**MORPHO-ANATOMICAL ADAPTIVE STRATEGIES OF PURPLE NUT SEDGE**

**(*Cyperus rotundus* L.) PLANTS AND COMPENSATING ROLE OF SILICON**

**AGAINST LEAD STRESS**

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Novelty statement

Silicon has been rarely used for improving plant growth under stress conditions. These results show that foliar application of silicon to nut sedge was effective in improving almost all morphological parameters of plant. So silicon application mitigated the effects of lead stress.

**Abstract**

Environmental pollution is the main serious threats for cultivated as well as native wild plants. Sewage waste is another burning issue that is increasing day by day in Pakistan due to rapid overpopulation and urbanization. It is important to study the mechanisms for the toxicity of heavy metals and morpho-anatomical behavior of plants in stress. The existing methods for remediation applicable to soils contaminated with heavy metals are expensive, environmental invasive and labor intensive. Lead (Pb) is present in all the components of environment like in soils, water, air and living organisms as contaminant pollutant. Silicon can develop the stress strategy in plants by promoting its growth and development in many plant species under heavy metals stress. Therefore the tolerance of *Cyperus rotundus* to heavy metal toxicity with and without application of silicon is to be determined. After establishment of the plants, stress was induced by applying the 50 ml of five different levels of heavy metal lead with and without foliar application of silicon. Morpho-anatomical attributes of plant were observed for evaluating the role of silicon in stability and adaptability of *Cyperus rotundus*. There were supporting results about the plasticity nature of plant mitigating the effect of heavy metal with the application of silicon.

**Keywords**: Hazardous metal; Foliar spray; Silicon; Mitigating; Adaptive strategies; Nut sedge

**Introduction**

Among the global issues, pollution is contaminating various components of ecosystem and environment as well environmental pollution is the main serious threats for cultivated as well as native wild plants. Industrialization over the past century has resulted in wide spread contamination of the environment with persistent organic and inorganic wastes (Cui *et al.* 2016; Liu *et al.* 2013; Wei and Yang 2010; Zhang *et al.* 2015). Transfer of pollution into non-pollution sites as dust, soil elements and sludge are cause of causing pollution of natural ecosystems (Rascio and Navari-Izzo 2011). In many countries, the issue associated with the dumping of wastes generated by pollution centers, both large and small, together with associated industrial activities have become alarming environmental issue (Ahmad *et al.* 2011; Ali *et al.* 2013). The realization that both treated and untreated dumping of wastes can result in the pollution of soils, surface water and ground water has caused to the development of a considerable regulatory measures and protocols purposed at mitigating or complete removal of pollution. Moreover, dumping of wastes is causing production more hazardous chemicals.

Sewage waste is another burning issue that is increasing day by day in Pakistan due to rapid overpopulation and urbanization. Untreated city wastes, industrial effluents and domestic wastes are polluting the environment, which ultimately affecting the food chain and adversely influencing the plant productivity and human health (Hernandez *et al.* 2008; Xin *et al.* 2016). So, among the global problems, environmental pollution is the main problem to seriously concerned because of its adverse effects on human health, animals, plants and exposed materials (Gulfraz *et al.* 2002). The distribution of soil pollution is more by man’s activities than other natural ways, and heavy metals pollution is more dominating pollution in soil environment (Fellet *et al.* 2014; Waqas *et al.* 2014; Yong *et al.* 2011).

A number of metals like Cu, Mn, Ni, Co, Cd, As, Pb, Fe and Zn are essential for the development of plants but at elevated concentrations can result in the reduction of plant growth (Gulfraz *et al.* 2003). The leaching of solutions into deeper soil layers may enhance its uptake by plants roots (Cui *et al.* 2012). Among these heavy metals, the high proportion of lead is responsible in decreasing the thickness of xylem and phloem (Chmielewska *et al.* 2005). The maximum proportion of lead also caused darkening of roots, stunned root growth, changes in morphology of plant body. It is reported that Pb is accumulated more in roots as compared to the shoots (Zu-li *et al.* 2011). The high concentration of lead affected the inhibition in shoot length, reducing the dry weight of plant body and decreasing the number of tillers (Bhatti *et al.* 2013). The mesophyll cells and vascular bundles are also affected by high concentration of lead. The mesophyll cells are scattered with less number of chloroplasts in the leaves. The decrease in diameter of roots, thickness of cortex and number of trichomes was also observed in the lead treated maize plants (Singh *et al.* 2015). Lead (Pb) influenced its toxicity on biochemical, morphological, and physiological aspect of plants. It has detrimental effects on cell division, plant growth, seed germination, chlorophyll production, transpiration, seedling development and root elongation (Krzeslowska *et al.* 2009; Maestri *et al.* 2010). However, the intensity of its effect depends on the lead concentration, duration, the range of plant stress and stage of plant growth. Plants achieved several internal adaptive methods to deal with metal toxicity including selective metal uptake, compartmentalization, and excretion (Jing and Liu 2010; Krzeslowska *et al.* 2010; Maestri, *et al.* 2010; Singh *et al.* 2010).

It is important to study the mechanisms for the toxicity, transfer and uptake of heavy metals. This is achieved by growing plants in limited polluted soils i.e. it reduced absorption of lead (Pb) as in vegetables or increased transfer when phytoextraction is required. Mostly legumes are considered good for growing in lead contaminated soils as compared to Liliaceae, Umbelliferae, Chenopodiaceae and Compositae, because of its reduce transfer of lead (Alexander *et al.* 2006).

Due to adverse effects of high metal concentration on most living organisms, techniques have been developed to cure the contaminated soils. Remediation of contaminated soils is based on physical (soil isolation, replacement and verification), chemical (soil immobilization and washing of pollutants) and biological (phytoevaporation, phytoextraction and phytostabilization) techniques (Khalid *et al.* 2017). The existing methods for remediation applicable to soils contaminated with heavy metals are expensive, environmental invasive and labor intensive. The selection of method must always be based on minimum environmental impact, maximum safety and as far as possible, recyclable end products (Zehra *et al.,* 2009). An environmental pollution remediation technique that is of less expensive but safe to human health and the environment would be a valuable addition to current remediation methods by utilizing the potential of plant species (Gardea-Torresdey *et al.* 2004; Qadir *et al.* 2004; Wanat *et al.* 2013) being known as Phytoremediation. Phytoremediation is categorized into phytodegradation, phytoextraction, phytovolatiliation, rhizofiltration and phytostabiliation.

Lead (Pb) is present in all the components of environment like in soils, water, air and living organisms as contaminant pollutant. The combustion of lead contaminated gasoline, smelting, or use of leaded media i.e. sewage sludge and fertilizers are the main source of lead to environment (Grover *et al.* 2010; Sammut *et al.* 2010).

Moreover, lead (Pb) is declared as the second being problematic element, after arsenic based on the toxicity, frequency of occurrence and its potential to cause hazardous problem for man by the Agency for Toxic Substances and Disease Registry (ATSDR 2003). The lead is widely studied in its several aspects like it transfer from soils to plants, use in biotesting or in phytoremediation (Arshad *et al.* 2008; Uzu *et al.* 2010).

The most beneficial serving element for plant growth and development is the silicon (Si). Which is the second most abundant element in soil after the oxygen (Luyckx *et al.* 2017) and readily absorbed; land plants contain it at an appropriate concentration, 1% to 10% or even higher in plant dry matter (Karathanasis 2002). Silicon can increase tolerance and resistance against biotic and abiotic stresses in many plants (Liang *et al.* 2015) by producing enzymatic and non-enzymatic antioxidants (Vaculik *et al.* 2012). Silicon can develop the stress strategy in plants by promoting its growth and development in many plant species (Bharwana *et al.* 2013; Liu *et al.* 2003; Liu *et al.* 2013a ; Vaculik *et al.* 2012; Zhang *et al.* 2012).

The family Cyperaceae commonly known as sedges has 5000 species and 104 genera (Goetghebeur 1998; Mishra and Chauhan 2013). It is the third largest family among the monocots evenly distributed in populated areas and in tropics.

Sedges are a group of plants which form clumps mounds or tussocks, they have very long narrow and evergreen leaves with or without Roots or stolons. Some of them are nearly ankle-height, many of them are knee height and some even can grow to waist height. They are particularly good growing in situations with poor soil, as they are remarkably tough and establish themselves well, often being capable of spreading quite extensively (Qureshi, 2004). Most sedges grow almost anywhere, but particularly like damp places and indeed there are several sedges which like really waterlogged soils (Parmar *et al.* 2013).

*Cyperus rotundus L*. is also called as Nut grass or Purple Nut sedge is a straight and evergreen sedge which have extensive root and root hairs system (Al-Snafi 2016). It is from family Cyperaceae that consists of about 3000 species, out of which approximately 220 are weeds and of which constitutes 42 % of genus *Cyperus*. In which *C. rotundus* and *C. conglomeratus* both are present (Omezine and Harzallah 2009).

**AIMS AND OBJECTIVES**

That species is widespread throughout the Lesser Cholistan Desert and many areas of the Punjab plain including polluted soils and industrial wastes. The present studies, therefore, conducted to evaluate the tolerance of *Cyperus rotundus* to heavy metal toxicity. Research studies have identified heavy metals as a potential threat to the environment, but little work has been carried out in remediation of these metals. Heavy metal toxicity in natural populations, in particular sedges have been least studied, especially with reference to phytoaccumulation.

