

Evaluation of toxic levels of fly ash in two of the fresh water fishes *Channa punctatus* and *Puntius sophora*

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Abstract

Present investigation deals with the assessment of toxic levels effects of fly ash for fishes, *Channa punctatus* and *Puntius sophore* which were determined under controlled laboratory conditions with pH maintained at 7.25 and temperature 25-30°C. During this study fishes were exposed to different concentrations by dissolving fly ash in gL of water. Fishes were exposed to different concentrations of fly ash for 96 hr LC50 determination but fish mortalities were recorded and monitored simultaneously for 24hr, 48hr, 72hr and ultimately at 96hrs of exposure with three replicates for both species per concentration. The toxic levels and 96hrs LC50 values for both fishes were calculated using finney's probit analysis method using 95% confident interval. 96hr LC50 for *Channa punctatus* recorded 5.75g/L and LC50 value for 96hrs for *Puntius sophore* was found to be 15.48g/L. *Puntius sophore* were found to be more tolerant to fly ash in comparison to *Channa punctatus*.

Key words:- Fly ash, 96hrs, LC50, toxic levels, Channa punctatus, Puntius sophore,

1. Introduction

Since times natural resources have been used beyond the level significantly being depleted, over used for the purpose of development. Among these natural resources coal is among considerably important natural resources which are used in the thermal power plants for production of electricity. Development in present, past and future as well is based on industrialization and urbanization which requires the backbone of electricity. In our state more than 11 thermal power plants produces electricity and utilizes coal as fuel which in turn produces fly ash in enormous quantities as by product. Fly ash is produced to about 26880 metric tonnes per day i.e. about 9.7 million tonnes of fly ash is produced

annually in Chhattisgarh state, out of which in Korba district alone four major thermal power plants produces 24000 metric tonnes of fly ash per day i.e. 8.7 million tonnes of fly ash produced annually (Fly ash utilization information centre, Korba). Production of fly ash in such voluminous quantities is itself a problem regarding its handling as it requires continuous and more over expensive efforts to deal with it. Fly ash also because of its physical properties such as being composed of minute powder like particles which are weightless possesses potential to cause environmental pollution such air, water and land pollution. Although depletion of this natural resource is itself a matter of concern for sustainable development and more over its combustion produces fly ash which is responsible for deterioration of environment due to the processes involved during its production. Fly ash produced is dumped into landfills, ash ponds and ash embankments for its disposal. Although government have implemented rules which are by laws enforces towards maximum utilization of fly ash in most of the government sanctioned projects which are also strategies for tackling with the problem of fly ash and its management (Parisara, State environment related issues). Fly ash causes air pollution which causes health hazards to organisms causing respiratory disorders have already been reported. Fly ash leakage and its spillage to the ground water causes deterioration of ground water quality as it consist of various metals.

Fly ash dumping sites have been monitored for assessment of its effect on organisms inhabiting fly ash dumping sites (Shrivastava, et. al., 2012) Water is also used for the disposal of fly ash by making ash ponds which not only unnecessarily acquires several acre of land but water being valuable resources for mankind, fly ash mixing to water also deteriorates and alters its quality which again is directly linked to

the welfare of organism in our ecosystem. (Shrivastava, et.al., 2014) Fly ash consists of various heavy metals such as molybdenum, mercury, selenium and cadmium etc. reported. Fly ash also needs attention as it possesses properties of sedimentation reported(Yang and Rose, 2003). There are wide array of heavy metals which have been reported to be harmful in degrading environment and posing serious threats to the terrestrial as well as aquatic flora and fauna on being continuous exposed to them (Shariff, 2001; Farombi, et.al., 2007) among these metals most of them are also present in fly ash so, it also need to be monitored for its role on aquatic organisms exposed to them. For study of toxic effects of compounds fishes are considered as the first choice for assessment of effects of substances because they are the survivors of water remaining in contact with all sorts of substances present in water, so there are more chances of being affected from mixing of influx of metals in water. All substances are poisonous. It is amount of any particular given substance that differentiates a poison from a remedy (Ausley, 2000).

