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EFFECT OF LEAD (PB) ON PHYSICOCHEMICAL CHARACTERISTICS OF *ARTEMISIA ANNUA* L

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Abstract

Artemisia annua L., often known as sweet wormwood, sweet Annie, sweet sagewort, and annual wormwood, is a widespread species of wormwood that originated in temperate Asia but has since spread naturally around the world. It is a member of the Asteraceae family. Chinese medicinal plant *Artemisia annua* L.is renowned for its antiplasmodial, antirheumatic, and anticancer effects. Currently, *Artemisia annua* L.is the source of artemisinin and semi-synthetic artemisinin derivatives, such as dihydroartemisinin, artesunate, artemether, and arteether, which are used to make combination therapies (ACTs, or artemisinin-based combination therapies) for the treatment of malaria. This study focuses on how lead (Pb) affects *Artemisia annua* L. physiochemical and bioactive characteristics. Effects of lead the variables that were examined—carotenoids, stomatal conductance, chlorophyll content, and photosynthesis—were all impacted by lead concentrations in various ways. For plants, animals, and humans alike, lead is an extremely dangerous environmental pollutant. The presence of Pb as an impurity in fertilizers over a long period of time is another factor in its occurrence in fields. Pb is actually present in many different environments, such as the soil, water, air, fertilizers, and so on. The results also showed that at various lead concentrations, germination percentage, shoot length, root length, leaf yield, and fresh weight decreased. In the plant treated with various doses of lead, the accumulation of CA activity, proline content, and CAT activity, among other things, was reduced.

Keywords: *Artemisia Annua* L. Antiplasmodial, Lead, Bioactive Parameters, Environmental Pollutant

INTRODUCTION

Artemisia annua L.*is* a member of the tribe Arthemideae and the family Asteraceae Asteroideae. It is also known as "*annua absinthe*" since it is an annual herb. The plant is grown in temperate areas of America, Asia, India, Central and Eastern Europe, Africa, Australia, and tropical regions**.** (Alesaeidi et al 2016 and Willox et al 2020). In temperate climates of Asia, including as China and Korea, it is widely utilized as a nutritional spice, herbal tea, and medicinal plant. (KO et al 2016). *Artemisia annua* L. plays an important role in combating the threats associated with malaria as the plant is used to produce anti-malarial drugs. *Artemisia annua* L. contains a valuable sesquiterpene lactone molecule, namely artemisinin (Naeem et al 2020). *Artemisia annua* L.essential oil has been widely used in ethno medicine, as a food preservative, and as an ingredient in cosmetics. *Artemisia annua* L. is a traditional Chinese medicine. It has been used in China for over 2000 years to treat many disorders including malaria. The isolation of artemisinin from *Artemisia annua* L. and the characterization for its antimalarial effect had been initiated by Chinese scientists in the 1970s (Marija et al 2023). It

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was one of the most important advances inside the fight against malaria in modern times. Many research have demonstrated the antibacterial, anticholesterol, antiviral, anti-inflammatory, anti-plasmodia, anticancer, anti-obesity, and anticonvulsant properties of this plant in addition to its antimalarial action. (Kondza et al. 2023).

Both natural processes and human activity are sources of heavy metals. Heavy metals of anthropogenic origin have been released into the environment at an increasing rate over the past few decades, with the majority of these metals increasingly building up in soils to potentially dangerous levels. (Shi et al 2018). In addition, several human activities (such as wastewater irrigation, pesticides, chemical fertilizers, urban wastes, and metal mining) have led to the accumulation and contamination of heavy metals in agricultural soils (Sun, et al 2020). Because heavy metals can enter the food chain and endanger human health, their buildup in agricultural soils has become a critical issue all around the world. Additionally, when there is an excessive buildup of heavy metals in the soil, it can cause agricultural failure as well as environmental and ecological damage. (Liu et al 2020). Heavy metals in both terrestrial and aquatic settings, lead (Pb) is one of the most prevalent heavy metal contaminants. Lead causes lipid peroxidation to occur and reactive oxygen species to be produced in a variety of plant species, which causes free radicals to form and inhibits metabolic processes like nitrogen absorption, photosynthesis, respiration, water uptake, and transcription. (Boussama et al., 1999). Lead causes two types of unfavorable processes in biological systems: (I) it inactivates several enzymes and (II) it can intensify the processes of reactive oxygen species (ROS) production leading to oxidative stress (Prasad et al., 1999). Reactive oxygen species produced can damage photosystem reaction center proteins by which photosynthesis could decrease and cause viability loss in the cells.

