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Effect of Heavy Metals on Biochemical Parameters in *Spinacea Oleracea L.*

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ABSTRACT

The study of heavy metals (Cd, Cr, and Ni) effect on biochemical parameters in Spinacea oleracea L. (Palak). Spinach (Spinacea oleracea L.) is an edible leafy vegetable plant in the family Amaranthaceae, spinach is an annual plant (sometimes biennial), Spinach shows the growth up to 30cm in tall. It can survive over winter and in temperate regions also. The leaves Spinach are alternate, ovate to triangular, and mostly variable in size that is about 2–12 cm long and 1–10 cm in broad, the larger leaves on the base of the plant and small leaves on the higher stem. Common spinach, S. oleracea, considered as long in the family Chenopodiaceae, but in 2003, that was merged into the family Amaranthaceae in the order Caryophyllales, spinach with raw contains 91% water, 4% carbohydrates, 3% protein, and it also contains negligible fat. Spinach is rich in carotenoids, body can convert it into vitamin-A. The present research study was conducted to know the toxicity nature of heavy metals in Spinacea oleracea L. leaf and its remediation. Pot culture experiments were conducted with three treatments till productivity levels at Greenhouse of Botanical Garden, Department of Botany, Osmania University, Hyderabad. The three treatments consist of Treatment I control without any addition of heavy metals to the soil, Treatment II heavy metals spiked into the soil and Treatment. III, 1 % of calcium hydroxide added along with heavy metals to the soil. The results showed when compared to treatment I and III the high concentrations of heavy metals (Ni, Cd and Cr) are found in leaf of Spinacea oleracea L. in (Treatment II). In addition, the plants grown in treatment III with 1% Calcium hydroxide treated soil, reversed the growth suppression and inhibited the heavy metal toxicity in plants as evidenced by reduced heavy metal concentration in leaf. The study concludes that leafy vegetable Spinacea oleracea L. affected with heavy metals can be treated by using calcium hydroxide.

1. Introduction

The Heavy metals are metallic chemical elements which have relatively high density and poisonous at low concentrations. They have properties like ductility, conductivity, stability as cations etc., along with atomic weight ranging more than 20, showing density higher than 5g cm^{-3} (Weast, 1984).

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The Heavy metals enter into the food chain through drinking water that is by lead pipes, air emission and various other ways (John, *et al*., 2009). They become bio accumulated and lead to serious ecological and health problem (Malik, 2004). Heavy metals are of great interest for research purpose with respect to toxicological importance to human health, plants and animals (Almeida, *et al.*, 2007, Azvedo, *et al.*, 2007 and Jarup, *et al*., 2003). Consumption of food crops contaminated by heavy metals is a major food chain route for human exposure (Khan, *et al*., 2008). Various factors like metal plating industries, combustion of fossil fuels, nickel mining and electroplating have played a major role in nickel contamination (Khodadoust, A.P. *et al.*, 2004). This heavy metal contamination can be reduced by using techniques like using cadmium enriched phosphate fertilizer or applying sewage sludge (Cd-rich biosolids) to soils used for crops meant for human consumption (Campbell, P. G. C. *et al*., 2006). The aim of present study to is assess the effect of heavy metals (Ni, Cd and Cr) on biochemical parameters of *Spinacea oleracea* L. leaf.

2. Materials and Methods

Spinacea oleracea L. seeds were purchased from the Indian institute of horticultural research (IIHR) Bangalore, Karnataka State, India. 20Kgs of black soil was used for the pot experiments, the soil consists of 15.4% of clay and 3.5% of total carbon was maintained at pH 6.5. The heavy metal solution was prepared in the laboratory by following the (APHA.1992) guidelines. The different concentrations of heavy metals prepared are cadmium (10ppm), chromium (20ppm), nickel (16ppm). These heavy metals were dissolved in 150 litres of distilled water and sprayed on 600kg of black soil and dried in shade for 10 days for proper mixing of heavy metals in soil.1.5 kg of 1% Calcium hydroxide was added to the 300kgs of soil spiked with heavy metals.

