Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; SP1: 1296-1299

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Impact of industrial wastewaters on the fish *Poecilia reticulate*: Integrating wastewater toxicity with ecological safety

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Abstract
Wastewater disposal from various sources are polluting the environment and disturbing our ecosystem at various levels. Industries are of special concerns due their effluent containing various contaminants which may have deleterious impact on environmental health. Concerning to current industrial pollution problem, the effect of industrial wastewater from Poolpur and Naini on different ecotoxicological parameters of selected fresh water fish Guppy spp *Poecila reticulata* was studied in laboratory conditions up to 96 hours. The increase space between gills and operculum, excessive excretion as well as increase surface activity were found in wastewater exposed fishes in comparison to control. The overall findings of the study indicates that industrial wastewaters are safe for crop irrigation but may have some negative impact on environment as on the basis of behavioural changes in effluent exposed fishes.

Keywords: *Poecila reticulata*, Behavioural changes, Irrigation, Wastewater

1. Introduction
The wastewater disposal is a common concern and major problem for our environment. The aquatic environment is very sensitive to wastewater disposal from various sources. The aquatic environment is globally one the most severely as well as directly affected segment of the environment due to both treated and untreated wastewater disposal from various sources (Abdel-Sabour, 2003; Singh *et al*., 2004). Industrial wastewaters are complex in nature and contain various pollutant causing deleterious impact on human health and environment (Petrie *et al*., 2015; Martin *et al*., 2012). Industries are also aware and not committed to treat properly their wastewater before disposal by following the recommended standards for wastewater disposal resulting severe pollution of different segments of environment resulting into poor impact on environment and health. Very few studies have been done on the basis of aquatic environment health and ecological safety perspectives. The fish's species are the best biological indicator of safe and healthy aquatic ecosystem due to their sensitivity to environmental contaminants (Kramer and Botterweg, 1993). Looking to the problem of pollution due to improper wastewater disposal and reuse at selected site area, the current study has been done with following objectives: Firstly to know the quality of wastewater of selected industries. Secondly, to know the possible impact of sampled industrial wastewaters on aquatic ecosystem and environmental health by using selected fish species as a tool of ecotoxicity evaluation which will be helpful in knowing the ecological safety of sampled wastewater of selected industries.

2. Material and Methods
Study Area: Study area is located at two sites. The first site is Phulpur industrial area of Allahabad district, Uttar Pradesh, India (figure-1). At Phulpur industrial site, Indian Farmers Fertiliser Cooperative Limited (IFFCO), a nitrogenous (Urea) fertilizer industry is discharging wastewater, which is being used for crop irrigation in IFFCO farm's land. Wastewater samples were collected from Naini, an industrial area of Allahabad district, Uttar Pradesh, India (figure-1). At Naini industrial site, Racron, a synthetic fibres (polyester yarn) manufacturing textile industry and their treated wastewater is disposed off and drain out by common drainage channel in the adjoining areas of farmers' field and were using for irrigating the crops.

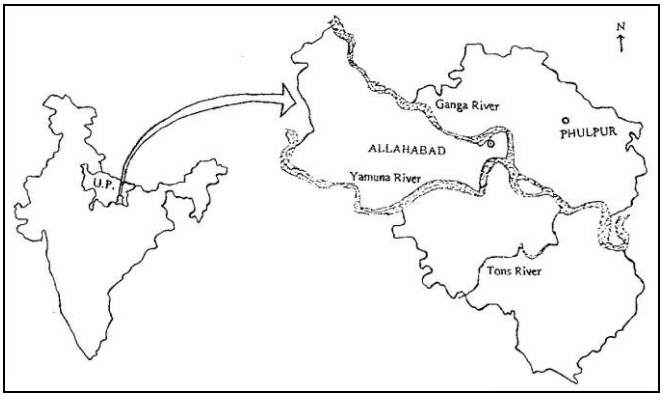

Fig 1: Location of sampling site at Phulpur and Naini, Allahabad district, Uttar Pradesh (India).

