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# FLUORIDE TOXICITY EFFECTS IN POTATO PLANT (SOLANUM TUBEROSUM L.) GROWN IN CONTAMINATED SOILS

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**Abstract:** This study aims to check the tolerance potential of *Solanum tuberosum* to accumulate fluoride (F). For this work *S. tuberosum* were grown for 87 days under five different concentrations of F viz. control, 11.05($T_1$), 22.11($T_2$), 44.21($T_3$), 110.53($T_4$) and 221.05($T_5$) mg per Kg NaF. Study results revealed that maximum reduction of root biomass (82.5 %) at the fluoride dose 95 mg NaF/Kg soil. However, growth ratio and tolerance index showed opposite trend with concentration of F. On the other hand, F accumulation pattern was recorded highest in leaves and % of total F translocation from soil to plant linearly decreases with increasing added fluoride in soil. The F accumulation in leaves, root, shoot and potato tuber is 3.96 mg NaF per Kg, 3.02 mg NaF per Kg, 2.8 mg NaF per Kg and 1.56 mg NaF per kg, respectively. It was inferred from this study that potato (*S. tuberosum*) accumulates fluoride at tissues level.
**Keywords** Bioaccumulation factor; Fluoride in plant; Phytotoxicity; *Solanum tuberosum;* Translocation factor.
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## INTRODUCTION

Fluoride is a strong electronegative element widespread in the environment, occurs in soil, air, water and the vegetation (Jha *et al.,* 2009). Water acts as predominant source of fluoride to cause fluorosis in the endemic areas, although some food materials also contribute significantly to the total intake of fluoride (Yadav *et al.,* 2009). Fluoride is considered as absolutely non-essential element for plants (Kabata- Pendias, 2001). However, it may be essential for animals and human (Jha *et al*. 2009). Stevens *et al.* (1997) also recorded that ionic species of fluoride in solution had a marked influence on the uptake of fluoride by plant roots with complexes species being more readily taken up by the roots than the free fluoride ions. Atmospheric pollution by fluoride is considered as a high phytotoxic risk due to its easy absorption through the stomata of plants leaves and subsequently causes toxicity to many plant species at relatively low concentration (WHO, 1984). The World Health Organization (WHO, 1984) and Bureau of Indian Standards (BIS, 2003) have laid down the maximum permissible limits of F in drinking water as 1.5 mg/L and 1.0 mg/L, respectively, but there is no stringent threshold limit of F in soil and plants above which the ingestion may be detrimental to human health. In acidic soil fluoride showed highest solubility due to its complexation with aluminum, but in alkaline condition, desorption of free fluoride due to repulsion by negatively charged surfaces. However, in neutral pH, fluoride readily bound to soil surface and is not available to plants. According to Arnesen (1997) plants can incorporate F from contaminated soil. The adsorbed F is translocated to the shoots causing physiological, biochemical, and structural damage and even cell death (Jha *et al.,* 2009) depending on the concentration of cell sap. Some plants accumulate F and even at higher concentration up to 4000 µg F per gram do not show any signs of toxicity (Rahman *et al.,* 2007). Most other plants show signs of toxicity at much lower concentration. The fluoride content of both leafy and root vegetables usually do not differ appreciably from those of cereals with an exception of spinach and onion (Jha *et al.,* 2009) usually enriched in fluoride and it is known as good accumulator of fluoride. Potato is one of the most important vegetable crops in India and consumed by the population as the ingredients of kitchen. Potato was therefore chosen as to study F accumulation, uptake and toxicity when gown on contaminated soils.

## EXPERIMENTAL

The present experiment was conducted by pot culture during 15th Nov 2011 to 10th Feb 2012. The Experimental design was framed through randomized block design (RBD). The pot soils were contaminated with graded concentration *i.e.* 0($T_1$), 11.05 ($T_2$), 22.11 ($T_3$), 44.21 ($T_4$), 110.53 ($T_5$) and 221.05 ($T_6$) mg NaFkg$^{-1}$ soil by adding sodium fluoride (NaF) to the pots and thoroughly mixed. Each treatment was replicated three times. Ten seeds of potato (*S. tuberosum)* were sown in each pot. The irrigation was applied with de-ionized water. All the plants were harvested at eighty seven (87) days. Half of the plant sample was used for determination of biomass and biochemical parameters. Whereas other half was segregated into shoots, roots, leaf and potato for dried, weighed, milled to pass through 0.2 mm sieve and kept for the total fluoride determination in the root, shoots, leaves and potato tubers. Similarly, soil samples collected from each pot after the harvest, were subject to analysis of pH, $CaCl_2$ extractable fluoride and total fluoride. The pH and EC (electrical conductivity) of the experimental soil was determined by using pH meter (SYSTRONICS-335, Systronics Pvt. Limited, Ahmedabad, India) and conductivity meter (Model 304-Systronics Pvt. Limited) CEC (cation exchange capacity) of the soil is determined by Schollenberger and Dreibelbis's (1930) method using spectrophotometer (Model 1203-Systronics Pvt. Limited). Textural analysis of the soil was carried out by international pipette method (hydrometer method) and soil moisture by gravimetric method, organic carbon (rapid titration method), available N (nitrogen), available P (phosphate), Bulk density, particle density and specific gravity were measured by following standard soil analysis manual.

