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## **Evaluating the Morphological And Biochemical Responses of Garlic (*Allium Sativum* L.) to Industrial Wastewater from Sugar and Textile Factories**

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*doi: 10.33472/AFJBS.6.13.2024.7918-7931***ABSTRACT:**

Wastewater has a toxic effect on crop yields. Efficacy may vary depending on wastewater composition and plant sensitivities. The present study aimed to investigate the quality of wastewater from the sugar and textile industries as well as their toxic effects on Garlic (*Allium sativum* L.). In this study, the morphological and biochemical effects on *Allium cepa* were evaluated with three replicates. Twenty-four pots filled with soil. Three concentrations of each wastewater as well as a control concentration were selected to study the toxicity of wastewater from the sugar and textile industries. Choice of concentration of sugar and industrial wastewater: 80% (A1), 90% (A2) and 100% (A3) and for the textile industry it is 80% (B1), 90% (B2) and 100% (B3). Different physicochemical properties of the two wastewaters were observed in which heavy metal concentrations were found more in textile wastewater. Morphological reductions in plant height, root and shoot length as well as fresh and dry weight of roots and shoots were observed in the wastewater treated samples. The morphological results are supported by genotoxicity studies. Biochemical results showed obvious results. A decrease in protein and chlorophyll and an increase in ascorbate peroxidase, proline and catalase were observed with increasing wastewater concentration. Overall, the spread of sugary wastewater and wastewater from the textile industry was found to have a negative impact on plant growth. However, the toxicity in textile wastewater is greater than in sugar industry wastewater. Textile wastewater is enriched with heavy metals compared to wastewater from the sugar industry.

**Keywords:** wastewater, heavy metals, morphological parameters

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**1. Introduction**

Complex chemical combinations, including both metallic and organic chemicals, can be present in industrial wastewater. Regulatory limits on physicochemical parameters related to the discharge of industrial wastewater into landscape waters have been established in many countries (Wepa, 2014). Metallic substances and components of sufficient thickness and which are dangerous or harmful to fasten are considered heavy metals. Because of their long-term

persistence in solution and their difficulty in converting to compounds that are insoluble in surface waters, heavy metals are among the most harmful natural water pollutants (Bilal et al., 2014). When wastewater from sugar mills is released throughout the climate without proper treatment, it causes bad odors and an unpleasant atmosphere. Farmers who used this wastewater for irrigation noticed a decrease in crop growth parameters, fruit yield rates and soil quality. Different metals such as chloride, nitrate, are present in the wastewater of sugar mills and are rejected by different companies and they are difficult to see, smell and taste because of these factors. Such toxic water is very dangerous for humans, animals and plants. Many workers are interested in how different types of industrial wastewater affect seed germination, growth and yield (Street et al., 2007). Due to the economic growth of the 1950s, which led to industrial land occupation encroaching on protected areas, domestic and industrial wastewater polluted the water bodies. In addition, streams and rivers near agricultural areas are contaminated with pesticides and chemical inputs (Dangond Araujo & Guerrero Dallos, 2006). Textile dyes are extremely toxic and possibly carcinogenic, associated with a significant number of industrial pollutants (Sharma et al., 2018). Organisms in the ecosystem are affected by this adverse change in the environment. The term “environmental pollution” refers to what happens in the environment. Environmental pollution is the most difficult problem (Gajewska et al., 2006). Rapid industrialization endangers biological forms. Industrial and domestic emissions are the main source of hazardous compounds (Hemavathi et al., 2015). The bulbous perennial plant known as garlic (*Allium sativum* L.) has a strong onion flavour and aroma, and it can reach a height of 1.2 meters. It is also referred to as rocambole, allium, stinking rose, rustic treacle, nectar of the gods, camphor of the poor, poor man’s treacle, and clove garlic, depending on the region in which it is grown and/or utilised. *A. Sativum* is the most often consumed bulb worldwide, second only to onions. It grows easily and may be found in both temperate and tropical climates (Bayan et al., 2014). Nonetheless, a number of health authorities have long acknowledged its medical and therapeutic benefits (Adaki et al., 2014). Plant systems have proven to be sensitive, affordable and effective among biological assays created to detect genetic mutations, genotoxicity, cytotoxicity and chromogenic potential. may be caused by environmental pollutants (Majer et al., 2005). Pigment destruction, depletion of cellular lipids and peroxidation of fatty acids are examples of biochemical damage caused by heavy metals (Tiwari et al., 2006). The objective of this study was to examine how soil contamination affects growth parameters. Explore the major devastating effects of soil pollution on all plant morphological and biochemical parameters. Toxicological testing of wastewater from textile and sugar industries at different concentrations and durations. Know the dangerous consequences of each dose rate (concentration) of industrial water.