1. **Collection of Wastewater samples:** Wastewater samples were collected from the identified site during the post monsoon season by using grab sampling methods for wastewater analysis, while tube well water samples (control) were also collected for comparative studies.
2. **Wastewater Quality Analysis:** Samples were processed and analyzed for the various physico-chemical parameters viz., pH, Electrical Conductivity (EC), Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Solid (TS), Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Total Hardness (TH), Ca, Mg, Cl, SO_4, K, Na, Na %, Sodium Adsorption Ratio (SAR) as per the standard methods of APHA (1998). For heavy metals (Cd, Cu, Co, Fe, Mn, Ni, Pd, Zn) analysis samples were processed by acid digestion with 1:4 mixtures of $HClO_4$ and HNO_3 and measured by using ICP- 8440 Plasmalab Latam Atomic Absorption Spectrophotometer (AAS).
3. **Fish Bioassay:**For the bioassay study with fresh water fish *Poecilia reticulate* commonly known as Guppy were collected from were collected from Saryu Hatchery (U.P. Matsya Vikas Nigam) Masodha, Faizabad. The fishes were collected from Saryu Hatchery (U.P. Matsya Vikas Nigam) Masodha, Faizabad. Static bioassay procedures, as outlined by the USEPA (2005) were followed. Ten fishes were acclimatized for 10 days before the treatment and were feed with artificial fish food. Fishes were not fed during toxicity studies. Numbers of survivors were noted at 24, 48, 72 & 96 hours upon exposure of fishes to 100% concentration of selected industries wastewater samples under the static experimental conditions. The exposed fishes were also studied for various behavioural changes (Gopal and Mishra, 1988).
4. **Statistical Analysis:** The data obtained after analysis was statistically analyzed by using analysis of variant (ANOVA), to test the statistical significance of the difference between the means of treatments with help of SPSS statistical software package.

3. Results and Discussion

1. Wastewater characteristics

The results of quality analysis of wastewater (IFFCO and Racron) along with control are presented in table-1 and 2. As per wastewater analysis results, the IFFCO wastewater has significantly ($p<0.01$) high EC, BOD, COD, phosphate, TS, TDS, TH, Ca, Cl, SO_4, K, Na, Na%, SAR and low DO. Whereas, analysed wastewater (IFFCO) has been found significantly ($p<0.01$) high in heavy metals (Zn, Ni, Pb, Mn and Fe) concentration than control (table-2). Wastewater of Racron was found significantly ($p<0.01$) high in pH, EC, BOD, COD, phosphate, TS, TDS, TH, Ca, Cl and SO_4. While concentration of heavy metals i.e. Zn, Ni, Pb, Mn and Fe were found significantly high in wastewater than control. The high values of water quality parameters along with heavy metals in both sites industrial wastewaters may cause the harmful impact on aquatic and terrestrial ecosystem due their disposal and reuse. The low DO, high BOD and COD may cause stress due to oxygen depletion and less availability of oxygen among aquatic organisms like fishes (Kumar and Gopal, 2001). The high levels of pH, EC, TS, TDS, TH, Cl and SO_4 in wastewater may also have various harmful impacts on fishes and soil fertility as well as cause of concern for humans (Singh *et* al., 2003; Yadav *et al*., 2005; Pandey and Singh, 2015; Pandey *et al*., 2001). The high phosphate may cause eutrophication of surrounding water bodies.

Table 1: Physico- chemical characteristics of wastewater samples collected from selected sites.

