Pol. J. Environ. Stud. Vol. 29, No. 2 (2020), 1535-1544
DOI: *10.15244/pjoes/101610* ONLINE PUBLICATION DATE: 2019-12-16

Original Research

Effects of Increasing Soil Fluoride on the Growth of Vegetation in the Vicinity of Brick Kilns: A Case Study from Rawalpindi, Pakistan

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Received: 25 August 2018
Accepted: 15 December 2018

Abstract

Increased fluoride emissions from brick kilns has affected the existence of plant species near brick kilns in Rawalpindi. For this purpose, six mesocosms in cardboard boxes were prepared, out of which two cardboard boxes were allocated as controls (no treatment given); while in four other cardboard box treatments of sodium fluoride, NaF was given. Fluoride concentrations of 30 ppm and 50 ppm were given to three different species of plants, i.e., Conyza Canadensis, Artemisia Absinthium and Cannabis Sativa. This study has evaluated the performance of different plants. The evaluation is done on several parameters such as chlorophyll level, carotenoid content and ascorbic acid. Similarly, other parameters checked are relative water content, above and below biomass, plant height, area of leaves and the number of leaves. Results showed that above- and below-ground biomass showed significant decreases in all selected species. Conyza Canadensis at 30 ppm and 50 ppm fluoride showed the greatest percentage reduction of leaf area with respect to control, i.e., 46.84% and 42.63%, respectively. Cannabis Sativa showed the greatest reduction in chlorophyll at 30 ppm (74.63%) and 50 ppm (59.73%). While artemisia absinthium did not show a significant decrease in both chlorophyll a and chlorophyll b.

Keywords: brick kilns, fluoride emissions, sodium fluoride, growth parameters, plant species, chlorophyll

Introduction

The diversity of plant species provides significant evidence of an expression of biodiversity and genetic diversity [1]. But, unfortunately, excessive fluoride emissions from anthropogenic activities is adversely affecting their existence, as it has been found that most of the plants are very much sensitive to different fluoride levels [2]. Anthropogenic sources of fluoride include burning of coal, oil refining, and production of steel, phosphate fertilizer plants and brick-making industries [3]. It has been reported that the total amount of fluorine released into air, surface water and land in the United States of America were found to be 39, 24 and 500 tons, respectively. In Canada as well, the total amount of fluoride emitted yearly into the environment

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specifically from industrial sources was approximately in excess of 23 500 tones [4]. Fluoride is reportedly considered one of the contaminants harmful to the environment produced by industries located in Pakistan [5]. Reportedly, the most common anthropogenic source affecting vegetation is brick kilns [6]. Moreover, brick kilns, which exist in South Asia, are often located inside and around cities on agricultural land and pollutants emitted from these brick kilns have been shown to directly affect the vegetation around the kilns [7]. Mean fluoride contents cannot exceed 5 mg/kg in plants grown in uncontaminated areas or with minimal anthropogenic activities. According to WHO Guidelines, minute injury could come about when plant species are exposed to around 0.2 mg/m^3 of fluoride [8].

Therefore, fluoride emissions possess a considerable potential for biodiversity loss and, in turn, ecological damage as they are not biodegradable and have the ability to accumulate slowly in the environment [9]. Depending on the sensitivity, plants exhibit noticeable alteration, which includes biochemical changes or metabolite accumulation in plant species. An accurate mechanism by which fluoride causes damage to plants is still little understood. However, certain physiological processes are known to be markedly affected by fluoride. For example, a reduced rate of photosynthesis and a gradual decrease in plant growth has been reported [10]. When there will be an abundant absorption of fluoride then this will lead to phytotoxicity and ultrastructural alterations in leaves, thus affecting the biochemical and physiological characteristics [11].

Fluoride Pollution and Growth of Plants

Fluoride is present in soil, water, air, and plants in different concentrations but it is not even considered essential for the normal growth of plants [12]. Most of the plants are very much sensitive to different fluoride levels. Fluoride is first taken up by plant roots and is then transported via xylematic flow of the transpiratory and storage organs. Bioaccumulation of fluoride in different parts of plants varies depending on its transfer from soil solution to roots and its movement from root to shoot [13].

Plants show more susceptibility to fluoride injury from the soil than the atmosphere [14]. Arya (1971) reported the worst injury to tomato plants when fluoride entered through the roots when a 250 ppm concentration of sodium fluoride solution was supplied [15]. Stevens et al. (1997) in their study revealed that fluoride ions in solution had a noticeable influence on the uptake of fluoride by plant roots, with complex species being more readily taken up by the roots than the free fluoride ions [16].

Fluoride Pollution and Pakistan

Ahmad et al. (2012) have highlighted the unfavourable effects of fluoride on vegetation as an evolving and unrecognized problem in South Asia. This is due to population growth and more construction, which is increasing the demand for bricks [17]. It is reported that 75% of the total global brick kilns (approx. 300,000) are located in countries including Pakistan, stand with 11000 brick kilns – most of which are located in Punjab Province, where the study area is located [18]. Like Pakistan, brick kilns in the South Asian region emit pollutants upon consuming 'coal' as an energy source that ultimately affects vegetation cover in the vicinity [19].