The soluble fluoride (0.01 M $CaCl_2$ extractable) was measured by the method adopted by Larsen and Wuddiwson (1971) and total F in the plant parts (roots, shoots, leaves and potato tuber (only internal part)) were determined following the method developed by Paul et al. (2011) with an F ion selective electrode (ORION 4 star). Biochemical analysis of plant Chlorophyll 'a', chlorophyll 'b', total chlorophyll and carotenoid were measured following Arnon (1949) method, Protein (Lowry *et al.,* 1951), sugar content (McCready *et al.,* 1950) and proline (Bates *et al.,* 1973) were measured following standard methods. After harvesting the plant, different parts (root, shoot, leaf and potato) were separated and washed with double distilled water and dried by gentle pressing with tissue paper for recording fresh weight. However, for dry weight, plant parts were then oven dried at 70 °C for 72 h till constant weight. Growth ratio (*GR*) and F tolerance index (*TI*) were calculated by using the following equations (Baker, 1987):

$$GR = \frac{[Plant\ biomass\ with\ F]}{[Plant\ biomass\ without\ F]} \times 100\% \quad (i)$$

$$TI = \frac{[Root\ length\ with\ F]}{[Root\ length\ without\ F]} \times 100\% \quad (ii)$$

Bioaccumulation factor (*BF*) and translocation factor (*TF*) were calculated (eq. iii, iv) for F as per Yoon *et al.,* (2006) and Marchiol *et al.,* (2004).

$$BF = \frac{[F\ concentration\ in\ shoot]}{[F\ concentration\ in\ soil]} \quad (iii)$$

$$TF = \frac{[F\ concentration\ in\ shoot]}{[F\ concentration\ in\ root]} \quad \text{(iv)}$$

The data were expressed as mean ± standard deviation. The comparison of the treatment means were done by ANOVA and the level of significance were determined at $p < 0.05$ and considered as significant. The data of the $CaCl_2$ extractable fluoride in soil and the added fluoride were subjected to the Pearson's correlation coefficient. All the graphs were constructed using computer software (Origin 6.0).

## RESULTS AND DISCUSSION

The experimental soil characterized by analyzing various physico- chemical parameters and the result is presented in Table 1. Figure 1 demonstrated that $CaCl_2$ extractable F always higher than that of soluble F. However, soluble fluoride higher in higher concentration of fluoride dose in the soil. On the other hand, total F concentration in soil and roots of plant are not well balanced. The product moment correlation coefficient showed positive relationship between the concentration of added fluoride and both $CaCl_2$ extractable ($r = + 0.992$, $p < 0.001$) and soluble fluoride ($r = + 0.995$, $p < 0.001$) in the solution. The $CaCl_2$ extractable and soluble F varied between 2.01 to 12.67 mg NaF per kg soils in the treatment range of 11.05 to 221.05 mg NaF per kg soil. Visible symptoms of fluoride toxicity did not appear even at a higher level of added fluoride. Moreover, at higher F dose, soil pH changes to alkaline which support to release higher fluoride from soil surface and subsequently plant availability increased (Saxena and Rani, 2012). Almost similar observations reported by Gupta and Banerjee (2011). However, higher level of fluoride enters to the plant to inhibit plant metabolism leading to necrosis, needle seratel, and tip burn disease (Mohan *et al.,* 2007). The effect of various F concentration on the growth of root and shoot length of *S. tuberosum* were measured in the form of growth ratio (GR). The growth ratio of *S. tuberosum* was evaluated in five concentrations of F (control, 11.05, 22.11, 44.21, 110.53 and 221.05 mg NaF per kg soil). The result showed that GR gradually decrease with increasing F concentration. However, TI increases with increasing F concentration up to 110.53 mg NaF per kg soil, but decrease at 221.05 mg NaF per kg soil. The Pearson correlation result showed inverse relationship between GR ($p < 0.004$) and TI with soluble F in soil (Table 2). The variation of root and shoot length is almost opposite up to 9.5 mg NaF per kg soil. Results revealed that shoot length decreased gradually with increasing the F concentration. However, root length gradually increased with increasing F concentration up to 95 mg NaF per Kg soil (results not shown). The variation of root and shoot length is also significantly affected by F. Almost similar results was demonstrated by Pant et al. (2008) for wheat (*Triticum aestivum*), Bengal gram (*Cicer arietinum L.*), mustard (*Brassica juncea*) and tomato (*Lycopersicon esculentum*). Saini et al. (2008) reported that both root and shoot growth decreased with increasing accumulation of NaF for *Prosopis juliflora*.

