



## **Impact of Elevated Soil Temperature on Physiological Parameters of Tea Plant Growing Adjacent to Gas Flaring Site**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors RK and KB was responsible for performing the experiment, data collection, statistical data analysis, interpretation of the results and manuscript preparation. Authors SCB and GKS conceived the idea. Author RK designed the experiment and edited the manuscript. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Gas flaring is now recognized as a major environmental problem. Smoking flare may be a significant contributor to overall particulate emissions. It also results in production of excessive heat in the nearby areas. The present investigation aims to study the impact of elevated soil temperature on physiological parameters of tea plant growing adjacent to Gas flaring site. The experimental plot was laid out in 5 x 2 factorial RCBD with two factors viz. different distances from the flare site and two seasons in two different tea gardens adjacent to Kathaloni OCS and Merbil Majuli OCS 6 in Dibrugarh district of Assam in 2019-20. Plant and soil samples were collected and estimation of some select plant physiological parameters was done. Significant increase in soil temperature and decrease in soil moisture content was observed in distance closer to the flare pit. The result of the study revealed that there was a gradual decline in relative turgidity, specific leaf weight, plucking point density, stomatal count, leaf area measurement, polyphenol content, chlorophyll content, caffeine content but an increase in water saturation deficit of the green tea leaves on plants existing at a distance moving closer to the flare pit. A significant increase in rain flushing season as compared to autumn was observed in all the plant parameters except water saturation deficit.

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**Keywords:** Gas flaring; soil temperature; relative turgidity; WSD; caffeine; polyphenol.

## 1. INTRODUCTION

Natural gas is a by-product of petroleum industry. Natural gas is made up of pure hydrocarbons. In the process of routine operations that are involved in the production of oil and gas, there is release of natural gas by-products called associated gas. Although composition of associated gases varies from location to location at fields, the basic components include methane, ethane, propane, isobutene, n-butane, isopentane, n-pentane, n-hexane, H<sub>2</sub>S, He, N<sub>2</sub> etc [1].

Associated gases are flared either as a means of disposal or as a safety measure to relieve pressure [2]. This process of burning of natural gases is called gas flaring. Gas flaring is defined as the controlled burning of natural gas that cannot be processed for sale or use because of technical or economic reasons [3].

Most flaring processes usually take place at the top of stack by burning of gases with the visible flame. Height of the flame depends upon the volume of released gas, while brightness and colour depend upon its composition. Gas flaring is now recognized as a major environmental problem. This problem can be caused by a rise in emission of greenhouse gases in the atmosphere. Gas flaring results in huge emissions of carbon dioxide (CO<sub>2</sub>), sulphur dioxide (SO<sub>2</sub>), hydrogen sulphide (H<sub>2</sub>S), nitrogen oxides (NO<sub>x</sub>) and volatile organic components (VOC). Smoking flare may be a significant contributor to overall particulate emissions. It also results in production of excessive heat in the nearby areas. An amount of about 150 billion cubic meters of natural gas is flared around the world, contaminating the environment with about 400 Million tones of CO<sub>2</sub> per year [4,5].

Occurrence of several crude oil drilling sites in and around tea plantation areas is common in upper Assam especially in the districts of Dibrugarh and Tinsukia. Soil and air contamination due to gas flaring in such tea gardens is very prevalent. Flaring may further contribute to local and regional environmental problems, such as acid rain with added impact on agriculture, forests and other physical infrastructure [6].

Gas Flaring contribute to local and regional environmental problems and often due to its

close proximity to tea plantations it affects the physical and chemical properties of tea growing soils which may reduce the growth and productivity of the tea plants and their resistance to biotic and abiotic stresses, making them more vulnerable to pathogens. There is inconsistent data and under-reporting of gas flaring by governments and companies, which has complicated the global effort to track progress on flaring reduction as there are some large uncertainties at the country and regional level. Very little research work has been done to study the effect of gas flaring on the physiology and growth of tea plants in Assam. Therefore specific study and a technical understanding were devised to study impact of elevated soil temperature on physiological parameters of tea plant growing adjacent to Gas flaring site.

