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Analysis of Eco-Toxicity Studies of Leaching of Metal Ions from Flyash-Sewage Sludge Mixture in Poecilia Reticulata (Guppy Fish)

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Abstract: ***This study aims to investigate accumulation of different heavy metal ions (Pb, Cd, Ni Cu and Zn) with control, leachate solution prepared from different sewage sludge concentration and optimised FA/SS mixture on fish species. Eco-toxicity studies were carried out on Poecilia reticulata (guppy fish) under various age groups (60, 90 and 120 days). The eco-toxicity studies revealed that deaths of fishes were increased with increased percentage of sewage sludge in the leachate solution. Fishes death is due to high concentration of heavy metal ions, which made the solution hazardous and the hazardous effect increases with high concertation of SS; however, no death of fishes was observed in control and optimized FA/SS (4:1) leachate solution. It may be concluded from the lab scale study that sewage sludge contains a large concentration of toxic heavy metal ions; however, a right amount of optimization with FA makes the leachate solution non-hazardous. The test results show that the optimised 4:1 FA/SS mixture is the best dose and leachate solution obtained from this ratio is non-hazardous in the aquatic environment. Hence, fly ash can act as a stabilizer for sewage sludge stabilization.***
Keywords: Fly ash, Stabilizer, Sewage sludge, Eco-toxicity, Heavy metals ions, Leaching

I. INTRODUCTION

The problem of heavy metal pollution is increasing day by day. It has been reported in the last few years that most of the aquatic system was contaminated with heavy metal pollution, which is a major environment problem and is of serious concern (Naeem et al., 2011). Heavy metal pollution is increasing in the environment because of the large use of heavy metals in a number of industries and other manufacturing processes, and further these heavy metals enter into the water bodies and from there to the aquatic fauna (Yang & Rose, 2003). The heavy metal pollutants are pollutants which have the ability to bio-magnify in the aquatic food chain and thereby increasing the heavy metal content in them; the process is called biomagnification (Rauf et al., 2009). Metals are of special concern in the environment because of their presence in different concentrations and their toxicity levels to the aquatic biota and flora (Javed, 2004). One of the major pollutants of aquatic environments causing damaging effects on the aquatic animals is considered because of the non-essential and non-biodegradable nature of cadmium (Hollis et al., 1999). It has been reported (Cherian & Goyer, 1989) that cadmium also enters aquatic environments through sewage sludge dissolution and with agricultural run-off as it is one of the main constituents of phosphate fertilizers. It was investigated that most of the heavy metal ions are toxic or carcinogenic in nature, posing serious health hazards to both humans as well as to the environment (Farombi et al., 2007).

Heavy metals have got major consideration in the eco-toxic studies because of their increased toxicity levels and accumulation capacity in the aquatic species (Javed, 2002). It was reported (Olaifa et al., 2004) that fish can accumulate a large quantity of heavy metals ions from the contaminated water. Not only this, it is reviewed (Papagiannis et al., 2004) that fish can accumulate high concentration of heavy metal and can be used as an indicator to predict the water contamination in the environment. This paper analyses the eco-toxicity studies of heavy metal ions from leachate solution prepared from different concentration of sewage sludge, and optimised dose FA/SS (4:1) on Poecilia reticulate (guppy fish) species.

II. MATERIALS AND METHODS

The sewage sludge sample was collected from the Wastewater Sewage Treatment Plant Okhla, New Delhi, (India). The collected sewage sludge sample was taken from the sludge drying beds and was further tested for the heavy metal contents in the laboratory of Thapar University, Patiala (India). At the laboratory, sewage sludge sample was crushed, homogenized by a high-speed blender, sieved and dried in an oven at 120ºC for ten hours.