**Table 1. Characterization of experimental Soil, values are mean ± SD**

| Parameters | Values |
|---|---|
| **pH (w/v:1/2.5)** | 6.5 ± 0.001 |
| **Electrical Conductivity ($mScm^{-1}$)** | 20 ± 0.2 |
| **Organic Carbon (%)** | 0.56 ± 0.03 |
| **Available N(kg/ha)** | 50.45 ± 0.11 |
| **Available P(kg/ha)** | 30.62 ± 0.01 |
| **Cation Exchange Capacity (meq/100g)** | 10.5 ± 0.41 |
| **Soil moisture (%)** | 9.41 ± 0.004 |
| **Bulk density($g/cm^3$)** | 1.25 ± 0.001 |
| **Porosity (%)** | 24 ± 0.44 |
| **Specific gravity ($g/cm^3$)** | 1.34 ± 0.06 |
| **Sand (%)** | 90.4 ± 1.01 |
| **Silt (%)** | 4.6 ± 0.23 |
| **Clay (%)** | 5.0 ± 0.19 |

The fluoride concentration in the shoot and leaves showed a linear trend with the added fluoride in the soil up to 95 mg NaF per kg soil except treatment 38 mg NaF per kg soil. However, F concentration in the root and potato tuber showed inconsistent result with the added

F in the soil. On the other hand, percentage of total F in different plant parts increase linearly with increasing added F in soil (Figure 2). Present study also highlighted the similar reduction of leaf and shoot biomass with increasing F concentration. However, root biomass increased with increasing F concentration up to 38 mg NaF per kg soil. Such reduction of biomass with increasing F concentration was also reported by Jha *et al.* (2009). Present study highlighted the lesser amount of F accumulation in potato probably due to maximum F translocated to the aerial part leading to high permeability through the endodermis (Keller, 1980). However, Vargava and Vargavas (2011) demonstrated in their paper that when wheat plants were exposed with 20 mg/L NaF, highest accumulation was recorded at roots (4.24 µg/g) and lowest in leaves (1.45 µg/g). This clearly indicates that F translocation in shoots is limited probably due to nonpermeability of endodermis tissue (Baunthiyal and Ranghar, 2013). Our earlier paper also highlighted the lower levels of fluoride in potato tuber compared to other vegetables (Pal *et al.,* 2012). However, it is also reported that, all vegetables do not support the accumulation of same level of fluoride in their body parts (Khandare and Rao, 2006).

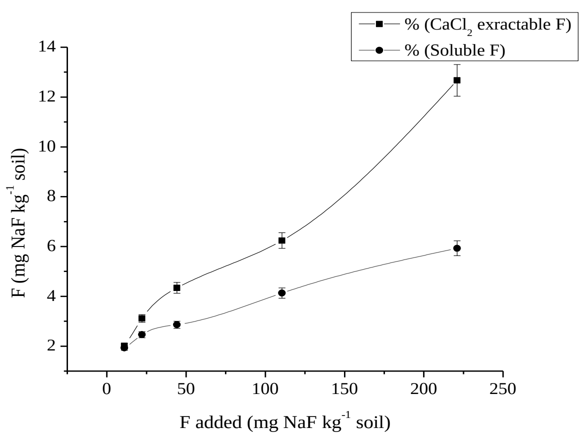


**Figure 1. Effect of added NaF on water soluble and $CaCl_2$ extractable F concentration in soil.** Both water soluble and $CaCl_2$ extractable F concentrations represent the mean of four samples. The bars represent the least significant difference (LSD) between the means at the 0.05 probability level.