## 2. MATERIALS AND METHODS

The present investigation was carried out during 2019-20 in two different tea gardens adjacent to Kathaloni OCS and Merbil Majuli OCS 6 in Dibrugarh district of Assam. Experimental design RCBD with 5 distances and 3 replications was laid out in both the locations. Collection of field data and plant samples were carried out in two seasons: Rain flush (S1) and autumn flush (S2). In each season samples were collected at four distances from the flare site with an interval of 20 m at distances D1 (11-31m), D2 (31-51m), D3 (51-71m) and D4 (71-91m) starting from the gas flaring point in both Merbil Majuli OCS-6 and Kothaloni OCS.

### 2.1 Soil Temperature and Soil Moisture

Soil thermometers were installed into the soil at 15 cm depth in the experimental plots and data was recorded at an interval of 2 hours from 6 am to 6 pm during both the flushing seasons. Soil samples were collected at different flushing seasons and distances from the flare pit from a depth of 0-30 cm from the ground by sampling auger and moisture content was determined by gravimetric method.

### 2.2 Plant Physiological Parameters

For sampling purpose, the fully expanded leaves of maintenance foliage were collected. For quality parameter like total polyphenol, caffeine and chlorophyll content, fresh two leaves and a bud were taken for estimation.

### 2.2.1 Relative turgidity

Relative turgidity was estimated using Relative water content technique given by Weatherly [7]. From the recorded values the relative turgidity was calculated using the following formula.

$$\text{Relative turgidity (RT)(\%)} = \frac{(\text{Fresh Weight} - \text{Dry Weight})}{(\text{Saturated Fresh Weight} - \text{Dry Weight})} \times 100$$

### 2.2.2 Water saturation deficit

Water saturation deficit was estimated using Relative water content technique given by Weatherly [7] using the following formula.

$$\text{Water saturation deficit (WSD)(\%)} = \frac{(\text{Saturated Fresh Weight} - \text{Fresh Weight})}{(\text{Saturated Fresh Weight} - \text{Dry Weight})} \times 100$$

### 2.2.3 Stomatal count

Stomatal count was estimated using Leaf impression method given by Beakbane and Mazumder [8]. The impression was taken from lower surface of fully expanded maintenance foliage using quick fix. The number of stomata per unit area of leaf was counted under high power microscope.

### 2.2.4 Specific leaf weight

Specific leaf weight was calculated by using the formula.

$$\text{Specific Leaf Weight (SLW)(g/cm}^2\text{)} = \frac{(\text{Dry Weight of leaves})}{(\text{Surface area of leaves (cm}^2\text{)})}$$

### 2.2.5 Leaf area measurement

Leaf area was determined using Millimeter graph paper method [9]. Leaf samples were taken and traced over graph paper and the grids covered by the leaves were counted to give the area.

### 2.2.6 Chlorophyll content

The extraction was done with methanol following the method of Taylor [10] to analyze chlorophyll pigments. The spectrophotometric observations of diluted solutions were recorded at wavelength 653 nm for chlorophyll-a and 666 nm for chlorophyll-b. The spectrophotometric values were converted into the actual quantities of chl-a and chl-b using the following formulas.

$$\text{Chl-a} = 15.65A_{666} - 7.34A_{653}$$

$$\text{Chl-b} = 27.05A_{653} - 11.21A_{666}$$

### 2.2.7 Caffeine content

The caffeine estimation and purification was done using chloroform following the method of Rapić [11] from freshly collected sample of two leaves and a bud. The amount of caffeine was calculated by taking weight of fresh tea leaves and weight of crude caffeine in gram, and then the total caffeine in tea leaves was expressed in percentage.

