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Toxicity Assay on Platinum Nano Particles to Fresh Water Fish "*Cirrhinus Mrigala*"

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Abstract: ***In the present study platinum nanoparticles were exposed to freshwater fish Cirrhinusmrigala and the toxicity(acute and sublethal) assay were investigated. The Lethal concentration value were noted. The median lethal concentration (LC 50) of platinum to an Indian major carp Cirrhinusmrigala for 24 hours and 96 hours were found to be 12 mg/L and 6 mg/L. The main toxic action of platinum on aquatic animals during acute and sublethal toxicity leads to mortality due to metal accumulation in fishes through the gills. During acute treatment of platinum, the fish Cirrhinusmrigala showed behavioral changes such as loss of balance, restlessness, abnormal swimming. During sublethal treatment of platinum, the fish Cirrhinusmrigalashowed the changes such as the gulping of air, opercular movement, erratic jumping.***

Keywords: Platinum nanoparticles, fish Cirrhinusmrigala, Bioassay(acute and sublethal).

1. Introduction

Nanotechnology is a rapidly growing science which deals with structures with at least one dimension of the size of one hundred nanometres and involves producing materials and devices of that size. Among these nanomaterials, nanoparticles (NPs) are now playing a crucial role in the field of nanotechnology. (Houška*et al*., 2008, 2009a, b). There is a wide array of fascinating nanoparticulate technologies capable of targeting different cells and extracellular elements in the body to deliver drugs, genetic materials, and diagnostic agents specifically to these locations (Zhang *et al*., 2006, Bhattacharya, 2007).While the commercialization of nanoparticles is rapidly expanding, their health and environmental impact is not well understood. Toxicity assays of platinum nanoparticles, were recorded in terms of mortality. Among the nanoparticles Pt NPs plays an important role in the toxic effects exhibited by the fishes as a consequence of nanoparticle exposure, accompanied by the metals inside the body (Asharani, *et al*., 2010).

Platinum are widely used in automobile catalytic converters and emitted into the environment and enter the aquatic ecosystem via runoff rainwater. Platinum (Pt) is a malleable, silvery white, noble metal widely but sparingly distributed over the earth's crust. It is found mainly as the isotopes with atomic weights of 194, 195 and 196, with a maximum oxidation state of +6, the oxidation states of +2 and +4 being the most stable. This platinum enters through water and causes pollution. Water pollution monitoring becomes a crucial problem as more and more contaminants enter the aquatic environment every year. The current trend is prediction of the toxicity level using various measurable attributes of the aquatic environment for bio monitoring (Pace, 2001). Pollution from toxic chemicals and their waste generates concern, because they effect human health, environment (water resources, air quality, and soil), ecology, (Pandey *et al.,* 2005; *Lee et al.,* 2009). Although aquatic ecosystems are equipped with a variety of physico-chemical and biological mechanism to eliminate or reduce adverse effects of toxic substances, toxicants may evoke changes in developments, growth, reproduction and behavior or may cause death of freshwater organisms (Offem and Ayotunde, 2008). A great deal of research has therefore been conducted to understand the effects of toxicants on the physiology of aquatic organisms especially in fish (Wood, 2001).

Toxicological studies of the pollutants upon aquatic organisms are very important from the point of environmental consequences (Krishnani*et al.,* 2003). Many contaminants originating from direct discharges or from hydrologic and atmospheric processes are ultimately deposited in ecosystems (Lamas *et* al., 2007). As direct analysis of such substances does not provide information about their effect on the ecosystem, the use of biomonitors is a highly recommended option because biomonitors respond specifically to the bioavailable pollutant loads (Ruelas-Inzunza and Paez-Osuna, 2000).To do so, the development of different biomarkers to investigate the in vivo effects of contaminants is a priority requirement to reveal the action mechanisms of toxicants (Oliveira Ribeiro *et al.,* 2006). Aquatic toxicology generally involves the measurement of contaminant levels to characterize the hazards imposed on the aquatic environment; however, this field of study includes information on how those contaminants can affect humans in and around these aquatic environments. Aquatic toxicity of xenobiotics often can be very helpful impredicting and preventing acute damage to aquatic life in receiving water as well as regulating toxic wastes discharges (Vutukuru*et al.,* 2005).Since fish are the chief source of protein to the people of India, in the present study Cirrhinusmrigala is used as an experimental animal for testing the toxicity of platinum in fresh water.