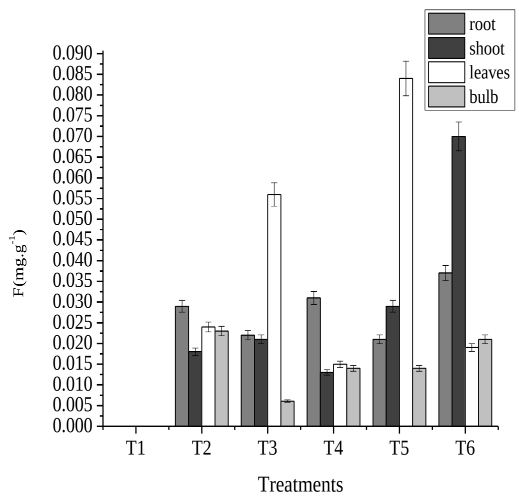


**Figure 2. F accumulation in different parts of potato plant. The bars represent the least significant difference (LSD) between the means at the 0.05 probability level.**

No visible symptom of phyto-toxicity of plants was noticed in the physical health of *S. tuberosum* with the fluoride range 0-190 mg NaF per kg soil. However, significant reduction in fresh weight of root, shoot and leaf was recorded with respect to control. About 76 % reduction of fresh shoot weight followed by 82.5 % reduction of fresh root weight and 81.56 % fresh leaf weight with respect to control was recorded at 95 mg NaF per kg fluoride in soil (result not shown). The wet biomass of *S. tuberosum* indicates the sequence: leaf > shoot > root for all studied F doses (Table 3). Number of potatoes/plant varies between 4 to 5. With increasing F level in soil, number of potato tuber/plant does not change. The average number of potato tuber/plant significantly varies with respect to control in higher concentration from 38 mg NaF per kg to 190 mg NaF per kg soil (results not shown). Similarly, individual weight of potato showed significant variation in all treatments except lower concentration (9.5 mg NaF per kg soil). Biochemical analysis of potato leaves indicate gradual decrease of total chlorophyll with increasing fluoride level in soil. Similarly Chl ‘a’ to ‘b’ ratio also showed linear decremented trend with applied F dose. Total chlorophyll level linearly decreased with increasing fluoride level. This is probably due to higher accumulation of fluoride in leaves and subsequently it can bind readily with $Mg^{2+}$, forming an $MgF^{+}$ complex. This kind of complexation may destroy the photosynthetic pigments, particularly the chlorophylls, thereby significantly decreasing the concentration of pigments. On the other hand, chl 'a' to 'b' ratio gradually increased with increasing F treatment clearly indicate that the chlorophyll 'b' is more sensitive to F that disrupt the balance between energy trapping in photosystem II and cause a decrease in electron transport. On the other hand, other parameters like carotenoid and protein showed similar decremental trend. Carotenoid is a non enzymatic antioxidant pigments which protects chlorophyll membrane and cell genetic composition against ROS under F stress condition. Present finding also indicate the drastic reduction of carotenoid in all studied concentrations with respect to control, which is probably due to quenching of triplet chlorophyll, replacing peroxidation and destruction of chloroplast membrane. However, proline level showed significant incremental trend with respect to control (Table 2). But sugar level in leaves initially increased up to F dose 38 mg NaF per kg soil, then drastically deceased (Table 2). Similarly, protein and sugar level in all the treated plants significantly reduced with respect to control. Similar reduction of protein and sugar synthesis under such fluoride stress was reported by Dey *et al.* (2012). From the Table 3 it is clear that F content in root significantly related with F level in potato tuber ($r = 0.860$, $p < 0.05$); and sugar content in potato tuber ($r = 0.837$, $p < 0.05$). However, F content in shoot positively influence the sugar ($r = 0.887$, $p < 0.05$) and proline ($r = 0.775$, $p < 0.05$) level in potato tuber. Similarly, proline level in potato tuber is also significantly influenced by F content in leaf and sugar level in potato tuber. On the other hand, sugar content in potato tuber significantly related with F level in tuber ($r = 0.912$, $p < 0.05$) (Table 5).

