



Potential Alternative to the Fish Acute Toxicity Test

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- The effect of pollutants on aquatic organisms, especially fishes, has been reported
- Plays a very important role as bio-indicators
- Acute fish toxicity (AFT) is mandatory for data requirement
- Fish, birds and frogs are also included as laboratory animals
- Research for animal alternatives is often neglected for these species, although fish is widely used

- Recently, use of animal alternatives for research has been extended to environmental testing.
- This is to reduce, refine, or replace (3Rs) the use of animals in testing where feasible.
- For AFT, mortality is the primary endpoint and it is often hypothesized that fish suffer distress and perhaps pain.
- Due to this reason AFT is not compatible with most current animal welfare legislation.

- Fish embryo toxicity test (FET) is mandatory test for whole effluent testing in Germany since 2005 and has already been standardized at the international level but it is yet to be implemented in India.
- In order to analyze the applicability of the FET, a comparative evaluation of both fish and fish embryo toxicity data was carried and results were compared to evaluate the correlation between the two data sets.

- **Test Samples**



The samples were collected from different location near Sachin, Gujarat.

- **Test System**



Zebrafish (*Danio rerio*)



Zebrafish embryos

Experimental Design

Test Condition	Fish Acute Toxicity Test	Fish Embryo Toxicity Test
Test System	Zebrafish	Zebrafish Embryos
Test Sample	Industrial effluents	Industrial effluents
Test Media	RO water	E3 Medium
Test Procedure	Semi-static	Semi-static
Number of Fish/Embryos	10/concentration	20/concentration
Test Chamber	Glass Tank	24 well plate
Test Duration	96 h	96 h
Observation	Every 24 h interval	Every 24 h interval

Experimental Design (Continued)

Month and Sampling Site	Fish Acute Toxicity Test	Fish Embryo Toxicity Test
	Test concentration (% v/v)	
May 2014: Site 1	6.03, 7.23, 8.68, 10.42 and 12.50	0.78, 1.56, 3.13, 6.25 and 12.50
May 2014: Site 2	48.23, 57.87, 69.44, 83.33 and 100.00	10.66, 18.66, 32.65, 57.14 and 100.00
May 2014: Site 3	0.38, 0.75, 1.50, 3.00 and 6.00	0.72, 1.22, 2.08, 3.53 and 6.00
Sept. 2014: Site 1	26.03, 36.44, 51.02, 71.43 and 100.00	19.75, 29.63, 44.44, 66.67, 100.00
Sept. 2014: Site 2	15.80, 23.70, 35.56, 53.33 and 80.00	11.85, 17.78, 26.67, 40.00, 60.00
Sept. 2014: Site 3	18.22, 25.51, 35.71, 50.00 and 70.00	17.51, 22.76, 29.59, 38.46, 50.00
Jan. 2015: Site 1	48.23, 57.87, 69.44, 83.33 and 100.00	13.49, 22.26, 36.73, 60.61, 100.00
Jan. 2015: Site 2	1.98, 2.96, 4.44, 6.67 and 10.00	0.85, 1.49, 2.61, 4.57, 8.00
Jan. 2015: Site 3	2.60, 3.64, 5.10, 7.14 and 10.00	0.21, 0.47, 1.03, 2.27, 5.00

