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Evaluation Of Flavonoid Content And Antioxidant Activity Of Spinacia Oleraceae L. Using Heavy Metal And Drought As Stress Conditions

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abstract
The present study focussed on how spinach (Spinacia oleracea L) changed in terms of flavonoid concentration and antioxidant properties under drought stress and heavy metal (mercuric chloride at 1 ppm, 5 ppm, and 9 ppm) conditions. After being cultivated in coco-peat, spinach seedlings were moved into red soil that had been mixed with vermicomposting. They were then carefully exposed to various stressors. ICP-OES for heavy metal accumulation, the SpectraVue leaf spectrometer for indicators such as TVI, CCI, ADI, and FRI, and the CI-340 photosynthesis system for photosynthetic rate (PN), leaf temperature, and PAR were used to study the physiological and biochemical reactions of the plants. The DPPH radical scavenging assay (RSA) was used to assess antioxidant capability; the results showed that RSA% increased with extract concentration but decreased under stress in comparison to controls. The present study showed that the amount of chlorophyll decreased with increasing amounts of heavy metals. The findings confirmed that while drought stress had a mild impact, larger quantities of heavy metals decreased antioxidant activity, flavonoid reflectance index, and chlorophyll content. By highlighting spinach's vulnerability to abiotic stress, which impacts its growth, photosynthetic efficiency, and nutritional value, this integrated method offers important insights on crop resilience and food safety in the face of environmental difficulties.
Key Words: *Spinacia oleracea, Antioxidant activity, DPPH, Photosynthesis, Heavy metal, mercury*

1. INTRODUCTION

Spinach (*Spinacia oleracea* L.) is an annual plant species belonging to the family Chenopodiaceae. The genus originated in South-west Asia, while S. *oleracea* was probably brought in 14th century by the Arabs to Spain, from where it spread to other countries by the 16th century (George 1985). Spinach is nowadays an important horticultural species, since its leaves have been used worldwide in human nutrition. In developed up to 80% of spinach produced is processed as canned or frozen food. The classification of numerous spinach cultivars is based on seed from (round or prickly); leaf texture (smooth or crinkled); leaf colour, shape and pose, and petiole length. Spinach – Family –Amaranthaceae and Subfamily – Chenopodioideae. A disadvantage of spinach is its high nitrate content (up to 3000mg NO3/kg), since during transport and storage nitrite arises by reduction in quantities which may cause methemoglobinemia. The nitrate content is higher in petioles than in leaf blades and can be influenced by fertilizers and other oxalic acid which is abundant in leaves, may also cause dietary disorder, due its ability to bind calcium ions [1].

Spinacia oleraceae L also known as spinach (English), was referred as palak (Hindi), pinni (India), pasalai (Tamil), mathuibucchali (Telugu), chhurika (Sanskrit), horenso (Japanese), bayam (Malaysian), spenat (Swedish), ispanak (Turkish), spinacio (Italian), sigeumchi (Korean), Buai leng (Thai) in the local languages in different parts of the world [2, 3].

The dark green leafy vegetable, *Spinacia oleraceae* referred as **power food** is packed with essential nutrients such as proteins, minerals, and vitamins [4,5]. In recent years, the people have started to use the medicinal plants as an alternative to the allopathic medicines. The recent studies with antioxidant substances have indicated that the occurrence of many diseases like Alzheimer's disease can be slowed down [6, 7, 8].

The leaves of the plants exhibit alternate, simple, ovate and varying in size from about 2-30 cm long and 1-15 cm broad. The larger leaves are found at the base of the plant and smaller leaves are found at apex of the flowering stem [9]. The flowers are very small, yellow to light green, 3-4 mm in diameter, which matures into a small dry fruit about 5-10 mm length. [10,11]

There are many diseases like Downy Mildew disease caused by *Peronospora farinosa*, White rust caused by *Albugo occidentalis*, Leaf spot disease caused by *Colletotrichum dematium*, Soil born disease, and Virus disease reported for this plant [12]. 13,14,15]. When *Spinacia* is affected by the diseases it responds by increasing the flavonoid biosynthesis. The same mechanism can be observed in the plant when there is increase of abiotic stress and heavy metals [13, 14, 15]. Flavonoids help the plants to overcome the oxidative stress caused by the heavy metals through ROS scavenging, metal chelation, membrane chelation and regulating stress responsive gene expression [16]. The Present study focusses on the effect of heavy metals and drought stress on the flavonoid content and anti-oxidant properties in *Spinacia oleracea*.

2. MATERIALS AND METHODS

2.1 Collection and Cultivation of Seeds: For cultivation activity, healthy and high-quality spinach (*Spinacia oleracea*) seeds were procured from a certified agricultural seed shop. The seeds were selected based on viability, purity and suitability for the local climate conditions. Coco-peat or coconut husk was carefully collected, sieved to remove any large particles and then evenly poured into the seed trays to ensure proper aeration and moisture retention for seed germination. Finally, the seeds were sown in coco-peat in a seed tray. After sowing, seeds began to germinate within 3 to 4 days, with small shoots indicating healthy seed development

2.2 Soil Collection and Preparation

Red soil was collected from a good environment condition with highly fertile productivity and not polluted. For soil analysis, wet digestion was performed using strong acids to convert elements into soluble form for Atomic Absorption Spectroscopy (AAS). The procedure involved weighing 1g of soil sample, grinding it well, transferring to a crucible, adding 10ml of freshly prepared acid mixture of HNO3: HCL (1:3), and boiling over a heating mantel for 4 to 5 hours until the sample dissolved and turned white.

2.3 Transferring Plants and Applying Stress

Each pot had three parts of red soil and one part of vermicomposting in the ratio of 3:1. Plants were transferred into pots gently to avoid damage. The experimental design included control groups and treatment groups with different concentrations of mercuric chloride (1 ppm, 5 ppm, and 9 ppm) as well as drought stress conditions. Mercury chloride solutions were prepared at different concentrations (1 ppm, 5 ppm, and 9 ppm) using appropriate dilutions of stock solution. Stress was applied twice a week by pouring 50ml of the respective solution to each potted plant.

