

Effect of Oxalic Acid on Cr³⁺ ion Toxicity on some Morphological and Biochemical Parameters of Sorrel Seedlings (*Hibiscus sabdariffa L*) Transplanted in Hydroponic Solution

Ogunleye, J. A¹; Dagari, M. S² and Balogun O. L³.

¹ Department of Pure and Industrial Chemistry, Bayero University Kano, Kano State

² Department of Pure and Industrial Chemistry, Federal University Gashua, Yobe state, Nigeria

³ Department of Integrated Science, Federal College of Education (Technical), Gombe.

.Correspondence author: adigunolawale2010@gmail.com and biochemicalbol@gmail.com.

Abstract: Chromium (Cr) toxicity depends on the oxidation state of the metal, which subsequently determines its uptake, translocation and accumulation. It severely affects mineral uptake and metabolic processes in plants even at low concentration. The aim of this research work is to investigate the effects of oxalic acid on Cr³⁺ ion toxicity on sorrel seedlings (*Hibiscus sabdariffa L*) transplanted in hydroponic solution. The growth of 8week old seedlings exposed to 0, 5, 10, 20 and 40mg/L Cr³⁺ ion and 20mM oxalic acid was monitored in a green house under controlled conditions. The results revealed that Increasing concentrations of chromium (III) ion caused deleterious reduction in growth: dry weight of shoots and root as well as lengths of roots and shoots of sorrel seedlings and chlorophyll content transplanted in both chelated (oxalic acid) and unchelated treatments compared to control. There was a significant difference ($p < 0.05$) in total chlorophyll and Carotenoid for both chelated and unchelated treatment compared to control. At increasing concentrations of chromium (III), all observed Parameters were found to be affected.

Key words: Hydroponic, Chelation, Sorrel, greenhouse, oxalic acid, chromium

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I. Introduction

Cr exists in several oxidation states but the most stable and common forms are Cr (III) and Cr (VI) species. However, a high concentration of chromium is highly toxic to plants (Chatterjee and Chatterjee (2000). Cr toxicity in plants depends on its valence state. Cr (VI) which is highly mobile is considered the most toxic form of Cr, occurs associated with oxygen as chromate (CrO₄²⁻), or dichromate (Cr₂O₇²⁻), oxyanions. Cr (VI) is rapidly reduced to Cr³⁺ in the presence of organic matters and in reducing environments. Cr (III) is less mobile, less toxic and is mainly found bound to organic matter in soil and aquatic environments (Becquer *et al* 2003).

The strong effect of Cr contamination in the physiology of plants depends on the oxidation state of the metal, which is responsible for its mobilization, subsequent uptake and resultant toxicity in the plant system. Cr is taken up by plants through carriers of essential ions such as sulphate. Symptoms of Cr toxicity in plants include decrease in seed germination, reduction of growth, decrease of yield, inhibition of enzymatic activities, impairment of photosynthesis, nutrient and oxidative imbalances, and mutagenesis. The root contains organic acids that bind to metals from highly insoluble forms in the soil and acids like citric acid and malic acids can act as essential ligands for metals (Rauser, 1999). Studies on the role of organic acids in Cr toxicity in *Lycopersicon esculentum* showed that in the presence of organic acids like carboxylic acid and amino acids, Cr uptake in roots is enhanced (Srivastava *et al.*, 1999). Organic acids like citric acid, aspartic acid and oxalic acid can convert inorganic Cr to organically bound Cr, making it soluble for a longer period of time and thereby available to plants (James and Bartlett, 1983). Under Cr stress, plant roots excrete different organic acids. (Zeng *et al.*, 2008). The organic acids may induce the solubility or the immobility of heavy metals depending upon the type and concentration of organic acids, soil properties and other environmental factors (Ding *et al.*, 2014). On the other hand, the released organic acids may protect the plant roots by limiting metal transport across the biological membranes due to metal ion complexes with organic anions (Kochian *et al.* ,2004). Generally, the organic acids are released as anions and their release is balanced by the release of cations. Mycorrhizae and organic acids (citric and oxalic) have been reported to play an important role in phytoremediation of Cr-contaminated soils by enhancing Cr uptake and increasing translocation to shoot (Chen *et al.*, 1994; Davies *et al.*, 2001). Significant increases in Cr accumulation from Cr (III)-treated maize plants in the presence of increasing concentrations of organic acid have been observed (Srivastava *et al.*, 1999a). Shahandeh and Hossner (2000b) have reported a high increase in Cr uptake aided by organic acids. Srivastava *et al.* (1999b) found that increasing concentrations of organic acids resulted in increased uptake of Cr without affecting the distribution