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Different standard solutions for metals including Pb, Cd, Ni and Cu and Zn were prepared by the analytical grade salt to analyse the heavy metal content with the help of atomic absorption spectrophotometer. The complete description of heavy metal concentrations in the digested sample of sewage sludge are shown in (Table 3). The fly ash sample was collected from the Thermal Power Plant, Panipat. (India) and was oven dried at 120ºC for ten hours. At the laboratory, the Physical and Chemical analysis of fly ash sample shown in (Table 1 and 2) was carried out in accordance with ASTM Standard C618, which is one of the most widely and internationally used methods for physical and chemical analysis of fly ash.

TABLE I
Physical Analysis of Fly Ash Samples

PARAMETER	VALUE
SPECIFIC GRAVITY (OD)	1.705±0.021
SPECIFIC GRAVITY	1.785 ±0.011 G/CC
DENSITY	1701 ±2 KG/M3
PERCENT ABSORPTION	4.712 ±0.054%
FINENESS MODULUS	7.522±0.23
PH (RAW FLY ASH SAMPLE)	7.5±0.02
ORGANIC CARBON	0.23±0.01

Source: (Kharub et al., 2012)

Table 2
Chemical Composition of Fly Ash

Constituent	Weight (%)
SiO_2	55.78
Al_2O_3	55.78
Fe_2O_3	9.65
Cao	3.25
SO_3	0.15
Na_2O	0.39

Source: (Kharub et al., 2012)

Table 3
Metal concentration in composite sewage sludge samples by atomic absorption spectrophotometer

S. No	Metals	Mean ±SE (mg/kg) Values
1	Fe	14284±0.025
2	Mn	200 ±0.04
3	Cu	132 ±0.03
4	Zn	1909 ±0.02
5	Cd	2.75 ±0.015
6	Co	ND
7	Ni	35.1 ±0.025

Source: (Kharub et al., 2012)

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III. ANALYTICAL METHOD

Leachate solution prepared from various concentrations of 5, 10, 15 and 20% (W/V) sewage sludge and the selected optimised 4:1 FA/SS mixture were used to check the eco-toxicity studies. Three different age groups of (60, 90 and 120 days) of fish species were taken for the eco-toxicity test. The survival rate was checked after 96-hour time period. The different concentration of metal accumulation in the fish species was checked after the digestion. The fishes were dissected into smaller pieces, put in petri dish oven-dried at 120ºC for 24 hours' time period until a constant weight is achieved. When the samples were confirmed completely dry, they were then ground to fine powder with the help of pestle and mortar and stored in air-tight plastic containers. After this process, dissected pieces of fishes were put in digestion flasks. The digestion of fishes was done with ultrapure HNO_3 and H_2O_2 in (1:1 v/v). The digestion flasks were heated to 130ºC on a hot plate until complete dissolution and further diluted with the help of distilled water. After this, the samples were analysed for different heavy metal ions (Pb, Cd, Ni, Cu and Zn) with the help of atomic absorption spectrophotometer (ECIL, India, Model AAS 4129).