3. Treatments

Treatment-I, 20 Kg of black soil was filled in 15 clay pots as control, Treatment-II, 20 Kg of black soil spiked with heavy metals was filled in 15clay pots, Treatment-III, 20 Kg of black soil spiked with heavy metals and 1% Ca (OH) 2 was filled in 15 clay pots. Seeds of *Spinacea oleracea* L. were sown in earthen pots at Greenhouse of Botanical Garden, Department of Botany, Osmania University Hyderabad, India. The plants were harvested till productivity level by following the standard protocol.

4. Biochemical Parameter Analysis

The Biochemical parameters were analyzed in fresh leaves of *Spinacea oleracea* L. The biochemical components like Chlorophyll (Arnon. 1949), Protein (Lowry *et al.,* 1957), Carbohydrates (Yoshida *et al*., 1976), Reducing sugars (Nelson, 1944), Non– reducing sugars (Loomis, 1980), Starch (McCready *et al*., 1950), Phenols (Swain *et al.,* 1959), Proline (Bates *et al*., 1973), DNA (Burton, 1968), RNA (Schmeider, 1957) and enzymes Catalse (Barber, 1976), and Peroxidase (Kar *et al*., 1976).

5. Results And Discussion

Table 1. *Treatment comparative study of Spinacea oleracea L. leaf on biochemical parameters.*

S.No	Biochemical parameter	Treatment-NO: I Control	Treatment No:II (Soil + Heavy metal)	Treatment No:III (Soil+Heavy metal +1% Ca(OH)2 treated)
		Mean ± S.D	**Mean ± S.D**	**Mean ± S.D**
1	Chlorophyll mg /gm	1.33±0.05	0.561±0.005	1.231±0.005
2	Catalase enzyme	1.8 ± 0.01	1.69± 0.01	1.72 ± 0.01
3	Peroxidase	5.32 ± 0.1	3.15 ± 0.3	3.44 ± 0.3
4	Proteins	29.69 ± 0.03	24.6 ±0.08	28.46 ± 0.03
5	Proline	1.73± 0.09	1.46± 0.03	1.65 ± 0.02
6	DNA	1.43 ± 0.02	1.15 ± 0.03	1.22 ± 0.02
7	RNA	1.73 ± 0.03	1.59 ± 0.02	1.69 ± 0.02
8	Phenols	1.921 ± 0.04	0.444 ± 0.007	0.976 ± 0.007
9	Starch	2.153 ± 0.07	0.929 ± 0.03	1.793 ±0.06
10	Total sugars	3.709 ± 0.001	1.82 ± 0.005	3.045 ± 0.001
11	Reducing sugars	0.804 ± 0.02	0.408 ±0.002	0.708 ± 0.002
12	Non-reducing sugars	0.331± 0.007	0.198 ±0.002	0.306 ± 0.005

5.1. Chlorophyll Content Mg / Gm

Chlorophyll is vital for photosynthesis, which allows plants to absorb energy from light (Carter J. Stein. 1996). Chlorophyll absorbs sun light and converts it to chemical energy (Yakar and Bilge 1987). The importance of chlorophyll for photosynthesis is that it captures light energy from the sun to produce glucose via a chemical reaction. Data presented in Table -I, Fig.1 show that chlorophyll content in leaf was significantly decreased in Treatment II which is 0.561±0.005 mg/gm when compared with Treatment –I 1.33±0.05 mg/gm and Treatment –III 1.231±0.005 mg/gm.