Therefore, research was conducted to study the effects of excessive emissions of fluoride from brick kilns in Rawalpindi. The effects on plant species of fluoride emissions from brick kilns were analyzed by the following parameters: 1) growth attributes, which include leaf area, number of leaves and plant height; 2) biochemical changes by examining the change in ascorbic acid, carotenoid content, chlorophyll level and relative water content; and 3) changes in above- and below-ground biomass of each of the selected plant species.

Materials and Methods

Overview of Study Area and Selected Species

The study was carried-out around a brick kiln site in Rawalpindi, Pakistan, which has a semi-arid sub-tropical climatic zone and experiences great variations in temperature. The annual temperature of Rawalpindi is 21.5ºC and annual rainfall is 941 mm [20]. Compared to the rest of the country, Rawalpindi remains most appropriate for the study as it is among the older cities in the country and therefore is a hub of industrial and commercial works [21]. In the initial phase of the study, brick kiln sites of Rawalpindi were visited and then plant species were selected on the basis of their common presence around brick kilns. Subsequently, the research study targeted the plant species, namely *Cannabis Sativus, Conyza Canadensis and Artemisia Absinthium* – commonly grown wild species around brick kilns in the study area. The presence of selected species cannot be ignored as they are commonly present around almost all the brick kiln sites of Rawalpindi (study area). So, it was necessary to check the effects of fluoride only on selected species which, actually, have a habitat along brick kiln sites. Each of the variety of species has its own significance for different purposes, too. For example, *Conyza Canadensis* has a property of reducing blood glucose levels and simultaneously improving glucose tolerance and hence is taken as a cure for diabetes [22]. Similarly, *Cannabis Sativus* and *Artemisia Absinthium* both are associated with a cure for different types of cancers [23-24].

Preparing Mesocosms and Collecting Seedlings of Selected Species

Soil pH affects the soil's physical, chemical, and biological properties and processes, as well as plant growth. The pH of the soil in the mesocosms of the current study was 7.2. The normal pH range for productive soil is from 6.5 to 8.4 [25]. The pH values of these soils indicate that the study areas have the required range for plant growth. The electrical conductivity of the soil in the mesocosms at the start of the experiment was found to be 0.108 dS/m, where different species of plants were grown, which means that conductivity value of the analyzed soil in this research work was within the normal range, i.e., 0-2 dS/m as reported by Zaku et al. in their research [26]. EC value of the soil in the mesocosms is an indication of nonsalinity. As high EC serves as a sign of salinity (EC>4 dS/m), which delays crop growth (inability to absorb water) and microbial activity [27].

For the purposes of data and processing, six mesocosms in total were prepared in 35.6 cm x 19.8 cm cardboard boxes. These mesocosms were filled with soil from the study site. Further, seedlings of *Cannabis Sativus, Conyza Canadensis* and *Artemisia Absinthium,* which were similar in size and at the same growth stage were collected and transferred into the prepared boxes. In each box, two seedlings of each species were planted. Two boxes were used as a control where no treatment was given. Similarly, two boxes were used for each treatment of fluoride, i.e., 30 ppm and 50 ppm of sodium fluoride (NaF). These levels of concentration of NaF have been selected in accordance with the previous studies conducted on the growth parameters of different plants, i.e., *Raphanus Sativus* and *Abelmoschus Esculentus* [28-29].

Preparation of Stock Solution and Treatment of Sodium Fluoride (NaF)

For treatment of NaF, 1000 ppm stock solution of sodium fluoride was prepared by dissolving 2.21 g of sodium fluoride in 1000 ML of distilled water[1]. By using this stock solution of 1000 ppm, two different dilutions of 30 and 50 ppm were prepared. Later, first treatment was given when the seedlings of each species attained stability and a certain height. Other two treatments were given with an interval of one week in order to check the effects of fluoride on plant growth patterns. So for the total period of six weeks, these mesocosms have been incubated under greenhouse conditions at a temperature of between 17 and 23ºC and relative humidity between 55% and 85%, and a 14 h photoperiod. Similarly, with distilled water (for quality control) plant species were watered regularly until harvesting.

Procedures for Determining Selected Parameters

Measurement of Growth Attributes

The first parameter was the measurement of 'growth attributes,' which include leaf area, number of leaves and plant height. Area of the leaf was measured by using the graphical method and after every treatment one leaf (from each treated plant species) was removed and placed on the graph. On the graph, number of square centimeters were counted. [30]. Then, the number of leaves was counted for each species and noted. Lastly, heights of plants were also measured by a commonly used procedure, i.e., measuring the height with tape from the base to the tip of the last leaf [31].