Mrigal, being a bottom feeder, and is more prone to the stress effect of toxic Platinum. To our knowledge, only limited data are available on the effects of acute and sublethal exposure of Platinum toxicity to major carps particularly on *Cirrhinusmrigala.* Information on the physiological response of *Cirrhinusmrigala*to acute and sublethal exposure of platinum toxicity will help for the better management. Hence in the present investigation an

attempt was made to study the impact of Platinum in fresh water fish Cirrhinusmrigala.

Toxicity monitoring is often used as a characterization tool to identify areas that are toxic and to provide a quantifiable measure of the observed toxicity. Toxicity is used to evaluate the concentration of chemical and the duration of exposure required to produce the criterion effect. However, toxicity tests can also be used to (1) assess long-term changes in pollutant loading to receiving streams, (2) demonstrate changes in spatial and temporal patterns in the biological quality of water that result from environmental remediation actions and improvements in treatment systems,(3) assess water quality in those areas where community sampling is not performed (e.g., springs and tributaries which may not have sufficient biota effectively monitor change), and (4) identify contaminants of concern when unexpected changes in water quality occur at sites where aquatic communities have stabilized. (Rand and Petrocelli, 1985).

Toxicity can be calculated by determining mortality rate after a fixed time as a function of increasing doses of the toxicant, different characteristic constants of the toxicant can be determined by using dose mortality test. Results of (acute) short-term test generally are expressed as (i) The percentage of organisms killed on immobilized in each test concentration and (ii) the LC 50 derived by observation (i.e. where 50% of the test organisms were cause in a concentration) interpolation on calculation (Parrish, 1985). The most important constant is the LC50 (median lethal concentration), which is the chemical value causing 50% mortality in the population being studied. According to Prague (1969) acute toxicity is measured as median lethal concentration (LC50) which kills 50 percent of test organisms in a fixed time. The LC 50 value is determined after 24 hours of exposure in tests of acute toxicity and in some cases after hours. As such, regulatory guidelines for aquatic pollutants in natural ecosystems have traditionally based on acute lethality test such as the 96hLC 50 (CCME., 1999: USEPA, 2001), although impacts on development, growth and reproduction have also been considered (Rand and Petrocelli,1985).

The effects of pollutants on a population can be better understood and predicted by studying the sublethal effects on an individual. It is therefore possible to use sublethal physiological responses to detect pollutant — caused disturbances at very early stage. Sublethal affects in fish allow us to define toxicity of the environment and understand the potential danger of pollutant imputs (Oliva *et al.*, 2009). Exposure of animals to sublethal levels of pollutant may inflict stress on the mechanism required for maintaining a healthy physiological state; these changes may result in physiological, biochemical and behavioral processes, which will show more accurate prediction of acceptable levels of pollutants in the environment (koeman and Strik, 1975; Waterpaugh and Beitinger, 1985).

Toxicity assay in platinum were determined during the acute and sublethal toxicity and the changes were noted in fish Cirrhinusmrigala. As it has been also observed in different fishes by many authors. Exposure of freshwater fish *Rainbow trout* exposed to colloidal silver nanoparticles during acute toxicity test showed sensitive changes, Yeo and Yoon, 2009. Silver nanoparticles to *Zebrafish* during acute and sublethal toxicity cause an increase in mortality (Asharani*et al*., 2010). *Rainbow trout* exposed to single walled carbon nanotubes (SWCNTs) during sublethal concentration showed decreased changes, Smith *et al*. (2007). Long-term chronic exposure of Pt compounds on human, affect the health in the body (KhaiwalRavindra, *et al*., 2004). Toxicity assays of platinum nanoparticles, using *Zebrafish* embryos to study their developmental and toxicity effects were carried out in Gold (Au-NP, 15-35nm), silver (Ag-NP, 5-35 nm) and platinum nanoparticles (Pt-NP, 3-10 nm) were synthesized using polyvinyl alcohol (PVA) as a capping agent. Toxicity was recorded in terms of mortality, acute and sublethal toxicity were observed and it was discussed. Decrease and increase in mortality were observed (Asharani*et al*., 2010). .In the present study exposure of metal platinum to fish *Cirrhinusmrigala* during acute and sublethal toxicity studies showed different changes such as loss of balance and erratic jumping, and it were discussed in the result and discussion part.