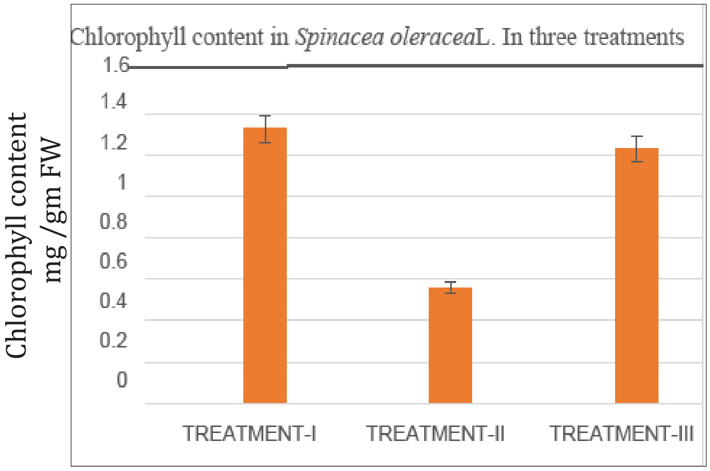

Figure 1. *Chlorophyll content in Spinacea oleracea L.*

5.2. Catalase Content

In green leaves a majority of CAT activity is found in peroxisomes (Foyer and Noctor 2000; Scandalios *et al*., 1997). Catalase (CAT, H2O2:H2O2 oxidoreductase; EC 1.11.1.6) by scavenging hydrogen peroxide to water and oxygen is an important enzyme of cell defense mechanisms against oxidative stress in plants (Dat *et al.,* 2003; Foyer and Noctor. 2000). Catalase is the most efficient enzyme as an antioxidative enzyme which lowers, hydrogen peroxide or superoxide to accumulate to toxic levels in plant growth (Bowler *et al.,* 1992; Brenan and Frenkel, 1977). Data presented in Table- I, Fig.2 show that catalase enzyme content were significantly decreased Treatment-II is 1.69**± 0.01** mg/gm as compared with Treatment – I 1.8 ± 0.01mg/gm and Treatment III 1.72 ± 0.01mg/gm.

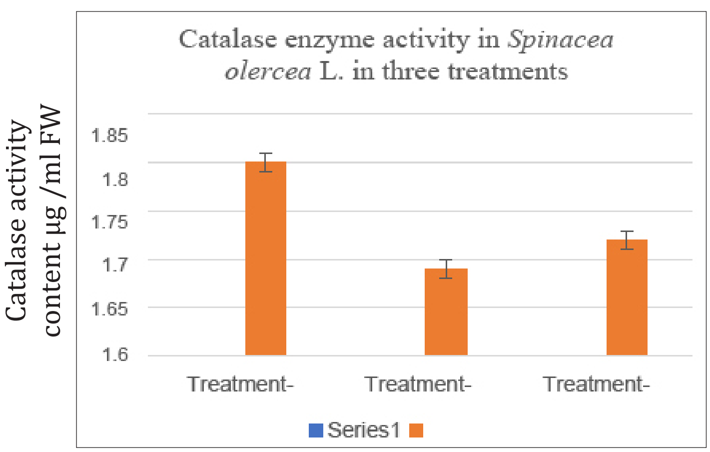

Figure 2. *Catalase enzyme activity in Spinacea olercea L. in three treatments*

5.3. Peroxidase Content

Peroxidases, a class of enzymes in animal, plant and microorganism tissues, catalyze oxidoreduction between H2O2 and various reductants. Peroxidase is involved in a large number of biochemical and physiological processes and may change quantitatively and qualitatively during growth and development (Shannon, 1969). Enzymes peroxidase and catalaseare highmolecular, which are capable of eliminating the hydrogen peroxide formed during non-enzymatic or enzymatic dismutation (Merzlyak, 1999). When plants are subjected to stress this tends to result in the release of reactive oxygen species (ROS). It is thought that peroxidase remove ROS, helping prevent damage. Data presented in Table 1 & Fig.3 show that Peroxidase enzyme content were significantly decreased in Treatment-II is 3.15 ± 0.3 mg/gm when compared with Treatment –I 5.32 ± 0.1 mg/gm and Treatment –III 3.44 ± 0.3 mg/gm'

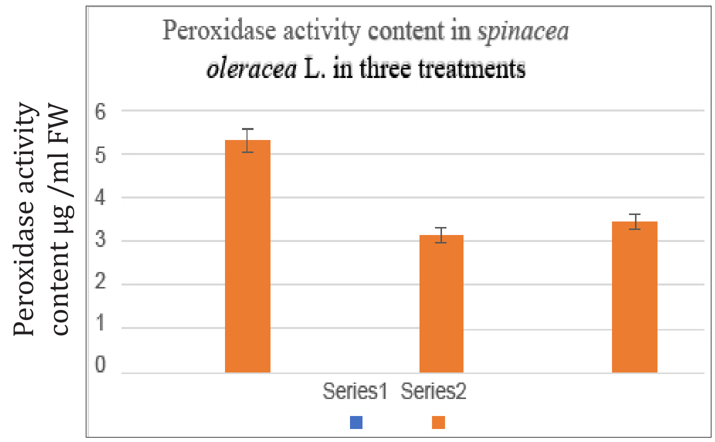

Figure 3. *Peroxidase activity content in spinacea oleracea L. in three treatments*

