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RESPONSE OF NICKEL POLLUTION ON PHYSIOLOGICAL AND BIOCHEMICAL ATTRIBUTES OF WHEAT (*TRITICUM AESTIVUM* L.) VAR. BHAKAR-02

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Abstract

For the determination of the effect of different nickel (Ni) concentrations on the physiological and biochemical attributes of wheat (*Triticum aestivum* L. var. Bhakar-02), an experiment was conducted in Botanic Garden of GCU, Lahore. Certified seeds of wheat crop were selected. Soil was mixed with different nickel chloride concentrations (50,100,150,200,250 and 300 mg/Kg of soil) to make the treatments from T1 to T6. The control plants were grown in soil without nickel salt. The study was carried out in order to find out the impact of nickel concentrations on different growth parameters such as plant height, number of green and senescent leaves, number of tillers per plant. All observed plants showed the reduction trend from T1 to T6 as compared to T0 (control). Along with these, biochemical analysis (chlorophyll a, b, total chlorophyll and carotenoids contents) and physiological parameters (photosynthetic rate, transpiration rate and stomatal conductance) of respective plants was carried out in laboratory in which decreasing trend was also recorded from T1 to T6 as compared to control. The present study was helpful in determining the toxic effects of Ni on physiological and biochemical parameters of wheat.

Introduction

The pollution may be defined as ‘‘the deterioration in the chemical, physical and biological properties of our surrounding environment’’. Soil pollution by heavy metals is a global problem causing vast areas of agricultural land to become non-arable and hazardous for both wildlife and human populations (Basta *et al*., 2001). Excess concentrations of some heavy metals in soils such as Cd^{+2}, Cr^{+6}, Cu^{+2}, Ni^{+2} and Zn^{+2} adversely affect the natural aquatic and terrestrial ecosystems (Gardea-Torresdey *et al.,* 1996; Mushtaq & Khan, 2010).

Nickel is released both from anthropogenic and natural sources in environment. No doubt, nickel is a micronutrient of plants, but nickel toxicity is considered more important than its deficiency (Nriagu, 1990; Pacyna & Pacyna, 2001). Nickel has a special place among the heavy metals. Unlike Cd, Pb, Hg, Ag, and several other metals that are not the components of plant enzymes, Ni is a constituent of urease, and small quantities of Ni (0.01 to 5 µg/g dry wt.) are essential for some plant species (Seregin & Kozhevnikova, 2006). Nickel is present in plant tissues in the range of 0.05-10 µg/g of dry matter (Marschner, 1995; Adriano, 2001). The nickel varies between different plant species due to difference in root to shoot transport and uptake (Marschner, 1995). The threshold concentration for nickel toxicity is lower than 100 µg/g (Adriano, 2001; Nieminen, 2004). Nickel is required in lower amounts in plants for their metabolism so this problem does not exist in most of plant species due to a large range between limiting and toxic levels of nickel (Welch, 1995; Gerendas *et al*., 1999).

Ni is a necessary micronutrient for leguminous plants as well as for all other higher plants as Eskew *et al*., (1983) described that soybeans without Ni element showed necrotic lesions. It was found that Ni deficiency resulted in reduction of growth and late nodulation. In the present study, supply of 1 µg/L resulted in low urea accumulation, growth depressions and necrosis. Clijsters & Van-Assche (1985) reported that heavy metals inhibit the photosynthesis. They also observed that field factors such as soil characteristics and air pollution should be considered for estimating the mechanism of action of heavy metals on photosynthesis in plants which were growing in polluted areas. Sheoran *et al*., (1990) observed the impacts of Ni^{+2} on photosynthetic rate in pigeon pea (*Cajanus cajan* L. cv. UPAS-120). Ni^{+2} used 30 days after sowing resulted in 32% reduction in net photosynthesis. Piccini & Malavoltae (1992) grew two beans cultivars (*Phaseolus vulgaris* L.) i.e., Carioca and IAPAR-14. Leaf chlorophyll was decreased 50% at 4 mg/L level. Pandolfini *et al*., (1996) studied the impact of Ni on growth and concentration of chlorophyll ‘‘a and b’’ in two wheat cultivars. The Ni application reduced the chlorophyll ‘‘a’’ concentration.