Experimental Procedure

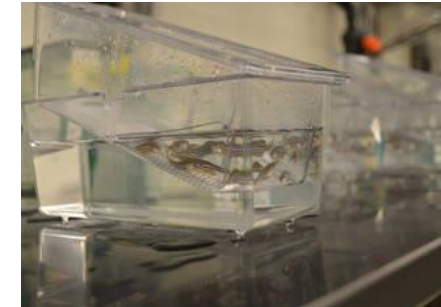
Procedure for Egg Collection



Breeding tank



Tanks with eggs



Separation of fish



Draining of water

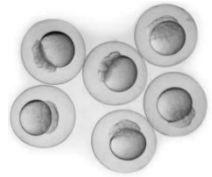


Collection of eggs

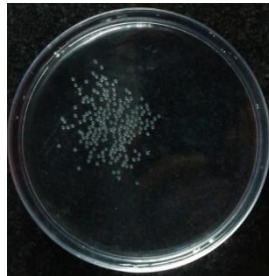


Selection of fertilised eggs

Experimental Procedure



Fertilised eggs



Exposure



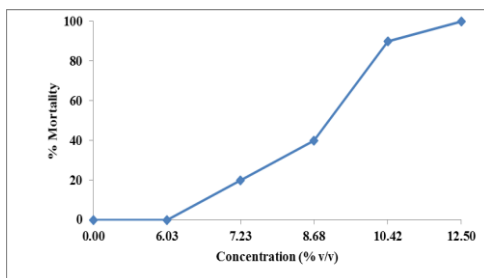
Transfer

	1	2	3	4	5	6
A	C	C	C	C	C	IC
B	C	C	C	C	C	IC
C	C	C	C	C	C	IC
D	C	C	C	C	C	IC

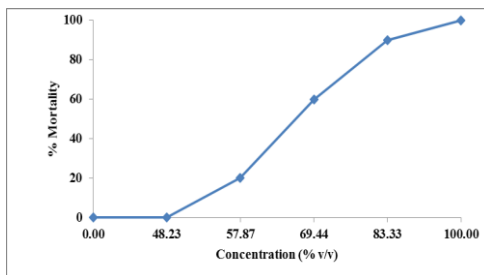
Fish Acute Toxicity Test

May 2014

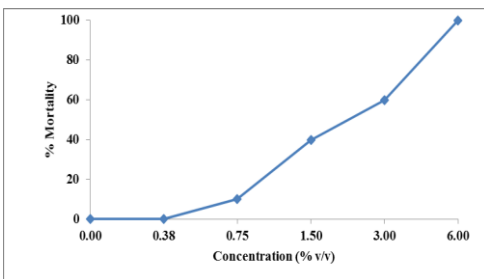
Site 1



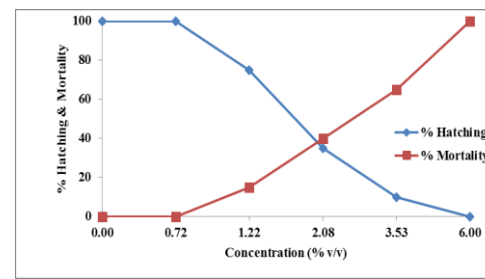
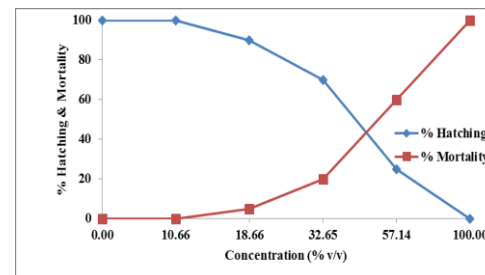
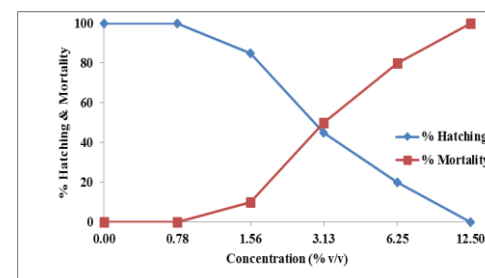
Site 2



Site 3



Fish Embryo Toxicity Test

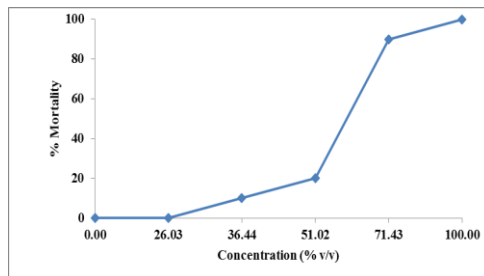


Results (Continued)

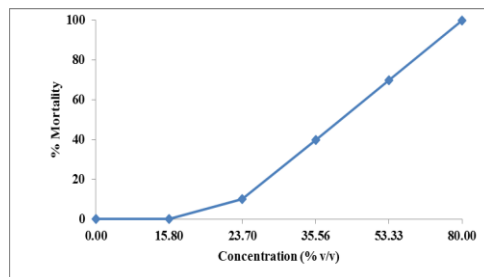
Fish Acute Toxicity Test

Sep 2014

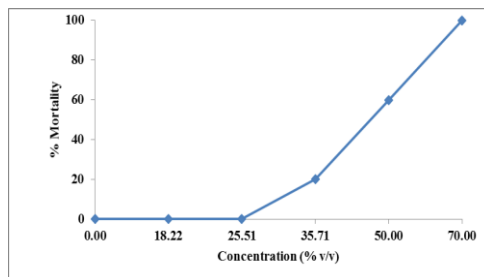
Site 1



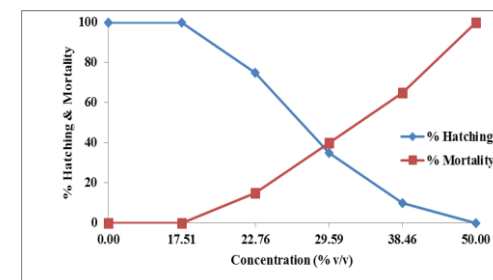
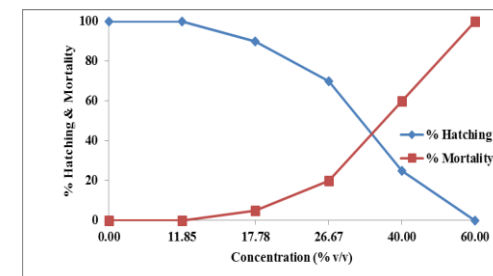
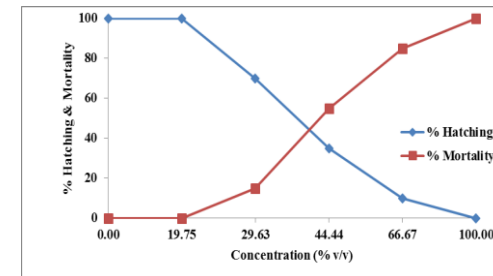
Site 2