2. Materials and Methods

Healthy specimens of *Cirrhinusmrigala* were obtained from Tamilnadu Fisheries Development Corporation Limited, Aliyar, Tamilnadu, India. Fish of the same age and size which hatched from the same lot of eggs were collected, the age of the fish being 2 to 3 month old. They were safely brought to the laboratory in well packed polythene bags containing aerated water and stocked in a large cement (36'x 18'x 19') tanks. Fish were acclimatization for about 20 days before the commencement of the experiment. During the acclimatization period, fish were fed *ad libitum* with rice bran and ground nut oil cake in the form of dough once in daily. Water was replaced every 24h after feeding in order to maintain a healthy environment for the fish. This ensures sufficient oxygen supply for the fish and the environmental is devoid of any accumulated metabolic waste. The feeding was withheld for 24h before the commencement of the experiment and to keep the specimens more or less in the same metabolic state.

Recruitment of Fish for Experiment

After acclimatization, fish with an average length about 7.0-8.0cm and average weight of 5.0-6.0g were selected. The fish were introduced into glass aquarium (26'×18'×18.5'cm) which was washed thoroughly and maintained in the laboratory. Fish belonging to both sexes were used. These fish served as the stock for experiment schedule.

Analysis of Water Quality

Tap water free from chlorine was used for the present study. The hydro- biological features such as temperatures, pH, salinity, dissolved oxygen, total alkalinity, and total hardness were estimated for each set of experiment as these factors have a significant influence on the biodegradability and toxicity of pollutants. Temperature was determined by using a thermometer and pH value by pen type ph meter (pH scan, Intec cybernetics Pvt. Ltd, Singapore).The salinity of water

was determined by Mohr's method using potassium chromate as an indicator. Dissolved oxygen was estimated by Winkler's method using starch as an indicator. Total alkalinity was determined by using methyl orange as an indicator. Total hardness was measured by using ammonia buffer solution and Erichrome black T as an indicator. The above physico- chemical analysis of water used in the present experiment was carried out as per APHA *etal*. (1998).

Determination of Median Lethal Concentration (LC50)

In the present study preliminary toxicity tests were carried out to find the median lethal tolerance limit of fish to platinum for 24h. Separate circular plastic tubs of 50 L of water capacity were taken and different concentrations of platinum were added. Then 10 healthy fishes were introduced into each tub. A control tub (no toxicant) with 50 litres of water and 10 fish were also maintained. Three replicates were maintained for each concentration groups. The mortality/survival of fish in control and platinum treated tubs was recorded after 24h and the concentration at which 50% mortality of fish occurred was taken as the median lethal concentration (LC50) for 24h which was 12 mg/L. A similar experimental set up was also maintained to determine the median lethal concentration of platinum to the fish *Cirrhinusmrigala*for 96h. The test water was renewed at the end of 24h and freshly prepared solution was added to maintain the concentration of platinum at a constant level. The median lethal concentration (LC50) of platinum for 96h was found to be 6 mg/L. The median lethal concentration of platinum was calculated by the probit analysis method (Finney,1978). In the present study the homogenicity of the fish population was tested using chi-square test (Busvine, 1971). Death was indicated by failure of the fish to respond to the gentle prodding with a gloss rod and cessation of the opercular movement.

3. Results

In the present investigation the median lethal concentration (LC 50) of platinum to an Indian major carp *Cirrhinusmrigala* for 24 hours and 96 hours were found to be 12 mg/L and 6 mg/L. The main toxic action of platinum on aquatic animals during acute and sublethal toxicity leads to mortality due to metal accumulation in fishes through the gills. During acute treatment of platinum, the fish *Cirrhinusmrigala* showed behavioral changes such as loss of balance, restlessness, abnormal swimming. During sublethal treatment of platinum, the fish *Cirrhinusmrigala*showed the changes such as the gulping of air, opercular movement, erratic jumping.