Tereza *et al*., (2000) assessed the metal stress on scented geranium (*Pelargonium* sp.). The photosynthetic efficiency was influenced on all levels of metals for tested plants. Ouzounidou *et al*., (2006) studied that Ni stress reduced the growth and photosynthesis in wheat. Nutrient status (Ca, Mg, Fe, K and Na), growth and photosynthesis showed a distinct decrease related to the period of Ni treatment. Ahmad *et al*., (2007) analyzed the mungbean that either it was an indicator, excluder or accumulator of Ni. The stress of Ni resulted in reduction of yield, growth and photosynthetic pigments and accumulation of cations i.e., Ca^{+2}, K^+ and Na^+ in mungbean. Gajewska & Sklodowska (2007) studied the influence of different Ni levels (50 and 100 µM) on chlorophyll in shoots of wheat. Chlorophyll in shoots was lowered in response to Ni. Wani *et al*., (2007) conducted an experiment to investigate the phytotoxic impacts of Ni on chickpea and found toxic and significantly reduced chlorophyll contents. Ali *et al*., (2008) exposed the plants of *Brassica juncea* to NaCl and/or $NiCl_2$. The plants exposed to NaCl and/or $NiCl_2$ exhibited a significant decline in level of pigments and photosynthetic parameters. Yadav *et al*., (2009) studied the impact of various Ni concentrations (100, 200, 500 and 1000 µM) on carotenoids and other pigments of radish seedlings (*Raphanus sativus* L.). Various pigments were also reduced by Ni treatments.

Materials and Methods

The present study was conducted in order to find out the response of nickel on biochemical and physiological attributes of wheat (*Triticum aestivum* L.) cultivar Bhakar-02. A pot experiment was carried out by mixing soil with different levels of nickel chloride per Kg of soil. Control plants were without nickel and higher levels up to T6 were in combination with nickel as 50, 100, 150, 200, 250 and 300 mg/Kg of soil. The physiological parameters viz., photosynthetic rate, transpiration rate and stomatal conductance at mid season and final season were determined by using IRGA (Infra Red Gas Analyzer) after Vernay *et al*., (2008). The biochemical analysis i.e., chlorophyll a, b, total chlorophyll at 645 nm and 663 nm wavelengths (Arnon, 1949) and carotenoids (Zofia *et al.,* 2006) contents at 440.5 nm wavelength were also determined at mid harvest by spectrophotometer.

One-way ANOVA was applied on the data for various parameters. Along with this Duncan's Multiple Range Test (DMRT) (Steel & Torrie, 1996) was further applied after ANOVA as post-hoc test at 5% probability level in order to find out the significance differences between treatments.

Results

The most commonly grown and recommended cultivar of wheat (*Triticum aestivum* L. cv. Bhakar-02) was grown for their entire life cycle and were managed according to local agricultural conditions in the Botanic Garden GCU, Lahore. Since, the commencement of the experiment with nickel chloride, early vegetative growth showed distinct difference in wheat among all the nickel treated pots after the completion of first week of emergence. The control potted plants (T0) were found healthier than all nickel treated plants viz., T1 to T6. The changes in wheat growth and metabolism in response to nickel concentration was determined by observing the physiological and biochemical attributes.

