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Evaluation of Zinc on pigment damage in *Crocus sativus L kashmirainus c.v*.

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ABSTRACT

Saffron plant (Crocus sativus L.) is a highly valued plant from which world's most expensive spice, saffron is derived. Saffron plant is grown in few Mediterranean countries like Spain, France, Iran and Turkey. In India saffron is mainly cultivated in Kashmir around the Pampore karewas. A number of medicinal properties have been ascribed to various parts of this highly valuable plant. From past few decades there has been constant increase in the growth of cement factories around the area where saffron is cultivated. The discharge of cement factories generally consists of particulate matter, sulphur dioxide and nitrogen oxides producing continuous visible clouds which ultimately settle on the surroundings as a result the whole ecosystem around the cement factories is subjected to extraordinary stress and abuse. This study was carried out, first of its type to evaluate the impacts of cement dust on growth and development of saffron. Five sampling sites were selected on the basis of distance from a cement factory (JK cement factory, Khrew) towards the control site (Lethpora, 12 km away from factory site). Current study was taken up for 2 years from March 2013–March 2015 during vegetative phase of saffron plant. From our findings it was observed that accumulation of Zinc beyond permissible levels in saffron plant and soil have adverse affects on growth and development of saffron plant vis-a-vis its primary and secondary metabolite constituents.

Keywords: Saffron, Cement, Kashmir, Metabolites

I INTRODUCTION

Saffron plant (*Crocus sativus* L.) is a flowering plant species of the Crocus genus in the Iridaceae family. It is best known for the spice saffron, which is produced from parts of the flowers, treasured for its golden-colored pungent stigmas. Saffron is the most precious and most expensive spice in the world derived from the stigma of the flower of saffron plant. Saffron contains many plant derived chemical compounds that are known to have several biological activities including antioxidant, anti-inflammatory, and antiproliferative (Abdullaev, 2002; Assimopoulou, et al., 2005; Chang, et al., 1964). Besides medicinal value, saffron is commercially important for its culinary uses and is one among the important cash crops of India.

In India saffron grows in Kashmir only in the Pampore region. In proximity to the saffron cultivating region a number of cement industries have been established during past few decades producing tones of cement annually. As India is the second largest cement producer in the world, so cement production of country is expected to increase at an alarming rate as per the Indian Cement Review, 2011. Cement is a fine, gray or white powder which is largely made up of cement kiln dust (CKD), a by-product of the final cement product, usually stored as waste in open-pits and landfills. Exposure to cement dust for a short period may not cause serious problems; however prolonged exposure can cause serious irreversible damage to plants and animals (Nemmar, et al., 2003; Vallejo, et al., 2003).

At the times when millions are spent on various studies to improve saffron quality and quantity, no attention has been payed on growth of cement factories, brick kilns and stone crushers around the area which is the main habitat to this valued plant. We have earlier done an exclusive study on impact of cement pollution on human health in the area and while carrying the study we felt that not only human health but plantation around the area is strongly affected by the pollution. In this backdrop we conducted a study to evaluate effect of cement pollution on saffron plant.

II MATERIALS AND METHODS

Study Sites

Five sites were selected for present study; these are listed in table 1.

Sites Selected	Characteristic Features		
	Geographical co-ordinates	**Altitude**	**Location**
Site I	34°02′ 08.61″N latitude and 075°01′02.2″E longitude	1650masl (5413.3 feet)	0 km away from the cement factory
Site II	34°02′37.7″N latitude and 074°57′31.0″E longitude	1648 masl (5406.8 feet)	3 km away from the cement factory
Site III	34°01′53.8″N latitude and 074°57′32.5″E longitude	1645 masl (5396.9feet)	5 km away from the cement factory
Site IV	34°02′15.0″N latitude and 074°55′30.0″E longitude	1645 masl (5396.6feet)	7 km away from the cement factory
Site V	34° 01’81.61’’N latitude and 074° 57’ 33.2’’E longitude	1642 masl (5387.1feet)	12 km away from the cement factory

Table 1: Characteristic feature of various sites

Sample collection

During the vegetative phase of the saffron plant, samples were collected, properly processed and stored as per requirement for different parameters to be analyzed.