The present research work aimed to study the morpho-anatomical characterization of plant for metal contamination, the potential role of plant species in phytoremediation. To alleviate and reduce the negative effects of heavy metals, various diagnosis have been evaluated. Silicon (Si) application has been known to enhance the tolerance of plants against abiotic stresses. However, the protective mechanism of Si under heavy metals contamination is poorly understood. The aim of this study is to assess the role of Si in counteracting heavy metal toxicity and also the supplemental role of Silicon to cope with the environmental hazards. The specific objectives are as follows:

* Study the growing capacity of *Cyperus rotundus* and its adaptability under heavy metal stress.
* Evaluate the compensation role of Silicon on the plants in the morpho-anatomical modifications in plant growing in heavy metal contaminated soil.

**MATERIALS AND METHODS**

In Southern Punjab province of Pakistan, the Cholistan is the vast desert spreading more 26000 km2. Its coordinates are 27o 42’ and 29o 45’ latitudes in North and 69o 52’ and 75o 24’ longitudes in East (Arshad *et al.,* 2008). It is mainly occupied by sand dunes, saline patches and some Dahars. In Lesser Cholistan Desert only two species of family Cyperaceae are present known as sedges.

**Experiment material:** The sedge, *Cyperus rotundus* Linn. was collected from the desert to study the effect of heavy metal and its accumulation in them. Fully grown plants was uprooted randomly and established in pots in nursery at The Islamia University of Bahawalpur. The experiment was carried out to find out the morphological and anatomical parameters of plants in the laboratory of Department of Botany.

**Treatments:** After establishment of the plants, stress was induced by applying the five different levels (0, 30, 60, 90 and 120 mg/L) of heavy metal Pb(No3)2 with and without foliar application of silicon (Na2SiO3; 1.0 mM). The 50 ml of each level of treatment of metal was given in the roots of plants without and with foliar spray of Sodium Silicate. Whole experiment was arranged with nine replicates. The heavy metal stress was given after every 10 days while normal irrigation was done with distilled water accordingly. The experiment was continued till its reproductive stage.

**Plant harvesting**

After the two months experiment completion, each plant was sampled from the pots with root carefully. The samples of *Cyperus rotundus* L. plants were collected from each treatment randomly and cleaned by washing them in the tap water to remove all dirt and soil particles from vegetative parts and preserved for the anatomical study.

The morphological parameters, height of plant, Internodal length, Length of inflorescence, Fresh weight of plant, Dry weight of plant, Number of vegetative branch, Number of leaves, number of fertile branches and Number of nodes were observed.

**Staining technique**

For the fixation purpose, the parts of plants i.e. leaves, Root and stem are cut down by scissors into pieces of almost 2-3cm. The parts were added in the FAA (mixture of formaldehyde, alcohol and acetic acid) for the time of 48 hours. FAA that is a preservative solution was formed by mixing the Ethyl alcohol 50%, Acetic acid 5%, Formalin 10% and Distilled water 35%. After 48 hours, the samples are transferred into the mixture of Alcohol and acetic acid that was prepared by the ratio of 3:1 of its ingredients. Double staining dehydration method was used for the formation of slides and capturing their images by the camera. The stains used in the staining technique include safranine and fast green (Ruzin 1999).

In double staining technique, the dehydration is very necessary for the formation of a clear slide. The clear images were clicked by using the camera (Nikon, 104, Japan) under 10X and 40X in the laboratory of “The department of Botany” The Islamia University Bahawalpur.

**Statistical analysis**

SPSS.20 software was used for analysis of variance and Significant differences within and between the treatments.

**Results**

**Morphological Attributes of Cyperus rotundus:**

**Height of Plants (cm):**

**Plants treated with Pb(NO3)3 and Pb(NO3)3 with Na2SiO3:**

The differences among the values of height of plants treated with sodium silicate (Na2SiO3) and different concentrations of lead nitrates (Pb(NO3)3) have been shown in the Fig. 1a. The plants of control group have the maximum height of plant as compared to the plants under stress. While there was decreasing in height of plants between the plants of different treatments. That shown decreasing trend as concentrations of metal increases. The height of plants applied foliar application of sodium silicate gave the compensation effect of metal as compared to the plants grown under metallic stress without foliar application. The height of plant of control *Cyperus rotundus* plant is 17.36 cm more from the height of all *Cyperus* *rotundus* plants treated with foliar application of Na2SiO3. Only maximum height of plant treated with 30 mg/L of lead nitrate with using foliar application of sodium silicate is 13.53 cm and in plant under same level of treated without sodium silicate has 12.43 cm of plant height. Lead nitrates suppressed the height of plant as by increasing the concentration of lead nitrate but there is promoting effect is observed in the height of plant of *Cyperus rotundus* under lead nitrate stress but with foliar application of sodium silicate. Comparison Fig. 1a clearly evident that 12.43 cm, 11.76 cm, 9.76 cm and 5.60 cm heights was observed in plants under stress condition of various treatments of lead nitrate. While plants grown in same levels of treatments but with foliar application of sodium silicate have 13.53 cm, 11.70 cm, 10.26 and 5.66 cm, that is greater in height as compared to plants grown without sodium silicates but less than the plants grown in control condition.

**Internodal length (cm):**

**Plants treated with Pb(NO3)3 and Pb(NO3)3 with Na2SiO3:**

The differences between the mean values of internodal of *Cyperus rotundus* grown under lead nitrate is given in Fig.1b. The plants of control group have the maximum internodal length of plant as compared to the plants under stress. While there was decreasing in internodal length of plants between the plants of different treatments. That shown decreasing trend as concentrations of metal increases. The internodal length of plants apply foliar application of sodium silicate gave the compensation effect of metal as compared to the plants grown under metallic stress without foliar application. The internodal length of control plants is maximum i.e 10.06 cm. There is clear difference in internodal length between the plants treated with only lead nitrate and with foliar application of sodium silicate. There is the decreasing trend in that morphological parameter. The maximum internodal length is 7.63 cm in plants treated with 30 mg/L while the minimum internodal length is evident in the plants treated with 120 mg/L i.e 4.53 cm as shown in Fig. 4.4. Other values are range between these two limits. The stimulating effect of silicon also observed that parameter. There is overall increase in internodal length of plants of *Cyperus rotundus* when plants are treated lead nitrates with foliar application of sodium silicate. The maximum observed value is 8.30 cm in level of 30 mg/L and minimum value is 4.83 cm was observed in the 120 mg/L lead treated with sodium silicate plants as in Fig 1b.

**Length of Inflorescence (cm):**

**Plants treated with Pb(NO3)3 and Pb(NO3)3 with Na2SiO3:**

The mean values of different morphological parameters of *Cyperus rotundus* graphically explained. In this way the mean values of length of inflorescence of plants treated with lead nitrate without and with sodium silicate are shown in Fig. 1c. The plants of control group have the maximum length of inflorescence of plant as compared to the plants under stress. While there was decreasing in length of inflorescence of plants between the plants of different treatments. That shown decreasing trend as concentrations of metal increases. The length of inflorescence of plants applied foliar application of sodium silicate gave the compensation effect of metal as compared to the plants grown under metallic stress without foliar application. The maximum length of inflorescence is observed in control plants which is 3.80 cm as compared to values of other plants. The length of inflorescence in 30 mg/L is 2.80 cm is larger than the plants grown in 60 mg/L, 90 mg/L and 120 mg/L solutions of lead nitrate without sodium silicate. The minimum length is observed in plants of 120 mg/L which is 0.96 cm as given in Fig. 4.7. The plants treated with lead nitrate and foliar application of sodium silicate is larger as the length of plants grown in only lead nitrate. The maximum length of inflorescence is 3.03 cm is in plants treated with 30 mg/L solution of lead nitrate with the foliar application of sodium silicate. That size is greater than the length of inflorescence of plants grown in higher concentration of solutions with same foliar application as given in Fig.4.7. The minimum length is 1.33 cm of plants grown in 120 mg/L solution of lead nitrate with foliar spray of sodium silicate. The length of inflorescence is 1.90 cm is also observed in the plants grown in 90 mg/L in same treatment of silicon which is slightly larger than the length of plants grown in 120 mg/L solutions.

**Fresh weight of plant (gm):**

**Plants treated with Pb(NO3)3 and Pb(NO3)3 with Na2SiO3:**

The mean values of fresh weight of plants treated with different concentrations of lead nitrate without and with sodium silicate are shown in Fig. 1d. The plants of control group have the maximum fresh weight of plant as compared to the plants under stress. While there was decreasing in fresh weight of plants between the plants of different treatments. That shown decreasing trend as concentrations of metal increases. The fresh weight of plants applied foliar application of sodium silicate has the compensation effect of metal as compared to the plants grown under metallic stress without foliar application. The maximum fresh weight is observed in plants of control group, which is 0.66 gm. The maximum fresh weight of plant is 0.53 gm from the 30 mg/L solution of lead nitrate without sodium silicate. That is maximum weight as compared to other plants treated with different concentrations of lead nitrate only. The minimum fresh weight of plant is 0.28 gm which was treated with 120 mg/L of lead nitrate without spraying sodium silicate. In it the sodium silicate is also help in over all increasing the weight of plants by mitigating the effect of heavy metals. So, the fresh weights of those plants are also increases grown in lead nitrate with foliar application of sodium silicate as given in Fig. 1d. The maximum fresh weight of plant is 0.61 gm grown in 30 mg/L solution of lead nitrate with applying sodium silicate that weight is also second maximum after fresh weight of control plants which is 0.66 gm. While the minimum mean fresh weight of those plants grown in 120 mg/L concentration of lead nitrate with foliar spay of sodium silicate is 0.32 gm. That fresh weight is also maximum weight as the fresh weight of those plants grown in lead nitrate without sodium silicate as shown in Fig.1d.

