

EFFECT OF IRRIGATION UPON IRON REDOX SPECIES WITHIN *BRASSICA OLERACEA* VAR. *CAPITATA* L.

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ABSTRACT

This research work is based on the study of different redox species of iron present in *Brassica oleracea* var. *capitata* by using recommended analytical methods reported for UV-Visible spectrophotometer with few modifications. Two methods viz 1,10-Orthophenanthroline (opt) and potassium thiocyanate (KSCN) methods were applied to determine total and ferric iron form, respectively, and the ferrous was calculated from difference of these two forms. For opt method hydroxylamine hydro chloride was used to reduce all ferric into ferrous. Buffer solution of sodium acetate was used to maintain pH between 5 and 9 and opt was the complexing agent to give orange red Fe (II)-opt complex. Amount of all reagents were set with respect to time for complexation. KSCN method, potassium thiocyanate was used as complexing agent and it gives blood red Fe (III)-SCN complex, so its amount was set with respect to time. Calibration curves drawn for both the Fe (II)-opt and Fe (III)-SCN methods in which correlation coefficients (r^2) showing good precision of the method was applied for sample analysis. Samples of *Brassica oleracea* were collected from Malir and Thatta fields irrigated with sewage and fresh water, respectively. Fresh and sewage water samples showed that total iron, ferric and ferrous was 17.0-49.5mg/100g, 9.5-27.4mg/100g, 7.5-14.3mg/100g, 5.18-12.8mg/100g, 1.00-3.24mg/100g, 2.89-9.4mg/100g, respectively. The results revealed that total iron is found to be high in fresh water samples whereas ferrous is found to be high in the samples irrigated in sewage water collected from Malir fields.

Key-words: Redox species, *Brassica oleracea*, Total Iron, irrigation, sewage water, fresh water, Malir, Thatta.

INTRODUCTION

Elemental nutrients primarily required to living being are classified into macro and micro based on their need Calcium, Phosphorous, Magnesium, Sodium, Potassium, Chlorine and Sulphur are termed as macro nutrients as body require these in larger quantities whereas metals from the first transition series are Vanadium, Chromium, Manganese, Iron, Cobalt, Nickel, Copper and Zinc and metals like Molybdenum and Tin are also known as micro or trace metals as their occurrence in the environment range from 0.1 to 10 ppm quantities in the environment matrices. Some of trace metals (iron, copper and zinc) are essential for plants and animals as well (Wintz *et al.*, 2002). The availability of copper, zinc, iron, manganese, molybdenum, nickel and cobalt are essential micronutrients (Reaves and Baker, 2000), but excess uptake to the plant requirements may exert toxic effects (Mark *et al.*, 2000; Monni *et al.* 2000). Plants as a natural source of concentrated mineral nutritional supplements. Vegetable are essential for human diet that contains important trace elements and nutrients (Abdullah and Chmielnicka, 1990). Vegetables are considered as ‘Protective supplementary food’, contain large quantities of minerals, vitamin, carbohydrate, essential amino acid and dietary fibers, which are required for normal growth (Huheey *et al.*, 2000).

Cabbage (*Brassica oleracea* var. *capitata*) is the most important green leafy vegetables grown worldwide. It belongs to the family of Cruciferae that also include cauliflower, kale, and broccoli. Cabbage is an essential source of vitamin C also containing some vitamin B potassium and calcium (Haque, 2006). Different cultivated types show great difference in cabbage with respect of the size, shape, and color of leaves as well as size, shape, color, and texture of the head (Singh *et al.*, 2006). Nieuwhof (1969) categorized the various forms of cabbage into white cabbage, red cabbage, and Savoy cabbage. The intake of cabbage has extremely increased in recent years, from 1.64 to 1.99 kg/person from 2006 to 2010 because of the benefits of these vegetables are becoming better known by the consumers. It is also necessary that consumers know that the variation in the antioxidant activity of *Brassica* vegetables is depended on many factors including growing conditions, cultivar, maturity at harvest, and post-harvest storage conditions (Singh *et al.*, 2007). Growth and yield of cabbage is influence by organic and inorganic source of nutrients. Use of inorganic fertilizers for crops is not encouraged due to residual impacts on health, whereas organic fertilizers carry no such problems and enhances the productivity of soil as well as crop quality and yield (Tindall,