Soluble F level showed significant ($p < 0.035$) positive relation with TF, but negative with GR ($r = -0.700$, $p < 0.004$) and BF ($r = -0.697$) (Table 4). *S. tuberosum* showed the bioaccumulation factor (BF) values 0.262- 1.63 at different F concentrations. The experimental plants showed a translocation factor (TF) of 0.42- 1.89 at different concentrations of F (Table 3). However, tolerance index was recorded highest at $T_4$ and growth ratio gradually decreases with increasing fluoride dose up to $T_4$ treatment (Table 3). The TF is an important factor in determining the capacity of hyper accumulation and assessment of soils with a high level of metal and non- metal contaminants. The TF shows the relative uptake of F ions by plants with respect to the presence of F in the soil solution. A ratio greater than 1 means hyperaccumulation of F in plants while a ratio less than 1 is hyperaccumulator (Gupta and Banerjee, 2011). In this study, TF of different concentrations of F may suggest that *S. tuberosum* can actively uptake F from soil and store them in its above ground parts, which makes this plant a remarkable phytoremediator. Previous field study results suggest that potato accumulates F in the range of 4.01 – 17.91 mg/kg dry biomasses. Yadav *et al.* (2012) reported that potato can accumulate 14.2 $\mu$g/g F. However, in our previous work (Pal *et al.,* 2012) much lower lever level of F (0.40 mg/kg) in potato at Junitpur and Nowapara village, Birbhum district were recorded. On the other hand, Gautom *et al.* (2010) reported that sarso leaves and spinach can accumulate 24.86 mg/kg and 23.12 mg/kg F, respectively. Moreover, the result of the present study showed that *S. tuberosum* has high ability to accumulate F (especially in the aerial parts) available in the soil. The strong F accumulation in the shoots combined with >1 BFs and TFs indicates the potentialities of *S. tuberosum* for field application in removal of F. Overall the study concludes that *S. tuberosum* showed tolerance towards F and maximum F accumulation in the aerial parts. Therefore, it is strongly suggest that the areal part may not be used for cattle feed and potato can be consumed. Moreover present results also indicate that *S. tuberosum* is a suitable candidate species for the removal of F in phytoremediation purpose.

**Table 2. Pigments, Protein, Sugar and Proline level in Potato leaf and Potato fiber**

| Treatment | Chl'a' (µg/g) | Chl'b' (µg/g) | T. Chl (µg/g) | Carotenoid (µg/g) | Potato leaf(µg/g) | | | Potato fiber(µg/g) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Protein | Sugar | Proline | Protein | Sugar | Proline |
| Control | 125ᵃ | 59ᵃ | 195.5ᵉ | 49ᵃ | 8.5ᵃ | 3.87ᶜ | 4.2ᶠ | 4.3ᵃ | 4.4ᵈ | 7.0ᶜ |
| $T_1$ | 64ᶜ | 29ᶜ | 100.1ᶠ | 29ᵈ | 3.6ᵉ | 1.16ᶠ | 5.3ᵉ | 3.9ᵃ | 8.0ᵇ | 7.4ᶜ |
| $T_2$ | 69ᵇ | 33ᵇ | 682ᵃ | 31ᶜ | 5.3ᵇ | 2.6ᵈ | 6.1ᵈ | 2.6ᵇ | 4.7ᵈ | 8.8ᵇ |
| $T_3$ | 36ᵈ | 16ᵈ | 487ᵇ | 14ᵉ | 4.6ᶜ | 2.1ᵉ | 7.9ᶜ | 2.0ᶜ | 7.5ᶜ | 8.9ᵇ |
| $T_4$ | 72ᵇ | 29ᶜ | 413ᶜ | 30ᶜ | 3.9ᵈ | 4.84ᵇ | 9.1ᵇ | 1.6ᶜ | 7.4ᶜ | 8.0ᵇ |
| $T_5$ | 122ᵃ | 58ᵃ | 346ᵈ | 40ᵇ | 3.6ᵉ | 8.1ᵃ | 11.1ᵃ | 0.66ᵈ | 10.0ᵃ | 12.0ᵃ |

Means followed by the same letter (a, b, c, d, e and f) within treatment are not significantly different at 5% using Duncan's multiple range test (DMRT). Means of three replicates are taken.

