PATNA WOMEN'S COLLEGE 1940

**IRIS**
**Journal for Young Scientists**
**ISSN** 2278 – 618X **(Print)**
**ISSN** 2278 – 6384 **(Online)**

http://www.patnawomenscollege.in/journal

# Bioremediation of fluoride spiked soil by earthworm *Eisenia fetida*

• **Surabhi Kumari** • **Kumari Barkha** • **Aditi Arora**
• **Sumeet Ranjan and Shahla Yasmin**

Received : November 2014
Accepted : March 2015
Corresponding Author : Shahla Yasmin

**Abstract :** *A study was conducted to find out the role of earthworm Eisenia fetida in bioremediation of fluoride spiked soil. Earthworms were cultured in the mixture of cowdung and soil (3:1 W/W) for a month. Four experimental setups were designed and named as Control A (without earthworms), Control B (with earthworms), Test A (without earthworms) and Test B (with earthworms). Fluoriated water was sprinkled in Test A and Test B for one month. Coriander plants were grown in Control A, Test A and Test B. Samples of plants, earthworm tissues and soil were tested on 10th, 20th and 30th day to compare the percent reduction and accumulation of fluoride in the different setups. The results showed that earthworms had accumulated fluoride.*

**Keywords:** *Bioremediation, Fluoride spiked soil, earthworm.*

**Surabhi Kumari**
B.Sc. III year, Zoology (Hons.),
Session : 2012-2015, Patna Women's College,
Patna University, Patna, Bihar, India

**Kumari Barkha**
B.Sc. III year, Zoology (Hons.),
Session : 2012-2015, Patna Women's College,
Patna University, Patna, Bihar, India

**Aditi Arora**
B.Sc. III year, Zoology (Hons.),
Session : 2012-2015, Patna Women's College,
Patna University, Patna, Bihar, India

**Sumeet Ranjan**
Research Scholar, Dept. of Zoology
Patna Women's College,
Patna University, Patna, Bihar, India

**Shahla Yasmin**
Head, Dept. of Zoology, Patna Women's College,
Bailey Road, Patna–800 001, Bihar, India
E-mail : shahla_apex@yahoo.co.in

## Introduction

Fluoride (F) enters into the human body via two major routes: (i) drinking water, (ii) nutritional supplement or food chain. When a fluoride compound is dissolved in water, the element fluorine will be present mainly as fluoride ions.WHO (1984) has prescribed the maximum permissible limit of fluoride in drinking water as 1.5mg/L. The Bureau of Indian Standards, has however, suggested the desirable limit of fluoride in drinking water as 1.0mg/L with a remark as "lesser the better". The amount of fluoride is considered lethal when taken orally as 35-70mg F/kg body weight. This is equivalent to 5-10g sodium fluoride (NaF) for a 70 kg adult and 1-2g NaF for a 15 kg child (Melberg and Ripa, 1983).

F has negative effects on human health. Excessive F intake over a long period of time may result in serious health problems, disfiguring dental fluorosis and disabling skeletal manifestations including crippling deformities, osteoporosis and osteosclerosis. Endemic fluorosis is known to be a global problem, occurring in all continents, and affecting many millions of people (Fawell et al., 2006). Gastrointestinal complaints, in the form of nausea, loss of appetite, pain in the stomach, constipation followed by intermittent diarrhoea

(Non-ulcer dyspeptic complaints) are considered as the early warning signs of fluoride toxicity manifestations (Susheela and Das., 1998; Susheela et al.,1992 ; Das et al., 1994 ; Gupta et al., 1994 ; Susheela , 1995 ; Dasarathy et al., 1996).

Bioremediation can be defined as a waste management technique that involves the use of naturally occurring organisms to remove or neutralize pollutants from a contaminated site. Due to their biological, physical and chemical actions, earthworms can be directly employed within bioremediation strategies to promote biodegradation of organic contaminants. Earthworms convert organic fraction of waste into available nutrients (Pattnaik and Reddy, 2010). Earthworms have also been shown to retard the binding of organic contaminants to soils. Studies have also shown that the earthworms accumulate heavy metals in their body tissue from contaminated substrates (Edward and Baxter, 1992; Gupta et al. 2005; Suthar et al. 2008).Thus, earthworms have been considered as important bio-accumulators of persistent pollutants like heavy metals (Gish and Christensen, 1973; Udovic et al. 2007;Suthar et al. 2008; Wang et al, 2009 ; Singh et al, 2014). Fluoride is more toxic for soil microorganisms than most of the heavy metals (Wilke 1987).

