

Hexavalent Chromium (Cr^{VI}) Uptake Induced Physiological Effects and Growth in *Amaranthus dubius* Mart. ex Thell

Simi. R, Subin. M.P*

PG Department of Botany and Research, S.N College, Thrissur- 680 566, Kerala, India

*Corresponding author: subinshiny@gmail.com

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Abstract The heavy metal chromium (Cr) is considered as one of the most hazardous metal ion which hampers crop productivity by contaminating both soil and water due to higher solubility. The present study evaluated the impact of different levels of hexavalent chromium (Cr^{VI}) stress on the morphological and physiological growth performance of *Amaranthus dubius*. The morphological growth parameters such as length of shoot, root and total plant, fresh and dry biomass accumulation, root/shoot ratio and total leaf number and leaf area/plant recorded progressive reduction along with the progressive increase in the Cr^{VI} stress, with certain exceptions with respect to the length of root and total plant at initial level Cr^{VI} stress and root/shoot ratio at higher level concentrations. With respect to physiological parameters, 1mg Cr/kg soil treatment caused a stimulatory action on stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and net photosynthetic rate (P_N) while further enhancement in the concentration of Cr^{VI} is inhibitory in action. The Cr^{VI} toxicity at higher level stresses of 50mg Cr/kg soil and above has significant deleterious effects and the tolerance index is reduced to 6.1 - 2.9% and inhibition of plant growth enhanced to 93.9 - 97.1% in terms of biomass yield compared to control plants.

Keywords: *Amaranthus dubius*, Cr^{VI} toxicity, physiological and morphological growth

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1. Introduction

The accelerated rate of industrialization and unscientific disposal of industrial effluence leading to heavy metal pollution is a major environmental problem. This situation is alarming in developing countries where untreated heavy metal containing wastewater from industries is extensively used for irrigation or is disposed off carelessly into open environments [1]. These metals are deposited in different soil profiles leading to long-term metal contamination. Metals are mostly absorbed by plants easily and prove toxic, which can be observed as deterioration in quality and retardation of growth and productivity [2]. Heavy metal chromium is a nonessential element and at higher concentrations they are toxic to plants. Hexavalent chromium (Cr^{VI}) is considered more toxic form which is a strong oxidant with a high redox potential accounting for a faster and over generation of reactive oxygen species (ROS) which disturb redox balance, affecting the water relations and physiology and hence inhibits growth performance in plants [3]. The requirement of Cr^{VI} compounds in diverse industries for metal plating, leathering, tanning, wood preservation etc. have led to widespread contamination. Even though, their increased

bioavailability in the agricultural land is actually a major concern regarding the productivity and quality of crop plants, in contrast to toxic metals such as cadmium, mercury and lead, the impact of Cr toxicity has not received much attention from scientists and researchers. The leafy vegetables represent an excellent component of the habitual diet in the tropical countries. It is known that leafy vegetables accumulate higher amounts of heavy metals in leaves than in roots, stems and fruits [4]. As the metal pollutants are non-degradable and are readily taken up by crop plants, these are likely to enter easily into the food chain causing serious health hazards to human beings [5]. Amaranth is one of the easily cultivable leafy vegetable which requires less attention and expense and also it is the cheapest leafy vegetable available in the market although it is rich in minerals, vitamins and essential micronutrients and is often referred to as "Poor man's vegetable". The present study is carried out to evaluate the impact of Cr^{VI} stress and toxicity on morphology, physiology and related growth performance and biomass yield of *Amaranthus dubius* Mart. ex Thell., a cultivable crop species (CO1) of *Amaranthus* which is best suited for tropical humid regions. It is an erect, annual herb growing up to an average of 90cm tall. The leaves are simple having ovate to rhomboid-ovate shaped lamina arranged spirally around the slender stem.

2. Materials and Methods

Healthy seeds of *Amaranthus dubius* after germination are transferred to plastic pots containing standard potting mixture prepared by using farmyard manure, red soil and sand in the ratio 1:1:1. One week after transplantation, healthy uniform sized seedlings are selected in triplicate blocks of 30 each for each treatment. Seven different concentration levels of hexavalent chromium (Cr^{VI}) are prepared from potassium dichromate salt ($\text{K}_2\text{Cr}_2\text{O}_7$) of analytical reagent grade and are 1mg, 5mg, 10mg, 20mg, 30mg, 50mg and 70mg Cr^{VI} /kg soil. The required concentrations of Cr^{VI} are applied to the potting mixture as aqueous solution in such a way that every 200ml contains required quantities of Cr^{VI} . 200ml of distilled water alone is used as control. The evaluation of physiological growth parameters are carried out on the 25th day of Cr^{VI} treatments while the morphological growth parameters are carried out on the 45th day of Cr^{VI} treatments.

2.1. Morphological Growth Parameters

- Length of shoot, root and total plant
- Total number of leaves and total leaf area/plant
Leaf area measurements are done using leaf area meter (Model LI - 3100C, LI-COR Biosciences, USA).
- Fresh and dry biomass
- Root/Shoot fresh and dry weight ratio
- The tolerance index (TI) [6]
 $\text{TI (\%)} = 100 \times (\text{Fresh biomass in metal treatment}) / (\text{Fresh biomass in control})$
- Inhibition of plant growth (IPG) [7]

$$\text{IPG (\%)} = \frac{\left(\frac{\text{Total biomass of control} - \text{Total biomass in metal treatment}}{\text{Total biomass in control}} \right) \times 100}$$

2.2. Physiological Growth Parameters

- Net photosynthetic rate (P_N)
- Stomatal conductance (g_s)

- Intercellular CO_2 concentration (C_i)

The study analyses the physiological parameters related to photosynthesis by using the LI-6400XT Portable Photosynthesis System (LI-COR, Lincoln, Nebraska, USA). The device is fitted with blue and red LEDs (LI-6400-02B) as a light source for the relevant photosynthetic measurements. All the leaf gas exchange properties were recorded under the sample chamber condition where photosynthetic photon flux density (PPFD) was at $1000 \mu\text{Mm}^{-2}\text{s}^{-1}$, leaf temperature was at 25°C , and the CO_2 level was at $380 \mu\text{M CO}_2 \text{ mol}^{-1}$. The leaf gas exchange measurements are measured on the second fully expanded leaf from the top of the plant during the period at 9:00 am –11:00 am. The data presented on each parameter is an average of six readings taken from six plants from each treatment.

