Effect of Lead-induced heavy metal stress and wastewater effluent on health and metabolism of *Allium cepa* L.

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Abstract
Plants, being fascicles in nature, have to evolve mechanisms to cope with both biotic and abiotic stress. In this current study, the adverse effects of wastewater and lead were analyzed to get an idea of the extent of damage they can induce to the onion plant (*Allium cepa* L.). Lead, being a heavy metal, is harmful to the plant's growth and development. Whereas wastewater contamination, on the other hand, is a matter of global concern as more and more aquatic and terrestrial ecosystems are becoming vulnerable due to the enhanced rate of pollution. In this present study, the impact of these pollutants was analyzed by performing polyphenol estimation, protein estimation, pigment estimation, and also the quality and integrity of DNA under these stress factors to assess the impact on the molecular level. The findings of this present study would facilitate understanding of not only the effect of stress but also how the onion plant is trying to withstand stress-induced negative impacts.

1. Introduction

Environmental stress suggests the unfavourable conditions of a plant, which can impede the growth and overall well-being of the plant. Environmental stress can be broadly classified into two types: Biotic and abiotic stresses. The biotic stresses include the stress induced on the plant by bacteria, fungi, nematodes, viruses, insects, etc., and the abiotic stresses include high temperatures, ultraviolet radiation, heavy metal contamination, and other adverse environmental conditions. In soil the natural concentration of lead is 10-50 ppm [1]. Consequently, lead and its derivatives accumulate for long term in the soil, persisting within the biological cycle for an estimated duration of 300-500 years [2] The low solubility, limited mobility, and resistance to microbial degradation or bioremediation process by microorganisms within the soil [3]. Plants differentially accumulate heavy metals within distinct organs through root interception mechanisms, absorbing them from contaminated soils. Elevated concentrations of heavy metals in edible plant tissues consistently pose potential

health risks to consumers due to exposure to hazardous chemicals. [4] These chemicals pose a serious threat to the consumers of these plants, as these heavy metals do not easily get removed from the system, and slowly they accumulate to make the system more toxic. So understanding the intricate responses of the plants to the provided stress is crucial for producing adaptive mechanisms and devising strategies to enhance stress tolerance.

On the other hand, wastewater can cause both biotic and abiotic stress to plants. The presence of diverse microorganisms within wastewater serves as a primary source of biotic stress for plants; these microorganisms infiltrate the plant tissues and disrupt cellular functions, triggering defense mechanisms. Moreover, it contributes to the imposition of abiotic stress on plants through oxidative stress. Within the wastewater, there are reactive oxygen species (ROS), which initiate the stress mechanisms in the plant. These ROS molecules cause serious damage to essential biomolecules, including DNA. Such DNA damage disrupts cellular integrity and functionality, which compromises the plant growth.

In this study, the plants (*Allium cepa* L.) were exposed to lead and wastewater to observe the changes in the physicochemical properties and the changes in the quality of the genes. In the process of doing so, three well-germinated onion plants were kept to grow in normal water, waste water collected from local water bodies, and lead nitrate solution, respectively, for 48 hours. After 48 hours, the roots and shoots of the plants were used to check the chlorophyll, carotenoid, polyphenol, and protein content of the plant. The polyphenols serve as major antioxidants within onion plants, comprising a significant portion of their secondary metabolites. However, their presence extends far beyond the realm of onions, as they are also present in various other plant-based foods in substantial amounts. Fruits, vegetables, cereals, tea, and coffee all share this valuable compound. [5] Chlorophyll stands as the primary pigment present in plants, which is responsible for photosynthesis. Carotenoids play an important role in plant health, and these accessory pigments also contribute to absorbing light energy for photosynthesis. Furthermore, carotenoids also serve as antioxidants alongside polyphenols. Apart from these, the quality of the DNA is also tested after the stress induced on the plants.

2. Materials and Methods

Allium cepa L. is taken as the model plant for this study. One untreated (control) set along with two treated sets- treatment with waste water, treatment with lead nitrate (100 μM) were maintained.

2.1 Estimation of Total Chlorophyll and Carotenoids.

To measure the amount of chlorophyll in the sample (Lichtenthaler *Et al*.1987) [6] was used with slight adjustments.

Different wavelength of light was used for different pigments - 662nm for chlorophyll A, 645nm for chlorophyll B, 470nm for carotenoids.

Content of different pigments were calculated from their respective absorbance from their specific wavelengths.

2.2 Estimation of Polyphenol

The plant materials were extracted using the technique of Brolis *Et al*., (1998) [7] with few minor adjustments.