Determining Biochemical Changes

The second parameter is determining 'biochemical change,' which includes change in ascorbic acid, carotenoid content, chlorophyll levels and relative water content (RWC). Initially, 'Chlorophyll a, b, total chlorophyll' level and 'carotenoid content' were determined by using the Arnon method (1949) [32]. For this purpose, fresh leaves weighing 0.5 g were cut down and crushed with 10 ml of 80% acetone. Then the extract was centrifuged at 2500 rpm for 10 minutes. Absorbance of leaf extract was measured through an ultraviolet (UV) spectrophotometer and recorded at wavelengths of 645 nm, 663 nm and 480 nm. The estimated chlorophyll levels were calculated through Arnon equations (1949) as:

$$\text{Chlorophyll a} = 0.0127 \times A663 - 0.00269 \times A645$$

$$\text{Chlorophyll b} = 0.0229 \times A645 - 0.00468 \times A663$$

$$\text{Total Chlorophyll} = 0.0202 \times A645 + 0.00802 \times A663$$

Then, the 'carotenoid content' was exclusively determined using the formula presented by Kirk and Allen (1965) [33]:

$$\text{Carotenoid (mg/g)} = A.480 + (0.114 \text{ x } A.663 - 0.638 \text{ x } A.645)$$

…where A 645, 638, 663 and 480 is absorbance at 645, 638, 663 and 480, respectively.

Ascorbic acid was determined by the titration method in which 2 mg of leaf sample was ground with 5% metaphosphoric acid. This sample was crushed with mortar and pestle until a slurry was formed. Then filtration was done through Whatman filter paper. And 10 ml of filtrate was titrated against the recommended

[1] 1 2.21 g Calculation:-
Molar mass of NaF: 41.98817 g/mol
Divide the total weight by atomic mass of fluorine gave 2.21 g
41.987 / 18.998 = 2.21

DCIP dye [34]. When the color was changed into pink then the volume of DCIP dye was noted. The DCIP solution was standardized against the known quantity of ascorbic acid. This was achieved by the titration of DCIP solution into a solution containing 1 ml ascorbic acid and 9 ml of 5% of metaphosphoric acid. The end-point of this titration was the conversion into pink color that was seen after only 15 seconds. The complete calculation method for determining an ascorbic acid is to divide 4.0 mg (the amount of ascorbic acid present in the standard solution) by the number of ml of dye titrated to determine the amount of ascorbic acid equivalent to 1.0 ml of dye:

$$\frac{\text{Ascorbic acid (mg)}}{\text{1.0 ml of dye}} = \frac{\text{4.0 mg of Ascorbic acid}}{\text{DCIP titrated (ml)}} \quad (1)$$

For the determination of ascorbic acid in and an aliquot of extract:

$$\frac{\text{Ascorbic acid (mg)}}{\text{Aliquot}} = \text{DCIP Titrated x Answer (1) (2)}$$

$$\text{Ascorbic acid mg/g} = \frac{\text{Answer of equation (2) x Total volume of extract}}{\text{Volume of aliquot}} \quad (3)$$

Then, lastly under biochemical change, the relative water content of selected species of plants was determined through the following formula derived by Chen and his colleagues [35]:

$$\text{Relative water content \%} = \frac{\text{Fresh Weight – Dry weight}}{\text{Fresh weight}} \times 100$$

Determining above- and below-Ground Biomass

The third and last parameter in the study was the determination of 'biomass' both above and below ground, for each species, at the end of experiment. For that purpose, destructive harvest was carried out at both above and below ground against all biomass species. Here, fresh biomass was determined just after harvesting. For determination of dry biomass, plants were dried in an oven for 48 hours at 65ºC and then weight was determined on a balance [36].

Statistical Analysis

All parameters' means were calculated and analysis of variance (ANOVA) was performed by using statistical package SPSS V. 21 to check significant differences between treatments. While for biomass and relative water content, the data was calculated in percentages reduction with respect to control.

Results and Discussion

Effects of the Treatment of NaF on Leaf Area

The results of the present study showed that in controlled plants the area of leaf was more than the treated plants, which were given 30 ppm and 50 ppm of sodium fluoride as shown in Fig. 1. Percentage reduction with respect to controlled plants was noticed in treated plants.

The percentage reductions in *Conyza Candensis* at 30 and 50 ppm were found to be 46.84% and 42.63%. While *Artemisia Absinthium* at both the concentrations of 30 ppm and 50 ppm of sodium fluoride showed a percentage reduction of 7.69%. The percentage reductions in *Cannabis Sativus* at 30 and 50 ppm of sodium fluoride were found to be 17.65% and 11.76%.

When the different concentrations of fluoride were analyzed, the result was not found to be significant (*Cannabis sativus* p-value = 0.60 and F = 0.55, *Artemisia Absinthium* p-value = 0.37 and F = 1.1 *Conyza Canadensis* p = 0.1 and F = 2.6). The trend of percentage reduction in all the selected plant species are in accordance with the study conducted on *Hypericum Perforatum* plants. Most interestingly, it was found that percentage reduction in 50 ppm was less than the percentage reduction in 30 ppm. These results are in accordance with the results of the study conducted on *Triticum Aestivum* [37-38].