2.3. Statistical Analysis of Data

Statistical analysis of data is carried out using Duncan's Multiple Range Test of SPSS statistics 20.

3. Results and Discussions

3.1. Length of Shoot, Root and Total Plant

The effect of different concentrations of hexavalent chromium (Cr^{VI}) on the length of shoot, root and total plant of *Amaranthus dubius* is depicted in the Figure 1. Cr^{VI} concentration treatments caused progressive reduction in the length of shoot, root and total plant over the control plants along with the increase in the concentration of treatments, with an exception in the case of 1mg Cr^{VI} /kg soil treatment with respect to root and total plant length, where an enhancement of 14.55% and 4.22% occurred for root and total plant length respectively over the control. The highest reduction of 74.66% for shoot length, 86.42% for root length and 78.83% for total plant length is obtained with 70mg Cr^{VI} treatment while the lowest reduction of 2.53% is obtained with 1mg Cr^{VI} treatment for shoot length whereas, 68.09% and 51.36% respectively for root and total plant length obtained with 5mg Cr^{VI} .

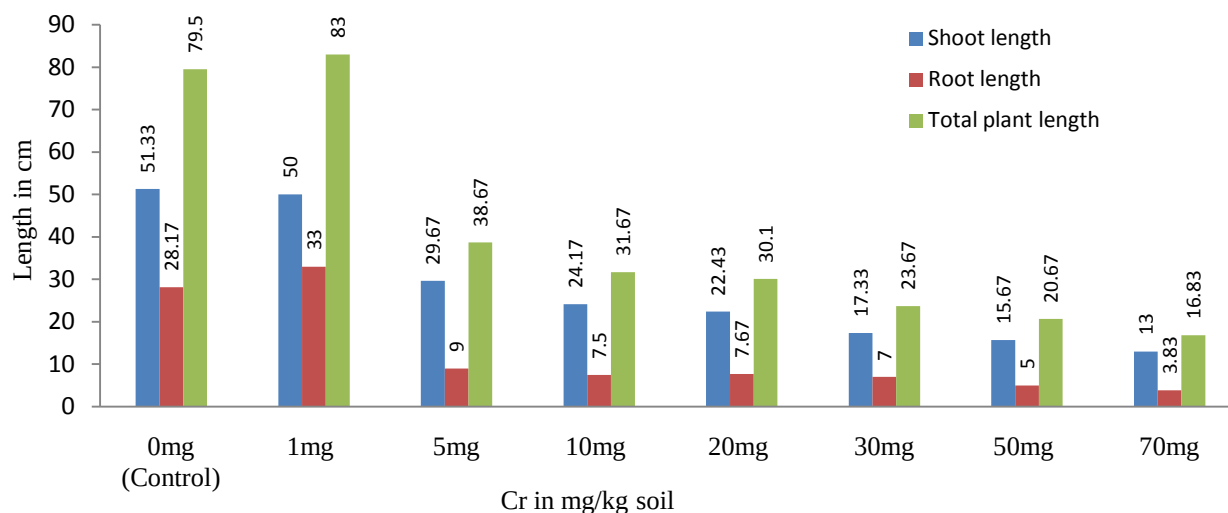


Figure 1. Effects of Cr^{VI} on *Amaranthus dubius* shoot, root and total plant length

Table 1. Duncan Analysis of Shoot Length Variation in *Amaranthus dubius* Treated with Cr^{VI}

Cr ^{VI} Treatment	Subset for alpha = 0.05				
	1	2	3	4	5
70mg	13.00				
50mg	15.67	15.67			
30mg	17.33	17.33	17.33		
20mg		22.43	22.43	22.43	
10mg			24.17	24.17	
5mg				29.67	
1mg					50.00
0mg (C)					51.33
Sig.	.253	.081	.079	.064	.705

Means for groups in homogeneous subsets are displayed.

The difference in shoot and total plant length obtained in 1mg Cr^{VI}/kg soil treated plants over control did not differ significantly but differed significantly higher (0.05 level) over the values of rest of the treated plants. The highest reduction in shoot and total plant length obtained in 70mg Cr^{VI} differed significantly (0.05 level) over control and plants treated with 1mg, 5mg, 10mg and 20mg Cr^{VI} while the differences over 30mg and 50mg Cr^{VI} are not significant (Table 1 & Table 3). The highest root length obtained in plants treated with 1mg Cr^{VI} is significantly higher (0.05 level) over control and other treated plants while the lowest root length obtained with 70mg Cr^{VI} treatment insignificantly differed over plants treated with 50 mg and 30mg Cr^{VI} but significantly lower (0.05 level) over rest of the treated plants (Table 2). Cr^{VI} stress has been reported to decrease the root growth in *Pisum sativum* [8]. The root is the part of the plant which is in direct contact with growing medium and the Cr^{VI} enters the root system along with carrier elements like sulphate, iron etc and accumulates in higher concentration compared to other regions of the plant due to immobilization of Cr^{VI} in vacuoles of root cells [9]. This toxic concentration in the root cells may disturb cell division and elongation and may also cause imbalances in Ca²⁺ transport between plasma membrane and cytoplasm [10]. These factors might have contributed to reduction in root length and overall growth of root system. The reduced root growth can also be attributed to toxicity induced extension of cell cycle in the roots [11]. The chromium toxicity induced inhibition of shoot length as well as total plant length is observed and reported previously in *Oryza sativa* L. [12]. The present study inferences are also in similar lines with these reports. The reduced uptake of water and minerals and their poor mobilization to shoot system imposed by poor root system

development, root injury and suberization of root under chromium stress could be the result of reduced shoot growth over control plants [13,14,15].