*Eisenia fetida* is commonly used in bioremediation studies. The present study therefore attempted to assess the remediation of fluoride from soil and also bioaccumulation of it in the tissues of earthworm species *Eisenia fetida*.

**Materials and Methods :**

Twenty earthen pots were taken in 4 sets (5 pots in each set). Cowdung and soil were mixed in 3:1 W/W ratio while in one set of pots only cowdung was taken. Earthworm species used was *Eisenia fetida*. The fluoridated water (10mg/L) was prepared with the standard fluoride solution.

Experimental setups were:

- Control–A : 50 coriander seeds + tap water (100ml twice a day).
- Control–B : 10 earthworms + tap water (100ml twice a day).
- Test–A :50 coriander seeds + fluoriated water (100ml twice a day).
- Test–B : 50 coriander seeds + 50 earthworms + fluoriated water (100ml twice a day).

Coriander seeds were used because being a leafy vegetable, it has been found to accumulate large amount of fluoride (Ranjan and Yasmin 2015)

Moisture content, pH and F levels were estimated on the first day of setup. The pots were left undisturbed for the germination of seeds. F level was measured in the germinates after 10th, 20th, and 30th day. With each harvest,moisture content and pH were also measured. On the 30th day the fluoride levels in the body tissues of earthworm from Test B and Control B were also measured.Ten adult earthworms were taken and dried at 105ºC for an hour. The dried earthworms were ground into fine powder with the help of mortar and pestle and 500mg of the powdered sample was taken for analysis.

Fluoride in water was measured by immersing the Ion-Selective Electrode Orion 9690 BNWP in the test tube.Samples of soil, coriander germinates and earthworm tissues were prepared for fluoride estimation by NaOH fusion method.Again fluoride was estimated in these samples with the Ion-Selective Electrode.

Statistical analyses were performed with ANOVA and t-test. The level of significance was taken as $p$ value $< 0.05$.

**Results and Discussion :**

Significant changes were found in the pH of control and Test–A setups after 30 days (Table 1). Moisture content was found to be high in the Test–A after 30 days as compared to zero day (Table 2). The fluoride content of the soil of Test–B (with earthworm) set up increased significantly after 30 days as compared to 0 day (Table 3). The concentration of fluoride in the soil of Control–A set up increased slightly after 20 and 30 days. This increased concentration of fluoride might have been incorporated in the soil through the tap water that was used for irrigation. There was significant accumulation of fluoride in the coriander grown in the control set up. In the Test–A, there was increased level of fluoride in soil because fluoriated water was used to irrigate the plants.The fluoride content in coriander plants grown in control and Test–A (without earthworm) setups after 30 days was significantly higher as compared to 0–day (Table 4). In the coriander plants of Test–A setups also, there was approximately 10–fold increase in the concentration of fluoride as compared to Control–A after 30 days.

There was insignificant change in the fluoride level in the earthworm tissues in the control pots from 0–day (2.50 ± 0.03, N=5) to 30 days (2.57 ± 0.12, N=5). The accumulation of fluoride in earthworm after 30 days (9.7 ± 0.32, N=5) was significantly greater in the fluoride spiked soil as compared to 0–day (2.27 ± 0.11 N=5). Accumulation of F in the soil of Test–A and Test–B was significantly greater as compared to control. However, the accumulation of fluoride in the coriander plants grown in the Test–A (without earthworm) was significantly higher as compared to control but lowered significantly in Test-B (with earthworm).The bioaccumulation factor (BAF) of fluoride in coriander in Test–A was 0.368, while that in Test–B was 0.317. Whereas, the BAF of F in earthworm was 0.143.

The accumulation of fluoride in the coriander plants grown in the Test–A (without earthworm) was significantly higher (28.59±0.34 N=5) as compared to control (3.75±0.09 N=5) but lowered significantly in Test–B (with earthworm) (21.47±0.28). Further, in the fluoride spiked soil with *Eisenia fetida*, there was increased level of fluoride in the tissues of earthworm. This shows that earthworm can accumulate fluoride.

