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Bioremediation of Iron-spiked water by earthworm Eisenia fetida

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Abstract: *Iron contamination of water can either be geogenic or, via industrial effluents and domestic waste. It is mostly present as ferrous (Fe^{2+}) in groundwater but is oxidised to ferric (Fe^{3+}) on exposure to air. In the present study, the efficiency of earthworm Eisenia fetida in bioremediation of iron contaminated water has been investigated. The samples were tested after an interval of 24 hours. The analysis of Fe concentration in Atomic Absorption Spectrophotometer showed that there was significant decrease in the concentration of Iron in both the conventional method (without worms) and through vermibed with earthworm Eisenia fetida. The test for bioaccumulation showed that the tissues of worms accumulated iron in higher concentration, which was significant at $p<0.05$. The result indicated that the earthworms can accumulate Fe in their body tissues and bioremediate the iron-spiked water.*

Keywords: *Iron, Bioremediation, Earthworms, Eisenia fetida.*

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Introduction:

Water is the most vital resource for all kinds of life, as it forms a medium in which physical and chemical transformations, especially those of biological significance, take place. Water pollution has become a global problem due to geogenic, or via industrial effluents, city sewage, chemical fertilizers of agriculture and various religious activities (Bajpai et al., 2002; Gadhia et al., 2014).

Heavy metals are the main pollutants in the environment and pose a big problem due to their toxicity and accumulation in the environment. About 80% of the diseases of the world population and more than one-third of the deaths in the developing countries are due to contamination of water (Achary, 2014). Man can control some undesirable chemical constituents in water before they enter the ground. But once the water enters the ground, man's control over the chemical quality of percolating water is very limited (Johnson et al., 1979).

The geochemical nature of Fe contamination of the aquifers of the Bihar region is of serious concern because groundwater is the primary source of drinking water. The quality of drinking water of Katihar district is very poor in comparison with other districts of Bihar. Due to high iron in the drinking water, dental caries and teeth colouring are prominent ailments of this district (Krishna et al., 2009).

Iron is mostly present in the soluble reduced divalent form as ferrous (Fe2+) in groundwater. Water containing iron is clear and colourless. However, on exposure to air or oxygen, such water becomes cloudy and turbid due to the oxidation of iron to the ferric (Fe3+) states which form colloidal precipitates.

The permissible limit of iron in groundwater is 0.3 mg/L (Achary, 2014). Iron is an essential element for haemoglobin, myoglobin and a number of enzymes and its deficiency leads to anaemia and loss of well-being. However, its overload causes severe health problems in human beings, such as, liver cancer, diabetes, cirrhosis of the liver, heart diseases and infertility, etc.

The use of earthworms for water bioremediation is a biological method, so that the pollutant concentrations in the water are reduced through bioaccumulation mechanisms in the body of the earthworms (Matschekoet al., 2002; Slizovskiy and Kelsey, 2010). These organisms can accumulate high concentrations of heavy metals in their bodies (Li et al., 2010).

Earthworms play an important role in the vermifiltration system by consumption, digestion and assimilation of high rates of organic solids present in wastewater. They also have the ability to tolerate a wide range of environmental stresses and possess high reproductive rate (Rajpalet al., 2014; Sinha et al., 2008).

Introduction of earthworms into the filtration system, with suitable bedding materials to break down organic pollutants, is called vermi filtration (Tomar and Suthar, 2011; Arora et al., 2016).

Vermiremediation is the uptake of metals from contaminated sites by earthworms and immobilisation. Earthworms accumulate heavy metals (Abdul Rida and Bouche, 1995; Morgan and Morgan, 1999). Vermifiltration technology, which uses earthworms as the means of aerobically treating water, is increasingly becoming an environmentally friendly water treatment technique (Manyuchi, 2013). Besides, bioaccumulation of heavy metals from waste by earthworms can prove to be very beneficial in getting rid of these toxic substances from our food chain.

In the present study, any bioremedial impact of Eisenia fetida against iron contaminated water has been assessed.

Materials and Methods:

Earthworm species used for research was Eisenia fetida. Two long plastic containers of 50 litres capacity and four containers of 10 litres capacity were taken. The methodology includes two experimental set-ups:-

Conventional method- The containers were kept on a stand one below the other. The topmost container of 10 litres, 'A', was used to fill iron-spiked water. The system consisted of simple 0.3 cm pipes with holes for trickling water that allowed uniform distribution of iron-spiked water on the different layers in container 'B'. The middle big container B comprised layers of big gravels (size- 3×2 cm), followed by small-sized gravels (size- 1×2 cm), which were kept in two consecutive layers followed by sand and sawdust and each layer was of 12 cm height (Sinha et al., 2008) (Fig. 1).

