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ORIGINAL ARTICLE

Study of Biochemical Characteristics of Spinach Irrigated with Industrial waste water of Bhiwadi, Rajasthan, India

Madhvi Saini, Kailash Chandra Sharma and Manju Sharma*
Department of Botany, University of Rajasthan, JLN Marg, Jaipur, Rajasthan, India. 301004.
*Address for correspondence: sharmamanju204@gmail.com

ABSTRACT
The present study was carried out to understand the effect of industrial waste water on biochemical contents of spinach. The plants of spinach were treated with 3 types of water samples i. Ground water ii. Treated water and iii. Untreated water and biochemical characters were analyzed. The parameters analyzed were fat, starch, total soluble sugar, pigments (chlorophyll a, chlorophyll b, carotenoids, and total chlorophyll) and antioxidative enzymes like catalase, peroxidase and super oxide dismutase (SOD). Results showed that fat and protein content is decreased in plants irrigated with waste water in comparison to that with ground water, Whereas starch , total soluble sugar, pigments and catalase, peroxidase and SOD contents were increased in plants irrigated with waste water in comparison to that with ground water .These phytochemical changes were attributed to the defensive reactions of plants against physiological stresses of waste water compounds.
Keywords: *industrial waste water, heavy metals, irrigation, spinach, biochemical constituents.*

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INTRODUCTION

The industrial development over the last five decades has resulted in an exponential increase in the production and consumption of chemicals. Production, use and disposal of numerous chemicals cause wide spread contamination of soils as well as ground waters and surface waters. [1]. Rapid growth of urban population and industrialization results in generation of huge quantities of waste water perennially. Instead of disposal of waste water into the environment , its wide use in both developed and developing countries including India and Pakistan may be for agricultural irrigation and other purposes after its treatment [2,3,4,5,6].
Waste water rich in organic materials and plant nutrients is finding agricultural use as a cheap way of disposal. As demand for fresh water intensifies, waste water is frequently being seen as a valuable resource and is an important alternative source of water for irrigation [7]. Industrial effluents are being used for irrigation in dry areas. The demand for water is continuously increasing in arid and semi-arid countries. Therefore, water of higher quality is preserved for domestic use while that of lower quality is recommended for irrigation [8]
Waste water from different sources not only provides water but also contains considerable amount of organic matter and plant nutrients (N, P, K, Ca, S, Cu, Mn and Zn) and has been reported to increase the crop yield [9,10,11,12,13,14,15,16] .These effluents not only contain nutrients that enhance the growth of crop plants but also have other toxic [17,18,19].
Vegetables constitute an important part of the human diet since they contain carbohydrates, proteins, as well as vitamins, minerals and heavy metals. Heavy metals are one of the important types of contaminants that can be found on the surface and in the tissue of fresh vegetables. 20,21]. Vegetables, especially those of leafy vegetables grown in heavy metals contaminated soils, accumulate higher amounts of metals than those grown in uncontaminated soils because of the fact that they absorb these metals through their leaves [22,23].The authors have reported presence of heavy metals beyond prescribed limits in the tissues of spinach plants grown in soils irrigated with industrial waste water of Bhiwadi. The present study was aimed at analyzing effect of the heavy metals present in the waste water of the said industrial area, on biochemical characteristics of Spinach. Peroxidase, Catalase and SOD enzyme activity, Carbohydrate,

Protein, Fat and Pigments content of Spinach irrigated with ground water and compare with plants irrigated with treated and untreated water samples.