2000). Cabbage, as other cruciferous vegetables, has high nutritional value and contains specific sulfur compound glucosinolates that increase its antioxidant activity. A single serving of cabbage contains nearly half of the daily Vitamin C requirement and also contain significant levels of manganese, iron, and vitamin B6. Cabbage also is high in dietary fiber and low in calories (Melody Rose, 2008).

Cabbage has good responsiveness on animal manure application in quantity of 40 t/ha. Organic fertilization increases the soil biological activity, improves nutrient mobilization and soil structure and enhances soil water retention. System relying on organic fertilizers as plant nutrient sources have different dynamics of nutrient availability than those using minerals fertilizers. Food is the key source of trace elements for human beings. The people of Pakistan, mostly low-income groups, eat a major quantity of vegetables. The necessary amount of vegetables and fruit in our daily diet must be 400g per person, assuming a standard portion size of 80g (Antonio, 2005).

As increasing food demands with time, there is need to adopt different method of irrigation by which more quantity of the crop can be obtained (Liu *et al.*, 2009). Irrigation with sewage water is another substitute being applied in the metropolitan. As sewage water contain macro and micronutrients like potassium, phosphorous, copper, zinc manganese, and iron etc. therefore sewage water is an alternative source of fertilizers and it may be used for irrigation purposes.

MATERIAL AND METHODS

Collection, pretreatment and preparation of samples

Already reported method of (AOAC, 2000) was applied with slightly modification. All the dilutions, preparation of primary and secondary standards were made using deionized distilled water. Certified analytical grade chemicals were used in the research work. *Brassica oleracea* var. *capitata* samples were collected from different districts of Karachi city. All the collected samples were cut into pieces followed by proper washing with distilled water. Moisture was removed through hot air oven at temperatures between 110-120°C for 5 to 6 hours. Dried samples were crushed with piston motor to fine powder. All the samples were analyzed in triplicates. Accurately about 3g of dried sample was weighed using Digital Weigh balance (Panther-USA) then placed in furnace ((Nabertherm, model no. B180) at 550°C for 12 hours. Once the white or grey ash was obtained, 4.0mL of 4M HCL was added to dissolve the samples. Then undissolved particles were centrifuged finally the solution were transferred to a 25mL volumetric flasks and volume was raised up to the mark by HCL solution. The samples were then kept for 24hours before complexation.

Analysis of samples

Determination of total Iron-Method 1

For opt method, hydroxyl amine hydro chloride was used to reduce all iron specie in to ferrous, buffer solution of sodium acetate was used to maintain pH between 5 and 9 and opt was the complexing agent to give orange red Fe (II)-opt complex. All parameters were optimized for complexation. Volume of reducing agent and buffer solution were set at 2ml and 4ml respectively, 30 minutes were found to be proper time for complexation, volume of opt was fixed on 2.0mL throughout experiment (Fig. 1 & 2). Before complexation the digested samples of cabbage were kept overnight (Rajan, 2011) after digestion and dissolved in HCl then all the work was performed in triplicates. Absorbance was determined by UV-Visible spectrophotometer (Jenway 6310) at 520nm. Total iron was determined using calibration curve method. For calibration curve method, standard solution of Fe (II) of different concentrations were used after treated with reagents as mention above.