Vermifiltration bed- The same set-up was made as with the conventional method. On the topmost layer, shredded filter paper was used as feed material and 150 gm of earthworms Eisenia fetida was introduced (Sinha et al., 2008)(Fig. 2). The worms were given around five days settling time in the shredded filter paper to acclimatize in the new environment. 24 hours retention period was provided to the earthworms to perform their action.

In both the beds, 20 litres of iron-spiked water was allowed to pass from A to B and then water was collected from the container 'C'.

Another set of experiments were set up for the conventional method and vermifiltration bed. But here, instead of sawdust, activated charcoal was used (Fig. 3 and Fig. 4).

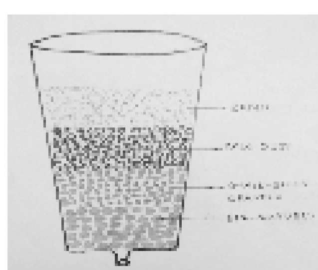

Fig. 1. First experimental set-up of Conventional method (with sawdust)

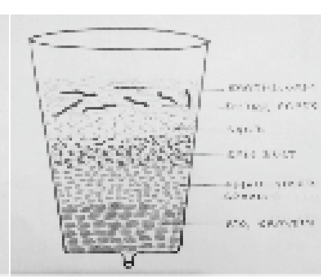

Fig. 2. First experimental set-up of Vermifiltration bed (with sawdust)

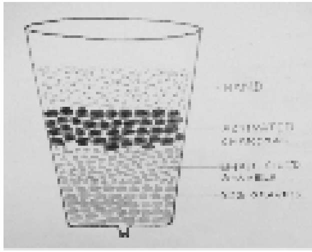

Fig. 3. Second experimental set-up of Conventional method (with activated charcoal)

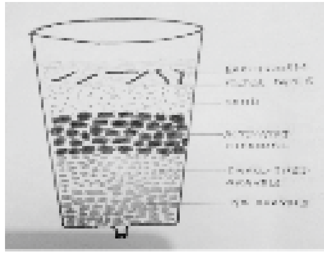

Fig. 4. Second experimental set-up of Vermifiltration bed (with activated charcoal)

The test water was analysed for its pH. Iron content in ppm was analysed using AAS (Atomic Absorption Spectrophotometer) Savant AA-AAS, GBC, Australia.

Iron-spiked water was prepared from a 100 ml bottle of iron (Central Drug House, New Delhi), which contained 1000 ppm concentration of iron in 1000 mg/L AAS in HNO_3 in double distilled water. The iron-spiked water passed completely through the set-up in 24 hours. A total of 8 series were repeated.

Estimation of Fe in water- Standard solutions of 1 ppm, 2 ppm, 4 ppm and 8 ppm concentrations were prepared in a 100 ml volumetric flask and the equipment was calibrated.Then, samples of control 'C' and test 'T' were collected at intervals of 24 hours for iron analysis.The samples were analysed using Savant AA-Atomic Absorption Spectrophotometer (AAS). The sample is injected through the nozzle and the acetylene gas burns the sample and generates colours of different intensities. The detector reads the colour intensity and displays the result in ppm.

Both control and tested earthworms were collected and their tissues were analysed to test for the bioaccumulation of Fe in their tissues.

Preparation of Earthworm Powder– Earthworms were washed and kept on moist filter paper in a petri dish and were left in the incubator for 48 hours at 25.7°C to flush out the matter from the gut. The earthworms were dried in the Newtronic Laboratory Oven (NW-DSU-52) at 180°C for 4 hours. The dried body tissues were ground with mortar and pestle to make them into powder which was further digested.

Acid Digestion Earthworm Powder–To the sample, 9 ml of freshly prepared acid mixture of 65% HNO_3 37% HCl was added. Then, the mixture was boiled gently over a water bath (95°C) for 4-5 hours (or until the sample had completely dissolved) (Ang and Lee, 2005).

