocol of Mahadevan and Sridhar (1982) is followed.
- Chlorophyll Index:** SPAD meter is used for the measurement of the Chlorophyll index.
- Catalase Activity:** the activity of enzyme Catalase was measured according to the protocol given by Aebi *et al* (1983).

## Results and Discussions

### 1. Total Soluble Proteins

For the estimation of Total Soluble Sugars, we follow the protocol given by Sadasuvam and Manickam (1992). For

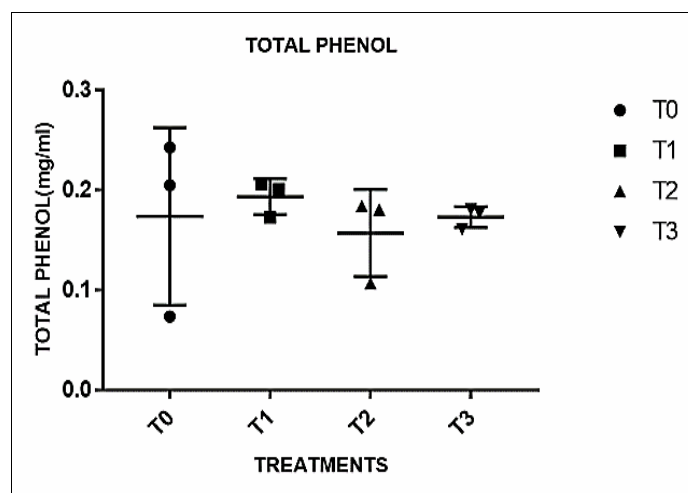
this, firstly we centrifuge our samples at 15000rpm for 15 min. and then observations were recorded by spectrophotometer at 595nm wavelength. The treatment T<sub>2</sub> (Sn) (50 ppm) shows a 60% decrease in protein content and T<sub>3</sub> (Mycorrhiza (150 spores per kg) + Sn (50 ppm) shows a 40% decrease as compared to T<sub>0</sub> and T<sub>1</sub>. T<sub>1</sub> (Mycorrhiza) shows a great increase in protein content i.e. 60%. The above study shows that there is a significant decrease in the protein content where there is Tin contaminated soil (Fig. 1).



**Fig. 1:** Total Soluble Proteins (TSP)

### 2. Total Phenols

To estimate the Total Phenols, we follow the protocol given by Mahadevan and Sridhar (1982). For this, we make observations on a spectrophotometer at 650 nm wavelength. According to this study, treatment having mycorrhiza shows a great increase in the total phenol content i.e. 80% as compared to the control and treatment with heavy metal (Sn) shows a 63% decrease in total phenols as compare to the control. Treatment having Sn + Mycorrhiza shows a slight decrease i.e. 10% as compare to control (Fig. 2).



**Fig. 2 :** Total Phenols

### 3. Chlorophyll Index

We use the SPAD meter to determine the chlorophyll index of the mustard leaves. For this, we put the instrument on 4-5 leaves of the plant and take its average as an index. Treatment first having Mycorrhiza shows a higher chlorophyll index (75%) as compared to all three. There is a significant decrease in treatment having Tin and Tin + Mycorrhiza i.e. 65% and 63% respectively as compared to control (Fig. 3).