Determination of ferric form of Iron-Method 2

For KSCN method, potassium thiocyanate was used as complexing agent and it gives blood red Fe (III)-SCN complex. 5ml KSCN was found sufficient for complex formation in thirty minutes (Fig. 3 & 4). Absorbance was determined by UV-Visible spectrophotometer (Jenway 6310) at 480nm. Fe (III) was determined using calibration curve method. For calibration curve method, standard solution of Fe (III) of different concentration were treated with reagent, potassium thiocyanate.

RESULTS AND DISCUSSION

Iron is found in human body as ferrous and ferric, both are important but ferrous is bioavailable. In this research work spectrophotometric technique with calibration curve method with some modifications was applied for

estimation of redox species of Iron. Total (T_{Fe}) and ferric (Fe^{+3}) iron forms were estimated by using opt and KSCN methods respectively, ferrous (Fe^{+2}) was found from difference of both species. For calibration curve method, eight standards of range e^{-04} , e^{-05} , e^{-06} and e^{-04} , e^{-05} were drawn for Fe (III)-SCN and Fe(II)-opt method, respectively. Summarized data of absorbance of standard solutions of different concentrations ranges with correlation coefficients (r^2) is presented in (Table 1) which shows good precision of the method applied for the sample analysis.

Table 1. Data for calibration curves drawn for Fe (II)-opt & Fe (III)-SCN Complexes of different concentration ranges with correlation coefficients (r^2).

| Fe (III) SCN | R^2 | Fe (II)-opt | | R^2 |
|---|--------|---|---|--------|
| | | Conc range for Fe (III) | Conc range for Fe (II) | |
| $1 \times 10^{-4} \dots 8 \times 10^{-4}$ | 0.7998 | $1 \times 10^{-4} \dots 8 \times 10^{-4}$ | | 0.9807 |
| $1 \times 10^{-5} \dots 8 \times 10^{-5}$ | 0.9810 | | $1 \times 10^{-5} \dots 8 \times 10^{-5}$ | 0.9740 |
| $1 \times 10^{-6} \dots 8 \times 10^{-6}$ | 0.9605 | | | |

Table 2. Concentration of Total Iron (T_{Fe}), Ferric (Fe^{+3}) and Ferrous (Fe^{+2}) in sample of *Brassica oleracea* collected from Thatta and Malir irrigated in fresh water and Sewage water, respectively.