Effects of the Treatment of NaF on Number of Leaves

The percentage reduction seen in *Conyza candensis* was 23% at 50 ppm of NaF, whereas there was not any marked percentage reduction seen in *Conyza candensis* at 30 ppm of sodium fluoride. *Artemisisa absinthium* did not show any reduction in the number of leaves at both 30 ppm and 50 ppm concentrations of NaF, whereas Cannabis sativa showed a decrease in number of leaves and percentage reduction was found to be 15% at both the concentration of 30 ppm and 50 ppm of sodium fluoride (shown in Fig. 2). These mentioned results are in accordance with the study of Singh et al. (2013) on growth parameters of *Raphanus Sativus* L., which

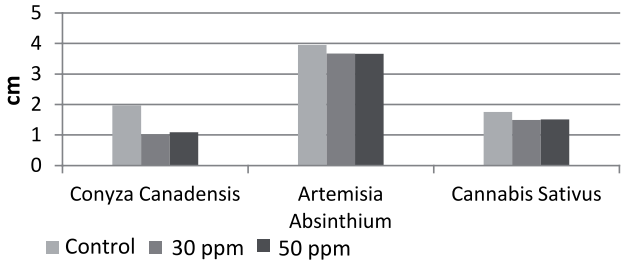

Fig. 1. Mean leaf area of *Conyza Canadensis, Artemisia Absinthium* and *Cannabis Sativus.*

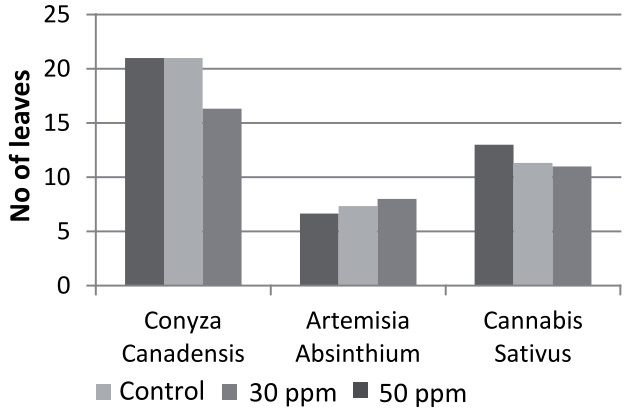

Fig. 2. Mean of number of leaves of *Conyza Canadensis, Artemisia Absinthium* and *Cannabis Sativus.*

revealed that treated plants with a range of sodium fluoride (NaF) also showed a reduction in number of leaves [39]. The differences between concentrations were analyzed in each species, but the results were not significant as the p-values of *Conyza Canadensis, Cannabis Sativus and Artemisia Absinthium* were 0.7313, 0.0955 and 0.3944, respectively.

Effects of the Treatment of NaF on Plant Height

The results of the present study showed that with increasing fluoride level, the heights of the plants decreased as shown in Fig. 3 below, whereas *Artemisia Absinthium* had not shown a percentage reduction in height during the experiment with respect to control plants. But it was noticed that the rate of growth was slowed after treatments were given. This behaviour of *Artemisia Absinthium* of increased height and diameter has also been seen in another study conducted by Papafotiou and colleagues [40]. While other species, i.e., *Conyza Canadensis* (percentage reduction of 7% at 30 ppm and 21% at 50 ppm) *and Cannabis Sativus (*percentage reduction of 26.92% at 30 ppm and 15.38% at 50 ppm) showed a reduction in heights with respect to control. The results of the study are consistent with the results of Singh et al. (2013). The results of different

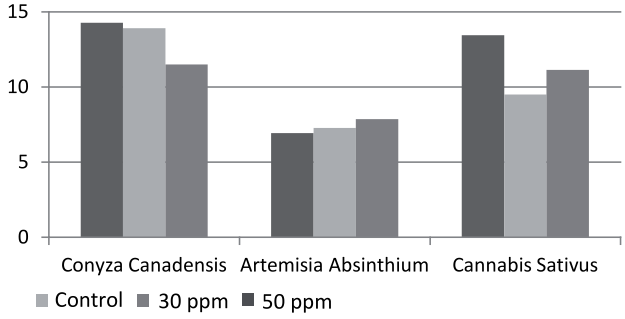

Fig. 3. Mean plant heights of *Conyza Canadensis, Artemisia Absinthium* and *Cannabis sativus.*

concentrations of fluoride were not found to significant (*Conyza Canadensis,* value of p = 0.18 F = 2.12, *Cannabis sativus* value of *p* = 0.13 F = 2.8, *Artemisia Absinthium* value of *p* = 0.4 F = 0.9) when analyzed. It was noticed that the heights of the treated plants were growing slowly as compared to the controlled plants, and this pattern of growth is in accordance with the above-mentioned studies.

