# Effect Of Multifactorial Stress On Plant Pigments, Flavonoids And Polyphenols In Methanolic Extract Of Pisum Sativum

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**ABSTRACT**

Climate change, heavy metal toxicity, and the widespread distribution of phthalates in the environment, all create serious threats to plant ecosystems and their vital pigments. The combined effects of these stresses are more severe than each of their own. When plants are exposed to heavy metals, phthalates, and physical pollution (UV light), their photosynthetic capacity declines and increase oxidative stress in plants.In light of these considerations, this study presents an in-depth examination of the cumulative impact of heavy metals (specifically nickel and cadmium), phthalates (dimethyl phthalate and di-n-octyl phthalate), and physical stressors (UV light ) on the pigments, specifically chlorophyll A, chlorophyll B, and carotenoids and phytochemicals such as flavonoids and phenolics of Pisum sativum . The results of this study suggest that plants treated with a combination of these stressors have considerable alterations in their pigment profiles, as well as its defense system and demonstrating the complicated impact of environmental factors on plant.

**KEYWORDS:** Heavy metals, Phthalates, UV light, Phytochemicals,spectrophotometer

## ABBREVIATIONS

Nickel (Ni), Cadmium (Cd), Dibutylthyl Phthalates (DBP), Di n octyl Phthalate (DOP), Total Flavonoid Content (TFC), Total Phenolic Content (TPC)

## INTRODUCTION

Climate change is currently recognized as the most severe threat to humanity and all other life forms on our planet. It has been linked to significant alterations in temperature ranges, weather occurrences, seasonal cycles, and related phenomena on a global scale. (Ullah et al., 2021). Experts from diverse scientific fields have issued warnings that the adverse consequences of climate change are expected to exacerbate in the future, especially if environmentally harmful human activities persist without restraint. (Chaudhry & Sidhu 2022)

Plants are greatly affected physically and chemically by stress, metal accumulation, and poor soil conditions. Additionally, climate change has direct effects on plant productivity (Khan *et al.*, 2022). Agriculture soil is polluted by human activities like mining, the use of herbicides, and the use of sewage waste water or industrial waste water, which are greater sources of organic and inorganic pollutants. (Akram *et al.,* 2018)

Climate change directly affects the accumulation and behavior of heavy metals in plants. Due to excessive rainfall, heavy metals transport to different sites and can reach agricultural land. (Wu *et al.*, 2017) At high temperatures, the production of ROS increases, which affects photosynthesis, respiration rate, and damage to plant cells, ultimately leading to plant death. (Tiwari *et al.,* 2019) Lower rainfall rates and high temperatures increase the evaporation in plants, which results in an increase in the accumulation and toxicity of heavy metals in plants and reduces root and shoot growth. (Malizia *et al.,* 2012) High temperatures and metal stress hinder the seed germination process. (Deng *et al.,* 2016) The agricultural sector faces significant challenges due to the growing frequency of droughts, excessive rainfall, temperature fluctuations, and increased salinity. (Ullah *et al.,* 2021)

Organic pollutants enter plants either from the soil or the air. The main sources of these chemicals entering plants are pesticide sprays, plastic mulch, biosolids, etc. Polychlorinated dibenzo-p-dioxins, organochlorine pesticides, phthalates, polychlorinated biphenyls, antibiotics, herbicides, and bisphenol A are the most common organic pollutants found in plants. (Zhang *et al.*, 2017) These organic pollutants translocate in plants and damage their chemical and biological features. (Obinna *et al.,* 2019) Phthalate has effects on the growth and development of plants, the germination of seeds, the metabolism pathway of plants, and the activities of antioxidant enzymes in plants. (Kumari *et al.,* 2020). The current investigation aims to assess the levels of photosynthetic pigments and total flavonoid content and total phenolics content in pea plant when exposed to multiple stressors simultaneously.

## MATERIALS AND METHODS

### *CHEMICALS*

Nickel (Ni), Cadmium Chloride ($CdCl_2$) Dimethyl phthalate (DMP), Di octyl phthalate (DOP), Methanol and Calcium carbonate ($CaCO_3$), Methanol, Auminum Chloride ($AlCl_3$), Folin-Ciocalten reagent, Sodium Acetate.