in plant parts. Recently there is huge interest in the use of phytoremediation for the remediation of soils, sediments and water contaminated by heavy metal (Terry and Banuelos,2000). Phytoremediation of Cr pollution can be achieved by extraction of the metal from polluted soils into harvestable plant tissues by the accumulation of the element in the root tissues or by the in situ detoxification of the metal through plant-based chelation, reduction, and/or oxidation mechanisms. There is scanty information on research work on the phytoextraction of Cr from contaminated soils and sediments. Very few plant species such as *Sutera fodina*, *Dicoma niccolifera* and *Leptospermum scoparium* have been reported to accumulate Cr to high concentrations in their tissues (Peterson 1975).

Sorrel (*Hibiscus sabdariffa. L*) belongs to the Malvaceae family, and is an annual or biennial plant cultivated in tropical and subtropical regions for its stem fibers, edible calyces, leaves and seeds. It is generally considered as a medicinal plant. The calyces or petals of the flower are extensively used to prepare herbal drinks, cold and warm beverages, as well as making jams and jellies (Rao, 1996; Tsai et al., 2002). The brilliant red color and unique flavor coupled with other organoleptic attributes make them valuable food products (El-Adawy and Khalil, 1994). Previous studies showed that Roselle seeds could be used as a potential source of proteins and oil (El-Adawy and Khalil, 1994; Touunkara *et al.*, 2011). Recently its possible use in phytoremediation has attracted attention, due to the fact that as a plant with small requirements, sorrel may be encountered in many ecosystems: The present study has the following objectives: (i) To determine changes in the photosynthetic pigments (chlorophyll *a*, chlorophyll *b* and Carotenoids) arising from Cr³⁺ stress;(ii) To determine the changes in the shoots and roots dry weights as well as changes in lengths of roots and shoots of sorrel seedlings.

II. Materials And Methods

2.1 Source of Sorrel (*Hibiscus sabdariffa L*) Seeds.

Sorrel (*Hibiscus sabdariffa. L*) seeds were obtained from International Institute of Tropical Agriculture (IITA) station, Tarauni, Kano with coordinates latitude 11°58'49"N, longitude 008°33'26.5"E and altitude 492.5m above sea level.

2.1.1 FIELD EXPERIMENT.

The field experiment was carried out at Department of Agronomy farm, Bayero University, Kano with coordinates latitude 8° 22, to 9° 25, north and longitude 11° 57 to 12° 00' east. The farm falls within the Sudan Savanna climatic zone. There are two seasons namely the dry season (harmattan) and the wet (rainy) season. The sorrel (*Hibiscus sabdariffa L*) seeds were planted and raised for Eight weeks. The map of the planting site is shown in figure 2.1