The percent accumulation of fluoride in earthworm was 74.14 and percent reduction in fluoride spiked soil (with earthworms) was 1.67.The value 1.67(% reduction in F in the soil) is less because the soil was irrigated with fluoriated water (10mg/L) twice a day for 30 days.

The present study showed that the earthworm, *Eisenia fetida* could bioremediate F. It was also observed that there was accumulation of F in the earthworm as well as in the coriander plant. Fluoride accumulation in plants has also been found by Roychowdhury (2013), Saini et al (2013), and Ranjan and Yasmin (2015).

The accumulation of fluoride in both earthworms and coriander plants suggest fluoride could enter the food chain and lead to its accumulation in the organisms of higher trophic levels. This could have harmful effects on the organisms belonging to higher trophic levels. Therefore, it is suggested that F rich water should not be used for irrigation.

**Table 1. Changes in pH in the experimental setup**

| | 0 Day | 10 Days | 20 Days | 30 Days |
|---|---|---|---|---|
| Control (N=5) | 7.36 ± 0.01 | 7.84±0.01 | 7.8 ±0.006 | 7.78±0.008* |
| Fluoride spiked soil (N=5) | 7.85±0.01 | 7.88±0.008 | 7.89±0.005 | 7.91±0.013* |
| Fluoride spiked soil with earthworm (N=5) | 7.81±0.006 | 7.72±0.037 | 7.71±0.037 | 7.71±0.01 |

Values are Mean±S.E.

*Significant change at P<0.05

**Table 2. Changes in moisture percent in the experimental setups**

| | 0 Day | 10 Days | 20 Days | 30 Days |
|---|---|---|---|---|
| Control (N=5) | 48.4±0.24 | 49.2±0.37 | 49.6±0.4 | 49.6±0.4 |
| Fluoride spiked soil (N=5) | 47.4±0.24 | 49.6±0.4 | 49.4±0.24 | 49.4±0.24* |
| Fluoride spiked soil with earthworm (N=5) | 48.6±0.24 | 48.6±0.24 | 48.6±0.24 | 49.2±0.2 |

Values are Mean±S.E.

*Significant change at P<0.05

**Table 3. Changes in F content (mg/L) in soil**

| | 0 Day | 10 Days | 20 Days | 30 Days |
|---|---|---|---|---|
| Control (N=5) | 67.2±0.06 | 67.5±0.17 | 68.32±0.57 | 68.9±0.56 |
| Fluoride spiked soil (N=5) | 67.36±0.08 | 70.14±0.23 | 72.73±0.41 | 77.64±0.99 |
| Fluoride spiked soil with earthworm (N=5) | 67.13±0.07 | 69.39±0.24 | 67.16±0.27 | 67.77±0.53* |

Values are Mean±S.E.

*Significant change at P<0.05

**Table 4. Changes in F content (mg/L) in coriander plants**

| | 10 Days | 20 Days | 30 Days |
|---|---|---|---|
| Control (N=5) | 3.51±0.02 | 3.59±0.05 | 3.75±0.09* |
| Fluoride spiked soil (N=5) | 21.37±0.04 | 28.09±0.12 | 28.59±0.34* |
| Fluoride spiked soil with earthworm (N=5) | 19.77±0.16 | 20.78±0.28 | 21.47±0.28* |

Values are Mean±S.E.

*Significant change at P<0.05

## Conclusion:

The present study revealed that fluoride is accumulated by the coriander plants and earthworm *Eisenia fetida*.Thus, earthworms can be used for bioremediation of F. But since, earthworms form 80% of soil fauna, this accumulated F can enter the food chain and affect the animals belonging to higher trophic levels. Therefore, it is suggested that F rich water should not be used for irrigation.

## Acknowledgements :

We are grateful to Dr. Sister Doris D'Souza A.C., Former Principal, Patna Women's College and the Research Committee for providing facilities and financial support under the Basific Scientific Research (BSR) Scheme.

## *References:*

Das T.K., Susheela A.K., Gupta I.P., Dasarathy S., Tandon R.K. (1994). Toxic effects of chronic F ingestion on this upper gastrointestinal tract.*J.Clin. Gastro.* 18 (3):194-199.

