

## Effect of Heavy Metals on Phenolic Content and Free Radical Scavenging Activity of *Ocimum Tenuiflorum L*



### Botany

**KEYWORDS:** Metal uptake, phenolic content, Free radical scavenging activity, *Ocimum tenuiflorum L*

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### ABSTRACT

In the pot experiment, *O. tenuiflorum L* reduced their root length, shoot length and dry biomass with increased concentrations of Cu, Cr and Cd over the control, and reduction trend observed for metal treatment was  $Cu > Cr > Cd$  after 80DAS. SOD-FSRA, H-RSA, DPPH-FRSA and phenolic content was more in leaf > shoot under Cu treated plant as compared with Cr and Cd. Maximum accumulation of Cu, Cr and Cd in the plant was 39.8ppm at 30 mg Kg<sup>-1</sup> Cu treated pot, 21ppm at 10 mg Kg<sup>-1</sup> Cr treated pot and 16ppm at mg Kg<sup>-1</sup> Cd treated pot respectively, recorded after 80DAS. Hence, the result of metals in plant indicates that more than the permissible limit (WHO) and should not advisable to use plant as a medicinal treatment without metal analysis.

### INTRODUCTION

The *Ocimum tenuiflorum L.* belonging to the Lamiaceae family and their aromatic leaves are used fresh or dried as a flavoring agent for foods, employed in folk medicine for its carminative, stimulant and antispasmodic properties etc. [1,2]. Heavy metal pollution of soils has dramatically increased in recent decades due to the discharge of waste and wastewater from anthropogenic sources [3]. It is mentioning that Cu, Cr and Cd toxicity is dependent on species, the concentration of metal supplied and exposure time [4,5]. In sensitive plant species or ecotypes, heavy metals were shown to inhibit growth and to interfere with important cellular processes such as photosynthesis and respiration [5]. Chromium toxicity exhibit both in plant as well as animal, in plants showed chlorosis and necrosis [6] and in animal causes poisoning [7]. Cadmium (Cd) is a very toxic heavy metal and an important environmental pollutant, which is present in the soil, water, air, food and in cigarette, smokes [8]. The toxic effects of cadmium are due to its inhibition of liver metabolic enzyme systems containing sulphhydryl groups and uncoupling of oxidative phosphorylation in mitochondria [9], which results in increased lipid peroxidation, hepatic congestion, ischemia and hypoxia [9]. It is well known that transition metals catalyze the formation of hydroxyl radicals (OH.) from the non-enzymatic chemical reaction between superoxide (O<sub>2</sub>.) and H<sub>2</sub>O<sub>2</sub> (Haber-Weiss reaction) [10]. Permissible levels of Cu, Cr are 0.5, 0.05 mg L<sup>-1</sup> respectively, and for Cd were not specify [11]. The medicinal plants are either naturally grown or cultivated in metal contaminated regions, there is a danger that the heavy metal accumulation by plants of medicinal value may cause serious health hazards to patients using metal adulterated herbal drugs. Therefore, the objective of this study was to examine the growth response, phenolic content free radical scavenging activities and metal accumulation in (*O. tenuiflorum L.*) under different heavy metals viz. Cu, Cr and Cd.

### MATERIAL AND METHODS

#### Set-up of Pot experiment

The soil was collected from an agricultural field of district Jaunpur (25.73°N 82.68°E), UP-India, chemical analysis of the soil showed that organic matter, total N and pH, were 3.65%, 0.16%, 7.2 and Cu, Cr and Cd were 1.32, 0.20 and 0.10 mg kg<sup>-1</sup>, respectively. Surface (0–20 cm) soil samples which were ground to pass through a 4.0mm mesh were used in the pot-experiment. The soil samples were air-dried, then mixed with basal fertilizers, at ratios of 100 mg N kg<sup>-1</sup> dry weight (DW) soil as NH<sub>4</sub>NO<sub>3</sub> and 30 mg P kg<sup>-1</sup> and 80 mg kg<sup>-1</sup> as K<sub>2</sub>HPO<sub>4</sub>. Eleven treatments of Cu, Cr and Cd were applied, namely C (the control) and treatments (concentrations of all three metals: 10, 30, 50, 70, 90, 110 and 150 mg kg<sup>-1</sup>, dry weight of soil). Each treatment