### 2.2.8 Total polyphenol content

Estimation of polyphenol was done using Folin-Ciocalteu reagent based on the reaction between phenols and an oxidizing agent phosphomolybdate which results in formation of blue complex [12]. The value of polyphenol was expressed in percentage.

### 2.2.9 Plucking point density

The method used for counting plucking points was similar to the method described by Barua and Dutta [13]. A square grid of 50 x 50 cm<sup>2</sup> area was placed at the top of a bush selected at random within the experimental plot. The number of plucking points were counted and expressed in number per 50 x 50 cm<sup>2</sup> area.

## 3. RESULTS AND DISCUSSION

### 3.1 Soil Moisture and Soil Temperature

The estimation of soil moisture and temperature is critical to the understanding of land surface-atmosphere interactions. The study revealed that soil temperature varies significantly ( $P=0.05$ ) among the different distances from the flare pit. Higher light intensities were recorded in distance closer to the flare and it decreases on moving away from the flare [51]. Although the air temperature outside the boundary wall was relatively low due to the insulating effect of the concrete walls, the mean soil temperatures varied between 29.2°C and 25.23°C for D1 and DC in both the experimental fields (Table 1 & 2). Since the flare intensity varies with distance from flare [14], it demarcates the increase in soil temperature in distance near the flare. The results fit the observation of [14-17].

The mean soil moisture level was also significantly ( $P=0.05$ ) different in all the different distances from the flare as shown in Table 1 & 2. A rapid decline in soil moisture content was observed in distances closer to the flare from 15.35% and 15.55% in DC to 11.44% and 11.74% in D1 in Kothaloni and Merbil respectively. The decrease in soil moisture corresponds with the increase in soil temperature. The relationship between soil temperature and moisture follow an inverse relationship, i.e., when the soil temperature increases, the soil moisture decreases. Similar observation was also made by Lakshmi [18] and Idso [19] where it was inferred that soil temperature shows an increase that corresponds to a decrease in the soil moisture.

### 3.2 Relative Turgidity, Water Saturation Deficit and Chlorophyll Content

Relative water content (relative turgidity) is an important parameter used to estimate the water potential under stress condition [20]. The mean relative turgidity were significantly ( $P=0.05$ ) different amongst seasons and different distances from the flare. Although the relative turgidity increases while going further from the flare, an initial leap was observed from D1 to D2 (Table 1 & 2). As Relative turgidity contains amount of available water in leaf, increasing stress causes to decreasing it. From such responses, it may be inferred that relative turgidity decreases with decrease in soil moisture content. [21]. Bansal and Nagarajans [22] and Clarke and Coig [23] showed that water stress-resistant cultivars have higher RWC, which is in agreement with the results of the present study.

In the present investigation, the relative turgidity was observed to be higher in rain flush as compared to autumn. This can be attributed to higher soil moisture content and atmospheric humidity in the rainy season as compared to autumn. The findings are in corroboration with work done by Morais [24] where RWC values were significantly affected by the season with significant decrease in autumn.

Water saturation deficit is very common in the production of most crops and numerous studies have indicated that they can have substantial negative impacts on plant growth and development [25]. There is some evidence that roots are the primary sensors of water deficit in the soil, causing the observed physiological and biochemical perturbations in the stems and the decline in growth to be generally interconnected

with changes in plant nutrition, carbon dioxide balance and water relations [26]. The mean water saturation deficit was significantly ( $P=0.05$ ) different amongst different seasons and distances from the flare pit.

At low soil moisture levels, the water saturation deficit was recorded to be the highest with 20.37% and 19.04% at D1 as compared to 15.96% and 14.51% at DC (Table 1 & 2). Alireza [26] observed that reduction of soil moisture content leads to increase in water saturation deficit which progressively inhibits plant growth. Aref [21] reported that the cultivars having the lowest water saturation deficit were recommended as more tolerant to drought stress. Mean water saturation deficit was found to be higher in autumn than rain flushing season. The water saturation deficit is rapidly reduced with increase in soil moisture and higher relative humidity of air as observed in rainy season [24].