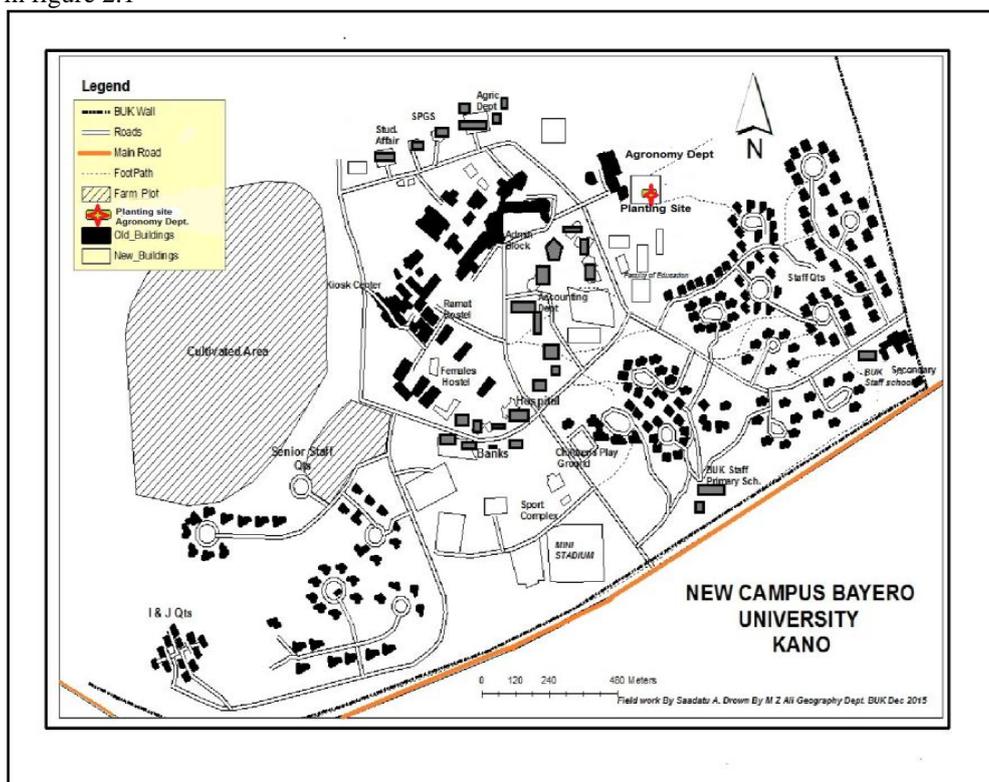


Figure2.1: Map of New site Bayero University, kano showing planting site.(Geography dept.BUK Dec. 2015)

The mean annual temperature of the areas was estimated to be 32°C. The lowest temperature of between 25°C- 28°C is recorded between the months of November- January, while the highest temperature of 36°C- 40°C is recorded in the months of March, April and May. The mean annual rainfall of the area is 850mm, with 4 to 5 months as rainy months (May to September). The mean annual humidity of the area is about 50% with the months of January, February and March recording the lowest humidity values of between 35% - 40% while the highest values of 85- 95 is recorded in the months of July, August and September.

2.1.3 Growth conditions and treatment

Eight week old Sorrel seedlings (*Hibiscus sabdariffa L.*) were carefully collected from Agronomy Departmental farm, Bayero University, Kano on 20th, June, 2014. They were washed with tap water to remove excess soil, and rinsed three times with deionised water before transplanting in hydroponic solution and kept in a green house at 65% constant relative humidity, 16/8 h day/night period under 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of light intensity, and day/night temperatures of 38/20°C. Plants were supplied with Hoagland nutrient solution (pH 6.0-6.3) which contained the following nutrients: 1mM KH_2PO_4 , 2mM $\text{MgSO}_4 \cdot 4\text{H}_2\text{O}$, 5mM KNO_3 , and 5mM $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and 4.6 μM H_3BO_3 , 0.8 μM $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.3 μM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, and 0.1 μM $\text{H}_2\text{MoO}_4 \cdot \text{H}_2\text{O}$. Iron was supplied as Fe-EDTA (1.8 mM). The treatment consists of three phases in a completely randomized design which composed of:

Phase I: Contain only the nutrient solution (hydroponic solution).

Phase II: Contain the nutrient solution and four levels (5, 10, 20 and 40mg/L) of added Cr^{3+} ion as $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$.

Phase III: Contain the nutrient solution and four levels (5, 10, 20 and 40mg/L) of added Cr^{3+} ion as $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ and constant concentration (20mM) of oxalic acid.

Each treatment in triplicates was allowed to stand for 10 days, after which the plants were harvested and subjected to physiological and biochemical analysis.

2.1.4. Plant Analysis

After ten days of exposure, the plants were harvested and washed thoroughly with tap water followed by distilled water and then wiped with tissue paper to remove the excess water in the roots. The plants were separated into roots and shoots, dried at room temperature for two weeks and then placed in a dark polythene bags for further analysis.