Heavy metal analysis

The soil samples were mainly collected from a depth of 15 cm. The soil samples were dried thoroughly and sieved through a <2 mm sieve. Then, the sieved soil samples were digested. While as plant samples were collected separately from different sites. The collected material was washed thoroughly with running tap water followed by washing with DDW (double distilled water) to remove the dust particles and possible parasites. They were shade dried, powdered and stored in closed air tight bottles for further experimentation.

Analytical procedure

A 1g of the powdered sample was weighed into a Teflon conical flask. 20 ml of the digestion mixture (a mixture of sulphuric acid, perchloric acid and nitric acid in ratio 1:4:40 by volume) was added and left to stand overnight. Thereafter, the flask was heated at 70^{o}C for about 40 minutes and then, the heat was increased to 120°C. The mixture turned black after a while and the digestion was complete when the solution became clear with appearance of white fumes. The digest was diluted with deionized 20ml of water and boiled for 15minutes. This was then allowed to cool, transferred into 100ml volumetric flasks and diluted to the mark with deionized water. The sample solution was then filtered using whatmann filter paper (pore size 0.45µ, Axiva) into a screw capped polyethylene bottle and stored for Zinc determination using Atomic Absorption Spectrophotometer (AAS, Perkin Elmer Germany) with a digital read out system. The standard stock solutions (1000 ppm) were diluted to obtain working standard solutions ranging from 1 ppm to 100 ppm and stored at 4°C. A calibration curve was plotted between measured absorbance and concentration (ppm). All the samples were analyzed in triplicate using Atomic Absorption Spectrophotometer (AAS, Perkin Elmer Germany) except Mercury (Hg) which was estimated by method of Kakar *et al* .(Mishra, 2007)

Pigments

Chlorophyll a, Chlorophyll b and total Chlorophyll were estimated according to the standard procedure (Strain, 1971). Carotenoids were estimated according to the standard procedure set forth by Vernon, 1960. Phaeophytins were estimated according to the standard procedure set forth by Duxbury and Yentech, 1956.

III RESULTS

Heavy Metal Analysis

The saffron soil and saffron plant samples (corms and needles) were analyzed for Zinc during the vegetative phase of the plant. From the analysis, following results (Table: 2). were obtained as mentioned below:

Table 2: Estimation of heavy metals (ppm) in saffron plant and soil during vegetative phase

Pigment Analysis

Sites	Vegetative Phase		
	Soil	**Corms**	**Needles**
Site I	$^{b}_{c}3.81\pm0.001$	0	$^{bc}_{c}3.99\pm0.002$
Site II	$^{c}_{bc}3.26\pm0.002$	0	$^{c}_{b}2.65\pm0.003$
Site III	$^{c}_{b}3.16\pm0.005$	$^{b}_{b}0.67\pm0.003$	$^{b}_{b}2.11\pm0.002$
Site IV	$^{c}_{a}2.84\pm0.001$	$^{a}_{a}0.43\pm0.001$	$^{a}_{a}0.24\pm0.001$
Site V	$^{b}_{a}2.65\pm0.001$	$^{a}_{a}0.39\pm0.001$	0

Values are represented as mean±SD (n=3), Data was analyzed by ANOVA using Duncan's multiple range test (SPSS17.0); the values with different superscript along the columns and different subscripts along the sites of soil, corms and needles are statistically significant at p<0.05.: Bold values indicate above permissible limits.

The estimation of chlorophyll 'a', chlorophyll 'b', total chlorophyll, pheophytin 'a', Pheophytin 'b', total Pheophytin and Carotenoids was estimated in the needles of saffron during both vegetative phase. The concentration of photosynthetic pigments analyzed showed a significant increase (P<0.05) with respect to factory site during vegetative phase (Table: 3) Thus from the

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observations it is clear that there was sharp decrease in pheophytin ‘a’ during 2014 when compared with the average values of 2013 at all five study sites and during this phase. While as in case of other pigments there was gradual decreasing trend while moving from cement factory site towards control (site I< site II< site III <site IV <site V).