| Fresh water samples Thatta | | collected from | Sewage water samples Malir | | collected from |
|-------------------------------|-----------------|-----------------|-------------------------------|-----------------|-----------------|
| Total Fe mg/100g | Fe III mg/100g | Fe II mg/100g | Total Fe mg/100g | Fe III mg/100g | Fe II mg/100g |
| 20.0 ± 1.00 | 12.5 ± 1.00 | 7.50 ± 0.06 | 9.50 ± 0.49 | 4.66 ± 0.64 | 4.84 ± 0.14 |
| 22.5 ± 0.50 | 12.0 ± 0.81 | 10.5 ± 0.60 | 7.10 ± 0.76 | 3.00 ± 0.27 | 4.10 ± 0.80 |
| 17.0 ± 0.66 | 9.50 ± 0.35 | 7.50 ± 0.64 | 6.70 ± 0.71 | 2.46 ± 0.83 | 4.24 ± 0.89 |
| 18.8 ± 0.06 | 10.8 ± 0.90 | 8.00 ± 0.72 | 6.29 ± 1.00 | 2.26 ± 0.90 | 4.03 ± 0.69 |
| 18.9 ± 0.17 | 11.0 ± 0.46 | 7.90 ± 0.57 | 6.64 ± 0.83 | 2.24 ± 0.05 | 4.40 ± 0.78 |
| 21.6 ± 0.17 | 11.5 ± 0.45 | 10.1 ± 0.67 | 5.72 ± 0.85 | 2.52 ± 1.00 | 3.20 ± 0.80 |
| 21.8 ± 0.006 | 12.0 ± 0.08 | 9.63 ± 0.70 | 9.09 ± 0.18 | 4.20 ± 0.72 | 4.89 ± 0.12 |
| 42.8 ± 0.45 | 28.5 ± 0.10 | 14.3 ± 0.40 | 5.18 ± 0.22 | 1.24 ± 0.27 | 3.94 ± 0.30 |
| 38.4 ± 0.52 | 22.9 ± 0.57 | 15.5 ± 0.49 | 4.62 ± 0.69 | 1.00 ± 0.16 | 3.62 ± 0.56 |
| 18.3 ± 1.00 | 10.0 ± 1.00 | 8.85 ± 1.00 | 9.18 ± 0.21 | 1.80 ± 0.96 | 7.38 ± 1.00 |
| 32.0 ± 0.26 | 18.8 ± 0.81 | 13.2 ± 0.90 | 7.90 ± 1.00 | 3.50 ± 1.00 | 4.40 ± 0.88 |
| 49.5 ± 0.35 | 19.7 ± 0.96 | 13.3 ± 0.77 | 6.62 ± 0.17 | 3.00 ± 0.59 | 3.62 ± 0.47 |
| 42.2 ± 0.29 | 25.4 ± 0.36 | 16.8 ± 0.64 | 5.46 ± 0.29 | 2.57 ± 0.88 | 2.89 ± 1.00 |
| 33.0 ± 0.81 | 19.7 ± 0.50 | 13.3 ± 0.91 | 6.00 ± 0.29 | 2.04 ± 0.90 | 3.96 ± 1.00 |
| 44.6 ± 0.93 | 35.4 ± 0.02 | 9.20 ± 0.92 | 12.8 ± 0.10 | 4.24 ± 0.85 | 8.46 ± 0.91 |
| 20.5 ± 0.45 | 15.0 ± 0.33 | 5.50 ± 0.77 | 11.9 ± 0.70 | 3.30 ± 0.76 | 8.60 ± 0.67 |
| 25.0 ± 0.26 | 13.0 ± 0.13 | 12.0 ± 0.35 | 7.80 ± 0.44 | 1.65 ± 0.11 | 6.23 ± 0.50 |
| 27.4 ± 0.50 | 14.3 ± 0.08 | 13.1 ± 0.47 | 7.50 ± 0.22 | 2.90 ± 0.76 | 4.60 ± 0.56 |
| 28.1 ± 1.00 | 14.6 ± 0.40 | 13.5 ± 1.00 | 7.40 ± 0.65 | 1.69 ± 0.37 | 5.71 ± 0.47 |
| 22.5 ± 0.50 | 15.5 ± 0.83 | 7.00 ± 1.00 | 11.5 ± 1.00 | 2.10 ± 0.14 | 9.40 ± 1.00 |
| 35.0 ± 0.90 | 25.0 ± 1.00 | 10.0 ± 0.60 | 10.0 ± 1.00 | 1.60 ± 0.80 | 8.40 ± 0.75 |
| 36.0 ± 1.00 | 27.4 ± 0.25 | 8.60 ± 1.00 | 10.4 ± 0.54 | 1.62 ± 0.74 | 8.78 ± 0.43 |

