Journal of Stress Physiology & Biochemistry, Vol. 21, No. 1, 2025, pp. 33-42 ISSN 1997-0838

ORIGINAL ARTICLE

Evaluating the Consequences of Fluoride Poisoning on Proximate Compositions of *Amaranthus dubius*

B. Sri Suyambulinga Perumal[1,2,*], P. Sri Renganathan[1],
D. Venkatesh[2], R. Padmavathi[3]

[1] *P. G. and Research Department of Chemistry, Rani Anna Government College for Women (Affiliated to Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli - 627 008, Tamil Nadu, India.*

[2] *Department of Science and Humanities (Chemistry), Vel Tech High Tech Dr.Rangarajan Dr.Sakunthala Engineering College, Avadi, Chennai - 600 062, India.*

[3] *Department of Science and Humanities (Chemistry), Vel Tech Multi Tech Dr.Rangarajan Dr.Sakunthala Engineering College, Avadi, Chennai - 600 062, India.*

*E-Mail: ***bsslperumal@gmail.com***

Received August 3, 2024

The effect of fluoride on proximate compositions such as contents of protein, carbohydrate and fat from leaves, stems, roots and seeds were studied using various concentrations of sodium fluoride in the water used to irrigate the plant *Amaranthus dubius.* The results showed that *Amaranthus dubius* which receives only water (control) had higher amount of protein (10.04μg/g) in its leaves and low amount of protein (0.63 μg/g) in the roots of *Amaranthus dubius* watered with 50ppm of sodium fluoride on the 55th day of growth. The amount of protein decreases when concentration of sodium fluoride increases. The amount of carbohydrate (μg/g) in leaves of all experimentally challenged *Amaranthus dubius* varied from 2.0μg/g - 42.3μg/g (minimum in roots to maximum in leaves) when estimated from 15 to 55 days. *Amaranthus dubius* watered with 50ppm concentration of sodium fluoride showed higher amount of carbohydrate which is much higher than the carbohydrate content present in the leaves of *Amaranthus dubius* grown as control. The maximum presence of fat was found in the leaves of *Amaranthus dubius* watered with 50ppm of sodium fluoride from day 15 to 55 (1.4μg/g-0.52 μg/g).

Key words: Fluoride, Amaranthus dubius, Protein, Carbohydrate, Fat

A growing amount of fluoride is making its way into the human food and drink chain when people consume tea, wheat, spinach, cabbage, carrots and other Indian items. Therefore, while calculating total fluoride intake, food fluoride concentration should not be disregarded. The concentration of fluoride in irrigation water and soil affects the amount of fluoride in food. Globally, child malnutrition, food insecurity and hunger are major issues. Lack of necessary nutrients is most prevalent among children and women, increasing the global disease burden of children by limiting healthy cognitive development, impeding physical growth and increasing vulnerability to infectious diseases (Hongyan *et al*., 2015). So, in order to address these issues, higher consumption of seeds and leafy vegetables is recommended, as these are regarded sources of many essential micronutrients, macronutrients and bioactive substances. These crops are underutilized but have commercial value. Using them can result in nutritionally enhanced food items (Mlakar *et al*., 2009). According to national research projects, in India, grain Amaranthus is acknowledged as an important crop for producing extremely nutritious food and feed. Both the grain and leafy vegetable parts of Amaranthus plants have numerous health benefits (Ebert *et al*. 2014). Amaranthus leaves also have anticancer properties, suppressing the proliferation of liver, breast, and colon cancer cell lines (Hongyan *et al.*, 2015). The seeds are abundant in protein and include necessary amino acids, primarily methionine and lysine; they also contain considerable amounts of squalene, a crucial precursor for all steroids (De-la-Rosa *et al.*, 2019).

In nations like India, excessive fluoride in the water is a serious endemic issue that affects plant and animal nutrition (Mondal *et al.*, 2012). Nearly every biological industry makes extensive use of compounds containing fluorine and environmental fluoride ion (F^-) contamination is frequent. Overconsumption of fluoride may be harmful to plants, animals and people's health (Mondal *et al.*, 2012). As a result, numerous research projects have been started to find out how fluoride affects plants. According to Ando *et al*. (1998), excessive fluoride deposition in plants results in evident leaf damage, fruit damage and decreased yield. Because it interferes with several metabolic processes, fluoride (F^-) is harmful to the majority of plants (Elloumi *et al.*, 2005). Overdosing on fluoride may affect vitamins, carbohydrates, lipids, proteins and carbs (Islam and Patel, 2011). Proline was gradually accumulated during the germination period and with increasing fluoride concentration due to stress-induced synthesis of proline-rich proteins, which caused the total soluble sugar and proline content in leaves to initially decrease and then increase (Yang and Miller, 1963; Greenway and Munns, 1980). Yu (1996) discovered that as fluoride concentration rose, the overall number of soluble sugars in mung bean (Vigna radiata) seedlings-particularly reducing sugars-decreased. According to Elloumi et al. (2005), the leaves' starch and sugar content had considerably dropped. Stress-induced increases in fluoride concentration caused the protein content of seedling leaves to gradually decline (Singh *et al.*, 1985). On the other hand, not much is known about the fluoride content of various cultivars of Amaranthus dubius. This study aims to explore how sodium fluoride affects lipid, protein and carbohydrate levels.