500mg plant tissue was obtained and they were uniformely mixed and dissolved in 5ml of an aqueous ethanol solution with a concentration of 50% and then 50 µl of this extract is aliquoted and blended with 250 µl of Folin-ciocalteu reagent and 750 µl of 10% Sodium Carbonate solution. The absorbance of this solution is measured using UV visible spectroscopy at 760nm wavelength. This measurement of the total polyphenol was done using the method given by Single ton *Et al.* 1999 [8] with minor modifications Talukder *Et al*. 2015 [9].

A Gallic Acid standard curve was prepared and was used for measuring polyphenol content.

2.3 Analysis of Protein content.

Protein content calculation was done by Ockutucu B. *Et al* 2017. [10]

1ml of plant extract was blended with 1ml of distilled water and 2ml of alkaline copper sulphate solution, then 500µl Folin's reagent is added and absorbance was measured using spectrophotometer at 660nm wavelength.

A BSA standard curve was prepared and was used for measuring protein content.

2.4 Plant DNA extraction for Agarose Gel Electrophoresis

Plant DNA was isolated by Edwards *Et al.* 1991 [11].

1 gram of plant tissue was obtained and homogenized in 2.4 millilitres of freshly prepared DNA extraction buffer. The homogenate was centrifuged at 1000 rpm for 10 minutes, and the supernatant was collected. An equal volume

of chloroform-saturated phenol was added, followed by centrifugation at 1000 rpm for 10 minutes. The aqueous phase was then collected and subjected to a second chloroform extraction by centrifugation. Following centrifugation, the clear upper aqueous layer was carefully collected, leaving the protein layer undisturbed. To this mixture, 1/10th volume of 3M ammonium acetate was added, and the volume was adjusted with isopropanol before centrifugation at 1000 rpm for 10 minutes to pellet the DNA. The pellet was dissolved in 200 µl of 70% chilled ethyl alcohol, followed by centrifugation at 1000 rpm for 10 minutes. The supernatant was removed, and the pellet was dissolved in 100 µl of sterile distilled water.

3. Result and Discussion

3.1 Chlorophyll and Carotenoid Content

The amount of Chlorophyll and carotenoids are shown in Table 1

Sample	Chlorophyll A (ng/ml)	Chlorophyll B (ng/ml)	Carotenoids (ng/ml)
Control sample	473	820	380
Sample treated with wastewater	470	490	230
Sample treated with $PbNO_3$	1002	1210	480

TABLE 1. Amount of plant pigments

(Table 1) shows the amount of chlorophyll and carotenoids in all the three samples. We can observe that the amount of chlorophyll A and B and the amount of Carotenoids are decreased at a considerable amount in the waste water sample. It can be deduced that this is happening due to the presence of some chemicals in the sample water is preventing the plant to produce these essential pigments and we can also observe that in the Lead Nitrate contaminated plant sample the amount of

Chlorophyll A and B and carotenoids have increased subsequently and this shows that to withstand the stress produced by the heavy metal the plant have increased its production of these pigments to withstand the stress (Fig. 1)

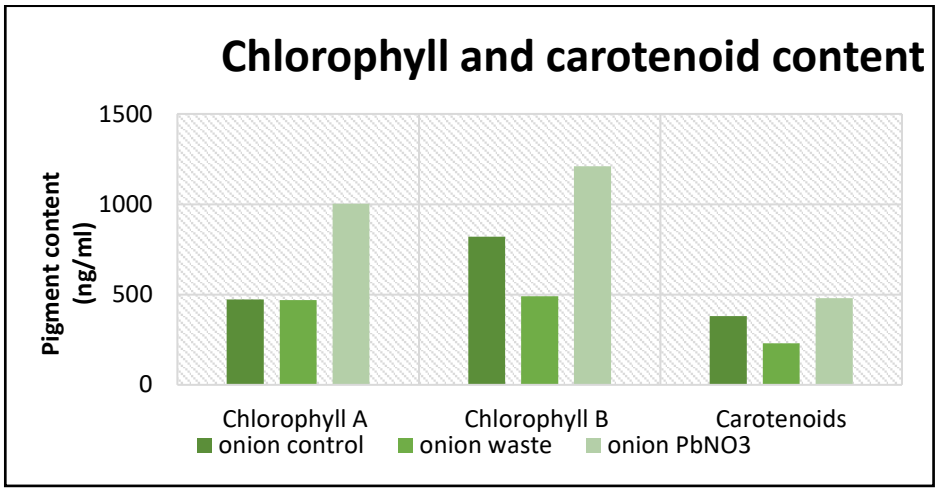
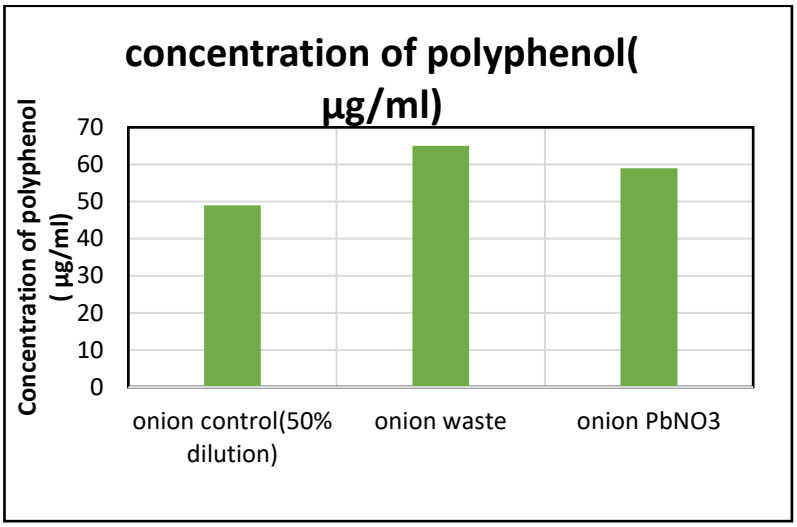