2.4 Analysis of Plant Samples using analytical techniques

2.4.1 SpectraVue Leaf Spectrometer

SpectraVue Leaf spectrometer was used to measure the Chlorophyll content indices (CCI, CPHLA, CPHLB, CPHLT) in Spinach leaf. It is a powerful spectrometer paired with a leaf probe attachment, onboard operating software, and display screen. Two broad-banded light sources are attached inside the device - one positioned in the leaf clamp for transmissive measurements and one placed inside the case for reflective measurements. The spectrometer module captures the light from the leaf probe attachment and projects the wavelength-dispersed light onto a CCD array. Each pixel of the CCD array corresponds to a specific wavelength of light. The operating software displays the light intensity of each pixel of the CCD array. Three spectroscopic measurements can be performed: Transmittance (T), Absorbance (A), and Reflectivity (R) (16).

2.4.2 CI-340 Handheld Photosynthesis System

This system measured the photosynthesis by determining the rate of CO_2 assimilation by calculating the difference in CO_2 concentration before and after it enters the leaf chamber, relative to a known leaf area. It also measured transpiration (the movement of water vapor from leaf tissue into the atmosphere) by calculating the rate of water vapor flux per one-sided leaf area. Stomatal conductance, which refers to the openness of leaf stomata that determines the rate of CO_2 assimilation and water vapor exit, was calculated by measuring the transpiration rate as a function of leaf temperature. The CI-340 can operate as either an open or closed system and can take both absolute and differential readings (17).

2.4.3 ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry)

ICP-OES uses an argon plasma to convert the sample into ions that emit light at characteristic wavelengths, which is then measured using an optical spectrometer. The Agilent 5800 ICP-OES with ICP-

Expert software was used for analysis, with specific parameters including 3 replicates, pump speed of 12, RF power of 1.20, and plasma flow of 12.0L/min. This technique provides fast analysis of multiple elements in a sample and is particularly useful for measuring heavy metal concentrations in plant tissues (18, 19).

2.4.5 High-Performance Liquid Chromatography (HPLC)

HPLC was also employed using a Shimadzu Prominence system, renowned for its high precision, reliable solvent delivery, and robust performance. This analytical method effectively separates, identifies, and quantifies components in complex mixtures, making it suitable for analyzing flavonoid content in the spinach samples.

2.5 Antioxidant Activity Analysis

2.5.1 DPPH Radical Scavenging Assay

The analysis of antioxidant propertied was performed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay (14, 20). The radical scavenging activity (RSA) was calculated using the formula:

$$\text{RSA\%} = \frac{Ao-A}{Ao} X100$$

Where Ao is the absorbance of the control and A is the absorbance of the sample.

3. RESULTS

3.1 Plant Height Determination

The mean height of the plants before and after the treatment was measured [Table 1 & 2]. Variances were observed in the height of the plants before and after the treatment. All the plants before the treatments were maintained the similar heights while those plants exhibited differences in the height after the treatments which showed clear evidence on the changes in the morphology of the plants.

Treatment	Mean Height (cm)
Control	2.415±0.526
1 PPM	2.240±0.390
5 PPM	2.095±0.181
9 PPM	2.055±0.246
Drought 1	2.165±0.201
Drought 2	1.935±0.255
Drought 3	2.080±0.357

Table 1: Mean plant heights before treatment application, showing relatively uniform initial growth across treatment groups.

Treatment	Mean Height (cm)
Control	3.03±0.56
1 PPM	3.215±0.5
5 PPM	6.515±5.95
9 PPM	4.5±3.40
Drought 1	2.085±0.32
Drought 2	2.165±0.18
Drought 3	1.935±0.23

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Table 2: Mean plant heights after treatment application, showing significant increases in heavy metal treated groups, particularly at 5 ppm concentration, likely representing a stress-escape response.

3.2 Antioxidant Activity Analysis

The antioxidant activity of the spinach extracts was evaluated using the DPPH radical scavenging assay and compared with a standard antioxidant, ascorbic acid. The results show that the radical scavenging activity increased with rising extract concentrations for all samples. However, plants under stress conditions, particularly those treated with higher concentrations of mercury chloride, showed reduced antioxidant capacity compared to control plants. This suggests that heavy metal stress negatively impacts the plant's ability to produce antioxidant compounds [Table 3].

Concentration (mg/ml)	Ascorbic acid	Control	1 ppm	5 ppm	9 ppm
0	0	0	0	0	0
0.25	97.6	85.9	84.6	82.7	82.4
0.5	98.2	90.8	90.1	85.9	83.5
0.75	98.4	93.0	91.8	89.3	85.4
1	98.6	95.3	94.2	92.1	90.1

Table 3: Radical Scavenging Activity (RSA%) at different extract concentrations for ascorbic acid (standard) and spinach extracts from different treatment groups.

3.3 Spectral Analysis

The spectral analysis results reveal significant changes in plant physiological parameters under different stress conditions. Chlorophyll content indices (CCI, CPHLA, CPHLB, CPHLT) showed a consistent decrease with increasing heavy metal concentration, indicating impaired chlorophyll synthesis or accelerated degradation. Similarly, the Flavanols Reflectance Index (FRI) decreased under stress conditions, suggesting reduced flavonoid production. Photosynthetic measurements showed that net photosynthetic rate (PN) was negatively affected by both drought and heavy metal stress, with the most severe reduction observed in plants treated with 9 ppm $HgCl_2$ These results collectively demonstrate that heavy metal stress has a more pronounced negative impact on spinach physiology than drought stress [Fig 1-12].

