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Physiological and biochemical alterations in sweet potato (*Ipomoea batatas* (L.) exposed to excess levels of chromium (VI)

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Abstract
The contamination of agricultural soils and water with chromium, mainly due to industrial and urban effluents, presents a substantial ecological risk. This study investigates the toxic effects of chromium (VI) on sweet potato (*Ipomoea batatas* (L.) Kiran) plants, grown in a controlled environment using refined sand pot culture with a pH range of 6.9–7.0. The research primarily examines the phytotoxic effects of elevated chromium (VI) concentrations, focusing on changes in growth, water transport, and metabolic activities.

The sweet potato plants were cultivated in a nutrient solution for 450 days before chromium (as dichromate) was introduced at concentrations of 0.00, 0.05, 0.10, and 0.25 mM. After 5-6 days of chromium (VI) exposure, visible signs of foliar toxicity emerged, starting with chlorosis (yellowing) and a loss of turgor in the older leaves, followed by similar symptoms in the middle leaves at the highest concentration (0.25 mM). Over time, the chlorosis intensified, leading to necrosis in patches, malformed leaf laminae, and weakened tendrils that lost their ability to coil. At 0.25 mM, chromium (VI) had a notably detrimental impact on sweet potato plants, leading to significant reductions in growth and development. It also hindered photosynthesis and disrupted metabolic and enzymatic processes. These results indicate that the consumption of crops with elevated chromium levels could pose health risks and reduce their nutritional value. Moreover, high chromium concentrations can interfere with plant growth and metabolic functions in the tested species.

Keywords: Chromium, *Ipomoea batatas*, phytotoxicity, relative water content, antioxidants

Introduction

Chromium, ranked as the seventh most abundant metal in the Earth's crust [1], has numerous industrial applications, but it is also recognized as a harmful heavy metal. It is used in processes like leather tanning and finishing [2], the production of refractory steel, manufacturing of dyes and pigments, drilling muds, electroplating, and as a catalyst in various chemical processes, including the production of chromic acid. Despite its wide range of uses, chromium, especially in its hexavalent form (Cr VI), poses significant toxicity risks. Research [3] indicates that the critical concentration of available chromium in soil, whether in the forms of Cr (III) or Cr (VI), is typically between 1 and 5 ppm. These two oxidation states, Cr (III) and Cr (VI), differ greatly in terms of mobility, bioavailability, and toxicity. Chromium can exist in several oxidation states ranging from -2 to +6, but Cr (VI) and Cr (III) are the most common and stable forms in nature [4].

The contamination of water sources with chromium presents severe health hazards for humans and animals [5]. Crops grown on chromium-contaminated soils can absorb the metal, leading to health risks through the food chain [6, 7]. It is important to note that chromium is not an essential element for plant growth, and its uptake by plants occurs through non-specific pathways. The toxicity of chromium largely depends on its speciation, which dictates how it is absorbed, transported, and accumulated in plants. The extent of chromium translocation and accumulation is influenced by factors such as the plant species, the oxidation state of chromium, and its concentration in the growth medium [7, 8].

A number of studies [5, 7, 8, 9] have explored the harmful effects of chromium on various agricultural crops. These effects can include stunted growth, poor seed germination, changes in plant physiology, reduced pigment levels, impaired nutrient uptake and translocation, decreased yield, compromised quality, altered antioxidant enzyme activity, and the induction of oxidative stress in plants [9, 10, 11]. The phytotoxicity of chromium is primarily determined by its speciation, which affects its uptake, movement, and accumulation within plants. The objective of this study is to investigate the phytotoxic effects, physiological changes, and metabolic disruptions caused by excessive chromium stress in sweet potato. This crop was chosen due to its widespread cultivation and its significant nutritional role as a key food source.

Materials and methods

Experimental design

Sweet potato (*Ipomoea batatas* (L.) Kiran) plants were cultivated in refined sand pot culture under controlled glasshouse conditions, with a pH range maintained between 6.8 and 7.0 and ambient temperature, as per the prescribed methods [12]. The experimental setup and growth medium were based on the protocol described in [13], modified to suit the Indian climatic conditions. The nutrient solution used in this study was adapted from the formulation employed at the Long Ashton Research Station, Bristol, U.K. The composition of the complete nutrient solution was as follows:

4 mM KNO_3, 4 mM $Ca(NO_3)_2$, 2 mM $MgSO_4$, 1.33 mM NaH_2PO_4, 100 µM Fe-EDTA, 10 µM $MnSO_4$, 30 µM H_3BO_3, 1 µM $CuSO_4$, 1 µM $ZnSO_4$, 0.2 µM Na_2MoO_4, 0.1 µM $NiSO_4$, 0.1 µM $CoSO_4$, 0.1 mM NaCl

Iron was supplied as Fe-EDTA (ferric ethylene diamine tetraacetic acid) chelate, as recommended for plant nutrient solutions [14]. The nutrient solution was refreshed daily,

except on Sundays, when each pot was flushed with distilled water to remove absorbed nutrients and any accumulated harmful substances from the root zone. The pH of the nutrient solution was kept within the range of 6.8 ± 0.2 before application to the plants throughout the study. Deionized water was used throughout the cultivation period, and the plants were watered as required to maintain optimal growth conditions.