Dasarathy S., Das T.K., Gupta I.P., Susheela A.K., Tandon R.K. (1996). Gastroduodenal manifestations in patients with skeletal Fluorosis.*J.Gastro.* 31:333-337.

Edward C.A., Baxter J.E. (1992). The use of earthworm in environmental management. *Soil Biology Biochem* 24(12): 1683-1689.

Fawell J., Bailey K., Chilton J., Dahi E., Fewtrell L., Magara Y. (2006). F in drinking water, *WHO, IWA publishing,* 1-144.

Gish C.H.D., Christesen R.E. (1973). Calcium, Nickel, Lead and Zinc in earthworms from roadside soil. *Environ Sci. Technol.* 7:1060-1062.

Gupta I.P., Das T.K., Susheela A.K., Dasarathy S., Tandon R.K. (1994). Toxic effects of chronic F ingestion on this upper gastrointestinal tract.*J.Clin. Gastro.* 18 (3): 194-199.

Gupta S.K., Tiwari A., Srivastava R., Murthy R.C., Chandre S. (2005). Potential of Eisenia fetida for sustainable and efficient vermicomposting of fly ash.*Water Air and Soil Pollut.* 163:293-302.

Melberg J.R., Ripa L.W. (1983). F metabolism.Fs in Preventive Dentistry – Theory and Clinical Applications.*Quintessence Publishing Co Limited*.p.81-102.

Pattnaik S., Reddy M.V. (2010). Assessment of

Municipal Solid Waste Management in Puducherry (Pondicherry). India. *Resources Conservation Recy*. 54: 512-520.:293-302.

Ranjan S. and Yasmin S. (2015). Assessment of F intake through food chain and mapping of endemic areas of Gaya district, Bihar, India. *Bull Env ContamToxicol*.94(2):220-224.

Roychowdhury T. (2013). F Accumulation in Food Chain and Daily Dietary intake from a F-Affected Area in Jharkhand District, India. *J. Int. Environmental Application and Science.* 8 (3): 455-461.

Saini P., Khan S., Baunthiyal M., Sharma V. (2013). Mapping of F endemic area and assessment of F accumulation in soil and vegetation. *Environ Monit Assess.* 185 (2): 2001-2008.

Singh S, Shalini, Roy AD, Ranjan S, Yasmin S (2014). Waste management and bioremediation using earthworms, *Eisenia fetida* and *Eudrilus eugeniae.* IRIS. 4:9-14.

Susheela A.K., Das T.K., Gupta I.P., Tandon B.K., Kockar S.K., Ghosh P., Deka R.C. (1992). F ingestion and its correlation with gastrointestinal discomfort.*Fluoride*.25(1):5-22.

Susheela A.K. (1995 ). Epidemiological studies of health risks from naturally contaminated with F: In proceedings of Rome Symposium: Assessing and managing health risks from drinking water contamination: Approaches and applications, ed: Eric G Richard and Giovanni A. Zapponi : *IAHS publication*. No. 233 : 123-124.

Susheela A.K., Das K. (1998). Chronic F toxicity : A scanning electron microscope study of duodenal mucosa. *Clinical Toxicology*. 26 (7): 467 – 476.

Suthar S., Singh S., Dhawan S. (2008). Earthworm as bioindicator of metal (Zn, Mn, Fe, Cu, Pb, Cd) in soils. Is metal accumulation affected by their ecological categories. *Ecol. Eng*. 32:99-107.

Udovic M., Plavc Z., Lestan D. (2007). The effect of earthworm or fractionation, mobility and bioavailability of Pb, Zn and Cd before & after soil leaching with EDTA.*Chemosphere*. 70:126-134.

Wang Q., Zhow D., Cang L., Li L., Zhu H. (2009). Indication of soil heavy metal pollution with earthworms and soil microbial biomass carbon in the vicinity of an abandoned copper mine in eastern Nanjing. China. *Eur. J. Soil Biol*. 45:229-234.

WHO (1984). Guidelines for Drinking Water Quantity (Vol. II): Health Criteria and Supporting Information, *World Health Organization*, Geneva, Switzerland.

Wilke B.M. (1987). F - induced changes in chemical properties and microbial activity of mull, moder and soils. *Bio Fertil Soils*. 5: 49-55.