**Dry weight of plant (gm):**

**Plants treated with Pb(NO3)3 and Pb(NO3)3 with Na2SiO3:**

The mean values differences of dry weight of plants are shown in the Fig. 1e. The dry weight of control plants is 0.25 gm which largest weight as compared to the all lead nitrate treated groups of plants as given in Fig. 1d. The plants of control group have the maximum dry weight of plant as compared to the plants under stress. While there was decreasing in height of plants between the plants of different treatments. That shown decreasing trend as concentrations of metal increases. The dry weight of plants applied foliar application of sodium silicate reduces the effect of metal as compared to the plants grown under metallic stress without foliar application. In it sodium silicate is also help to increase the overall dry weight of all lead nitrate treated plants. The plants in which only lead nitrate solutions are used, the plants have 0.140 gm which is the maximum dry weight of 30 mg/L solution of lead nitrate among the others plant of same treatment. The minimum dry weight is 0.053 gm is observed in the plants which are treated with 120 mg/L solution of lead nitrate. While the those plants which grown in lead nitrate with foliar application of sodium silicate have maximum dry weight as compared to the same levels of treatment without foliar spray. The maximum mean values of dry weight of those plants grown in 30 mg/L solution of lead nitrate with foliar application have 0.22 gm. That dry weight is smaller with the control plants dry weight but greater as compared to the other plants with same levels of treatment solutions. The minimum dry weight is 0.09 gm observed from the plants grown in 120 mg/L of lead nitrate with foliar application of sodium silicate.

**Number of vegetative branch:**

**Plants treated with Pb(NO3)3 and Pb(NO3)3 with Na2SiO3:**

The mean values of differences of number of vegetative branch of plants of *Cyperus rotundus* treated with lead nitrate without and with sodium silicate are given in Fig. 1f. The plants of control group have the maximum height of plant as compared to the plants under stress. While there was increasing in number of vegetative branch of plants between the plants of different treatments. That shown increasing trend as concentrations of metal increases except in plants grown in metal level of 120 mg/L. The number of vegetative branch of plants applied foliar application of sodium silicate reduce the effect of metal as compared to the plants grown under metallic stress without foliar application. The control plants have no. of vegetative branch 2.33, which is maximum mean value among all the other plants. The plants grown in 30 mg/L of lead nitrate has mean value of no. of vegetative branch is 1.33 which is less as other plants. While, the plants grown in 90 mg/L have 2.0, which is slightly less than the control group of plants. The plants grown in 60 mg/L and 120 mg/L solutions of lead nitrate has equal value of no. of vegetative branch i.e 1.67. In that parameter sodium silicate has not any significance effect, so the plants grown in 30 mg/L of lead nitrate with foliar spray of sodium silicate have 1.33 value of no. of vegetative branch which equal to the plants grown in 90 mg/L solution but less than the other plants. That also equal to the value of plants of 30 mg/L solution of lead nitrate without sodium silicate as shown in Fig. 1f. While the plants from 60 mg/L solution of lead nitrate with foliar spray of sodium silicate have value of 2.0 of no. of vegetative branch, that is slightly less than control plants, equal to the value of plants from 90 mg/L solution of lead nitrate without sodium silicate and larger than the other plants. The plants grown in 120 mg/L solution of lead nitrate with application of sodium silicate have 1.67 values, which equal to the plants grown in 60 mg/L and 120 mg/L concentrations of lead nitrate without sodium silicate. That value is larger than the plants of 30 mg/L and 90 mg/L solutions grown.

**Number of leaves:**

**Plants treated with Pb(NO3)3 and Pb(NO3)3 with Na2SiO3:**

The difference among mean values of number of leaves of *Cyperus rotundus* grown in lead nitrate without sodium silicate and with sodium silicate is shown in Fig. 1g. The plants of control group have the maximum number of leaves per plant as compared to the plants under stress. While there was decreasing in number of leaves of plants between the plants of different treatments. That shown decreasing trend as concentrations of metal increases except the plants grown in the level of 120 mg/L concentration. The number of leaves of plants applied foliar application of sodium silicate the mitigating the effect of metal as compared to the plants grown under metallic stress without foliar application. The plant grown in control environment has the 5.34 maximum numbers of leaves. That value is larger than the values of plants grown in all concentrations of lead nitrate without sodium silicate as given in Fig. 1g. The plants grown in 30 mg/L solution of lead nitrate have 5.0 numbers of leaves which is slightly less from the value of control plants and larger from other plants grown in different concentrations of lead nitrate. The plants grown in 90 mg/L solution of lead nitrate without foliar application of sodium silicate have 4.29 no. of leaves which is smallest value from the others plants of same group grown in different concentrations of lead nitrate. While the plants grown in 60 mg/L and 120 mg/L solutions of lead nitrate have 4.63 and 4.64 respectively. These values are quite similar. So there the number of leaves show no difference widely by changing the different concentrations of lead nitrate to plants. In it silicon also gives promoting effect in increasing the no. of leaves by mitigating the effect of heavy metal. Like plants grown in 30 mg/L concentration of lead nitrate with foliar spray of sodium silicate have 6.0 no. of leaves which is largest value among the both group of plants grown without and with foliar application of sodium silicate. The plants grown in 90 mg/L of lead nitrate with foliar spray of sodium silicate has the 4.50 number of leaves which is the smaller than the value of other plants. While the plants grown in 120 mg/L solution of lead nitrate with foliar application of sodium silicate have 5.34 no. of leaves which is equal to the plants of control environment, larger than plants of 90 mg/L and smaller from other plants as shown in Fig. 1g.

**Number of fertile branches:**

**Plants treated with Pb(NO3)3 and Pb(NO3)3 with Na2SiO3:**

The mean values of fertile branches of plants *Cyperus rotundus* are grown in different concentrations of lead nitrate without and with sodium nitrate are given in Fig. 1h. The plants of control group have the maximum number of fertile branches as compared to the plants under stress. While there was increasing in number of fertile branch of plants between the plants of different treatments. That shown increasing trend as concentrations of metal increases except in plants grown in metal level of T1. The number of fertile branch of plants applied foliar application of sodium silicate suppress the effect of metal as compared to the plants grown under metallic stress without foliar application as in the plants of T4. The mean value of fertile branch of control plants is 2.33 which is the maximum value. The plants grown in 60 mg/L solution of lead nitrate without sodium silicate have 2.0 values, which is slightly smaller than the value of control plants and larger than the other. The plants grown in 30 mg/L and 120 mg/L solutions of lead nitrate without the foliar application of sodium silicate both have similar value of 1.33 of no. of fertile branches. While, the plants grown in 120 mg/L of lead silicate without sodium silicate have 1.67 mean value of no. of fertile branches. When the plants are grown with foliar application of sodium silicate with similar metal effect, plants have certain positive effect on the no. of fertile branches. Like the plants treated with 30 mg/L and 60 mg/L of lead silicate with foliar spray of sodium silicate have same mean value of fertile branch as 1.33. Similarly the plants grown in 90 mg/L and 120 mg/L also have same value of no. of fertile branches as 2.33. That value is also similar to the control plants but larger than other plants.

**Number of nodes:**

**Plants treated with Pb(NO3)3 and Pb(NO3)3 with Na2SiO3:**

The difference among the mean values of number of nodes of plants treated with different concentration of lead nitrate without and with sodium silicate is given in Fig. 1i. The plants of control group have the maximum number of nodes as compared to the plants under stress. While there was increasing in number of nodes of plants between the plants of different treatments. That shown increasing trend as concentrations of metal increases except in plants grown in metal level of T1. The number of nodes of plants applied foliar application of sodium silicate mitigates the effect of metal as compared to the plants grown under metallic stress without foliar application as in the plants of T4. The group of control plants has 2.33 mean value of maximum number of node as compared to the plants of all others group. The plants grown in 30 mg/L and 120 mg/L solution of lead nitrate have similar mean value of number nodes that is 1.33. This is the minimum number of nodes as to others. While the plants grown in 60 mg/L and 90 mg/L also have similar mean value of number of nodes i.e 1.67. The plants grown in different concentrations of lead nitrate with sodium silicate application have following mean values. The plants grown in 30 mg/L and 60 mg/L concentrations of lead nitrate with foliar application of sodium silicate have similar value of mean value of node that is 1.33. These values are the minimum values among the plants of other group. The plants grown in the 90 mg/L of lead nitrate with foliar application of sodium silicate has 1.67 mean value of number of node which is similar to the other plants grown in lead nitrate without sodium silicate application. The number of nodes of plants grown in 120 mg/L solution of lead nitrate with foliar application sodium silicate has 2.0. That value is larger from the all values of other plants but slightly smaller than the plants of control environment as given in Fig. 1i.

**Analysis of variance of morphological parameters of Cyperus rotundus:**

**Plants treated with Pb(NO3)3 and Pb(NO3)3 with Na2SiO3:**

The Table of analysis of variance for different morphological parameters of plants of *Cyperus rotundus* treated with different concentrations of lead nitrate without sodium silicate application is shown in Table 1. The means of different morphological parameters when analysis on the basis of different significance levels, the height of plants (cm), internodal length (cm), length of inflorescence (cm), fresh weight of plant (gm), dry weight of plant (gm) and number of leaves shown the highly significance at the level of 0.05 and 0.01. While the number of vegetative branch, number of fertile branch and number of nodes shown non-significance results with reference to the effect of lead nitrate metal without application of sodium silicate.