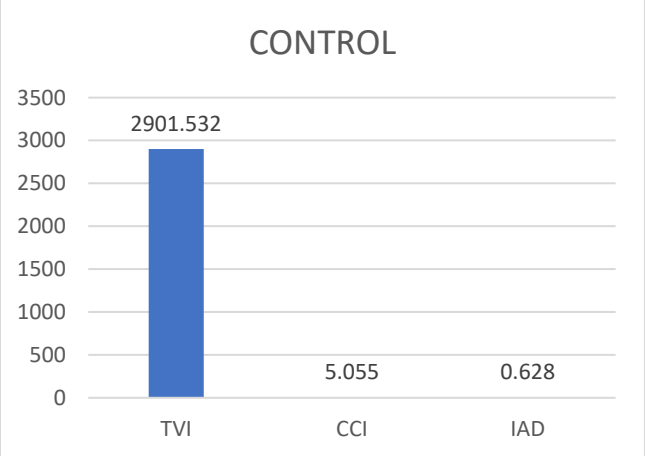

Fig 1

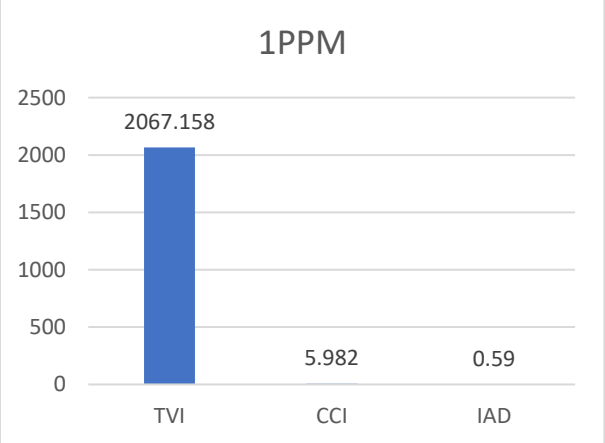

Fig 2

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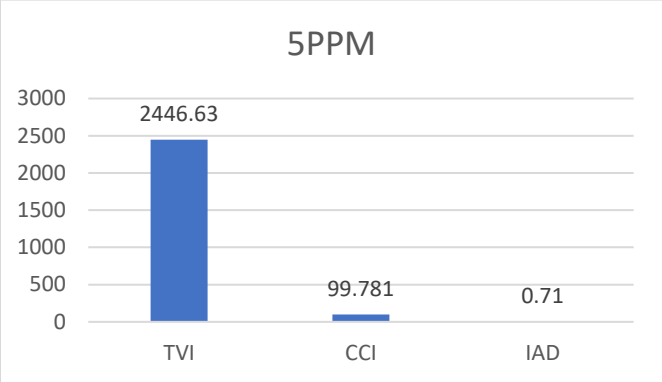