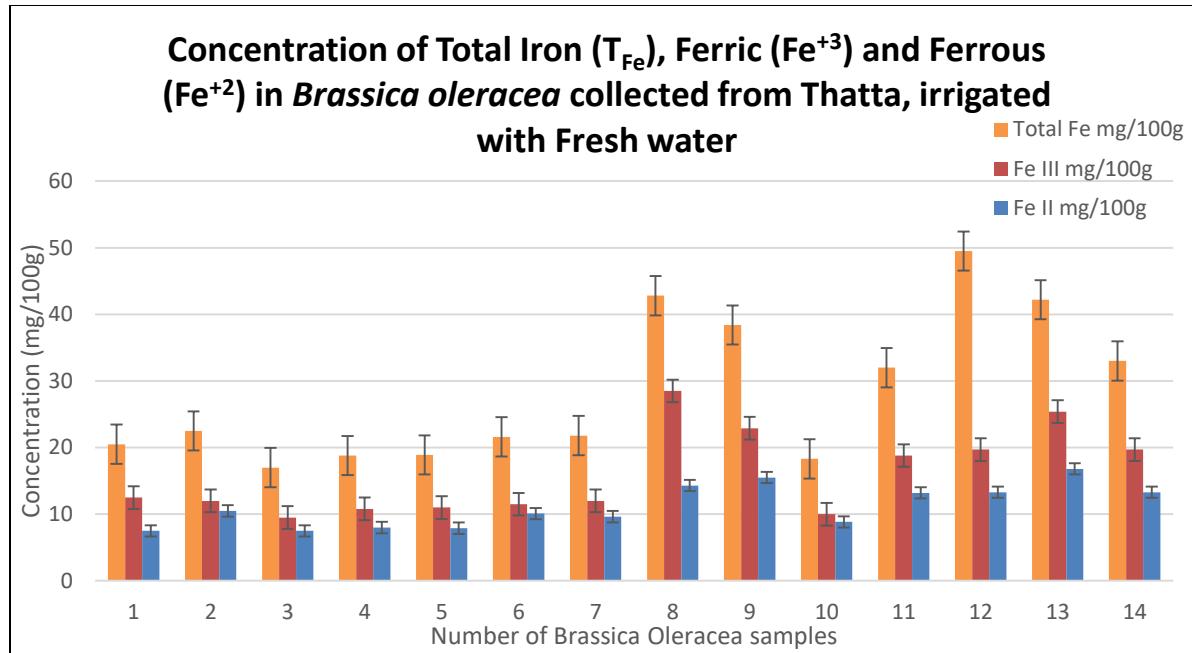


Fig.5. Concentration of Total Iron (T_{Fe}), Ferric (Fe^{+3}) and Ferrous (Fe^{+2}) in *Brassica oleracea* collected from Thatta irrigated in fresh water.

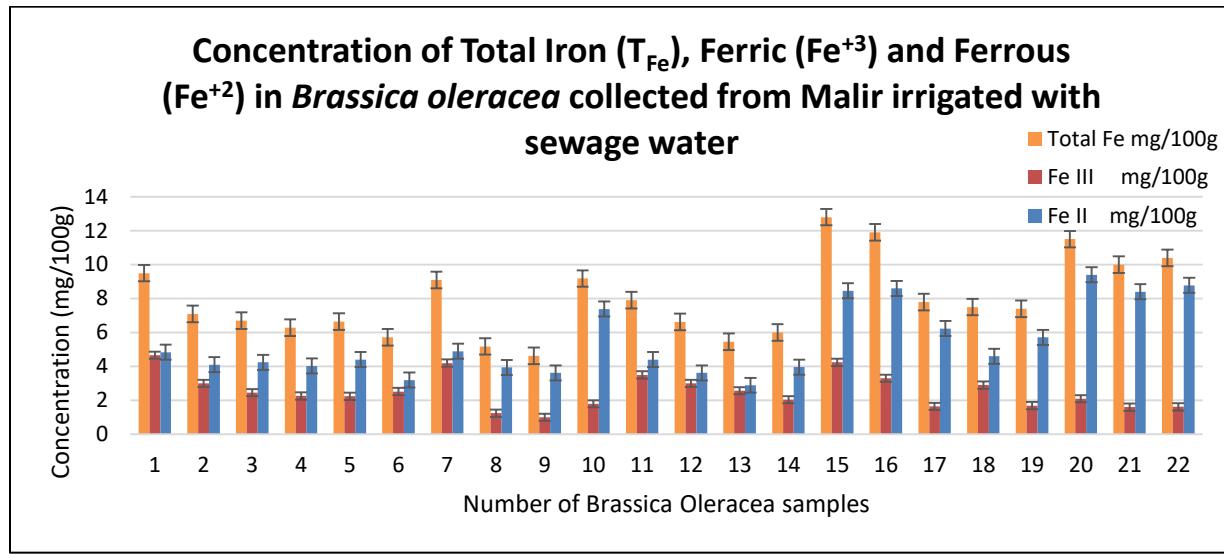


Fig. 6. Concentration of Total Iron (T_{Fe}), Ferric (Fe^{+3}) and Ferrous (Fe^{+2}) in *Brassica oleracea* collected from Malir irrigated in sewage water.