IV. RESULTS AND DISCUSSIONS

A. Eco-toxicity Studies

The eco-toxicity studies of heavy metal ions were carried out on Poecilia reticulate (Guppy fish). The fish species were purchased from fish aquarium Sheravali gate, Patiala and were acclimated to the laboratory conditions for 7 days. However, the fish were not fed during the last 48 h of adaptations and throughout the toxicity test duration of 96 h for each metal to be analyzed. The toxicity tests with three age groups of fish, viz. 60, 90 and 120-day-old fish, were conducted in glass aquaria at room temperature conditions (26 ±1°C). Ten fishes per glass chamber of 5 L water capacity were added in a control sample (without leachate solution), in leachate solution prepared from different concentrations (5, 10, 15 and 20%) of sewage sludge and optimised 4:1 FA/SS mixture. The total mean values in terms of weight and total length of different age group of fishes are shown in (Table 4). The bio-available concentrations of different heavy metal ions in leachate solution of sewage sludge (5, 10, 15 and 20%) weight by volume are shown in (Table 5). The Poecilia reticulate (guppy fish) for 60 days was kept in the leachate solution of the optimised dose FA/SS mixture (4:1) for 96 hours, and the metal concentration in the fishes were observed in the leachate solution of sewage sludge (5%, 10%, 15 % and 20%). The accumulation for Pb-metal observed was 2.36µg/g with 5% leachate, 5.13 µg/g with 10%, 6.72 µg/g with 15% and 13.14µg/g with 20% leachate which increased in 120 day old fish to 26.10 µg/g with 5% leachate, 38.23 µg/g with 10% leachate, 54.14 µg/g with 15% and 79.65 µg/g with 20% leachate solution, respectively as shown in (Table 6). The development of metal toxicity involves the initial binding of metals, followed by the internal partitioning of metal between detoxified and metabolically active forms (Luoma and Rainbow, 2005). A lesser accumulation for Cd-metal was observed which was 0.4 µg/g with 5% leachate, 0.98 µg/g with 10% leachate, 1.05 µg/g with 15% leachate, 2.3 µg/g with 20% leachate solution among 60-day old guppy fish as shown in (Table 6). In 90 day old fish it was 2.90 µg/g with 5% leachate, 4.24 µg/g with 10% leachate, 7.14 µg/g with 5% leachate, 9.81µg/g with 20% leachate which increased to 9.68 µg/g with 5% leachate, 16.2 µg/g with 10% leachate, 22.24 µg/g with 15% leachate, 39.18 µg/g with 20% leachate solution in 120 day old fish. Cd is biologically non-essential, non-biodegradable, and its compounds have the potential to cause toxicity to the fish. The continuous exposure to Cd at low level may cause significant impacts on biological processes in fish (Karlsson-Norrgran and Runn, 1985). It has been reported that the toxicity level of a fish depends on various factors such as the fishes which are exposed, their genetics, age and the development stage of the fish species (Stoskus et al., 1999). The 60-day old fish were significantly more sensitive to the toxicity of water-borne Cd and Co, followed by that of the 90 and 120-day age groups. Therefore, the sensitivity of fish towards various metals decreased with age due to their ability to concentrate heavy metals that exerted significant impact on the tolerance limits of fish (Giguere et al., 2004). It has been observed from the experimental results that out of the five selected heavy metal ions (Pb, Cd, Ni, Cu and Zn) the maximum accumulation of the Zn metal- ion was observed which was 0.98 µg/g initially in the control sample which increased with increased percentage of the leachate solution that is 92 µg/g with 5% leachate, 159 µg/g with 10% leachate, 197 µg/g with 15% leachate , 270 µg/g with 5% leachate in 90 day old fish which was increased to 160 µg/g with 5% leachate, 236 µg/g with 10% leachate, 342 µg/g with 15% leachate, 378 µg/g with 20% leachate solution (Monika, 2013). It has been reported by (Murugan et al., 2008) that bioaccumulation is the total metal present inside the fish species in various organs/tissues. The bioaccumulation of different metals Cr, Ni and Mn were reported in different tissues of cyprinid fish (L. ambratus) (Nussey et al., 2000). The main point of entry of heavy metal in fish is through the gill, which further aggravates lesions and gill damage as reported by (Bols et al., 2001), whereas, the detoxification process is carried out by the liver and the kidney (Vinodhini and Narayanan, 2008).

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Table 4 - Total weight and length values of Poecilia reticulate (Guppy fish) species with three age groups (60 day, 90 day, 120 day) Used for the eco-toxicity test

Age group	Fish Species	Total weight of oven dried Fish Sample(mg)	Total length of Fish (mm)
60 day	Poecilia reticulate (Guppy fish)	40	19
90 day	Poecilia reticulate (Guppy fish)	60	22
120 day	Poecilia reticulate (Guppy fish)	120	25

Table 5 - Bio-available [1]concentrations of metals present in the sewage sludge leachate samples