The leaf chlorophyll (chlorophyll a and b) was significantly ( $P=0.05$ ) different amongst different seasons and distances from the flare pit. Chlorophyll increased with increasing distance from the flare point and from rain flush to autumn flush. The chlorophyll content reduced with decreasing leaf water potential and relative leaf water content (Table 1 & 2).

Loss of chlorophyll reduces the amount of photons absorbed by leaves and can finally reduce photosynthesis rate and consequent increase in excitation energy, which may lead to reduction of oxygen, forming reactive oxygen species [27]. It is well established that photosynthetic pigments such as chlorophyll a and b are sensitive to high-temperature stress. Heat stress results in plant leaf pigment loss and significantly damages photosynthetic activities [28]. Total chlorophyll content was found to be significantly ( $P=0.05$ ) higher in rain flush season than in autumn flush. The results in the present study are in conformity with the findings of Alireza [26] where they reported that water stress (excess water and water deficit) significantly decreases leaf chlorophyll (chlorophyll a, b and total chlorophyll) concentrations. Water deficit can destroy the chlorophyll and prevent making it [29,30].

### 3.3 Stomatal Count, Leaf Area Measurement and Specific Leaf Weight

Stomata are the primary anatomical structures involved in water loss and gas exchange in

plants. The study on stomatal count revealed that the mean stomatal count was significantly ( $P=0.05$ ) different amongst different seasons and distances from the flare pit as shown in Table 1 & 2. With the increase in distance, the stomatal count increases along the distance. On the other hand stomatal count was found to decrease from rain to autumn season. Similar findings were observed by Tezara. [31] that reduction of leaf water content leads to reduction of stomatal frequency which progressively inhibits conductance and photosynthesis. Moreover, Khan [32] also observed that low numbers of stomata may be attributed to higher drought stress as reported in other plants. As season changes major decrease in stomata occur, possibly due to occlusion of stomatal pores by wax or accumulation of abscisic acid as dormancy approaches for leaf senescence in autumn.

The mean leaf area measurement reduces with gradual decrease in distance from the flare and were significantly ( $P=0.05$ ) different amongst different distances from the flare pit. The increase in water depletion levels of soil led to a significant decrease in the leaf area values of the tea plant at a significant level of 5% (Table 1 & 2). The reason for the reduction of leaf area with increased levels of water stress is the occurrence of reduction in the growth of the leaves due to the decrease in the process of division and elongation of cells as a result of the decrease in the relative water content of leaves. This was attributed to an increase in the leaf water pressure causing a reduction in the opening of stomata which reduces the process of carbon representation. Accordingly production of plant pigments including chlorophyll reduces the production of carbohydrates which negatively affects the leaf area [33,34].

The mean specific leaf weight was significantly ( $P=0.05$ ) different amongst different seasons and distances from the flare pit. A notable reduction in the specific leaf weight was observed in distances closer to the flare. Dry weight of leaf and leaf area significantly decreased under drought and combined stress as compared to control (Table 1 & 2). Decrease in dry weight and leaf area leads to subsequent reduction in specific leaf weight. Similar observation was recorded by Misganaw [35] where it was reported that heat and water stress affected the biomass

of all alfalfa cultivars compared with the control and significantly reduced the shoot fresh weight and thus specific leaf weight.

### 3.4 Caffeine Content

Caffeine is the most studied at the molecular biochemistry level and is known to be regulated by light conditions, altitude and stress [36,37,38]. The present study confirmed that the amount of caffeine content decreases significantly ( $P=0.05$ ) at distances closer to the flare and from rain to autumn season. Caffeine content ranged from 2.301 % to 3.333 % with increase in distances from the flare (Table 1 & 2). Caffeine was found to be highest in control distance. Season variation in caffeine was also observed with decrease in caffeine content from rain to autumn flush. Therefore it can be concluded from the above findings that reduction in the caffeine content takes place under increase in temperature, water deficit and change in harvestable season.

