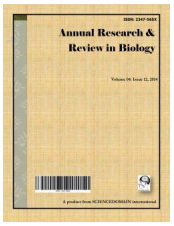

Annual Research & Review in Biology

25(3): 1-8, 2018; Article no.ARRB.40018
ISSN: 2347-565X, NLM ID: 101632869

Impact of Industrial Wastewaters on Fresh Water Fish, *Cyprinus carpio* (Linnaeus, 1785): A Tool for Ecological Risks Monitoring

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Authors' contributions

This work was carried out in collaboration between all authors. Author RP designed the study, performed the statistical analysis, wrote the protocol, and wrote all the draft of the manuscript. Authors MT and JS managed the literature searches and analyses of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2018/40018
Editor(s):
(1) Layla Omran Elmajdoub, Department of Zoology, Faculty of Science, Misurata University, Misurata, Libya.
(2) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) Bruno Fiorelini Pereira, Federal University of West of Bahia, Brazil.
(2) Vesela Yancheva, Plovdiv University, Bulgaria.
(3) Marcia Regina Salvadori, Biomedical Institute –II University of São Paulo, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/23915

Original Research Article

Received 18th January 2018
Accepted 27th March 2018
Published 31st March 2018

ABSTRACT

Background: In the era of industries, the problem of pollution of aquatic resources has become aggravated due to continuous wastewater disposal. The Aquatic ecosystem health can be considered as an indicator of the environmental state. Furthermore, fishes are an ideal indicators for contaminated environment.
Aims: To assess the sensitivity of common carp fishes against industrial wastewaters exposure on the basis of lethal effects and behavioral changes as a tool for ecotoxicology for knowing the possible effects upon environment due to selected industries wastewater.
Methodology: In this study, the effects of industrial wastewaters for different ecotoxicological parameters (mortality and behaviour changes) on common carp were studied under static conditions during different time interval (24, 42, 72 and 96 h) followed by sampled industrial wastewater quality analysis for physicochemical parameters.

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Results: Increased space between the gills and operculum, excessive excretion, as well as increased fish surface activity was found in the wastewater exposed fish compared to control.
Conclusion: The overall findings from the present study indicate that the sampled industrial wastewaters are safe for crop irrigation but may have some adverse impact on the biota due to the observed behavioral and histological changes in wastewater exposed fishes.

Keywords: Behavioral changes; Cyprinus carpio; industrial wastewaters; irrigation.

1. INTRODUCTION

The wastewater disposal is a well known problem for our environment. The aquatic environment is very sensitive to water disposal. Due to wastewater disposal from various industrial, agricultural and domestic sources, the aquatic environment is one of the most severely as well as directly affected segment of the environment around the world [1-5]. Industries are the major sources of pollution due to the nature of their operations which require high volume of water that resulting into the higher wastewater generation [6]. Uptake of effluents through food chain in aquatic organisms may cause various serious physiological disorders such as hypertension, sporadic fever, renal damage, cramps, etc. [7]. Industries are also aware and committed to treat properly their wastewater before disposal by following the recommended standards for wastewater disposal but very few studied have been done on the basis of aquatic environments ecological health and safety perspectives. The treated wastewaters may also cause some health effects upon their disposal and reuse due to traces of some contaminants in long run [8]. Sensitive fish species are the best biological indicator for healthy aquatic ecosystem [9,10]. Based on this, the main objectives of the study are: firstly to study the quality of selected industries wastewaters. Secondly, to study the possible impact of wastewater on cropland and aquatic ecosystem by using common carp which may be useful for development of ecotoxicity evaluation tool and understanding the ecological safety concerns due wastewater disposal or use of selected industries wastewater.

2. MATERIALS AND METHODS

2.1 Study Area

The study area is located at two sites. The first site is Phulpur industrial area of Allahabad district, Uttar Pradesh, India (Fig. 1). At Phulpur industrial site, Indian Farmers Fertiliser Cooperative Limited (IFFCO), a nitrogenous (Urea) fertilizer industry discharges wastewater, which is used for crop irrigation in the IFFCO farm's land. Wastewater samples were collected from second site Naini, an industrial area of Allahabad district, Uttar Pradesh, India (Fig. 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 the agricultural fields and is used commonly using for irrigating the crops.

2.2 Collection of Wastewater Samples

Wastewater samples were collected from the identified site during the post monsoon season by using grab sampling method and followed APHA [11] standards for wastewater sampling, processing and handling in addition tube well water samples as control were also collected for comparative studies.

2.3 Wastewater Quality Analysis

The 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 [11]. 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).