### *EXPERIMENTAL SETUP*

Pot experiments were conducted using a completely randomized design. In October 2023 seeds were purchased from local market, washed and sterilized them so all impurities run out then seeds of pea plants were sown. When Plants grown out those were subjected to various abiotic stresses, including organic, inorganic, and physical stressors, having 100ppm concentration. After 15 days of stress exposure plants were uprooted and thoroughly washed with water to ensure the removal of impurities. Different parts of plants, leaves, stem, roots were separate out and a detailed analysis was carried out.

***SOLVENT EXTRACTION***

0.3gm of plants material (root, stem, leaves) was taken and crushed it in mortar and pestle and add 10 ml of chilled methanol. Centrifuge at 6000rpm for 10mins and saved for further analysis.

***PIGMENT ANALYSIS***

Chlorophyll extraction was performed by homogenizing fresh leaves (0.3g) of Pea. The leaves were crushed using 10 ml of chilled methanol and add calcium carbonate in trace amount. The extract was centrifuged at 6000 rpm for 10 minutes, and the supernatant was analyzed for atomic absorbance at 665nm, 649 nm, and 470nm. Chlorophyll A, Chlorophyll B, and carotenoids wemeasured using the following equations: (Ma *et al.,* 2022).

$$Chlorophyll\ a\ content = 13.5(\mathrm{A665}) - 6.88(\mathrm{A649})$$

$$Chlorophyll\ b\ content = 24.96(\mathrm{A649}) - 7.32(\mathrm{A665})$$

$$\mathrm{Carotenoid\ content} = 1000[\mathrm{A470}) - 2.05(\mathrm{chl\,a}) - 11.48(\mathrm{chlb})]/245$$

### *TOTAL FLAVONOID CONTENT*

Aluminum chloride colorimetric method was used to determine total flavonoid method.400 µL methanol,100 µL 10%of $AlCl_3$ was added in 100 µL plant extract then add 100 µL sodim acetate and kept in dark for 30mins. At510nm absorbance was noted using spectrophotometer. (Nguyen *et al.,*2020)

### *TOTAL PHENOLIC CONTENT*

Total phenolic content was analyzed by Folin–Ciocalteu method using Marichali *et al*., 2014 method with some modifications.100µL of plant exatct as taken and dilute with 400 µL distill water. Add 150 µL Folin–Ciocalteu reagent and leave at room temperature for 5-10min. Add 500 µL 20'% sodium carbonate and remain for 1 hour. Absorbance was taken at 650nm using spectrophotometer.(Sharma et al 2020)

## RESULTS AND DISCUSSION

### *CHLOROPHYLL CONTENT*

Result of this study shows In fig.1. that content of chlorophyll a, chlorophyll b and carotenoids significantly decreases in stressed plants. In present study fig.1. untreated plant chlorophyll a, chlorophyll b and carotenoids content was observed 10.3,3.7 and 4.3 respectively. When plants were subjected to UV light for 24 hours value was recorded 2.1,1.6 and 0.7 respectively. Plant treated with mixture of all stressors shows maximum reduction 1.3,0.9 and 1.09.

Photosynthetic pigments serve as the primary parameter for assessing a plant's photosynthetic capacity and revealing the harm inflicted by external stressors on plants. (Luo *et al.,* 2021) In a study with increase in dose and time of phthalate noticeable decline in plant pigment was seen. (Zhang et al., 2015) Variations in pigment levels are closely associated with observable signs of plant distress and can provide insights into the plant's photosynthetic performance. (Chandra & Kang, 2016). Any decrease in carotenoid levels can have significant implications for the stability and protection of chlorophyll pigments, potentially leading to their vulnerability to photooxidative destruction. (Ramel et al., 2012). Carotenoids are known to serve as antioxidants and photoprotective agents in plants. (Yao et al.,2022) In salt stress plants Ni shows more decline in chlorophyll a, chlorophyll b and carotenoids than Cd. Yasar, *et al.,*2012 reported that increase in NaCl concentration decrease in chlorophyll content. In phthalates stressed plants, plant pigments decrease in DBP more than DOP. A study observed that the contents of chlorophyll A and chlorophyll B were inhibited to some extent when exposed to DBP for various periods of time. (Yao *et al.,* 2022) A study shows under the influence of cadmium stress, observed a reduction in both total chlorophyll content and chlorophyll a content. (Liu et al., 2023) (Ren et al,.2021) Physical stressors have also direct impact on plant pigments. (Khaleghi, *et al.,* 2012) Under drought stress conditions, plants experience a significant reduction in the functionality of their photosynthetic reaction centers. (Efeoğlu *et al.,* 2009)