Chromium was supplied in the form of potassium dichromate (AR grade) at concentrations of 0.00, 0.05, 0.10, and 0.25 mM, which was added to the nutrient solution after the plants had grown for 50 days. The plants were monitored regularly for visible foliar symptoms and changes in growth parameters during the course of the experiment.

Analytical tools and adopted methods

Relative Water Content (RWC) was assessed on day 56 (6 days after chromium treatment) in the middle leaves of the plants, following the procedure outlined [15]. Measurements were conducted between 9 and 11 AM, when the sand in the pots remained saturated with the nutrient solution. The ambient temperature during these measurements ranged from 30 to 40°C, with humidity levels between 60 and 70%. On day 20 after the metal treatment, leaf area (measured in cm^2) was used as an indicator of plant growth and quantified using a Delta-T leaf area measurement system. On day 70 (20 days post-treatment), various physiological parameters were analyzed in mature young leaves. These included the concentrations of chlorophylls (a and b), Hill reaction activity, sugars, starch, nitrogen, phenols, and the activity of key enzymes such as catalase, peroxidase and ribonuclease. Additionally, the soluble protein content in crude leaf extracts was measured (Table 1). All measurements were conducted in triplicate, and the data were subjected to statistical analysis. The standard error of the mean is provided alongside the mean values [16].

Table 1: Analytical test and reference methods used for analysis of tested parameters

Sr. No.	Parameter	Reference of Method
1	Relative water content	[15]
2	Chlorophyll contents	[28]
3	Hill activity	[29]
4	Protein	[30]
5	Starch	[31]
6	Nitrogen	[32]
7	Sugars	[33]
8	Catalase	[34]
9	Peroxidase	[35]
10	Ribonuclease	[36]

Quality control and quality assurance

Standard calibration reference materials were used for quality control and assurance of the analytical techniques.

Results

Visual phytotoxic symptoms of chromium (cr)

The present study assessed the damage caused by excess levels of chromium (Cr) supply to sweet potato plants grown in refined sand pot culture with varying levels of Cr (0.05, 0.10, and 0.25 mM) supplied as potassium dichromate (AR grade salt), along with control pots. The toxic effects of different levels of chromium (VI) stress were observed in terms of visible phytotoxic symptoms and growth behavior of the plants.

Day 5-6: Symptoms of excess Cr (0.25 mM) were visible on older leaves, manifesting as chlorosis.

Day 7-8: The toxicity symptoms progressed to wilting of the affected leaves, which hung down from the petiole, especially at the 0.10 and 0.25 mM Cr levels.

Day 9-10: Older leaves turned golden yellow, with a reduction in leaf size and number, and intensified chlorosis. Necrotic patches began to form, coalescing into larger necrotic areas.

Subsequent Days: Chlorotic leaves became permanently wilted and dry, leading to premature leaf fall. These symptoms gradually spread to the middle and younger leaves. Day 15-17: The growth of chlorosis was less pronounced in plants treated with lower Cr levels (0.05 and 0.10 mM), with symptoms of toxicity more delayed compared to higher Cr concentrations.

Effects of cr on biomass, grain yield, leaf area, and relative water content

The effects of Cr on the biomass, grain yield, leaf area, and relative water content (RWC) of sweet potato plant leave are presented in Table 2.

Biomass: The dry biomass of sweet potato decreased progressively with increasing Cr (VI) concentration in the nutrient solution. At 0.25 mM Cr, biomass was reduced compared to control plants at day 70.

Leaf Area: By day 70 (20 days after Cr treatment), leaf area had decreased with increasing Cr levels. At 0.25 mM Cr, leaf area was reduced compared to control plants.

Relative Water Content (RWC): RWC decreased significantly with increasing Cr treatment. The reduction in RWC was associated with visible wilting of leaves.

Effects of cr on photosynthesis, sugars, nitrogen, starch, and phenols

The concentration of photosynthetic pigments, sugars (both reducing and non-reducing), starch, nitrogen, and phenols in the leaves of sweet potato plants under varying levels of Cr (VI) treatment are shown in Table 3.

Photosynthetic Pigments: The concentrations of chlorophyll decreased significantly with increasing Cr exposure.

Sugars: Reducing sugars increased significantly in Cr-treated plants compared to controls, with the highest concentrations at the 0.25 mM Cr treatment. Total sugars also showed an increasing trend with higher Cr levels, whereas non-reducing sugars decreased slightly.