Table 2. Duncan Analysis of Root Length Variation in *Amaranthus dubius* Treated with Cr^{VI}

Cr ^{VI} Treatment	Subset for alpha = 0.05				
	1	2	3	4	5
70mg	3.83				
50mg	5.00	5.00			
30mg	7.00	7.00	7.00		
10mg		7.5	7.5		
20mg			7.67		
5mg			9.00		
0mg (C)				28.17	
1mg					33.00
Sig.	.060	.119	.235	1.00	1.00

Means for groups in homogeneous subsets are displayed.

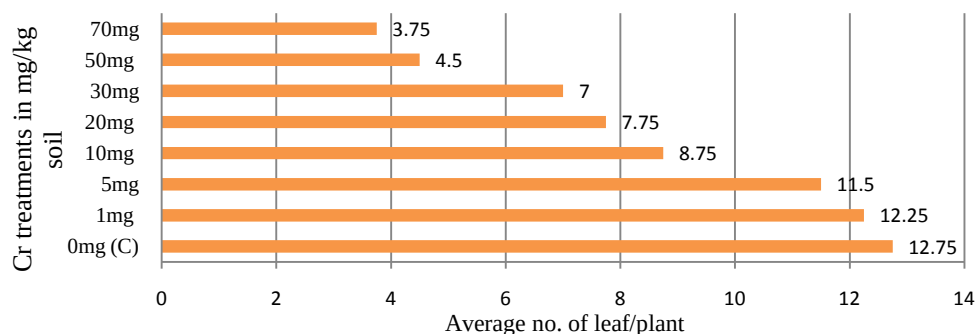
Table 3. Duncan Analysis of Total Plant Length Variation in *Amaranthus dubius* Treated with Cr^{VI}

Cr ^{VI} Treatment	Subset for alpha = 0.05			
	1	2	3	4
70mg	16.83			
50mg	20.67			
30mg	23.67	23.67		
20mg		30.10	30.10	
10mg		31.67	31.67	
5mg			38.67	
0mg (C)				79.50
1mg				83.00
Sig.	.117	.070	.054	.385

Means for groups in homogeneous subsets are displayed.

3.2. Total Leaf Number and Leaf Area/Per Plant

The analysis of the data shown in Figure 2 and Figure 3 revealed a progressive reduction in total leaf number and leaf area/plant in *A. dubius* along with the increase in concentration of Cr^{VI} treatments, with the highest values being recorded in 1mg Cr^{VI} treatment while the lowest values in the highest concentration treatment of 70mg Cr^{VI}. The differences in leaf number caused due to 1mg and 5mg Cr^{VI} treatment did not differ significantly over the control while the differences obtained in all other concentration treatments are significantly lower. Similarly, the average leaf area/plant of 345.74 cm² obtained in 1mg Cr^{VI} treatment insignificantly differed over control value of 362.12 cm² while values of others are significantly lower (Table 4 & Table 5).

**Figure 2. Effects of Cr^{VI} treatments on total number of leaf/plant in *Amaranthus dubius***

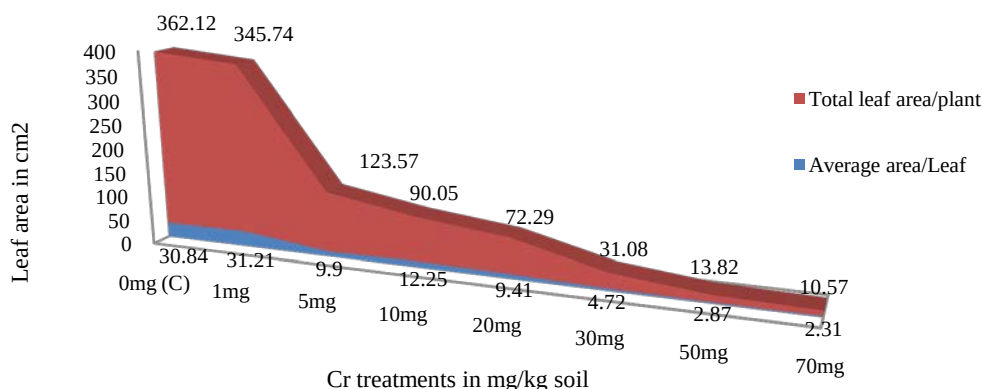


Figure 3. Effects of different concentrations of Cr^{VI} on leaf area/plant in *Amaranthus dubius*

Table 4. Duncan Analysis on the Total Number of Leaf/Plant in *Amaranthus dubius* Treated with Cr^{VI}

Cr ^{VI} Treatment	Subset for alpha = 0.05			
	1	2	3	4
70mg	3.75			
50mg	4.50			
30mg		7.00		
20mg		7.75	7.75	
10mg			8.75	
5mg				11.50
1mg				12.25
0mg (C)				12.75
Sig.	.334	.334	.201	.132

Means for groups in homogeneous subsets are displayed.

Table 5. Duncan Analysis on the Total Leaf Area/Plant in *Amaranthus dubius* Treated with Cr^{VI}

Cr ^{VI} Treatment	Subset for alpha = 0.05			
	1	2	3	4
70mg	10.57			
50mg	13.82			
30mg	31.08	31.08		
20mg	72.29	72.29	72.29	
10mg		90.05	90.05	
5mg			123.57	
1mg				345.74
0mg (C)				362.12
Sig.	.066	.070	.113	.585

Means for groups in homogeneous subsets are displayed.

The leaf morphological as well as physiological characteristics of plants are generally highly sensitive to heavy metal stress and the major visible impacts include reductions in leaf parameters. It is reported that reduction in leaf number and leaf area in *Brassica juncea* under Cr^{VI} stress and could be used as an indicator of heavy metal toxicity [16]. Similar observation is also reported in *Citrullus vulgaris* exposed to Cr^{VI} stress [17]. The inferences of the present study are in agreement with these reports and further revealed the reductions in leaf parameters are more or less progressive and positively correlated with increase in Cr^{VI} stress. The reductions in the leaf number and leaf area of Cr^{VI} treated plants over the control could be due to the inhibitory effect of chromium toxicity on two major physiological processes, cell division and expansion, which in turn can be attributed to the adverse effects on hydraulic conductance and water potential of plant tissues and restrictions in the

uptake of mineral nutrients like nitrate, potassium etc [18,19,20].