Chlorophyll is the more important photosynthetic pigment of green plants which give them greenish colour normally. The variations in pigment contents of wheat (*Triticum aestivum* L. cv. Bhakar-02) are shown in Table 1. The chlorophyll 'a' contents showed that control plants showed higher value of pigment at 645 nm and 663 nm of wavelengths and consecutive higher treatments showed lower quantity of chlorophyll 'a' as well as chlorophyll 'b' is considered T0 also depicted the same results i.e., the decreasing trend appeared from control to higher nickel levels (T1, T2, T3, T4, T5 and T6). This reduction is due to the changes in the photosynthetic apparatus of wheat by nickel toxicity which affects it directly. The total chlorophyll contents of wheat (*Triticum aestivum* L. cv. Bhakar-02) were in same line with chlorophyll 'a' and 'b' values. The total contents of wheat showed greater reduction at T6 than control. The successive reductions of its values are showed in Table 1. The graphical representation of total chlorophyll presented showed that % reduction values are lower in control because reduction are negligible in control as compared to higher treatments. The % reductions of T6 are higher than any other treatment while other treatments from T1 to T5 showed less reduction than T6. The percentage (%) reduction in concentration of chlorophyll pigments (a, b and total chlorophyll) and carotenoids contents are compared in Figs. 1 & 2 respectively.

Table 1. The impact of different nickel concentrations on pigments of wheat (*Triticum aestivum* L.) var. Bhakar-02 during growth assessment.

Treatments	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophylls (mg/g)	Carotenoids
T0	1.85a ± 0.21	1.74a ± 0.23	3.59a ± 0.23	6.56a ± 0.23
T1	1.78a ± 0.08	1.56a ± 0.25	3.34a ± 0.34	6.36a ± 0.42
T2	1.45b ± 0.23	1.40b ± 0.20	2.85b ± 0.45	5.67b ± 0.17
T3	1.31bc ± 0.34	1.28b ± 0.09	2.59c ± 0.21	5.236c ± 0.21
T4	1.02c ± 0.14	0.98c ± 0.08	2.0d ± 0.23	4.67d ± 0.30
T5	0.97cd ± 0.23	0.74c ± 0.09	1.71cd ± 0.70	3.35e ± 0.22
T6	0.59d ± 0.15	0.45d ± 0.01	1.04e ± 0.89	2.43f ± 0.34

Treatments means followed by letters in every column are statistically different from each other at P=0.05 according to DMRT (Duncan's Multiple Range Test)

The physiological attributes viz., photosynthetic rate, transpiration rate and stomatal conductance are tabulated (Table 2). Ni impacted badly photosynthesis balance as well as transpiration rate and stomatal conductance. The photosynthetic rate was lower in harvest II as compared to harvest I because as time passed from harvest I to II, the ability of cells to photosynthesis is reduced due to nickel toxicity in higher nickel treatment plants especially. The values showed reduction from control to higher nickel levels in both harvests. As nickel concentration in soil increased, their uptake increased with greater effects on these physiological parameters. The transpiration rates were also lower in higher treated plants than control without nickel in both harvests. The reduction showed the same trend in plants.

The stomatal conductance of wheat were also lowered in harvest II due to the same reason of nickel toxicity. The reductions were more cleared in T6 than control. The % reductions of photosynthetic rate, transpiration rate and stomatal conductance were higher in final harvest than mid harvest. But the reduction trend from higher treated plants to lower plants was same in both harvests as indicated in Figs. 3, 4 & 5 respectively.