## **2. Material and Methods**

### **2.1 Experimental work**

#### **2.1.1 Wastewater collection**

Water samples were gathered and kept in water bottles from the Sugar industry, Pattoki, Kasur, and the Textile industry, Lahore, Pakistan.

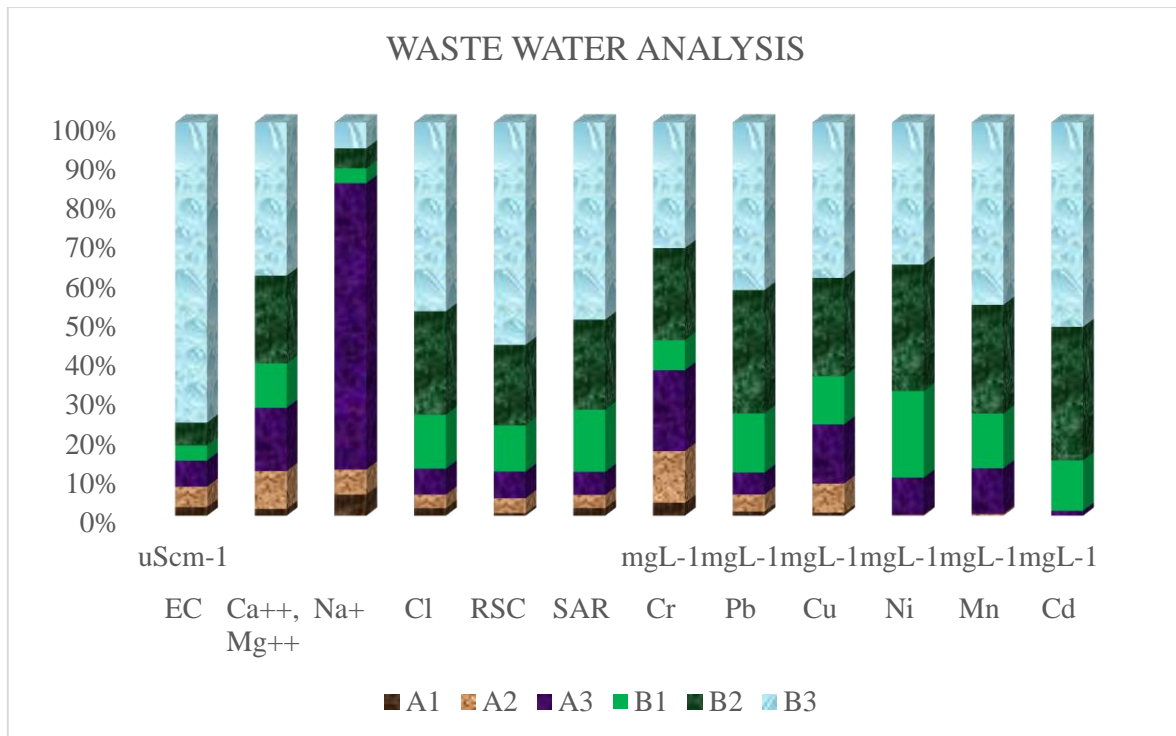


Figure 1: waste water analysis

**Experiment Site:**

In the nursery close to the University of Education, Bank Road Campus in Lahore, the experiment was conducted.

