

**Effects of Fluoride-Contaminated Ground water on Morphological and Biochemical Characteristics of *Spinacia oleracea*****Anjana Bhatiya<sup>1\*</sup> and Archana Pancholi<sup>2</sup>****Abstract**<sup>1</sup>School of Studies in Botany Samrat Vikramaditya Vishwavidhyalya Ujjain (M.P.)<sup>2</sup>Swami Vivekananda Govt. P.G. College Neemuch (M.P.)\*Corresponding author-**Anjana Bhatiya**

This study explores the effects of fluoride-contaminated groundwater, simulated by varying concentrations of sodium fluoride (NaF), on the morphological and biochemical characteristics of *Spinacia oleracea* (spinach). Pot experiments were conducted using NaF concentrations of 0, 4, 8, 10, and 12 mg/L to reflect a range of environmental exposures. The impact of fluoride was assessed at multiple developmental stages by evaluating seed germination, seedling vigor, mature plant growth parameters, and key biochemical indices. The results revealed a clear dose-dependent inhibition of seed germination, seedling growth, and mature plant development, with significant reductions in root and shoot length, biomass, leaf area, and grain yield at higher fluoride concentrations. Morphological symptoms of fluoride toxicity, such as chlorosis, wilting, and stunted growth, were observed in treated plants. Biochemically, increasing NaF exposure led to marked decreases in chlorophyll and protein content and calorific value, while fluoride accumulation in plant tissues significantly increased. These findings indicate that high levels of fluoride in irrigation water can severely impair spinach growth and reduce nutritional quality, posing a threat to crop productivity and food safety in regions affected by fluoride-rich groundwater. The results underscore the importance of monitoring water quality and adopting mitigation strategies in agriculture.

**Paper Rceived date**

05/12/2025

**Paper date Publishing Date**

10/12/2025

**DOI**<https://doi.org/10.5281/zenodo.18037932>**Keywords:** Fluoride toxicity, *Spinacia oleracea*, groundwater contamination, morphological traits, biochemical parameters, phytotoxicity, environmental stress, seed germination**Introduction:**

Groundwater contamination with fluoride is a pressing environmental and agricultural issue, particularly in regions where groundwater serves as the primary source for both irrigation and



drinking water. Naturally occurring fluoride is found in various geological formations and can leach into groundwater, especially in arid and semi-arid regions where water tables are deep and evaporation rates are high (Amini et al., 2008). While low concentrations of fluoride (typically less than 1.5 mg/L, as recommended by the World Health Organization) are known to provide dental health benefits and may even enhance plant growth in trace amounts, excessive fluoride is toxic to both humans and plants (Barbier et al., 2010; WHO, 2011). Chronic exposure to high fluoride levels can lead to fluorosis, a debilitating condition affecting bones and teeth in humans, and also disrupts plant physiological processes, ultimately affecting crop yield and quality (Gupta et al., 2009; Ranjan et al., 2009).

Plants irrigated with fluoride-rich water are particularly at risk, as they can absorb and accumulate fluoride in their tissues. The extent of fluoride uptake and its phytotoxic effects depend on several factors, including soil properties, plant species, growth stage, and the duration and concentration of exposure (Kumar et al., 2009). Among vegetables, leafy crops such as *Spinacia oleracea* are highly susceptible because of their high transpiration rates and the edible nature of their foliage. Consequently, the accumulation of fluoride in spinach not only impairs plant growth but also poses risks to food safety for consumers (Singh et al., 2013).

Previous studies have reported that excess fluoride interferes with a wide range of physiological and biochemical processes in plants. Notably, it can inhibit seed germination, reduce root and shoot elongation, and impair leaf expansion, leading to stunted growth and lower productivity (Bhargava & Bhargava, 2013). The underlying mechanisms of fluoride toxicity involve disruption of cell division, inhibition of enzyme activities, and interference with nutrient uptake and transport (Barbier et al., 2010; Aske & Iqbal 2014). Biochemically, fluoride exposure has been shown to decrease chlorophyll synthesis, resulting in chlorosis (yellowing of leaves) and reduced photosynthetic efficiency. Additionally, protein content and overall metabolic activity are suppressed, likely due to the inhibitory effects of fluoride on key enzymes and protein synthesis pathways (Gupta et al., 2009; Kumar et al., 2009).