In ecotoxicological research basically the attention is focussed towards the aquatic environment because it is the final destination, the receiving body for almost all sources of wastes produced in the environment and discharged.(O' Halloran et.al., 1998; Canli, 1999;). In ecotoxicological researches the use of vertebrates and organisms to detect the toxic effects of a particular pollutant is also providing precious information about the hazardous affects of particular pollutant under study that if could impose which also be related to the other species (Pritchard, 1993). Earlier these techniques were employed to protect to protect the aquatic environment from the overexploiting activities of humans either by reducing the disposal of wastes into water bodies and also other sources of pollution caused by man so that the pollutants concentration might be reduced which in turn causes reduction in oxygen contents dissolved in water (Hodson, et.al., 1984;; Norwood,2003; Alexander, 2008; Mansour, 2002). Later more attention is required to draw because these pollutants interacting with other substances in the aqueous medium can bring enhanced toxic effects and cause damages (Blum&Speece 1990; Ramade, 1987). In these types of toxicology studies it becomes utmost important to analyse and understand the concentration at which the presence of a particular substance under study can substantially cause detrimental effects to the organisms on being exposed for continual and long period of time and at the same time to understand the pattern it is undergoing bioaccumulation and biomagnifications(Vander Oost, et.al., 2003; Papagiannise, et. al., 2004) fly ash have been reported to cause alterations in the physicochemical parameters of water after getting

mixed to the water bodies(Shrivastava, et. al., 2012). It is also a matter of fact that some of the elements are essential if it is present in trace amounts, but on other hand their presence in large quantities can also become highly toxic if it reaches to higher levels can cause several physiological, behavioural alterations(Mc.Geer, et.al., 2000; Monteiro, et. al., 2005; Wagh and Khalid, 1985; Yacoob and Javed, 2012). In this beacon fly ash also needs to be monitored in toxicity studies it is considered to conduct acute toxicity tests for bio monitoring the health of organisms. These researches are conducted for various time periods by monitoring the mortality in organisms. These acute toxicity tests can be used in biomonitoring the aquatic ecosystem and also determining safe guards as these tests calculate the toxic levels by providing data of amounts at which they can be considered as threatless(Mohapatra and Rengarajan, 1997). In this context present work evaluates the toxic levels of fly to fresh water fishes *Channa punctatus* and *Puntius sophore*.

2. Material and Method

Fly ash was mixed with water to prepare solution to obtain desired concentration. Initially pilot experiments were conducted to select and obtain the concentration at which the fishes were killed. Similar ranges were selected to acquire 11 different concentrations for each tests and ten fishes per test solutions i.e. n=10 no. of fishes per test solution were introduced in containers. Fresh water fish *Channa punctatus* of height ranging between 10 to 12 cm and weight 10.2 – 12.2gms and *Puntius sophore* selected of height 9 to 11cms and weight 9.5- 11.5 gms were chosen to be used as test fishes in study. The fishes were brought from market place and brought to laboratory in live conditions where fishes were acclimatized to the laboratory conditions for 12 days at room temperature of 30°C. During acclimatization fishes were fed with fish food. Feeding to the fishes was stopped before 24 hrs prior to the commencement of the experimentation. During the entire period of experimentation after every 24hrs, 48hrs, 72hrs and 96hrs of time period mortality in the no. of fishes per test solution from both the species in live and dead conditions were noted (Sprague, 1969).

3. Observation

The objective of present investigation is to have data for the range of concentration of fly ash producing adverse effect in test species of fishes and to have the range of time period of exposure, so that the toxicity of fly ash could be established on the basis of dose, responses relationship(Rand and Petrocelli, 1985; Moore and Ramamoorthy, 1984). Dosage of fly ash were provided to fish species under experimentation. It was observed during entire experimentation period

that due to the successive or gradual increase in the concentration of fly ash the rate of mortality in fishes were increased. The mortality percentage were noted and were calculated. The data obtained were converted to probits according to (Finney probit analysis method, 1971). The observed calculations of LC50 values for 95% confidence limits for fly ash in fish species *Channa punctatus* at regular time intervals of 24hrs, 48hrs, 72hrs and 96hrs are given in Table(II) and for fish species *Puntius sophore* were given in (Table IV).