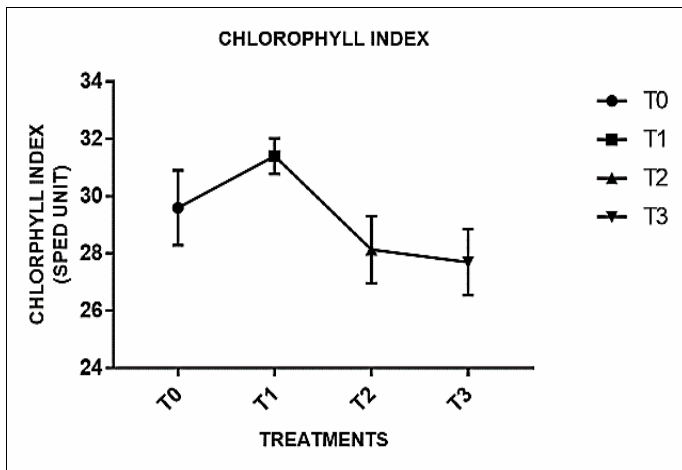


Fig. 3 : Chlorophyll Index

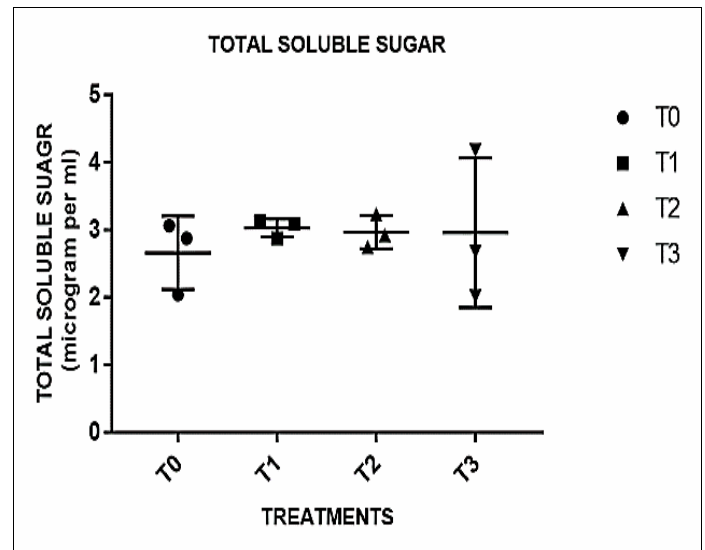


Fig. 5 : Total Soluble Sugars

4. Catalase

To estimate the catalase activity, we perform the protocol of Aebi *et al.* (1983). For determining catalase activity, we take reading on a spectrophotometer at 240nm wavelength. My study shows that there is a slight increase in the treatment having Mycorrhiza and a slight decrease in the treatment having metal stress i.e. 9.50% and 10% respectively as compared to the control. Treatment having Tin + Mycorrhiza shows a 12% increase in catalase as compare to control. The treatment shows a slight decrease in catalase where there is Tin (Sn) contaminated soils. Whereas other results are close to each other. The above results show that Tin does not affect the catalase activity of the plants too much extent (Fig. 4).

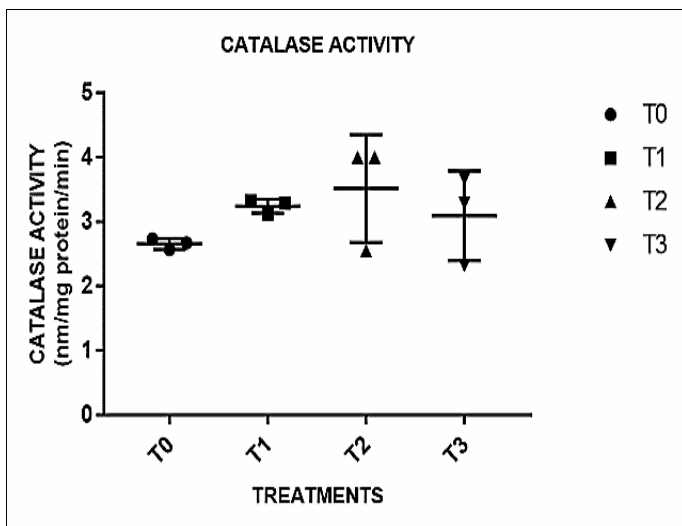


Fig. 4 : Catalase activity

5. Total Soluble Sugars

For the estimation of Total Soluble Sugars, we follow the protocol of Sadasuvam and Manickam (1992). To determine the sugar content observations are taken on a spectrophotometer at 620nm wavelength. According to my study, treatment having Mycorrhiza and Tin + Mycorrhiza shows a significant increase in Total Soluble Sugars i.e. 43% as compare to control. In the treatment of having mental stress, there is a 41% decrease in Sugar content as compare to treatments having Mycorrhiza in the soil. This shows that there is a visual increase in the Sugar level of the plants growing in the soils having Mycorrhiza (Figure 5).

Conclusion and Recommendations

Under natural conditions, Tin does not show any toxicity to the plants as well as humans. But a significant increase in the anthropogenic activities or industrialization of its concentration in the soil increases. Its higher concentration affects the growth and quality of the food products and ultimately human health. The above study gives results that treatment having high Sn concentration shows a decrease concerning biochemical and treatment having Mycorrhiza alone shows a comparatively good increase. On higher concentration, Sn affects the plant cell membrane first, enters the plant tissues and subsequently affects the quality of the produce. Tin stress slightly affects the catalase activity of the mustard plant while Mycorrhiza shows good results for all the biochemical. Mycorrhiza shows better results in the mitigation of Tin (Sn) stress therefore, this study recommends Mycorrhiza to mitigate Tin stress in the mustard plant.

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Author Contributions

The study was designed by P.K. and M.S. the biochemical protocolizations were established, the experiment was carried out and the data analyzed and interpreted were collected. The paper has been written by the author.

Conflict of Interest Statement

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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