**Garlic (*Allium sativum* L.) bulbs collection**

The plant garlic bulbs are bought from a nearby store and cleaned with tap water to remove any dirt.

**Soil Preparation**

Each treatment had three duplicates made. The soil was poured into twenty-four pots. Hoeing the soil for three days made the surface permeable. Seven bulbs were planted in each planter. Bulb planting was carried out in August. The bulbs were planted such that they were a half inch below the soil surface. To ensure that bulbs had enough soil contact after planting, a little pressure was applied to the soil. The soil around newly planted bulbs was then thoroughly irrigated to close up any air spaces. Water was applied to bulbs once daily.

**Assessment of Growth Parameter**

The evaluation of plant growth metric involved three duplicates of each plant. Plant height, root & shoot length, fresh and dry weight of root & shoot, chlorophyll, protein, antioxidant enzymes (APX, CAT), and proline content observed.

**Experimental treatment**

Garlic (*Allium sativum* L.) was tested for its reaction to sugar and effluent from the textile industry. Three levels of effluent from the sugar industry were selected: 80% (A1), 90% (A2), and 100% (A3) & control (A0). Three levels of effluent from the textile industry were selected: 80% (B1), 90% (B2), and 100%.

### **Morphological analysis**

All Morphological parameters including plant height, root length, shoot length, fresh & dry weight of root & shoot noticed.

### **Biochemical Analysis**

#### **Chlorophyll content**

Amount of chlorophyll determined by using a spectrophotometric method (Arnon, 1949). Using a mortar and pestle, simply 1 gram of leaves removed, grinded it well & formed into a liquid paste before being placed in a centrifuge tube. A volume of 30 ml made up of 80% acetone was held for 15 minutes to allow for full extraction. For five minutes, tubes were centrifuged at 4500 rpm. With the use of a UV Spectrophotometer, the supernatant was collected and placed in a cuvette to measure its absorbance at 645nm and 663nm.

#### **Estimation**

The Bradford (1976), method was used to estimate the protein content. This is how the Bradford solution was created: 50 mL of 95% ethanol were used to dissolve 0.1g of Coomassie brilliant blue. Then, 100mL of O- phosphoric acid (85%) was added, and 1 litre of distilled water was added to the mixture. After adding 0.1ml of the produced extract to 5mL of Bradford solution, the mixture was vortexed. By using a UV/VIS spectrophotometer, read the absorbance at a specified wavelength (595 nm). Bovine serum albumin stock solutions diluted to different concentrations which are 100mg to 500mg, from 7.5mg per 15 mL of the stock solution were used to create the standard curve for bovine serum. This curve is used to identify proteins in unidentified samples.

#### **Ascorbate Peroxidase**

Nakano & Asada (1987), used to measure the activity of Ascorbate peroxidase. The oxidation of the ascorbate by 1mM H<sub>2</sub>O<sub>2</sub> in a mixture containing 0.25M ascorbate was monitored by measuring the drop in A<sub>290</sub> after 1min of incubation following the addition of 37.5L of enzyme extract protein in a 1mL reaction mixture to evaluate the activity of the APX. The units of the activity were mg<sup>-1</sup> Protein.

#### **Catalase (CAT)**

Catalase CAT activities were evaluated using the Chance & Maehly (1955), method. 1mL of H<sub>2</sub>O<sub>2</sub> (5.9mM) and 1.9mL of concentrations of the phosphate buffer (50mM; pH 7.0) were contained as a result of the CAT reaction solution (2ml). The enzymes extract of 100μL were added for the start of reaction. Spectrophotometric measurements for two minutes after every 20 seconds showed a decrease in reaction solution absorbance at 240 nm. Based on changes in optical density (OD) per minute, the CAT enzyme activity was measured per unit. The protein analysis was extract as indicated, and the activity of the two catalysts indicated about the based of leaf protein.

#### **Proline**

The procedure (Bates et al., 1973) was used to determine proline. Salfosalicylic acid was combined with fresh material (100 mg) that had been crushed in liquid nitrogen. Glacial acetic acid and ninhydrin in equal parts were mixed into the filtrate. 30 minutes were spent letting the toluene sit at room temperature in a test tube. Utilizing toluene as a blank, we performed a spectrophotometric measurement of the optical density at 520 nm. D-Proline standard cure was employed for proline concentration.