The Table of analysis of variance for morphological parameters of plants grown in different concentrations of lead nitrate with foliar application of sodium silicate is given in Table 2. The means of different morphological parameters when analysis on the basis of different significance levels, the height of plants (cm), internodal length (cm), length of inflorescence (cm), fresh weight of plant (gm) and dry weight of plant (gm) are shown the highly significance at the level of 0.05 and 0.01, while the number of leaves is only significance at the level of 0.05.

The number of vegetative branch, number of fertile branch and number of nodes are shown non-significance results with reference to the effect of lead nitrate with foliar application of sodium silicate.

**ANATOMICAL ATTRIBUTES OF ROOT:**

**Diameter of Root (µm):**

**Plants treated with Pb(NO3)2 and Pb(NO3)2 with Na2SiO3:**

The difference among the values of root diameter of plants treated with Sodium Silicate i.e.Na2SiO3 and different concentrations of lead have been shown in the Plate 1 and Fig. 2a. The plants of T2 with silicon group have the maximum diameter of root as compared to the plants of T0 and other plants under stress. While there was decreasing in diameter of root of plants between the plants of different treatments. That shown decreasing trend as concentrations of metal increases. The diameter of root of plants applied foliar application of sodium silicate has the compensation effect of metal as compared to the plants grown under metallic stress without foliar application. The root diameter of control plant is 3.63 µm. While the plant that got treatment of T1 without silicon has the root diameter of 3.9 µm is less than the root diameter 4.1 µm of a plant that was given the treatment of T1 with silicon. The root diameter on treatment of T2 without silicon is 3.26 µm less than the diameter of root 3.4 µm of a plant treated by T2 with silicon. The plant treated with T3 without silicon has the root diameter 2.8 µm more than the 2.43 µm root diameter that is noted in a plant treated with T3 with silicon. The plants of T4 without silicon have the root diameter of 2.4 µm less than the root diameter 2.53 µm of a plant T4 with silicon. The plants that get the maximum root diameter of 4.1 µm from the plants that got treatment of T2 with silicon.

**Epidermal Thickness (µm):**

**Plants treated with Pb(NO3)2 and Pb(NO3)2 with Na2SiO3:**

The difference among the values of epidermal thickness of root of plants treated with Sodium Silicate i.e. Na2SiO3 and different concentrations of Lead have been shown in the Plate 1 and Fig. 2b. The plants of T4 with silicon group have the maximum epidermal thickness as compared to the plants of T0 and other plants under stress. While there was increasing in epidermal thickness in plants between the plants of different treatments except plants of T2 without silicon& T2 with silicon. That shown increasing trend as concentrations of metal increases. The epidermal thickness of plants applied foliar application of sodium silicate has the compensation effect of metal as compared to the plants grown under metallic stress without foliar application. But in T3 with silicon group plants are not follow that trend. The plant of T1 has 0.53 µm epidermal thickness less than 0.56 µm epidermal thickness of a plant that got T1 with silicon. The epidermal thickness of a plant of T2 without silicon is 0.43 µm similar to the epidermal thickness 0.43 µm of plant T2 with silicon. The root epidermal thickness of 0.46 µm is recorder in the plant that is treated T3 without silicon that is more than the epidermal thickness of 0.43 µm of a plant that get treatment T3 with silicon. The plant treated by T4 with silicon has epidermal thickness of Root 0.5 µm that is less than the epidermal thickness of root 0.65 µm of a plant treated T4 with silicon. The maximum epidermal thickness of root 0.65 µm is noted in plant treated by T4 with silicon.

**Endodermis Thickness (µm):**

**Plants treated with Pb(NO3)2 and Pb(NO3)2 with Na2SiO3:**

The difference among the values of endodermis thickness of root of plants treated with Sodium Silicate i.e. Na2SiO3 and different concentrations of Lead have been shown in the Plate 1 and Fig. 2c. The plants of T0 group have the maximum endodermis thickness as compared to the plants of growing under stress. While there was decreasing in endodermal thickness in plants between the plants of different treatments except plants of T2 with silicon. That shown increasing trend as concentrations of metal increases. The endodermal thickness of plants applied foliar application of sodium silicate has the mitigating effect of metal as compared to the plants grown under metallic stress without foliar application. The plant that got treatment of T1 without silicon has 0.4 µm endoermal thickness equal to 0.4 µm endodermal thickness of a plant that get T1 with silicon. The endodermal thickness of a plant treated by T2 without silicon is 0.33 µm less than the endodermal thickness 0.43 µm of plant treated with T2 with silico. The Root endodermal thickness of 0.33 µm is recorder in the plant that was treated by the T3 without silicon that is less than the endodermal thickness of 0.4 µm of a plant that get treatment of T3 with silicon. The plant treated by T4 without silicon has endodermal thickness of root 0.3 µm that is slightly less than the endodermal thickness of root 0.36 µm of a plant treated with T4 with silicon. The maximum endodermal thickness of root 0.43 µm is noted in plant treated with T2 with silicon.

**Vascular Bundles Thickness (µm):**

**Plants treated with Pb(NO3)2 and Pb(NO3)2 with Na2SiO3:**

The difference among the values of vascular bundle thickness of Root of plants treated with Sodium Silicate i.e. Na2SiO3 and different concentrations of Lead have been shown in the Plate 1 and Fig. 2d. The plants of T0 group have the maximum vascular bundles thickness as compared to the plants of growing under stress. While there was decreasing in vascular bundles thickness in plants between the plants of different treatments except plants of T3 with silicon. That shown slightly increasing trend as concentrations of metal increases. The vascular bundle thickness of plants applied foliar application of sodium silicate has the reverse the effect of metal as compared to the plants grown under metallic stress without foliar application. The vascular bundle thickness is 1.40 µm of a plant treated with T1 without silicon less than 1.70 µm vascular bundle thickness of plant treated with T1 with silicon. The vascular bundle thickness is 0.96 µm of a plant treated with T2 without silicon less than 1.04 µm vascular bundle thickness of plant treated with T4 with silicon. The vascular bundle thickness is 0.79 µm of a plant treated with T3 without silicon solution less than 0.9 µm vascular bundle thickness of plant treated with T3 with silicon. The vascular bundle thickness is 0.6 µm of a plant treated with T4 without silicon is almost equal to 0.61 µm vascular bundle thickness of plant treated with T4 with silicon.

**Area of epidermal cell (µm2):**

**Plants treated with Pb(NO3)2 and Pb(NO3)2 with Na2SiO3:**

The difference among the values of epidermal cell area of Root of plants treated with Sodium Silicate i.e. Na2SiO3 and different concentrations of Lead nitrate have been shown in the Plate 1 and Fig. 2e. The plants of T2 without silicon group have the maximum area of epidermal cell as compared to the plants of growing in control and others under stress. While there was decreasing in epidermal cell area in plants of T0 between the plants of different treatments without using of foliar spray of lead. That shown slightly increasing trend as concentrations of metal increases. The area of epidermal cells of plants applied foliar application of sodium silicate has the enhancing effect with metallic stress as compared to the plants grown under metallic stress without foliar application. The area of epidermal cell is 0.49 µm2 of a plant treated with T1 without silicon less than 0.68 µm2 area of epidermal cell of plant treated with T1 with silicon. The area of epidermal cell is 0.53 µm2 of a plant treated with T2 without silicon less than 0.64 µm2 area of epidermal cell of plant treated with T2 with silicon. The area of epidermal cell is 0.53 µm2 of a plant treated with T3 without silicon less than 0.64 µm2 area of epidermal cell of plant treated with T3 with silicon. The area of epidermal cell is 0.56 µm2 of a plant treated with T4 without silicon is almost equal to 0.63 µm2 area of epidermal cell of plant treated with T4 with silicon. The maximum area of epidermal cell in root is 0.68 µm2 of a plant that got treatment with T2 without silicon.

**ANATOMICAL ATTRIBUTES OF LEAF:**

**Area of leaf (µm2):**

**Plant treated with Pb(NO3)2 and Pb(NO3)2 with Na2SiO3:**

The difference among the values of area of leaf of plants treated with Sodium Silicate i.e. Na2SiO3 and different concentrations of Lead have been shown in the Plate 2 and Fig.3a. The plants of T0 group have the maximum area of leaf as compared to the plants growing under metallic stress. While there was decreasing in area of leaf of plants between the plants of different treatments. That shown decreasing trend as concentrations of metal increases except the plants of T4 with silicon which shown slightly increase in leaf area. The area of leaf of plants applied foliar application of sodium silicate has the mitigating effect of metal as compared to the plants grown under metallic stress without foliar application. The area of leaf is 4.09 µm2 of a plant treated with T1 without silicon less than 4.33 µm2 area of leaf of plant of T1treated with lead and foliar apply of silicon. The area of leaf is 3.49 µm2 of a plant treated with T2 without silicon less than 4.01 µm2 area of leaf of plant treated with T2 with silicon. The area of leaf is 2.64 µm2 of a plant treated with T3 without silicon less than 2.95 µm2 area of leaf of plant treated with T3 with silicon. The area of leaf is 2.3 µm2 of a plant treated with T4 without silicon is almost equal to 2.3 µm2 area of leaf of plant treated with T4 with silicon. The maximum area of leaf is 4.33 µm2 of a plant that got treatment with T1 with siliconbetween the metallic stress plants.