3.3. Fresh and Dry Weight of Shoot, Root and Total Plant

The Cr^{VI} treatments in *A. dubius* plants caused differences in the fresh weight of shoot and root components and in the total plant over control (Figure 4). The fresh weight decreased progressively over the control as the concentration of treatment increased. The highest decrease of 97.08% for shoot component, 97.66% for root component and 97.16% for total plant over control is obtained with 70mg Cr^{VI} treatment while the lowest decrease of 10.53%, 14.06% and 10.99% is obtained respectively for the shoot, root and total plant in 1mg Cr^{VI} treatment. A similar trend is also obtained with dry weight measurements where the highest reduction of 98.19%, 97.96% and 98.16% recorded respectively for shoot, root and total plant and the lowest reduction of 9.99%, 14.74% and 10.52% recorded respectively for shoot, root and total plant (Figure 5). The differences in the fresh biomass accumulation of shoot component as well as the total plant obtained in 1mg Cr^{VI} treatment over the control plants are not significant but significantly higher over the values of rest of the treated plants (Table 6 & Table 8). The decreases in the root fresh biomass in all Cr^{VI} treated plants are significantly lower over control (Table 7). The lowest shoot fresh biomass recorded by 70mg Cr^{VI} treatment is significantly lower over 1mg, 5mg and control treatments while that of root component as well as the total plant differed significantly lower only over control and 1mg Cr^{VI} treatments.

Table 6. Duncan Analysis of Shoot Fresh Weight Variation in *Amaranthus dubius* Treated with Cr^{VI}

Cr ^{VI} Treatment	Subset for alpha = 0.05		
	1	2	3
70mg	.99		
50mg	2.15	2.15	
30mg	3.43	3.43	
20mg	4.45	4.45	
10mg	4.53	4.53	
5mg		6.37	
1mg			30.33
0mg (C)			33.90
Sig.	.162	.099	.122

Means for groups in homogeneous subsets are displayed.

Table 7. Duncan Analysis of Root Fresh Weight Variation in *Amaranthus dubius* Treated with Cr^{VI}

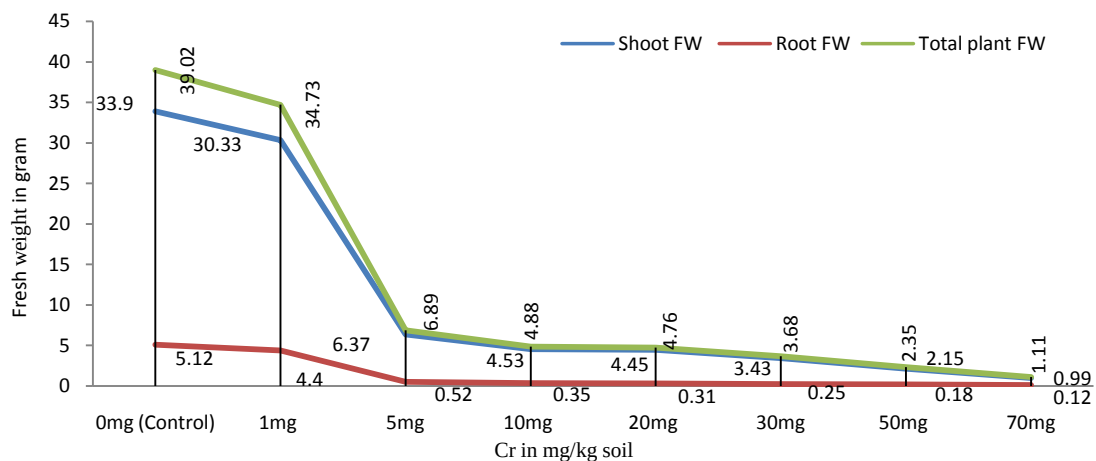
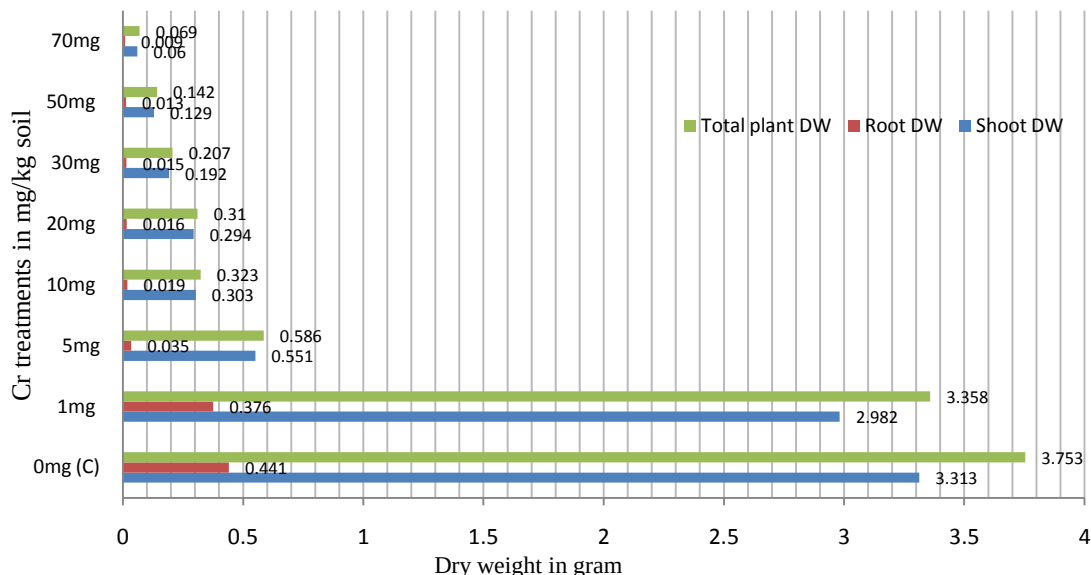
Cr ^{VI} Treatment	Subset for alpha = 0.05		
	1	2	3
70mg	.12		
50mg	.18		
30mg	.25		
20mg	.31		
10mg	.35		
5mg	.52		
1mg		4.40	
0mg (C)			5.12
Sig.	.282	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Table 8. Duncan Analysis of Total Plant Fresh Weight Variation in *Amaranthus dubius* Treated with Cr^{VI}

Cr ^{VI} Treatment	Subset for alpha = 0.05	
	1	2
70mg	1.11	
50mg	2.35	
30mg	3.68	
20mg	4.76	
10mg	4.88	
5mg	6.89	
1mg		34.73
0mg (C)		39.02
Sig.	.053	.103

Means for groups in homogeneous subsets are displayed.