Fig 3

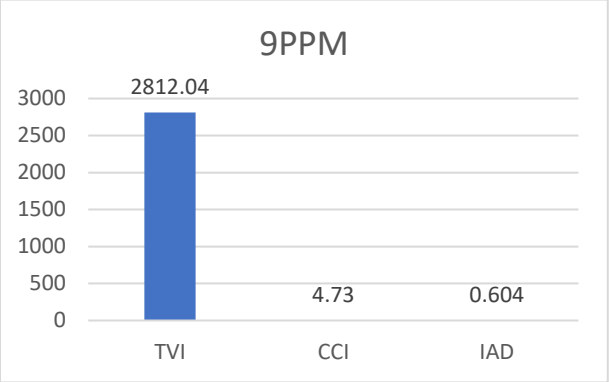

Fig 4

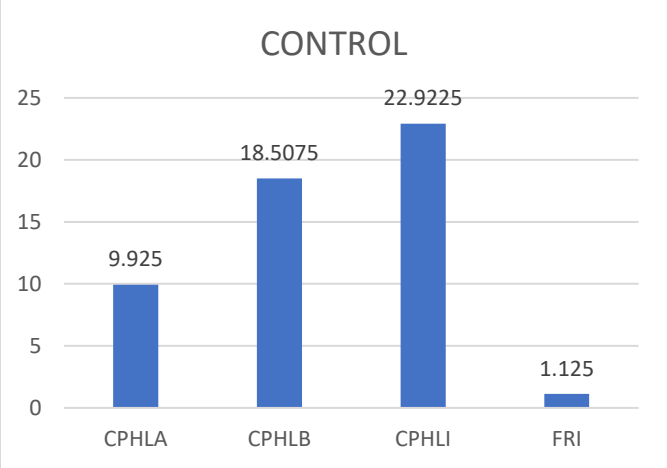

Fig 5

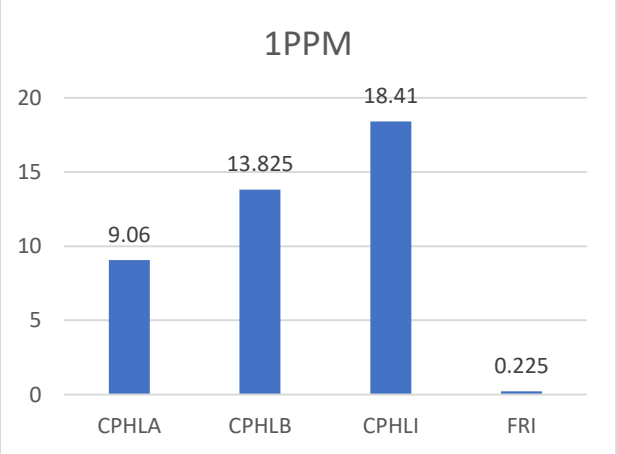

Fig 6

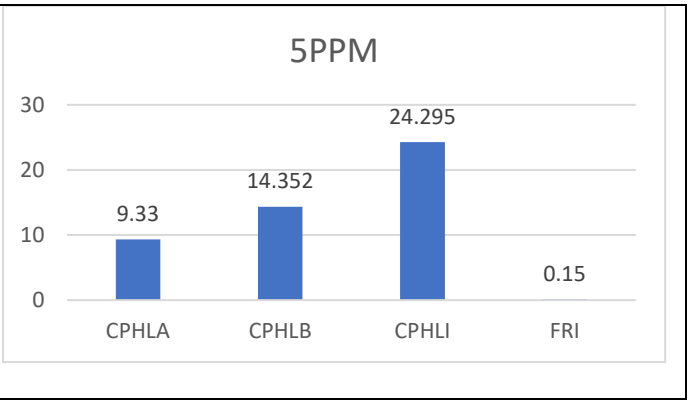

Fig 7

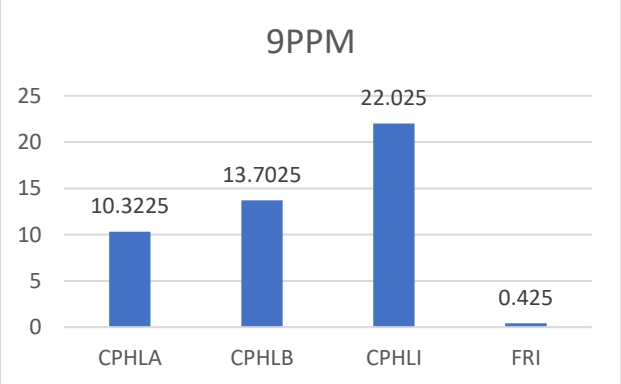

Fig 8

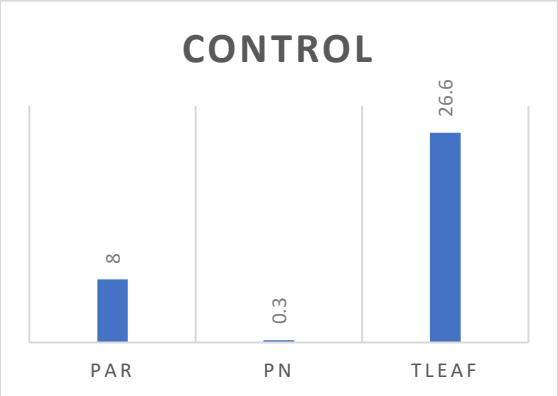

Fig 9

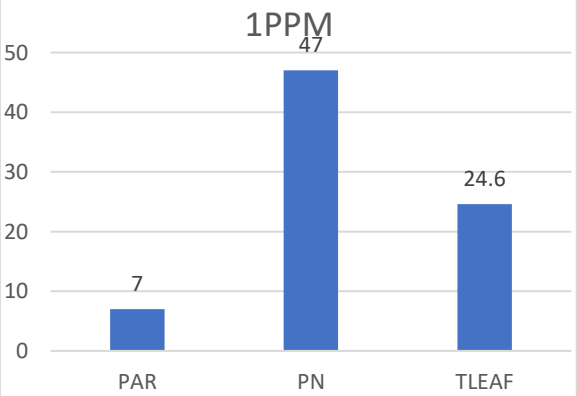

Fig 10

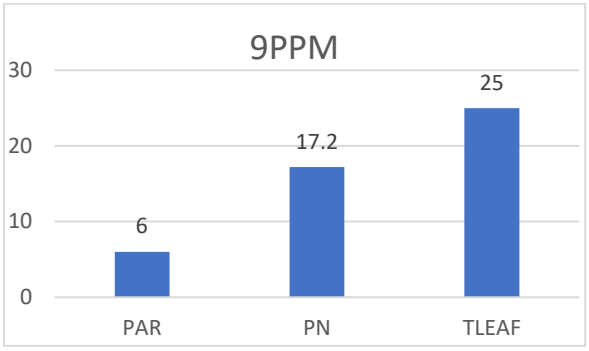

Fig 11

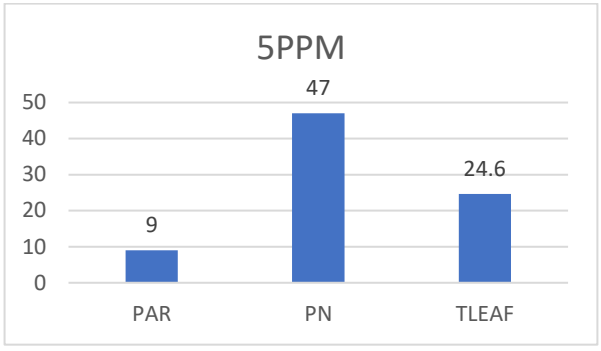

Fig 12

Figure1-12: Photosynthetic parameters measured across different treatment groups. The graph shows declining photosynthetic rates (PN) with increasing stress intensity, particularly in heavy metal treatments, indicating compromised photosynthetic efficiency.

3.4 Analysis of Heavy Metal Accumulation in Spinach

The ICP-OES analysis results confirm the uptake and accumulation of mercury in spinach tissues. Control plants showed minimal mercury content (<0.10 ppm), while plants treated with increasing concentrations of $HgCl_2$ showed proportionally higher mercury accumulation in their tissues. Plants treated with 1 ppm $HgCl_2$ accumulated 0.31 ppm mercury, those treated with 5 ppm accumulated 2.12 ppm, and those treated with 9 ppm accumulated 5.2 ppm [Table 4]. This demonstrated that spinach readily absorbed and accumulated heavy metals from the soil, which has important implications for food safety when grown in contaminated environments.

Sample (leaf)	Concentration (ppm)
Control	<0.10
1PPM	0.31±0.05
5PPM	2.12±0.12
9PPM	5.2±0.11

Table 4: Mercury content in spinach leaf powder as measured by ICP-OES, showing increasing accumulation with higher treatment concentrations. This confirms the uptake and bioaccumulation of mercury in plant tissues.

3.5 HPLC Analysis of Flavonoid Content

The quercetin standard showed a prominent peak at retention time 9.844 (95.531%), which served as a reference for identifying and quantifying this specific flavonoid in the spinach samples [Table 7, Figure 14]. Minor peaks were also observed at retention times 10.793 (1.188%) and 10.879 (3.258%). The HPLC analysis of control spinach samples revealed a complex profile of flavonoid compounds with 23 distinct peaks. Major peaks were observed at retention times of 7.020 (13.792%), 7.143 (10.311%), 7.995 (11.697%), 8.059 (14.858%), and 8.491 (10.574%) [Table 6, Figure 13]. The presence of multiple peaks indicates a diverse range of flavonoid compounds in healthy spinach. Samples from plants treated with 1 ppm, 5 ppm, and 9 ppm $HgCl_2$ showed alterations in their flavonoid profiles compared to control plants [Tables 8, 9, 10; Figures 15, 16, 17]. While the overall pattern of peaks remained similar, there were notable changes in peak areas and relative proportions of different compounds. For example, in the 9 ppm treatment, the peak at retention time 7.029 decreased to 9.430% (compared to 13.792% in control), indicating reduced content of specific flavonoids under heavy metal stress [Table 5].