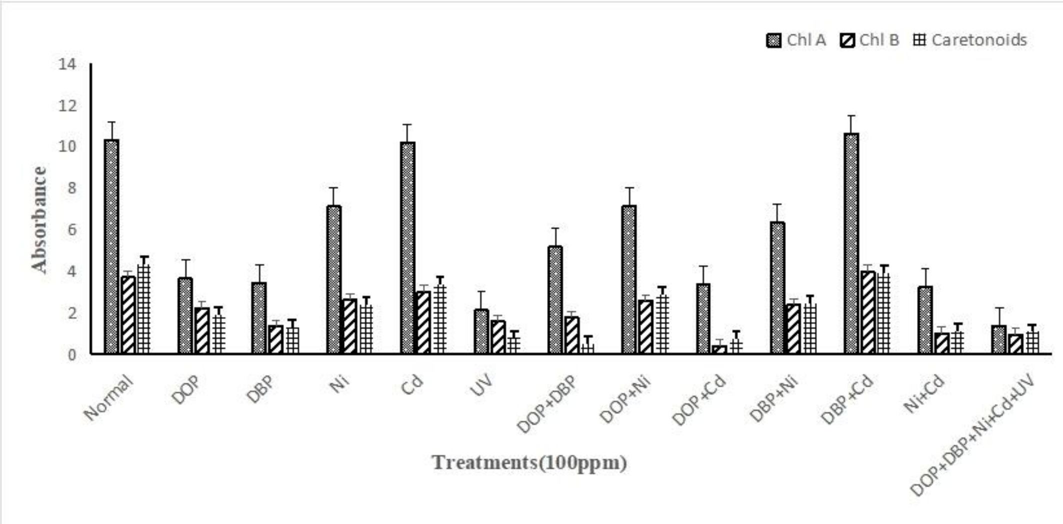


Figure 1. The impact of multifactorial stress on plant pigments of Pisum sativum

***TOTAL FLAVONOIDS***

In fig.2 total flavonoid content in roots, stem and leaves of stressed plants were examined separately. with reference to fig 2 total flavonoid content in normal plant was 0.019, 0.009 and 0.301 in roots, stems and leaves, maximum increase 0.411 ,0.09 and 0.39 respectively was observed in plants having combined stress.Santos, *et al***.,** 2014 studied that pea plant shoot have considerable amount of flavonoid.

Flavonoid play important role against stress caused by Uv light that damage plants by increase in oxidative stress. (Agati, *et al.*,2009) Significant increase of total flavonoid content in Arial part of plant was observed while in roots and shoot flavonoid content increase in trace. Ikram *et al.,* 2020 reported that increase in flavonoid content was observe in roots and leaves of Atriplex canescens under chromium and zinc stress. Increase in TFC was reported in stem and roots of cucumber plants after Cd stress. (Sun,et al., 2022) Growth rate decreases and content of flavonoid increases in coriander under stress conditions. (Fattahi *et al.,* 2021)