The amount of total iron (T_{Fe}) in fresh water sample found to be high as compared to samples of sewage water as shown in (table 2). Same result was concluded by Rani *et al.* (1970) in the sample of coriander and spinach samples where in sewage irrigated sample total Fe was found 628.73 and 895.71 mg/kg and in ground water irrigated sample total Fe was found 935.44 and 547.05mg/kg, respectively. While recent literature has now reverse result, continuous irrigation with sewage has significantly build-up Fe in the sewage irrigated soil sample (Al Omron *et al.*, 2012; Anwar *et al.*, 2016). T_{Fe} of fresh water in the present study sample ranged between 17.0-49.5mg/100g and sewage water found between 4.62mg-12.8mg/100g. It may because of high concentration of heavy metals present in sewage water that may affect uptake of iron due to metal-metal synergism and antagonism (Chibuike *et al.*, 2014). The behaviors of metal in soils are very complex, which involve adsorption-desorption, complexation-dissociation, oxidation-reduction, ion exchange, and other carrier transport role (Swartjes *et al.*, 2007). While Root

uptake, root selectivity, ion interactions, rhizosphere processes, leaf uptake from the atmosphere, and plant partitioning are important processes that ultimately govern the accumulation of metals and metalloids in edible vegetable tissues (McLaughlin, 2010). Along with pH, Soil type is an important factor in nutrient uptake as clays and organic soils hold nutrients and water much better than sandy soils making it less available for the plant.