Table 2: Calculation of log concentration / probit regression line for 24h experiment in fish *Cirrhinusmrigala*were exposed todifferent concentration of platinum

S. No	Concentration PPM of Platinum	No.of Fish used	% Dead	Log Conc	Empirical Probit	Expected Probit	Working probit	Weight coefficent	Weight					
				X		Y	y		W	WX	Wy	WX^2	Wy^2	WXy
1	4	10	46	0.602	4.60	4.66	4.89	0.620	6.20	3.732	30.314	2.24	148.25	18.24
2	8	10	50	0.903	4.66	4.90	4.98	0.625	6.25	5.643	31.12	5.09	155.00	28.10
3	12	10	54	1.079	4.70	5.10	5.10	0.630	6.30	6.797	32.13	7.33	163.86	34.66
4	16	10	58	1.204	4.75	5.20	5.20	0.627	6.27	7.549	32.60	9.08	169.54	39.25
5	20	10	62	1.301	4.80	5.25	5.28	0.624	6.24	8.118	32.94	10.56	173.96	42.86
									31.26	31.839	157.7	34.3	810.61	163.11
									SW	SWX	SWY	SWX^2	SWY^2	SWXY

X = 1.264 x2 = 1.0412
Y = 3.820 Fiducial limits at 5% level = 0.426
b = 0.249 Lower limit m1 = 0.361
variance 'V' = 0.0361 Upper Limit m2 = 1.321
LC50 24 h value = 12 ppm Regression equation Y = 2.433 + 0.361X
Values are means of five individual observations

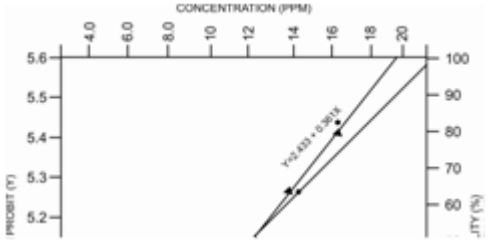

Figure 1: Log concentration of Platinum Vs. percent mortality of fish *Cirrhinusmrigala*and determination of LC50 (24h)
value calculated from Table. 2

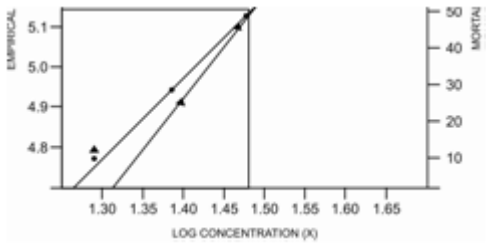

Table 3: Calculation of log concentration/probit regression line for 96hr experiment in fish Cirrhinusmrigala were exposed to different concentration of platinum.

S. No	Concentration PPM of Platinum	No.of Fish used	% Dead	Log Conc	Empirical Probit	Expected Probit	Working probit	Weight coefficent	Weight					
				X		Y	y		W	WX	Wy	WX^2	Wy^2	WXy
1	2	10	46	0.3010	4.40	4.40	4.492	0.615	6.15	0.185	27.06	0.55	128.09	8.31
2	4	10	50	0.602	4.50	4.50	4.546	0.619	6.19	3.126	27.85	2.243	128.92	16.54
3	6	10	54	0.778	4.60	4.61	4.652	0.623	6.23	4.846	28.72	3.74	134.02	22.54
4	8	10	58	0.903	4.80	4.80	4.795	0.627	6.27	5.661	30.09	5.11	144.16	27.11
5	10	10	62	1.0	4.90	4.92	4.845	0.630	6.30	6.30	30.87	6.30	147.86	30.52
									31.14	20.11	144.59	17.94	679.05	105.02
									SW	SWX	SWY	SWX^2	SWY^2	SWXY

X = 0.043 x2 = -0.031
Y = 2.980 Fiducial limits at 5% level = -0.036
b = 0.023 Lower limit m1 = -0.028
variance 'V' = 0.126 Upper Limit m2 = 1.468
LC50 24 h value = 6 ppm Regression equation Y = +2.341 + 0.459 X
Values are means of five individual observations