### Statistical analysis

IBM SPSS statistics was used to analyse the experimental work data. ANOVA was applied to analyse mean values of data of the wastewater from the sugar and textile industries. Duncan's multiple range tests were used to compare all data at a significant value of 5% level.

## 3. Results

### Morphological Parameters

As shown in Figure 2, a larger increase in all morphological parameters was noted under pressure (A1 and B1) at 80% wastewater concentration. In (A3 and B3), at 100% concentration, the smallest measure of all morphological parameters was recorded. All morphological parameters were reduced in samples A1, A2, A3, B1, B2 and B3 at concentrations of 80%, 90% and 100% compared to the control value A0. The highest concentration increase of 80% in all morphological parameters was recorded under stress (A1 and B1). In (A3 and B3), at 100% concentration, a decrease in all morphological parameters was observed. All parameters were higher in A1, A2, A3, B1, B2 and B3 than the control value A0. Plant height, root and shoot length, fresh and dry weight of dead roots and shoots at 100% concentration in sugar and textile industry wastewater. At a concentration of 80%, plant height decreased less than at a concentration of 100% of wastewater. Minimize all morphological parameters observed at 100% wastewater concentration. The minimum reduction observed at the stress concentration was 80%.

### Chlorophyll and Protein Content

As shown in Figure 3, more chlorophyll and protein content increases were observed under stress (A1 and B1) at 80% wastewater content. In (A3 and B3), at 100% concentration, a very small increase in chlorophyll and protein content was noted. Chlorophyll and protein content were lower in samples A1, A2, A3, B1, B2 and B3 at concentrations of 80%, 90% and 100% compared to the control value A0. An increase in chlorophyll and protein content was observed in (A1 and B1) at 80% stress concentration. In (A3 and B3), at 100%, the chlorophyll and protein content decreased significantly. Chlorophyll and protein content in samples A1, A2, A3, B1, B2 and B3 were lower than the control value A0.

### Antioxidant Enzymes

As shown in Figure 3, APX and CAT increased under all pressures (A1, A2, A3, B1, B2, B3) at concentrations of 80%, 90% and 100% compared with control A0. The maximum increase of both antioxidant enzymes was observed at 100% of wastewater concentration. A minimal increase was observed at a stress concentration of 80%.

### Proline

As shown in Figure 3, proline increased significantly at the concentration of 100% wastewater. A slight increase was observed in the stress concentration of 80%. Proline content increased under all stress (A1, A2, A3, B1, B2, B3) at concentrations of 80%, 90% and 100% compared to control A0.