Fig 1: Chlorophyll A, Chlorophyll B and carotenoid content in *Allium cepa* L. under different treatments



3.2 Polyphenol Content

The amount of Polyphenol is shown in Table 2

Sample	Concentration of polyphenol(µg/ml)
Control sample (50% dilution)	49
Sample treated with wastewater	65
Sample treated with $PbNO_3$	59

TABLE 2. Amount of polyphenol

(Table 2) shows the amount of polyphenol content in all the three samples. We can observe that the amount of polyphenol content is higher in the waste water sample and this proves that the plant tried to fight back the stress in order to do so it produced a greater amount of polyphenol whereas in the Lead Nitrate contaminated plants the polyphenol content is slightly lower than the waste water contaminated but it is higher than the control which proves that it tried to fight back but it could not as effectively as the waste water contaminated sample (Fig. 2).

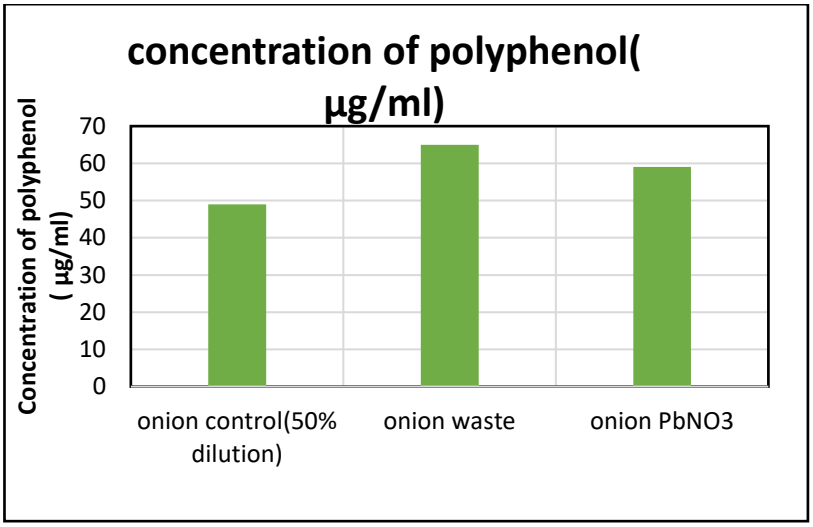


Fig 2: Polyphenol content of *Allium cepa* L. under different treatments.

3.3 Protein Content

The amount of protein is shown in Table 3

Sample	Concentration of protein (milimole/litre)
Control sample (50% dilution)	1.2
Sample treated with wastewater	1.4
Sample treated with $PbNO_3$	1.9

TABLE 3. Amount of protein

(Table 3) shows the amount of protein in all the three samples. We can observe that the amount of protein has increased in the wastewater and the lead nitrate treated sample, in lead nitrate the protein content has increased more so we can deduce that in the lead nitrate treated sample protein production has increased to fight back

the externally induced stress. Wastewater treated sample has also produced a greater amount of protein to fight back but it could not surpass the stress limit like the lead treated sample did (Fig. 3).