**Thickness of upper epidermis(µm):**

**Plant treated with Pb(NO3)2 and Pb(NO3)2 with Na2SiO3:**

The difference among the values of upper epidermal thickness of leaf of plants treated with Sodium Silicate i.e. Na2SiO3 and different concentrations of Lead nitrate solution have been shown in the Plate 2 and Fig.3b. The plants of T0 group have the minimum thickness of upper epidermis as compared to the plants growing under metallic stress. While there was increasing in thickness of upper epidermis of plants between the plants of different treatments. That shown increasing trend as concentrations of metal increases. But the plants growing in treatment of T2 without silicon and with silicon have equal thickness of upper epidermis. The thickness of upper epidermis applied foliar application of sodium silicate has the compensating effect of metal as compared to the plants grown under metallic stress without foliar application. The values are recorded under the ocular lense having the power of 10X. The thickness of upper epidermis of control (T0) group plant is 0.43 µm. The thickness of upper epidermis is 0.7 µm of a plant treated with T1 without silicon less than 0.83µm thickness of upper epidermis of plant treated with T2 with silicon. The thickness of upper epidermis is 0.9 µm of a plant treated with T1 with silicon equal to 0.9 µm2 thickness of upper epidermis of plant treated with T2 without silicon. The thickness of upper epidermis is 1 µm of a plant treated with T3 without silicon equal to 1 µm thickness of upper epidermis of plant treated with T3 with silicon. The thickness of upper epidermis is 1 µm of a plant treated with T4 without silicon is less than 1.03 µm2 thickness of upper epidermis of plant treated with T4 with silicon. The maximum thickness of upper epidermis is 1.03 µm of a plant that got treatment with T4 with silicon.

**Thickness of lower epidermis (µm):**

**Plant treated with Pb(NO3)2 and Pb(NO3)2 with Na2SiO3:**

The difference among the values of lower epidermal thickness of leaf of plants treated with Sodium Silicate i.e. Na2SiO3 and different concentrations of Lead nitrate have been shown in the Plate 2 and Fig.3c. The plants of T0 group have the minimum thickness of lower epidermis as compared to the plants growing under metallic stress. While there was fluctuating in thickness of lower epidermis between the plants of different treatments. That shown abrupt trend in thickness of lower epidermis as concentrations of metal increases except the plants of T3 without silicon which shown increase in thickness of lower epidermis. The thickness of lower epidermis of plants applied foliar application of sodium silicate has the reversing effect of metal as compared to the plants grown under metallic stress without foliar application. The values are been recorded under the ocular lense having the power of 10X.The thickness of lower epidermis of control (T0) group plant is 0.1 µm. The thickness of lower epidermis is 0.1 µm of a plant treated with T1 without silicon solution less than 0.16 µm thickness of lower epidermis of plant treated with T1 with silicon. The thickness of lower epidermis is 0.2 µm of a plant treated with T2 without silicon is more than 0.13 µm thickness of lower epidermis of plant treated with T2 with silicon. The thickness of lower epidermis is 0.2 µm of a plant treated with T3 without siliconequal to 0.2 µm thickness of lower epidermis of plant treated with T3 with silicon. The thickness of lower epidermis is 0.2 µm of a plant treated with T4 without silicon is less than 0.2 µm2 thickness of lower epidermis of plant treated with T4 with silicon. The maximum thickness of lower epidermis is 0.2 µm of a plant that got treatment with T4 with silicon.

**Thickness of Leaf Lamina (µm):**

**Plant treated with Pb(NO3)2 and Pb(NO3)2 with Na2SiO3:**

The difference among the values of thickness of leaf lamina of plants treated with Sodium Silicate i.e.Na2SiO3 and different concentrations of Lead have been shown in the Plate 2 and Fig.3d. The plants of T0 group have the maximum thickness of leaf lamina as compared to the plants growing under metallic stress. While there was decreasing in thickness of leaf lamina of plants between the plants of different treatments except plants growing in treatments of T3 without silicon and with silicon which have equal thickness of leaf lamina. That shown decreasing trend as concentrations of metal increases. The thickness of leaf lamina of plants applied foliar application of sodium silicate has the mitigating effect of metal as compared to the plants grown under metallic stress without foliar application. The values are been recorded under the ocular lense having the power of 10X. The maximum thickness of leaf lamina of control group plant is 5.63 µm. The thickness of leaf lamina is 4.83 µm of a plant treated with T1 without solution less than 5.3 µm thickness of leaf lamina of plant treated with silicon. The thickness of leaf lamina is 4.66 µm of a plant treated with T2 without silicon is less than 4.93 µm thickness of leaf lamina of plant treated with T2 with silicon. The thickness of leaf lamina is 4.13 µm of a plant treated with T3 without silicon equal to 4.13 µm thickness of leaf lamina of plant treated with T3 with silicon. The thickness of leaf lamina is 3.3 µm of a plant treated with T4 without silicon is equal to 3.3 µm thickness of leaf lamina of plant treated with silicon. The maximum thickness of leaf lamina is 5.3 µm of a plant that got treatment with T1 with silicon between the plants treated with treatments.

**Density of Stomata:**

**Plant treated with Pb(NO3)2 and Pb(NO3)2 with Na2SiO3:**

The difference among the values of stomatal density per square millimeter of leaf of plants treated with Sodium Silicate i.e. Na2SiO3 and different concentrations of Lead nitrate have been shown in the Plate 2 and Fig.3e. The plants of T0 and T1 with silicongroup have the maximum density of stomata as compared to the plants growing under metallic stress. While there was decreasing in density of stomata of plants between the plants of different treatments except the plants growing in T4 without silicon and with silicon have equal stomatal density. That shown decreasing trend as concentrations of metal increases. The density of stomata of plants applied foliar application of sodium silicate has the reducing the effect of metal as compared to the plants grown under metallic stress without foliar application. The values are been recorded under the ocular lense having the power of 40X.The stomatal density in leaf is 16 of a plant treated with T1 without silicon less than 18 stomatal density in leaf of plant treated with T1 silicon. The stomatal density in leaf is 14 of a plant treated with T2 without silicon solution is less than 17 stomatal density in leaf of plant treated with T2 with silicon. The stomatal density in leaf is 12 of a plant treated with T3 without silicon equal to 13 stomatal density in leaf of plant treated with T3 with silicon. The stomatal density in leaf is 9 of a plant treated with T4 without silicon solution is equal to 10 stomatal density in leaf of plant treated with T4 with silicon. The maximum stomatal density in leaf is 18 of a plant that got treatment with T1 with silicon.

**Area of vascular bundles (µm2):**

**Plant treated with Pb(NO3)2 and Pb(NO3)2 with Na2SiO3:**

The difference among the values of area of vascular bundles of leaf of plants treated with Sodium Silicate i.e. Na2SiO3 and different concentrations of Lead nitrate have been shown in the Plate 2 and Fig.3f. The plants of T0 group have the maximum area of vascular bundle as compared to the plants growing under metallic stress. While there was decreasing in area of vascular bundles of plants between the plants of different treatments. That shown decreasing trend as concentrations of metal increases. The area of vascular bundle of plants applied foliar application of sodium silicate has the mitigating effect of metal as compared to the plants grown under metallic stress without foliar application. The values are been recorded under the ocular lense having the power of 10X. The vascular bundle area in leaf is 2.07 µm2 of a plant treated with T1 without silicon less than 2.12 µm2 vascular bundle area in vascular bundle of plant treated with T1 with silicon. The vascular bundle area in leaf is 1.50 µm2 of a plant treated with T2 without siliconis less than 1.73 µm2 vascular bundle area in leaf of plant treated with T2 with silicon. The vascular bundle area in leaf is 1.23 µm2 of a plant treated with T3 without silicon more than 1.15 µm2 vascular bundle area in leaf of plant treated with T3 with silicon. The vascular bundle area in leaf is 0.85 µm2 of a plant treated with T4 without silicon is less than 0.87 µm2 vascular bundle area in leaf of plant treated with T4 with silicon. The maximum vascular bundle area in leaf is 2.12 µm2 of a plant that got treatment with T1 with siliconbetween the plants got treated.

**Area of epidermal cell (µm2):**

**Plant treated with Pb(NO3)2 and Pb(NO3)2 with Na2SiO3:**

The difference among the values of area of upper epidermal cell of leaf of plants treated with Sodium Silicate i.e. Na2SiO3 and different concentrations of Lead nitrate have been shown in the Plate 2 and Fig.3g. The plants of T0 group have the maximum area of epidermal cell as compared to the plants growing under metallic stress. While there was decreasing in area of epidermal cell of plants between the plants of different treatments. That shown decreasing trend as concentrations of metal increases except the plants of T4 without silicon and T4 with siliconwhich shown equal values of area of epidermal cell. The area of epidermal cell of plants applied foliar application of sodium silicate has the compensating the effect of metal as compared to the plants grown under metallic stress without foliar application. The values are been recorded under the ocular lense that has the power of 40X. The area of upper epidermal cell in leaf is 2.96 µm2 of a plant treated with T1 without silicon less than 3.05 µm2 area of upper epidermal cell in leaf of plant treated with T1 with silicon. The area of upper epidermal cell in leaf is 2.15 µm2 of a plant treated with T2 without silicon is less than 2.41 µm2 area of upper epidermal cell in leaf of plant treated with T2 with silicon. The area of upper epidermal cell in leaf is 2.15 µm2 of a plant treated with T3 without silicon less than 2.49 µm2 area of upper epidermal cell in leaf of plant treated with T3 with silicon. The area of upper epidermal cell in leaf is 1.85 µm2 of a plant treated with T4 without silicon is almost equal to 2.35 µm2 area of upper epidermal cell in leaf of plant treated with T4 with silicon. The maximum area of upper epidermal cell in leaf is 3.05 µm2 of a plant that got treatment with T1 with silicon between plants growing under stress.