Table 3: Estimation of various pigments (µg/g) in saffron plant during vegetative phases

Sites	Concentration µg/g													
	Vegetative phase													
	2013							2014						
	Chlorophyll (a)	Chlorophyll (b)	Total Chlorophyll	Pheophytin (a)	Pheophytin (b)	Total Pheophytin	Carotenoids	Chlorophyll (a)	Chlorophyll (b)	Total Chlorophyll	Pheophytin (a)	Pheophytin (b)	Total Pheophytin	Carotenoids
I	^{a}2. 2 ±0.1	^{a}1.7 ±0.2	^{a}3. 9 ±0.1	^{a}3.3 ±0.2	^{a}3. 7 ±0.05	^{a}6.9 ±0.4	^{a}1.6 ±0.05	^{a}0.57 ±0.6	^{b}2.6 ±0.7	^{a}3.1 ±1.1	^{a}0.99 ±0.3	^{a}0.9 ±0.4	^{b}3.7 ±0.6	^{a}1.3 ±0.1
II	^{a}3. 5 ±0.05	^{a}2. 4 ±0.2	^{b}5. 7 ±0.08	^{a}4. 5 ±0.09	^{b}6.3 ±0.2	^{b}9. 3 ±0.09	^{b}2.3 ±0.1	^{b}1.6 ±0.6	^{a}1.5 ±0.2	^{a}3.1 ±0.8	^{a}1.8 ±0.3	^{a}1.9 ±0.6	^{a}2.4 ±0.2	^{b}2.9 ±0.7
III	^{c}6. 5 ±0.3	^{c}4. 8 ±0.06	^{d}11.3 ±0.3	^{b}7.1 ±0.07	^{c}9. 7 ±0.1	^{c}13.4 ±0.3	^{c}4.5 ±0.04	^{a}0.9 ±0.6	^{a}1.9 ±0.4	^{a}2.9 ±0.2	^{a}1.7 ±0.7	^{a}1.4 ±0.6	^{a}2.5 ±0.7	^{b}2.5 ±0.4
IV	^{bc}5. 5 ±0.1	^{b}3.5 ±0.01	^{c}8.7 ±0.1	^{b}8.5 ±0.1	^{d}12.6 ±0.1	^{d}18. 5 ±0.09	^{bc}3.2 ±0.2	^{b}1.6 ±1.1	^{b}2.1 ±0.4	^{a}3.5 ±0.9	^{a}1.5 ±1.1	^{a}1.1 ±0.8	^{b}3.5 ±0.3	^{b}2.5 ±0.5
V	^{c}7. 2 ±0.009	^{bc}4. 5 ±0.2	^{d}11. 4 ±0.2	^{b}8.9 ±0.2	^{e}13. 3 ±0.1	^{d}18. 4 ±0.2	^{b}2.7 ±0.03	^{c}3.5 ±0.2	^{b}2.5 ±0.08	^{b}4.9 ±1.4	^{a}2 ±0.05	^{b}7.5 ±0.1	^{c}13.2 ±0.06	^{c}4. 8 ±0.09

Values are represented as mean±SD (n=3), Data was analyzed by ANOVA using Duncan’s multiple range test (SPSS-17.0); the values with different superscript along the columns are statistically significant at $p<0.05$.

IV DISCUSSION

Unplanned developmental activities over the globe have altered the environmental quality both at micro as well as at macro levels which is accompanied by some form of pollution, which threatens not only animal and plant life but the very existence of the human race. Cement industry though socioeconomically an important sector gives rise to substantial quantity of dust emissions which causes an adverse impact on the environment. Cement factories are major source of pollutants in the neighborhood areas. Dust is a major component produced during production of cement. It usually results from frequent vehicular movements inside construction plants and other earth-moving processes, including excavation. Other activities related to cement and dust production include quarrying operations, grinding and blending of components during production, kiln operation stacks, packaging, transportation and storage of raw materials, besides kiln operation also serves as the largest source of dust. Dust is considered as a major environmental problem because it can cause severe pollution(Mehraj *et al.*, 2013). The dust and other pollutants emitting from the cement factories, apart from getting deposited in the upper crust of the soil and changing its composition and chemistry, get deposited on the foliage, block the stomata or small pores through which plants breathe, affect the photosynthetic rate, pollination and other processes vital to the plant life.