5.4. Proteins

They are complex combinations of smaller chemical compounds called *amino acids*. Proteins are involved in processes such as catalyzing chemical reactions (enzymes), facilitating membrane transport, intracellular structure and energy generating reactions involving electron transport. A challenge lies in the fact that proteins have a finite life span and must be constantly translated from mRNA in order for plant growth and development to continue. Data presented in Table-I Fig.4 show that protein content were significantly decreased in plant treated with Treatment-II 24.6 ±0.08 mg/gm when compared with Treatment –I 29.69 ± 0.03 mg/gm in plant material and Treatment –III 28.46 ± 0.03 mg/gm.

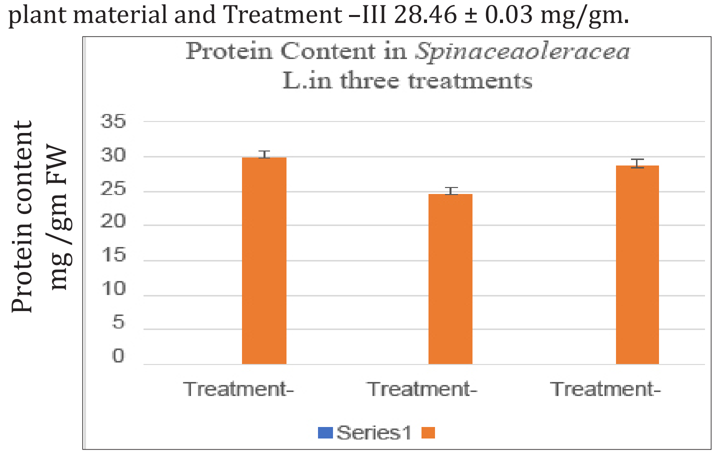

Figure 4. *Protein Content in Spinacea oleracea L.in three treatments*

5.5. Proline Content

Proline provide protection to plants from stress by contributing to cellular osmotic adjustment, ROS detoxification, protection of membrane integrity and enzymes/protein stabilization (Ashraf *et al.,* 2007; Bohnert *et al*., 1996; Yancey, 1994). Proline can act as a signaling molecule to modulate mitochondrial functions, influence cell proliferation or cell death and trigger specific gene expression, which can be essential for plant recovery from stress. Data presented in Table- I and Fig.5 show that Proline content were significantly increased in Treatment-II is 1.73± 0.09 mg/gm when compared with Treatment –I 1.46± 0.03 mg/gm and Treatment –III 1.65 ± 0.02mg/gm.

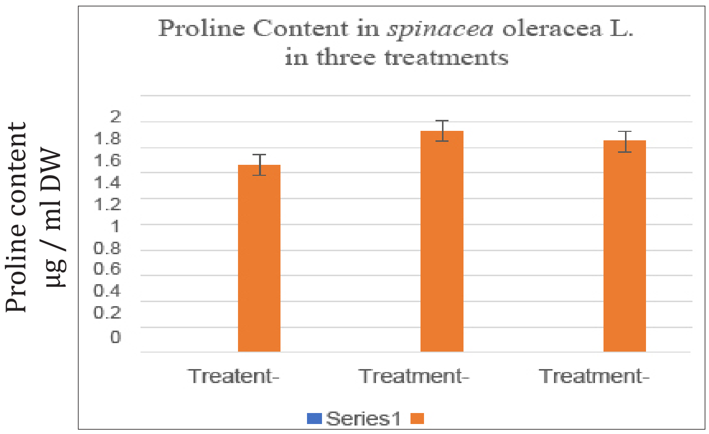

Figure 5. *Proline Content in spinacea oleracea L. in three treatments*

5.6. DNA Content

The DNA segments that carry genetic information are called genes, but other DNA sequences have structural purposes, or are involved in regulating the expression of genetic information The DNA segments that carry genetic information are called genes in eukaryotes such plants and animals. Data presented in Table-I Fig.6 show that DNA content were significantly decreased in Treatment-II is 1.15 ± 0.03 mg/gm when compared with Treatment –I 1.43 ± 0.02 mg/gm and Treatment –III 1.22 ± 0.02 mg/gm.

