RESEARCH REVIEW JOURNALS
Volume-04
Issue-04
April -2019

ISSN: 2455-3085 (Online)
RESEARCH REVIEW International Journal of Multidisciplinary
www.rrjournals.com [UGC Listed Journal]

Effect of Lead Chloride on Biochemical Attributes of *Tinospora cordifolia*

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ARTICLE DETAILS

Article History
Published Online: 15 April 2019

Keywords
Tinospora cordifolia; Lead (Pb); Biochemical Contents.

ABSTRACT

Accumulation of heavy metals in soils is caused by different man-made activities including manufacturing, agricultural, mining, and waste removal practices. These metal ions dissolved in irrigation water contaminate the cultivated soils and initiates toxic impact on living system when their concentration in soil is high. Lead (Pb) is one of the most important heavy metals frequently available in the environment. In plants, its accumulation has been reported in stem, leaves, roots and seeds, which increases with increase in Pb levels in the growth medium. Lead influences plants by hampering a variety of physiological processes including nutrient uptake. Present study was conducted to investigate toxic effects of $PbCl_2$ on biochemical attributes of *Tinospora cordifolia*. Plants were treated with different $PbCl_2$ concentrations. The results of this experiment showed that the $PbCl_2$ stress decreased the biochemical contents such as chlorophyll, carbohydrate and proteins in all the treated plants. So $PbCl_2$ as a heavy metal has detrimental effect on *Tinospora* cordifolia physiology.

1. Introduction

Heavy metal pollution of air and agricultural soils is one of the most important ecological problems on world scale. In India and other areas heavy metal contamination issues are increasing day by day with many documented cases of metal toxicity in mining industries, smelters, coal burning power plants and agriculture (Nagajyoti et al;2010). From plant products these metals are transferred to human diet and affects the human health (Rauser and Meuwly 1995).

Among these heavy metals, lead is one of the most toxic heavy metal. According to the environmental protection agency (EPA), Pb is the most common heavy metal contaminant in the environment Pb contamination has resulted from mining and smelting activities, Pb containing paints and gasoline. Pb is available to plants from soil and aerosol sources (Sharma and Dubey, 2005). It is a nonessential element in metabolic processes and may be toxic or lethal to organisms even when absorbed in small amounts . It is readily taken up by the root system of many plant species and its toxicity generally is considered to be 2-20 times higher than that of other heavy metals.

Pb absorption is regulated by pH, cation exchange capacity of the soil, as well as by exudation and physicochemical parameters (Lane *et al.,* 1977 and McGrath, 1995). Absorption by roots from the soil occurs via the plasma membrane, probably involving cationic channels such as calcium channels. Roots are capable of accumulating significant quantities of this heavy metal and simultaneously restrict its translocation to the shoot (Lasat, 2000). The retention of Pb in roots involves binding to the cell wall and extracellular precipitation, mainly in the form of Pb carbonate, which is deposited in the cell wall. At low concentration, Pb can move through root tissue, mainly via the apoplast and radially through the cortex where it accumulates near the endoderm.

Plants undergo significant morphological and metabolic changes in response to metal stress. Visible symptoms of metal toxicity in the plants are the expression of metal-induced alterations at the structural and ultra structural levels. These changes at the cell, tissue and organ levels, in turn, are the result of a direct interaction of the toxic metals with structural components at these sites (Singh and Sinha, 2004). In heavy metal stress epicuticular waxes on leaf surface and opening of stomata was affected (Mehrotra, 2005).A high lead level in soil induces abnormal morphology in many plant species. For example, lead causes irregular radial thickening in pea roots, cell walls of the endodermis and lignification of cortical parenchyma (Paivoke, 1983). Lead also induces proliferation effects on the repair process of vascular plants (Kaji et al., 1995). Lead administrated to potted sugar beet plants at rates of 100–200 ppm caused chlorosis and growth reduction (Hewilt, 1953). Low amounts of lead (0.005 ppm) caused significant reduction in growth of lettuce and carrot roots (Baker, 1972). Inhibitory effects of Pb^{2+} on growth and biomass production may possibly derive from effects on metabolic plant processes (Sharma and Dubey, 2005). Also it has been reported that Pb phytotoxicity affects plant growth and several metabolic activities in different cell components including decreased seed germination percent, length and dry mass of roots and shoots disruption mineral nutrition (Sharma and Dubey, 2005), reduction in cell division (Eun *et al.,* 2002), inhibition and enhancement/activation of photosynthetic pigment contents as well as antioxidant enzymatic activities (Ekmekci et al., 2009). The primary cause of cell growth inhibition arises from a lead-induced simulation of indole-3-acetic acid (IAA) oxidation. Lead is also known to affect photosynthesis by inhibiting activity of carboxylating enzymes (Stiborova et al., 1987) . The effect of Pb exposure induced biochemical and molecular changes in *T.cordifolia* species has not been studied yet. Therefore, we studied about the biochemical contents. The aim of the present study was to evaluate the hazardous effects of lead on biochemical attributes of *Tinospora cordifolia*, one of the important medicinal plants.