Wastewater characteristics	Control	IFFCO wastewater	Racron wastewater
pH	7.21 ±.05	7.10 ±.12	7.82 ±.04**
EC (dSm^{-1})	0.89 ±.00	1.3 ±.00**	2.14 ±.03**
DO (mg/L)	3.9 ±.02	2.8 ±.03**	4.2 ±.06**
BOD (mg/L)	1.26 ±.04	12.5 ±.3**	13.2 ±.05**
COD (mg/L)	6.7 ±.04	42.8 ± 1.4**	28.7 ± 2.1**
Temp (°C)	26 ±.00	28 ±.00	26 ±.00
TA (mg/L)	174.4 ± 4.1	68.4 ± 2.3**	87.2 ± 3.7**
Phosphate (mg/L)	2.62 ±.01	15.6 ±.00**	24.2 ±.08**
TS (mg/L)	1020 ± 8	1610 ± 8**	1320 ± 11**
TDS (mg/L)	780 ± 6	1490 ± 5**	1310 ±.00**
TSS (mg/L)	240 ± 6	120 ± 5**	10 ±.00**
TH (mg/L)	420 ± 2.5	480 ± 3**	461.4 ±.27**
Ca (mg/L)	10.2 ± 1.2	184.2 ± 13.3**	120.8 ±.51**
Mg (mg/L)	122.4 ±.42	10.1 ± 5.2**	1.4 ±.02**
Cl (mg/L)	610.2 ± 12.6	4012.4 ± 8.9**	1210.7 ± 12.8**
SO_4 (mg/L)	12.1 ±.00	910 ± 4.5 **	62.4 ± 3.25 **
K (mg/L)	3.24 ±.02	12.4 ±.1**	1.3 ±.00**
Na (mg/L)	65.52 ± 3.54	234 ± 3**	44.51 ± 2.52**
Na (%)	32.53 ± 1.8	53.1 ± 2.1**	36.77 ± 1.1**
SAR	1.005 ±.13	2.970 ±.17**	0.712 ±.05**

* Above values are average ± SD of three determinations.
** Indicates $p < 0.01$ at significant level (1%).

Table 2: Results of the analysis of heavy metals (mg/L) in wastewater samples collected from selected sites.

*Metals	Control	IFFCO wastewater	Racron wastewater
Cd	<0.002 ±.00	<0.002 ±.00	<0.002 ±.00
Zn	0.06 ±.001	0.32 ±.001**	0.26 ±.002**
Ni	0.018 ±.002	0.044 ±.006**	0.039 ±.007**
Pb	0.19 ±.004	0.27 ±.001**	0.32 ±.007**
Cu	<0.001 ±.00	<0.001 ±.00	<0.001 ±.00
Mn	<0.01 ±.00	0.027 ±.00**	0.018 ±.003**
Co	ND	ND	ND
Fe	0.137 ±.003	1.005 ±.002**	0.223 ±.004**

* Above values are average value ± SD of three determinations.
** Indicates $p < 0.01$ at significant level (1%).

2. Effect of wastewater exposure on fish

The results of toxicity assessment of wastewater (IFFCO and Racron) on the survival (%) of *Poecilia reticulata* are presented in table-3. The result showed that survival % of fish in wastewater of IFFCO and Racron industry were found lower in comparison to control after 72h and 96h exposure which indicates the toxic effect on wastewater due to high levels of pollutants (pH, EC, TS, TDS, TH, Cl and SO_4 followed by high concentration of heavy metals i.e. Zn, Ni, Pb, Mn and Fe) in the wastewater. The comparison of results of fish survival (%) after exposure of wastewaters of IFFCO and Racron were found safe for crop land irrigation as per prescribed standards (CPCB, 1993). Similar findings were also reported by Joshi *et al* (1996). The industrial wastewater can be also reuse by for their industrial processing according to quality wastewater (Lu *et al*., 2010).

Table 3: Percentage Survival (%) of *Poecilia reticulate* on exposure of wastewater.

S. No.	Sources of wastewater collection		Percentage Survival (%)			* Standard value (96 h) for irrigation of cropland
		24 h	48 h	72 h	96 h	Survival (%)
1.	Control	100	100	100	100	90
2.	IFFCO wastewater	100	100	90	90	
3.	Racron wastewater	100	100	90	90	

***** Source: CPCB standard by The Gazette of India: No. 174. 19-5-1993.

Results of behavioural changes such as fin movement, distance between gills and operculum, increase surface activity and excretion due to the wastewaters (IFFCO and Racron) exposure on fishes is presented in Figure-2. Results indicates that rate of fin movement increases directly in proportion to exposure duration (24h, 48h, 72h and 96h) and found significantly high ($p<0.01$) in comparison to control at 100 % concentration. It indicates that exposed fishes showed the behavioural changes due to the stress caused by pollutants (low DO, high EC, SO_4, phosphate and heavy metal concentrations) in wastewater. The rate of fin movement was found highest in Racron wastewater exposed fishes than IFFCO wastewater. The distance between gills and operculum wastewater was found highest in wastewaters exposed fishes than control due to the presence of stress causing pollutants in wastewater like heavy metals (Raina *et al*., 1998). In case where fishes were exposed to wastewaters (IFFCO and Racron), space between gills and operculum were found more in IFFCO wastewater followed by Racron wastewater when compared with control. The results indicate the presence of pollutants i.e. heavy metals in wastewaters (Khumyakari *et al*, 2001). Apart from the common changes, some specific changes were also observed in wastewaters exposed fishes. The Racron wastewater exposed fishes showed excessive excretion followed by IFFCO wastewater. The excessive excretion by fishes indicated the presence of heavy metals in wastewaters. Similar findings were reported by Luckey *et al* (1975). Increase surface activity of the fishes was found in IFFCO wastewater exposed fishes followed by control in comparison to Racron wastewater exposed fishes. It may be attributed due to decrease in DO level of wastewater.