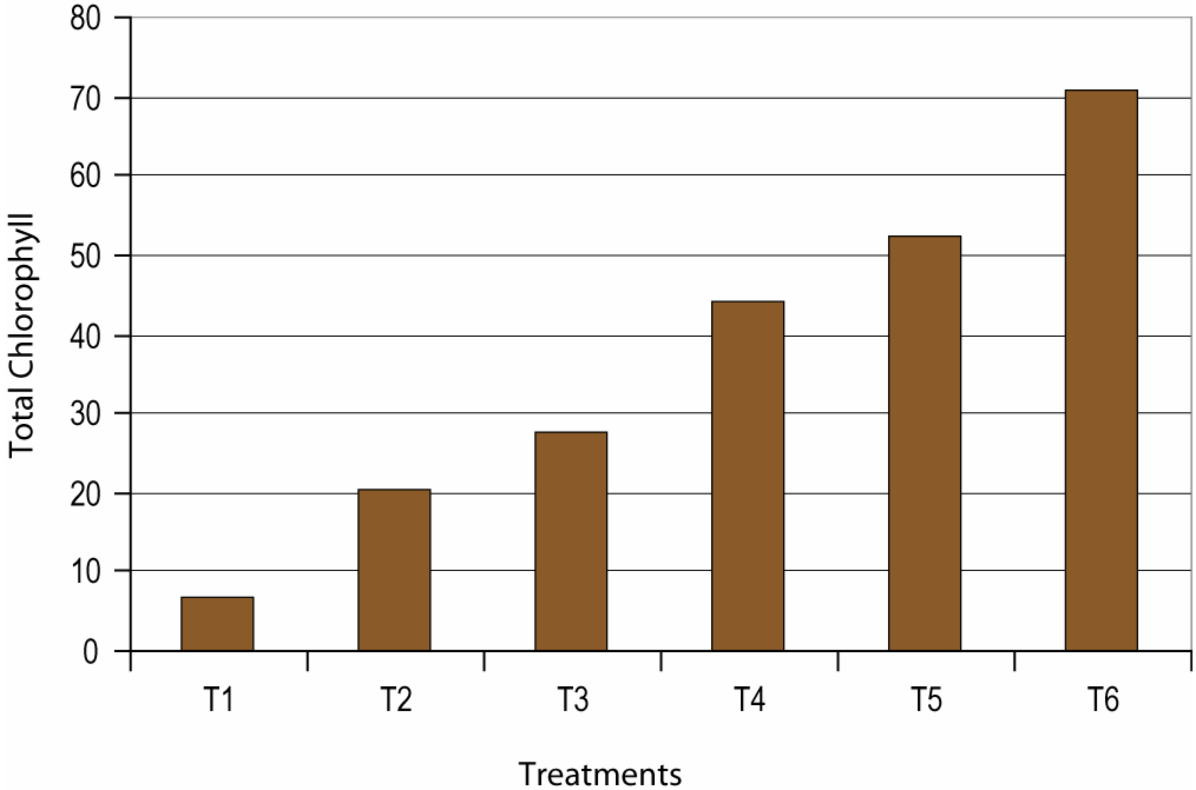

Fig. 1. Impact of various nickel concentrations (percent decrease vs. control) on total chlorophyll contents of wheat (*Triticum aestivum* L.) cv. Bhakar-02.