2. Materials and Methods:

Sample Collection: The healthy and authentic seeds of *Tinospora cordifolia* were purchased from MFP PARC (Vindhya herbal) Bhopal (MP) Bhopal. Seeds were sterilized with 1% $Hgcl_2$ for 10 min, then washed several times with distilled water.

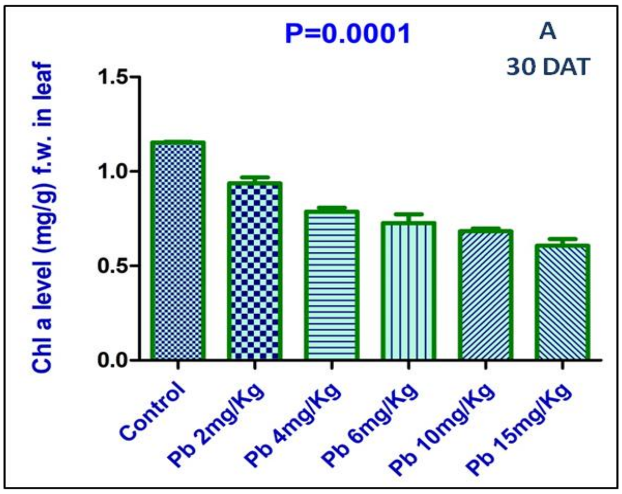
***Pot culture and Treatments*:**

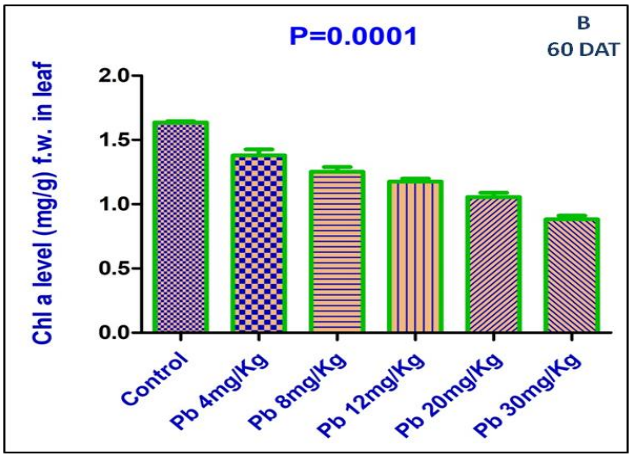
The pot experiment was conducted at Govt. M.L.B Girls P.G (Autonomous) College Bhopal. The pots of equal size were filled with homogenous soil. The seeds were sown in pots filled with soil. These pots were kept in green house and regularly watered to allow growth. After 30 days later, the first heavy metal treatment of each selected dose (2mg, 4mg, 6mg, 10mg and 15mg kg^{-1} of soil) of Pb was applied in pots. The second dose was given at 30 days later i.e., at 90 days after sowing.

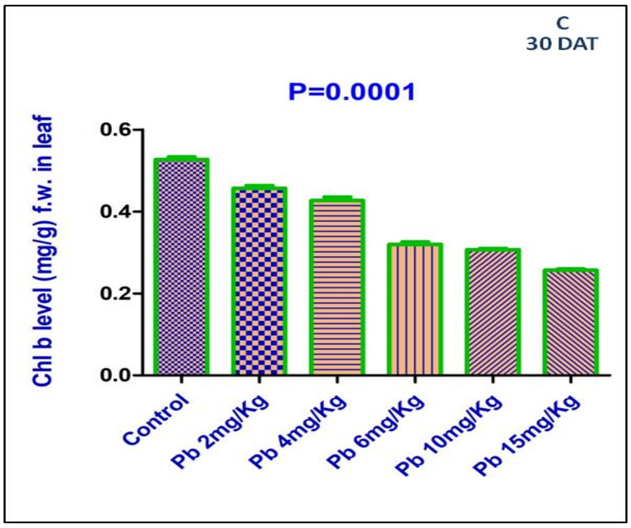
Biochemical analysis

The control (untreated) and lead-treated leaf tissue of plants were analyzed for total chlorophyll and carotenoid content (Arnon, 1949) and carbohydrate content (Anthrone method). Total protein content in the leaf tissue of plants was determined (Lowry 1951) using UV Spectrometer . BSA standard stock solution was used for calibrating the equipment . All experiments were carried out in triplicate and the results were analyzed using one way ANOVA and Dunnett's test.