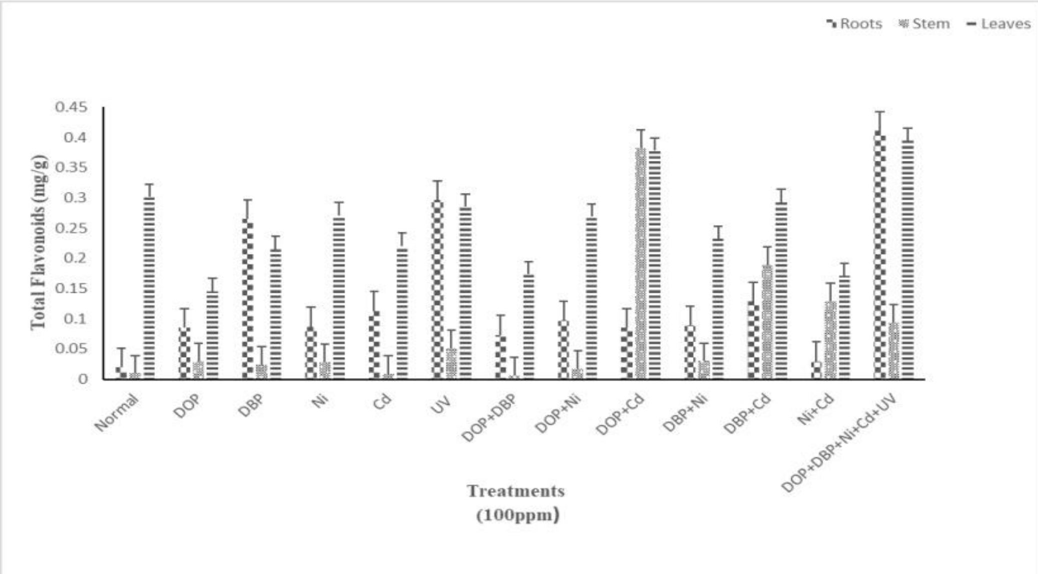


Figure 2. The impact of multifactorial stress on Total Flavonoid Content of Pisum sativum

***TOTAL PHENOLIC CONTENT***

In current study phenolic content in untreated plant 0.015,0.010 ,0.045 in roots, stem and leaves were recorded respectively which increase with in stress containing plants.UV light have more adverse effect on polyphenols than chemical stress. Maximum stress in plants interact with UV stress was seen 0.024,0.039 and 0.0591 respectively as compare to control.  Polyphenols in plants are antioxidant in nature and become more active in plants having stress conditions.with increase in DAP concentration level of  polyphenols increase in plants.(Sharma et al 2020). When combines stress was given it decrease the polyphenols 0.0008,0.004 and 0.0073 respectivety as a study shows that combine effect of cd and Se decreases the polyphenols in plants.(Sun, et al., 20220) A study on *C. sativum* plant shows that Increase in Polyphenolics results decrease in oxidative stress caused by stress .(Marichali, et al., 1993)

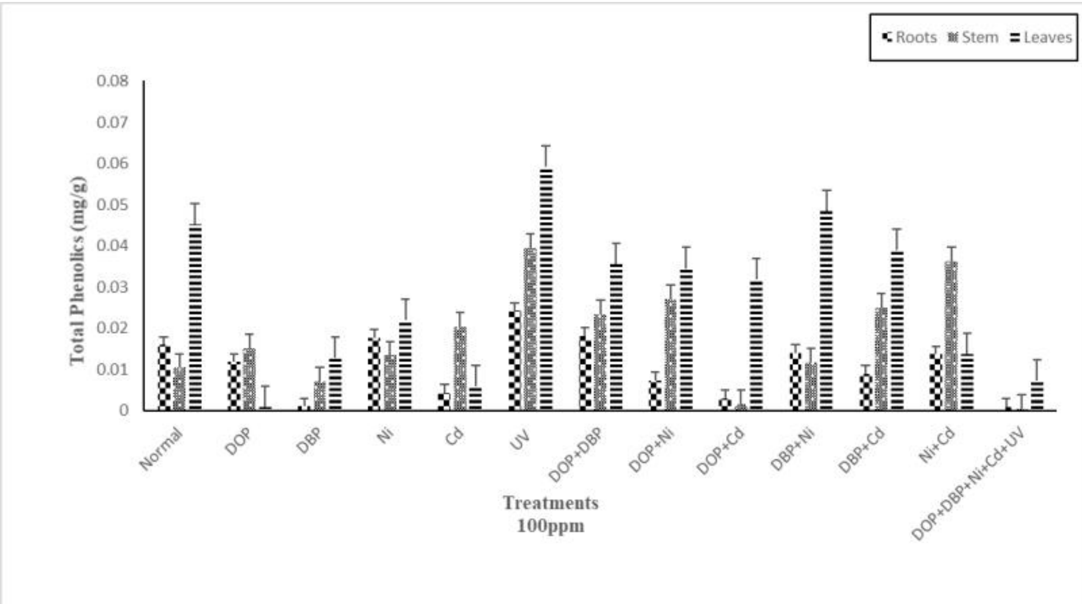


Figure 3. The impact of multifactorial stress on Total Phenolic Content of Pisum sativum

## CONCLUSION

Findings from the study consistently demonstrate that exposure to heavy metals, phthalates, physical stressors, and combinations of chemical and physical stressors can exert a substantial influence on the levels of pigments, flavonoids and phenolics within plants. The notable variations underscore the profound impact of multifactorial stresses on the photosynthetic mechanisms, phytochemicals and the overall health of plants.

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## DISCLOSURE STATEMENT

The authors declare no potential conflicts of interest.

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