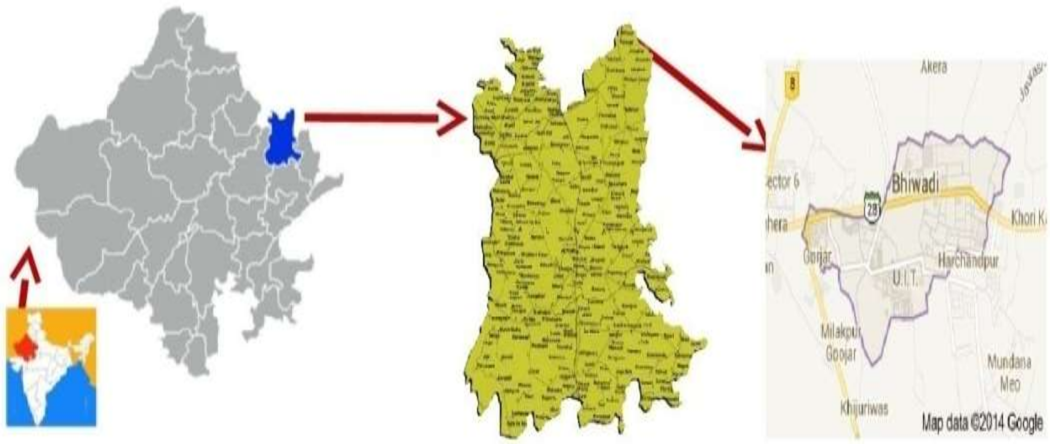
MATERIAL AND METHODS

Experimental plant

Beta palonga (Basu and Mukharjee) (Spinach).

Study area

Bhiwadi termed as gateway of Rajasthan, is an industrial hub and located in Tijara tehsil of Alwar Distt.. It is part of Delhi NCR region .It is 55 km away from Indira Gandhi International airport, New Delhi and 200 km from Jaipur. It has about 1850 small and large industries including MNC industrial units and steel, furnance, engineering, pharmaceuticals and textiles etc. units.



India Rajasthan Alwar Bhiwadi

Fig 1 : showing study area map

Collection of plants and water samples

The study was conducted with waste water released from industries at Bhiwadi, Alwar. Three categories of water samples were collected. (i) Waste water samples were collected from the main outlet point of combined effluents from all the industries is termed as untreated water/ inlet of CETP. (ii) Second category of samples were collected from the water treated by CETP (common effluents treatment plant) and termed as outlet samples. (iii) Under the third category, water samples of ground water were collected from bourwell of Bhiwadi and termed as GW samples (figure I A,B,C)

Three plots of 6.5 × 4.5 m^2 size were prepared. Genetically uniform seeds of spinach were sown in each plot. Uniform irrigation schedule was followed at all the three plots to maintain similar moisture condition throughout the growth of plants. Names of the 3 plots were given as inlet, outlet and GW plot respectively (figure I:D,E,F). Plant samples collected were washed with distilled water to remove dust particles. Samples were air dried and then grounded into fine powder, sieved and stored in polythene bags. Both fresh and powdered leaf materials were used for estimation of different biochemical contents.

Experiments

Fresh leaves were used for chlorophyll, protein and all the three antioxidative enzymes viz., peroxidase, catalase and SOD (super oxide dismutase). Chlorophyll a, chlorophyll b , carotenoids and total chlorophyll was estimated by Arnon's method [24]. Protein content in leaves was measured by method described by Lowry *et al.* [25]. Peroxidase, catalase and SOD in leaf was estimated by methods used by Putter [26], Aebi [27] and Beauchamp and Fridovich [28] respectively.

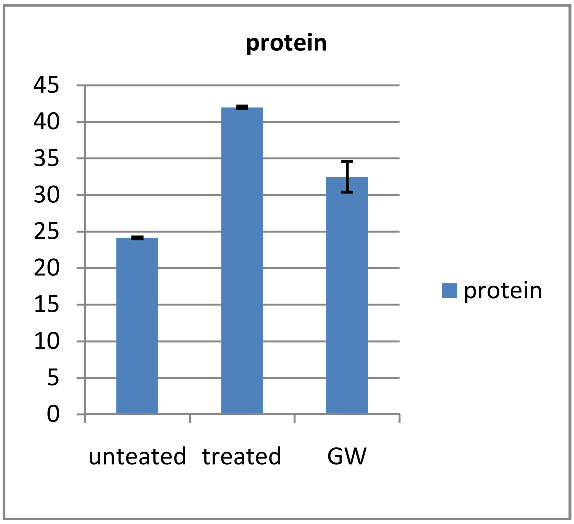
For estimation of fat, starch content and total soluble sugar dry leaf powder was used. Fat content in leaf was measured by method described by Jayaraman [29]. Total soluble sugar was estimated in leaf by phenol- sulphuric acid method (Dubois *et al.* [30]. Starch content was measured in leaf by method used by Mc Cready *et al.* [31].

RESULTS AND DISCUSSION

Table 1. Showing comparison of biochemical characters of plants irrigated with untreated water, treated water and ground water.