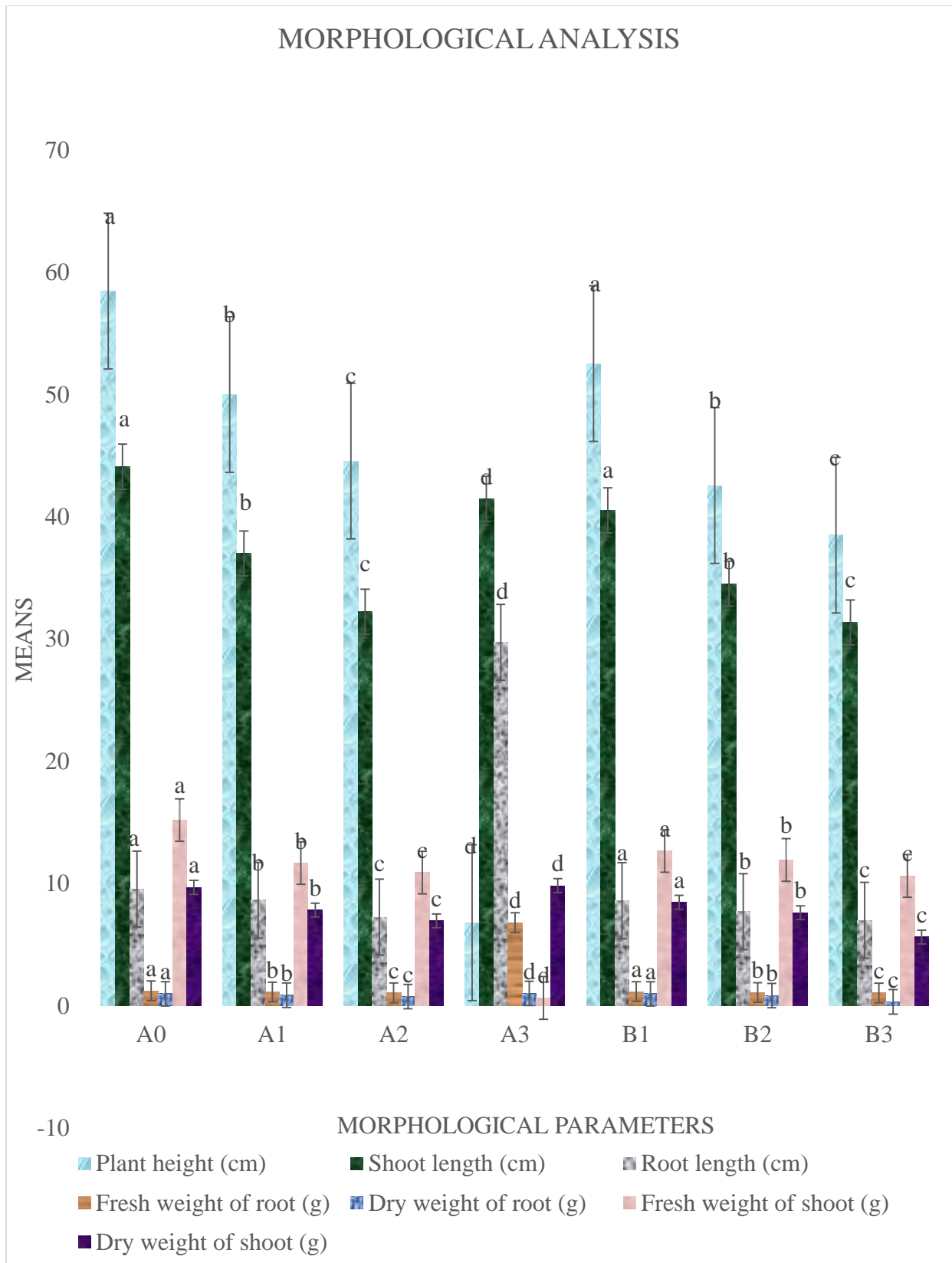


Figure 2: Effect of sugar & textile industrial wastewater on morphological parameters