4. Result and Discussion

Concentration of fly ash and Percentage of mortality for *Channa punctatus*

Table (I)

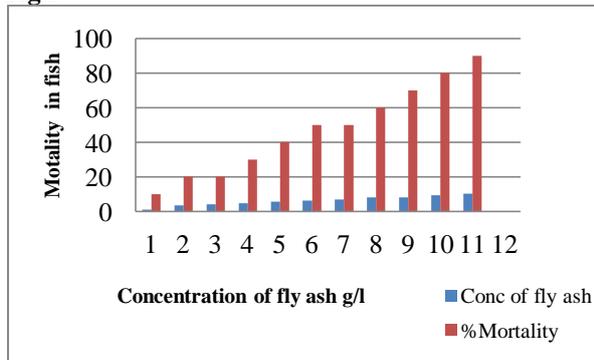
24Hrs values			48Hrs values			72Hrs values			96Hrs values		
Conc. of fly-ash (g/L)	Log conc.	Percentage of fish mortality	Conc. of Fly ash g/L	Log. Conc.	Percentage of fish mortality	Conc. of Fly ash mg/L	Log. Conc	Percentage of fish mortality	Conc. of fly ash mg/L	Log. Conc.	Percentage of fish mortality
1.09		10	1.09		10	1.09		10	1.09		10
3.36		10	3.36		10	3.36		10	3.36		20
4.35		10	3.35		20	4.35		20	4.35		20
4.68		20	4.68		20	4.68		20	4.68		30
5.69		20	5.69		20	5.69		30	5.69		40
6.25		30	6.25		30	6.25		40	6.25		50
6.97		30	6.97		30	6.97		50	6.97		50
7.98		30	7.98		40	7.98		60	7.98		60
8.09		40	8.09		50	8.09		70	8.09		70
9.39		50	9.39		60	9.39		80	9.39		80
10.36		60	10.36		70	10.36		90	10.36		90

Table(II) LC50 values of fly ash concentration in *Channa punctatus*

S.No.	Duration	LC50 values
1	24 Hrs	16.98
2	48Hrs	12.022
3	72Hrs	9.33
4	96Hrs	5.75

Concentration of fly ash and Mortality in fish *Channa punctatus*

Fig I



Concentration of fly ash and Percentage of mortality for fish *Puntius sophore*

Table (III)

24Hrs values			48Hrs values			72Hrs values			96Hrs values		
Conc. of fly-ash	Log conc.	Percentage of fish mortality	Conc. of Fly ash (g/L)	Log. Conc.	Percentage of fish mortality	Conc. of Fly ash (g/L)	Log.conc	Percentage of fish mortality	Conc. of fly ash (g/L)	Log. Conc.	Percentage of fish mortality
10.50		10	10.50		10	10.50		10	10.50		10
12.50		10	12.50		10	12.50		10	12.50		20
13.36		10	13.36		20	13.36		20	13.36		30
14.14		20	14.14		20	14.14		20	14.14		30
14.50		20	14.50		20	14.50		30	14.50		40
15.25		30	15.25		30	15.25		40	15.25		40
16.72		30	16.72		30	16.72		50	16.72		50
17.50		40	17.50		40	17.50		60	17.50		60
18.45		40	18.45		50	18.45		70	18.45		70
19.14		50	19.14		60	19.14		80	19.14		80
20.60		60	20.60		70	20.60		90	20.60		90

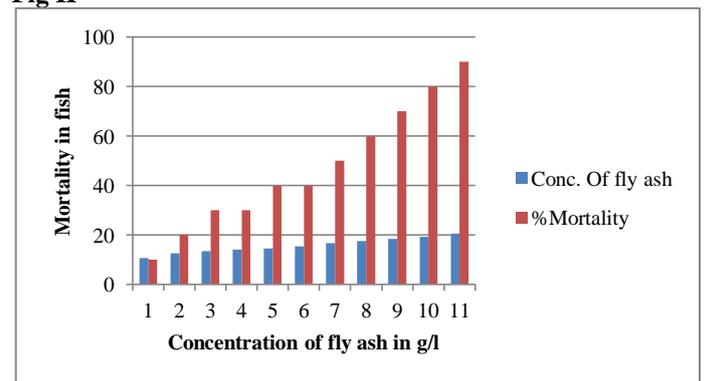
Table(IV)