MATERIAL AND METHODS

Experimental design

Seeds of *Artemisia annua* L. will be purchased from the local market Uttrakhand Dehradun. Before sowing, the seeds of *Artemisia annua* L. (variety CIM-Arogya) were surface sterilized with 0.02% HgCl2 solution for 5 min with frequent shaking and then washed with de-ionized water. Initially the seeds of CIM-Arogya variety were sown in the seed beds (1m × 1m). After one month of sowing, the seedlings of uniform size were transplanted to earthen pots (one seedling per pot). The pots were watered a little after transplantation. Prior to transplantation, 5 kg homogenous mixture of soil and farmyard manure (4:1) was filled in each pot (25 cm diameter × 25 cm height). A uniform recommended basal dose of N, P and K was applied before sowing. Treatments used were 0, 15, 30, and 45 Pb mg/kg $^{-1}$. Three replicates with a single healthy plant were maintained for each treatment of the study.

Determination of Physical attributes

Germination percentage: We counted the number of seeds that sprouted in each pot daily until the seventh day, knowing that germination is successful when the radical length reaches 5 mm.

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Radical length of germination-stage seeds was measured in centimeters after the seventh day.

We used the formula: to determine the percentage of germination.

$$G\ (\%) = (\text{number of seeds that germinated} / \text{total seeds}) / 100$$

Determination of fresh weight, root length, shoot length and dry weight

Each treatment's plants were carefully uprooted, and the shoot height was measured. To get rid of any foreign objects that had adhered, plants were cleaned with tap water. The weight of each fresh shoot was measured after the plant's roots were cut off. The shoots were air dried at 80C for 48 hours, after which their dry weights were noted. Weighing the plants' total leaves allowed us to calculate their leaf yield.

Biochemical attributes

Total amounts of carotenoids and chlorophyll

Total amounts of carotenoids and chlorophyll the approach of Lichtenthaler and Buschmann (2001) was used to estimate the total amounts of chlorophyll and carotenoids in the leaves. Using a mortar and pestle, the fresh tissue from the interveinal leaf part was ground. Using a spectrophotometer (Shimadzu UV1700, Tokoyo, Japan), the optical density (OD) of the pigment solution was measured at 662, 645, and 470 nm to determine the contents of chlorophyll a, chlorophyll b, and total carotenoids, respectively. By combining the amounts of chlorophyll a and b, the total amount of chlorophyll was calculated. Each photosynthetic pigment's content was given as mg g^{-1} leaf FW.

Net photosynthetic rate and stomatal conductance

These parameters were determined employing the youngest fully expanded randomly selected leaves from the five replicates of each treatment. Measurements were made on sunny days at 1100 hours using the Infra-Red Gas Analyzer (IRGA, Li-Cor 6400 Portable Photosynthesis System Lincoln, Nebraska, USA) both at 100 and 120 DAP.

Carbonic anhydrase activity (CA)

The method outlined by Dwivedi and Randhawa (1974) was used to evaluate the activity of the carbonic anhydrase (E.C. 4.2.1.1) enzyme in fresh leaves that were randomly picked. Approximately 200 mg of the leaves (chopped leaf fragments) were put in Petri dishes. The leaf pieces were submerged for 20 minutes at 4°C in a 10 mL solution of 0.2 M cystein hydrochloride. The leaf fragments were immediately transferred to a test tube containing 4 mL of phosphate buffer (pH 6.8) after the solution adhering to them was removed with the aid of blotting paper. To it, 4 mL of 0.2 M sodium bicarbonate solution and 0.2 mL of 0.022% bromothymol blue were added. The reaction mixture was titrated against 0.05 N HCl using methyl red as indicator. The enzyme activity was expressed as µmol CO2 kg-1 leaf FW s^{-1}.