was carried out in six replicates. The tested topsoil samples were mixed thoroughly with CuCl<sub>2</sub>, CrCl<sub>2</sub> and CdCl<sub>2</sub>·2.5H<sub>2</sub>O at the above-mentioned concentrations, filled into plastic pots (20 cm in diameter, 15 cm in height, 2.5 kg air-dried soil per pot) and equilibrated for one month. Seeds of *O. tenuiflorum L.* were surface sterilized in 2% (w/v) sodium hypochloride for 1 min, washed several times with sterilized distilled water (SDW), and soaked in SDW for overnight [18]. Twenty soaked seeds were sowed directly into the 0.8% agar plate and incubated for three days in dark room. Three similar sizes of sprouted seed were placed in the each treatment pots in the greenhouse with natural light (10–12h; photoperiod) and temperature (20–33°C). The tested soils were watered to reach 60% of the water-holding capacity and this level was maintained by watering daily throughout the experiment. The plants were harvested after 80DAS (days after showing) and root length, shoot length and dry biomass were observed, rest plant samples were kept at 40°C for further analysis.

#### Determination of superoxide, Hydroxyl and DPPH- free radical scavenging activity

Superoxide radicals were examined by the method [12], 40µL aliquot of *O. tenuiflorum L.* extracts was mixed with 3ml of reaction buffer solution (1.3 mm riboflavin, 13 mM methionine, 63 µM nitro blue tetrazolium and 100µM EDTA, pH 7.8). The reaction solution was illuminated for 15 min at 25 °C. The reaction mixture, without sample, was used as a control. The scavenging activity was calculated as follows: scavenging activity (%) = (1-absorbance of the sample/absorbance) × 100. The Hydroxyl radical scavenging activity (HR-SA) was determined according to the method [13] in extracts of shoot and leaf of *O. tenuiflorum L.* spectrophotometrically at 412 nm. 100 µl of extracts was taken in different test tubes. 1.0 ml of Fe-EDTA solution (0.1% ferrous ammonium sulfate and 0.26% EDTA), 500 µl of DMSO (0.85% v/v in 0.1 M Phosphate buffer, pH 7.4) were added to these tubes, and the reaction was initiated by adding 500 µl of 0.22% ascorbic acid, and incubated at 80-90°C for 15 min. The reaction was terminated by the addition of 1 ml of ice cold TCA (17.5 %w/v). 3 ml of Nash reagent were mixed and raised to 1 L with distilled water was added to all of the tubes and left at room temperature for 15 min for the color development. The percentage of HR-SA was calculated by using the formula: % of HR-SA = 1-absorbance of sample/absorbance of blank × 100. Scavenging activity against DPPH radicals was assessed according to the method [14]. 100µM DPPH-methanol solution was mixed with 1 ml of 100µ M DPPH methanol solution. After the solution was incubated for 30 min at 25 °C in dark, the decrease in the absorbance at 517nm was measured. Control contained methanol instead of antioxidant solution, while blanks con-

tained methanol instead of DPPH solution in the experiment. Ascorbic acid and BHT were used as positive controls. The inhibition of DPPH radicals by the samples was calculated according to the following equation: DPPH-scavenging activity (%) = [1-(absorbance of the sample-absorbance of blank)/absorbance of the control] ×100.

**Total phenolic content**

Total phenolic compounds in *O. tenuiflorum* L. were quantified by using Folin-ciocalteu's method. 50 µl of Folin-ciocalteu's reagent (50% v/v) were added to 10µl of sample extract. It was incubated for 5 min. After incubation 50µl of 20 % (w/v) sodium carbonate and water was added to final volume of 400 µl. Blank was prepared by replacing the reagent by water to correct for interfering compounds. After 30 min of incubation, the absorbance was measured using spectrophotometer at 760 nm. Total phenol contents were expressed in terms of gallic acid equivalent (gm/100g of dry mass), which is used as a reference compound.