Beyond physiological symptoms, fluoride toxicity manifests as visible morphological changes. Plants exposed to elevated fluoride often exhibit symptoms such as leaf tip burn, marginal necrosis, wilting, and a general reduction in leaf area and biomass accumulation (Ranjan et al., 2009). These effects not only compromise plant health and vigor but also reduce the nutritional and market value of affected crops. The cumulative impact of fluoride toxicity thus poses a significant threat to sustainable agriculture, especially in areas where groundwater contamination is widespread and monitoring or remediation practices are limited (Amini et al., 2008; Singh et al., 2013).

Despite the recognition of fluoride's phytotoxic effects, comprehensive studies examining its impact on both morphological and biochemical characteristics of spinach under controlled



conditions remain limited. Most existing research has focused on single growth stages or isolated parameters, providing an incomplete picture of the full extent of fluoride-induced damage. Therefore, the present study aims to systematically evaluate the effect of fluoride-contaminated groundwater, simulated by sodium fluoride treatments, on seed germination, early seedling development, mature plant morphology, and key biochemical indices such as chlorophyll, protein content, calorific value, and fluoride accumulation in *Spinacia oleracea*.

#### Materials and Methodology:

Earthen pots of equal size were sterilized, filled with sandy loam soil mixed with natural fertilizers, and irrigated with distilled water. Sodium fluoride (NaF) solutions at 4, 8, 10, and 12 mg/L were applied to study their effects on selected vegetables, parallel to field experiments. Growth parameters recorded included seed germination, seedling traits (root, shoot, coleoptile length and biomass), mature plant growth, leaf area, and phenotypic changes. Biochemical indicators such as chlorophyll, protein, calorific value, and fluoride accumulation were also assessed. Seeds were surface-sterilized, germinated under controlled conditions, and seedlings measured after seven days. Mature plant measurements and biochemical analyses followed standard methods, including Arnon, Lowry, bomb calorimetry, and ion-selective electrode techniques, with all experiments performed in triplicate.

$$\text{G.P.} = \frac{\text{Number of normally germinated seeds}}{\text{Total number of seeds}} \times 100$$

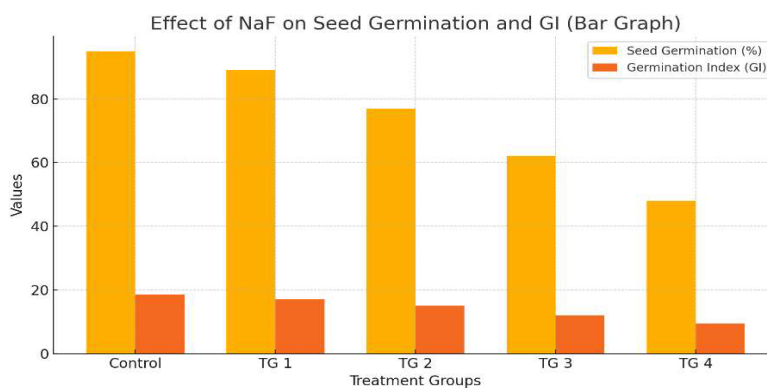
#### Results:

##### Effect of Fluoride on Seed Germination Percentage and Germination Index:

Exposure to increasing concentrations of sodium fluoride (NaF) in *Spinacia oleracea* resulted in a clear, dose-dependent reduction in both seed germination percentage and germination index after 48 hours. The control group, with no added fluoride, exhibited the highest seed germination (95%) and germination index (18.5). As the NaF concentration increased, both parameters declined progressively, with the most severe reduction observed at 12 mg/L, where only 48% of seeds germinated and the germination index dropped to 9.5. These results indicate that fluoride contamination significantly hampers the early stages of spinach seed development, potentially by impairing metabolic processes required for germination (Table 1).

**Table 1: Effect of Fluoride-Contaminated Groundwater (NaF) on Seed Germination Percentage and Germination Index of *Spinacia oleracea* after 48 Hours (Values are presented as mean  $\pm$  standard error (SE) from three replicates)**

Treatment Group	NaF (mg/L)	Seed Germination (%)	Germination Index (GI)
Control	0	95 $\pm$ 2	18.5 $\pm$ 0.4
Treated Group 1	4	89 $\pm$ 3	17.2 $\pm$ 0.3
Treated Group 2	8	77 $\pm$ 4	15.0 $\pm$ 0.5
Treated Group 3	10	62 $\pm$ 5	12.1 $\pm$ 0.7
Treated Group 4	12	48 $\pm$ 6	9.5 $\pm$ 0.9



**Figure1 : Graph comparing the seed germination percentage and the germination index (GI) across different NaF concentrations for *Spinacia oleracea***