Proline (PRO) content estimation

The method of (Bates et al 1973) was used to estimate the PRO content (mg g^{-1} FW). The plant material was homogenized in an aqueous sulfosalicylic acid solution at a concentration of 3%

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before the homogenate was centrifuged at 10,000 rpm. The PRO content was estimated using the supernatant. Two milliliters each of acid ninhydrin and glacial acetic acid were added to the reaction mixture. The material was boiled for one hour at 100°C. The PRO content was extracted using 4 mL of toluene after the reaction had been stopped using a cold bath, and the absorbance at 520 nm was then measured.

Lipid peroxidation (TBARS Content)

Oxidative damage to leaf lipids was estimated by the content of total 2-thiobarbituric acid reactive substances (TBARS) expressed as equivalents of malondialdehyde (MDA). The total content of TBARS was estimated using the method of (Cakmak and Horst 1991). TBARS were extracted from 0.5 g of chopped fresh leaves, grinding the latter with 5 mL of 0.1% (w/v) trichloroacetic acid (TCA). Following the centrifugation at 12,000×g for 5 min, an aliquot of 1 mL of the supernatant was added to 4 mL of 0.5% (w/v) of tetrabutylammonium (TBA) in 20% (w/v) TCA. Samples were incubated at 90°C for 30 min. Thereafter, the reaction was stopped using an ice bath. The content was centrifuged at 10,000×g for 5 min, and the absorbance of the supernatant was recorded at 532 nm with the help of a spectrophotometer and the values were corrected for non-specific turbidity by subtracting the absorbance at 600 nm. TBARS content was expressed as nmol g^{-1} FW.

Catalase activity (CAT)

The method suggested by Chandlee and Scandalios (1984) was slightly modified in order to quantify the activity of catalase (CAT). 2.6 mL of 50 mM potassium phosphate buffer (pH 7.0), 0.4 mL of 15 mM H_2O_2, and 0.04 mL of the enzyme extract made up the test mixture. The extract was centrifuged at 12,500 g for 20 minutes at 4 °C. The supernatant was used for enzyme assay. The assay mixture contained 2.6 mL of 50 mM potassium phosphate buffer (pH 7.0), 400 µL of 15 mM H_2O_2 and 40 µL of enzyme extract. The decomposition of H_2O_2 was followed by the decline in absorbance at 240 nm. The enzyme activity was expressed in units per milligram protein (U = 1 mmol of H_2O_2 reduced per minute per mg protein).

Estimation of Artemisinin content

The artemisinin content of the leaves was estimated using the high-performance liquid chromatography (HPLC) technique as described in Zhao and Zeng (1986). The amount of artemisinin in the leaf was determined using the artemisinin standard curve (Sigma-Aldrich, USA). One gram of dried leaf from each treatment is utilized, changed to form compound Q260, and quantified using the HPLC method to determine the amount of artemisinin in the plant. The material was extracted for 24 hours at 70 rpm using petroleum ether (20 mL). This was followed by three further washes with petroleum ether (20 ml). Under reduced pressure, the entire batch of petroleum ether washings was collected. Derivatized artemisinin was analyzed and quantified using a reverse-phase column (C18; 5 m; 4.6 mm; 250 mm) and a premix of methanol: 10 mM phosphate buffer (pH, 6.5) in a ratio of 60:40 with the mobile phase at constant flow at a rate of 1 ml/min, with the detector set at 260 nm.

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Statistical analysis

Descriptive statistics had been presented as mean ± standard error of the mean. Two-way ANOVA (Analysis of Variance) were used to test for differences between treatments. Duncan's multiple range test (DMRT ≤ 0.05%) changed into extensively utilized to perceive one of a kind treatments observed by ANOVA. The level of statistical importance was taken into consideration as 5%, and the statistical program SPSS (ver: 22) became used for all statistical calculations. In addition, principal component analysis (PCA) was completed the usage performed of Minitab 19.2.0 software.