**Plant and soil metal analysis**

The plants were immersed in a 0.01M HCl solution to remove any external Copper, Chromium and Cadmium and rinsed with deionized (DI) water for 1 min. After that, plants were dried at

100°C for 10 min, then at 70°C in an oven until completely dry. The plant and soil samples were digested with a solution of 3:1 HNO<sub>3</sub>:HClO<sub>4</sub> (v/v). The concentrations of Cu, Cr and Cd were determined using the atomic absorption spectrophotometry by the method [15].

**Statistical analyses**

The data obtained were subjected to ANOVA, and means were compared with Duncan's multiple range test. All statistical analyses were conducted using SPSS (Version 14; IBM, Armonk, NY, USA).

**RESULTS AND DISCUSSION**

In this study, with increased concentrations of Cu, Cr and Cd showed, root length, shoot length, and dry biomass of *O. tenuiflorum* L. were reduced over the control, and the trends of reduction was observed for metal Cu>Cr>Cd after 80DAS. Reduction was significantly observed at ≥ 50 to 150 mg Kg<sup>-1</sup> in Cu, ≥ 30 to 110 mg Kg<sup>-1</sup> in Cr and ≥ 30 to 70 mg Kg<sup>-1</sup> in Cd treated plant (Table 1). Copper acts as structural element in certain metalloproteinase, many of which are involved in electron transport in chloroplasts and mitochondria as well as in oxidative stress response [16]. Dry biomass reduction was more as compared with growth of shoot and root for all the metals (table 1).

**Table 1.**

Table 1: Effects of different concentrations of Cu, Cr and Cd on growth of *Ocimum tenuiflorum* L. after 80DAS (pot-experiment).

Treatment	Copper			Chromium			Cadmium		
	RL	SL	Dry BM	RL	SL	Dry BM	RL	SL	Dry BM
Control	12.3±2.6a	43.9±4.8b	11.2±3.2a	11.9±3.1a	42.4±4.5a	11.5±2.2a	12.6±2.5a	44.8±4.5a	12.3±1.2a
10 mg Kg <sup>-1</sup>	12.2±2.1a	41.6±4.2a	10.7±2.3ab	11.1±2.4a	42.1±4.3a	11.1±2.8a	12.2±1.4a	42.4±4.3b	11.5±1.8a
30 mg Kg <sup>-1</sup>	11.5±2.8a	40.1±3.6ab	10.0±3.7ab	9.7±2.8b	22.8±3.5b	8.2±2.4ab	8.7±2.8b	35.5±2.8bc	8.25±1.1b
50 mg Kg <sup>-1</sup>	10.9±2.1b	37.6±4.5ab	9.4±2.2bc	8.5±2.9bc	18.7±3.2bc	6.3±1.8c	6.5±1.9c	22.8±2.5c	6.34±1.9cd
70 mg Kg <sup>-1</sup>	9.1±2.8ab	35.2±3.9bc	8.6±2.1c	6.2±2.2c	13.5±2.1c	5.2±1.9cd	4.2±1.2d	18.5±2.1d	5.15±1.3cd
90 mg Kg <sup>-1</sup>	8.2±2.8bc	33.5±5.7bc	6.5±2.2cd	5.6±2.0cd	13.2±1.8cd	4.0±1.4d	ns	ns	ns
110 mg Kg <sup>-1</sup>	8.1±2.2c	32.6±4.2cd	6.0±2.6d	4.2±1.2d	10.5±2.5d	3.2±1.0e	ns	ns	ns
130 mg Kg <sup>-1</sup>	6.5±1.4cde	22.2±3.8d	5.2±1.4de	ns	ns	ns	ns	ns	ns
150 mg Kg <sup>-1</sup>	4.5±1.2e	21.3±4.5d	5.1±1.2e	ns	ns	ns	ns	ns	ns

RL=Root length (cm), SL= Shoot length (cm), Dry BM=Dry biomass (g) and ns= plant not survive. Data are expressed as mean ± SD. Figures followed by different letters in a same line are significantly different at P < 0.05, n= 6.