#### Effect of Sodium Fluoride on Seedling Growth Parameters:

After 72 hours of exposure to NaF, spinach seedlings showed marked declines in root and shoot lengths, total weight, dry weight, and seedling vigor index. The control seedlings had the longest roots and shoots, and the highest vigor index (741). Fluoride-treated seedlings displayed progressively shorter roots and shoots, with root reduction reaching 58.1% and shoot reduction 47.4% at 12 mg/L NaF. Correspondingly, total seedling weight and dry weight decreased, as did coleoptile length. The seedling vigor index dramatically dropped to 246 at the highest fluoride concentration, reflecting the substantial inhibitory effect of fluoride on overall seedling health and early growth potential (Table 2).

**Table 2: Effect of Sodium Fluoride (NaF) on Seedling Growth Parameters of *Spinacia oleracea* after 72 Hours**

Treatment Group	NaF (mg/L)	Root Length (cm)	Shoot Length (cm)	Root Reduction (%)	Shoot Reduction (%)	Coleoptile Length (cm)	Total Weight (mg)	Total Dry Weight (mg)	Seedling Vigor Index (SVI)
Control	0	6.2 ± 0.2	7.8 ± 0.3	0	0	3.5 ± 0.1	115 ± 4	28 ± 1	741
Treated Group 1	4	5.5 ± 0.3	7.1 ± 0.2	11.3	9.0	3.2 ± 0.2	104 ± 3	25 ± 1	670
Treated Group 2	8	4.7 ± 0.2	6.2 ± 0.2	24.2	20.5	2.8 ± 0.1	92 ± 4	22 ± 2	529
Treated Group 3	10	3.9 ± 0.2	5.4 ± 0.2	37.1	30.8	2.5 ± 0.1	80 ± 3	18 ± 1	398
Treated Group 4	12	2.6 ± 0.4	4.1 ± 0.3	58.1	47.4	1.9 ± 0.1	62 ± 5	12 ± 1	246

#### **Effect of Sodium Fluoride on Growth Parameters After 30 Days:**

Long-term exposure to increasing NaF concentrations led to significant reductions in root length, shoot length, root and shoot weights, and grain yield in spinach plants after 30 days. The control group maintained the highest values for all parameters, while plants treated with 12 mg/L NaF had root and shoot lengths reduced by more than half and exhibited the lowest root and shoot biomass (1.1 g and 3.2 g, respectively) along with a sharp decline in grain yield (0.7 g/plant). These trends indicate that chronic fluoride exposure severely restricts the development and productivity of spinach plants (Table 3).

#### **Effect of Sodium Fluoride on Morphological Characteristics:**

Morphological assessment of spinach after 30 days revealed pronounced fluoride toxicity symptoms with increasing NaF levels. Control plants were robust, with well-branched roots, healthy shoots, and the highest number of leaves and leaf area. Even at 4 mg/L, mild reductions in root length and chlorosis began to appear. At higher concentrations, roots became thinner, less branched, with brownish tips, and shoots displayed increasing levels of chlorosis and wilting. The number of leaves, their weight, and total leaf area steadily decreased, with the most severe stunting

and leaf damage at 12 mg/L NaF, highlighting the negative impact of fluoride on plant morphology and foliage development (Table 4).

**Table 3: Effect of Sodium Fluoride (NaF) on Growth Parameters of *Spinacia oleracea* After 30 Days. Values presented as mean  $\pm$  standard error (n = 3).**

Treatment Group	NaF (mg/L)	Root Length (cm)	Shoot Length (cm)	Root Weight (g)	Shoot Weight (g)	Grain Yield (g/plant)
Control	0	18.2 $\pm$ 0.7	25.4 $\pm$ 0.8	2.8 $\pm$ 0.1	7.3 $\pm$ 0.2	2.1 $\pm$ 0.1
Treated Group 1	4	16.7 $\pm$ 0.6	23.5 $\pm$ 0.7	2.5 $\pm$ 0.1	6.6 $\pm$ 0.2	1.8 $\pm$ 0.1
Treated Group 2	8	14.3 $\pm$ 0.8	20.4 $\pm$ 0.9	2.0 $\pm$ 0.1	5.8 $\pm$ 0.3	1.5 $\pm$ 0.1
Treated Group 3	10	11.8 $\pm$ 0.7	16.8 $\pm$ 0.8	1.5 $\pm$ 0.1	4.6 $\pm$ 0.2	1.1 $\pm$ 0.1
Treated Group 4	12	8.9 $\pm$ 0.6	13.2 $\pm$ 0.9	1.1 $\pm$ 0.1	3.2 $\pm$ 0.2	0.7 $\pm$ 0.1