S.NO.		Untreated water	Treated water	Ground water
1.	Protein (mg/gm)	24.15±0.16	41.99 ±0.199	32.5±2.1
2.	Lipid (mg/gm)	27.66±2.05	23±2.30	29.33 ±2.86
3.	Starch (mg/gm)	28.8±0.9	26.1±0.9	15.3±0.9
4.	Total Soluble sugar (mg/gm)	29.22±0.22	30.5±1.22	20.4±0.29
5.	catalase	1.1011±0.047	0.3653±0.115	0.2040±0.0214
6.	peroxidase	2.4992±0.265	0.9724±0.145	0.5953±0.242
7.	SOD	430.09±66.4	142 ±14.45	57.86±11.68
8.	Chlorophyll a (mg/gm)	0.9222±0.027	0.9087±0.012	0.7821±0.016
9.	Chlorophyll b (mg/gm)	0.2896±0.023	0.2679±0.023	0.1886±0.014
10.	Total chlorophyll (mg/gm)	18.95±0.187	18.20±0.132	15.51±0.095
11.	Carotenoids(mg/gm)	0.4096±0.023	0.3916±0.0101	0.3098±0.014

Figure :2:A,B,C : collection sites of untreated water, treated water and ground water respectively. D,E, F : plants grown in untreated water ,treated water and ground water respectively



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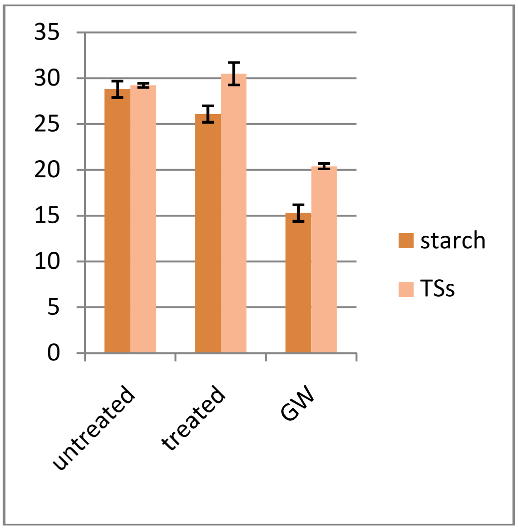

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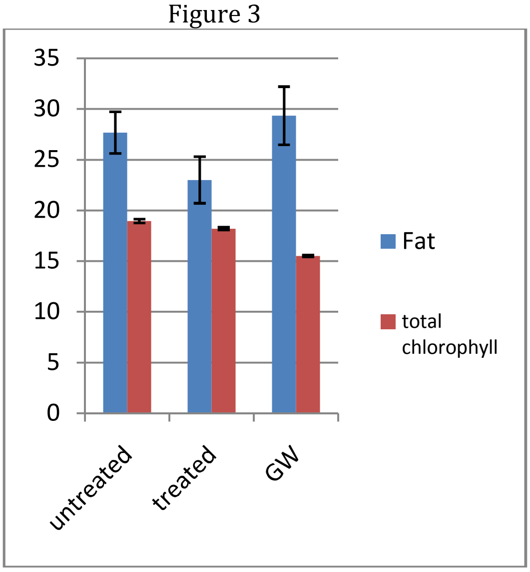