3. Results:







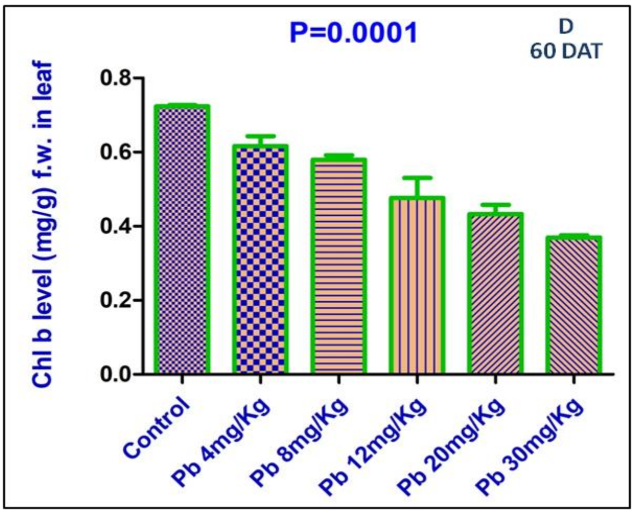
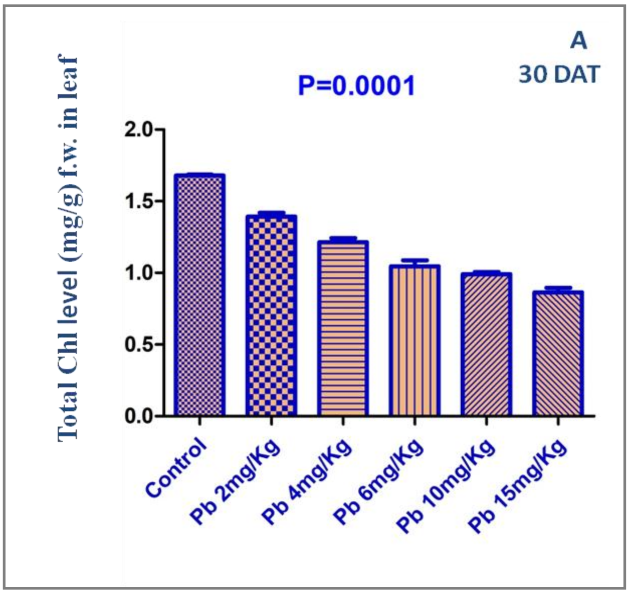


Figure-1: Chlorophyll'a' and 'b' content in the leaves of *Tinospora cordifolia* treated with varying doses of lead and estimated at 30 (Control, 2mg, 4mg, 6 mg ,10mg and 15 mg kg^{-1} soil) and 60 (Control , 4mg, 8mg, 12 mg ,20mg and 30mg kg^{-1} soil) days after treatment (DAT).



