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# Cu-Induced oxidative stress modifies the metabolic profile of *Allium cepa* roots

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**Abstract**
Heavy metals (Cu, Fe, Zn etc) are capable of imparting oxidative stress in life systems and generally responsible for various ailments such as cancer. Alternation of metabolic profile is one of the critical factors for causing such diseases. In this backdrop our study aims to study the Cu-induced metabolic changes in *Allium cepa* roots. *A. cepa* has been extensively used as model by researchers for various studies due to its easy availability as well as robustness and consistency of the outcomes. $CuSO_4$ was used as a sole source of $Cu^{2+}$ ions. *A. cepa* roots were treated with 25-250 μM $CuSO_4$ for 24 h and volatile metabolic constituents of the root extract (hexane extract) were assessed by GC-MS analysis. The result showed that when compared with the control, Cu could modify the volatile constituents of *A. cepa*.

**Keywords:** Cu, *Allium cepa*, heavy metal, metabolic profile, GC-MS

## Introduction
The term ‘Heavy metal’ is a collective term given to a group of metals and metalloids with atomic density greater than 4 g/cm$^3$ or 5 times or more, greater than water such as Cd, Cu, Zn, Ni, Co, Cr, Pb etc (Huton and Symon, 1986) [1]. These heavy metals are potentially responsible for contamination of agriculture soils and various causes such as long-term use of chemical fertilizers especially phosphatic fertilisers, disposal of sewage sludge to the agriculture lands, dust from smelters, industrial waste water and bad irrigation practices are assumed to be responsible for such contamination (Bell *et al*., 2001; Schwartz *et al*., 2001; Passariello *et al*., 2002) [2-4]. They are also considered as pollutants of the soil as they induce toxic effect on the plants grown on such soils through the mechanism of oxidative stress. Crop plants growing on these soils show a reduction in growth, performance, and yield leading to depletion in food reserve. Some of the direct toxic effects caused by high metal concentrations include inhibition of cytoplasmic enzymes as well as cellular damage. (Assche and Clijsters 1990; Jadia and Fulekar 2009) [5, 6].

Copper (Cu) is an essential metal for the growth and development of plants. It plays a crucial role as micronutrient in many physiological functions in plants including photosynthetic electron transport chain, mitochondrial respiration, oxidative stress responses, cell wall metabolism, and hormone signalling (Marschner 1995; Raven *et al*.,1999) [7, 8]. However, Cu is toxic to the plant when treated in excess. It inhibits plant growth and interferes with cellular processes like photosynthesis and respiration in plants (Marschner 1995; Prasad and Strzalka., 1999) [7, 9]. When plants are grown in the presence of high level of Cu, they show a low content of biomass and chlorotic symptoms, as well. Cu catalyses the formation of hydroxyl radicals (OH) from the non-enzymatic chemical reaction between superoxide ($O_2^-$) and $H_2O_2$ (Haber-Weiss reaction) (Halliwell and Gutteridge 1984) [10]. Oxidative stress induced by the high concentrations of Cu poses metabolic alteration in plants leading to cell death.

*Allium cepa* L. commonly called as onion is a plant belonging to the family Alliaceae subf. Allioideae It is a large genus, comprising more than 800 species (Li *et al*. 2010) [11]. It is one of the most important genus of this family. Its close relatives include garlic, leek, chive etc. This species contains 16 large chromosomes (2n=16) which have clear and characteristic morphological feature, for this reason it used in mitotic and genotoxic studies. *Allium* species are used as food flavouring agents. It is an excellent *in vivo* model to study genotoxicity. It is easy to handle and large enough, moreover its roots grow very soon for us to study the effect of certain chemicals on it.

In the present study *A. cepa* is used as a system to study the metabolic alteration induced by toxic effect of Cu at the concentrations range 25-250 μM. The GC-MS based method is used for comparative analysis between control (without treatment) and treating taking various organic volatile compounds as markers, which are responsible for the biological activity, flavour and odour in the onion.

## Materials and Methods

### Materials

Chemicals

Copper (II) sulphate pentahydrate (AR, CAS No.: 7758-99-8, Himedia), Hexane (GC-MS grade, AR, CAS No.: 7757-82-6, Himedia), Sodium sulphate (AR, CAS No.: 110-54-3, Himedia), Double distilled water.