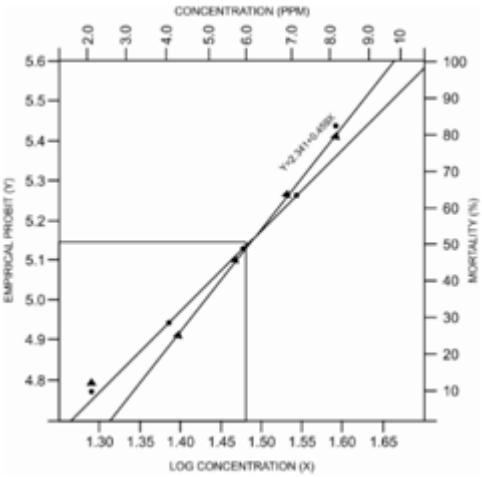

Figure 2: Log concentration of Platinum Vs. percent mortality of fishCirrhinusmrigalaand determination of LC50 (96h)
value calculated from Table. 3

4. Discussion

An important tool for understanding and evaluating the potential hazard of chemicals to aquatic organisms is the chronic or full life cycle toxicity test; a chronic toxicity test can indicate concentration of a chemical that would interfere with normal growth, development and attainment of reproductive potential of an aquatic organisms: concentrations that produce chronic effects are lower than those produce readily observable acute effects such as mortality (therefore, chronic toxicity test can provide a more sensitive measure of chemical toxicity than acute toxicity test. (Petrocelli,1985). Giesy and Graney (1989) reported that the ultimate goal of toxicity testing is to monitor or predict the effects of single compounds, elements or mixtures on the long-term health of individual organisms, populations, communities or, ecosystems. Sprague (1970) is of the opinion that description of sublethal effects of pollutant will probably be more profitable application. Sublethal effects, such as reproductive and endocrine end points, are gaining prominence in chronic ecotoxicity testing regimes (Hutchinson *et al.*, 2003). Bio monitoring using chronic toxicity assay may sensitively indicate the pollution stress posed by the pollutants at sublethal levels (Zhou *et al.*, 2008).

Platinum (Pt), seem to spread in the environment and accumulate in living organisms, they may pose a threat to animals and humans. From the vechicle catalysts through rain water it enters into the aquatic environment. For many years automotive exhaust catalysts have been used to reduce the significant amounts of harmful chemical substances generated by car engines, such as carbon monoxide, nitrogen oxides, and aromatic hydrocarbons. Although they considerably decrease environmental contamination with the above-mentioned compounds, it is known that catalysts contribute to the environmental load of platinum metals (essential components of catalysts), which are released with exhaust fumes. Contamination with platinum metals stems mainly from automotive exhaust converters, but other major sources also exist. (Pawlak*et al*., 2014).

The increasing emphasis on improving the quality of aquatic ecosystems and monitoring surface waters has highlighted the need to know what factors causes the environmental deterioration. Further the above authors stated that such changes are often manifested by impairment of vital functions viz., nerve and muscle functions, respiration, circulations, immune defense, osmoregulation and hormonal regulation. Recent years have witnessed significant attention being paid to the problems of environmental contamination by a wide variety of pollutants, including salts and metals (Mendil*et al.*, 2005). Nutrient discharges from a wide variety of anthropogenic sources including fertilizers, urban/ sewage run-off, textile mills, livestock waste and land fill leachate often end up in aquatic ecosystems (Dave and Nilsson, 2005). This is of primary concern as escalating anthropogenic activities, associated with population growth and Industrialization, is increasing the nutrient levels in ground and surface waters globally and recognized as a global issue (Camargo *et al*, 2005b; Kroupova*et al.*, 2008. Accumulation of platinum metal in the environment has been increased over the time.

Catalytic converters of modern vehicles are considered to be the main sources of platinum pollution, since the correlation is between the Pt ratios in various environmental compartments and in converter units. The present literature survey shows that the concentration of the metal has increased significantly in the last decades in diverse

environmental matrices; like airborne particulate matter, soil, roadside dust and vegetation, river, coastal and oceanic environment. Generally, Platinum are referred to behave in an inert manner and to be immobile. However, there is an evidence of spread and bioaccumulation of these elements in the environment. Platinum content of road dusts can be soluble, consequently, it enters the waters, sediments, soil and finally, the food chain. The effect of chronic occupational exposure to Pt compounds is well-documented, and certain Pt species are known to exhibit allergenic potential. However, the toxicity of biologically available anthropogenic Pt is not clear. Osterauer,et al., 2009.