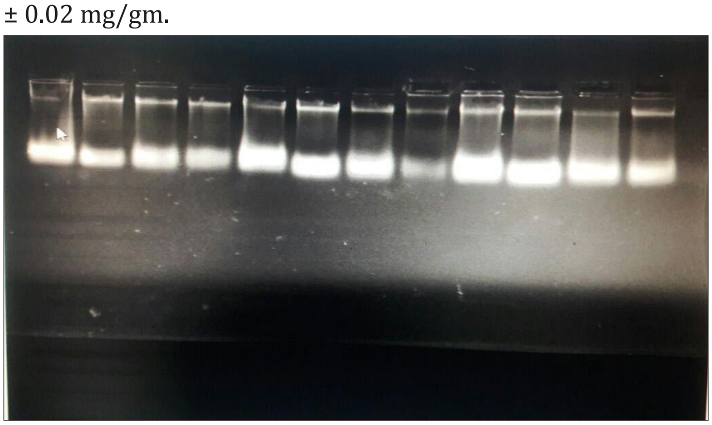

Figure 6. *Electrophoretic gel picture of DNA is isolated from leaves of Spinacea oleracea L. in three treatments*

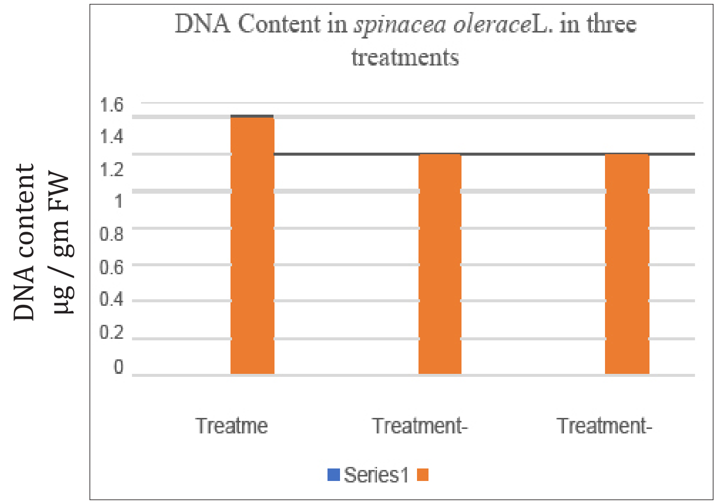

Figure 7. *DNA Content in spinacea olerace L. in three treatments*

5.7. RNA Content

Ribonucleic acid or RNA is a nucleic acid polymer consisting of nucleotide monomers that plays several important roles in the processes that translate genetic information from deoxyribonucleic acid (DNA) into protein products. Some RNA molecules play an active role within cells by catalysing biological reactions, controlling gene expression, or sensing and communicating responses to cellular signals. One of these active processes is protein synthesis, a universal function whereby mRNA molecules direct the assembly of proteins on ribosomes. This process uses transfer RNA (tRNA) molecules to deliver amino acids to the ribosome, where ribosomal RNA (rRNA) links amino acids together to form proteins. Data presented in Table-I Fig.7 show that RNA content were significantly decreased in Treatment-II which is 1.59 ± 0.02 mg/gm in plant material as compared with Treatment –1.73 ± 0.03mg/gm in plant material and Treatment –III 1.69 ± 0.02 mg/gm