Statistical Analysis-The results are presented as mean±SE. Total variation present in a set of data was analysed through one-way Analysis of Variance (ANOVA) and t-test. The level of significance was taken as $p<0.05$. Graph Pad Prism Programme (GraphPad Software, Inc., San Diego, USA) was used for the graphs.

Results and Discussion:

In the present study, it was observed that the pH of the Fe-spiked water decreased after its treatment with the conventional method (without worms) and when treated with earthworm ***Eisenia fetida***.The results were significant. It was noted that there was a slight change in the body colour of the earthworms ***Eisenia fetida*** when they were treated with iron-spiked water.

First experimental set-up (with sawdust):

Table 1. Changes in the concentration of Fe (ppm) in the experimental set-up (with sawdust)

S.No.	Spiked Fe 'F'(ppm)	Control 'C' (ppm) (without worms)	Test 'T' (ppm) (with worms)
1.	*10*	*6.36±0.08*	*5.46±0.08*
2.	*10*	*6.46±0.08*	*6.43±0.01*
3.	*10*	*6.36±0.08*	*4.36±0.08*
4.	*10*	*6.40±0.05*	*3.06±0.08*

Values are Mean±SE (n=3). *Significant at $p<0.05$

Table 2. Bioaccumulation of Fe (ppm) in the tissues of *Eisenia fetida* (with sawdust)

S.No.	Control Eisenia fetida (C.Ef.) (ppm)	Test Eisenia fetida (T.Ef.) (ppm)
1.	*0.591±0.0008**	*1.477±0.002**
2.	*0.582±0.0008**	*2.031±0.0005**
3.	*0.593±0.0009**	*4.951±0.0005**
4.	*0.584±0.0008**	*1.362±0.001**

Values are Mean±SE (n=3). *Significant at $p<0.05$

In the present study, we observed that there was decrease in the concentration of Fe in the Fe-spiked water (F) after its treatment with the conventional method (without worms) and with the earthworm ***Eisenia fetida.*** However, there was significant reduction in iron concentration when treated with the earthworm ***Eisenia fetida.*** This was probably due to the introduction of the earthworm since they accumulate iron in their body tissues (Table 1).

Table 1 and fig. 1 show that there was decrease in the concentration of Fe in the Fe-spiked water (F) after its treatment with the conventional method (without worms) which were 36%, 35.4%, 36.4% and 36% at the end of first, second, third and fourth series, respectively. The results were not significant at p<0.05 when compared to Fe-spiked water.

Table 1 and fig. 1 show that there was decrease in the concentration of Fe in the Fe-spiked water (F) after its treatment with Earthworm (Eisenia fetida) which were 45%, 35.7%, 56.4% and 69.4% at the end of first, second, third and fourth series, respectively. However, the results were not significant at p<0.05 when compared to Fe-spiked water.

Table 2 and fig. 2 show that the accumulation of Fe in the tissues of earthworm bodies was 59.98%, 35.12%, 88%, 57.12% higher when compared to the untreated or controlled earthworm at the end of first, second, third and fourth series, respectively, and was significant at p<0.05.

Change in the concentration of Fe (ppm) in the Fe spiked water (F) after its treatment with the conventional method (with sawdust) and bioaccumulation of Fe in the tissues of earthworm Eisenia fetida are shown through the graph in Figure 1 and Figure 2, respectively.

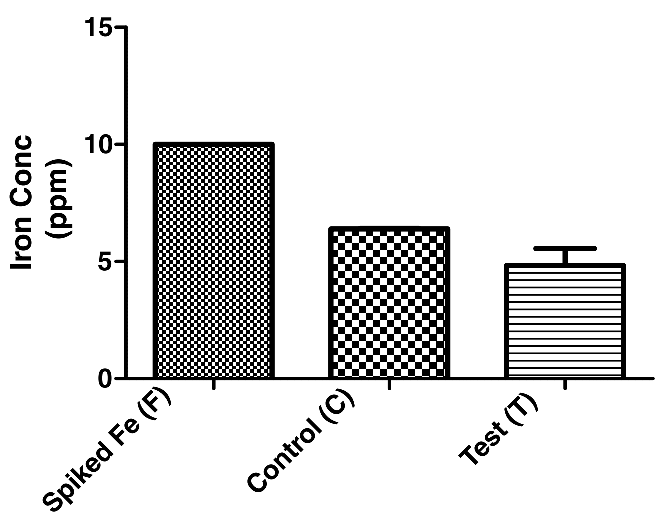

Fig. 1. Change in the concentration of Fe (ppm) in the experimental set-up(with sawdust)