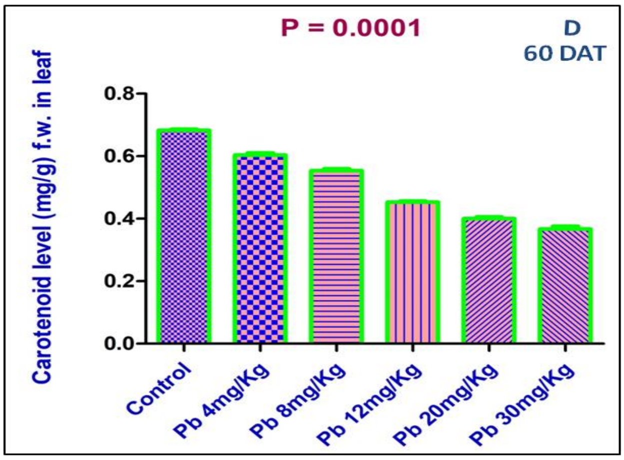
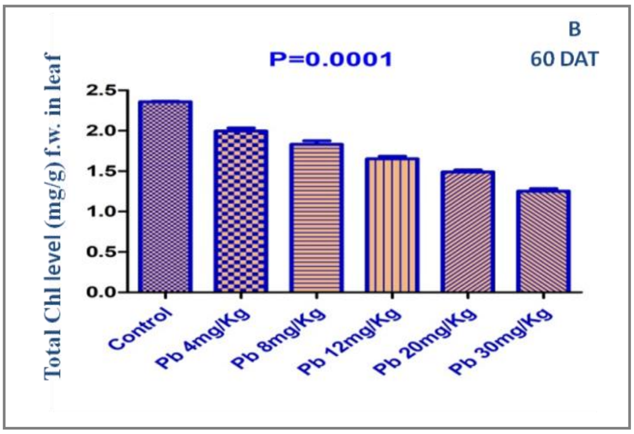
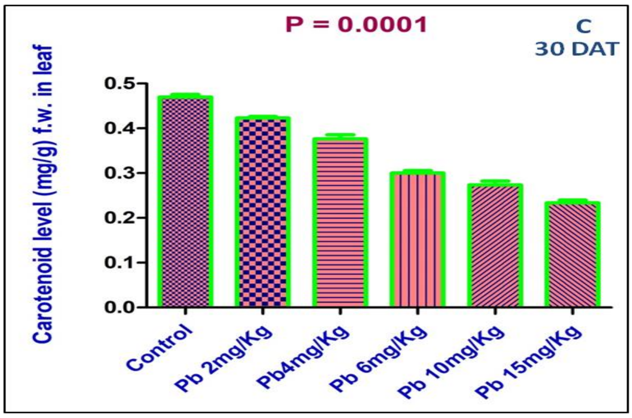
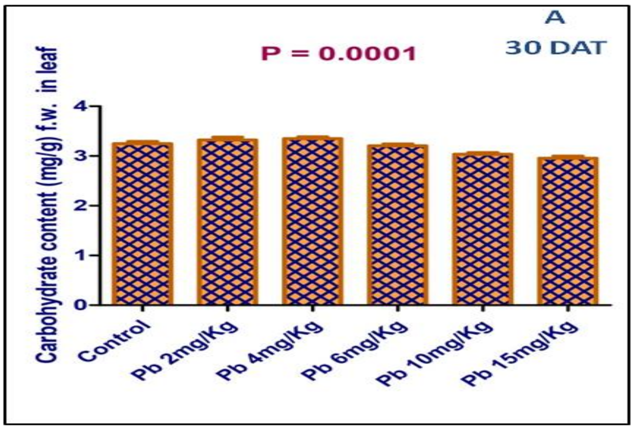
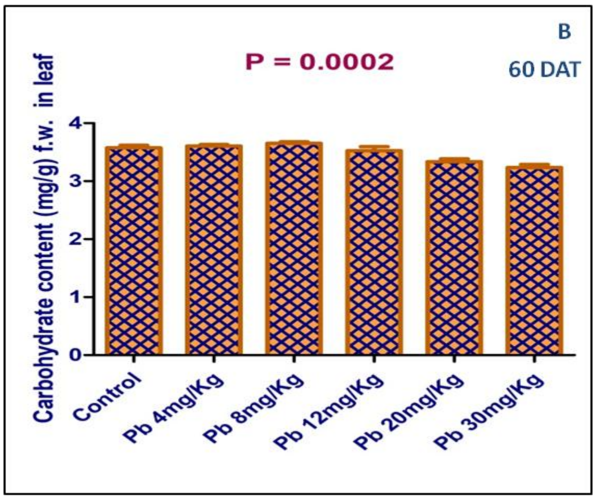


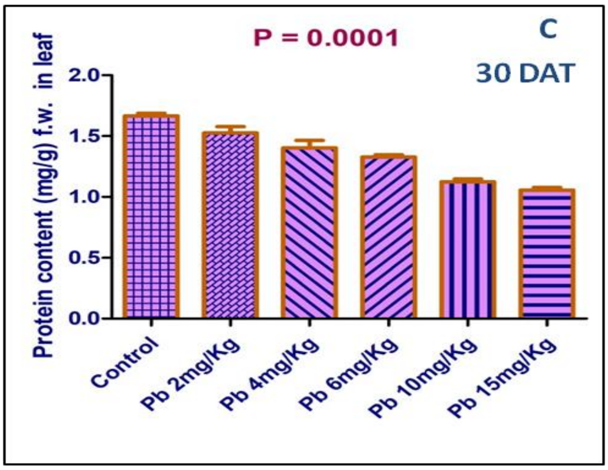
Figure-2: Total Chlorophyll and carotenoid content in the leaves of *Tinospora cordifolia* treated with varying doses of lead and estimated at 30 (Control, 2mg, 4mg, 6 mg ,10mg and 15 mg kg^{-1} soil) and 60 (Control , 4mg, 8mg, 12 mg ,20mg and 30mg kg^{-1} soil) days after treatment (DAT).