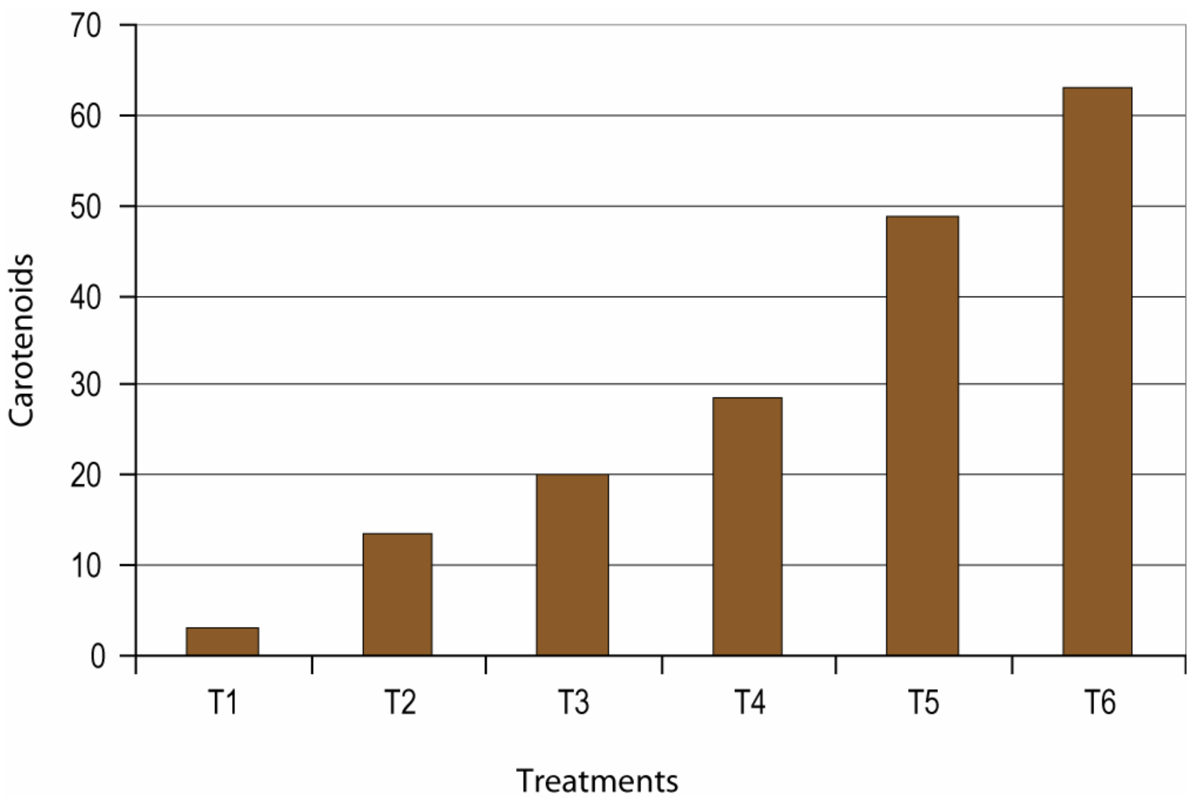

Fig. 2. Impact of various nickel concentrations (percent decrease vs. control) on carotenoids of wheat (*Triticum aestivum* L.) cv. Bhakar-02.