Sewage Sludge concentrations (W/V)[2]	Metal Concentration in mg/L				
	Pb (mg/L)	Cd (mg/L)	Ni (mg/L)	Cu (mg/L)	Zn (mg/L)
5%	1.75	0.15	1.21	3.27	47.7
10%	3.89	0.45	2.42	6.54	95.4
15%	5.25	0.52	3.63	9.84	143.1
20%	7	0.69	4.84	13.08	190.8

Table 6- Accumulation of different metal ions in Poecilia reticulate (guppy fish) with different age groups during 96 h exposures

Guppy fish Age (days)	Leachate Source W/V	No. of fish exposed	Pb(µg/g) Dry Fish weight	Cd(µg/g) Dry Fish weight	Ni(µg/g) Dry Fish weight	Cu(µg/g) Dry Fish weight	Zn(µg/g) Dry Fish weight	No of fish died
60 days	Control[3]	10	0.56	0.001	0.64	0.95	0.98	0
	4:1 FA/SS	10	0.53	0.003	0.67	0.92	1.02	0
	5%	10	2.36	0.4	4.06	8.02	92	7
	10%	10	5.13	0.98	9.10	20.38	159	9
	15%	10	6.72	1.05	24.04	30	197	10
	20%	10	13.14	2.3	40.44	50.42	270	10
90 days	Control	10	0.76	0.004	0.94	1.90	2.08	0
	4:1 FA/SS	10	0.78	0.006	0.99	1.89	2.01	0
	5%	10	6.56	2.90	14.06	18.06	128	8
	10%	10	11.14	4.24	26.05	36.68	198	9
	15%	10	21.68	7.14	34.21	42	265	10
	20%	10	39.24	9.81	51.16	61.12	388	10
120 days	Control	10	0.94	0.009	1.52	2.01	1.95	0
	4:1 FA/SS	10	0.96	0.011	1.45	2.05	1.99	0
	5%	10	26.10	9.68	23.04	35.01	160	7
	10%	10	38.23	16.2	29.23	46.24	236	9
	15%	10	54.14	22.24	46.52	60	342	10
	20%	10	79.65	39.18	65.67	79.23	378	10

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[1]The fish's were dissected in to smaller pieces, oven-dried at 120ºC for 24 hours' time period until a constant weight is achieved. The digestions of fish's were done with ultrapure HNO_3 and H_2O_2 in (1:1 v/v) samples were digested until complete dissolution. The samples were analysed for different heavy metal ions (Pb, Cd, Ni, Cu and Zn) with AAS (ECIL, India, Model AAS 4129).

[2]These are the weight by volume concentrations of different volumes of sewage sludge used in different percentages mixed with distilled water and the leachate have the above listed metal concentration in them.

[4] Control – The water sample without fly ash and sewage sludge mixture.

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V. CONCLUSIONS

The experimental findings of the present study concluded that no death of fishes was observed after 60, 90,120 days, in control samples and the samples with optimised dose of leachate solution (FA/SS, 4: 1). It confirmed that the leachate solution with optimum dose (FA/SS, 4:1) is the best dose and will not cause eco-toxicity to the fishes. The samples with leachate solution of different doses of sewage sludge (5%, 10%, 15% and 20%) showed increased death rate due to heavy metal toxicity after 96 hours of exposure on different days (60, 90 and 120 days). It further confirmed that leaching of heavy metal ions increases with increase in content of sewage sludge in the leachate solution, which poses toxicity to guppy fish and hence death of fishes. The experimental results showed that leachate solution with optimised dose of fly ash and sewage sludge (4:1) was safe in respect of eco-toxicity effects. So, it is concluded that the disposal of sewage sludge without its stabilization can be dangerous to the environment and to the aquatic biota. For the proper and safe disposal of sewage sludge, firstly, it is recommended to properly treat sewage sludge. After treatment, it should be stabilized with the proper amount of fly ash depending upon the characteristics of sewage sludge in order to reduce the hazardous effect of various heavy metal ions present in sewage sludge.