LC50 values of fly ash concentration in *Puntius sophore*

S.No.	Duration	LC50 values
1	24 Hrs	19.49
2	48Hrs	18.19
3	72Hrs	16.59
4	96Hrs	15.48

Concentration of fly ash and Mortality in fish *Puntius sophore*

Fig II



Now a day all over the world each countries are globally due to various sources like anthropogenic activities, leaching, mining, sewage disposal, disposal of industrial wastes are responsible for influx if wide array of pollutants in aquatic environment. It is also a known fact that presence of metals as pollutants are very dangerous to the extent

that although in very trace quantities but due to their presence for long time and in continuity have proven to cause harmful effects to the aquatic organisms. Wide literatures are available on research reporting wide array of substances metals discharged in water causes alterations in biological, physiological, behavioural affecting fish species at tissue, organs at organismic level as whole (Mazon and Fernandes, 1999; Yousouf and Shahabi, 1999; Mansour and siddiky, 2002 ; Sehar et. al., 2014; Mahboob et. al., 2014; Shahid et. al.,2016). Most of these metals due to the complexities they possesses in their molecular nature and due to the pattern of their interaction they possess harm to the inhabitants of aqueous environment. The concentration of the contaminants in the water bodies adversely affects the survival of the aquatic organisms. As reported by (Jones and Reynolds, 1997) substances at high concentration when it reaches upper limit it may cause deleterious effects leading to the death of the inhabitants, where death is the ultimate result to be taken under consideration in toxicity studies. In toxicological studies the LC50 determination has been proved to be an extremely useful tool in determination of a particular toxicant. Most of these studies are based on determination of lethal concentration of a substance during short term study or acute toxicity test. Although there are vast literatures are available on the study of toxicity tests of heavy metals. But almost very least or insufficient data are available on the study of effects of fly ash on fish. The available data are insufficient to derive appropriate understanding of effect of fly ash or damage caused by stimulus provided by fly ash concentration to the responses of aquatic organisms. So it is an important field to assess the effects of fly ash in order to have appropriate knowledge from observing the pattern of stimulus provided by fly ash to the fish. So that effective measures can be implemented to combat this environmental pollutant.

It has been observed during the entire period of study that on continued exposure to fly ash in fishes oxygen consumption increased and they become less active showing escape to the environment a kind of stressed behaviour. It has been reported by (Rafia and Devi, 1995) that there increase in oxygen consumption in fish *Mystix gulio* due to presence of copper and zinc in different concentration. Altered oxygen consumption and stressed tupe of behaviour also observed by (Shreena and Logaswamy, 2008).

LC 50 values for various chemical substances specially for heavy metals have been estimated and literatures are available on LC50 values for various substances such as malathion has been recorded 6.61ppm as per standards (Shastry and Sachdev, 1994). It has been described general toxic effect (Alderdice, 1967) to the acute toxicity in which maximum dose of substance is given for short period

of time termed as acute toxicity, such mechanisms show clear absolute damage and small doses of substance is given for long period of time for analysing chronic toxicity. Acute toxicity is generally lethal where as chronic toxicity of two types lethal and sub lethal. LC 50 is the term which is applied to concentration of a substance administered to test organism at which half of the i.e. 50% of the animal survived. No. Of literatures are available on investigation performed on chemicals, pesticides to see the harmful effects on aquatic organisms. In our present study we have applied the term short term or acute toxicity study and long term or chronic toxicity study for convenience. Acute toxicity determined (Leblond and Hontella, 1999) of metals mercury, zinc and cadmium in rainbow trout fishes and reported fishes to be more susceptible to mercury followed by zinc and cadmium. (Chinni and Yallapragada, 2000) performed acute toxicity tests with metals (Pb, Zn, Cd and Co) carried on *penaeus indicus* post larvae and found LC50 values for copper, cadmium, zinc and lead were 2.535, 3.119, 6.223 and 7.223 mg/L^{-1} respectively. Acute toxicity test performed individually or in combination being tested on different species of fishes to assess the LC50 values of Cd, Pb and Zn on fish *Mugil cephalus* by (Rajkumar, 2011) found these heavy metals to be toxic.