**Table 3. Wet biomass of Root, Shoot and Leaf, Bioaccumulation and Translocation factor, Tolerance index and Growth rate of Potato plant**

| Treatment | Leaf fresh mass(g) | Shoot fresh mass (g) | Root fresh biomass (g) | BF | TF | TI | GR |
|---|---|---|---|---|---|---|---|
| Control | 57.03ᵃ | 33.03ᵃ | 6.59ᵃ | - | - | - | 100ᵃ |
| $T_1$ | 21.89ᵇ | 22.07ᵇ | 1.27ᵈ | 1.63ᵃ | 0.62ᵈ | 0.99ᶜ | 62ᵇ |
| $T_2$ | 16.74ᶜ | 12.95ᶜ | 1.44ᶜ | 0.949ᵇ | 0.95ᶜ | 1.1ᶜ | 38ᶜ |
| $T_3$ | 12.28ᶠ | 10.63ᵈ | 1.56ᶜ | 0.294ᵈ | 0.42ᵉ | 1.14ᵇ | 29ᵈ |
| $T_4$ | 14.68ᵈ | 7.92ᶠ | 1.15ᵈ | 0.262ᵉ | 1.38ᵇ | 1.41ᵃ | 22ᶠ |
| $T_5$ | 13.68ᵉ | 8.7ᵉ | 2.65ᵇ | 0.317ᶜ | 1.89ᵃ | 0.98ᶜ | 26ᵉ |

Means followed by the same letter (a, b, c, d, e and f) within treatment are not significantly different at 5% using Duncan's multiple range test (DMRT). Means of three replicates are taken.

**Table 4. Correlations between T.Chl, T.F in Plant, Soluble F, BF, TF, GR, TI, Chl 'a' to 'b' ratio**

| | T.Chl | T.F P | SF | BF | TF | GR | TI |
|---|---|---|---|---|---|---|---|
| **T.FP** | -0.540 | | | | | | |
| **S F** | -0.818* | 0.793 | | | | | |
| **BF** | 0.979* | -0.400 | -0.697 | | | | |
| **TF** | -0.614 | 0.944* | 0.904* | -0.448 | | | |
| **GR** | 0.976* | -0.499 | -0.700 | 0.979* | -0.527 | | |
| **TI** | -0.405 | 0.312 | 0.050 | -0.498 | 0.057 | -0.557 | |
| **Chl'a'/'b'** | -0.243 | 0.249 | 0.020 | -0.346 | -0.033 | -0.338 | 0.888* |

* $p < 0.05$; BF (Bio-concentration Factor), TF (Translocation Factor), TI (Tolerance Index), GR (Growth Ratio), TFP (total fluoride in plant), SF (soluble fluoride) and Chl a/b (chlorophyll a/b).

**Table 5. Correlation Coefficient between each pair variable: % F trans., F root, F shoot, F leaves, F blub, Average wt. potato, Potato sugar, Potato protein and Potato proline**

| | % FT | FR | FS | FL | FB | AWP | PS | PP |
|---|---|---|---|---|---|---|---|---|
| **FR** | 0.281 | | | | | | | |
| **FS** | -0.180 | 0.683 | | | | | | |
| **FL** | -0.117 | 0.082 | 0.141 | | | | | |
| **FB** | 0.426 | 0.860* | 0.597 | 0.061 | | | | |
| **AWP** | -0.593 | 0.581 | 0.609 | -0.048 | 0.387 | | | |
| **PS** | 0.027 | 0.837* | 0.775 | -0.033 | 0.912* | 0.687 | | |
| **PP** | -0.659 | -0.590 | -0.283 | -0.283 | -0.354 | 0.163 | -0.141 | |
| **PPr** | -0.120 | 0.795 | 0.887* | 0.887* | 0.741 | 0.709 | 0.909* | -0.262 |

* $p < 0.05$; Fluoride (F) in FR (root), FS (shoot) , FL (leaves), FB (bulb), AWP (average weight of potato), PS (sugar in potato), PP (protein in potato) and PPr (proline in potato), FT (fluoride tranlocation).

Very recently Saxena and Sewak (2015) reported that F contents in cereals, pulses and vegetable varies from 1.7 – 14.03 mg/kg, 2.34 – 6.2 mg/kg and 1.79 – 7.33 mg/kg, respectively. However, Ranja and Yasmin (2015) reported that leafy vegetables can accumulate higher level of F.

## CONCLUSION

The result of the present study showed that *S. tuberosum* has high ability to accumulate F (especially in the aerial parts) available in the soil. The strong F accumulation in the shoots combined with > 1 BFs and TFs indicates the potentialities of *S. tuberosum* for field application in removal of F. Overall the study concludes that *S. tuberosum* showed tolerance towards F and maximum F accumulation in the aerial parts. Therefore, it is strongly suggest that the areal part may not be used for cattle feed and potato can be consumed. Moreover present results also indicate that *S. tuberosum* is a suitable candidate species for the removal of F in phytoremediation purpose.

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