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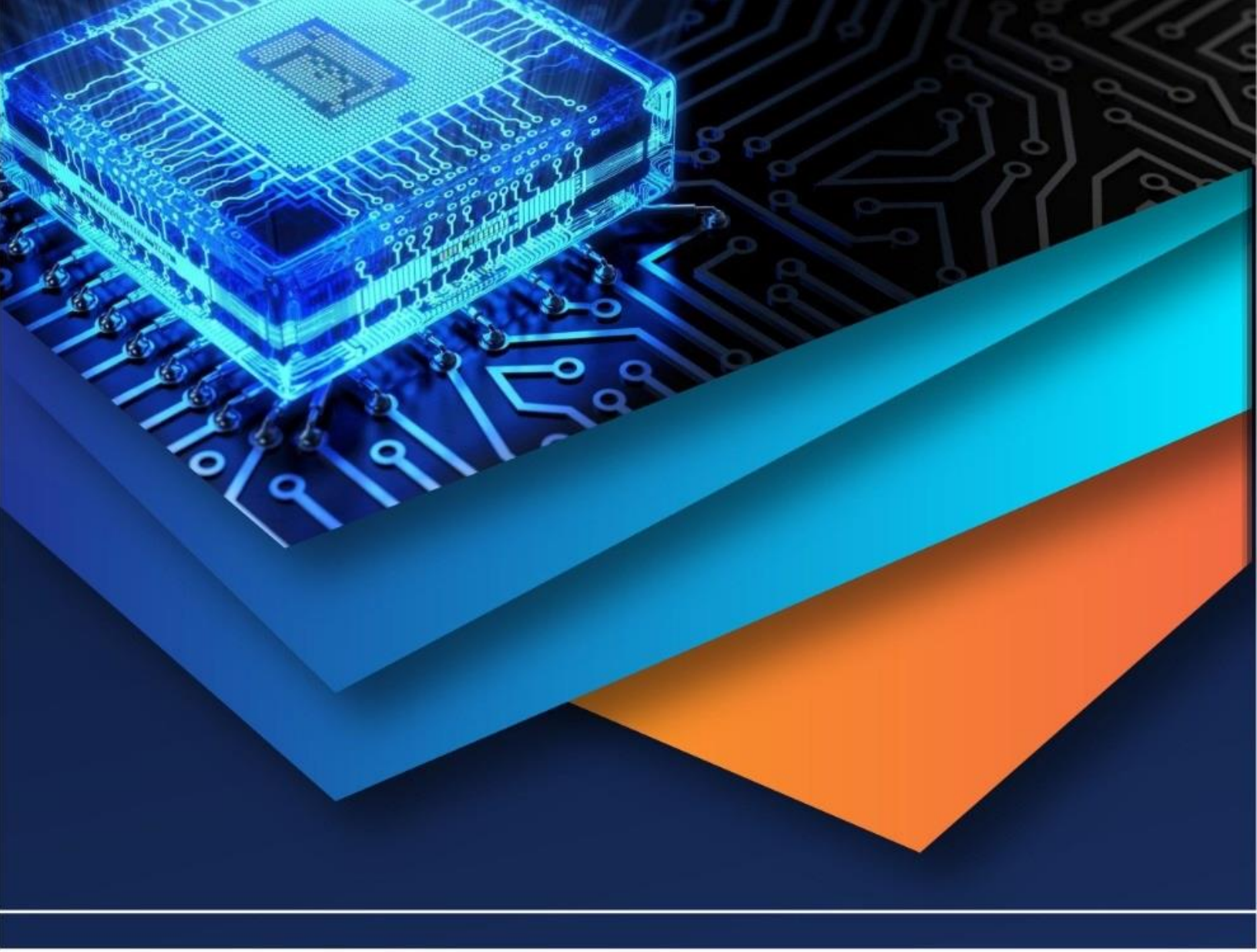

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