In an experiment conducted by Kumar [36] it was reported that salinity and drought stress alter caffeine levels in young leaves of *Coffea canephora* by altering the caffeine degradation route and perhaps to a smaller extent even the regulation of transcripts of the genes involved in the biosynthesis pathway. Salinity and drought stress converge in similar physiological conditions in terms of water deficit.

### 3.5 Total Polyphenol Content

Significant ( $P=0.05$ ) variation in total polyphenol content was recorded in tea bushes near the flare pit compared to the tea bushes in the control. Decrease in total polyphenol content was observed with season variation from rain to autumn in the tea bushes (Table 1 & 2). Phenolic compounds in fresh tea leaves are composed of predominantly flavan-3-ols (catechins) and flavonol glycosides and phenolic [39,40]. In a study by Caffin [41], the levels of EGCG and ECG, the main catechin gallates in tea flushes, were higher in the leaves harvested in warmer months in Australia. The authors suggested that fresh leaves harvested during warmer months contained higher catechin gallate levels and had greater potential for making high quality BT than those harvested at other times of the year.

**Table 1. Effect of gas flaring on soil temperature, soil moisture and plant physiological parameters of tea (Kothaloni OCS)**

Distances	Soil temperature (°C)		Soil moisture (%)		Relative turgidity (%)		Water saturation deficit (%)		Stomatal count (stomata mm <sup>-2</sup> )		Specific leaf weight (g cm <sup>-2</sup> )		Leaf area (cm <sup>2</sup> )		Chlorophyll content (mg g <sup>-1</sup> )		Caffeine content (%)		Total polyphenol content (%)		Plucking point density	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
D1 (11-31 m)	29.3	29.0	11.51	11.37	86.37	72.87	13.62	27.12	21.33	20.33	0.008	0.007	36.66	33.91	0.997	0.927	2.321	2.280	22.213	20.973	32.0	30.0
D2 (31-51 m)	29.0	29.3	12.94	11.69	90.25	76.18	9.74	23.81	21.33	20.33	0.008	0.006	43.73	38.56	1.167	1.150	2.437	2.383	22.470	21.237	34.0	32.3
D3 (51-71 m)	27.7	27.7	12.34	12.30	89.11	78.93	11.30	21.54	21.33	20.33	0.010	0.009	45.57	44.39	1.583	1.387	2.903	2.557	23.207	22.323	38.0	34.0
D4 (71-91 m)	27.3	27.3	15.21	14.25	89.64	78.41	11.00	21.02	21.33	20.67	0.011	0.010	49.65	48.94	2.193	2.087	3.240	3.160	23.320	22.833	40.0	38.3
D <sub>c</sub> (150-170 m)	26.7	27.0	15.38	15.32	89.86	79.16	10.34	21.58	20.67	22.33	0.012	0.010	50.66	50.66	2.343	2.270	3.417	3.250	23.487	23.060	42.0	41.0
CD (factor D)	0.6		1.88		2.45		2.41		0.45		0.002		8.507		0.115		0.046		0.848		1.218	
CD (factor S)	N/A		N/A		1.55		1.53		0.29		0.001		N/A		0.073		0.029		0.536		0.770	

\*Significant at 5% probability level

**Table 2. Effect of gas flaring on soil temperature, soil moisture and plant physiological parameters of tea (Merbil Majuli OCS-6)**