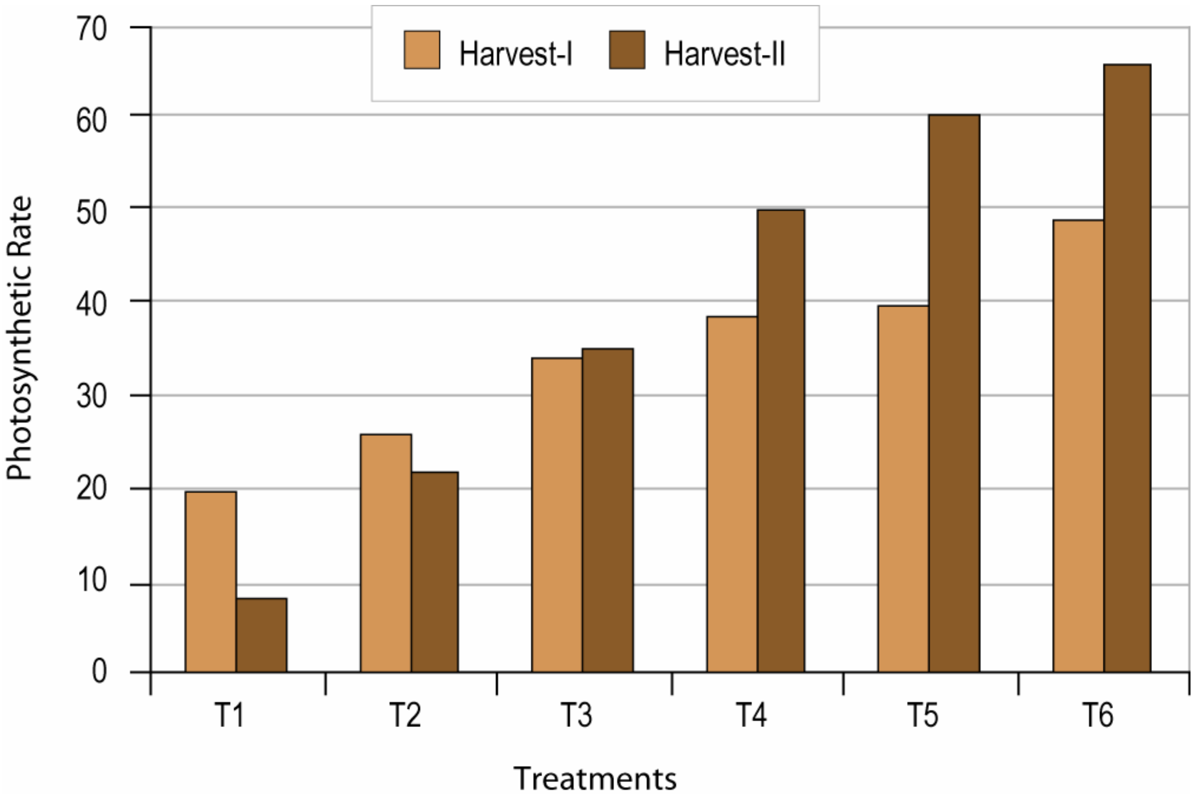

Fig. 3. Impact of various nickel concentrations (percent decrease vs. control) on photosynthetic rate of wheat (*Triticum aestivum* L.) var. Bhakar-02 during two harvests.