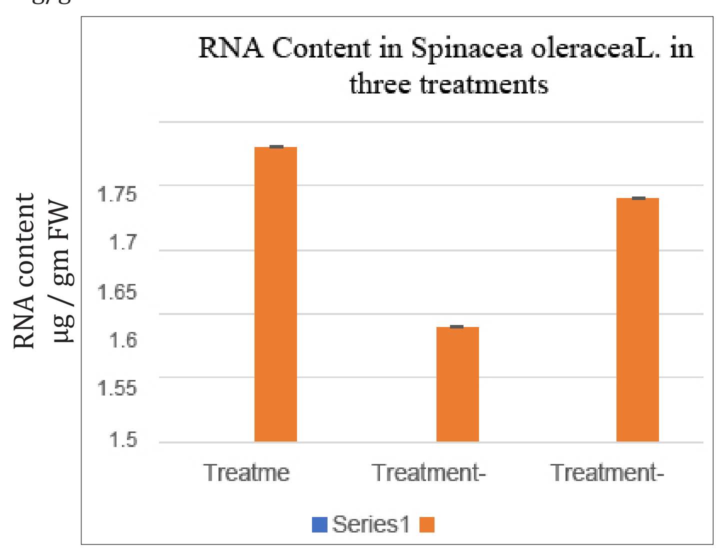

Figure 8. *RNA Content in Spinacea oleraceaL. in three treatments*

5.8. Phenols Content

Phenolic compounds are crucial for plants growth and reproduction, and are produced as a response to environmental factors (light, chilling, pollution etc) and to defend injured plants (Valentine *et al.*, 2003) Phenolics acids are the most important groups of secondary metabolites and bioactive compounds in plants (Kim *et al.*, 2003). Data presented in Table-I Fig.8 show that phenols content were significantly increased in Treatment-II is 0.444 ± 0.007 mg/gm when compared with Treatment –I 1.921 ± 0.04 mg/gm and Treatment –III 0.976 ± 0.007 mg/gm.

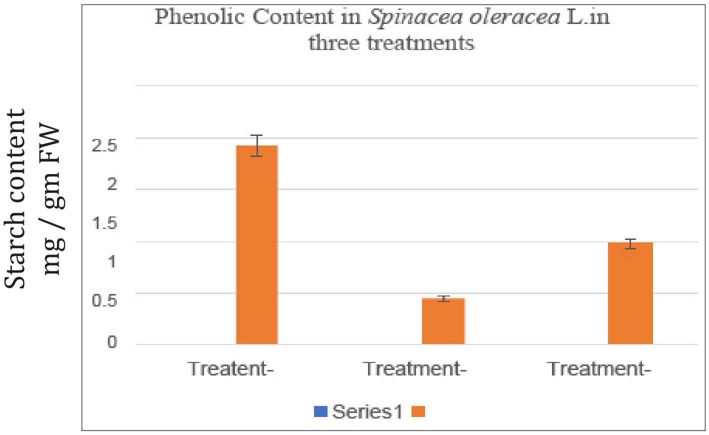

Figure 9. *Phenolic Content in Spinacea oleracea L.in three treatments*

5.9. Starch Content

Starch is the main form by which plants store carbohydrate and is a major photosynthetic product in many species. Starch is the major energy reserve for plants; it is located mainly in the seeds, roots or tubers, stem pith, and fruit. The functional properties of native starch are determined by the granule structure. This is important for normal growth in a diurnal cycle and is finely controlled to suit the growth conditions (Gibon et al., 2009). Data presented in Table- I Fig.9 show that starch content were significantly decreased plant treated with Treatment-II is 0.929 ± 0.03 mg/gm when compared with Treatment –1. 2.153 ± 0.07 mg/gm and Treatment – III 1.793 ±0.06 mg/gm.