MATERIALS AND METHODS

***Collection of Amaranthus dubius* seeds**

The experiment was carried out in natural climates and soil bed condition with cultivars of Red Lettuce seeds of *Amaranthus dubius*. The seeds were collected from Agriculture department, Government of Tamilnadu, Tirunelveli. The seeds were stored in a refrigerator for long-term storage. For short-term storage, the seeds were kept in a cool, shady and dry place.

Maintenance of *Amaranthus dubius* plant

The experimental setup was done with seven pots in triplicates, each one has 4kg of soil and kept aside for 3 days. The *Amaranthus dubius* seeds were wetted with distilled water for 2 hours and 20 seeds were sowed in each pot with adequate space between them. Each pot was marked indicating the experimental setup and numbered from 1 to 7 as per the watering of pot with various concentrations of sodium fluoride. Control pot was watered with tap water and others were watered with 1, 2, 5, 10, 25 and 50mg/l of sodium fluoride solution. The pots were watered at early morning every day. The watering process was continued as per the requirement of the experiment.

Estimation of protein (µg/g) from leaf, stem, root and seed of *Amaranthus dubius*

Using Chen *et al.* (2007) protocol, protein was extracted. The leaf, stem, root and seeds of *Amaranthus dubius*, which were subjected to varying concentrations of sodium fluoride, were pulverized into a fine powder using liquid nitrogen, weighing around 0.25 g in total. After that, 2 ml of extraction buffer-which included 0.1M 2-mercaptoethanol, 15% (v/v) glycerol, 2% (w/v) SDS, and 80 mM Tris-HCL-was added and thoroughly mixed once more. For two minutes, the samples were centrifuged at 13,500 rpm. Each sample's supernatant was removed, heated for ten minutes at 95 to 100 degrees Celsius in a water bath and then allowed to cool to room temperature. The protein content of the resulting supernatant was measured using Bradford's (1976) method, which relies on the protein's binding of the dye Coomasie Blue G. The dye's maximal absorption is shifted from red to blue by this binding. When compared to a standard curve, the solution's absorbance at 595 nm is proportionate to the protein concentration. By measuring the absorbance of the unknown tubes and comparing them to the standard protein curve, the amount of protein in each was found. The following equation was used to determine the solution's concentration:

Concentration of unknown = mg of Protein determined by assay / 0.1 ml

Estimation of carbohydrate (µg/g) from leaf, stem, root and seed of *Amaranthus dubius*

Fresh leaves, stem, roots and seeds of *Amaranthus dubius* were collected and dried in a forced-clraft oven at 100ºC for an hour after being treated with different sodium fluoride concentrations and a control. The samples were dried at 70°C until they reached a consistent weight. They were then packed into glass bottles and powdered to fit through a 40-mesh sieve. The bottles were carefully sealed and kept for the purpose of analyzing carbohydrates. 100 mg of each sample were weighed into a boiling tube in order to extract the carbohydrates. The sample was then hydrolyzed for three hours in a boiling water bath using 5.0 ml of 2.5 N HCl.

Carbohydrate present in 100 mg of the sample = (mg of glucose/ OD of test sample) x 100

Estimation of fat (µg/g) from leaf, stem, root and seed of *Amaranthus dubius*

Amaranthus dubius fresh leaves, stem, roots, and seeds were dried separately in a forced-cluster oven for one hour at 100 degrees Celsius, then ground into a powder that could pass through a 40-mesh sieve. The Official Method of the AOAC (2012) was used to estimate fat. After 16 hours of ether extraction in a Soxhlet-style extractor, the lipid extract is dried at 95 to 100 °C and weighed. The following formula was used to determine the percentage of fat in the plant samples.

(%) of crude fat = F-T/S X100

Where F = weight of cup + fat residue (g); T = weight of empty cup (g); S = test portion weight (g)

RESULTS AND DISCUSSION

A lower rate of protein synthesis could be the cause of a decrease in protein with a higher concentration of F^-, according to Eyini *et al.* (1999). The work by Dey *et al.* (2012) also showed a nonmonotonic elevation in the level of proline, which is a crucial stress marker for reduced protein synthesis, with greater exposure to NaF and $CaCl_2$. According to Singh *et al.* (1985) stress caused the protein content in seedling leaves to gradually decline as fluoride concentration increased. In the present investigation, the protein content in the leaves, stem and root decreases while the plant continues to grow (Tables & Figures 1, 2 and 3). In case of seed, the protein content is high in seeds obtained from plants that receive only water (Table 4 & Figure 1).