Nitrogen: Protein nitrogen (protein N) decreased with increasing Cr exposure, while non-protein nitrogen (non-protein N) increased. Overall, total nitrogen content decreased with increasing Cr levels.

Starch: Maximum starch content decreased at 0.25 mM Cr treatment, indicating impaired starch synthesis.

Phenols: The concentration of phenolic compounds increased with increasing Cr levels. At 0.25 mM Cr, maximum phenol concentration increased compared to control plants.

Effects of cr on protein content and enzyme activities

Biochemical parameters such as protein content and the activities of enzymes (catalase, peroxidase, ribonuclease) and Hill reaction activity in the leaves of sweet potato exposed to excess Cr (VI) are presented in Table 4.

Protein Content: Protein concentration progressively decreased with increasing Cr exposure. At the level of 0.25 mM Cr exposer the lowest protein content was observed.

Catalase: Catalase activity decreased with increasing Cr (VI) exposure.

Peroxidase: Peroxidase activity increased gradually with higher Cr concentrations.

Ribonuclease: Ribonuclease activity also increased progressively from 0.05 to 0.25 mM Cr.

Hill Reaction: Hill reaction activity in the leaves decreased with increased in the Cr concentrations.

Discussion

Visual phytotoxic symptoms under cr (vi) exposure

Throughout the experimental period, sweet potato plants displayed clear visual signs of chromium-induced toxicity. Within 5-6 days of initiating chromium (0.25 mM) treatment, chlorosis appeared in older leaves. By Day 7-8, these leaves exhibited wilting, and in some cases, they detached from the petiole, particularly at higher chromium concentrations (0.10 and 0.25 mM). By Days 10-11, the affected leaves turned golden yellow, accompanied by a decrease in both leaf size and number, and a worsening of chlorosis. The chlorotic leaves soon developed large necrotic patches, resulting in permanent wilting, leaf desiccation, and premature leaf shedding.

These observations align with previous studies [17, 18], which reported similar effects in plants like *Citrullus* and cauliflower exposed to chromium stress. Our findings further support the idea that excessive chromium triggers a progressive deterioration in plant health, negatively affecting vital physiological functions such as photosynthesis and nutrient absorption, eventually leading to plant death.

Impact of chromium stress on biomass, leaf area, and relative water content (rwc)

Increasing chromium concentrations led to a marked decrease in biomass, grain yield, leaf area, and relative water content (RWC) in sweet potato plants (Table 2). The reduction in biomass can be attributed to inhibited growth in both shoots and roots, restricting the transport of water and nutrients to the aerial parts of the plant. These findings are consistent with those of [7], who reported similar decreases in leaf area in response to chromium exposure. Excess chromium disrupts critical metabolic processes, including germination, growth, and chlorophyll production, leading to stunted growth, chlorosis, and ultimately plant death [19, 20].

RWC significantly decreased with increasing concentrations of chromium (0.05, 0.10, and 0.25 mM), with higher concentrations resulting in greater water loss and visible wilting. This suggests that chromium negatively impacts the hydration status of plants, a finding also reported by several researchers [8, 9], who observed decreased water potential and RWC in chromium-treated plants.

Table 2: Effect of variable chromium stress on biomass, grain, leaf area and relative water content of sweet potato plants. Values are means ± SE (n=5)

Days of growth	Days after metal supply		mM chromium				LSD (*P*=0.05)
			Control	0.05	0.10	0.25	
70	20	Biomass: g plant^{-1}	86.35 ±4.25	79.41 ±2.94	52.67 ±1.83	37.28 ±0.96	6.23
70	20	Leaf area: cm^2	91.65 ±7.25	77.24 ±6.52	62.17 ±5.88	54.32 ±0.4.86	3.18
56	6	RWC: %	97.16 ±8.14	91.26 ±6.41	67.38 ±6.05	43.62 ±4.71	4.26

Biochemical changes in chlorophyll, sugars, starch, nitrogen, and phenols under chromium stress

The levels of key biochemical markers: chlorophyll, sugars, starch, nitrogen, and phenols were notably altered under chromium treatment (Table 3). A significant reduction in chlorophyll content was observed at 0.25 mM chromium compared to control plants. Chlorophyll is crucial for photosynthesis, and its reduction is commonly associated with chromium-induced damage to chloroplasts, disruption of chlorophyll synthesis, and impairment of photosynthetic electron transport [11, 19]. These findings are agreement with those of [7, 21], who reported similar chlorotic and necrotic symptoms under chromium stress.