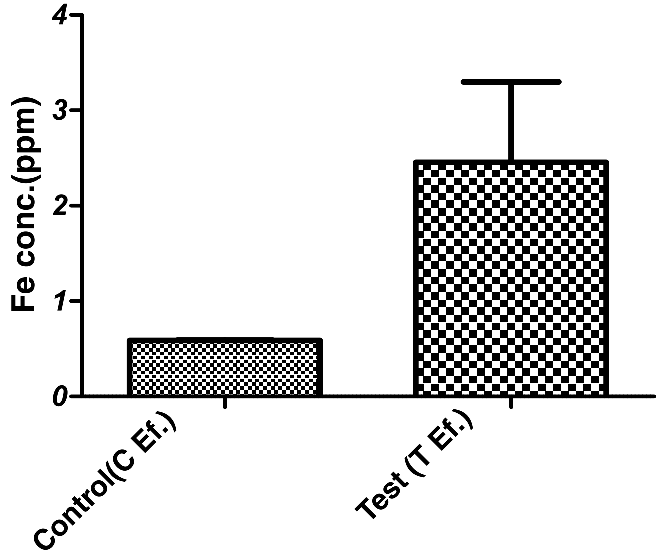

Fig. 2. Bioaccumulation of Fe (ppm) in the tissues of Eisenia fetida (with sawdust)

Second experimental set-up (with activated charcoal):

Table 3. Change in the concentration of Fe (ppm) in the experimental set-up (with activated charcoal)

S. No.	Spiked Fe 'F' (ppm)	Control 'C' (ppm)	Test 'T' (ppm)
1.	10	0.53±0.008*	0.53±0.008*
2.	10	0.54±0.008*	0.54±0.008*
3.	10	0.01±0.004*	1.09±0.005*
4.	10	1.10±0.004*	2.20±0.004*

Values are Mean±SE (n=3). *Significant at p<0.05

Table 4. Bioaccumulation of Fe (ppm) in the tissues of *Eisenia fetida* (with activated charcoal)

S.No.	**Control** *Eisenia fetida* **(C.Ef.) (ppm)**	**Test** *Eisenia fetida* **(T.Ef.) (ppm)**
1.	0.590±0.0008*	0.796±0.001*
2.	0.585±0.0008*	0.845±0.0004*

*Values are Mean±SE (n=3). *Significant at p<0.05*

In the second experimental set-up, the entire series of filtration was repeated four times, but the analysis of accumulation of Fe in the tissues of earthworm was done after an interval of two series of filtration. In this set-up, there was a sharp decline in the concentration of Fe,which was significant in both control (C) and when treated with the earthworm ***Eisenia fetida*** (T). This was in comparison to the presence of iron in Fe-spiked water

(F). It was probably due to the introduction of activated charcoal (Table 3).

Table 3 and fig. 3 show that there was a sharp decline in the concentration of Fe in the Fe-spiked water (F) after its treatment with the conventional method (without worms) which were 94%, 94%, 99% and 99% at the end of first, second, third and fourth series, respectively. The results were significant at $p<0.05$ when compared to Fe-spiked water.

Furthermore, a decrease in the concentration of Fe in the Fe-spiked water (F) after its treatment with earthworm (***Eisenia fetida***) which were 94%, 94%, 89% and 78% at the end of first, second, third and fourth series, respectively were reported (Table 3 and Fig. 3). However, the results were significant at $p<0.05$ when compared to Fe-spiked water.

Table 4 and fig. 4 show that the accumulation of Fe in the tissues of earthworm bodies was 25.8% and 30.76%, higher when compared to the untreated or controlled earthworm at the end of second and fourth series, respectively, which was significant at $p<0.05$.

The variation in concentration of Fe in the water and bioaccumulation of Fe in the tissues of earthworm ***Eisenia fetida*** is shown through the graph in Figure 3 and Figure 4, respectively.