2.2. Determination of Morphological and Biochemical Parameters.

Changes in root and shoot lengths and weights were determined using the following relation:

Change in shoot length (ΔShl) = (shoot length for a given treatment) – (shoot length for control) cm.

Change in root length (ΔRtl) = (root length for a given treatment) – (root length for control) cm.

Change in shoot weight (ΔShW) = (shoot weight for a given treatment) – (shoot weight for control) g/DW.

Change in root weight (ΔRtW) = (root weight for a given treatment) – (root weight for control) g/DW.

2.2.1 Determination of Pigment Content (Chlorophyll a, Chlorophyll b and Carotenoid)

The estimation of pigments content in both control and treated plants were carried out according to the method of Lichtenthaler and Wellburn (1983)

Two grams of dried leaf tissue of each sample were homogenized using 80% acetone. The homogenate was centrifuged for 10 minutes and supernatant was collected. The residue was again extracted and the supernatant was pooled together. The extraction process was repeated until the residue became colourless. Volume of the combined supernatant was noted. The absorbance of solution was measured at 646nm, 663nm and 470nm for Chlorophyll a, Chlorophyll b and Carotenoid respectively. The Concentrations of chlorophyll a, b and total chlorophyll content were calculated using equation 1.

$$\text{Chlorophyll a } (\mu\text{g/mL}) = 12.21(A_{663}) - 2.81(A_{646})$$

$$\text{Chlorophyll b } (\mu\text{g/mL}) = 20.13(A_{646}) - 5.03(A_{663})$$

$$\text{Total chlorophyll} = a + b$$

$$\text{Carotenoid } (\mu\text{g/mL}) = (1000A_{470} - 3.27(\text{Chl a}) - 104(\text{chl b}))/227$$

2.3. Spectrophotometric determination of Cr^{3+} ion in Roots and Shoots of Harvested Sorrel Seedlings.

After 10 days exposure, the sorrel seedlings were harvested and washed first with tap water, followed by 1% HNO_3 and finally rinsed with deionised water (Wong and Lau 1985). The roots and shoots were separated and oven dried at 80°C for 48 hours. They were grinded with wooden mortar and pestle to a fine powder. A washed dried porcelain crucible was ignited on a hot electric plate for 5minutes. 2g of shoot and 0.5g of root were accurately weighed into the crucible and gently heated on hot electric plate until the smoking ceased. It was then transferred and ashed to constant weight in a muffle furnace at 550°C for 4hours. The ash

was cooled in a dessicator, dissolved in 0.10M HNO₃, filtered into 50cm³ volumetric flask and made to mark. The Cr³⁺ ion content in the roots and shoots was analyzed using atomic Absorption Spectrophotometer (BUCK SCINTIFIC VGP 210) at 359.7nm. The concentration of Cr³⁺ ion was reported as mg g⁻¹ dry weight.

2.4. Statistical Analysis

All data were analyzed using Excel 2010 program for window and significance test were performed using One-way ANOVA at 95% confidence level. Data were expressed as mean followed by SD. Statistical significance was assumed at $P < 0.05$.

III. Results and Discussions

3.1 Change in Root and Shoot Length of *Hibiscus sabdariffa* L. Seedlings

Increasing concentrations of Cr³⁺ ion caused significant reduction in root and shoot length. In this work, root and shoot length were significantly decreased for both chelated and unchelated treatment as compared to control. Mohammed *et al* (2014) observed that treatment with low concentrations of Cr (100ppm) significantly suppressed root length in four (4) jute varieties. Reduced growth in terms of root and shoot lengths at increasing doses of Cr³⁺ ion might be due to adverse effect of this metal on Sorrel plants. Panda and Patra (2000) found that 1μM of Cr increased the root length in seedlings growing under nitrogen (N) nutrition levels; but decreased root length at higher Cr concentration in all the N treatments

Figure 3.1 shows changes in root and shoot length of sorrel seedlings (*Hibiscus sabdariffa* L) against same Cr³⁺ concentrations