Treatment group	Major Peak Retention Times (min)	Corresponding Area Percentages
Control	7.020, 7.143, 7.995, 8.059, 8.491	13.792%, 10.311%, 11.697%, 14.858%, 10.574%

1 PPM	7.044, 7.161, 7.995, 8.079, 8.505	14.149%, 8.177%, 12.323%, 20.502%, 8.801%
5 PPM	7.025, 7.146, 7.995, 8.064, 8.489	10.544%, 7.833%, 13.517%, 19.413%, 8.374%
9 PPM	7.029, 7.146, 8.005, 8.070, 8.495	9.430%, 6.509%, 11.711%, 16.688%, 9.670%

Table 5: Comparison of major HPLC peaks across different treatment groups, showing changes in flavonoid composition under stress conditions. The data reveals stress-induced alterations in the relative proportions of different flavonoid compounds.

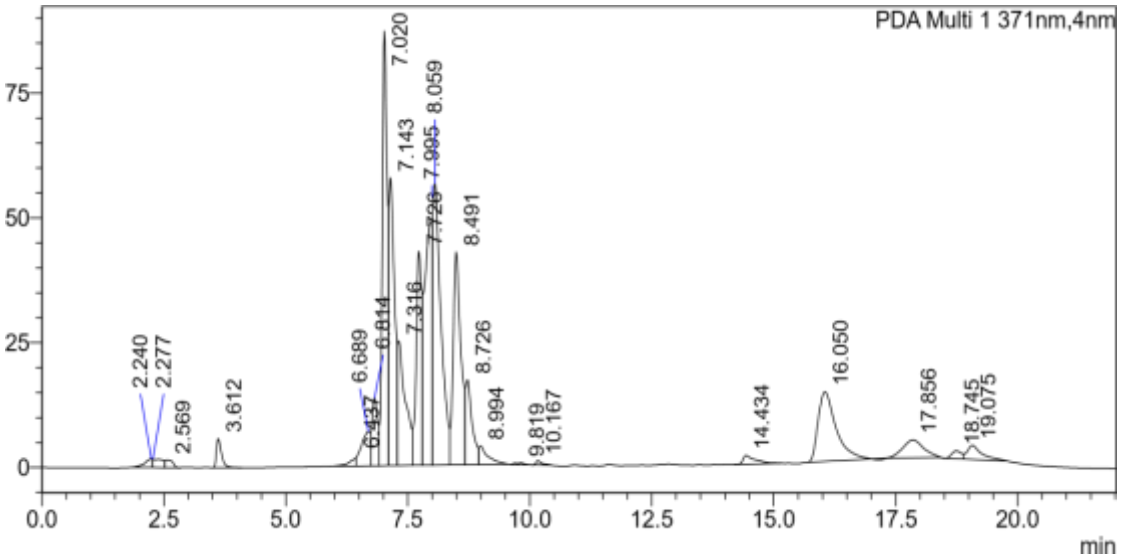

Figure 13: HPLC chromatogram of control spinach extract showing multiple peaks representing different flavonoid compounds. The retention times and peak areas provide quantitative information about specific compounds present in the sample

Peak#	Ret. Time	Area	Height	Area%
1	2.240	19182	1832	0.432
2	2.277	23996	1678	0.540
3	2.569	15804	1527	0.356
4	3.612	43402	5865	0.977
5	6.437	15878	1581	0.357
6	6.689	88792	6908	1.999
7	6.814	108523	13580	2.443
8	7.020	612667	87051	13.792
9	7.143	457998	57619	10.311
10	7.316	275799	24960	6.209
11	7.726	335802	42879	7.560
12	7.995	519581	48871	11.697
13	8.059	660014	56382	14.858
14	8.491	469691	42628	10.574
15	8.726	160246	17019	3.607

16	8.994	47411	3710	1.067
17	9.819	3704	341	0.083
18	10.167	6677	842	0.150
19	14.434	30226	1734	0.680
20	16.050	356160	13928	8.018
21	17.856	109721	3611	2.470
22	18.745	21933	1585	0.494
23	19.075	58845	2828	1.325
Total		4442054	438960	100.000

Table 6. The retention times and peak areas exhibited in HPLC chromatogram of control spinach extract

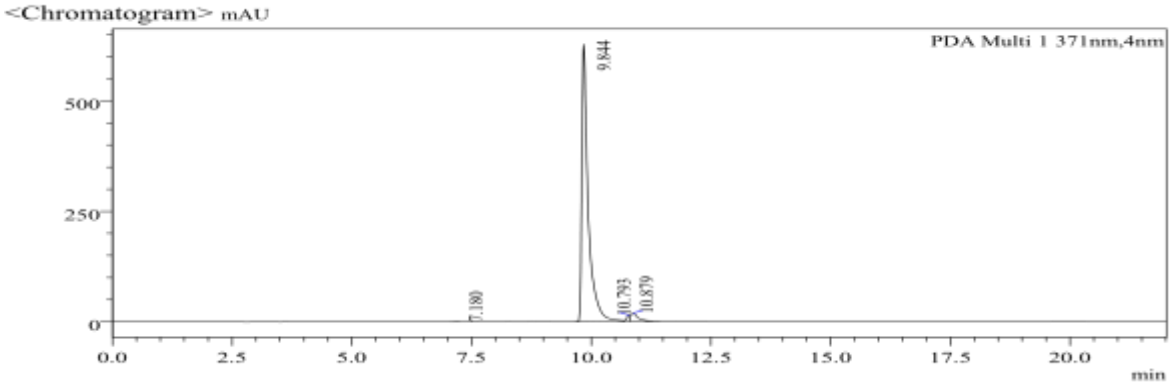

Figure 14: HPLC chromatogram of quercetin standard used for identification and quantification of flavonoids in spinach samples. The prominent peak at retention time 9.844 represents pure quercetin.