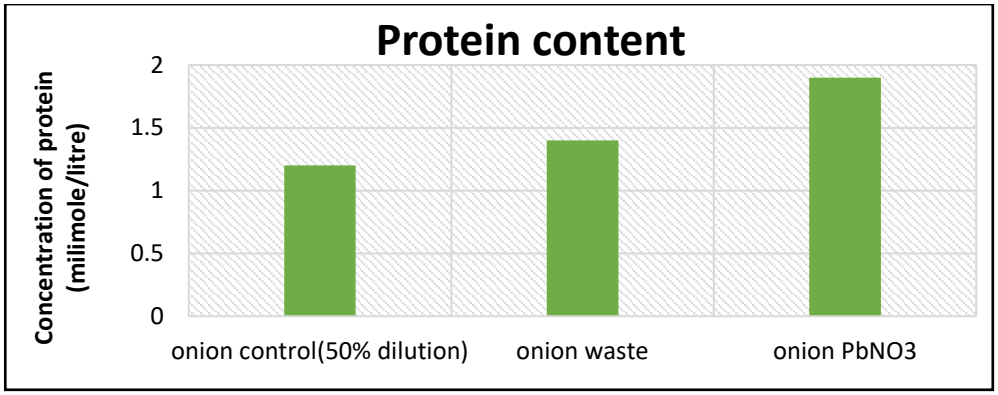


Fig 3: Protein content of *Allium cepa* L. under different treatments.

3.4 Analysis of quality of DNA by Gel electrophoresis

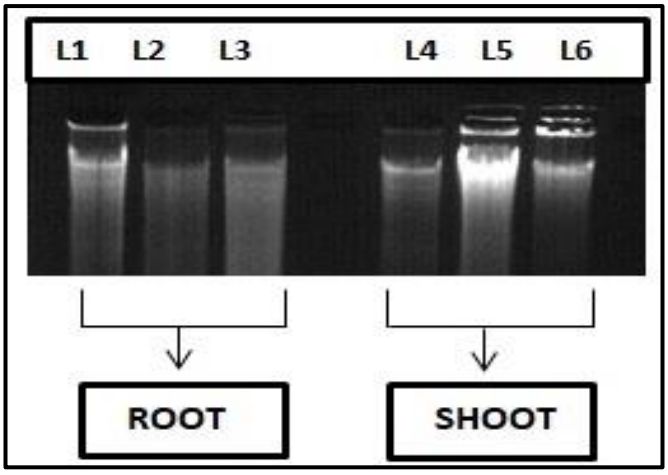


Fig 4: Gel electrophoresis of *Allium cepa* L. under different treatments

L1- DNA of the root tissue of the control sample.

It can be observed from the gel electrophoresis analysis that the sample in lane 1 exhibits peak intensity which indicates minimal damage of the DNA and thus have better quality and integrity of the DNA.

L2-DNA of the root tissue of the sample treated with wastewater.

From the gel electrophoresis analysis in lane 2 it can be concluded that the sample has incurred significant damage likely attributed to stress induced by exposure to wastewater which is evident from the notably reduced intensity of

DNA content within the gel, indicating severe deterioration in the integrity and quality of the DNA.

L3- DNA of the root tissue of the sample treated with $PbNO_3$.

Analysis of the gel indicates that the DNA sample in lane 3 has compromised quality and integrity, likely attributable to stress induced by the metal ions in the PbNO3 solution. This inference is proved by the smeared gel samples. However, if compared to lane 2 the damage inflicted is relatively lesser, suggesting the PbNO3 solution has caused less harm to the DNA sample than the wastewater solution.

L4-DNA of the shoot tissue of the control sample.

The lane 4 of the gel does not have the best intensity which suggests that the DNA of the shoot in the control sample is neither much damaged nor it has the best quality and integrity, indicating a minimal damage probably due to some impurities in the water that it was left to grow upon.

L5- DNA of the shoot tissue of the sample treated with wastewater.

Evaluation of the gel reveals that in lane 5 the DNA is showing the highest peak intensity and pronounced smearing, which suggests significant DNA damage resulting from the exposure to wastewater. Despite the damage the Quantity of the DNA content is high which contributes to the observed intensity peak relative to the other samples

L6- DNA of the shoot tissue of the sample treated with $PbNO_3$.

The lane 6 of the gel has less intensity and is moderately smeared which proves that the DNA in the shoot of the plant is to some extent intact, which results in minimal damage in the integrity of the DNA.

4. Conclusion

In this study, we investigated the impact of the biotic and abiotic stress imposed upon our sample *Allium cepa* L. by subjecting them to waste water and lead nitrate solutions. In our findings we see clear changes in the biochemical and molecular level of the sample. The biochemical changes has the changes in the level of the plant pigments, polyphenol content and the protein content of the plant and the molecular changes include the changes in the DNA bands in the plants as it was also observed in previously researched data in

[12] the DNA and chromosome gets damaged from the lead. The main reason for the damage in DNA of the plant can be due to the reactive oxygen species (ROS) which induces a oxidative stress on the plant tissues as it was also observed in [13], As the DNA of the shoot of the sample is comparatively better than the DNA in the root of the treated samples we can also get the understanding of roughly how much time is needed for the toxins to reach the whole plant body.

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