Reduction in the plants growth and dry biomass by Cr was more than the Cu and less then Cd (table 1), similar finding was earlier reported [6,7]. Excess amount of Cr in plants, exhibit the adverse effect on biomass and growth by change in concentration of essential mineral nutrients in citrullus and brassica juncea L. [17]. The greater impact of heavy metal Cd, was observed on the root growth as compared to shoot leading to a greater reduction in its length and fresh weight [3,15]. The reduction in plant growth during stress is due to low water potential, hampered nutrient uptake and secondary stress such as oxidative stress[7,13,15]. Several studies suggest that antioxidants could prevent accumulation of these reactive oxygen species [13,15,18]. In the present study, SOD-FSRA, H-RSA and DPPH-FRSA was more in leaf then shoot under all metal treatment, and the activity trends for the metals was Cu>Cr>Cd (Figure 1 B&C). Cu, Cr and Cd are efficient catalyst in the formation of reactive oxygen species [4,7,15].

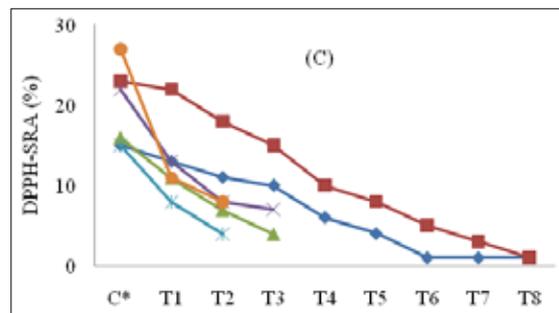
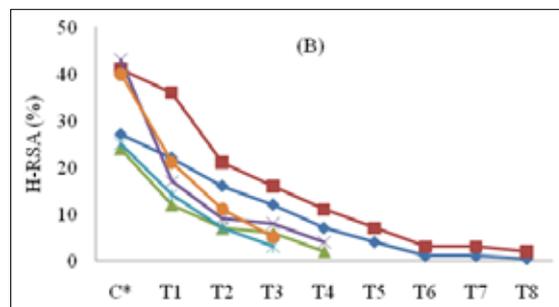
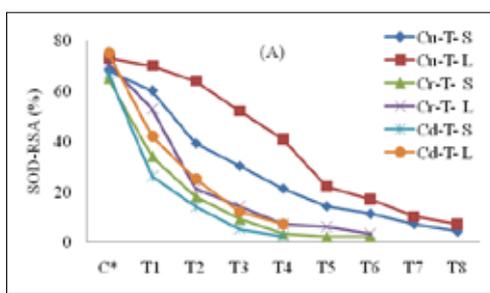


Figure 1. (A)= Percent of Superoxide radical activity (SOD-RSA %), (B) = Percent of Hydroxyl radical scavenging activity (HR-SA %) and (C)= DPPH-Free radical scavenging activity (DPPH-FRSA %) in root, shoot and leaf of *O. tenuiflorum* L. under different treatments of metals (Cu, Cr and Cd) after 80DAS. Results are mean of six replicates. Cu-T-S (Copper treated shoot), Cu-T-L (Copper treated leaf), Cr-T-S (Chromium treated shoot), Cr-T-L (Chromium treated leaf), Cd-T-S (Cadmium treated shoot) and

Cd-T-L (Cadmium treated leaf). C\*=Control, T1, T2, T3, T4, T5, T6, T7 and T8= 10, 30,50,70,90,110,130 and 150mg Kg-1 respectively.