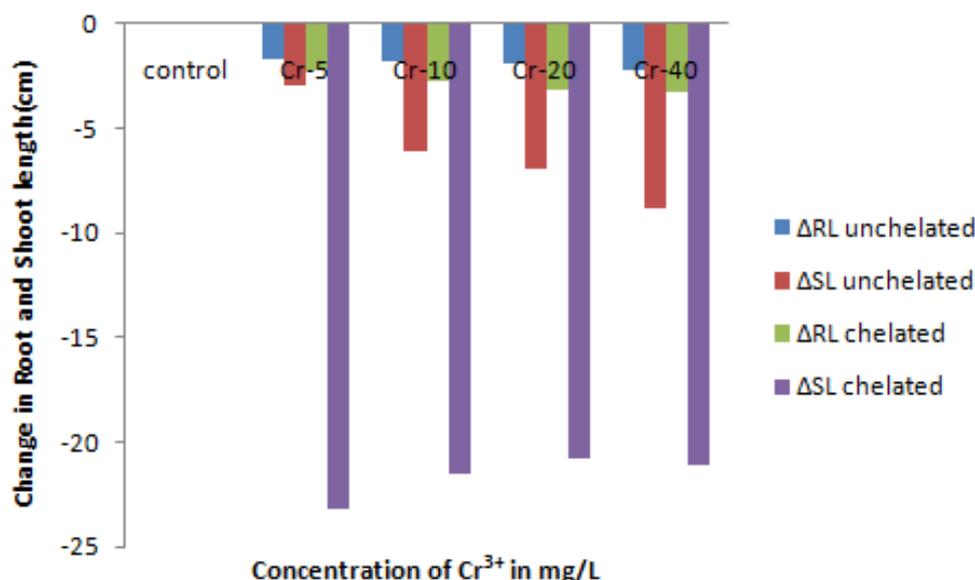


Figure 3.1: Change in root and shoot length of sorrel seedlings (*Hibiscus sabdariffa* L) in 20mM oxalic acid and unchelated treatment of same Cr³⁺ concentrations

3.2 Change in Root and Shoot dry weight of (*Hibiscus sabdariffa* L) Seedlings

In this study, there is a decrease in root and shoot dry weight in both chelated (20mM oxalic acid) and unchelated treatment of same concentrations. The decrease in root and shoot dry weight of sorrel seedlings may be due to leaching of the oxalic acid and higher toxicity of Cr³⁺ ion in solution culture because of the solubility of Cr³⁺ ion supplied and its availability for plant uptake in the hydroponic medium. Cr³⁺ ion phytotoxicity occurs at lower Chromium concentrations when Cr is supplied in hydroponic culture relative to Cr supplied in soil. For example, Turner and Rust (1971) observed that the initial symptoms of Cr toxicity on plants occurred with the addition of as little as 0.5mgkg⁻¹ Cr to the nutrient culture and as much as 60 mgkg⁻¹ to the soil culture. The application and effects of chelators which depend on dose and time also resulted in the removal of essential metal nutrients from the media, leading to deficiencies in the plants (Ruley *et al.*2006).

Figure 3.2 shows change in root and shoot dry weight of sorrel seedlings (*Hibiscus sabdariffa* L) against same Cr³⁺ concentrations

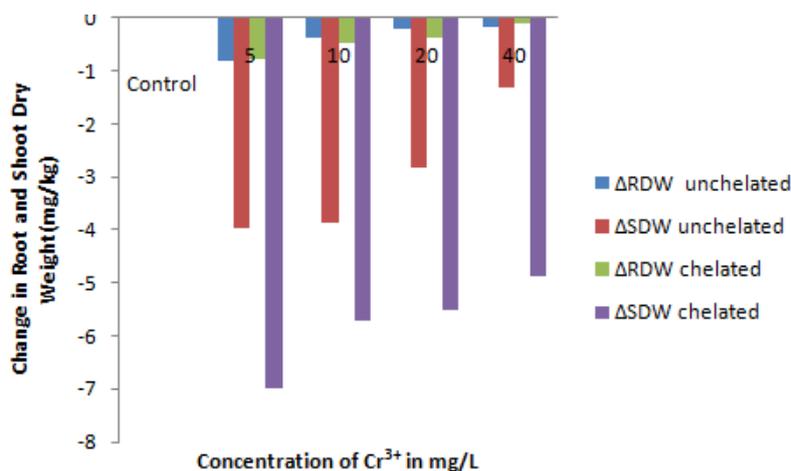


Figure 3.2: Change in root and shoot Dry weight of sorrel seedlings (*Hibiscus sabdariffa* L) seedlings in 20mM oxalic acid and unchelated treatment of same Cr³⁺ concentrations