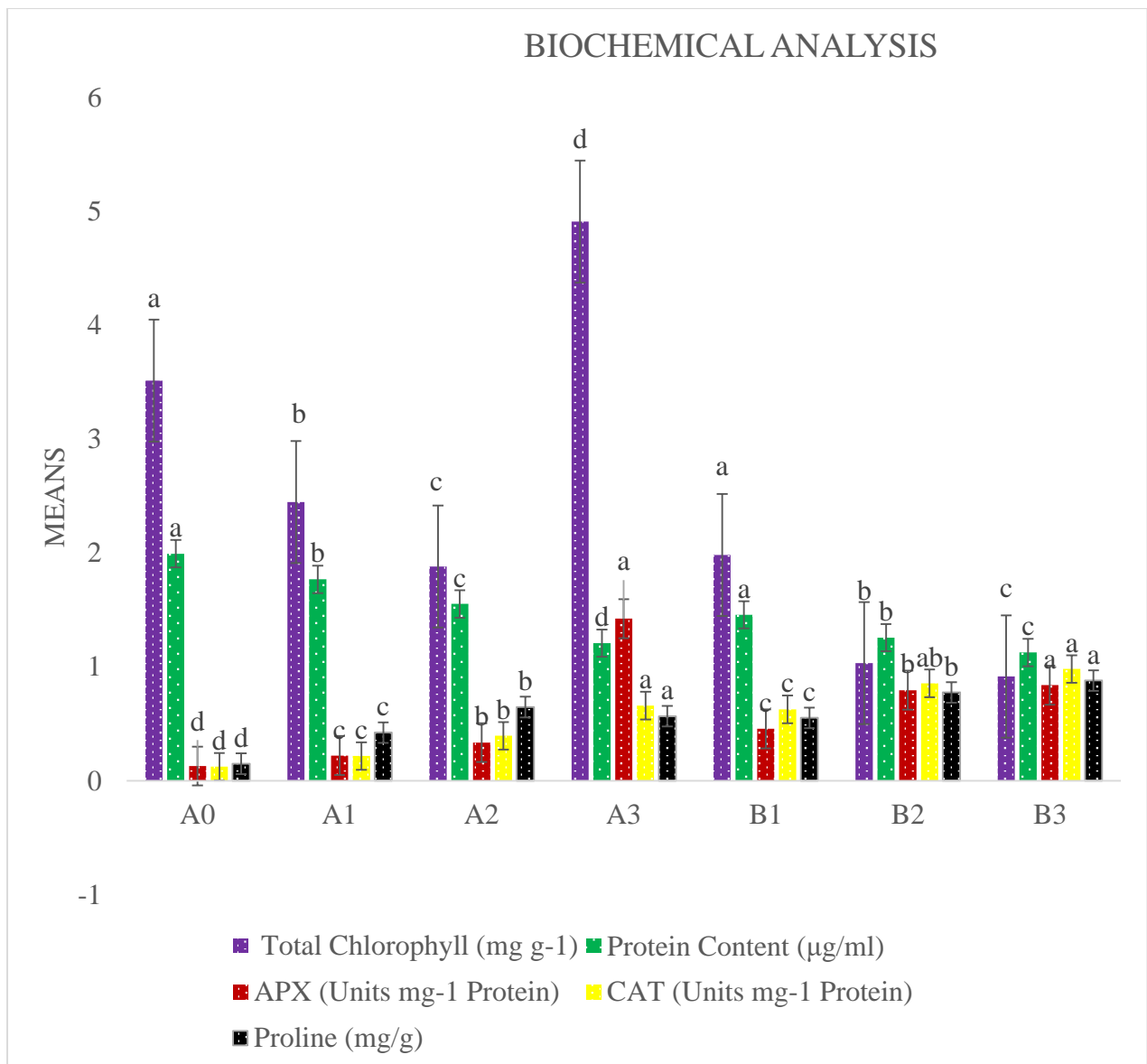


Figure 3: Effect of sugar and textile Industrial wastewater on biochemical parameters of chlorophyll, protein, antioxidant enzymes and proline content

#### 4. Discussion

The results of our morphological analysis of plant height, shoot length, root length, fresh and dry weight of shoots and roots are in agreement with the results found in the literature. The mean of all measured characteristics and the standard error are significantly different from the mean of the control. Levels of all morphological traits decreased. At all concentrations, irrigation with sewage significantly reduced the height of both varieties, with undiluted wastewater having the worst effect (Hajhashemi et al., 2020). At 50% dilution they increased significantly, but in raw water they decreased significantly and at 75% dilution they did not change significantly (Hajhashemi et al., 2019). Excessive accumulation of various nutrients, including calcium, silicon, sodium, zinc and magnesium, from wastewater can destroy plant uptake and inhibit various metabolic functions. Kochian et al., 2005 Cole et al., 2016; Rengel et al., 2015). ). The growth and yield of all crops are also impaired by heavy metal stress as metabolism and entire biochemical cycles are compromised (Shanker et al., 2005). Heavy metal pollution in wastewater induces deficiencies of essential elements in plants by reducing