Effects of the Treatment of NaF on Chlorophyll a, b and Total Chlorophyll

At 30 and 50 ppm concentrations of sodium fluoride in the current study, *Conyza Canadensis* showed a decrease in chlorophyll of about 62.5% and 55.5%, respectively. Whereas at 30 and 50 ppm, chlorophyll b showed a percentage decrease with respect to control of 59.26% and 26% respectively (as can be seen in Fig. 5). It was noticed only in the middle of the second week that *Conyza Canadensis* at both 30 ppm and 50 ppm showed a value of an increasing trend in chlorophyll a concentration, after which transformation occurred into persistent decreasing values until the end of the experiment. Results showed that *Artemisia Absinthium* at 30 ppm and 50 ppm of sodium fluoride showed a decrease in the level of chlorophyll 'a' of about 53.85% and 32.69% respectively. But, in the case of chlorophyll b, it was seen that there was an increase in chlorophyll b level at 30 and 50 ppm as shown in Fig. 5. In the case of *Cannabis Sativa,* in the controlled condition, it showed an increase in chlorophyll level. But at 30 ppm and 50 ppm, chlorophyll a decreased about 74.63% and 59.7% with respect to control. Similarly, Chlorophyll b at 30 and 50 ppm showed a decrease of about 64.7% and 47% respectively in *Cannabis Sativa*. Total chlorophyll content of *Cannabis Sativus, Conyza Canadensis and Artemisia Absinthium* had an overall decreasing trend with the passage of time, as shown in Fig. 6.

The differences between concentrations were analyzed in each species. We found that in *Conyza Canadensis* there was a significant decrease in chlorophyll a (p = 0.02, F = 6.0143, Std. deviation for control, 30 and 50 ppm were found to be 0.204, 0.025 and 0.153 respectively) and chlorophyll b (p = 0.0369, F = 6.0143, Std. deviation for control, 30 and 50 ppm were found to be 0.042,0.066 and 0.101 respectively). Similar results have been seen in *Cannabis Sativus* (for chlorophyll a, value of p = 0.0038 F = 16.2181, Std. deviation for control = 0.178, 30 ppm = 0.067 and 50 ppm = 0.051 while for chlorophyll b value of p = 0.007 F = 12.4688, Std. deviation for control = 0.060 30 ppm =0.015 and 50 ppm = 0.021), whereas *Artemisia Absinthium* did not show a significant decrease when it was analyzed at different concentrations. Results from the present study are in agreement with the study of Sreedevi and Damodharam, (2013) in which chlorophyll a, chlorophyll b, and total chlorophyll content in leaves of *Cicer aritinum* decreased at 75 ppm of sodium fluoride

[41]. The reason for this reduction of chlorophyll content in the current study may be due to the breakdown of chlorophyll molecule during stress. Another reason can be inhibition of biosynthesis of chlorophyll, which is a major symptom of chlorosis induced by fluoride [42].

Effects of the Treatment of NaF on Carotenoid Content

Carotenoids are accessory pigments in photosynthetic systems and protect chlorophyll against oxidative stress [43]. Similarly, in the present work carotenoid content was found to be decreasing at the concentration of 30 ppm and 50 ppm of sodium fluoride. But, it has been seen at the same time that carotenoid content increased in the initial weeks with the growth of plants. The percentage reduction in *Conyza Candensis, Artemisia Absinthium,* and *Cannabis Sativus* were found to be 6.5%, 14.29% and 16.67% in both 30 and 50 ppm of sodium fluoride. Whereas it was revealed that *Artemisia Absinthium* had not shown any reduction, in carotenoid content at 50 ppm of NaF, as shown in Fig. 7. There was no significant reduction found in the result when different concentrations of fluoride were analyzed in each species. As the value of p of *Conyza Canadesis, Artemisisa Absinthium* and *Cannabis Sativus* was found to be greater than 0.05, i.e., 0.83, 0.85 and 0.50 respectively.

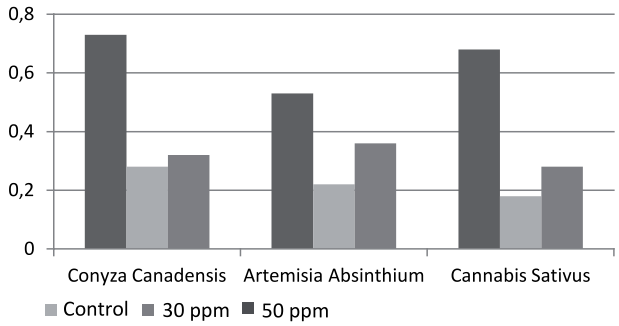

Fig. 4. Mean chlorophyll a concentration in *Conyza Canadensis, Artemisia absinthium* and *Cannabis sativus.*