Peak#	Ret. Time	Area	Height	Area%
1	7.180	1389	253	0.023
2	9.844	5757855	626718	95.531
3	10.793	71628	13718	1.188
4	10.879	196346	18692	3.258
Total		6027218	659382	100.000

Table 7. The retention times and peak areas exhibited in HPLC chromatogram of quercetin standard

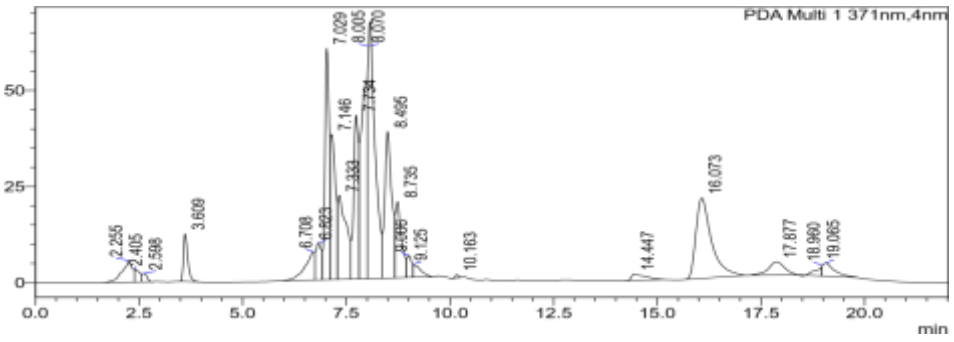

Figure 15: HPLC chromatogram of spinach extract treated with 9 ppm mercury showing multiple peaks representing different flavonoid compounds. The retention times and peak areas provide quantitative information about specific compounds present in the sample

Peak#	Ret. Time	Area	Height	Area%
1	2.255	93993	5562	2.005
2	2.405	27448	3145	0.585
3	2.598	17634	2131	0.376
4	3.609	91144	12382	1.944
5	6.708	117688	7339	2.510
6	6.823	91621	9600	1.954
7	7.029	442122	60168	9.430
8	7.146	305180	37720	6.509
9	7.333	306761	21790	6.543
10	7.734	351252	42603	7.492
11	8.005	549094	51711	11.711
12	8.070	782447	66779	16.688
13	8.495	453376	38040	9.670
14	8.735	197969	19805	4.222
15	9.006	45736	5752	0.975
16	9.125	40732	3167	0.869
17	10.163	4256	775	0.091
18	14.447	39866	1673	0.850
19	16.073	544604	20888	11.615
20	17.877	93886	3434	2.002
21	18.960	23547	2206	0.502
22	19.065	68261	3409	1.456
Total		4688619	420077	100.000

Table 8. The retention times and peak areas exhibited in HPLC chromatogram of spinach extract treated with 9 ppm mercury

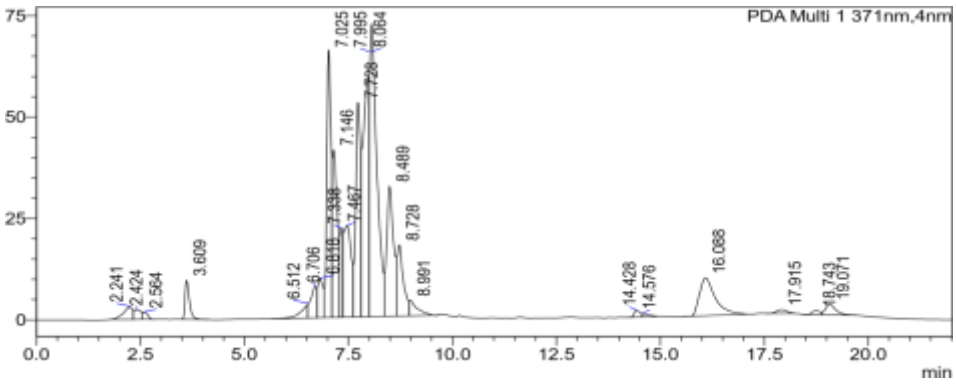

Figure 16: HPLC chromatogram of spinach extract treated with 5 ppm mercury showing multiple peaks representing different flavonoid compounds. The retention times and peak areas provide quantitative information about specific compounds present in the sample

Peak#	Ret. Time	Area	Height	Area%
1	2.241	47661	3278	1.085
2	2.424	28174	2375	0.641
3	2.564	15238	1800	0.347
4	3.609	71080	9664	1.618
5	6.512	38552	3161	0.878
6	6.706	82491	7623	1.878
7	6.818	81893	9743	1.864
8	7.025	463196	65962	10.544
9	7.146	344101	41297	7.833
10	7.338	109024	21972	2.482
11	7.467	283022	22607	6.442
12	7.728	428283	52859	9.749
13	7.995	593837	58658	13.517
14	8.064	852817	72214	19.413
15	8.489	367865	32059	8.374
16	8.728	189432	17542	4.312
17	8.991	48811	3781	1.111
18	14.428	16670	1693	0.379
19	14.576	8991	785	0.205
20	16.088	248072	9345	5.647
21	17.915	16651	950	0.379
22	18.743	11939	962	0.272
23	19.071	45330	2645	1.032
Total		4393132	442973	100.000

Table 9. The retention times and peak areas exhibited in HPLC chromatogram of spinach extract treated with 5 ppm mercury