The present study findings indicate that on the 55th day of growth, *Amaranthus dubius* that receives only water (control) had the highest amount of protein (4.88, 2.56, 2.15 and 3.13µg/g) in its leaves, stems, roots and seeds respectively. When comparing the control and *Amaranthus dubius* that were irrigated with 50 ppm of sodium fluoride (1.58, 0.82, 0.63, 1.35µg/g) on the 55th day of growth, there was a significant difference. The amount of protein reduces in every experimental setup as the sodium fluoride concentration rises. Among the plant parts tested for protein, leaves have the highest concentration of protein. According to Imran and Donald (2021), fluoride had a detrimental effect on all aspects of protein synthesis by reducing ribosome count and destroying the structure of ribosomal proteins. We may use our results to draw the same conclusion. Because of the slowed rate of amino acid synthesis caused by fluoride stress, the protein content in the leaves, stems, roots and seeds of *Amaranthus dubius* under stress exhibited a considerable reduction when compared to the control plants.

Stressors have a direct impact on plant sugar levels. When compared to the non-affected area, the reducing sugar of the vegetables and leaves in the fluoride affected area was much lower. This might be the result of reduced photosynthesis, which would reduce the build-up of photo assimilation in leaves and fruits under fluoride stress and lessen the susceptibility of various crop plants (Imran and Donald, 2021). Tables 1, 2, 3 and 4 & Figures 1, 2 and 3 exhibit the findings of the current investigation on the estimation of carbohydrates from the leaves, stem, root and seeds of *Amaranthus dubius* that were irrigated with varying sodium fluoride concentrations.

In order to test the fluoride toxicity of the submerged plant *Hydrilla verticillata*, Gao et al. (2018) treated the plants for 7, 14, 21, and 28 days at varied fluoride (F^-) concentrations (0, 10, 20, and 40 mg/L). Compared to the control, the carbohydrate content of *H. verticillata* leaves increased during exposure to 10 mg/L fluoride. Additionally, they reported that *H. verticillata*'s carbohydrate content decreased as a result of fluoride's harmful effects, which peaked at 20 mg/L. In contrast our present study *Amaranthus dubius* planted as a control exhibited a lower carbohydrate content in its leaves, stems, roots, and seeds compared to the plants irrigated with 50 ppm concentration of sodium fluoride, which naturally produced more carbohydrate. As a result, *Amaranthus dubius* watered with 50 ppm sodium fluoride are richer in carbohydrate amount than control plants. Plants that are watered with different concentrations of sodium fluoride have higher carbohydrate concentrations. Our present study can be supported by the statement of Imran and Donald (2021) who stated that, since formation of reducing sugars such as glucose, fructose, and mannose in leaves is thought to be inhibited by fluoride, the tendency of plants exposed to fluoride to decrease the concentrations of such sugars in their leaves indicates the possible conversion of these sugars to non-reducing sugars, such as sucrose and raffinose or sugar alcohols. Under these conditions, increased levels of non-reducing sugars in tissues might be a mechanism adopted by plants to reduce fluoride toxicity. Therefore, it can be concluded that, fluoride toxicity increases the levels of non-reducing sugar and the plant *Amaranthus dubius* deposited those sugars in its parts such as leaves, stems, roots and also in seeds.

Major unsaturated fatty acids (UFAs) found in most plants are comprised of three C18 species: oleic (18:1), linoleic (18:2) and α-linolenic (18:3) acids. These simple substances are essential to many vital functions in plants (Choudhury et al., 2017). Saturated fatty acids increased in proportion to water fluoride levels, as reported by Wang et al. (2000). Among all the experimental configurations in this study, the control plant's *Amaranthus dubius* leaves, stems, roots, and seeds had the lowest fat content (0.09, 0.04, 0.02, and 0.07µg/g, respectively) and the concentration increased with increased fluoride content. The results are presented in tables 1, 2, 3 and 4 & Figures 1, 2 and 3.

In the present study, the *Amaranthus dubius* that was irrigated with 50 ppm sodium fluoride had the highest lipid content (0.52, 0.17, 0.15 and 1.2µg/g) when compared to the leaves, stems, roots and seeds of control plant on 55th day of plant growth. The lipid content was high in leaves when compared to the other parts of the plant. This finding can be supported by the statements of He el al. (2018), who stated the terrestrial plants as sessile organisms and they must endure stressors. As a result, they have developed complex coping mechanisms to mitigate or prevent these consequences. As one of the universal defense mechanisms against a variety of biotic and abiotic stressors, unsaturated fatty acids (UFAs) are gaining attention (He et al., 2018). Among the plant parts tested, seeds have higher fat content than leaf, stem and root.