**Figure 4. Effects of Cr^{VI} on the fresh weight of *Amaranthus dubius* shoot, root and total plant****Figure 5. Effects of Cr^{VI} on the dry weight of *Amaranthus dubius* shoot, root and total plant**

The reduction in dry biomass of shoot component and total plant in 1mg Cr^{VI} treated plants showed no significant differences over control while the reductions in other treated plants are significant over control and 1mg Cr^{VI} treated plants. With respect to root biomass, the reductions in all the treatments are significant over control. The highest decrement recorded by 70mg Cr^{VI} treatment showed no significant differences over 50mg, 30mg, 20mg, 10mg and 5mg Cr^{VI} treatments (Table 9, Table 10 & Table 11).

The Cr toxicity has been reported to reduce biomass of leaves, shoot, root, and total plant in *Helianthus annuus* [21]. The major factors responsible for the decreased fresh and dry biomass of shoot, root and total plant of *A. dubius* in the study might be due to Cr^{VI} toxicity induced water stress, restricted availability of essential nutrients like Zn, Mn, Cu, Fe, Na and Ca, reduction in photosynthetic pigments etc. which lead to reduced growth rate under disturbed cell cycle and cell division [22,23].

Table 9. Duncan Analysis of Shoot Dry Weight Variation in *Amaranthus dubius* Treated with Cr^{VI}

Cr ^{VI} Treatment	Subset for alpha = 0.05	
	1	2
70mg	.0597	
50mg	.1290	
30mg	.1917	
20mg	.2943	
10mg	.3033	
5mg	.5510	
1mg		2.9820
0mg (C)		3.3113
Sig.	.058	.146

Means for groups in homogeneous subsets are displayed.

Table 10. Duncan Analysis of Root Dry Weight Variation in *Amaranthus dubius* Treated with Cr^{VI}

Cr ^{VI} Treatment	Subset for alpha = 0.05		
	1	2	3
70mg	.009		
50mg	.013		
30mg	.015		
20mg	.016		
10mg	.019		
5mg	.035		
1mg		.376	
0mg (C)			.441
Sig.	.418	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Table 11. Duncan Analysis of Total Plant Dry Weight Variation in *Amaranthus dubius* Treated with Cr^{VI}

Cr ^{VI} Treatment	Subset for alpha = 0.05	
	1	2
70mg	.069	
50mg	.142	
30mg	.207	
20mg	.310	
10mg	.323	
5mg	.586	
1mg		3.358
0mg (C)		3.753
Sig.	.073	.122

Means for groups in homogeneous subsets are displayed.

3.4. Root/Shoot Weight Ratio (R/S Weight Ratio)

The Cr^{VI} treatments have caused a reduction in R/S ratio in terms of both fresh weight (FW) and dry weight (DW) over control, with the exception of R/S dry weight ratio in 70mg Cr^{VI} which recorded an insignificant increase of 11.36% over control (Figure 6). The results generally indicate that the reductions are progressive up to 20mg Cr^{VI} treatment and then progressive enhancement up to 70mg Cr^{VI}. The highest reduction of 55.61% of fresh weight ratio and 60.29% of dry weight ratio are recorded in 20mg Cr^{VI} treatment over control while the lowest reduction of 2.99% for fresh weight and 4.14% for dry weight are recorded in 1mg Cr^{VI} treatment. With the exception in 1mg Cr^{VI} treated plants with respect to R/S fresh weight ratio and 1mg and 70mg Cr^{VI} treated plants with respect to R/S dry weight ratio, the decrease in R/S weight ratio of all the treated plants differ significantly lower over control (Table 12 & Table 13). Generally, each plant type has an inherent normal R/S ratio under healthy environmental conditions and it clearly indicates normal balanced growth of the plant, but any decrease in the R/S ratio from the normal level indicates that plant has encountered some kind of stresses. The root system is the part which is directly in contact with the toxic soil environment and as the Cr^{VI} metal is toxic and non-essential for the plant, it is getting sequestered in vacuoles of root cells which in turn may cause disturbed cell division and elongation of root cells followed by the decline in the root growth and impairment of the root penetration leading to reduction in R/S ratio [24]. However after certain level of Cr^{VI} concentration, the further enhancement caused a gradual increase from the lowered R/S ratio due to initial level concentrations. This might be due to stunned shoot growth which is as a result of severe Cr^{VI} toxicity induced root injury. The root injury may cause considerable reduction in absorption as well as translocation of root-zone resources such as water and nutrients into the shoot system for their utilization into above ground biomass yield by making use of CO₂ and sunlight.