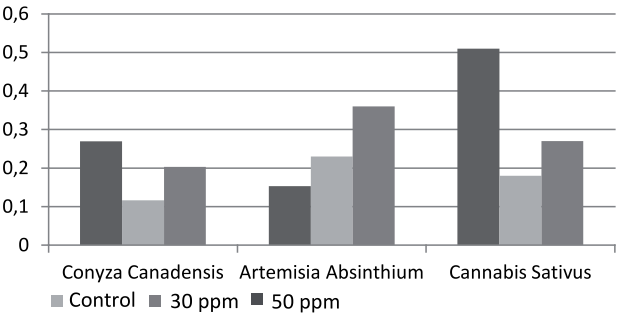

Fig. 5. Mean of chlorophyll b concentration in *Conyza Canadensis*, *Artemisia Absinthium* and *Cannabis Sativus.*

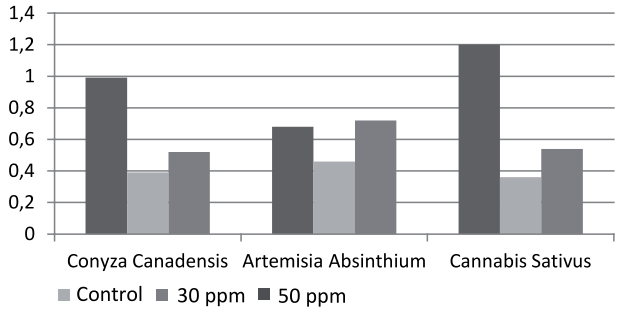

Fig. 6. Mean of total chlorophyll of *Conyza Canadensis*, *Artemisia Absinthium* and *Cannabis Sativus.*

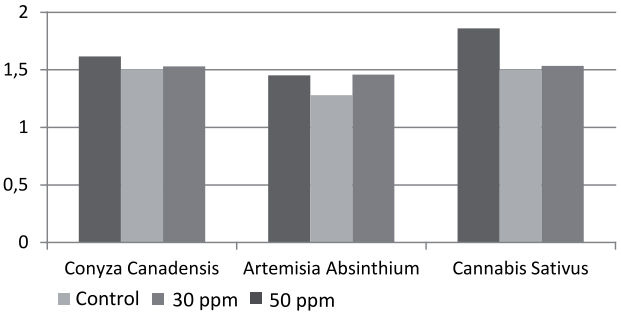

Fig. 7. Mean carotenoid concentrations in *Conyza Canadensis, Artemisia Absinthium* and *Cannabis Sativus.*

Our results are in accordance with the study on *Oryza Sativa L*. to fluoride stress, which was carried out by [44]. In their results, the carotenoid content decreased by up to 50% of the controlled species. As carotenoid content prevents the production of single oxygen by inhibiting the excited states of chlorophyll molecule and this condition maybe due to the fluoride toxicity which affects carotenoid content.

Effects of the Treatment of NaF on Ascorbic Acid

Ascorbic acid is an antioxidant that plays an important role in protecting against physiological stress. There is a rapid increase in ascorbic acid when there is some salt stress on plants. Thus this increase indicates that ascorbic acid is involved in many physiological processes, but there have been a lot of differences in views regarding the mechanisms through which ascorbic acid reduces the damaging effects of such stresses in plants [45]. Similar results of increasing ascorbic acid have been seen in the present study, where the percentage increase was noticed with respect to control (as can be seen in Fig. 8 below). At 30 ppm, *Conyza Canadensis* showed a percentage increase of 24.41% and *Cannabis Sativus* showed a percentage increase of 27% with respect to control. A similar increasing trend of ascorbic acid followed when 50 ppm of sodium fluoride was also given. But in *Artemisia Absinthium* ascorbic acid increased only when 50 ppm of sodium fluoride was

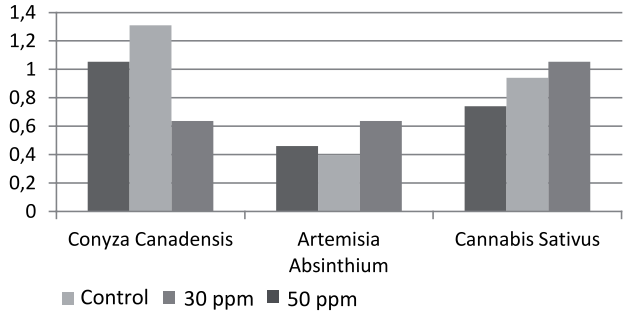

Fig. 8. Mean ascorbic acid concentrations in *Conyza Canadensis, Artemisia Absinthium* and *Cannabis Sativus.*

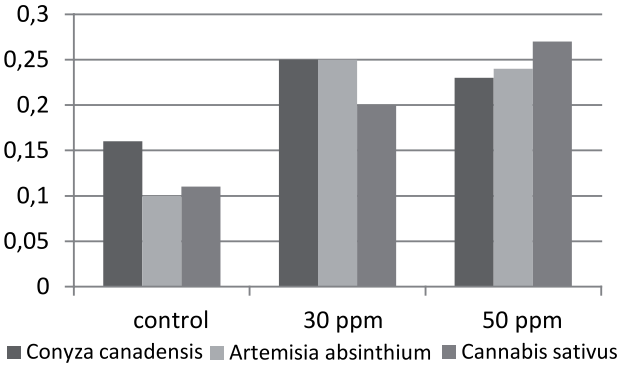

Fig. 9. Relative water content of aboveground biomass.