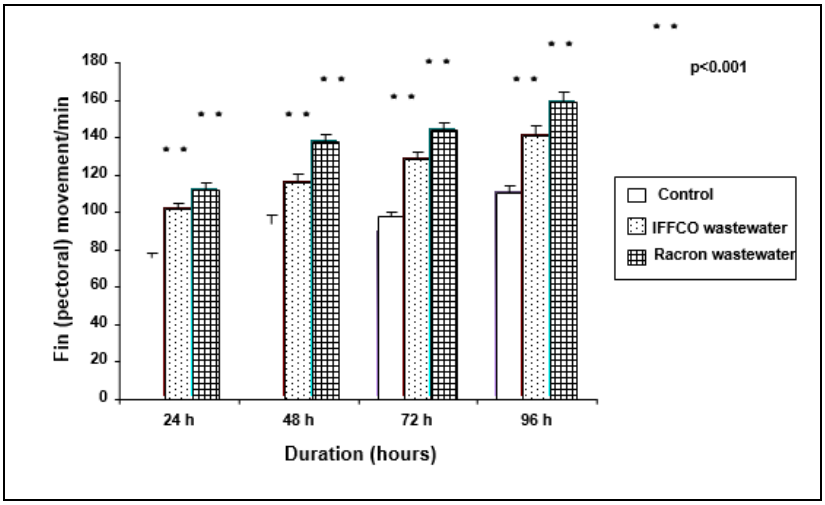

Fig 2a: Fin movement of *Poecilia reticulata* after wastewater exposure.

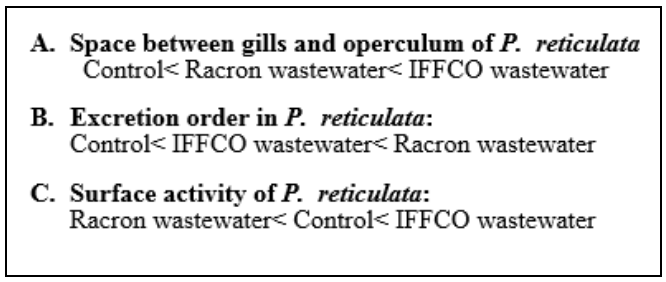

Fig 2b: Behavioural changes in *Poecilia reticulata* after wastewater exposure.

4. Conclusion

Overall, results of wastewater analysis of IFFCO and Racron industry, for biological safety assessment performed by various bioassays showed both positive and negative impact on test organism as compared to control. As the results showed that wastewaters were significantly differ in terms of their physico-chemical parameters and heavy metals than control, but were in safe limits prescribed by CPCB (1993) and EPA (2004), for irrigation. The results of studies on IFFCO and Racron wastewater exposed fishes survival (%) indicated no significant negative impact of wastewater on exposed fishes than control. The comparison of wastewaters exposed fish survival (%) data with recommended standards (CPCB, 1993) for cropland irrigation also proved the suitability and safety of wastewaters for irrigation. Whereas, behavioural changes among fishes due to wastewaters (IFFCO and Racron) exposure which were found significantly high ($p<0.01$) in case of fin movements. Wastewater exposed fishes showed negative impact of wastewater on fish sensitivity due to presence of possible pollutant (dissolved salts and heavy metals) in wastewaters. So, the results found on the basis of performed bioassays showed the suitability of IFFCO and Racron wastewater for cropland irrigation but may have negative impact upon aquatic environment as well as human health in long run due to heavy metals contamination.

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