The concentration of fat in seeds of *Amaranthus dubius* treated with 50ppm of sodium fluoride was found to be 1.2(µg/g). The finding was supported by the study of Koiwai et al. (1983). Koiwai et al. (1983) studied the fatty acid composition of leaves and seeds of 56 varieties of Nicotiana species. On a dry weight basis, the total fatty acid content of green leaves ranged from 2.1 to 4.4%, and that of seeds from 25 to 40%. With 50–63% of the total fatty acid content, linolenate was the most prevalent fatty acid in the leaves of every species that was investigated. Most species' seeds were primarily composed of linoleate, which made up 69–79% of the total fatty acid content.

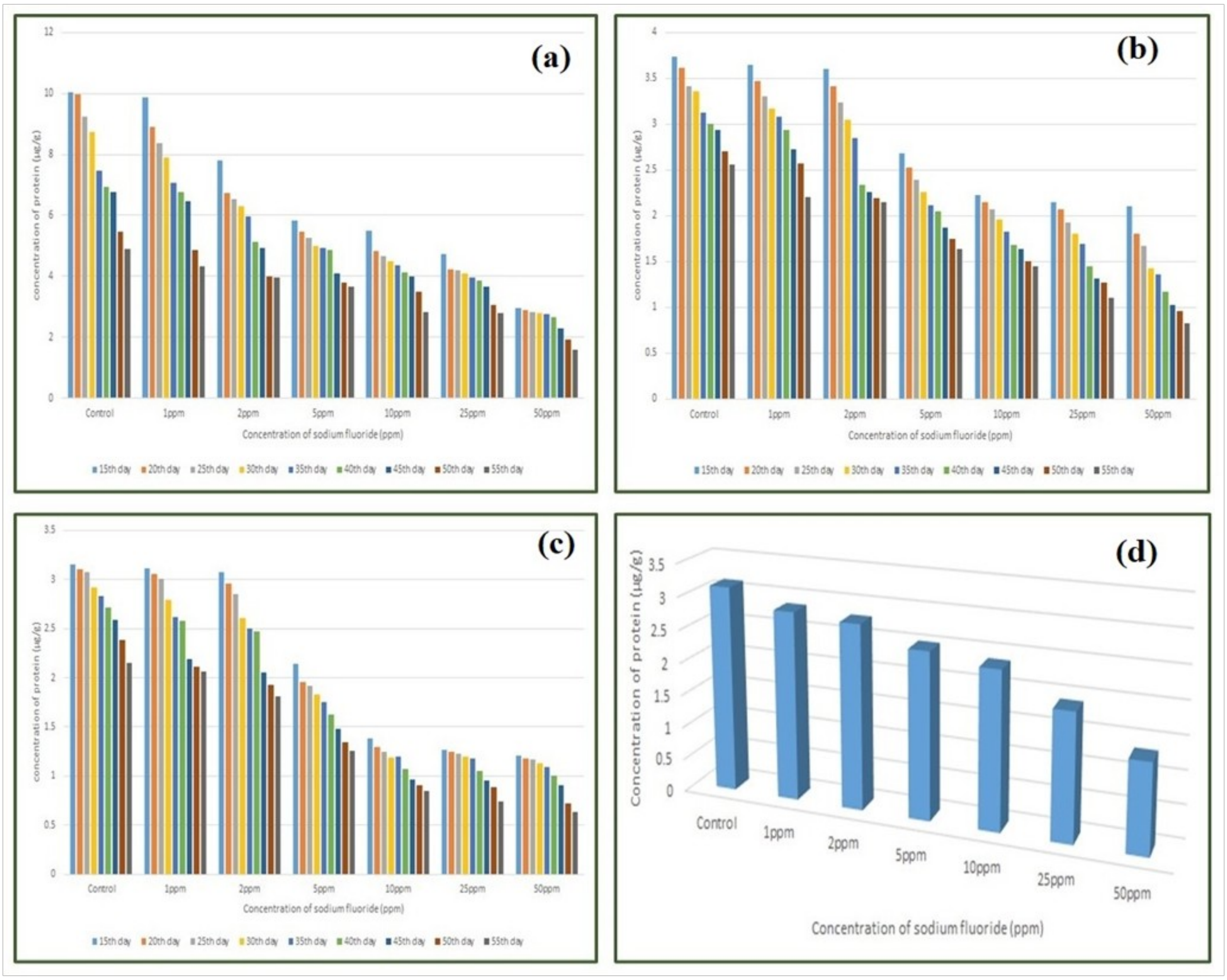

Figure 1: Protein content (µg/g) of *Amaranthus dubius* (a) leaves (b) stem (c) roots and (d) seeds watered with various concentrations of sodium fluoride harvested on various days

Table 1: Protein content (µg/g) of *Amaranthus dubius* leaves watered with various concentrations of sodium fluoride and harvested on various days