RESULT

The effect of different concentrations of Pb (0, 15, 30 and 45 mg kg-^{1} Pb in soil) reduced the values of growth attributes in this study (Table 1). At highest Pb load, 'CIM-Arogya' showed decrease in germination percentage (91%, 89%, 86% and 79%) at 0 mg kg-^{1}, 15 mg kg-^{1}, 30 mg kg-^{1} and 45 mg kg-^{1} respectively Shoot length in control samples became 84.4. At Pb 15 mg kg-^{1,} shoot length became recorded as 98.1. The suggest shoot length recorded at 30 mg kg-^{1} and 45 mg kg-^{1} was 76.2 and 64.7, respectively. The root length in the control sample was 28.9. When the concentration increased to 15 mg/kg, the root length extended to 35.1. At Pb 30 mgkg-^{1} and Pb 45 mgkg-^{1} root length become recorded as 25.2 and 21.8 respectively. Under maximum Pb stress, 'CIM-Arogya' showed decrease in leaf yield/plant (64.41, 69.61, 56.39 and 52.19) at 0 mg kg-^{1}, 15 mg kg-^{1}, 30 mg kg^{-1} and 45 mg kg-^{1} or At highest Pb load, 'CIM-Arogya' showed decrease in fresh weight (443.1, 352.3, 284.1 and 230.3) at 0 mg kg-^{1}, 15 mg kg-^{1}, 30 mg kg-^{1} and 45 mg kg-^{1} respectively. Under highest Pb stress, 'CIM-Arogya' confirmed decrease in plant dry weight (72.48, 74.28, 58.39 and 50.22) at 0 mg kg-^{1}, 15 mg kg-1, 30 mg kg^{-1} and 45 mg kg-^{1} or Pb treatment considerably reduced the photosynthetic rate and stomatal conductance of *Artemisia annua* L. CIM-Arogya' comparison to the manipulate (Table 2). In 'CIM-Arogya' it decreased the rate of photosynthesis to 15.13, 14.39, 14.11 and 13.18 at 0 mg kg^{1}, 15 mg kg-^{1}, 30 mg kg-^{1} and 45 mg kg-^{1} respectively and stomatal conductance to 0.26%, 0.23%, 0.18% and 0.16% at 0 mg kg-^{1}, 15 mg kg-^{1}, 30 mg kg-^{1} and 45 mg kg-^{1} in that order. Chlorophyll and carotenoids were extensively reduced compared to the control. Carbonic anhydrase (CA) activity was significantly inhibited by increasing Pb levels. Pb stage reduced the activity of CA “CIM-Arogya” by (212.5, 201.3, 192.1 and 179.4) at 0 mg kg-^{1}, 15 mg kg-^{1} and 30 mg kg-^{1} and 45 mg kg -^{1}, respectively. However, proline level changed into higher at 45 mg Pb kg-^{1} soil increased proline content material with the aid of (8.29, 8.72, 9.47 and 10.79) at 0 mg kg-^{1}, 15 mg kg-^{1} and 30 mg kg -^{1} or 45 mg kg-^{1}. Pb-treated *Artemisia aanua* L.confirmed a higher level of lipid peroxidation in leaves, indicating significant oxidative stress. Compared to the control, the maximum level of Pb to a significant increase in lipid peroxidation (TBARS content). It increased leaf TBARS content by means of “CIM-Arogya” (7.04, 9.83 and 11.76 and 16.12) at 0 mg kg-^{1}, 15 mg kg-^{1} and 30 mg kg-^{1} and 45 mg kg-^{1} in that order. The activity of antioxidant enzymes (CAT) turned into rapidly stimulated at 45 mg Pb kg-^{1} soil. The activities of these enzymes have been comparatively lower at lower Pb levels. Compared to control, the maximum level of Pb improved CAT activity by (8.34, 9.89, 10.54 and 11.94) at 0 mg kg-^{1}, 15 mg kg-^{1} and 30 mg kg-^{1} and 45 mg kg -^{1} respectively