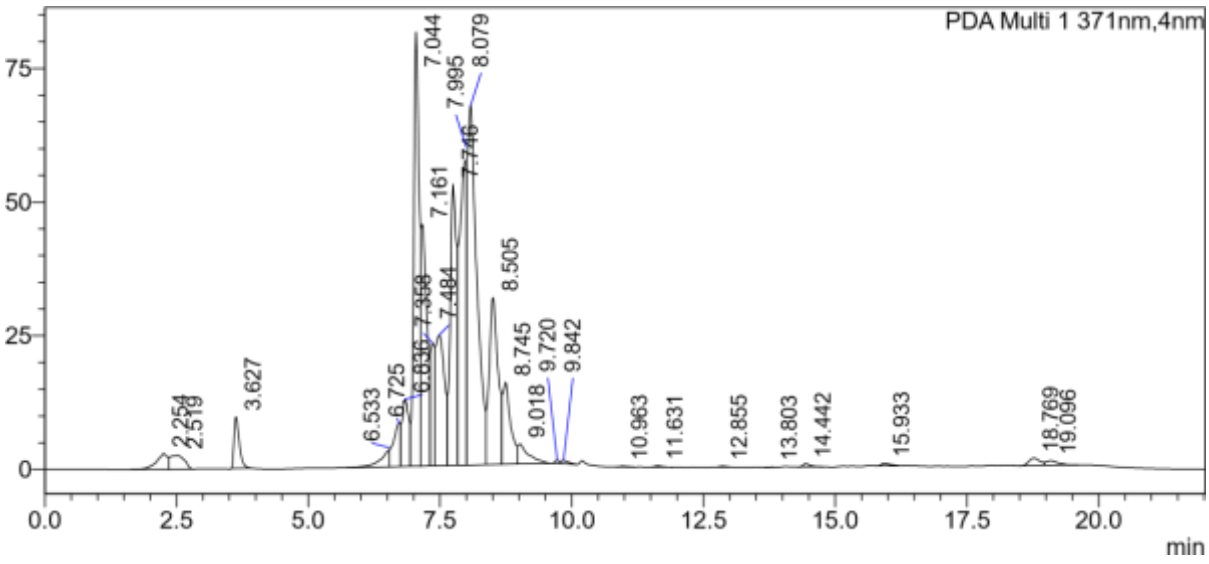

Figure 17: HPLC chromatogram of spinach extract treated with 1 ppm mercury showing multiple peaks representing different flavonoid compounds. The retention times and peak areas provide quantitative information about specific compounds present in the sample

Peak#	Ret. Time	Area	Height	Area%
1	2.254	44044	2961	1.059
2	2.519	46771	2619	1.125
3	3.627	66480	9617	1.598
4	6.533	36990	3104	0.889
5	6.725	76066	8133	1.829
6	6.836	110808	12584	2.664
7	7.044	588504	81185	14.149
8	7.161	340117	45179	8.177
9	7.358	128525	22964	3.090
10	7.484	285775	24416	6.871
11	7.746	430236	52567	10.344
12	7.995	512540	56949	12.323
13	8.079	852736	67255	20.502
14	8.505	366068	31234	8.801
15	8.745	161311	15346	3.878
16	9.018	52387	3759	1.260
17	9.720	3798	631	0.091
18	9.842	6186	661	0.149
19	10.963	1368	161	0.033
20	11.631	2271	253	0.055
21	12.855	1721	193	0.041
22	13.803	395	67	0.009

Table 10. The retention times and peak areas exhibited in HPLC chromatogram of spinach extract treated with 1 ppm mercury

4. DISCUSSION

This study investigated the effects of abiotic stressors on the growth, flavonoid content, and antioxidant activity of *Spinacia oleracea* (spinach), particularly heavy metal stress caused by mercuric chloride ($HgCl_2$) and drought stress. Changes in morphological, physiological, and biochemical markers demonstrate spinach's obvious susceptibility to environmental stress.

4.1 Initial Growth and Stress Response

Prior to treatment, initial assessments of plant height revealed comparatively consistent growth between the control and treatment groups, suggesting regular circumstances for germination and cultivation. Following stress application, heavy metal-treated groups showed increased plant height (especially at 5 ppm and 9 ppm concentrations). This is probably a stress-escape response where plants invest in shoot elongation when conditions are unfavourable. However, physiological and biochemical constraints were frequently seen in these higher plants.

4.2 Heavy Metal Accumulation and Physiological Impact

ICP-OES analysis verified heavy metal build-up, which is consistent with earlier findings that spinach easily absorbs and accumulates heavy metals. Key indices such as the flavonols reflectance index (FRI) and chlorophyll content index (CCI) decreased with increasing $HgCl_2$ concentrations, according to spectroscopic analyses performed with the SpectraVue leaf spectrometer [19]. This suggests that heavy metals have a detrimental effect on flavonoid accumulation and chlorophyll synthesis. Since these pigments are essential for photosynthesis and photo- protection, it is possible that heavy metal stress reduces a plant's capacity to absorb light and defend itself from oxidative damage.

4.3 Comparative Stress Effects

These indicators decreased less in plants under drought stress than in plants under heavy metal stress, suggesting that spinach is more resilient to mild water constraint than to heavy metal contamination. This pattern was also seen in photosynthetic performance as determined by the CI-340 handheld system: even while net photosynthetic rate (PN) and associated metrics dropped under all stress conditions [19], the decline was more pronounced in the 9 ppm $HgCl_2$ group, suggesting that heavy metal stress causes more metabolic disruption.

4. 4 Antioxidant Activity

Both drought and heavy metal stress resulted in reduced RSA% when compared to controls, according to the antioxidant activity measured by the DPPH radical scavenging assay; however, antioxidant activity rose with larger extract concentrations. This suggests that under stress, plants maintain their antioxidant response capacity, albeit with a reduced capacity. It's interesting to note that plants under drought showed a less noticeable decline in antioxidant activity than plants subjected to heavy metals, which suggests that drought is a less severe stressor for spinach than exposure to mercuric chloride [20].

4. 5 HPLC Analysis of the plant samples

The HPLC analysis provided detailed information about the flavonoid composition of spinach under different stress conditions. The chromatograms revealed a complex mixture of compounds in all samples, with significant variations in peak areas and relative proportions between treatment groups. The results suggested that heavy metal stress alters the flavonoid profile of spinach, with some compounds showing reduced content while others might be induced as part of the plant's stress response. These changes in flavonoid composition likely contributed to the observed differences in antioxidant activity between treatment groups [20,21].

5 CONCLUSION

Together, these findings demonstrate that although spinach has built-in defences against abiotic stress, such as retaining some antioxidant activity and growing taller shoots, heavy metal stress, particularly at higher concentrations, impairs photosynthetic efficiency, pigment content, and flavonoid accumulation more than drought stress. Higher levels of heavy metal build-up not only jeopardize plant health but also increase the risk of health problems for consumers, which may have an impact on food safety and nutritional quality. Overall, this study emphasizes how crucial it is to keep an eye on environmental pollutants and water availability when growing spinach because these elements have a direct impact on plant growth, metabolism, and antioxidant qualities 4all of which are vital for crop productivity and human nutrition.