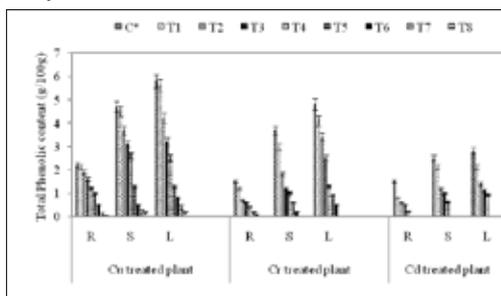


Figure 2. Total phenolic content (g/100gm) of root= (R), shoot= (S) and leaf =(L) of *O. tenuiflorum* L. under different concentration of Copper (Cu), Chromium (Cr) and Cadmium (Cd) after 80DAS. Data are significantly different at  $P < 0.05$ ,  $n = 6$ . C\*=Control, T1, T2, T3, T4, T5, T6, T7 and T8= 10, 30,50,70,90,110,130 and 150mg Kg-1 respectively.

#### Figure 1. and Figure 2.

Hence, the presence of excess metals can cause oxidative stress in plants and subsequently increase the antioxidant responses due to increased production of highly toxic oxygen free radicals. Phenolic content was found to be minimum under Cd treated plant as compared to Cr and Cu, and it was more in leaf as compared to shoot and root (Figure 2). 86%, 76% and 67% reduction was observed in root, shoot and leaf respectively at 70 mg Kg-1 Cd treated plant after 80DAS over the control. Cadmium as a non-essential element for living organisms has a very high mobility in soil-plant systems, with propensity to adversely affect both human health and the functioning of ecosystems [15]. 73%, 72% and 72% reduction was observed in root, shoot and leaf respectively at 70 mg Kg-1 over the control. Omer [2] reported that oil and phenolic content altered under stress like salinity of different basil species. In the case of the Cu treated plant showed, least reduction of phenolic compound among all the metals in root, shoot and leaf, 45%, 44% and 56% at 70 mg Kg-1. The phenols contain hydroxyls that are responsible for the radical scavenging effect mainly due to redox properties [19]. However, the antioxidant capacity of the plant extracts is mainly dependent on phenolic compounds. So far, in the *Ocimum* species the *O. basilicum* and *O. sanctum* have been reported for

their secondary metabolite content [20]. Maximum accumulation of Cu, Cr and Cd in the plant was 39.8ppm at 30 mg Kg-1 Cu treated pot, 21ppm at 10 mg Kg-1 Cr treated pot and 16ppm at mg Kg-1 Cd treated pot respectively, recorded after 80DAS (Figure 3).

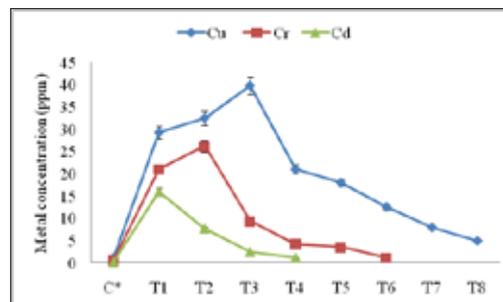


Figure 3. Copper, Chromium and Cadmium concentration in plant of *O. tenuiflorum* L. under different concentration metals treatments after 80DAS. Data are significantly different at  $P < 0.05$ ,  $n = 6$ . C\*=Control, T1, T2, T3, T4, T5, T6, T7 and T8= 10, 30,50,70,90,110,130 and 150mg Kg-1 respectively.

#### Figure 3.

Copper was more accumulating in plant as compared with Cr and Cd, because Cu play important role in several enzymatic activity as well metabolic activity in plant [5,16]. Distribution of Cr in crops had a stable character which did not depend on soil properties and concentration of this element; the maximum quantity of element contaminant was always contained in roots and a minimum in the vegetative and reproductive organs [6]. A result indicates that the levels of metals are more than the permissible limit [11] and should not advisable to use plant as a medicinal treatment without metal analysis.