**Area of Stomata (µm2):**

**Plant treated with Pb(NO3)2 and Pb(NO3)2 with Na2SiO3:**

The difference among the values of stomatal area in leaf of plants treated with Sodium Silicate i.e.Na2SiO3 and different concentrations of Lead nitrate solution have been shown in the Plate 2 and Fig.3h. The plants of T0 and T1 with silicon group have the maximum and almost equal to the area of stomata as compared to the plants growing under metallic stress. While there was decreasing in area of stomata of plants between the plants of different treatments. That shown decreasing trend as concentrations of metal increases except the plants of T2 with silicon which shown slightly increase in area of stomata. The area of stomata of plants applied foliar application of sodium silicate has the mitigating effect of metal as compared to the plants grown under metallic stress without foliar application. The values are been recorded under the ocular lense that has the power of 40X. The area of stomata in leaf of control (T0) group plant is 0.56 µm2.The area of stomata in leaf is 0.37µm2 of a plant treated with T1 without silicon less than 0.55 µm2 area of stomata in leaf of plant treated with T1 with silicon. The area of stomata in leaf is 0.29 µm2 of a plant treated with T2 without silicon is less than 0.33 µm2 area of stomata in leaf of plant treated with T2 with silicon. The area of stomata in leaf is 0.24 µm2 of a plant treated with T3 without silicon slightly less than 0.27 µm2 area of stomata in leaf of plant treated with T3 with silicon. The area of stomata in leaf is 0.19 µm2 of a plant treated with T4 without silicon is almost equal to 0.19 µm2 area of stomata in leaf of plant treated with T4 with silicon. The maximum area of stomata in leaf is 0.55 µm2 of a plant that got treatment with T1 with silicon among plants growing in metallic stress.

**ANATOMICAL ATTRIBUTES OF STEM:**

**Diameter of Stem (µm):**

**Plant treated with Pb(NO3)2 and Pb(NO3)2 with Na2SiO3:**

The difference among the values of diameter of stem of plants treated with Sodium Silicate i.e. Na2SiO3 and different concentrations of Lead have been shown in the Plate 3 and Fig.4a. The plants of T0 group have the maximum diameter of stem as compared to the plants growing under metallic stress. While there was decreasing in diameter of stem of plants between the plants of different treatments. That shown decreasing trend as concentrations of metal increases, while plants of T0 and T1 with silicon have equal value of stem diameter. The diameter of stem of plants applied foliar application of sodium silicate has the minimizing the effect of metal as compared to the plants grown under metallic stress without foliar application. The values have been recorded under the ocular lense that has the power of 40X. The diameter of stem of control (T0) group plant is 2.96 µm. The diameter of stem is 2.83 µm of a plant treated with T1 without silicon less than 2.96 µm diameter of stem of plant treated with T1 with silicon. The diameter of stem is 2.6 µm of a plant treated with T2 without silicon is less than 2.7 µm diameter of stem of plant treated with T2 with silicon. The diameter of stem is 2.43 µm of a plant treated with T3 without silicon slightly more than 2.36 µm diameter of stem of plant treated with T3 with silicon. The diameter of stem is 2 µm of a plant treated with T4 without silicon is almost equal to 2.03 µm diameter of stem of plant treated with T4 with silicon. The maximum diameter of stem is 2.96 µm of a plant that get treatment with T1 with silicon.

**Thickness of epidermis (µm):**

**Plant treated with Pb(NO3)2 and Pb(NO3)2 with Na2SiO3:**

The difference among the values of thickness of epidermis of stem of plants treated with Sodium Silicate i.e.Na2SiO3 and different concentrations of Lead nitrate have been shown in the Plate 3 and Fig.4b. The plants of T0, T1 without silicon, T1with silicon and T4 with silicon group have the minimum and equal value of thickness of epidermis as compared to the plants growing under metallic stress. While there was increasing in thickness of epidermis of plants between the plants of different treatments. That shown increasing trend as concentrations of metal increases except the plants of T4 with silicon which shown decrease in thickness of epidermis. The thickness of epidermis of plants applied foliar application of sodium silicate has the reducing the effect of metal as compared to the plants grown under metallic stress without foliar application. The values have been recorded under the ocular lense that has the power of 10X. The epidermal thickness of stem of control (T0) group plant is 0.16 µm. The epidermal thickness of stem is 0.16 µm of a plant treated with T1 without silicon similar to 0.16 µm epidermal thickness of stem of plant treated with T1 with silicon. The epidermal thickness of stem is 0.2 µm of a plant treated with T2 without silicon is less than 0.23 µm epidermal thickness of stem of plant treated with T2 with silicon. The epidermal thickness of stem is 0.2 µm of a plant treated with T3 without silicon solution slightly less than 0.23 µm epidermal thickness of stem of plant treated with T3 with silicon. The epidermal thickness of stem is 0.23 µm of a plant treated with T4 without silicon is more than 0.16 µm epidermal thickness of stem of plant treated with T4 with silicon. The maximum epidermal thickness of stem is 0.23 µm of a plant that got treatment with T4 without silicon.

**Thickness of Xylem (µm):**

**Plant treated with Pb(NO3)2 and Pb(NO3)2 with Na2SiO3:**

The difference among the values of thickness of xylem in stem of plants treated with Sodium Silicate i.e. Na2SiO3 and different concentrations of Lead have been shown in the Plate 3 and Fig.4c. The plants of T0 group have the maximum thickness of xylem as compared to the plants growing under metallic stress. While there was decreasing in thickness of xylem of plants between the plants of different treatments. That shown decreasing trend as concentrations of metal increases except the plants of T2 with silicon which shown slightly increase in thickness of xylem. The thickness of xylem of plants applied foliar application of sodium silicate has minimizing the effect of metal as compared to the plants grown under metallic stress without foliar application. The values have been recorded under the ocular lense that has the power of 10X. The xylem thickness in stem of control (T0) group plant is 1.23 µm which is maximum value. The xylem thickness in stem is 0.8µm of a plant treated with T1 without silicon is less than 0.9 µm xylem thickness in stem of plant treated with T1 with silicon. The xylem thickness in stem is 0.86 µm of a plant treated with T2 without silicon is less than 0.93 µm xylem thickness in stem of plant treated with T2 with Na2SiO3 spray. The xylem thickness in stem is 0.76 µm of a plant treated with T3 without silicon slightly more than 0.7 µm xylem thickness in stem of plant treated with T3 with Na2SiO3 spray. The xylem thickness in stem is 0.66 µm of a plant treated with T4 without silicon solution is more than 0.6 µm xylem thickness in stem of plant treated with T4 with Na2SiO3 spray. The maximum xylem thickness in stem is 0.9 µm of a plant that got treatment with T1 with Na2SiO3 foliar spray.

**Thickness of Phloem (µm):**

**Plant treated with Pb(NO3)2 and Pb(NO3)2 with Na2SiO3:**

The difference among the values of thickness of phloem in stem of plants treated with Sodium Silicate i.e.Na2SiO3 and different concentrations of Lead nitrate have been shown in the Plate 3 and Fig.4d. The plants of T0 group have the maximum thickness of phloem as compared to the plants growing under metallic stress. While there was decreasing in thickness of phloem of plants between the plants of different treatments. That shown decreasing trend as concentrations of metal increases. The thickness of phloem of plants applied foliar application of sodium silicate has reducing the effect of metal as compared to the plants grown under metallic stress without foliar application. The values have been recorded under the ocular lense that has the power of 10X. The phloem thickness in stem of control group plant is 0.43 µm. The phloem thickness in stem is 0.4 µm of a plant treated with T1 without silicon solution is similar to 0.4 µm phloem thickness in stem of plant treated with T1 with Na2SiO3 spray. The phloem thickness in stem is 0.36 µm of a plant treated with T2 without silicon solution is less than 0.4 µm phloem thickness in stem of plant treated with T2 with Na2SiO3 spray. The phloem thickness in stem is 0.33 µm of a plant treated with T3 without silicon is equal to 0.33 µm phloem thickness in stem of plant treated with T3 with Na2SiO3 spray. The phloem thickness in stem is 0.26 µm of a plant treated with T4 without silicon spray is equal to 0.26 µm phloem thickness in stem of plant treated with T4 with Na2SiO3 spray. The maximum phloem thickness in stem is 0.4 µm of a plant that got treatment with T1 with Na2SiO3foliar spray.