Site 3



Fish Embryo Toxicity Test

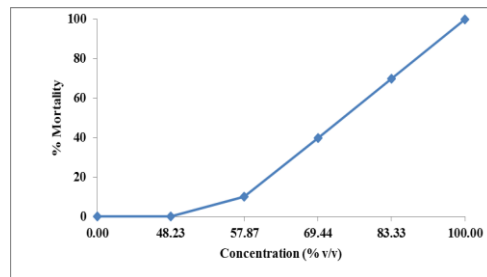


Results (Continued)

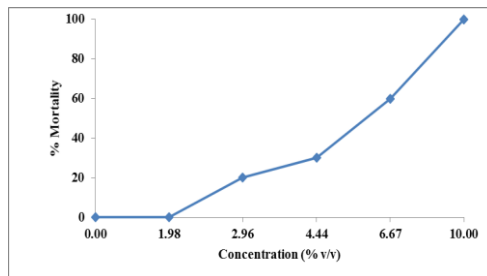
Fish Acute Toxicity Test

Jan 2015

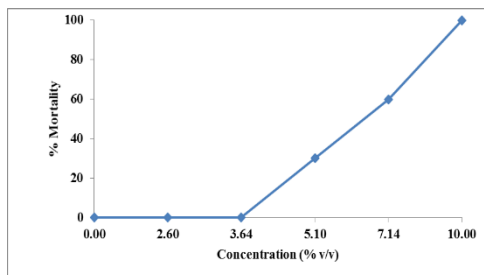
Site 1



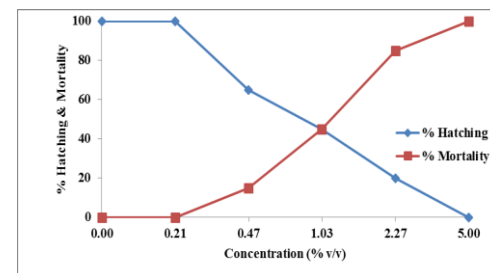
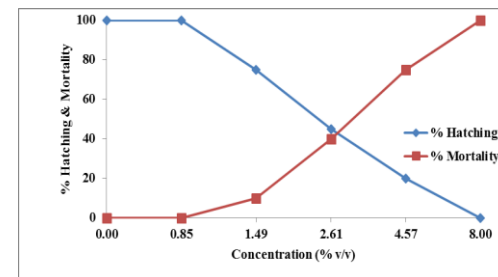
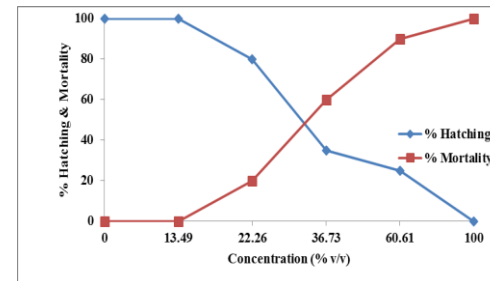
Site 2



Site 3



Fish Embryo Toxicity Test



Results (Continued)

Fish Acute Toxicity Test

Sign of Toxicity
Loss of Equilibrium
Lying on bottom
Swimming at surface
Lethargy
Mortality



Normal

Fish Embryo Toxicity Test

Sign of Toxicity
Absence of heartbeat
slow heartbeat
yolk sac oedema
pericardial oedema
Scoliosis
non-detachment of tail
lack of somite formation
coagulation



- ▲ = Pericardial oedema
- ↙ = Yolk sac oedema
- * = Scoliosis



Coagulation

Results (Continued)

Month and Sampling Site	Fish Acute Toxicity Test	Fish Embryo Toxicity Test
	LC ₅₀ (% v/v)	
May 2014: Site 1	8.59	3.44
May 2014: Site 2	66.74	50.64
May 2014: Site 3	2.21	2.60
Sept. 2014: Site 1	54.92	43.72
Sept. 2014: Site 2	41.36	36.65
Sept. 2014: Site 3	46.25	33.03
Jan. 2015: Site 1	74.33	32.94
Jan. 2015: Site 2	5.85	3.08
Jan. 2015: Site 3	6.40	1.07

- The current research revealed that all of the industrial effluent tested had acute toxicity effect on zebrafish and its embryos.
- On the basis of the study, the effluent tested here may cause increasing embryotoxicity in the zebrafish embryos.
- The result of acute fish toxicity test and fish embryo toxicity test are more comparable with each other.
- The embryo has been proved in this research as a sensitive organism compare to adult zebrafish.

- Our study confirmed that zebrafish embryos are a valuable model for assessing environmental pollutant as an alternative for the adult zebrafish acute test.

Acknowledgement

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Thank You