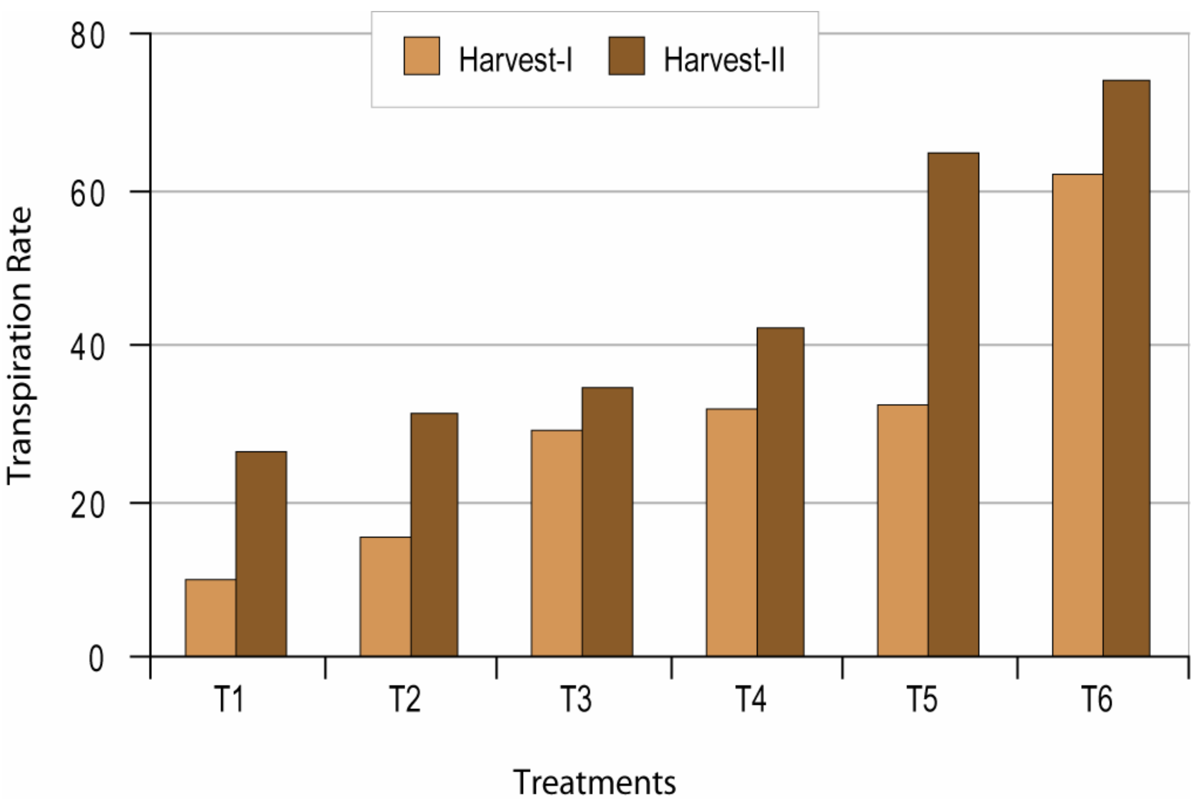

Fig. 4. Impact of various nickel concentrations (percent decrease vs. control) on transpiration rate of wheat (*Triticum aestivum* L.) var. Bhakar-02 during two harvests.

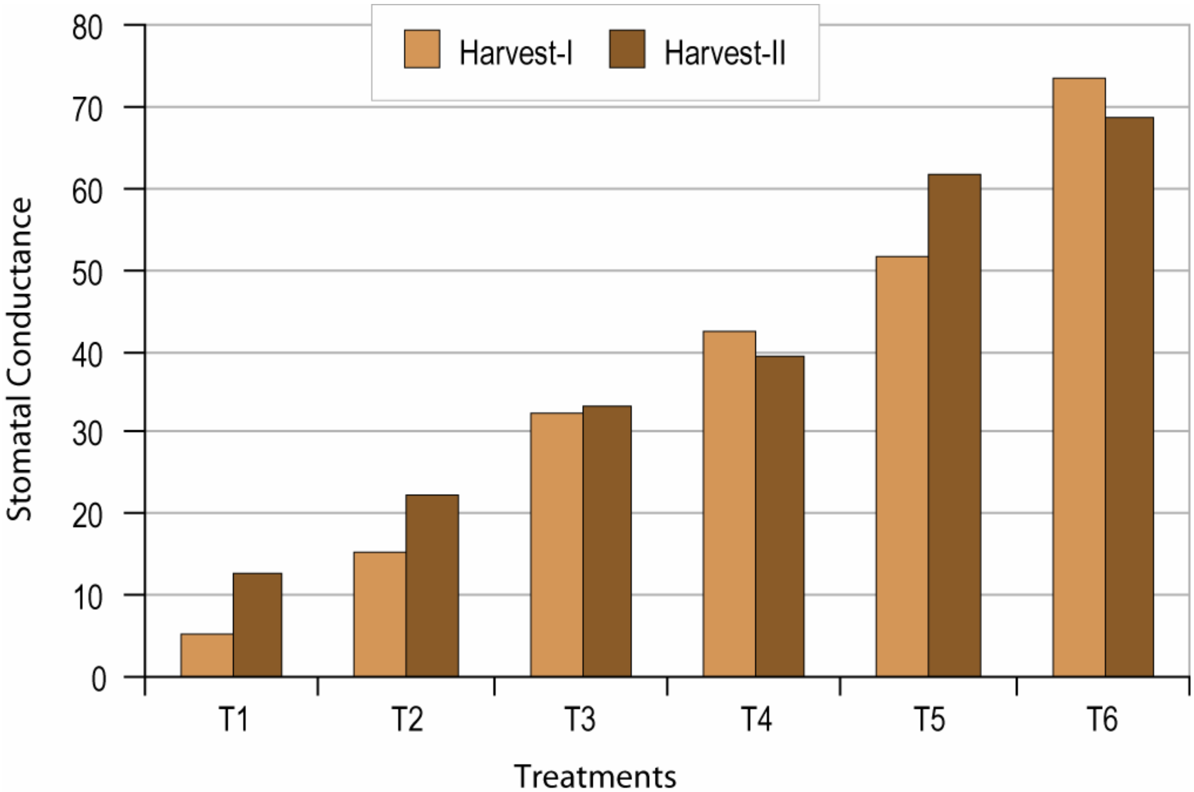

Fig. 5. Impact of various nickel concentrations (percent decrease vs. control) on stomatal conductance of wheat (*Triticum aestivum* L.) var. Bhakar-02 during two harvests.