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Yield and quality attributes

Crop plant yield was significantly increased at lower Pb level (15 mg kg-^{1} soil) regardless of stage. However, with the increasing level of Pb in the stages, it decreased regardless of the *Artemisia aanua* L .variety. Compared to the control, 45 mg pb kg-^{1} soil (peak pb level) reduced the yield of 'CIM-Arogya' (317.1, 372.6, 299.4 and 269.7) at 0 mg kg-^{1}, 15 mg kg -^{1} respectively 30 mg kg^{-1} and 45 mg kg-^{1}. The cultivar 'CIM-Arogya' contained the highest artemisinin content below the maximum Pb level; expanded artemisinin content by material by way of (528.3, 573.6, 651.8 and 724.1) at 0 mg kg-^{1}, 15 mg kg-^{1} and 30 mg kg-^{1} and 45 mg kg-^{1} respectively. Pb-treated *Artemisia annua* L.of the variety also produced the highest Artemisinin yield compared to the control. Even through all levels of Pb significantly increased artemisinin production, leaf artemisinin content material turned into higher in 'CIM-Arogya'. The pb level of Artemisinin yield by CIM-Arogya' by (0.031, 0.039, 0.043, 0.049) at 0 mg kg-^{1}, 15 mg kg-^{1} and 30 mg kg-^{1} and 45 mg kg-^{1} respectively.

Table 1: Physical parameters of *Artemisia annua* L

Pb (mg Kg -^{1})	Germination %	shoot length/plant (cm)	Root length / plant (cm)	Leaf yield/ plant (g)	FreshS weight/plant (g)	Dry weight /plant (g)
0	91	84.4	28.9	64.41	443.1	72.48
15	89	98.1	35.1	69.61	352.3	74.28
30	86	76.2	25.2	56.39	284.1	58.39
45	79	64.7	21.8	52.19	230.3	50.22

Table 2: Chemical parameters of *Artemisia annua* L.

Pb (mg kg – ^{1})	Net photosynthetic rate (µmol m-2s-1)	stomatal conductance (m mol m-2s-1)	Total chlorophyll content (mg g-1)	Total carotenoids content (Mg g-1)
0	15.13	0.26	1.04	0.438
15	14.39	0.23	0.97	0.461
30	14.11	0.18	0.83	0.413
45	13.18	0.16	0.77	0.384

Table 3: Bioactive parameters of *Artemisia annua* L.

Pb(mg kg – ^{1})	CA activity (µM Co^{2} kg^{-1} leaf FW s^{-1})	Proline content (mg g^{-1} FW)	TBRAS CONTENT (nmol g^{-1} FW)	CAT activity U mg^{-1} protein)
0	212.5	8.29	7.04	8.34
15	201.3	8.72	9.83	9.89
30	192.1	9.47	11.76	10.54
45	179.4	10.79	16.12	11.94

Table 4: Yield and quality attributes of *Artemisia aanua* L.

Pb (mg kg – ^{1})	Herbage yield (g)	Artemisinin content (µg^{-1}DW)	Artemisinin yield (g plant^{-1} DW)
0	317.1	528.3	0.031
15	372.6	573.6	0.039
30	299.4	651.8	0.043
45	269.7	724.1	0.049

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DISCUSSION

Crop plants suffer severe damage and productivity losses as a result of being sessile, making them vulnerable to different environmental cues. According to reports, lead (Pb) and other heavy metals were the elements that people have used the most recently. As a result, these elements have significantly contaminated surface soils by binding to humic matter in organically rich soil and iron oxides in mineral soil. According to some reports, Pb and its compounds gather in soils and sediments, where they may stay bioavailable for a very long time (Alloway 1954). Because Pb is strongly absorbed by humic matter at pH 4 and above, it has been hypothesized that the amount of organic matter in the soil affects the chemical behavior of Pb (Bunzl et al 1976, Kerndorff and Schnitzer 1980). As reported earlier (Jones et al 1973, Li et al a 2007), most of the lead in the soil appears to be generally unavailable to the plant tops, considering that from the Pb (Pb2+) absorbed by the roots of plants, very little is translocate from the roots to the above-ground plant organs, which was shown to depend on the physiological status of the plant (Koeppe 1977) and may cause toxicity, leading to changes in the metabolism of the plant (Pourrut et al 2011, Ashraf et al 2017). Different plant species have different tolerance levels to Pb contamination, which could depend on the Pb concentration (Yoon et al 2006), as well as the root Pb storage potential and transport of a particular plant species (Rout et al 2001).