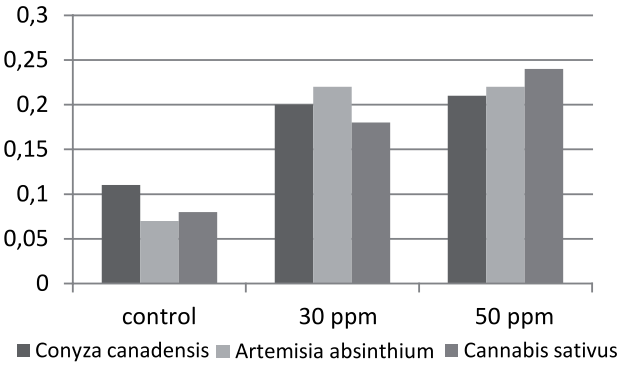

Fig. 10. Relative water content of belowground biomass.

provided to them and there was not as much increase in 30 ppm of NaF, whereas *Conyza Canadensis* at 50 ppm of sodium fluoride did not show an increase in ascorbic acid, but a visible injury in leaves was seen during the experiment. The differences between concentrations of fluoride were analyzed in each species and the results were not found to be significant in all three species (*Conyza Canadensis,* value of p = 0.1 F = 3.36, *Cannabis Sativus* value of p = 0.3 F = 1.0, *Artemisia Absinthium* value of p = 0.16 F = 1.4) at all concentrations.

Effects of the Treatment of NaF on Relative Water Content (RWC)

RWC of a leaf is the presence of water relative to its turgidity. Water is a requirement for plant life. A high level of water content in a plant body will assist in the maintenance of physiological balance under harsh conditions such as fluoride toxicity stress when the transpiration rates are usually high. Plants with high relative water content under polluted conditions may be tolerant of pollutants [46]. The results of the present study showed that with increasing fluoride content, the relative water content increased. In controlled conditions, *Cannabis Sativus, Conyza Canadensis* and *Artemisia Absinthium* had a relative water content of 20%, 16%, and 10% respectively. At 30 and 50 ppm, *Cannabis Sativa* showed an increasing trend of 25% and 27% respectively. While *Conyza Canadensis* at 30 and 50 ppm of sodium fluoride showed an increasing trend of 25% and 58.6% respectively. Similarly, *Artemisia Absinthium* at 30 and 50 ppm showed an increasing trend of 11% and 23% respectively.

A similar trend has been shown by below-ground biomass, where the relative water content in treated plants was found to be greater than controlled plants. This increased trend has also been seen in the study conducted on *Olea Europaea L*. The reason for increased relative water content is stated that high accumulation of proline and soluble sugars plays a role in the activation of water uptake to maintain cellular tissue turgor [47-48]. Some other studies have revealed that at higher concentrations above relative water content of plants decreased [49].

Effect of the Treatment of NaF on Biomass

Fresh Weight (above and below Biomass of Treated Species)

The present study showed that there had been a reduction in the above- and belowground biomass of treated species. Control and treated plants were more or less similar, but as the treatment progressed then reductions in the biomass of *Brassica Napus L.* were seen. However, a significant reduction in fresh weight of root, shoot, and leaf was recorded with respect to control [50]. Similarly, in the present study, it was noticed that there was more reduction in the aboveground biomass than the belowground biomass of *Conyza Candensis, Artemisisa Absinthium,* and *Cannabis Satavis.* Mentioned reductions in studies are implied to be due to the fact that fluoride causes a reduction in root length and shoot length due to unbalanced nutrient uptake by seedlings in the presence of fluoride. The results of our study showed that treated plants with 30 and 50 ppm concentrations of sodium fluoride had a decrease in fresh and dry weight in aboveground biomass. At a 30 ppm concentration of sodium fluoride, aboveground biomass of Cannabis sativa was reduced by 23.4%. While at 50 ppm concentration of sodium fluoride there was a reduction of 29.63% of aboveground biomass of Cannabis sativa. Similarly, *Conyza Canadensis* at 30 ppm and 50 ppm concentrations of sodium fluoride