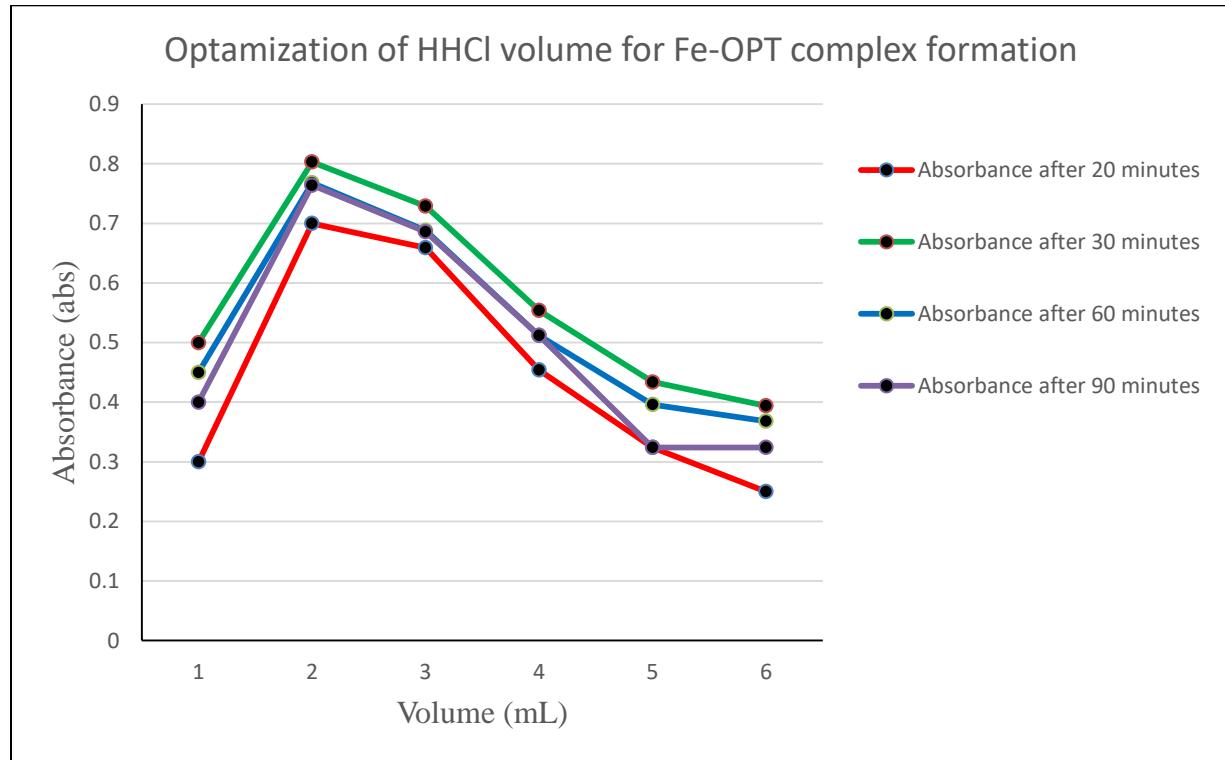


Fig.1. Effect of Volume of Hydroxylamine hydrochloride (HCl) on Fe-OPT complex Absorbance with Different Time Intervals.

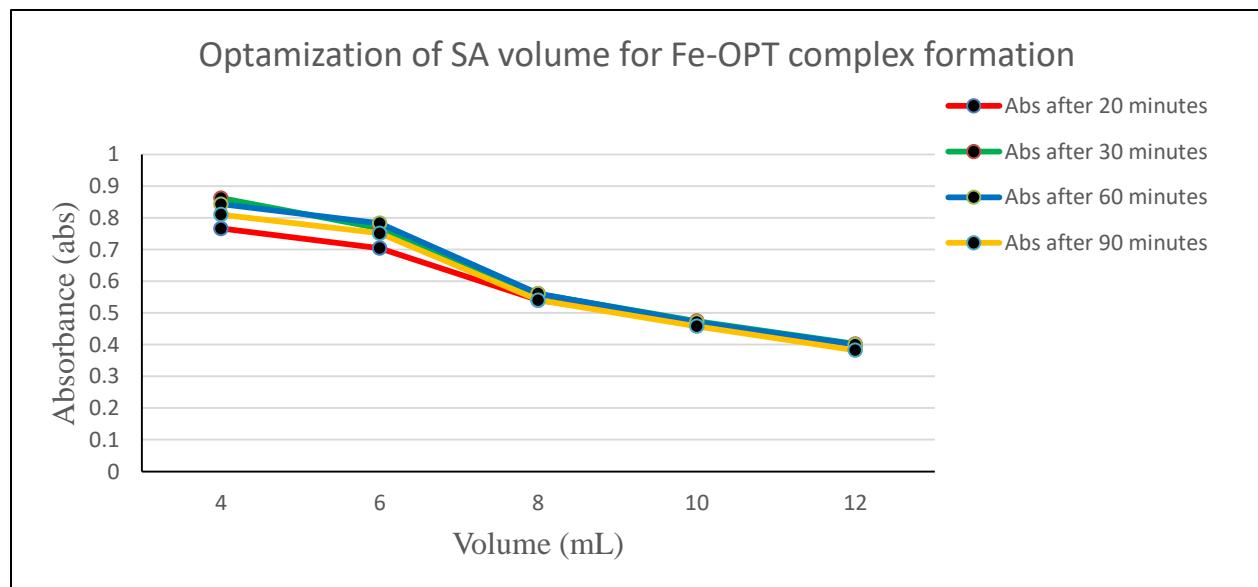


Fig.2. Effect of Volume of Sodium acetate (SA) on Fe-OPT complex absorbance with Different Time Intervals