Test System:

Medium sized onion bulbs

### Method

Almost 1.5 Kg of commercial variety of medium sized onion bulbs were bought from the local market. Outer skin and scales were peeled off and they were placed in sand for the roots to grow. The sand was frequently moisturised by sprinkling water. The onions were placed for 72 hours. When the roots grew up to a length of almost 2.5 cm, they were subjected to $CuSO_4$ treatment followed by hexane extraction.

Treatment regime: Twenty (20) best onions were selected based on root formation criteria (~2.5 cm) and each set of four onions were dipped in respective concentrations of $CuSO_4$ solutions (25, 50, 100 and 250 µM) including the control (without $CuSO_4$). The treatment was given for 24 hours maintaining 12 hrs day: 12 hrs night cycle. Post treatment the roots were finely cut and were dried at 50˚C temperature. Extraction was performed using GC-MS grade hexane maintaining the solvent volume and dry weight ratio constant.

### Identification of volatile compounds

Gas chromatography/ mass spectrometry (GC/MS) analysis was performed on Shimadzu GC interfaced with a mass spectrometry using SH-Rxi™-5Sil (Shimadzu) column. The oven temperature ranged from 70ºC to 260ºC. A 49 min method was programmed at 5ºC/minute ramping with initial hold time for 1 minute and final hold time for 10 min. The carrier gas was He at a flow rate of 1 ml/minute. The injection port was set at 250ºC. MS operating parameters include electron impact ionization at 70 eV with mass range of 50-600 amu. 2 µL of sample of each condition was injected. Data was analysed by comparing with NIST mass spectral library 2017 (Version 1.0) and considering Kovat index, obtained from online server (El-Sayed and Ashraf 2008) [12].

## Results and Discussion

Cu has been reported to exert toxic effect in the *A. cepa* system by various authors. Yidiz *et al*. 2009 [13] showed that $CuSO_4$ significantly reduced the mitotic index of the onion root cells. Presence of heavy metals such as Cu, Cd etc in the wastewater was effectively increased the antioxidant enzymatic activity in *A. cepa* (Fatima *et al*. 2005) [14]. It was evident from these studies that $CuSO_4$ is capable of altering the metabolic activity in *A. cepa* which in turn can affect the phytochemical profile of the plant as found in our study.

The volatile organic compounds from hexane extract were identified by GC-MS analysis (Table 1). The analysis of onion roots allowed the identification of eleven compounds. The mass spectroscopy results showed the presence of a number of volatile organic compounds in the control set compared to that of the treated ones. O-xylene was a compound present in all the sets except 25 and 50 µM at a retention time of 3.86 minute. It is noteworthy that eicosane, tetracosane and pentacosane were only present in the control and not identified in any other test concentrations. The absence of these compounds indicated the direct effect of copper on the metabolic Constituents onion. Docosane was found in all the test concentrations including the control, except 50 µM condition. Similarly, Hexacosane was identified only in control and 100 µM concentration but not in other treatments (Figure 1 and 2).

**Table 1:** List of volatile compounds identified by GC-MS

| Compound Name | Class | KI | RT | Control | Treatment (µM) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | 25 | 50 | 100 | 250 |
| o-Xylene | Aromatic hydrocarbon | 888 | 3.85 | 3.40 | -- | -- | 1.74 | 5.95 |
| Hexadecanoic acid (Palmitic acid) | Fatty acid | 1984 | 28.29 | 2.35 | -- | -- | ND | ND |
| Eicosane | | 2000 | 32.77 | 1.38 | ND | ND | ND | ND |
| Heneicosane | | 2100 | 34.49 | 3.78 | 4.24 | 3.52 | 4.5 | 5.94 |
| Docosane | | 2200 | 36.14 | 7.79 | 8.36 | ND | 9.78 | 5.94 |
| Tetracosane | | 2400 | 37.73 | 14.63 | ND | ND | ND | ND |
| Pentacosane | Alkane | 2500 | 39.27 | 16.21 | ND | ND | ND | ND |
| Hexacosane | | 2700 | 40.97 | 16.79 | 18.65 | 16.11 | ND | 16.73 |
| Nonacosane | | Not Available | 45.56 | 10.83 | ND | 6.09 | 6.89 | ND |
| Tetracontane | | Not Available | 48.16 | 6.09 | 6.51 | 9.61 | 4.71 | ND |
| Hexa- triacontane | | 3600 | 50.20 | 4.16 | ND | 5.83 | ND | ND |