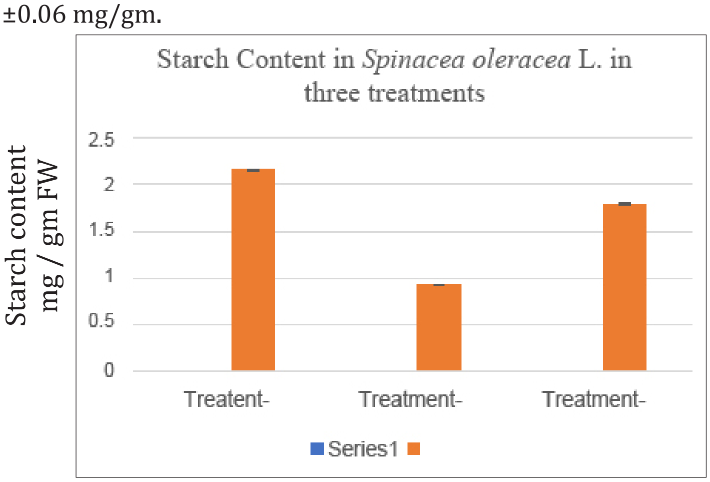

Figure 10. *Starch Content in Spinacea oleracea L. in three treatments*

5.10. Total Sugars Content

Carbohydrates are formed by green plants from carbon dioxide and water during the process of photosynthesis. A source of energy for the body e.g. glucose and a store of energy, e.g. starch in plants. The components of other molecules example of DNA, RNA, glycolipids, glycoproteins and ATPs. Data presented in Table -I Fig.10 show that carbohydrates content were significantly decreased in plant treated with Treatment-II 1.549±0.015 is mg/gm when compared with Treatment-I 2.588±0.024 mg/gm and Treatment –III 2.543±0.023 mg/gm.

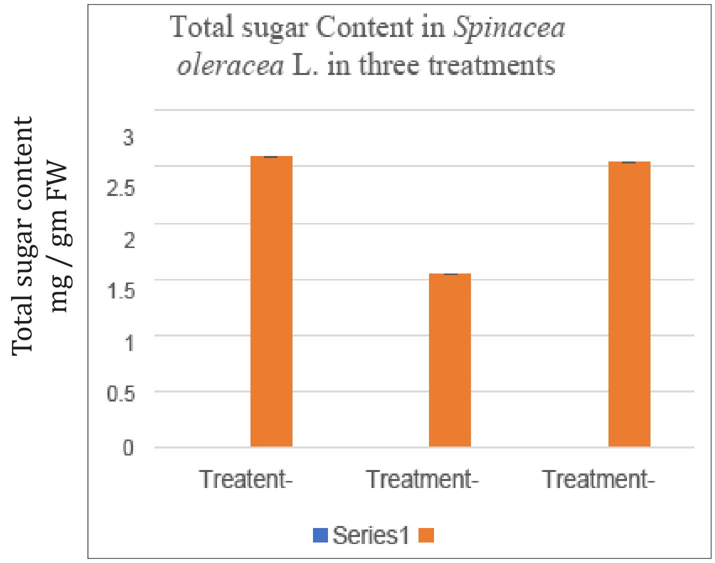

Figure 11. *Total sugar Content in Spinacea oleracea L. in three treatments*

5.11. Reducing Sugar Content

Glucose is a carbohydrate, and is the most important simple sugar in human metabolism. Glucose is made during photosynthesis from water and carbon dioxide, using energy from sunlight, is a very important source of power for cellular respiration. Glucose may be stored in plants as the polymers starch and cellulose. Sugars such as glucose, fructose, and sucrose are recognized as signaling molecules in plants (Rolland *et al.*, 2006; Bolouri Moghaddam *et al.*, 2010). In addition to their typical roles as carbon and energy sources (Koch, 2004). Invertases play crucial roles in the regulation of sucrose levels, sink strength, and sucrose:hexose ratios linked to sugar signaling. Vacuolar, cell wall, and neutral/alkaline invertases can be discerned (Koch, 2004; Xiang *et al.*, 2011). Sugar signaling might also be of great importance in plant (defense) responses under biotic and abiotic stresses. Data presented in Table-I Fig.11 show that Reducing sugars content were significantly decreased in plant treated with Treatment-II is 0.408 ±0.002 mg/gm when compared with Treatment –I 0.804 ± mg/gm and Treatment –III 0.708 ± 0.002mg/gm.