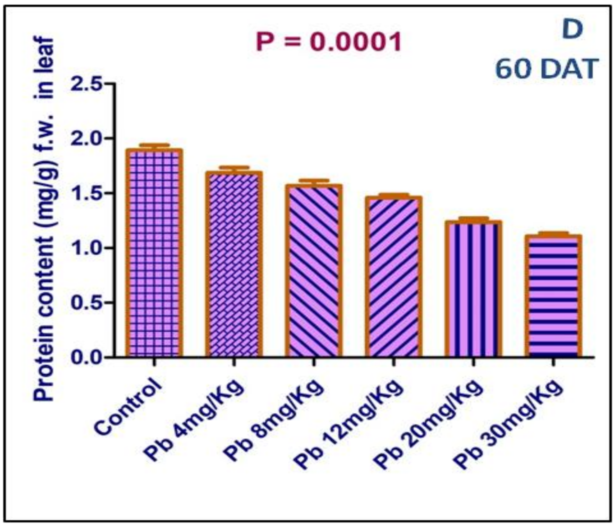
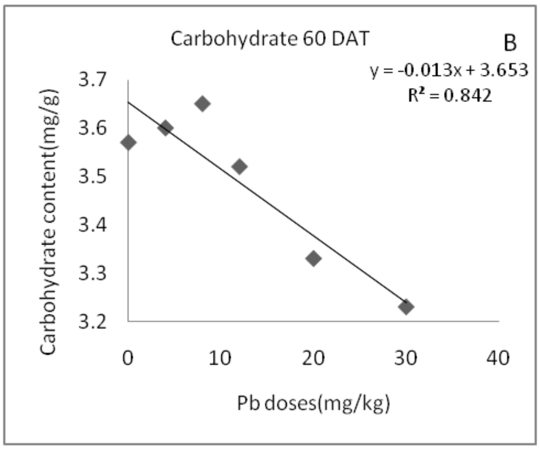
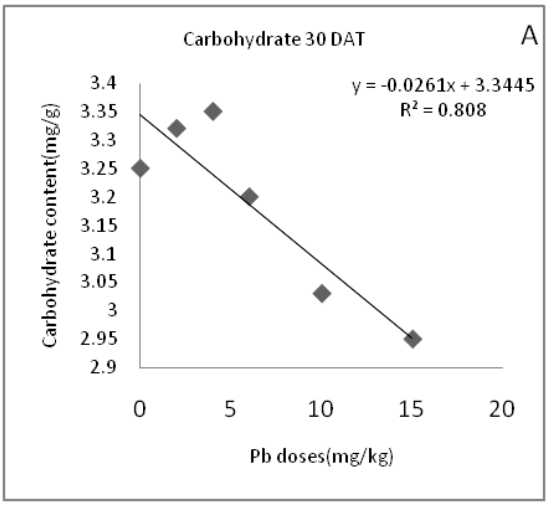
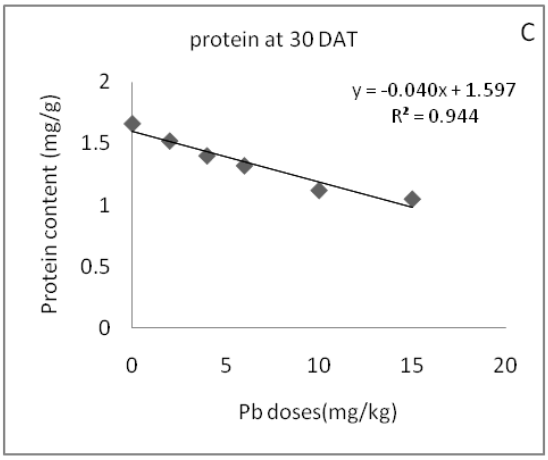


Figure-3: Carbohydrate and protein content in the leaves of *Tinospora cordifolia* treated with varying doses of lead and estimated at 30 (Control, 2mg, 4mg, 6 mg ,10mg and 15 mg kg^{-1} soil) and 60 (Control , 4mg, 8mg, 12 mg ,20mg and 30mg kg^{-1} soil) days after treatment (DAT).