**Area of Vascular Bundles (µm2):**

**Plant treated with Pb(NO3)2 and Pb(NO3)2 with Na2SiO3:**

The difference among the values of area of vascular bundles in stem of plants treated with Sodium Silicate i.e.Na2SiO3 and different concentrations of Lead nitrate have been shown in the Plate 3 and Fig.4e. The plants of T0 group have the maximum area of vascular bundle as compared to the plants growing under metallic stress. While there was decreasing in area of vascular bundle of plants between the plants of different treatments. That shown decreasing trend as concentrations of metal increases. The area of vascular bundle of plants applied foliar application of sodium silicate has reducing the effect of metal as compared to the plants grown under metallic stress without foliar application. The values have been recorded under the ocular lense that has the power of 10X. The area of vascular bundles in stem of control (T0) group plant is 1.85 µm2. The area of vascular bundles in stem is 1.08 µm2 of a plant treated with T1 without silicon solution less than 0.92 µm2 area of vascular bundles in stem of plant treated with T1 with Na2SiO3 spray. The area of vascular bundles in stem is 0.93 µm2 of a plant treated with T2 without silicon solution is less than 1.49 µm2 area of vascular bundles in stem of plant treated with T2 with Na2SiO3 spray. The area of vascular bundles in stem is 0.89 µm2 of a plant treated with T3 without silicon solution slightly more than 0.84 µm2 area of vascular bundles in stem of plant treated with T3 with Na2SiO3 spray. The area of vascular bundles in stem is 0.45 µm2 of a plant treated with T4 without silicon solution is almost equal to 0.46 µm2 area of vascular bundles in stem of plant treated with T4 with Na2SiO3 spray. The maximum area of vascular bundles in stem is 1.49 µm2 of a plant that got treatment with T2 with Na2SiO3 spray.

**Area of Epidermal Cell (µm2):**

**Plant treated with Pb(NO3)2 and Pb(NO3)2 with Na2SiO3:**

The difference among the values of area of epidermal cell in stem of plants treated with Sodium Silicate i.e. Na2SiO3 and different concentrations of Lead nitrate have been shown in the Plate 3 and Fig.4f. The plants of T0 group have the minimum area of epidermal cell as compared to the plants growing under metallic stress. While there was increasing in area of epidermal cell of plants between the plants of different treatments. That shown increasing trend as concentrations of metal increases. The area of epidermal cell of plants applied foliar application of sodium silicate has reducing the effect of metal as compared to the plants grown under metallic stress without foliar application. The values have been recorded under the ocular lense that has the power of 40X. The area of epidermal cell in stem of control (T0) group plant is 0.32 µm2. The area of epidermal cell in stem is 0.28 µm2 of a plant treated with T1 without silicon solution less than 0.42 µm2 area of epidermal cell in stem of plant treated with T1 with Na2SiO3 spray. The area of epidermal cell in stem is 0.31 µm2 of a plant treated with T2 without silicon solution is less than 0.38 µm2 area of epidermal cell in stem of plant treated with T2 with Na2SiO3 spray. The area of epidermal cell in stem is 0.43 µm2 of a plant treated with T3 without silicon solution slightly more than 0.42 µm2 area of epidermal cell in stem of plant treated with T3 with Na2SiO3 spray. The area of epidermal cell in stem is 0.38 µm2 of a plant treated with T4 without silicon solution is less than 0.50 µm2 area of epidermal cell in stem of plant treated with T4 with Na2SiO3 spray. The maximum area of epidermal cell in stem is 0.50 µm2 of a plant that get treatment with T4 with Na2SiO3 spray.

**Discussion**

The major reason of environmental pollution is industrial revolution. Being a developing country we are completely dependent on industries for the betterment of Pakistan and basic needs of nation. The industrial effluents include toxic solid waste, liquid wastage and contaminated smoke. The major pollutants include heavy metals like Lead that is hazardous for all kind of life. The addition of these heavy metals in the biomass of any organism adversely affects the food chain. These heavy metals cause reduction in plant growth, biomass and affect the anatomical characters of plant structures. If the contaminated plants are eaten by the animals, it causes different disorders like cancer. So, it is necessary to remove these heavy metals from the environment (Pandey *et al.* 2011). These heavy metals can be removed from the environment by using phytoremediation techniques. Many investigations showed that *Cyperus rotundus* such as has the capability to cope the tress environment like salinity and drought conditions. The main objective of this study is to explore the ability of the plant to withstand in the soils contaminations with heavy metals and to reclaim the polluted soil through phytoextraction. Another objective of the study was to assess the role of silicon to alleviate heavy metals effect in the plant. The results of various researches also proved that silicon is very helpful to alleviate the heavy metals. So, Silicon can be applied on plants to improve the phytoremediation process (Garba *et al.,* 2018).

**Effect of Lead without and Lead with Silicon on Morphological Attributes:**

The raising level of lead reduces the shoot length, number of leaves, dry weight and fresh weight of the plant body. While the foliar spray of solution of silicates reduce the toxic effect of lead on the plant body. But at high levels of lead, Silicon shows a little or no positive effect. The plant which is treated with 30mg/L solution of Lead with silicon spray has maximum root length, shoot length, leaves number per plant, fresh weight and dry weight of plant. The minimum values of morphological characters are noted in the plants which are treated with 120mg/L solution of Lead nitrate.

It is evident from that studies increased level of lead causes reduction in root length (Fahr *et al.* 2013), shoot length of the plants (Nas and Ali 2018), leaf area (Kastori *et al.* 1998), dry weight and fresh weight (Lamhamdi *et al.* 2013) and overall biomass of the plant (Talanova *et al.* 2000). These findings are demonstrated in cowpea, wheat and spinach plants.

The compensating effect of silicon was also observed in plant of cotton and it was found that silicon reduce the effect of lead that cause reduction in shoot length. The plants treated with silicon and lead has more plant height than the plant that is treated with only lead solution. The plants also have more leaf per plant, fresh and dry weight of plant those treated with chromium with silicon as compare to those plants that is treated only with lead solution (Bharwana *et al.* 2013).

The high level of lead gave negatively affects on the leaf morphophysiological characteristics in European hackberry and it also decrease the fresh weight and dry weight of egg plant (Yilmaz *et al.* 2009; Hataminan *et al.* 2020). It is also evident that the application of nano particles of silicon as well as normal silicon increase the biomass of the rice plants under lead stress as compare to the plant that were grown under lead stress only. The results shown that silicon mitigate the toxic effects of lead on rice plants (Liu *et al.* 2015).

**Effect of Lead Nitrate and Lead Nitrate with Sodium silicate on Anatomical Attributes:**

The results indicated that increasing level of heavy metals impose a negative effect on the anatomical attributes of the plant. Such as Lead reduce the diameter of root, epidermal cell length, diameter of cortex cell, xylem and phloem thickness in the Root. In leaf, the epidermal thickness increase while xylem thickness, phloem thickness, density of stomata and area of stomata decrease with raising level of lead. In stem also due to elevation of lead concentration, diameter of stem, thickness of vascular bundles, length of epidermal cell decrease but the epidermal thickness increase. While the foliar spray of silicon reduces the toxic effect of lead on the plant anatomical structures. But at high level of Lead, Silicon shows a little or no positive effect.

The increasing levels of lead reduce the diameter of rhizome of ginger while application of silicon reverse the effect of lead stress by increasing rhizome growth. The results supported the above results that the increasing lead concentrations increase the thickness of epidermis of Root and roots in a sea grass (Chen *et al.,* 2019). The increase in heavy metals increase the epidermal thickness and silicon also enhance the thickness of Epidermis was also evaluated by Vaculik *et al.* (2012).

It also concluded that the vascular bundles thickness is reduced under the influence of heavy metals i.e. Lead and Chromium in *Zea mays* plant roots, the area of epidermal cell increase in root with increase in the heavy metal conc. in the soil. The effect of silicon on the passion fruit plant increases the growth and diameter of the stem of plant body (Singh *et al.* 2015; Gomes *et al.* 2011; Costa *et al.* 2016).

There is an increase in stomatal density on the application of different silicon sources in micropropagated banana plantlets, the effect of silicon increases the thickness of vascular bundles in the crops and increase their productivity. It was concluded a different result there is no change in area of stomata due to application of Silicon on leaves. Although, Silicon spray decrease the transpiration rate in plants. The plants species also increase their xylem thickness by uptaking the silicon. Silicon infacts hardened the walls of xylem (Ma *et al.* 2001; Asmar *et al.* 2013; Mitani and Ma 2005; Gao *et al.* 2006).

The heavy metals like Cu and Ag increase the epidermal thickness of the stem in Potamogeton plant. It was evaluated that application of silicon strengthens the stem due to addition of more lignin in the plant body. The application of silicon increases the thickness of epidermis in soyabean plant (Al-Saadi *et al.* 2013; Hussain *et al.* 2020).

**Conclusion**

By that study we concluded that plant *Cyperus rotundus* shows morpho-anatomical plasticity in heavy metals stress. But these adaptabilities made the plant to continue. The application of silicon also equipped it more successful under stress environment of heavy metals and mitigated the severe effects of heavy metals. So, the silicon played helpful role by enhancing the more adaptive morpho-anatomical modifications in plants in heavy metals challenging environment.