With the ongoing technological advancements in industrialization and urbanization process, release of toxic contaminants like heavy metals in the natural resources has become a serious problem worldwide. Metal toxicity affects crop yields, soil biomass and fertility. Presence of any heavy metal, like mercury, arsenic, nickel, cobalt, cadmium, copper, lead and chromium in air, soil and water released from cement factories can cause bioaccumulation affecting the entire ecosystem (Finnegan & Chen, 2012; Joshi & Swami, 2009; Sethy & Ghosh, 2013).As a trend concentration of Zinc should increase at polluted site but the results are somehow different as the Zn concentration was found minimum at cement factory site and maximum at control site (Pena, et al., 2011; Sfaxi-Bousbih, et al., 2010; Singh, Nath, & Sharma, 2007). The reverse trend is due to the fact that high concentration Cu has a tendency to replace Zn (Ryu, Park, & Lee, 2003).

Photosynthesis is of fundamental importance in utilizing the carbon locked up in plant material as a source of bio fuel energy. Because of the fundamental biological significance of photosynthesis and its importance in agricultural productivity, a considerable amount of research has been directed toward understanding the effects of environmental factors on the process(S. Jan, 2011). The chlorophyll pigments are essential component for photosynthesis and occur in chloroplast, as green pigment in all photosynthetic plant tissues. A decrease in chlorophyll has been used as an indicator of an air pollution injury. The increase in chlorophyll content of saffron needles, from the cement factory site to control site obtained in the present study is in line with the previous findings and is possibly due to the decrease in the deposition of cement dust with increasing distance from the factory (Farmer, 1993). Chlorophyll content in a cement-polluted environment, were associated with a decrease in the levels of stomatal and cuticular transpiration of encrusted leaf surfaces (A. Jan, 2009). It might also be due to chloroplast damage by incorporation of cement kiln dust into leaf tissues. The cement kiln dust decreases chlorophyll content, confirming the earlier findings (Uysal, Ozdilek, & Ozturk, 2012). The same trend was observed for pheophytins during the study period (site I< site II< site III< site IV< site V). Chlorophyll a is degraded to Pheophytin under SO_2 affect by replacing Mg^{2+} ions with two atoms of H^+, from chlorophyll molecules. In chlorophyll b, SO_2 removes the phytol group of the chlorophyll b molecules. Dust from a cement factory seems to cause substantial changes to leaf physiology, possibly leading to reduced plant productivity. The present results are consistent with those reported earlier that cement dust decrease the total leaf pheophytins (Nanos & Ilias, 2007).

Carotenoids are a class of natural fat-soluble pigments found principally in plants, algae and photosynthetic bacteria, for their critical role in the photosynthetic process. They act as accessory pigments in higher plants. They are tougher than chlorophyll but much less efficient in light gathering, help the valuable but much fragile chlorophyll and protect chlorophyll from photoxidative destruction(Joshi & Swami, 2009). Carotenoids protect photosynthetic organisms against potentially harmful photoxidative processes and are essential structural components of the photosynthetic antenna and reaction center (Joshi & Swami, 2009). Present investigation revealed the decreased carotenoid content in cement factory site when compared to control site. The result are in agreement with findings of who showed that plant species subjected to air pollution showed highest decrease in carotenoid contents and same observations were reported by many other workers (Joshi & Swami, 2009). The deposition of cement dust on herbaceous plants and fruit crops can cause effects that range from blocked stomata, reduced number of plant leaf and injury to complete reduction in vegetative growth and reproductive structures.

V CONCLUSION

From this study it can be concluded that there is decrease in chlorophyll, pheophytins and carotenoid content in saffron needles at cement factory was apparently due to high cement pollution. An increase in zinc at reference sites when compared with polluted site is due to high accumulation of copper at polluted site, which replaces Zinc.

Thus decrease in the primary and secondary metabolite production of saffron plant will ultimately lead to qualitative and quantitative degradation in the saffron. The current study makes a strong case to take stringent steps against establishing of cement factories in the area and for policy law makers to rethink about the already adopted policies, so as to ensure that further damage to this important cash crop is avoided.

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