Plants parts	Days	Concentrations of sodium fluoride (ppm)						
		Control	**1ppm**	**2ppm**	**5ppm**	**10ppm**	**25ppm**	**50ppm**
Leaves	15	10.04	9.88	7.80	5.84	5.48	4.72	2.96
	20	9.98	8.89	6.72	5.45	4.82	4.23	2.89
	25	9.25	8.37	6.52	5.26	4.67	4.18	2.83
	30	8.74	7.90	6.29	5.01	4.48	4.09	2.79
	35	7.48	7.05	5.95	4.92	4.35	3.96	2.75
	40	6.92	6.77	5.12	4.85	4.11	3.85	2.64
	45	6.76	6.46	4.92	4.10	4.00	3.67	2.30
	50	5.45	4.85	4.00	3.80	3.48	3.05	1.93
	55	4.88	4.32	3.95	3.66	2.82	2.80	1.58
Stem	15	3.74	3.65	3.60	2.68	2.22	2.15	2.10
	20	3.61	3.47	3.41	2.52	2.15	2.07	1.80
	25	3.41	3.30	3.24	2.39	2.07	1.93	1.67
	30	3.36	3.17	3.05	2.26	1.96	1.80	1.42
	35	3.12	3.08	2.85	2.11	1.82	1.69	1.36
	40	3.00	2.94	2.34	2.05	1.68	1.45	1.17
	45	2.94	2.72	2.26	1.87	1.64	1.31	1.03
	50	2.70	2.57	2.19	1.75	1.50	1.27	0.96
	55	2.56	2.20	2.15	1.64	1.45	1.10	0.82
roots	15	3.15	3.11	3.07	2.14	1.38	1.27	1.21
	20	3.10	3.06	2.96	1.96	1.30	1.25	1.18
	25	3.07	3.01	2.85	1.92	1.25	1.23	1.17
	30	2.92	2.79	2.61	1.83	1.19	1.20	1.13
	35	2.83	2.62	2.50	1.75	1.20	1.18	1.09
	40	2.72	2.58	2.47	1.63	1.07	1.05	1.00
	45	2.59	2.19	2.05	1.48	0.97	0.96	0.91
	50	2.38	2.11	1.93	1.34	0.91	0.89	0.72
	55	2.15	2.06	1.81	1.46	0.85	0.74	0.63

Table 2: Carbohydrate content (µg/g) in leaves of *Amaranthus dubius* watered with various concentrations of sodium fluoride harvested on various days

Plants parts	Days	Concentrations of sodium fluoride (ppm)						
		Control	**1ppm**	**2ppm**	**5ppm**	**10ppm**	**25ppm**	**50ppm**
Leaves	15	15.0	16.8	17.5	20.1	22.4	24.0	29.3
	20	17.3	18.6	19.4	21.7	23.8	25.5	30.6
	25	20.0	21.2	22.0	22.8	25.6	26.2	31.9
	30	22.0	24.0	25.3	26.6	27.5	29.0	33.2
	35	24.1	25.7	26.9	28.9	30.6	31.8	36.0
	40	26.3	27.4	29.1	32.0	32.4	33.5	38.5
	45	27.8	29.8	31.4	33.6	34.9	35.6	40.6
	50	30.1	31.5	32.8	36.1	37.1	38.0	42.3
	55	32.4	33.1	34.2	38.4	40.5	41.2	44.0
Stem	15	2.6	2.9	3.2	3.5	3.7	4.1	6.0
	20	3.1	3.6	4.1	4.6	4.9	6.2	7.7
	25	4.7	5.0	5.8	6.9	8.3	9.8	10.5
	30	5.2	5.5	6.0	7.5	9.4	10.6	11.4
	35	6.8	7.1	7.8	8.8	10.5	12.5	13.3
	40	7.4	7.6	8.5	9.6	11.9	13.9	15.5
	45	8.2	8.9	9.3	11.2	12.2	15.2	17.2
	50	9.5	10.2	11.0	12.8	14.6	16.5	18.3
	55	10.3	11.9	12.4	14.1	15.7	17.2	19.0
roots	15	2.0	2.5	3.3	4.1	6.0	8.2	10.4
	20	3.5	3.7	4.1	5.4	7.1	9.0	11.5
	25	4.6	4.9	5.3	6.3	7.9	9.8	12.4
	30	5.0	5.2	5.9	7.0	8.5	10.3	13.7
	35	5.8	6.1	6.8	7.8	9.4	11.0	15.0
	40	6.3	6.7	7.4	8.4	10.5	11.7	15.8
	45	6.8	7.3	8.3	10.0	11.2	12.6	16.3
	50	7.5	8.9	9.7	11.4	12.3	13.5	16.9
	55	8.2	10.1	11	12.6	13.1	14.6	17.4