It is possible that the applied Pb concentration had a significant impact on the growth-related parameters of *rice* as well as the major yield components of *rice* in a concentration-, genotype-, and duration-dependent manner based on the inhibitory effect of Pb on the morphological aspects of *rice* plants, including the major yield components of *rice*, particularly in *rice* cultivars identified as Pb sensitive (Ilmi, Yasmen, and Amber Barka), and much less in those identified as Pb tolerant ((Tunnae and Mashkab), On the other hand, heavy metals have reportedly been shown to have detrimental effects on plant growth and development, cell division, and both (Singh et al 2016). In addition, Ilmi, Yasmen, and Amber Barka were recently reported to be sensitive towards drought and dehydration stresses (Al Azzawi et al 2020). A similar response was observed when Ilmi and Yasmen were exposed to Pb stress. The negative effects on plant growth and development could be attributed in part to the obstruction of nutrient uptake from the roots due to the high Pb application (Ashraf et al 2017). Additionally, (Kibra 2008) supported that *rice* cultivars grown in soil contaminated with 1 mg Hg Kg^{-1} Pb exhibited a significant reduction in plant height, number of tillers per plant, and number of panicles per plant. In the same way, another study suggested that the toxic effect of heavy metals on the growth parameters of plants could be attributed to a reduction in the alteration of the activity of some antioxidant enzymes or mineral nutrition uptake or the photosynthetic process.

Plants stress in complicated physiological and biochemical reprogramming and resource redistribution in response to stress induction brought on by either biotic or abiotic stress stimuli, in our case, lead-induced oxidative stress. This causes a number of antioxidant (enzymatic and non-enzymatic) systems to become active and the production of a number of biological components. The Pb treatment in the current study demonstrated that all *rice* cultivars

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(including both Pb-tolerant and -sensitive rice cultivars) considerably increased their catalase (CAT) activity as a treatment. In general, an increase in CAT activity is anticipated to contribute to a reduction in ROS buildup, notably H_2O_2. The Ilmi (0.6 and 1.2 mM Pb), Yasmen, and Amber Barka cultivars, which were also identified as the most sensitive cultivars in terms of their phenotypic responses to Pb stress, were shown to have a much more dramatic H_2O_2 buildup pattern in this study. In a recent study, we also found that drought stress in Arabidopsis plants—a model plant for dicots—increased CAT activity in both drought-tolerant and -sensitive genotypes (Kibra 2008). It is therefore believed that CAT activity alone may not be sufficient to provide the required level of tolerance towards Pb stress. Rather, our study favors the hypothesis that a coordinated action involving various antioxidant (enzymatic and nonenzymatic) systems and well-organized signaling cascades would be more beneficial to plants in providing the expected degree of tolerance, while tending to maintain a balanced reduction–oxidation state within the cells. In addition to CAT, the activity of peroxidase (POD) and that of polyphenol oxidase (PPO) increased concomitantly with an increase in Pb concentration. Furthermore, the Pb-tolerant *rice* cultivars Tunnae and Mashkab that exhibited a balanced phenotypic growth and improved tolerance towards Pb stress showed a significant increase in POD activity. Moreover, Tunnae showed a significant increase in PPO as well as an increase in superoxide dismutase (SOD) activity.