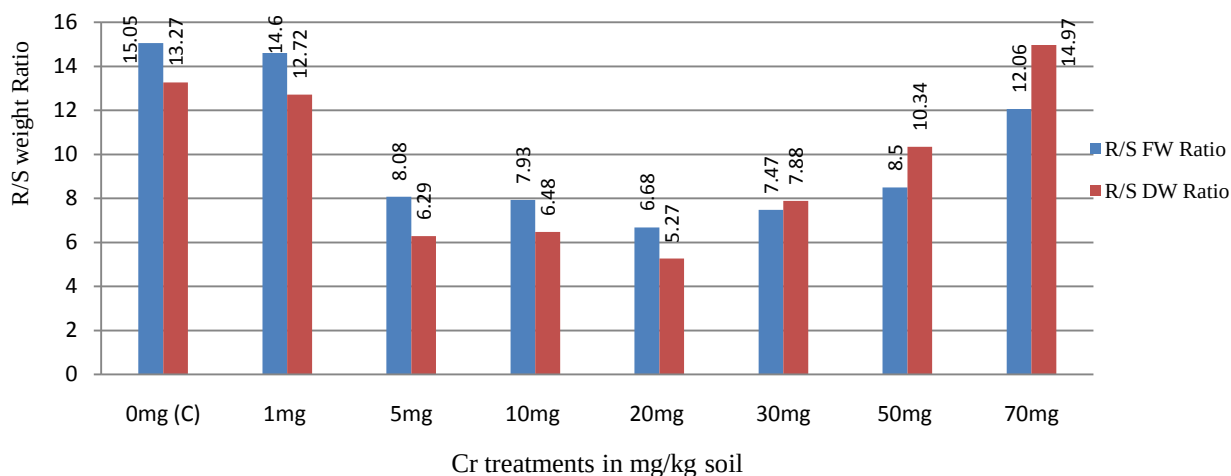
**Figure 6.** Effects of different concentrations of Cr^{VI} on Root/Shoot fresh and dry weight ratio in *Amaranthus dubius*

Table 12. Duncan Analysis of R/S Fresh Weight Ratio Variation in *Amaranthus dubius* Treated with Cr^{VI}

Cr ^{VI} Treatment	Subset for alpha = 0.05		
	1	2	3
20mg	6.68		
30mg	7.47		
10mg	7.93		
5mg	8.08		
50mg	8.50		
70mg		12.06	
1mg			14.60
0mg (C)			15.05
Sig.	.098	1.000	.638

Means for groups in homogeneous subsets are displayed.

Table 13. Duncan Analysis of R/S Dry Weight Ratio Variation in *Amaranthus dubius* Treated with Cr^{VI}

Cr ^{VI} Treatment	Subset for alpha = 0.05			
	1	2	3	4
20mg	5.27			
5mg	6.29	6.29		
10mg	6.48	6.48		
30mg		7.88		
50mg			10.34	
1mg				12.72
0mg (C)				13.27
70mg				14.97
Sig.	.295	.173	1.000	.060

Means for groups in homogeneous subsets are displayed.

Table 14. Tolerance Index (TI %) and Inhibition of Plant Growth (IPG %) in *Amaranthus dubius* Treated with Cr^{VI}

Treatment Cr ^{VI} / kg soil	Tolerance index (TI %)				Inhibition of plant growth (IPG %)			
	Fresh biomass	SD±	Dry biomass	SD±	Fresh biomass	SD±	Dry biomass	SD±
0mg (C)	100.00	0.0	100.00	0.0	0.00	0.0	0.00	0.0
1mg	88.92	2.7	89.39	2.4	11.11	3.3	10.61	2.9
5mg	17.64	0.28	15.65	0.5	82.37	0.4	84.36	0.6
10mg	12.18	3.5	8.45	2.3	87.80	4.2	91.61	2.8
20mg	12.07	1.6	8.17	1.1	87.97	1.9	91.86	1.4
30mg	9.39	0.8	5.50	0.6	90.63	1.0	94.50	0.7
50mg	6.07	0.5	3.82	0.3	93.93	0.7	96.94	1.6
70mg	2.89	0.5	1.86	0.4	97.07	0.6	98.13	0.5

3.5. Tolerance Index (TI %) and Inhibition of Plant Growth (IPG in %)

The details of tolerance index (TI %) and inhibition of plant growth (IPG %) in terms of fresh and dry biomass accumulation in *A. dubius* under Cr^{VI} stress is depicted in the Table 14. The highest TI of 88.92% and 89.39% and the lowest IPG of 11.11% and 10.61% respectively in terms of plant fresh biomass and dry biomass in the study is obtained in 1mg Cr^{VI} treatment. The data revealed that the further increase in Cr^{VI} stress is causing a progressive decline in the TI % and a corresponding progressive enhancement in IPG % and finally the lowest TI of 2.89% and 1.86% and likewise the maximum IPG of 97.07% and 98.13% respectively obtained in the highest concentration of 70mg Cr^{VI}. The decrease in the tolerance index and the increase in the inhibition of plant growth recorded in all the Cr^{VI} treated plants differed significantly over control, both in terms of fresh and dry biomass accumulation (Table 15, Table 16, Table 17 & Table 18). The highest TI as well as the lowest IPG recorded by plants treated with 1mg Cr^{VI} significantly differed over other treated plants. Similarly, the lowest TI recorded by 70mg Cr^{VI} treated plants are significantly lower compared to others while the highest IPG value obtained by these plants is insignificant over 50mg Cr^{VI} treated plants but significant over rest of the treated plants.

The inferences obtained from the analysis of different morphological growth parameters in the study clearly indicated reduction in tolerance index and inhibition of growth and this could be due to Cr^{VI} toxicity induced damage and poor development of root system, which causes restricted absorption of water and essential elements and their poor mobilization into different regions of plants to carryout various physiological growth processes to yield biomass. The study revealed the existence of a positive correlation between the inhibition of growth and the intensity of Cr^{VI} stress. This can be attributed to the

differences in Cr^{VI} toxicity and that induced extension of cell cycle and related inhibition in cell division and enlargement which in turn may be due to the differences in alteration or imbalance in physiological processes. The Cr^{VI} toxicity can disturb biosynthesis of photosynthetic pigments, reduction in stomatal conductance and gas exchange activities, disruption in electron transport system etc which are capable of undesirably affecting the rate of photosynthesis, ATP production and energy level of cells etc.