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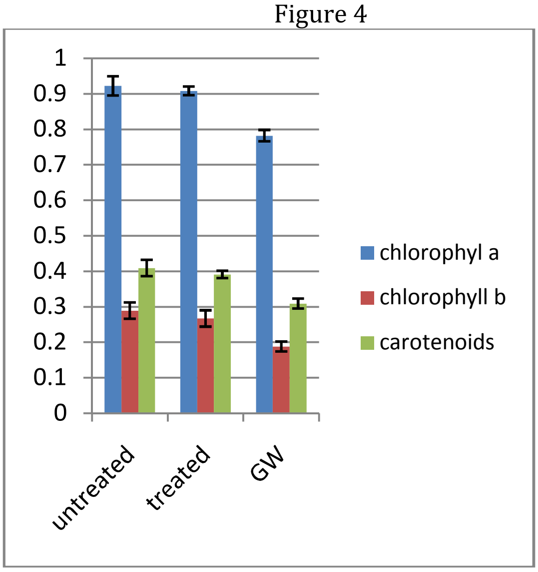
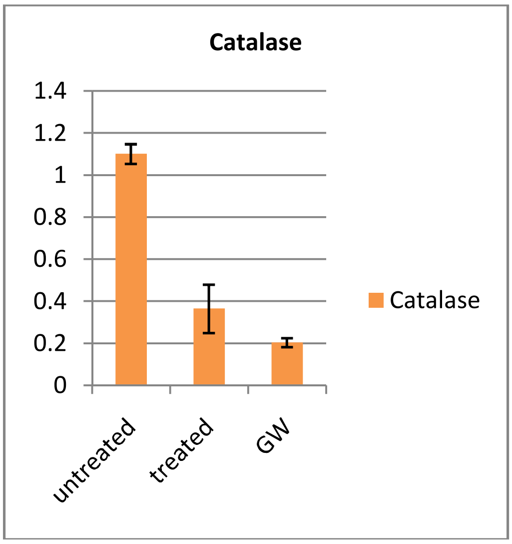


Figure 6

Figure 3,4,5,6 showing protein, starch , total soluble sugar , fat, total chlorophyll ,chlorophyll a, b, and carotenoids in plants irrigated with untreated water, treated water and ground water respectively.



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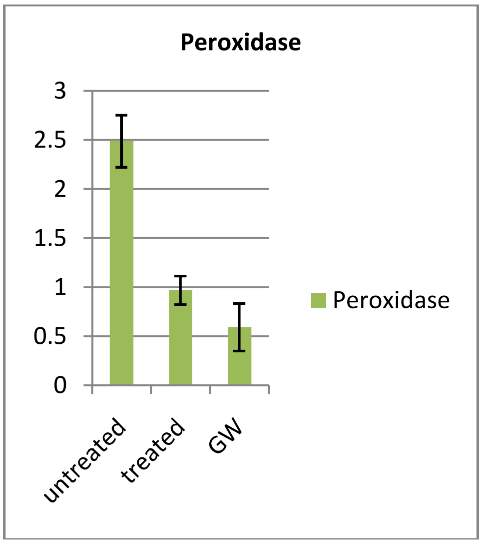

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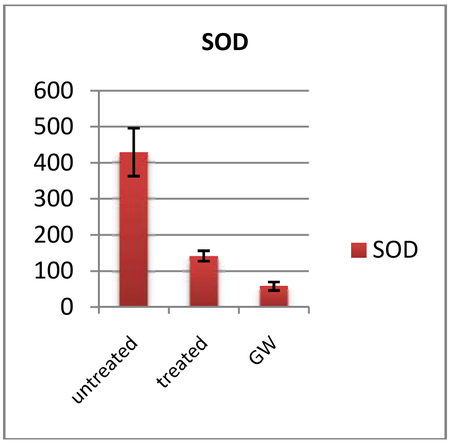

Figure 9

Figure 7,8,9 showing catalase, peroxidase and SOD contents in plants irrigated with untreated water, treated water and ground water respectively.

Amount of protein was 32.5±2.1, 41.99 ±0.199, 24.15±0.16 mg/gm in plants irrigated with ground water, treated water and untreated water respectively (Table 1). This reveals that protein amount was higher in plants irrigated with treated water followed by protein found in plants irrigated with ground water and minimum amount of protein was present in plants irrigated with untreated water. Reduction in protein content in plants and observed that this could be attributed to effect on nitrate reductase activity [32, 33]. Singh *et al.* [33] also reported the similar trends of proteins amount in *Pistia stratiotes* L.

Amount of starch and total soluble sugar 15.3±0.9, 26.1±0.9, 28.8±0.9 mg/gm;20.4±0.29, 30.5±1.22, 29.22±0.22 mg/gm respectively (Table I) were observed in plants irrigated with ground water, treated water and untreated water respectively. Significant reduction was observed in starch amount in plants grown in untreated water followed by starch content of plants grown in treated water and minimum starch content in plants grown in ground water , whereas total soluble sugar amount was higher in plants irrigated with treated water followed by plants grown in untreated water and ground water respectively.