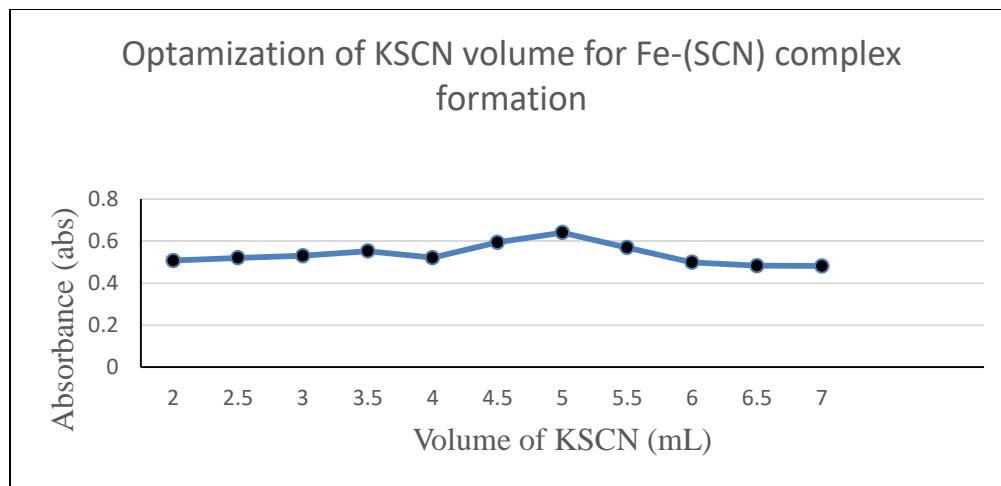


Fig.3. Optimization of volume of KSCN for Fe-(SCN) Complex Formation.

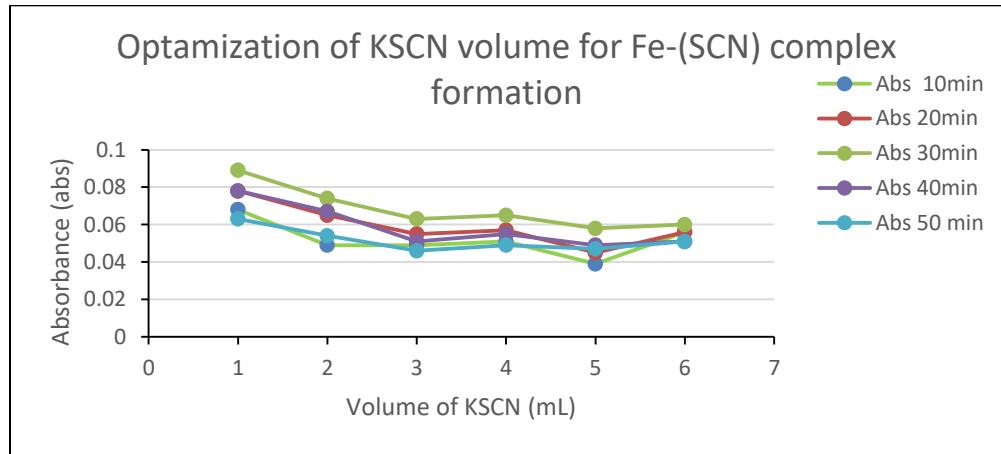


Fig.4. Effect of potassium thiocyanate volume on Fe-(SCN) Complex formation with different time intervals.

It is found that samples of sewage water have less ferrous (Fe^{+2}) as compared to fresh water irrigated cabbage (Fig. 5 & 6). Antioxidants have ability to reduce species and sewage contain synthetic phenolic antioxidants (Liu *et al.*, 2015) moreover cabbage is naturally abundant in antioxidants, presence of these reducing species is responsible for more Fe^{+2} in cabbage of sewage water (Chun *et al.*, 2004).

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