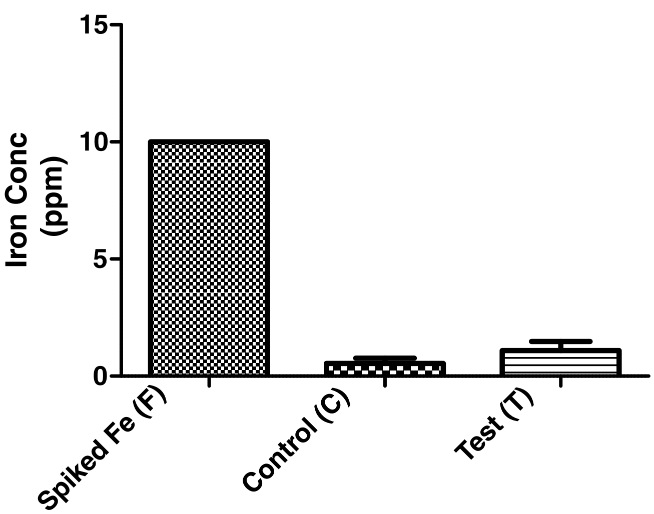

Fig. 3. Change in the concentration of Fe (ppm) in the experimental set-up (with activated charcoal)

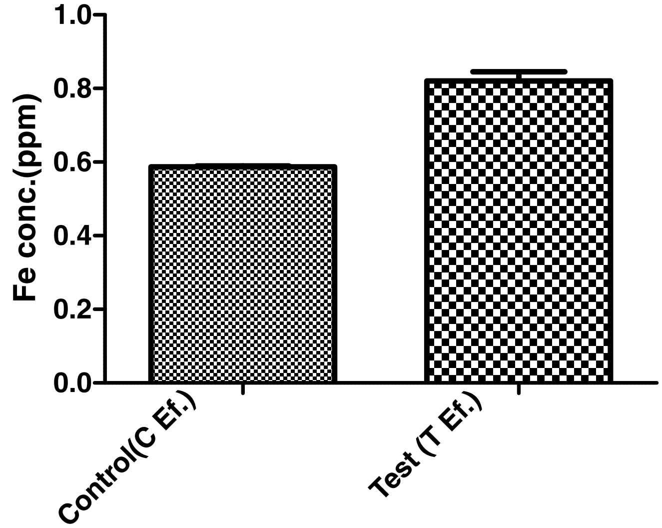

Fig. 4. Bioaccumulation of Fe (ppm) in the tissues of *Eisenia fetida* (with activated charcoal)

In this study, there was a lowering of pH in the treated water indicating that the earthworms cause a shift in the pH from alkaline to neutral. Works of Elvira et al. (1996) and Mitchell (1997) are in agreement with the studies, in as far as the lowering of pH due to vermifiltration is concerned. Earthworms can accumulate a high concentration of heavy metals in the body (Shahmansouriet al., 2005; Li et al., 2010; Brewer and Barrett, 1995; Bamgboseet al., 2000). Similarly, in the present work, it was observed that earthworm Eisenia fetida helped in bioremediation of iron from iron-spiked water.

Some plants, like water hyacinth (Echhorniacrassipes), have the ability to accumulate non-essential metals, such as, Cd and Pb, and this ability could be harnessed to remove non-essential heavy metals from the polluted soil (Wen et al., 2004; Rogars et al.,2000; Liao et al., 2004). The present study showed that the earthworm Eisenia fetida is of great help in bioremediation of iron-spiked water. Similar findings were reported by More and Patole (2012).

The chloragogen cells in earthworm appear to accumulate heavy metals absorbed by the gut and immobilize the metals in small spheroidal chloragosomes and vesicles found in these cells (Sinha et al., 2008).

There was a decrease in the concentration of iron in water treated by earthworms as compared with the conventional method (without worms) (Table 1 and 3). The present findings clearly show that earthworms can accumulate the heavy metals like iron in their body tissues (Table 2 and 4). This is in agreement with the previous study by Verma et al., 2016.

Earthworms can offer an inexpensive and effective bioremediation alternative to complex and costly clean-up methods. Vermifiltration Technology (VFT) is most cost-effective and ecofriendly.

Conclusion:

From the present study, it can be concluded that earthworms can bio-accumulate heavy metals, like iron, in their body tissues. Earthworms can be successfully used for bioremediation of heavy metals from polluted water. Vermifiltration technology is eco-friendly and inexpensive, as compared to other complex and costly methods used for removal of heavy metals.

Exposure to the heavy metals through ingestion or uptake of drinking water, particularly where water has been reused, can lead to accumulation in animals, plants and humans. Excess of iron can cause dental diseases, kidney problems, mental retardation and neuro-problems among humans, and retarded growth in plants.

Acknowledgements:

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