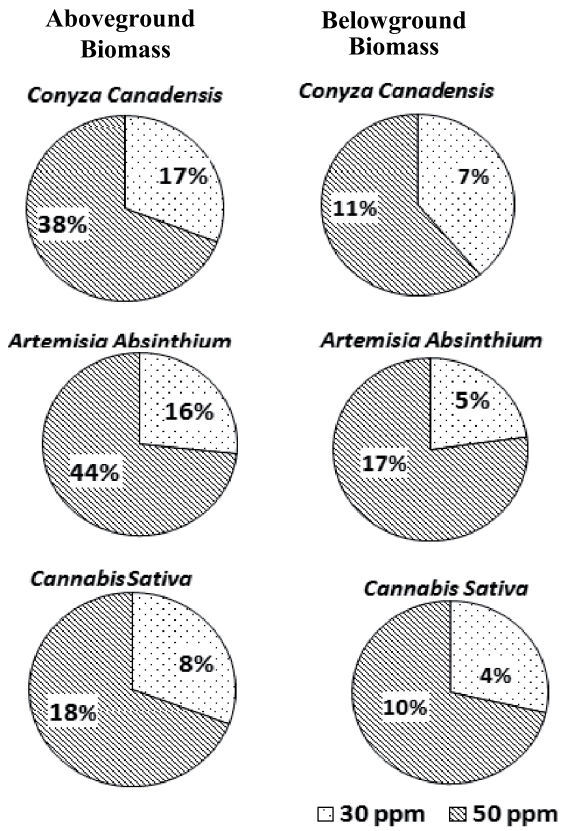

Fig. 11. Fresh weights of above- and belowground biomass.

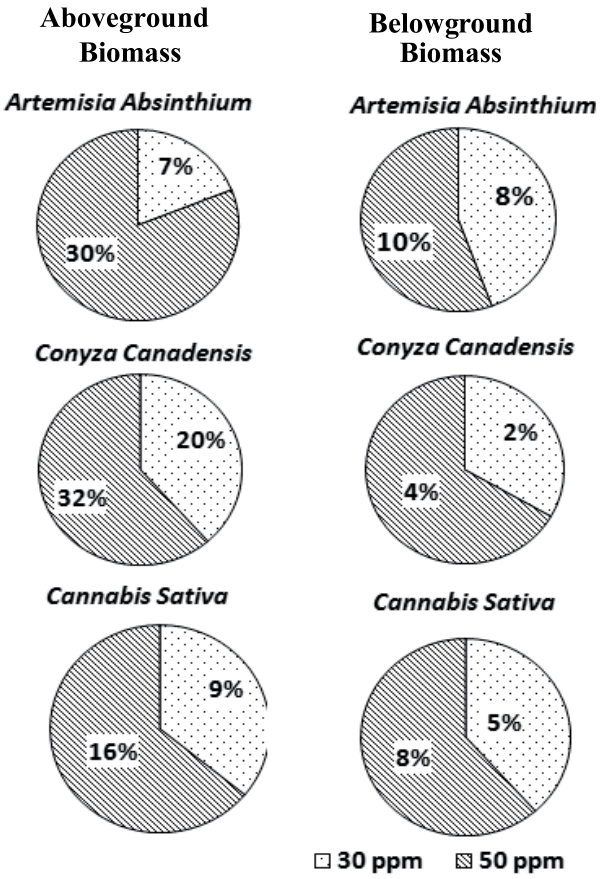

Fig. 12. Dry weight of above- and belowground biomass.

showed a reduction of 23.3% and 57.58 % of fresh aboveground biomass respectively. While *Artemisia Absinthium* at 30 ppm and 50 ppm concentrations of sodium fluoride showed a reduction of 11.64% and 21.19% respectively.

Dry Weight of above- and below- Ground Biomass

Likewise, the results of aboveground biomass; it also showed that treated plants have a decrease in fresh and dry weight in belowground biomass as well. At 30 ppm concentration of sodium fluoride, belowground biomass of *Cannabis Sativa* was reduced by 25%, while at 50 ppm concentrations of sodium fluoride there was a reduction of 33.7% in belowground biomass of *Cannabis Sativa*. Similarly, *Conyza Canadensis* at 30 ppm and 50 ppm, the concentration of sodium fluoride, showed a reduction of 16.67% and 25% respectively, while *Artemisia Absinthium* at 30 ppm and 50 ppm concentrations of sodium fluoride showed reductions of 33.33% and 40%, respectively.

Conclusions

We studied and evaluated the effects of different concentrations of fluoride in wild plants that are common near brick kilns. Under fluoride toxicity, we have found that 50 ppm and 30 ppm concentrations of fluoride, given three times within an interval of a week, affected different growth attributes of selected plants such as area of leaves, heights of plant, carotenoid content, and chlorophyll level. Whereas untreated plants have grown healthy, which shows that increasing fluoride levels in the environment are affecting the plant's species – especially those which are near brick kilns. Overall, this study suggests that in 30 ppm of fluoride (given three times with an interval of a week), plants can still grow, but above this range major physical as well as chemical changes can be observed in those affected plants.

Acknowledgements

The authors of this study are grateful to the journal editor highlighting the structural changes required to improve the outlook of this manuscript. Special thanks also go to anonymous reviewers for their detailed reviews and comments that helped the authors refine the findings and results of this study presented in this manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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