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**Table 1: Analysis of variance for the plants of *Cyperus rotundus* grown in different concentrations of lead nitrate without foliar application of sodium silicates on the morphological parameters.**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sources of variation** | **Degree of freedom** | **Mean squares** | | | | | | | | |
| **Height of plant (cm)** | **Internodal length (cm)** | **Length of inflorescence (cm)** | **Fresh weight of plant (gm)** | **Dry weight of plant (gm)** | **No. of vegetative branch** | **No. of leaves** | **No. of fertile branch** | **No. of node** |
| Treatment | 4 | 544.833\*\* | 12.864\*\* | 3.521\*\* | 0.065\*\* | 0.020\*\* | 0.433ns | 0.942\*\* | 0.567ns | 0.500ns |
| Error | 10 | 0.075 | 0.037 | 0.018 | 0.001 | 0.001 | 0.467 | 0.027 | 0.467 | 0.333 |
| Total | 14 |  |  |  |  |  |  |  |  |  |

**Table 2: Analysis of variance for the plants of *Cyperus rotundus* grown in different concentrations of lead nitrate with foliar application of sodium silicates on the morphological parameters.**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sources of variation** | **Degree of freedom** | **Mean squares** | | | | | | | | |
| **Height of plant (cm)** | **Internodal length (cm)** | **Length of inflorescence (cm)** | **Fresh weight of plant (gm)** | **Dry weight of plant (gm)** | **No. of vegetative branch** | **No. of leaves** | **No. of fertile branch** | **No. of node** |
| Treatment | 4 | 55.44\*\* | 11.954\*\* | 2.77\*\* | 0.071\*\* | 0.015\*\* | 0.567ns | 1.040\* | 0.900ns | 0.933ns |
| Error | 10 | 0.073 | 0.053 | 0.036 | 0.002 | 0.01 | 0.467 | 0.079 | 0.733 | 0.467 |
| Total | 14 |  |  |  |  |  |  |  |  |  |

\*, \*\*=significance level at 0.05 and 0.01 respectively, ns= non-significant

Means followed the same letters are not significantly different at *p* =0.05

1. **(b) (c) (d)**

**(e) (f) (g) (h) (i)**

**Figure 1. Effect of different levels of Lead Nitrate without and with Sodium Silicate on the morphological parameters of *Cyperus rotundus* (a-i).**

**(To= Control, T1= 30mg/L, T2= 60mg/L, T3= 90mg/L, T4= 120mg/L, Lead Nitrate and Lead Nitrate with Sodium Silicate)**

1. **(b) (c) (d)**

**(e)**

**Figure 2. Effect of different levels of Lead Nitrate without and lead nitrate with Sodium Silicate on anatomical attributes of Root of *Cyperus rotundus* (a-e)*.***

**(To= Control, T1= 30mg/L, T2= 60mg/L, T3= 90mg/L, T4= 120mg/L, Lead Nitrate without and Lead Nitrate with Sodium Silicate)**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | To | T1 | | T2 | | T3 | | T4 | |
| Without | With | Without | With | Without | With | Without | With |
| 10X | IMG20200615113417.jpg | D:\M phil thesis\Images\rhizome pics\IMG_20200822_103851.jpg | IMG20200615113019.jpg | IMG20200615130757.jpg | IMG_20200622_111026.jpg | D:\M phil thesis\Images\T3Cd\IMG_20200624_120326.jpg | D:\M phil thesis\Images\rhizome pics\IMG_20200822_104638_1.jpg | IMG_20200622_111107.jpg | IMG_20200624_113051.jpg |
| 40X  Dermal region | IMG20200615113417.jpg | D:\M phil thesis\Images\rhizome pics\IMG_20200822_103913.jpg | IMG20200615113019.jpg | IMG20200615130757.jpg | D:\M phil thesis\Images\T2CdS\IMG_20200624_113335.jpg | D:\M phil thesis\Images\T3Cd\IMG_20200624_120348.jpg | D:\M phil thesis\Images\rhizome pics\IMG_20200822_103851.jpg | IMG_20200624_113335.jpg | IMG_20200624_102046.jpg |
| 40X  Vascular bundle | IMG20200615113417.jpg | D:\M phil thesis\Images\T1Cd\IMG_20200624_102241.jpg | IMG20200615113019.jpg | IMG_20200624_140959.jpg | D:\M phil thesis\Images\T2CdS\IMG_20200624_113351.jpg | D:\M phil thesis\Images\T3Cd\IMG_20200624_120422.jpg | IMG_20200624_102125.jpg | IMG_20200622_111441.jpg | IMG_20200624_102125.jpg |

**Plate 1. Anatomy of root under Lead stress**

**(To= Control, T1= 30mg/L, T2= 60mg/L, T3= 90mg/L, T4= 120mg/L, Lead Nitrate without and Lead Nitrate with Sodium Silicate)**

1. **(b) (c) (d)**

**(e) (f) (g) (h)**

**Fig: 3 Effect of different levels of Lead Nitrate without and lead nitrate with Sodium Silicate on anatomical attributes of leave of *Cyperus rotundus* (a-h)*.***

**(To= Control, T1= 30mg/L, T2= 60mg/L, T3= 90mg/L, T4= 120mg/L, Lead Nitrate without and Lead Nitrate with Sodium Silicate)**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | To | T1 | | T2 | | T3 | | T4 | |
| Without | With | Without | With | Without | With | Without | With |
| 10X | IMG20200615140332 | D:\M phil thesis\slides set for plates\Leaves\T1P\IMG_20200618_134821.jpg | D:\M phil thesis\slides set for plates\Leaves\T1PS\IMG_20200622_112613.jpg | D:\M phil thesis\slides set for plates\Leaves\T2P\IMG20200618144902.jpg | D:\M phil thesis\slides set for plates\Leaves\T2PS\IMG_20200622_120645.jpg | D:\M phil thesis\slides set for plates\Leaves\T3P\IMG20200618145056.jpg | D:\M phil thesis\slides set for plates\Leaves\T3PS\IMG_20200622_121052.jpg | **D:\M phil thesis\slides set for plates\Leaves\T4P\IMG20200618145347.jpg** | **D:\M phil thesis\slides set for plates\Leaves\T4PS\IMG_20200622_121237.jpg** |
| 40X | IMG20200615140651 | D:\M phil thesis\slides set for plates\Leaves\T1P\IMG_20200618_134854.jpg | D:\M phil thesis\slides set for plates\Leaves\T1PS\IMG_20200622_112639.jpg | D:\M phil thesis\slides set for plates\Leaves\T2P\IMG20200618144951.jpg | D:\M phil thesis\slides set for plates\Leaves\T2PS\IMG_20200622_120750.jpg | D:\M phil thesis\slides set for plates\Leaves\T3P\IMG20200618145402.jpg | D:\M phil thesis\slides set for plates\Leaves\T3PS\IMG_20200622_121203.jpg | D:\M phil thesis\slides set for plates\Leaves\T4P\IMG20200618145435.jpg | D:\M phil thesis\slides set for plates\Leaves\T4PS\IMG_20200622_121356.jpg |

**Plate 2. Anatomy of Leaf under Lead Stress**

**(To= Control, T1= 30mg/L, T2= 60mg/L, T3= 90mg/L, T4= 120mg/L, Lead Nitrate without and Lead Nitrate with Sodium Silicate)**

1. **(b) (c)**

**(d) (e) (f)**

**Fig: 4 Effect of different levels of Lead Nitrate without and lead nitrate with Sodium Silicate on anatomical attributes of stem of *Cyperus rotundus* (a-f)*.***

**(To= Control, T1= 30mg/L, T2= 60mg/L, T3= 90mg/L, T4= 120mg/L, Lead Nitrate without and Lead Nitrate with Sodium Silicate)**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | To | T1 | | T2 | | T3 | | T4 | |
| Without | With | Without | With | Without | With | Without | With |
| 10X | D:\M phil thesis\Images\T 0\IMG20200615121529.jpg | D:\M phil thesis\Images\T4P\IMG20200618142901.jpg | D:\M phil thesis\Images\T3PS\IMG20200622131350.jpg | D:\M phil thesis\Images\T1PS\IMG_20200622_110413.jpg | D:\M phil thesis\Images\T1Cd\IMG_20200624_122843 - Copy.jpg | D:\M phil thesis\Images\T2PS\IMG_20200622_122737.jpg | D:\M phil thesis\Images\T2Cd\IMG_20200624_123924.jpg | D:\M phil thesis\Images\T2CdS\IMG_20200624_140536.jpg | D:\M phil thesis\Images\T3PS\IMG20200622131218.jpg |
| 40X  Dermal region | D:\M phil thesis\Images\T 0\IMG20200615121804.jpg | D:\M phil thesis\Images\T4P\IMG20200618142955.jpg | D:\M phil thesis\Images\T3PS\IMG20200622131411.jpg | D:\M phil thesis\Images\T1PS\IMG_20200622_110511.jpg | D:\M phil thesis\Images\T1Cd\IMG_20200624_122927.jpg | D:\M phil thesis\Images\T2PS\IMG_20200622_122820.jpg | D:\M phil thesis\Images\T2Cd\IMG_20200624_123939.jpg | D:\M phil thesis\Images\T3PS\IMG20200622131253.jpg | D:\M phil thesis\Images\T2CdS\IMG_20200624_140555.jpg |
| 40X  Vascular bundle | D:\M phil thesis\Images\T 0\IMG20200615121844.jpg | D:\M phil thesis\Images\T4P\IMG20200618143110.jpg | D:\M phil thesis\Images\T3PS\IMG20200622131437.jpg | D:\M phil thesis\Images\T1Cd\IMG_20200624_122951.jpg | D:\M phil thesis\Images\T1PS\IMG_20200622_110457.jpg | D:\M phil thesis\Images\T2PS\IMG_20200622_122644.jpg | D:\M phil thesis\Images\T2CdS\IMG_20200624_140549.jpg | D:\M phil thesis\Images\T2Cd\IMG_20200624_123951.jpg | D:\M phil thesis\Images\T3PS\IMG20200622131247.jpg |

**Plate 3. Anatomical structures of Stem under Lead stress**

**(To= Control, T1= 30mg/L, T2= 60mg/L, T3= 90mg/L, T4= 120mg/L, Lead Nitrate without and Lead Nitrate with Sodium Silicate)**