Table 15. Duncan Analysis of Tolerance Index Based on the Fresh Biomass in *Amaranthus dubius* Plants Treated with Cr^{VI}

Cr ^{VI} Treatment	Subset for alpha = 0.05						
	1	2	3	4	5	6	7
70mg	2.89						
50mg		6.07					
30mg			9.39				
20mg				12.07			
10mg				12.18			
5mg					17.64		
1mg						88.92	
0mg (C)							100.00
Sig.	1.000	1.000	1.000	.930	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Table 16. Duncan Analysis of Tolerance Index Based on the Dry Biomass in *Amaranthus dubius* Plants Treated with Cr^{VI}

Cr ^{VI} Treatment	Subset for alpha = 0.05					
	1	2	3	4	5	6
70mg	1.86					
50mg		3.82				
30mg		5.50				
20mg			8.17			
10mg			8.45			
5mg				15.65		
1mg					89.39	
0mg (C)						100.00
Sig.	1.000	.076	.759	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Table 17. Duncan Analysis of Inhibition of Plant Growth (%) in Terms of Fresh Biomass in *Amaranthus dubius* Treated with Cr^{VI}

Cr ^{VI} Treatment	Subset for alpha = 0.05					
	1	2	3	4	5	6
0mg (C)	0.00					
1mg		11.11				
5mg			82.37			
10mg				87.80		
20mg				87.97		
30mg				90.63	90.63	
50mg					93.93	93.93
70mg						97.07
Sig.	1.000	1.000	1.000	.129	.068	.081

Means for groups in homogeneous subsets are displayed.

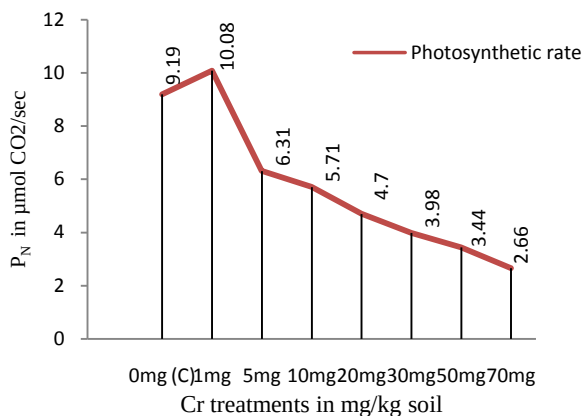
Table 18. Duncan Analysis of Inhibition of Plant Growth (%) in Terms of Dry Biomass in *Amaranthus dubius* Treated with Cr^{VI}

Cr ^{VI} Treatment	Subset for alpha = 0.05					
	1	2	3	4	5	6
0mg (C)	0.00					
1mg		10.61				
5mg			84.36			
10mg				91.61		
20mg				91.86		
30mg				94.50	94.50	
50mg					96.94	96.94
70mg						98.13
Sig.	1.000	1.000	1.000	.059	.092	.393

Means for groups in homogeneous subsets are displayed.

3.6. Net Photosynthetic Rate (P_N)

The highest average net photosynthetic rate of 10.08 $\mu\text{mol CO}_2\text{m}^{-2}\text{sec}$ obtained in *A. dubius* plants treated with lowest concentration of 1mg Cr^{VI}. This is about 8.83% insignificant increases over control while the increase over rest of the Cr^{VI} treated plants is significant. However, further increase in Cr^{VI} stress caused progressive reduction in the P_N value and the lowest P_N value of 2.66 $\mu\text{mol CO}_2\text{m}^{-2}\text{sec}$ is obtained in the highest concentration of 70mg Cr^{VI}, which is about 71.06% decrease over control (Figure 7). This lowest P_N value has no significant differences over the P_N value obtained in 50mg and 30mg Cr^{VI} stress while differed significantly lower over control and rest of the Cr^{VI} stress (Table 19).

**Figure 7.** Rate of photosynthesis in *Amaranthus dubius* against different concentrations of Cr^{VI} stress**Table 19. Duncan Analysis of Net Photosynthetic Rate in *Amaranthus dubius* Treated with Cr^{VI}**

Cr ^{VI} Treatment	Subset for alpha = 0.05				
	1	2	3	4	5
70mg	2.66				
50mg	3.44	3.44			
30mg	3.98	3.98			
20mg		4.70	4.70		
10mg			5.71	5.71	
5mg				6.31	
0mg (C)					9.197
1mg					10.08
Sig.	.064	.077	.134	.365	.186

Means for groups in homogeneous subsets are displayed.

There are reports on the inhibition of growth and productivity in plants due to heavy metal stress induced disturbances in electron transport system and reduction in net photosynthetic rate [25,26]. Previous studies have recorded Cr^(VI) toxicity induced depletion of net photosynthetic rate in *Lolium perenne* [27] and in *Oryza sativa* [28]. The observations of the present study are in agreement with these reports. The reduction in net photosynthetic rate and growth recorded in *A. dubius* plants subjected to Cr^{VI} stress can be attributed to their inhibitory action on ATP production and energy level of cells by affecting the primary photosynthetic reactions. This might be due to the disturbances on electron carriers and the flow rate of electron from water to PS-II reaction centre by Cr^{VI} toxicity.

3.7. Stomatal Conductance (g_s)

The stomatal conductance of *A. dubius* under Cr^{VI} stresses are depicted in Figure 8. The highest stomatal conductance in the study is recorded in plants treated with the lowest Cr^{VI} stress while the maximum reduction in the highest Cr^{VI} stress and the data revealed that the reductions are progressive from the highest stomatal conductance of 0.077 m mol m⁻²s⁻¹ obtained in the study towards the lowest value of 0.023 m mol m⁻²s⁻¹, along with the increases in Cr^{VI} stress. The increase in stomatal conductance of 2.6% recorded by plants treated with 1mg Cr^{VI} over control is insignificant while its differences over other Cr^{VI} treated plants are significantly higher. Similarly, the lowest stomatal conductance recorded by 70mg Cr^{VI} treated plants did not differ significantly over 50mg Cr^{VI} treatment whereas the differences over rest of the treated plants are significantly lower (Table 20). While evaluating the impact of Cr stress on fluorescence parameters, the decrease in stomatal conductance followed by fall in the rate of CO₂ assimilation reported earlier in plants [29] and these observations are in similar lines with present study inferences. The reduction in stomatal conductance under Cr^{VI} stress inferred in the study might be the result of toxicity induced water imbalance and poor stomatal opening caused by loss in turgor pressure inside the guard cells of stomata [30]. The production and accumulation of abscisic acid (ABA) molecules following water stress and their involvement in signal pathways to control water loss by stomatal closure might be the key factor in the decrease of stomatal conductance.