Table 2. Impact of different nickel concentrations on some physiological attributes of wheat (*Triticum aestivum* L.) var. Bhakar-02 during two growth assessment stages.

Treatments	Photosynthetic rate ($\mu Mm^{-2}s^{-1}$)		Transpiration rate ($\mu Mm^{-2}s^{-1}$)		Stomatal conductance ($\mu Mm^{-2}s^{-1}$)	
	Harvest I	Harvest II	Harvest I	Harvest II	Harvest I	Harvest II
T0	17.42a ± 0.92	10.65a ± 0.13	1.44a ± 0.18	0.99a ± 0.06	1.95a ± 0.42	1.12a ± 0.15
T1	14.0b ± 0.45	9.75b ± 0.24	1.3a ± 0.16	0.734a ± 0.16	1.85a ± 0.44	0.98b ± 0.09
T2	12.88c ± 0.18	8.31c ± 0.53	1.22a ± 0.05	0.689a ± 0.13	1.65a ± 0.23	0.87b ± 0.27
T3	11.48d ± 0.68	6.84d ± 0.51	1.02a ± 0.12	0.654a ± 0.04	1.32a ± 0.15	0.75b ± 0.16
T4	10.69e ± 0.70	5.31f ± 0.42	0.98b ± 0.04	0.572a ± 0.12	1.12a ± 0.04	0.68b ± 0.05
T5	10.49e ± 0.54	4.23g ± 0.18	0.97b ± 0.18	0.352a ± 0.12	0.94b ± 0.05	0.43b ± 0.12

Treatments means followed by letters in every column are statistically different from each other at P=0.05 according to DMRT (Duncan's Multiple Range Test)

Discussion

Impact of nickel on biochemical and physiological parameters of wheat cv. Bhakar-02 has been described in results. The response of higher amount of Ni to these parameters was found toxic as these concentration exceeds the micronutrient values of Ni which are of permissible amount.

Chlorophyll is the key chemical compound present in the plants which is necessary for photosynthesis. The present work clearly showed that control plants were healthier than treated ones and have higher amount of chlorophyll a,b, total chlorophyll and carotenoids. But at higher nickel levels, concentration increased in soil, the amount of chlorophylls and carotenoids decreased. Total chlorophyll decreased by 6%-70% in wheat (*Triticum aestivum* L. Bhakar-02). The carotenoids contents are also reduced in both cultivars. This reduction occurred due to the disturbance in chlorophyll synthesis and uptake of magnesium.

The following workers findings were same like present research as Pandolfini *et al*., (1996) observed the nickel effects on chlorophylls of two wheat cultivars in which drought sensitive cultivars showed greater reduction of chlorophyll a and b than drought tolerant cultivar in response to higher nickel levels. Ouzounidou *et al*., (2006) observed the response of wheat to nickel in which they found that chlorophyll contents decreased with increasing nickel levels.

Different Ni levels also remarkably affected physiological growth of the plants. The toxicity of nickel affected the process of photosynthesis by damaging ultra-structure of chloroplast, less synthesis of chlorophyll, carotenoids and decreased activities of enzymes in Calvin cycle, CO_2 deficiencies and changes in thylakoid membrane. The present work showed that photosynthetic and transpiration rate and stomatal conductance were decreased by increasing concentrations of nickel in wheat (*Triticum aestivum* L. Bhakar-02). This reduction was more pronounced during second harvest than first harvest.

Wallnofer *et al*., (1984) observed that accumulation of toxical elements in plants resulted to disturbance of physiological processes as photosynthesis and transpiration. While Morgutti *et al*., (1984) concluded that excess nickel concentration altered the fundamental physiological processes like photosynthetic and transpiration activities. These observations were same like the present findings.

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