3.3 Change in pigment content of *Hibiscus sabdariffa* L) Seedlings

Change in Chlorophyll a (ΔChl a) = (Chlorophyll a of a Particular treatment- Chlorophyll a in Control)

Change in chlorophyll b (ΔChl b) = (Chlorophyll b of Particular treatment) – (Chlorophyll b in Control)

Change in Carotenoid (ΔCR) = (Carotenoids of particular treatment) – (Carotenoid in control)

The decrease in total chlorophyll, chlorophyll a and b, and carotenoids has been well documented under Cr stress in plants by several researchers (Tripathi and Smith, 2000; Panda *et al.*, 2003). In this study, a significant decrease was observed in the chlorophyll content (Chl a, and Chl b) with the application of Cr³⁺ with and without oxalic acid ($p < 0.05$). There were several possible explanation for this decrease. Cr possesses the capacity to degrade aminolevulinic acid dehydratase, an important enzyme involved in chlorophyll biosynthesis, thereby affecting aminolevulinic acid (ALA) utilization (Vajpayee *et al.*, 2000). Cr, mostly in its hexavalent form can replace Mg ions from the active sites of many enzymes and deplete chlorophyll content (Vajpayee *et al.*, 2000). However, (Vajpayee *et al.*, 2000) in contrast reported that an increase in carotenoids content was seen under Cr treatment in *Vallisneria spiralis* and other aquatic plants. The increase in carotenoids content may act as an antioxidant to scavenge ROS generated as a result of Cr toxicity.

Figure 3.6 shows change in Chl a, Chl b and Carotenoid in sorrel seedlings (*Hibiscus sabdariffa* L) against same Cr³⁺ concentrations

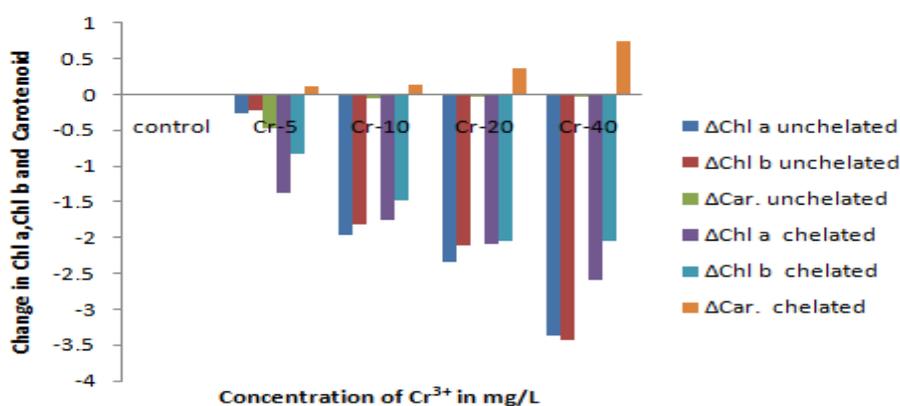


Figure 3.6: Change in Chl a, Chl b and Carotenoid in sorrel seedlings (*Hibiscus sabdariffa* L) in 20mM oxalic acid and unchelated treatment of same Cr³⁺ concentrations.

IV. Conclusion

Chromium toxicity has a significant effect on the root growth and form. The toxicity is concentration and medium dependent. It can be concluded from this study that Cr toxicity behaves differently at different levels of Cr supply. Consequently, Chromium concentration in the plants increases with an increase in the concentration of chromium in the media. Phytotoxicity symptoms which include chlorosis, necrosis was observed depending on the concentration of Cr³⁺ ion. The addition of oxalic acid lessens the effect of chromium toxicity by mitigating oxidative stress through its chelating property compared to chromium treatment only.

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