In order to endure abiotic challenges, plants engage a variety of adaptive response mechanisms, such as the accumulating of proteins and solutes, as well as the enzymatic antioxidant system. Here, we observed a substantial decrease in the overall protein content across all *rice* cultivars and Pb concentrations. Proline, also known as the stress amino acid, is a different non-enzymatic antioxidant that functions as an osmoprotectant and often accumulates in response to abiotic stress. (Yamada et al 2005). Recent studies reported that proline was differentially accumulated when rice cultivars were exposed to Pb and copper (Cu) stress (Chen et al 2001). Our data indicated that the accumulation of proline was much higher in the Pb-tolerant *rice* cultivars Tunnae and Mashkab under 1.2 mM Pb treatment, therefore suggesting a possible role of proline in the adaptive response mechanism towards Pb stress tolerance in *rice*

Many studies have reported a change in the accumulation of photosynthetic pigments, such as chlorophyll, in response to abiotic stresses (Rolly et al 2020). Chlorophylls help plants get the nourishment and energy they need to finish their life cycle in conditions of normal plant growth. Since Pb poisoning would disrupt the photosynthetic process and energy supply within the cell, the observed decrease in chlorophyll would be indicative of this. This disturbance would then have an impact on the synthesis of soluble carbohydrates. As a result of the Pb stress, the soluble carbohydrates like sucrose, glucose, and fructose were found to be differently impacted. When Pb was applied at 1.2 mM, for example, we saw a considerable rise in sucrose concentration. The levels of glucose and fructose were found to be much lower under the same circumstances, indicating that sucrose predominates over glucose and fructose in the adaptive response process toward Pb tolerance. Similar to this, earlier studies found that exposure to heavy metals changed how carbs are produced, how sugar is metabolized, and how different osmolytes aggregate. (Rodríguez et al 2009, Kumar et al 2015). It appears that each antioxidant system may not be sufficient on its own to offer the necessary level of resistance to lead (Pb)

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stress. Instead, the cumulative effects of the results point to the synergistic actions of both enzymatic and non-enzymatic antioxidant systems within the cell as the cause of the tolerance to lead (Pb)-induced oxidative stress, which supports optimal growth and productivity of *rice* plants.

Treatment of shade doesn't make a noticeable difference in plant height, but treatment of manure that nested in the shade effect did make a big difference. The combination of goat manure and no shade, as can be seen, produced the maximum plant height, with an average of 178.67 cm and an increase in plant height of 22% above the treatment without fertilizer. Additionally, at the levels of 50% and 75% shade, goat manure fertilizer produced an average of the highest plants with discernible effects. With a 35% increase in plant height from the treatment without fertilizer, the greatest yield at 50% shade was 177.33 cm, and at 75% shade, the plant height was 181.33 cm with a 34% increase from the treatment without fertilizer. Manure fertilizer is used to provide the vital nutrients needed for the cultivation of *Artemisia annua* L. With sufficient soil nutrients then productivity of biomass will increase. Goat manure containing 1.19% N, 0.92% P and 1.58% K2O. From the various elements, nutrient elements nitrogen (N) is the most influential element on plant growth, especially vegetative growth (Hikmah et al 2008). Manure has a nitrogen content that serves to form amino acids that are the result of protein degradation and function to form growth hormone (Dewi et al 2016). The availability of carbohydrates from the photosynthesis process, which uses chlorophyll and involves nitrogen components, affects cell division, which affects the increase of plant height (Dapoigny et al 1997). In our investigation, Artemisia annua treated with various doses of lead had similar outcomes. *Artemisia annua* L. flowers contain artemisinin and other anti-fungal, anti-microbial, and antioxidant substances. Age at harvest, pH levels of fertilizer and soil, geographic location, plant clones, and extraction techniques all have an impact on these chemical components (Bilia et al 2014). According to (Ferreira et al 2005) When flowering plants have finished blooming, the concentration of artemisinin peaks. The weight of the bloom is not significantly affected by the shade treatment, but the blossoming age is significantly affected by the application of manure fertilizer in the shade.