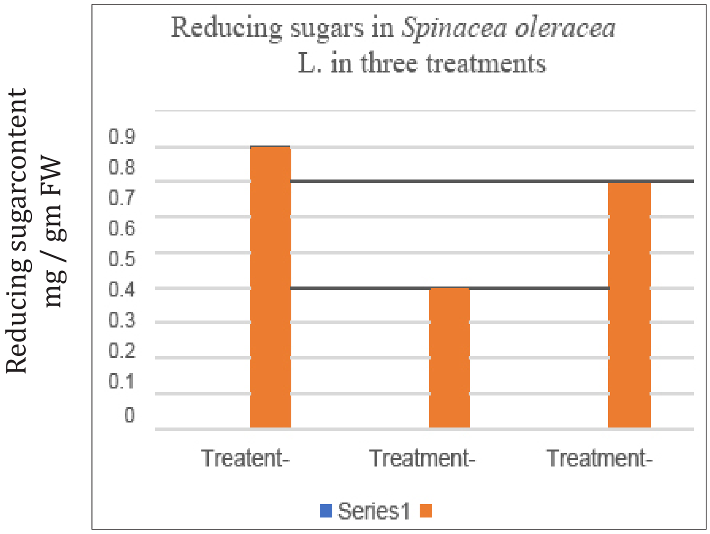

Figure 12. *Reducing sugars in Spinacea oleracea L. in three treatments*

5.12. Non-Reducing Sugar Content

Sucrose is an important carbohydrate in most plants. It has multiple functions such as regulating photosynthesis and respiration, serving as storage compound and helping to maintain the osmotic pressure in the cytosol. It provides protection to plants from stress by contributing to cellular osmotic adjustment, ROS detoxification, protection of membrane integrity and enzymes/protein stabilization (Ashraf *et al.,* 2007; Bohnert *et al.,* 1996; Yancey, 1994). Data presented in Table-I Fig.12 show that Nonreducing sugars content were significantly decreased in plant treated with Treatment-II is 0.198 ±0.002 mg/gm when compared with Treatment –I 0.331± 0.007 mg/gm and Treatment –III 0.306 ± 0.005 mg/gm.

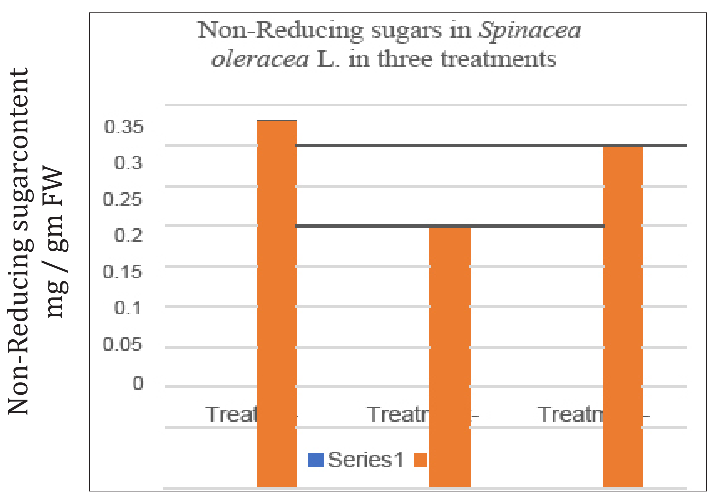

Figure 13. *Reducing sugars in Spinacea oleracea L. in three treatments*

6. Conclusion

It reveals that there is a decrease in biochemical contents in Treatment II when compared with Treatment I and Treatment III of *Spinacea oleracea* L. It was also observe that the phenol and proline parameters are increased in Treatment II and the heavy accumulation in the leaf parts, where these are indicators of heavy metal where as in Treatment I and Treatment III phenol and proline contents decreased. Remaining biochemical contents such as Chlorophyll, Protein, Total sugar/Carbohydrates, reducing sugars, Non •reducing sugars, Starch, DNA and RNA and Enzymes Catalase, and Peroxidase content is decrease in Treatment –II compared with Treatment I and Treatment III. It may conclude that direct relationship between heavy metals and biochemical in plant *Spinacea oleracea* L. It may conclude that there is relationship between heavy metal and biochemical parameters. Heavy metal content in the plants, growing in polluted areas can be reduced when the soils are treated with of 1% Ca (OH)2.

Conflict of Interest: The authors declared no conflict of interest.

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