ND –not determined; KI- Kovat Index; RT- Retention Time; -- - no peak detected

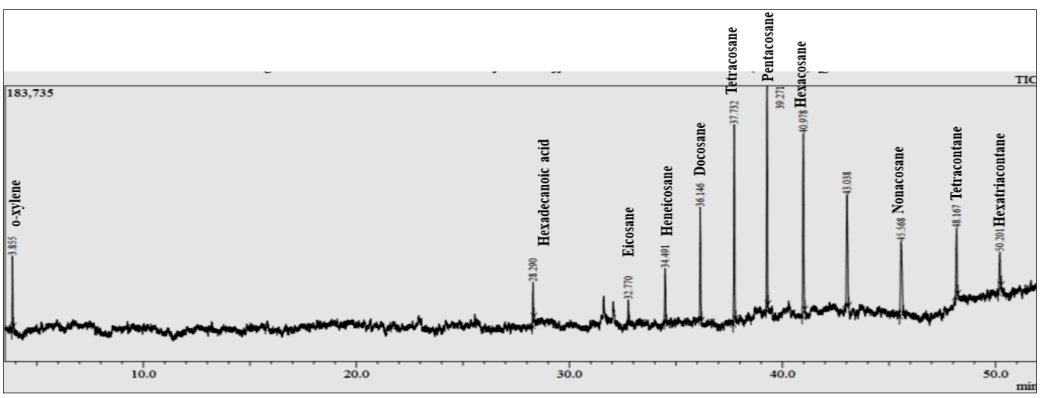


**Fig 1:** Mass spectrum of volatile compounds in Control

In agreement with the finding of Lekshmi *et al*. 2014 [15] hexadecanoic acid is also detected from the *A. cepa* root extract in our study. Further, volatile flavour components such as eicosane, docosane, tetracosane and pentacosane those were identified in the present study were also reported in *Allium grayi*, a plant belonging to the same family of *Allium cepa* (Hashimoto *et al*. 1984) [16]. Alkanes such as tetracosane and hexa-triacontane determined in the hexane extract also detected in the essential oil from *Spondias mombin* L.(Cajá), *Spondias purpurea* L. (Ciriguela) and *Spondia* sp. (Lima *et al*. 2016) [17].

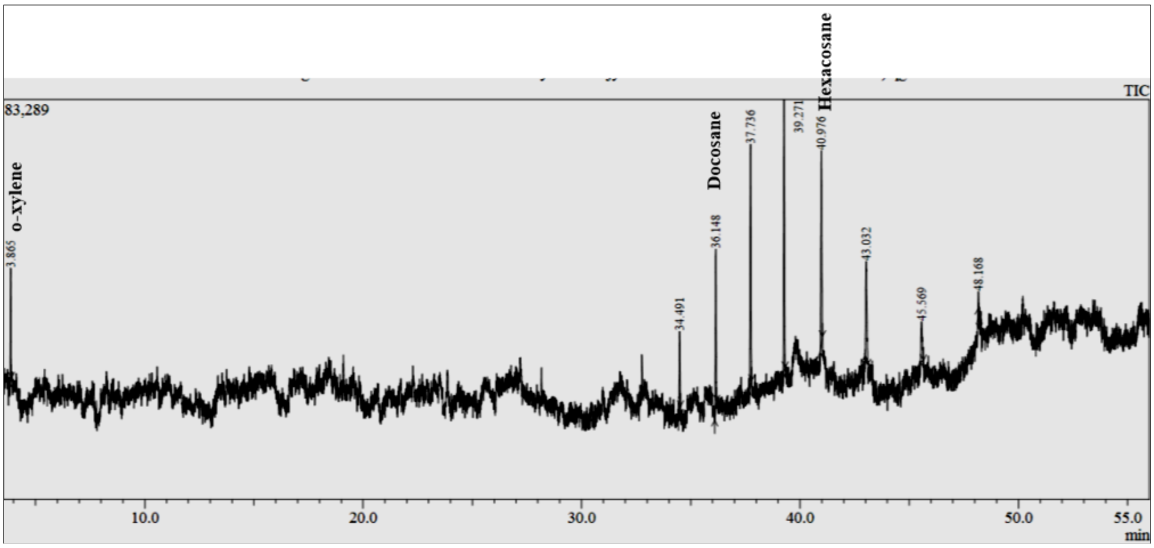


**Fig 2:** Mass spectrum of volatile compounds in 250µM Concentration of Test solution

## Conclusion

In the present study an easy and an effective method for the analysis of volatile compounds in onion roots using GC-MS was performed. It can be concluded from outcome of the current investigation, that treatment with $CuSO_4$ for 24 h, can alter the metabolic profile of the *Allium cepa* system as determined by GC-MS analysis.

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