Fish mortality due to presence of various harmful substances in aquatic ecosystem such metals, pesticides mainly depend on the sensitivity of the fish species to the toxicants, its time period of exposure and the concentration of toxicants. The determination of LC50 concentration of a particular substance is important before any further studies to be performed on physiological alteration in animals. In present study fish *Channa punctatus* exposed to fly ash and the toxic level were expressed in terms of LC50 value. The LC50 values evaluated for *Channa punctatus* were found to be 16.98, 12.02, 9.3 and 5.75g/l at for time period of 24, 48, 72 and 96hrs respectively showed in table II . Toxicity experiements performed where fish *Puntius sophore* exposed to fly ash and toxic levels were evaluated as shown in table IV 19.49, 18.19, 16.59 and 15.48 for duration of 24, 48, 72 and 96hrs respectively. The percentage of survival of fish observed decreasing with the increasing concentration and time period of exposure. Present study shows relationship between the time period of exposure and concentration of fly ash. Numerous studies had been performed to assess toxicity of various heavy metals and trace elements upon different species of fishes. Similar study conducted by (Rajkumar, 2011) on *Mugil cephalus* exposed to metals such as Cd, Pb and Zn and found copper to be more toxic than other metals. (Naji, et. al., reported 96hr LC50 of cobalt as 327.5mg L^{-1} . (Yacoob and Javed, 2012) reported major Indian carps like *Labeo rohita*, *Cirrhinus mrigala* and *Catla*

catla to be more sensitive to cobalt than cadmium. Although very less or almost no data are available on toxic level of concentration of fly ash to fish. Directly or indirectly many of these metals like Zn, Cu, Pb and Cd etc and many other metals reported to present in fly ash as their constituents (Rohatgi, et.al., 1995; Sushil and Batra, 2006). In present study *Channa punctatus* were found more sensitive to increased concentration of fly ash where as *Puntius sophore* were quite tolerant in comparison to *Channa punctatus*. It is also clear from earlier studies that LC50 value for various pollutants to fresh water species varies from species to species and are also dependent and influenced by number of factors such as size, time period of exposure etc. the responses were observed on increasing concentration of dose and also on increased period of exposure such that responses arise at threshold dose. Present investigation table I indicates fly ash concentrations and mortality percentage of fish *Channa punctatus* for time periods 24, 48, 72 and 96hrs. Present work table III shows doses of fly ash to which fishes *Puntius sophore* exposed for time intervals 24, 48, 72 and 96hrs. These responses were reported on the basis of dose response relationship concept. (Malik, et.al., 2012; Pathan, et.al., 2009). In present study Fig. I indicates increased mortality in fish species *Channa punctatus* with increased dose of fly ash. Fig.II shows increased number of fish death due to increased concentration of fly ash in *Puntius sophore*.

Toxicity test performed on fishes *Labeo rohita*, *Cirrhinus mrigala* and *Catla catla* on exposure to metals such as Cadmium and cobalt was found to be toxic to these major Indian carps (Yacob and Javed, 2012).

96hrs LC50 values for copper, cobalt on fish *Cyprinus carpio* (Naji et.al., 2007) recorded to be 327.5mgL^{-1} . Similar results were recorded by (Gundogdee, 2008) on fish *Onchorynchus mykiss* determines 96hrs LC50 values for zinc and copper ions and other metals to be toxic for fish were found more sensitive towards metals. A lot more data on LC50 values, lethal concentration are available as numerous studies have been performed on heavy metals toxicity to aquatic organism especially on different species of fish, but as data is either least available or insufficient data is available on fly ash. On exposure to fly ash altered and disturbed behavioural responses observed in *Channa punctatus* and *Puntius sophore*. Similar observations reported in fishes *Rasbora daniconius* and *Barbus ticto* were reported 0.35 and 0.175ppm respectively conducted in laboratory experiment reported by (Wagh and Khalid, 1985). (Shah, 2002) reported fishes showing abnormal disrupted behaviour such as frequently jumping towards the surface, jerky body movement lose of balance coming to the surface for engulfing air.

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