the uptake or immobilization of elements in the roots (Benavides et al., 2005; Lefevre et al., 2014); Sharma & Dubey, 2005). At 100% concentration, both *Corchorus olitorius* and *Senna occidentalis* showed significant inhibition of seedling growth parameters (stem length and root length) (Sagaya et al., 2020). Many researchers have found that irrigation of various crops with high concentrations of industrial wastewater leads to growth inhibition (Yasmin et al., 2011; Oladele et al., 2011; Pandey, 2006). ; and Uaboi-Egbenni et al., 2009). Root and shoot lengths showed a decreasing trend for increasing wastewater concentrations (60%, 80% and 100%), confirming the toxicity of wastewater to turnip plants. Excessive amounts of calcium, magnesium, chloride and hardness from sugar mill wastewater affect plant growth. Much larger wastewater has a significant impact on root length (Vijaaragavan et al., 2011). The above results corroborate the results of Kaushik et al., (2004), who found that the wastewater from sugar mills is clearly dangerous for the growth and development parameters of wheat plants. The maximum reduction was observed in the corn mill, ranging from 75% to 100% of sugar mill wastewater. The fresh and dry weight of corn increased when the EMS concentration was minimal and decreased when the mill effluent ratio was higher. Excessive amounts of chloride, calcium, sulfate and phosphate in sugar mill wastewater have a significant negative impact on crop growth (Hussain et al., 2013). According to Ayyasamy et al., (2008) and Kazemi & Eskandari (2011), wastewater from sugar mills is an abiotic stress that limits plant growth parameters. Many agricultural crops, including chickpeas studied by Baskaran et al., (2009), wheat by Kaushik et al., (2004), two chickpeas and maize by Ayyasamy et al., (2008), maize plants studied by Ezhilvannan et al., (2011), and turnip plants by Vijayaragavan et al., (2011), showed evidence of reductions in effluents of other growth parameters. together. Organic and inorganic substances restrict energy flow, retard growth and development of germinated seeds, toxicity of wastewater reduces cell size and intercellular space and hardens the soil, shortening of roots, stems, and shoots and reducing fresh and dry weight (Baskaran et al., 2009). The results of our biochemical analysis are in agreement with those published in the literature. The mean of all measured properties and standard errors (total chlorophyll, protein content, ascorbate peroxide (APX), catalase (CAT) and proline content) were significantly different from the control. . Both protein and total chlorophyll content decreased but APX, CAT and proline increased significantly compared with control. The examined plants demonstrated a gradual and significant decrease in chl a, chl b, along with an increase and accumulation of metals present in irrigation water, consistent with growth responses. According to Hashem et al., (2013), a symptom of cytotoxicity associated with heavy metal accumulation may be a decrease in the concentration of photosynthetic pigments. They showed a significantly reduced degree of undiluted (Hajihashemi et al., 2019). The total chlorophyll content in plants decreases due to prolonged exposure to high concentrations of industrial wastewater. After 8, 16, 24 and 32 days, high concentrations of textile industry wastewater showed a decrease in total chlorophyll content (from 50% to 75%) (Aarti & Mittal, 2017). Sugar mill wastewater reached levels harmful to plants at concentrations of 60%, 80% and 100%, and a decrease in photosynthetic pigment was observed. Chlorophyll evaluation showed that irrigation with sugar mill wastewater had a significant impact on chlorophyll a, b and total plant chlorophyll (Vijayaragavan et al., 2011). Chlorophyll concentrations in both cultivars were significantly reduced in response to EMS in the experiment (Hussain et al., 2013). According to some studies Munir et al., (2013), Nagajyoti et al., (2008), Baskaran et al., (2009), Vijayaragavan et al., (2011), irrigation wastewater from the sugar industry is known to have a significant effect reduce chlorophyll pigment in some plants. Many researchers have reached similar conclusions (Gurpreet et al., 2012; Pandey & Tripathi, 2011). Tomato researchers (Cho & Park, 2000) hypothesized that inhibition of chlorophyll production is responsible for the decrease in chlorophyll concentrations in the presence of metals. Researchers using lettuce, cucumbers, and legumes have shown that heavy metal treatment tends to reduce the amount of all pigments