6. REFERENCES

[1] Nešković, M., and Ćulafić, L., 1988, "Spinach (Spinacia oleracea L.)," Biotechnology in Agriculture and Forestry, pp. 370–385, https://doi.org/10.1007/978-3-642-73520-2_18.

[2] Subhash, G. P., Virbhadrappa, S. R., and Vasant, O. K., 2010, "Spinacia oleracea Linn: A Pharmacognostic and Pharmacological Overview," Int. J. Res. Ayurveda Pharm., 1, pp. 78–84.

[3] Rao, K. N., Tabassum, B., Babu, S. R., Yaja, A., and Banji, D., 2015, "Preliminary Phytochemical Screening of Spinacia oleracea L.," World J. Pharm. Pharm. Sci., 4, pp. 532–551.

[4] Segheloo, A. E., Gharneh, H. A., Mohebodini, M., Janmohammadi, M., Nouraein, M., and Sabaghnia, N., 2014, "The Use of Some Morphological Traits for the Assessment of Genetic Diversity in Spinach (Spinacia oleracea L.) Landraces," Plant Breed. Seed Sci., 69, pp. 69–80.

[5] Tehseen, M., Hina, S., Nisa, A., and Ahmad, A., 2014, "Antioxidant Potential of Differently Irrigated Soil Grown Varieties of Spinach," World Appl. Sci. J., 32, pp. 1235–1241.

[6] Feng, Y., and Wang, X., 2012, "Antioxidant Therapies for Alzheimer's Disease," Oxid. Med. Cell. Longev., 2012, pp. 1–17, https://doi.org/10.1155/2012/472932.
[7] Nascimento, N. L., Costa, I. H., and Freitas, R. M., 2014, "Nutritional Aspects and Their Influences on the Pathophysiology of Alzheimer's Disease: A Systematic Review," Rev. Cienc. Med., 23, pp. 33–40.
[8] Ye, X., Tai, W., and Zhang, D., 2012, "The Early Events of Alzheimer's Disease Pathology: From Mitochondrial Dysfunction to BDNF Axonal Transport Deficits," Neurobiol. Aging, 33, pp. 1122.e1–1122.e10, https://doi.org/10.1016/j.neurobiolaging.2011.11.004.
[9] Nayak, A. K., Pal, D., Pany, D. R., and Mohanty, B., 2010, "Evaluation of Spinacia oleracea L. Leaves Mucilage as an Innovative Suspending Agent," J. Adv. Pharm. Technol. Res., 1, pp. 338–341, https://doi.org/10.4103/0110-5558.72430.
[10] Mane, P. C., Kadam, D. D., Chaudhari, R. D., Varpe, K. A., Sarogade, S. D., Thorat, V. T., et al., 2015, "Phytochemical Investigations of Spinacia oleracea: An Important Leafy Vegetable Used in Indian Diet," Cent. Eur. J. Exp. Biol., 4, pp. 1–4.

[11] Dande, P. R., Sharma, G. M., Sharma, R. M., and Chakraborthy, G. S., 2010, "Pharmacognostical Studies of Leaves of Spinacia oleracea Linn.," Int. J. Pharm. Sci. Res., 1, pp. 41–46.
[12] Correll, J. C., et al., 1994, "Economically Important Diseases of Spinach," The American Phytopathological Society. https://web.archive.org/web/20040328132308id_/http://comp.uark.edu:80/~morelock/images/EIDS_grey.pdf
[13] Abhiram S, Xavier J., 2023, "Comprehensive phytochemical, anti-oxidant and GC-MS analysis of Strobilanthes jomyi Biju P, Josekutty, Rekha, Wood JRI.," Asian J of Plant Sci., pp. 22:227–38. https://scialert.net/abstract/?doi=ajps.2023.227.238
[14] Sardar, A., Shahid, M., Natasha et al., 2020, "Risk assessment of heavy metal(loid)s via Spinacia oleracea ingestion after sewage water irrigation practices in Vehari District," Environ Sci Pollut Res., 27, pp. 39841–39851. https://doi.org/10.1007/s11356-020-09917-4
[15] Nair A, Xavier J., 2025, "Phytochemical characterization by GC-MS and in vitroevaluation of antioxidant potential of Walsura piscidia Roxb leaves extract," Plant Science Today, 12(3): pp. 1-9. https://doi.org/10.14719/pst.8653
[16] Swastini, D. A., Martien, R., Fachiroh, J., and Nugroho, A. E., 2023, "Bibliometric Analysis of Basella spp. as an Antioxidant," BIO Web Conf., 75, p. 01001.
[17] Reddy, G. J., Madhavi, T. R., Rao, C. A., Nithya, S., and Rao, P. N., 2023, "An Updated Phytochemical and Pharmacological Review on Malabar Spinach (Basella alba or Basella rubra)," GSC Biol. Pharm. Sci., 24(2), pp. 161–169.
[18] CID Bio-Science, 2025, "CI-710S SpectraVue Leaf Spectrometer - CID Bio-Science," https://cid-inc.com/plant-science-tools/leaf-spectroscopy/ci-710-miniature-leaf-spectrometer/#Theory.
[19] Kamal, W. J., and Xavier, J., 2023, "Effect of Heavy Metals on the Pigmentation and Photosynthetic Capability in Jacobaea maritima (L.) Pelser & Meijden," Plant Sci. Today, 10(4), pp. 192–197, https://doi.org/10.14719/pst.2490.
[20] Harshitha, K. R., and Xavier, J., 2025, "Phytochemical Profiling and Evaluation of Antioxidant and Antiinflammatory Activities of Ipomoea alba L.," Plant Sci. Today, February, https://orcid.org/0000-0003-4044-1583.
[21] Dzakovich MP, Le EA, Tak AL and Chacko SK, 2025, "A comprehensive UHPLC-MS/MS and extraction method reveals flavonoid profile and concentration are diverse in spinach (Spinacia oleracea L.)," Front. Plant Sci., doi: 10.3389/fpls.2025.1496200