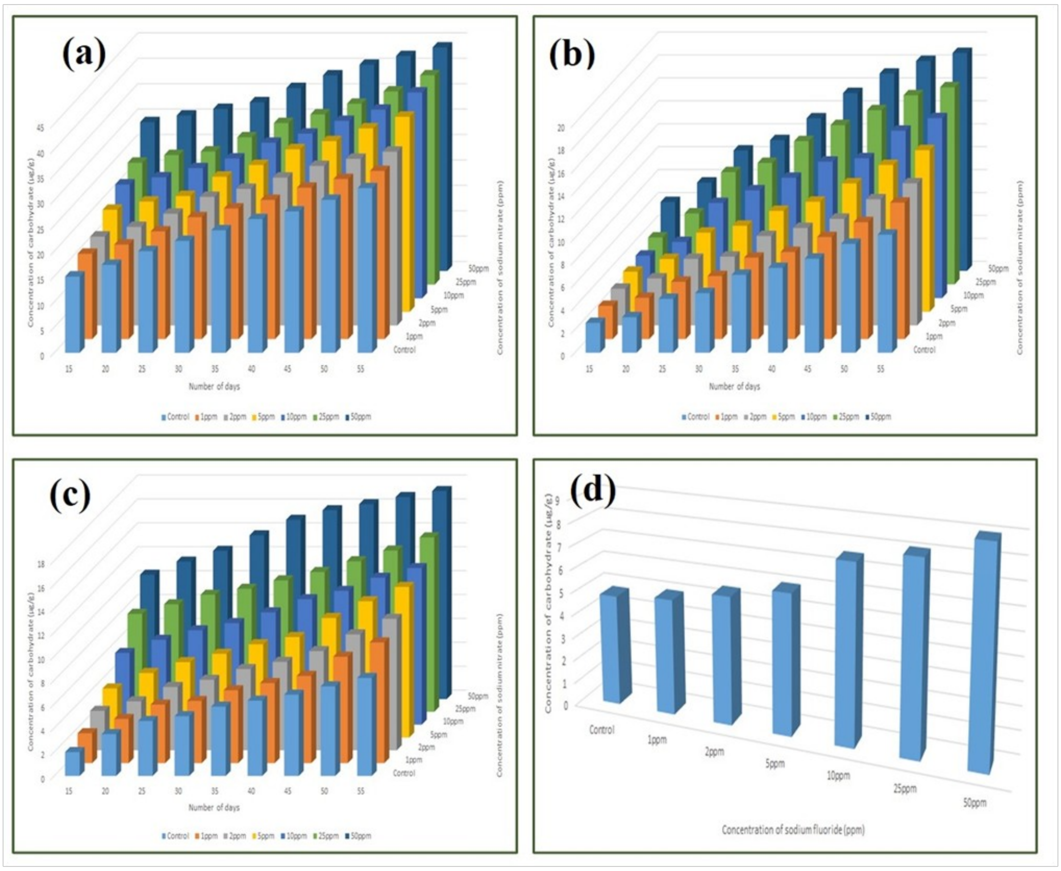

Figure 2. Carbohydrate content (µg/g) of *Amaranthus dubius* (a) leaves (b) stem (c) roots and (d) seeds watered with various concentrations of sodium fluoride harvested on various days