In contrast, the levels of reducing sugars and total sugars increased in chromium-treated plants, indicating a shift in carbohydrate metabolism. The rise in reducing sugars suggests an accumulation of carbohydrates in leaves, likely due to chromium's inhibition of carbohydrate export from the leaves. This observation supports the theory that chromium disrupts the vascular system of plants [22]. Non-reducing sugars, however, were not significantly affected, consistent with studies in barley [23] and pea plants [7]. Starch content also decreased significantly in the leaves of sweet potato plants exposed to chromium, a reduction in starch synthesis that mirrors findings in other plants under chromium stress, such as *Citrullus* species [17].

Chromium exposure also affected nitrogen content in sweet potato plant leaves, reducing protein and total nitrogen levels, while non-protein nitrogen increased. These changes reflect chromium's disruption of nitrogen uptake and

assimilation processes [24]. An increase in phenolic compounds was also observed, which may be a response to oxidative stress, as phenols are involved in scavenging free radicals and chelating metal ions [25]. This increase suggests that phenols might act as a protective mechanism, enhancing the plant's antioxidant capacity.

Table 3: Variable chromium treatment on concentration of chlorophyll, sugar fraction, starch, nitrogen and phenols in leaves of sweet potato plant leaves. Values are mean ±SE (n=5)

Parameters evaluated	mM chromium				LSD (P=0.05)
	Control	0.05	0.10	0.25	
Chlorophyll: mg g^{-1} fresh wt					
a	0.862 ±0.06	0.628 ±0.03	0.412 ±0.02	0.362 ±0.01	0.04
b	0.395 ±0.05	0.295 ±0.03	0.284 ±0.03	0.192 ±0.04	0.03
Sugars: % fresh weight					
Reducing	0.31 ±0.02	0.29 ±0.02	0.38 ±0.03	0.44 ±0.03	0.04
Non reducing	0.08 ±0.01	0.07 ±0.01	0.06 ±0.01	0.05 ±0.01	0.00
Nitrogen: % fresh weight					
protein nitrogen	1.35 ±0.05	1.09± 0.05	0.82± 0.03	0.52± 0.03	0.05
non protein nitrogen	0.317± 0.02	0.281 ±0.04	0.343 ±0.03	0.297 ±0.03	0.04
Starch: % fresh weight	1.265 ±0.12	1.117 ±0.10	0.715 ±0.09	0.435 ±0.05	0.07
Phenols: % fresh weight	0.002± 0.00	0.005± 0.00	0.006± 0.001	0.008± 0.001	0.001

Chromium-phytotoxic changes in enzyme activity, hill reaction, and protein content

Further biochemical responses to chromium exposure were evaluated by measuring enzyme activities and protein content (Table 4). Catalase activity increased with chromium exposure, peaking at 0.25 mM. This rise in catalase activity aligns with its role in detoxifying reactive oxygen species (ROS) produced under chromium-induced oxidative stress [19]. Similarly, peroxidase activity increased under chromium stress, contributing to the detoxification of H_2O_2, thus indicating a defensive response against oxidative damage [26].

Ribonuclease activity also increased in response to chromium, particularly at the higher concentrations (0.25 mM), potentially due to chromium-induced RNA degradation in plant cells. This result contrasts with previous studies but is in line with findings in other species under stress conditions [9].

In contrast, Hill reaction activity, which measures photosynthetic electron transport, decreased significantly under chromium stress. This reduction suggests that chromium impairs the light reactions of photosynthesis, further corroborating the observed decline in chlorophyll content and overall photosynthetic efficiency [27].

Finally, the protein content in sweet potato plant leaves decreased with increasing chromium exposure, consistent with prior reports that metal stress often leads to reduced protein synthesis, likely due to damage to ribosomes and other protein synthesis machinery [18].

Conclusions

This study underscores the considerable effects of chromium (VI) stress on sweet potato, revealing a variety of visual phytotoxic symptoms, impaired plant growth, and disruptions in vital metabolic pathways. The results show that increasing chromium concentrations negatively impacted plant biomass production, yield quality, and overall plant health. Higher chromium exposure interfered with plant metabolism, primarily by competing for nutrients, inhibiting key enzymes, and displacing essential nutrients from their functional sites within plant cells. These disruptions led to symptoms such as chlorosis, necrosis, and stunted growth, ultimately compromising both physiological and biochemical functions of the plants.

Moreover, the accumulation of chromium in crops represents a significant health risk to both humans and animals, especially if these crops are consumed. This highlights the potential dangers linked to the use of chromium-contaminated irrigation water or effluent in agricultural practices. While the observed toxicity is clear, the precise physiological and biochemical mechanisms behind Cr (VI) toxicity in intact plant systems remain inadequately understood, suggesting the need for further investigation.

In conclusion, this study offers important insights into how chromium (VI) affects plant growth and development and contributes to the understanding of the risks posed by chromium contamination in agricultural environments. The findings also stress the necessity of developing effective strategies to manage chromium pollution, ensure crop safety, and reduce potential health risks for humans and animals.

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