**Table 4: Effect of Sodium Fluoride (NaF) on Morphological Characteristics of *Spinacia oleracea* After 30 Days. Values presented as mean  $\pm$  standard error (n = 3).**

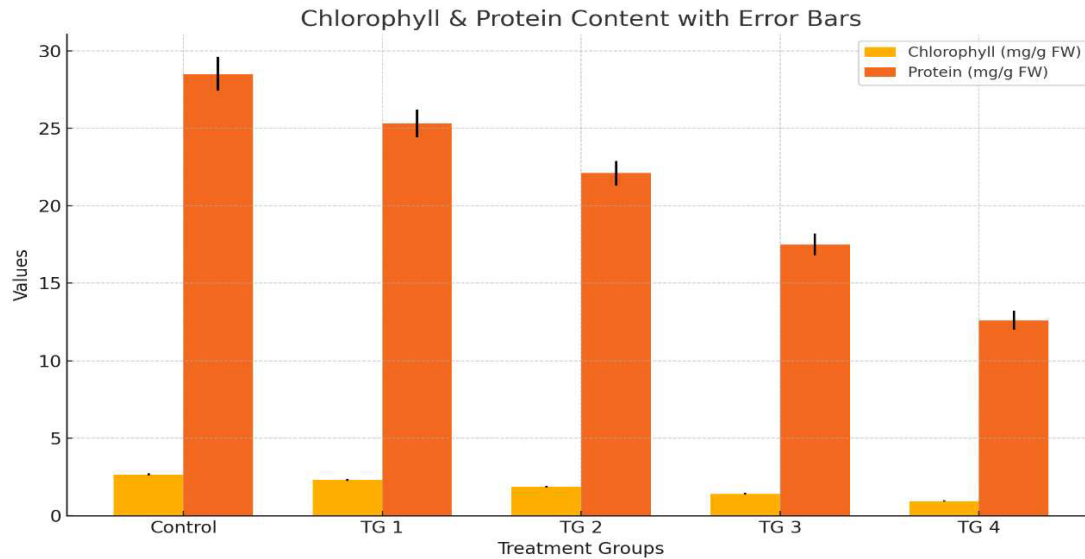
Treatment Group	NaF (mg/L)	Root Phenotype	Shoot Phenotype	No. of Leaves	Leaf Weight (g)	Leaf Area (cm <sup>2</sup> )
Control	0	Normal, well-branched	Robust, green	19 $\pm$ 1	5.3 $\pm$ 0.2	78.6 $\pm$ 3.2
Treated Group 1	4	Slightly reduced length	Mild chlorosis	17 $\pm$ 1	4.7 $\pm$ 0.2	71.4 $\pm$ 2.8
Treated Group 2	8	Thinner, less branched	Noticeable chlorosis	14 $\pm$ 1	3.9 $\pm$ 0.2	59.2 $\pm$ 3.1
Treated Group 3	10	Short, poorly branched	Chlorosis, mild wilting	11 $\pm$ 2	2.8 $\pm$ 0.3	43.7 $\pm$ 2.5
Treated Group 4	12	Stunted, brownish tips	Severe chlorosis, wilting	8 $\pm$ 1	1.6 $\pm$ 0.2	29.5 $\pm$ 2.0