Distances	Soil temperature (°C)		Soil moisture (%)		Relative turgidity (%)		Water saturation deficit (%)		Stomatal count (stomata mm <sup>-2</sup> )		Specific leaf weight (g cm <sup>-2</sup> )		Leaf area (cm <sup>2</sup> )		Chlorophyll content (mg g <sup>-1</sup> )		Caffeine content (%)		Total polyphenol content (%)		Plucking point density	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
D1 (11-31 m)	29.33	30.33	11.71	11.93	83.33	80.95	16.66	19.04	17.00	16.00	0.007	0.007	31.61	31.15	0.55	0.48	1.44	1.32	22.24	21.09	22.00	21.00
D2 (31-51 m)	28.66	29.00	12.32	12.30	84.17	81.28	15.82	18.71	18.00	16.00	0.007	0.007	32.17	32.04	0.62	0.51	2.66	1.98	22.51	21.06	23.00	22.00
D3 (51-71 m)	27.33	27.66	12.88	1.75	84.76	82.25	15.23	17.74	19.00	18.00	0.010	0.010	32.60	31.68	0.76	0.68	2.64	2.22	23.27	22.35	24.00	22.00
D4 (71-91 m)	26.33	26.66	15.47	14.16	85.89	84.00	14.40	15.99	20.00	19.00	0.010	0.010	32.84	32.50	0.99	0.77	2.64	2.30	23.36	22.82	24.00	23.00
D <sub>c</sub> (150-170 m)	25.23	26.22	15.55	14.26	86.77	85.78	13.23	14.51	21.00	20.00	0.010	0.010	33.65	33.10	0.99	0.78	2.74	2.36	23.40	22.83	26.00	24.00
CD (factor D)	0.857		1.742		1.020		1.430		0.813		0.002		0.808		0.145		0.684		0.936		0.772	
CD (factor S)	N/A		N/A		0.720		1.010		0.575		0.001		N/A		0.102		N/A		0.662		0.553	

\*Significant at 5% probability level/competing interests

Investigation the effect of manufacturing season on the antimicrobial activity of tea Chou [42], found that the catechin content in tea leaves varied with season; and the tea product made from tea leaves harvested in summer exhibited the strongest antimicrobial activity due to the highest content of catechin. Similar experiment conducted by Ghabru and Sud [43] reported that the samples of summer flush season invariably had high level of TP. It could be due to the fact that high temperature and longer day time during summer flush season may help tea shoots to accumulate higher level of TP. The levels of total phenolics in fresh tea shoots were reported to be lower in cooler months that increased throughout the warmer months [44].

### 3.6 Plucking Point Density

The plucking point density was significantly ( $P=0.05$ ) different among the different distances studied and also significantly ( $P=0.05$ ) different between the seasons. However, the distance x season interaction was not significant. The plucking point density of tea bushes observed was reduced with seasonal variation and increase in heat and water stress caused by close proximity to the flare (Table 1 & 2).

Variations between seasons result from the effect of climate on shoot growth rates influenced by air and soil temperatures and rainfall, saturation deficit of the air being the dominant factor [45,46]. Kumar and Bera [47] reported that the field observations from their experiment have shown that the density of dormant shoots are less during periods of higher leaf yield (rush crop) and high during periods of lower leaf yield.

An increase in water availability results in a significant increase in growth of potted tea plants on the basis of both plant height and new leaves [48]. Altered water availability is a key driver of plant performance [49,50] and significantly impacts both growth and secondary metabolite concentrations of tea plants [49,51,52,53,54].

The reduced growth of plants under drought treatment in this study concurs with the widely accepted recognition that lower soil moisture content reduces photosynthesis, growth and survivability of plants [55,56].

## 4. CONCLUSION

Increase in the soil temperature and reduced soil moisture due to proximity to gas flare caused a gradual decline in relative turgidity, stomatal

count, specific leaf weight, leaf area measurement, chlorophyll content, caffeine content, total polyphenol content, plucking point density but an increase in water saturation deficit in the tea bushes growing in the area. All the plant physiological and growth parameters under the study were recorded to be higher in rain flush than autumn flush except water saturation deficit which was higher in the autumn flushing season.

Natural gas is a vital component of the world's supply of energy. It is one of the cleanest, safest, and most useful of all energy sources. Flaring it leads to degradation of this natural energy. Proper mitigation of natural gas should be done to stop the resulting environmental degradation. Therefore, engineering an ergonomic technique for a better use of natural gas produced by the petroleum industry is a need of the hour.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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