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#### REFERENCE

- Shetty S, Udupa S, Udupa L, Somayaji N, 2006. Wound healing activity of *Ocimum sanctum* Linn with supportive role of antioxidant enzymes. *Indian J Physiol Pharmacol*, 50,163-168.
- Omer EA, Said-Al Ahl HAH, and Hendawy SF, 2008. Production, chemical composition and volatile oil of different basil species/varieties cultivated under Egyptian soil salinity conditions. *Res. J. Agric. Biol.Sci.*, 4(4), 293-300.
- Ghosh M, Singh SP, (2005). A comparative study of cadmium phytoremediation by accumulator and weed species. *Environmental Pollution*, 33, 365-371.
- Lombardi L, Sebastiani L, 2005. Copper toxicity in *Prunus cerasifera*: growth and antioxidant enzymes responses of in Vitro grown plants. *Plant Science*, 168, 797-802.
- Kramer U, Clemens S, 2006. Functions and homeostasis of zinc, copper, and nickel in plants. In: Tamas M, Martoinoia E (eds) *Molecular Biology of Metal Homeostasis and Detoxification from Microbes to Man*. Berlin, Springer, pp 214-272.
- Cervantes C, Campos-Garcia J, Debars S, Gutierrez-Corona F, Loza-Tavera H, 2001. Carlos-Tarres-Guzman, M. and Moreno-Sanchez, R. Interaction of chromium with Microgenesis and plants. *FEMS Microbiol. Rev.*, 25, 335-347.
- Stojs SJ, Bagachi D, Hassoun E, Baguchi M, 2000. Oxidative mechanisms in the toxicity of chromium and cadmium ions. *J Environ Pathol Toxicol Oncol.*, 19,201-13.
- Del Raso NJ, Foy BD, Gerhart JM, Frazier JM, 2003. Cadmium uptake kinetics in rat hepatocytes correction for albumin binding. *Toxicol Sci.*, 72,19-30
- Swiergosz R, Zaurzewska M, Sawicka-Kapusta K, Bacia K, Jankowska I, 1998. Accumulation of cadmium and its effect on bank vole tissues after chronic exposure. *Ecotoxicol Environ Saf.*, 41,130-136.
- Halliwell B, Gutteridge JMC, 1984. Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochemical Journal*, 219,1-14.
- World Health Organization (WHO) and UNICEF, 2006. Rapid Assessment of Drinking Water Quality. Country Report, Nigeria, 82.
- Giannopolites C N, Ries S K, 1997. Superoxide dismutase. I. Occurrence in higher plants. *Plant physiology*, 59, 309-314.
- Singh R P, Murthy C K N, Jayaprakash G K, 2002. Studies on the antioxidant activities of pomegranate (*Punica granatum*) Peel and seed extracts using in vitro models. *Journal of agricultural and food chemistry*, 50, 81- 86.
- Larrauri J A, Sanchez-Moreno C, Saura- Calixo F, 1998. Effect of temperature on the free radical scavenging capacity of extracts from red and white grape pomace peels. *Journal of agricultural and food chemistry*, 46, 2694-2697.
- Sun RL, Zhou QX, Sun FH, Jin CX, 2007. Antioxidative defense and proline/phytochelatin accumulation in a newly discovered Cd-hyperaccumulator, *Solanum nigrum* L. *Environ. Exp. Bot.*, 60,468- 476.
- Puig S, Andres-Colas N, Garcia-Molina A, Penarrubia L, 2007. Copper and iron homeostasis in *Arabidopsis*: responses to metal deficiencies, interactions and biotechnological applications. *Plant Cell and Environment*, 30, 271-290.
- Ghani A, 2011. Effect of chromium toxicity on growth, chlorophyll and some mineral nutrients of brassica juncea L. *Egypt. Acad. J. biolog. Sci.*, 2(1),9-15.
- Upadhyay S K, Singh J S, Saxena AK and Singh D P, 2012. Impact of PGPR inoculation on growth and antioxidants status of wheat plant under saline condition. *Plant Biology*, 4, 605-611.
- Rice-Evans CA, Miller N J, Paganga G, 1997. Antioxidant properties of phenolic compounds. *Trend. Plant Science*, 4,152-159.
- Lukmanul Hakkim F, Gowri Shankar C, Girija S, 2007. Chemical composition and antioxidant property of holy basil (*Ocimum sanctum* L.) leaves, stems and inflorescence and there in vitro callus cultures. *Journal of Agricultural and Food chemistry*, 55,9109-9117. | |