Among the available biomonitors, fish are frequently used as they offer several specific advantages in describing the natural characteristics of aquatic systems and in assessing changes to habitats (Chovanec*et al.*, 2003). Firstly, they are long lived and integrate fluctuations of pollutants over time; moreover the most persistent compounds will be most abundant in the tissues of older organisms. Secondly, they are always present in thewater, thereby enabling the continuous monitoring of pollutants and allowing spatial integration of pollutant data (Lamas *et al.*, 2007). Most tests so far have been carried out on fish, not only because this group is considered to be the best understood for the aquatic environment, but also (and perhaps mainly) because of its direct interest to man (Cairns, 1982). Also, it appears that many of the species routinely used for toxicity testing are, among their fellows in the natural environment, those with the lowest ecological needs and the largest tolerances regarding many environmental variables(euryspecies); this implicitly makes them the easiest to maintain and handle in the laboratory.

The effects of short-term exposure to platinum on survival, opercular movement and post-treatment growth of coho salmon fry *(Oncorhynchuskisutch)* was investigated. Employing a static water acute toxicity bioassay with platinum as $PtCl_4 2HCl \cdot 6 H_2O$, at 8.5±0.2°C, and a water hardness of 55.9±3.5 mg $l.^{-1}$ (as $CaCo_3$), the 24, 48, and 96-h LC_{50} values were 15.5, 5.2, and 2.5 mg Pt^{4+} $l.^{-1}$ respectively. Rates of opercular movement for fish exposed to platinum increased with increasing concentrations to a level of 1.0 mg $l.^{-1}$. No further significant increases were evident above this level. Hypoactivity of fish exposed to 0.3 mg $l.^{-1}$ and higher was evident during the acute toxicity bioassay and much of the post-treatment study. Post-treatment rate of growth for fish exposed to sublethal concentration of platinum for 96 h was less than that of the controls. Paul Ferreira and Richardwolke, 1979 *Rainbow trout* to platinum nanoparticles during acute toxicity due to rapid opercular movement and gulping of air Yeo and Yoon, 2009. Changes occurred when *Zebra fish* exposed to platinum during acute and sublethal toxicity and this may be due to short-term defense mechanism designed to prevent the toxicant reaching the sensitive gill epithelium, mucus accumulation on the gill surface can cause impairment of the gill functions (Asharani*et al*., 2010), *Rainbow trout* exposed to sub-lethal concentrations of single walled carbon nanotubes (SWCNTs) showed signs of gill stimulation and elevated mucus secretion. Toxicity assays of platinum nanoparticles, using *Zebrafish* embryos to study their developmental and toxicity effects were carried out in Gold (Au-NP, 15-35nm), silver (Ag-NP, 5-35 nm) and platinum nanoparticles (Pt-NP, 3-10 nm) Toxicity was recorded in terms of mortality. This leads to hatching delay, phenotypic defects and metal accumulation. The addition of Ag-NP resulted in a concentration dependant increase in mortality rate. Both Ag-NP and Pt-NP induced hatching delays, as well as a concentration dependant drop in heart rate, touch response and axis curvatures. Ag-NP also induced other significant phenotypic changes including pericardial effusion, abnormal cardiac morphology, circulatory defects and absence or malformation of the eyes. In contrast, Au-NP did not show any indication of toxicity. This was probably due to the small size of the Pt nanoparticles compared to Ag-NP and Au-NP, thus resulting in fewer metal atoms being retained in the embryos. Among the nanoparticles studied, Ag-NPs were found to be the most toxic and AuNPs the non-toxic. The toxic effects exhibited by the zebrafish embryos as a consequence of nanoparticle exposure, accompanied by the accumulation of metals inside the body calls for further investigations (Asharani*et al*., 2010). In the present investigation platinum exposed to freshwater fish *Cirrhinusmrigala* during acute and sublethal toxicity the changes were observed.During acute treatment of platinum, the fish *Cirrhinusmrigala* showed behavioral changes such as loss of balance, restlessness, abnormal swimming. During sublethal treatment of platinum, the fish *Cirrhinusmrigala*showed the changes such as the gulping of air, opercular movement, erratic jumping which leads to mortality due to metal accumulation in fishes through the gills.

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