Earlier studies have shown that stress induces the decline in protein contents in plants but increase in soluble sugar content [34, 35, 36]. The enrichment of soluble sugars, in addition to its osmotic index, may protect the membranes from drying out. It has been shown that certain sugars such as trehalose, by binding to membrane lipids, could stabilize the membrane structure [37].

Bekhouche [38] and Bedouh and Bekhouche [39] working in different varieties of Wheat and in onion respectively irrigated with waste water observed a high activity peroxydatique resulting in increased levels of soluble sugars. The data presented here showed an increase in soluble sugars in spinach irrigated with industrial waste water. Bekhouche and Bedouh and Bekhouche giving an explanation to this stated that the accumulation of osmolytes (sugars and proline) turns out to be a reliable indicator of stress tolerance in general. The data presented here seems to support this.

Fat amount estimated was 29.33 ±2.86, 23±2.30 and 27.66±2.05 mg/gm in plants irrigated with ground water, treated water and untreated water respectively (Table I). Amount of fat was estimated higher in ground water plants followed by untreated water plants and minimum in treated water plants. Similar results were also observed by Bhardwaj [40] studying effects of waste water on tomato and chillies. Lipid contents of organisms reduced with increasing concentrations of effluent. This might be due to the utilization of lipids for energy demand under the stress conditions [41, 42].

Amount of chlorophyll a , chlorophyll b , carotenoids, and total chlorophyll observed were 0.7821±0.016, 0.9087±0.012, 0.9222±0.027 mg/gm; 0.1886±0.014, 0.2679±0.023, 0.2896±0.023 mg/gm ; 0.3098±0.014, 0.3916±0.0101, 0.4096±0.023 mg/gm ; 15.51±0.095, 18.20±0.132, 18.95±0.187 mg/gm respectively (Table I) in plants irrigated with ground water, treated water and untreated water respectively. Amount of chlorophyll a ,chlorophyll b , carotenoids and total chlorophyll were higher in plants irrigated with untreated water followed by chlorophyll a of plants irrigated with treated water and minimum in plants irrigated with ground water. significant increment was observed in amount of chlorophyll a in plants irrigated with untreated water in comparison to plants irrigated with ground water.

Singh *et al.*[43] observed that total chlorophyll and carotenoid were higher in plants of waste water irrigated sites. Carotenoid is photosynthetic pigment, also functions as non enzymatic antioxidant protecting plants from oxidative stress by changing the physical properties of photosynthetic membranes with involvement of xanthophyll cycle [44]. An increase in carotenoid content was suggested to be a defense strategy of the plants to combat metal stress [45].

Amount of Catalase, Peroxidase and SOD (super oxide dismutase) enzyme were estimated in plants grown in the water samples. 0.2040±0.0214, 0.3653±0.115, 1.1011±0.047; 0.5953±0.242, 0.9724±0.145, 2.4992±0.265; 57.86±11.68, 142 ±14.45, 430.09±66.4 respectively (Table I) were observed in plants grown in ground water, treated water and untreated water respectively. Amount of catalase, peroxidase and SOD enzyme were higher in plants irrigated with untreated water followed by plants irrigated with treated water and the lowest in plants irrigated with ground water . Significant increment was observed in amount of catalase in plants irrigated with untreated water in comparison to plants irrigated with ground water.

Increased activity of Catalase and SOD was observed in 30% effluent treated seeds than control as well as treated effluent [1]. An enhanced activity of catalase was seen as detoxifying enzyme and the induction of mechanism of tolerance [46]. The induction of the stress response leads to expression of a group of proteins referred to as stress proteins, which are thought to protect the cell are supported [47].

Antioxidative enzyme, peroxidase also showed increment in its activity in plants grown at waste water irrigated sites as compared to those at ground water irrigated ones. Peroxidase activity showed positive and significant relationship with all the metal concentrations in plants [43]. Peroxidases play a significant role in defense against oxidative stress and are suggested to be indicators of metal toxicity [48]. Positive relationship suggests that with increase in the heavy metal concentrations, there were increase in the oxidative modifications to cellular components of the plants [49].

CONCLUSION

The water with industrial effluents of the studied site used for agriculture purpose of Spinach, enhance abiotic stress to the plant as evidenced by increased amount of the antioxidative enzymes along with starch and total soluble sugar and chlorophyll contents and decrease in fats and proteins.

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