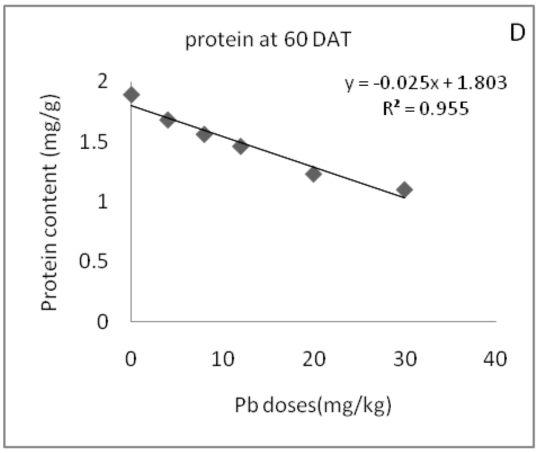


Figure 4: Linear regression and square of correlation coefficients between carbohydrate content and dosees of lead (A, B), between protein content and doses of lead (C, D), in the leaves of *Tinospora cordifolia* and estimated at 30 (Control, 2mg, 4mg, 6 mg ,10mg and 15 mg kg^{-1} soil) and 60 (Control , 4mg, 8mg, 12mg, 20mg and 30mg kg^{-1} soil) days after treatment (DAT).

4. Discussion:

The objective of the present research was to find out the effect of $PbCl_2$ on the photosynthetic pigments, carbohydrate and protein contents of *Tinospora cardifolia.* With the increase in concentration of Pb the chlorophyll, carotenoid and protein content of treated plants adversely affected. The inhibitory effect was concentration dependent (Fig 1, 2, 3), however the carbohydrate content was increased slightly in the first two and at higher concentrations it gets decreased. This is evident from these results that lead stress predominantly affects biochemical metabolism. The biochemical contents of the plant such as total chlorophyll, carotenoid and proteins decreased with increased concentration of $PbCl_2$ (Fig.1,2, 3). Under the metal stress, the levels of photosynthetic pigments, namely Chlorophyll 'a' and Chlorophyll 'b' and Carotenoids decrease as the concentrations of Pb in soil increases (Bhardwaj *et al.,* 2009). The decline in the levels of these pigments clearly shown the metal interference with pigment metabolism. Similar observations were made by Mukherji and Maitra (1976) in rice where Pb toxicity resulted in lowering Chl a/b ratio. Lead was found to inhibit δ amino levulinic acid dehydratase activity in mung bean resulting in a decrease in Chlorophyll Content (Prasad and Prasad, 1987) . Pb also distorts the membrane structure of chloroplasts, which ultimately leads to decrease in Chlorophyll Content. The reduction in protein content may be caused by the increased protein degradation process as a consequence of enhanced protease activity that is found to increase under stress condition (Palma *et al.,* 2002). It is also likely that lead may have induced disintegration of proteins due to the toxic effects of reactive oxygen species that led to reduce the protein content (Davies *et al.,* 1987). Protein content under lead stress may be affected due to enhanced protein hydrolysis resulting in decreased concentration of proteins (Melnichuk *et al.,* 1982) and catalytic activity of lead (Bhattacharya and Choudhuri;1997.) The results related to the carbohydrate content are depicted in Fig 3 (A, B)which revealed that lower concentrations of Pb increased the carbohydrate content; however, higher concentrations of Pb showed a decrease in carbohydrate content after 30 days and 60 days respectively. The correlation coefficients determined between carbohydrate content and varying doses of lead were negative (Figure 4 A, B). The correlation coefficient between protein content and doses of lead in the fresh leaf tissues of *T.cordifolia* were strongly negative at both intervals. Our results corroborate with the findings of Ahmad *et al.,* (2006) who found that an increase in soluble sugars at low concentrations of salt stress and decrease at higher concentrations in *Pisum sativum.*

5. Conclusion:

Results showed that $PbCl_2$ has detrimental effect on *Tinospora cordifolia* physiology. The findings of this study will help in revealing lead toxicity mechanism which subsequently will facilitate to manipulate the conditions for lead detoxification in plants growing in lead contaminated environment. Furthermore research is needed in this area so that the exact mechanism of inhibition of various physiological parameters of *Tinospora cordifolia* caused due to lead stress will be evaluated.

Acknowledgement: *Financial support for this work was provided by the University Grants Commission, New Delhi (India). Masrat Jan is grateful to the UGC New Delhi for the same.*

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