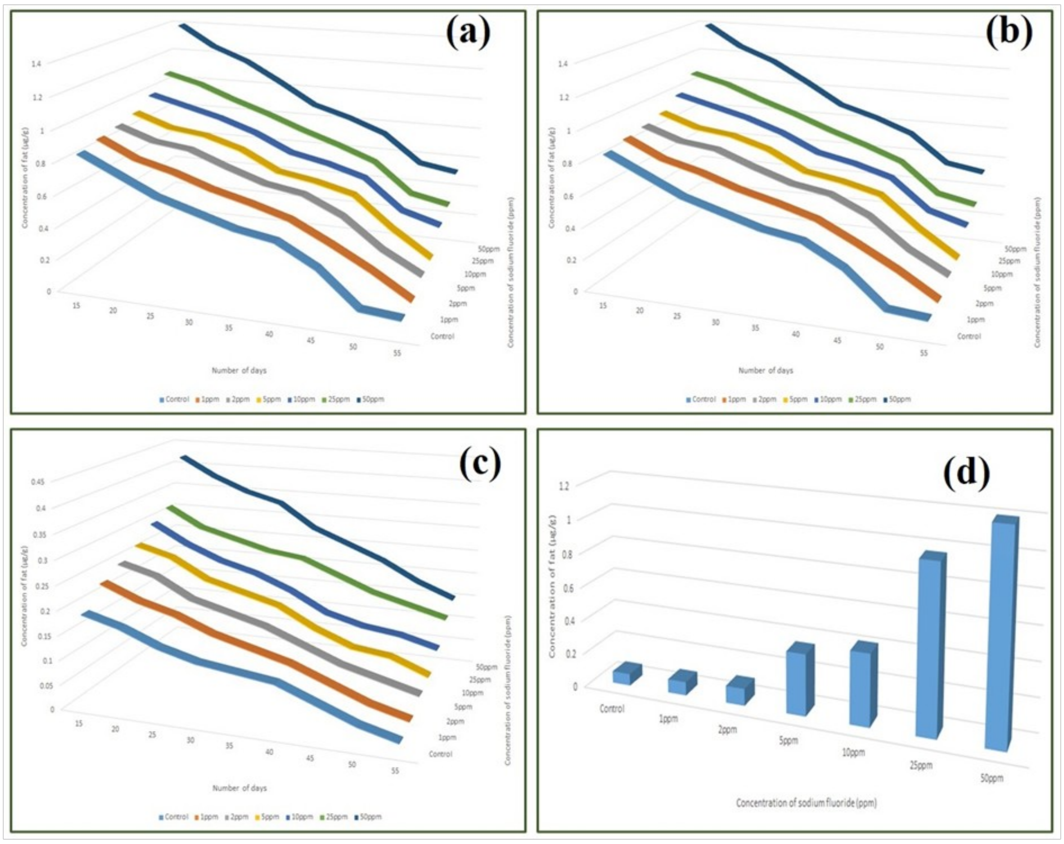

Figure 3: Fat content (µg/g) of *Amaranthus dubius* (a) leaves (b) stem (c) roots (d) seeds watered with various concentrations of sodium fluoride harvested on various days

Table 3: Fat content (µg/g) in leaves of *Amaranthus dubius* watered with various concentrations of sodium fluoride harvested on various days

Plants parts	Days	Concentrations of sodium fluoride (ppm)						
		Control	**1ppm**	**2ppm**	**5ppm**	**10ppm**	**25ppm**	**50ppm**
Leaves	15	0.85	0.88	0.90	0.93	1.0	1.1	1.4
	20	0.74	0.77	0.82	0.85	0.94	1.04	1.26
	25	0.63	0.71	0.79	0.82	0.88	0.95	1.17
	30	0.56	0.63	0.70	0.75	0.80	0.87	1.04
	35	0.49	0.57	0.62	0.63	0.69	0.78	0.90
	40	0.45	0.50	0.58	0.59	0.64	0.70	0.83
	45	0.32	0.38	0.47	0.53	0.57	0.61	0.74
	50	0.11	0.25	0.29	0.34	0.38	0.42	0.56
	55	0.09	0.10	0.16	0.18	0.31	0.37	0.52
Stem	15	0.56	0.75	0.81	0.85	1.0	1.1	1.21
	20	0.52	0.63	0.73	0.79	0.86	0.94	1.10
	25	0.47	0.52	0.58	0.63	0.73	0.83	1.06
	30	0.39	0.46	0.50	0.54	0.67	0.71	0.90
	35	0.32	0.39	0.42	0.48	0.60	0.66	0.84
	40	0.28	0.31	0.38	0.41	0.52	0.58	0.72
	45	0.21	0.26	0.29	0.32	0.44	0.49	0.63
	50	0.19	0.20	0.22	0.24	0.33	0.38	0.45
	55	0.04	0.06	0.07	0.08	0.09	0.11	0.17
roots	15	0.19	0.23	0.25	0.27	0.30	0.32	0.42
	20	0.17	0.20	0.23	0.25	0.26	0.28	0.38
	25	0.14	0.18	0.19	0.21	0.23	0.26	0.35
	30	0.12	0.15	0.17	0.19	0.21	0.24	0.33
	35	0.11	0.13	0.15	0.17	0.18	0.23	0.28
	40	0.10	0.11	0.12	0.13	0.14	0.20	0.25
	45	0.07	0.08	0.09	0.10	0.12	0.17	0.22
	50	0.04	0.05	0.07	0.09	0.11	0.15	0.18
	55	0.02	0.03	0.05	0.06	0.09	0.13	0.15

Table 4: Protein, carbohydrate and fat content (µg/g) of *Amaranthus dubius* seeds watered with various concentrations of sodium fluoride

S.No	Concentration of sodium fluoride (ppm)	Amount of protein (µg/g)	Amount of carbohydrate (µg/g)	Amount of fat (µg/g)
1	Control	3.13	4.8	0.07
2	1ppm	2.86	5.0	0.08
3	2ppm	2.79	5.5	0.10
4	5ppm	2.51	6.0	0.36
5	10ppm	2.37	7.6	0.42
6	25ppm	1.91	8.1	0.97
7	50ppm	1.35	9.0	1.2

CONCLUSION

Based on the above data, the relationship between *Amaranthus dubius* and sodium fluoride would further remain very attractive to be studied, especially to study the effect of fluoride on other biological factors of *Amaranthus dubius*. It was fascinating to observe how fluoride related to the proximate components, including protein, carbohydrates and fat of the leaves, stems, roots and seeds of *Amaranthus dubius* cultivated in different sodium fluoride concentrations. Higher sodium fluoride concentrations tended to cause plants to have more varied protein, carbohydrate and fat contents. Ultimately, this data demonstrated that sodium fluoride had an effect on *Amaranthus dubius* and significantly affected the plant's nutritional composition.

CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

REFERENCES

Ando M, Tadano M, Asanuma S, Tamura K, Matsushima S, Watanabe T, *et al.* (1998) Health effects of indoor fluoride pollution from coal burning in China. *Environment Health Prospect*. 106:239-244.

AOAC (2012) Official Method of Analysis: Association of Analytical Chemists. 19th Edition, Washington DC, 121-130.

Bradford MM. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*.72:248-54.

Chen, F, Dong, J, Wang, F, Wu, F, Zhang, G, Chen, G, Li, Z, Chen, J and Wei, K. (2007) Identification of barley genotypes with low grain Cd accumulation and its interaction with four microelements. *Chemosphere*, 67: 2082-2088.

Choudhury, F. K., Rivero, R. M., Blumwald, E., Mittler, R. (2017). Reactive oxygen species, abiotic stress and stress combination. *Plant J*. 90, 856–867.

Plummer David T. (1990) An Introduction to Practical Biochemistry, 179 Third Edition

De-la-Rosa AP, de-León-Rodríguez A, Laursen B, Fomsgaard IS. (2019) Influence of the growing conditions on the flavonoids and phenolic acids accumulation in amaranth (Amaranthus hypochondriacus L.) leaves. *Terra Latinoamericana*. 37(4):449-57.

Dey U, & Mondal NK, Das K, Datta JK. (2012). Dual effects of fluoride and calcium on the uptake of fluoride, growth physiology, pigmentation, and iochemistry of Bengal gram seedlings Cicer arietinum L.). *Fluoride*. 45. 349-353.

Ebert AW, Wu TH, Yang RY. (2014) Amaranth sprouts and microgreens–a homestead vegetable production option to enhance food and nutrition security in the rural-urban continuum. In Proceedings of the Regional Symposium on Sustaining Small-Scale Vegetable Production and Marketing Systems for Food and Nutrition Security, Bangkok, Thailand, 2014, 25-27.

Elloumi N, Abdallah FB, Mezghani I, Rhouma A, Boukhris M. (2005) Effect of fluoride on almond seedlings in culture solution. Fluoride. 38:193-198.

Eyini M, Sujanandini K, Pothiraj C, Jayakumar M, Kil B-S. (1999) Differential response of Azolla microphylla Kaulf. and Azolla filiculoides Lam. to sodium fluoride. *J Plant Biol;* 42:299-301.

Greenway H, Munns RA. (1980) Mechanism of salt tolerance in non-halophytes. *Annual Review of Plant Physiology*. 69:514.

Gao, Y. X., Wang, L., Hu, X. Q., Zhang, Z. L., Liu, B. G., Zhang, X. H., & Wang, G. X. (2021). Rapid adaptive responses of rosette-type macrophyte Vallisneria natans juveniles to varying water depths: The role of leaf trait plasticity. *Ecology and Evolution*, 11, 14268–14281.

He, M., He, C. Q., Ding, N. Z. (2018). Abiotic stresses: General defenses of land plants and chances for engineering multistress tolerance. *Front. Plant Sci.* 9, 1771.

Hongyan L, Deng Z, Liu R, Zhu H, Draves J, Marcone M et al, Tsao R. (2015) Characterization of phenolics, betacyanins and antioxidant activities of the seed, leaf, sprout, flower and stalk extracts of three Amaranthus species. *Journal of Food Composition and Analysis*. 37:75-81.

Imran, A., & Donald, S. (2021). Accumulation of fluoride in vegetable and associated biochemical changes due to fluoride contamination in water in Western Rajasthan Districts. *Journal of Soil Science and Plant Physiology*, 3(2), 136.

Islam, M.; Patel, R. (2011) Thermal activation of basic oxygen furnace slag and evaluation of its fluoride removal efficiency. *Chem. Eng. J.*, 169, 68–77.

Mlakar SG, Turinek M, Jakop M, Bavec M, Bavec F. (2009) Nutrition value and use of grain amaranth: potential future application in bread making. *Agricultura*. 6(2):45-53.

Mondal NK, Pal KC, Kabi S. (2012) Prevalence and severity of dental fluorosis in relation to fluoride in groundwater in the villages of Birbhum district, West Bengal, India. *Environmentalist*. 32: 72–84.

Singh G, Kaur P, Sharma R. (1985) Effect of CCC and kinetin on certain biochemical parameters in wheat under different salinity levels. *Plant Physiology and Biochemistry*. 12:104-111.

Wang, Y., Xiao, K., Liu, J., Dallner, G., Guan, Z. (2000). Effect of long-term fluoride exposure on lipid composition in rat liver. *Toxicology*. 146. 161-9. 10.

Yang SF, Miller GW. (1963) Biochemical studies on the effect of fluoride on higher plants. Metabolism of carbohydrates, organic acids and amino acids. *Journal of Biochemistry*. 88:505-509.

Yu MH. (1996) Effects of fluoride on growth and soluble sugars in germinating Mung bean (*Vigna radiata*) seeds. *Fluoride*. 29:3-6.