and to some extent alter their ratio ( Vassilev et al., 2007). According to Zhao & Wu (2017), Zn is a trace element involved in various physiological functions. However, excessively high levels of Zn in plants have dangerous effects on plants, as Mg displaces zinc inside chlorophyll molecules, inhibiting and restricting growth (Küpper & Andresen, 2016). According to Kumar et al., (2012), this could be due to chlorophyll degradation under stress or to inhibition of chlorophyll production. The amount of chlorophyll in plants is reduced by various abiotic stressors (Ahmad et al., 2006). Similar results were observed when *Cyamopsis tetragonoloba* was exposed to the waste of a sheet manufacturing company (Selvaraj et al., 2012). Mineral ions can be connected to wastewater causing a lesser variation in pigment content. The development of a specific type of chlorophyllase catalyst, which is responsible for chlorophyll degradation, is one of the potential causes of the decrease in chlorophyll content (Gupta et al., 2004; Saravanamoorthy & Kumari, 2005). According to Saravanamoorthy & Kumari (2005), increased chlorophyllase activity or decreased de novo chlorophyll synthesis may be related to chlorophyll pigment degradation under EMS stress. After 16, 24 and 32 days, *Tagetes erecta*'s soluble protein concentration decreased to a concentration of 75°C. The most subtle macromolecule affected by wastewater is protein concentration. In the present study, it was found that the soluble protein content of *Tagetes erecta* decreased when treated with high concentration of textile industry wastewater (Aarti & Mittal, 2017). In addition, the resulting protein content decreased as the wastewater concentration gradually increased to 60%, 80% and 100% (Vijayaragavan, 2011). According to Palma et al., (2002), the increased protease activity under stress conditions may be responsible for the decreased protein content of *L. polyrhizes*. Due to the inhibition of protease activity in wastewater, the free amino acid and protein content decreased at high salt concentrations (Joshi & Tandon, 2003). According to the present study, irrigation with wastewater significantly increased CAT & APX activity (Hashem et al., 2013). Various metal-treated plants have shown significant increases in CAT and APX activities in response to heavy metal exposure (MacFarlane and Burchett, 2001; Cho and Park, 2000). . In this study, it was found that APX and CAT activities in maize varieties were significantly increased due to EMS (Hussain, 2013). To counteract oxidative damage induced by ROS, wastewater-exposed plants exhibited a greater function of antioxidant catalytic activity (Baskaran et al., 2009; Noman et al., 2012). APX activity continued to increase when SME was added to the culture medium. Both varieties showed a consistent increase in wastewater-induced CAT activity (Hussain et al., 2013). Compared with the control distilled water, the rice varieties had increased proline accumulation in the raw water. According to the results of our study, plants had the lowest proline content at 25% (Akhtar et al., 2018), while proline accumulation increased in crude form compared with the control. According to Hussain et al. (2013), plants exposed to EMS accumulated proline faster. According to Ramasubramanian et al., (2006) and Matysik et al., (2002), proline accumulation is often used as a biochemical indicator of plant stress tolerance. Proline content has been shown to scavenge hydroxyl radicals and the molecular oxygen state, in addition to its role as metal chelators and osmolytes, protects cells against ROS-induced cellular damage (Yılmaz & Parlak, 2011). Stress causes various changes in plants and to manage these changes, trees have a defense system consisting of natural products (Muhammed et al., 2013). The most important component of this plant defense system is proline. Due to abiotic stressors, proline content occurs in plant leaves (Kumar, 2006). Proline accumulation confers resistance to abiotic stressors (Jamil et al., 2009).

## 5. Conclusion

Research results support the idea that heavy metal-containing effluents from different industries, such as the rubber and steel industries, can have different negative effects on plants. Lead, cadmium, chromium, mercury and arsenic are examples of heavy metals that are toxic

to plants and animals. Their accumulation in soil and water can also have a negative effect on plant growth over time. It is concluded that the effluent concentrations of sugar and textile industries have a negative effect on the amount of *Allium cepa*. In summary, the results show that wastewater from the sugar and textile industries has a negative impact on the development of facilities. Future agricultural research may benefit from plants exposed to wastewater from the sugar and textile industries.

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