Higher As concentrations had a negative impact on physiological characteristics, leaf and herbage yield, and overall growth, although they increased the activity of antioxidant enzymes and the amount and yield of artemisinin. Surprisingly, regardless of stages, the plant variety ('CIM-Arogya') produced more leaves and had higher fresh and dry weights at low As levels (15 mg As kg^{-1} soil). *Artemisia annua* L. plants' ability to grow was hindered by the presence of greater As levels in the soil, with 45 mg of As kg^{-1} of soil having the most harmful effects. The growth of plants and crop yield are significantly impacted by excess As in the soil. To reduce the toxicity of As, plants have developed a number of ways (Verbruggen et al 2009). Over the past decade, exploration of As-transporter proteins has led to the understanding of critical role in As metabolism in plants, indicating that As-moduled signal transduction pathways may lead to fast growth-reduction processes (Zhao et al 2010, Siddiqui et al 2015). According to reports, plants exposed to As-treated soils may experience a number of detrimental morphological and reproductive alterations, including a loss of both fresh and dry biomass in the roots and shoots as well as a reduction in yield and fruit production (Biswas et

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al 2015, Shaibur et al 2008, Miteva et al 2005) reported significant decrease in shoot and root growth of tomato plants at higher As-levels. According to Shaibur and Kawai 2009, Rai et al 2011b, Rai et al 2014), a reduction in plant biomass at higher As-level (4,500 µg L^{-1}) may be brought on by *Artemisia annua* L. and *mustard spinach* exhibiting decreased enzyme activity or oxidative stress brought on by As.

They hypothesized that *Artemisia annua* L. high As absorption could be the cause of the species' declining biomass. The rate of photosynthesis and stomatal conductance in the variety were lower under the peak As-level (45 mg As kg^{-1} soil) under the control. 'CIMArogya' reduction in leaf chlorophyll concentration. On the other hand, independently of the phases, As application at 15 mg As kg^{-1} soil considerably increased the level of carotenoids content in the variety. According to, the absence of adaptive changes of pigment synthesis at high As-levels may be the cause of a considerable decrease in the synthesis of photosynthetic pigments. This was backed by several employees (Stoeva and Bineva 2003, Stoeva et al 2005)), who noted that the rate of CO_2 fixation and functional activity of Photosystem II were reduced in plants under As-stress. Results agree with the earlier findings that reveal decline in contents of photosynthetic pigments as a result of As exposure (Mishra et al 2014, Srivastava et al 2007, and Srivastava et al 2013).

Factually, As interferes with the functioning of enzymes of carbohydrate and nitrogen metabolism, which may result in impaired growth and reduced biomass (Chandrakar et al 2016, Jha et al 2004, and Singh et al 2009). In the current study, plant exposure to varied As-levels decreased the CA activity. Prior to B and Al application to *Artemisia annua* L.Plants, the decline in CA activity was also seen. The variety as-treated plants produced the highest level of leaf-proline content compared to the control.

As evidence for our findings, *Hydrilla verticillata*, a *Spinacea oleracea* species, has previously been found to have an AS-mediated rise in proline level (Pavlik et al 2010), *Vigna mungo*, *Oryza sativa* (Kumar et al 1982) and *Artemisia aanua* L. eternal As-treated plants showed signs of severe oxidative stress through a greater rate of lipid-peroxidation (TBARS concentration) in the leaves. No of the type or stage, lipid peroxidation in plants was also at its highest level at the peak As-level. As concentrations grew, antioxidant enzyme activity and proline content in the leaves increased, indicating ROS generation and oxidative stress in AS-treated plants.

As per (Foyer and Noctor 2011), The ascorbate-glutathione pathway, which is made up of the enzymes APX and GR (glutathione reductase), is a crucial process involved in preventing ROS formation at the cellular and organellar (chloroplast and mitochondria) levels in plants. It may be agreed that enhanced activities of ROS scavenging enzymes (CAT, POX and SOD) in As-treated plants might be due to increased generation of ROS as a result of oxidative stress. An observed increase in the activity of SOD, the major O2 - scavenger, finds support from the previous data regarding Indian *mustard* (Diwan et al 2007, khan et al 2009).

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