**Effect of Sodium Fluoride on Biochemical Parameters**

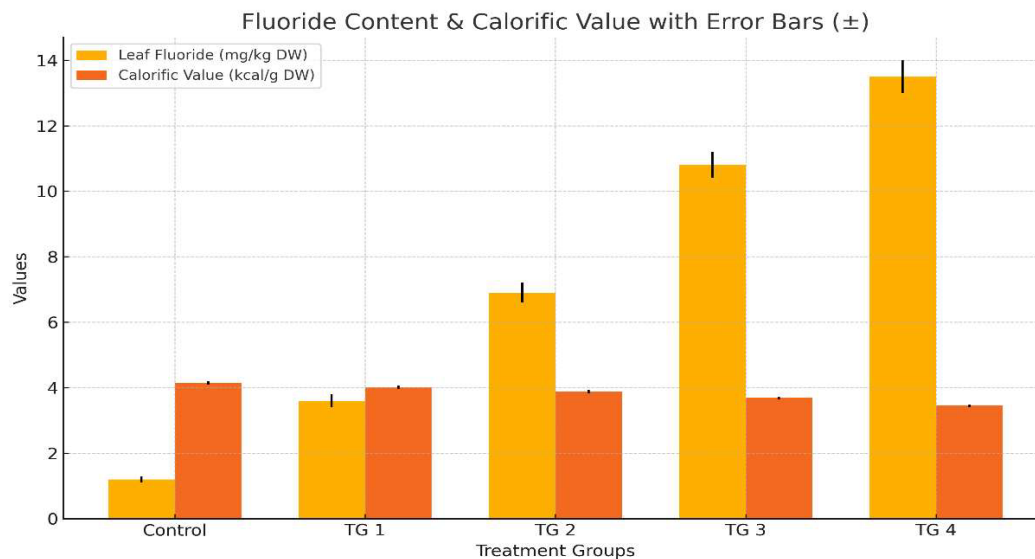
Biochemical analysis of spinach leaves after 30 days under fluoride stress showed significant reductions in chlorophyll and protein content, alongside increased fluoride accumulation and decreased calorific value. The control group had the highest chlorophyll (2.65 mg/g FW) and protein levels (28.5 mg/g FW), while plants exposed to 12 mg/L NaF showed drastic declines in both (0.95 mg/g FW and 12.6 mg/g FW, respectively). Fluoride accumulation in leaves increased sharply with rising NaF, reaching 13.5 mg/kg DW at the highest dose. Calorific value also declined, suggesting diminished nutritional quality and energy content due to fluoride toxicity (Table 5).

**Table 5: Effect of Sodium Fluoride (NaF) on Biochemical Parameters of *Spinacia oleracea* Leaves (After 30 Days). Values presented as mean  $\pm$  standard error (n = 3). FW = Fresh Weight; DW = Dry Weight.**

Treatment Group	NaF (mg/L)	Chlorophyll Content (mg/g FW)	Protein Content (mg/g FW)	Leaf Fluoride (mg/kg DW)	Calorific Value (kcal/g DW)
Control	0	2.65 $\pm$ 0.08	28.5 $\pm$ 1.1	1.2 $\pm$ 0.1	4.15 $\pm$ 0.06
Treated Group 1	4	2.30 $\pm$ 0.07	25.3 $\pm$ 0.9	3.6 $\pm$ 0.2	4.02 $\pm$ 0.05
Treated Group 2	8	1.87 $\pm$ 0.06	22.1 $\pm$ 0.8	6.9 $\pm$ 0.3	3.88 $\pm$ 0.05
Treated Group 3	10	1.41 $\pm$ 0.05	17.5 $\pm$ 0.7	10.8 $\pm$ 0.4	3.69 $\pm$ 0.04
Treated Group 4	12	0.95 $\pm$ 0.04	12.6 $\pm$ 0.6	13.5 $\pm$ 0.5	3.45 $\pm$ 0.04



**Figure 2:** Effect of different conc. of NaF on Chlorophyll and Protein content of *Spinacia oleracea* Leaves. Values presented as mean  $\pm$  standard error (n = 3).



**Figure 3:** Effect of different conc. of NaF on Leaf Fluoride (mg/kg DW) and Calorific Value (kcal/g DW) of *Spinacia oleracea* Leaves. Values presented as mean  $\pm$  standard error (n = 3).

**Discussion**

The findings of this study demonstrate that fluoride-contaminated groundwater, simulated with sodium fluoride (NaF), imposes significant adverse effects on both morphological and biochemical characteristics of *Spinacia oleracea*. The observed dose-dependent reduction in seed germination percentage and germination index aligns with previous reports indicating that fluoride disrupts early seed metabolic activity, likely by interfering with key enzymes and cellular energy processes (Kumar et al., 2009; Aske & Iqbal 2013; Bhargava & Bhargava, 2013). Seedlings exposed to higher NaF concentrations exhibited substantial decreases in root and shoot lengths, fresh and dry biomass, and vigor index. These results are consistent with studies on other crops, which also documented inhibition of cell elongation and division, resulting in stunted early growth (Gupta et al., 2009).

At the mature plant stage, chronic fluoride exposure led to marked reductions in root and shoot growth, leaf area, and biomass accumulation, as well as grain yield. Morphological symptoms such as chlorosis, wilting, and stunted or poorly branched roots became increasingly apparent at higher fluoride concentrations. These symptoms correlate with fluoride's disruption of nutrient uptake, photosynthetic efficiency, and membrane integrity (Barbier et al., 2010). The decrease in leaf number, weight, and area further reflects impaired photosynthetic capacity, which is essential for plant growth and productivity.