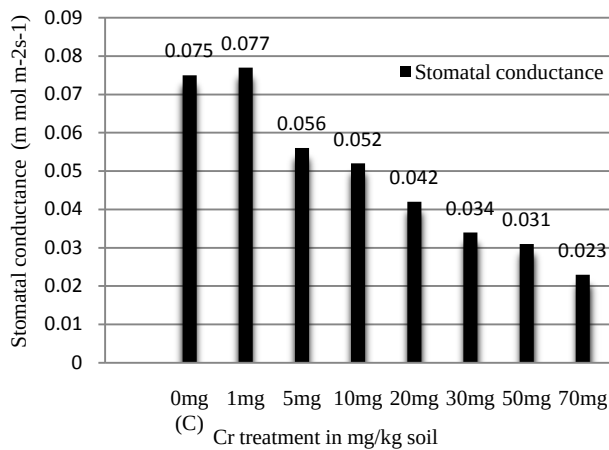


Figure 8. Stomatal conductance in *Amaranthus dubius* against different concentrations of Cr^{VI} stress

Table 20. Duncan Analysis of Stomatal Conductance in *Amaranthus dubius* Treated with Cr^{VI}

Cr ^{VI} Treatment	Subset for alpha = 0.05				
	1	2	3	4	5
70mg	.023				
50mg	.031	.031			
30mg		.034			
20mg			.042		
10mg				.052	
5mg				.056	
0mg (C)					.075
1mg					.077
Sig.	.056	.536	1.000	.251	.566

Means for groups in homogeneous subsets are displayed.

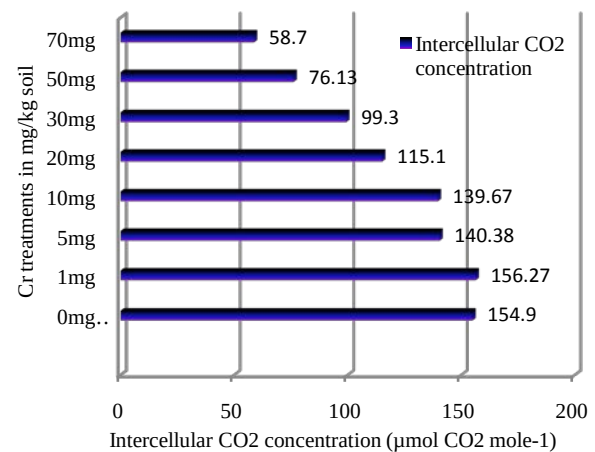


Figure 9. Intercellular CO₂ concentration in *Amaranthus dubius* against different concentrations of Cr^{VI} stress

Table 21. Duncan Analysis of Intercellular CO₂ Concentration in *Amaranthus dubius* Treated with Cr^{VI}

Cr ^{VI} Treatment	Subset for alpha = 0.05			
	1	2	3	4
70mg	58.70			
50mg	76.13	76.13		
30mg		99.30	99.30	
20mg			115.10	
10mg				139.67
5mg				140.38
0mg (C)				154.90
1mg				156.27
Sig.	.145	.055	.186	.205

Means for groups in homogeneous subsets are displayed.

3.8. Intercellular CO₂ Concentration (C_i)

The intercellular CO₂ concentration (C_i) levels in *A. dubius* plants under Cr^{VI} stresses are found to respond in similar lines with stomatal conductance and net photosynthetic rate (Figure 9). The C_i value of 156.27 μmol CO₂ mole⁻¹ recorded in 1mg Cr^{VI} treated plants is about 0.88% increase over control. Further enhancement in Cr^{VI} stress caused a progressive reduction and the lowest C_i value of 58.7 μmol CO₂ mole⁻¹ recorded in 70mg Cr^{VI} treated plants is about 62.10% decrease over control. The C_i values recorded by plants treated with 1mg, 5mg and 10mg Cr^{VI} insignificantly differed among themselves and over control plants while they differed significantly higher over the values of 20mg, 30mg, 50mg and 70mg Cr^{VI} treated plants. The lowest C_i value recorded by 70mg Cr^{VI} treated plants did not significantly differ over 50mg Cr^{VI} treated plants while the differences over rest of the treated plants are significant (Table 21). Cr^{VI} stress has been reported to negatively interfere with assimilation of CO₂ and which affects intercellular CO₂ concentration and net photosynthetic rate in *Triticum aestivum* L. [31]. The present study indicates the existence of a negative correlation between the degree of Cr^{VI} stress and C_i value, which is evident from the progressive reduction in CO₂ concentrations along with the increasing Cr^{VI} stress. The decrease of C_i value clearly revealed the toxic and inhibitory effect of Cr^{VI} stress on stomatal conductance, which affects the essential gas exchange activities [32].

4. Conclusion

The present investigation clearly revealed the hexavalent chromium (Cr^{VI}) stress has significantly deleterious effects on the morphological growth parameters and photosynthesis related characteristics like net photosynthetic rate, stomatal conductance and intercellular CO₂ concentration in *Amaranthus dubius* plants. The reduced water potential and enhanced diffusive resistance due to root injury and poor root system development induced by Cr^{VI} toxicity might have led to the restrictions in the availability of water and essential elements necessary for relevant physiological growth processes. This could have contributed to the disruption in mitochondrial electron transport, oxidative stress, chloroplast structural alteration, inhibition of chlorophyll biosynthesis, reduction in stomatal conductance and intercellular CO₂ concentration which ultimately lead to decreased net photosynthetic rate and reduced biomass yield. Although several literatures are available to understand the interaction of toxic Cr^{VI} with essential metal ions which are relevant in biosynthetic and physiological pathways in plants, their involvement and mechanism of causing toxicity at bio-molecular level is still poorly understood.

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