Biochemical analyses provide further insight into the physiological basis of fluoride toxicity. The substantial decline in chlorophyll content under increasing NaF exposure may be attributed to fluoride-induced inhibition of key enzymes involved in chlorophyll biosynthesis, as well as enhanced oxidative stress and chloroplast damage (Kumar et al., 2009). Reduced protein content suggests suppressed metabolic activity and impaired nitrogen assimilation, which are vital for plant growth and stress response (Gupta et al., 2009). The decrease in calorific value indicates a reduction in the overall nutritional quality of spinach, which is a serious concern for food security in fluoride-affected regions. The pronounced accumulation of fluoride in leaf tissues observed in this study raises food safety concerns, as spinach is widely consumed and can serve as a direct source of fluoride exposure for humans (Amini et al., 2008).

Collectively, these findings reinforce the urgent need for monitoring and managing water quality in agricultural settings, especially in regions where groundwater fluoride exceeds recommended limits. Potential mitigation strategies include blending high-fluoride water with safer sources, adopting fluoride-tolerant crop varieties, and implementing phytoremediation or soil amendments to limit fluoride uptake (Barbier et al., 2010; Singh et al., 2013). Further research is warranted to elucidate the molecular mechanisms of fluoride toxicity and to develop effective agricultural interventions for minimizing risks to crop yield and human health.



#### Conclusion:

This study highlights the detrimental impact of fluoride-contaminated groundwater on the growth, morphology, and biochemical attributes of *Spinacia oleracea*. Elevated fluoride levels significantly hindered germination, growth, yield, and nutritional quality, while increasing fluoride accumulation in edible tissues. These results underscore the necessity of regular groundwater monitoring and the adoption of mitigation strategies to safeguard crop productivity and food safety in affected regions.

#### References:

1. Amini, M., Mueller, K., Abbaspour, K. C., Rosenberg, T., Afyuni, M., Møller, K. N., ... & Johnson, C. A. (2008). Statistical modeling of global geogenic fluoride contamination in groundwaters. *Environmental Science & Technology*, 42(10), 3662–3668. <https://doi.org/10.1021/es071958y>
2. Aske, D. K., & Iqbal, S. (2013). Evaluation of Physical chemical Characteristics of Ground water at Alirajpur, Madhya Pradesh, India. *Researcher*, 5(1), 89-95.
3. Aske, D. K., & Iqbal, S. (2014). Laboratory study of fluoride toxicity on wheat (*Triticum aestivum* Var. lok-1), *Science Research Reporter*, 4(2):159-162.
4. Barbier, O., Arreola-Mendoza, L., & Del Razo, L. M. (2010). Molecular mechanisms of fluoride toxicity. *Chemico-Biological Interactions*, 188(2), 319–333. <https://doi.org/10.1016/j.cbi.2010.07.011>
5. Bhargava, D., & Bhargava, A. (2013). Effects of fluoride on growth and metabolism of plants. *International Journal of Science and Research*, 2(8), 260–263.
6. Gupta, S., Banerjee, S., Saha, R. N., & Datta, J. K. (2009). Impact of fluoride on photosynthesis, growth and accumulation of free proline in rice (*Oryza sativa* L.). *Research Bulletin of the Panjab University Science*, 59, 85–92.
7. Kumar, S., Tripathi, R. D., Rai, U. N., & Singh, N. (2009). Impact of fluoride on photosynthetic pigments, lipid peroxidation, and antioxidant enzymes in *Azolla pinnata*. *Biologia Plantarum*, 53(3), 563–566. <https://doi.org/10.1007/s10535-009-0101-z>
8. Ranjan, R., Ranjan, A., Mehrotra, N., & Singh, K. (2009). Effect of fluoride toxicity on growth parameters in Spinach (*Spinacia oleracea*). *Indian Journal of Environmental Science*, 13(1), 37–40.
9. Singh, M., Chauhan, M., & Tandon, P. K. (2013). Effects of fluoride on growth and metabolism in plants: A review. *International Journal of Pharmaceutical Sciences Review and Research*, 21(1), 154–161.



## **International Educational Applied Research Journal**

**Peer-Reviewed Journal-Equivalent to UGC Approved Journal**

**A Multi-Disciplinary Research Journal**

---

10. World Health Organization. (2011). Guidelines for drinking-water quality (4th ed.). Geneva: WHO Press.