2.4 Fish Bioassay

For the bioassay study common carps were collected from Saryu Hatchery (U.P. Matsya Vikas Nigam) Masodha, Faizabad. Static bioassay procedures, as outlined by the USEPA [12] were followed. Ten fishes were acclimatized for 10 days before the treatment and were fed

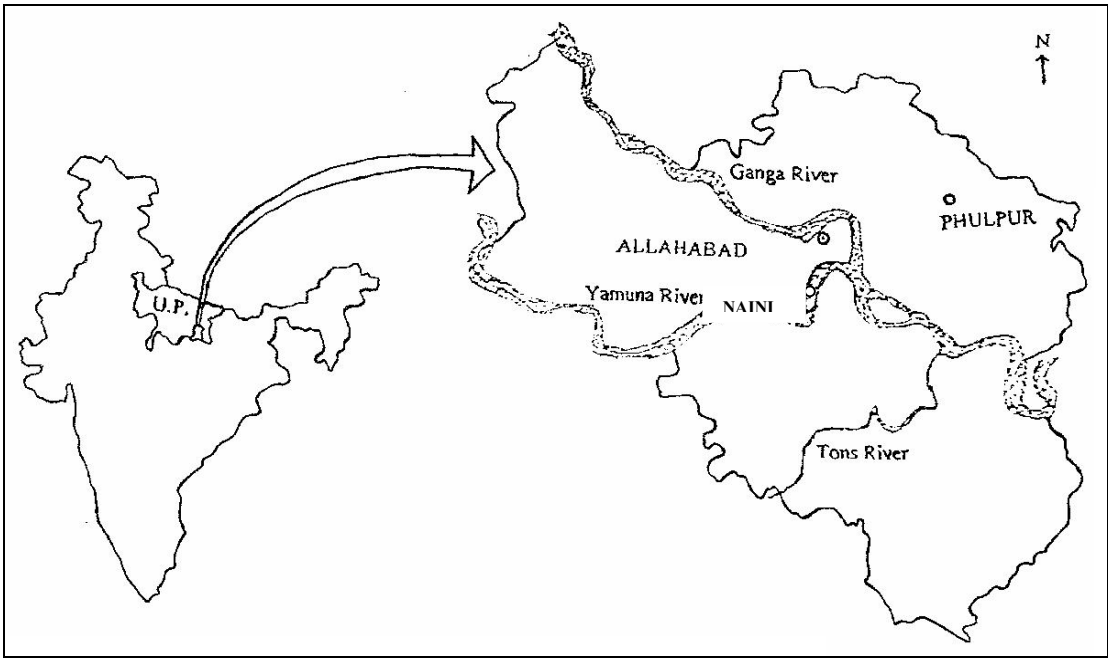

Fig. 1. Location of sampling site at Phulpur and Naini, Allahabad district, Uttar Pradesh (India)

with artificial fish food under laboratory condition of Department of Environmental Sciences, Dr Ram Manohar Lohia Avadh University, Faizabad (Uttar Pradesh), India. Fish were not fed during the toxicity studies. Numbers of survivors were noted at 24, 48, 72 and 96 h upon exposure of fishes to 100% concentration of selected industries wastewater samples under the static experimental conditions. The exposed fish were also monitored on a regular basis for various behavioural changes [13].

2.5 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

3.1 Wastewater Characteristics

The results of quality analysis of wastewater (IFFCO and Racron) along with control are presented in Table 1 and 2. As evident by 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 (Fe, Mn, Ni, Pb and Zn) 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. Fe, Mn, Ni, Pb, and Zn were found significantly high in wastewater than control. The concentration of heavy metals is known to cause toxic effects both individually and in mixed form. However, the damage may be greater when the metals are present as a mixture due to increase in total metals concentrations as well as mixed effects of metals [14]. The heavy metal concentrations in wastewater found in traces along with high level of physico-chemical parameters were also observed by Roopadevi and Somashekar [15]. The high values of different water quality parameters along with heavy metals in both wastewaters may cause the harmful impact on environment after their disposal for long time [16-18].

3.2 Effects of Wastewater Exposure on Fish

The results of toxicity assessment of wastewater on the basis of survival (%) of fish common carp are presented in Table 3. The results revealed that survival (%) of fish on exposure of wastewater of IFFCO and Racron industry along

Table 1. Physicochemical 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 (^oC)	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%)*

with control were found 100% after the 96h exposure. The results of fish survival (%) were also compared with standard value prescribed for irrigation by wastewater [CPCB, 1993] and found under safe limit. The results of behavioral changes such as fin movement distance between gills and operculum, increased surface activity and excretion due to the wastewaters (IFFCO and Racron) exposure on the fish are presented in Fig. 2a and b. The results indicate that rate of fin movement increases directly in proportion to exposure duration (24 h, 48 h, 72 h and 96 h) and found significantly high ($p<0.01$) in comparison to the control at 100% concentration of industrial wastewaters.

Table 3. Percentage Survival (%) of *Cyprinus carpio* 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	100	100	
3.	Racron wastewater	100	100	100	100	

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

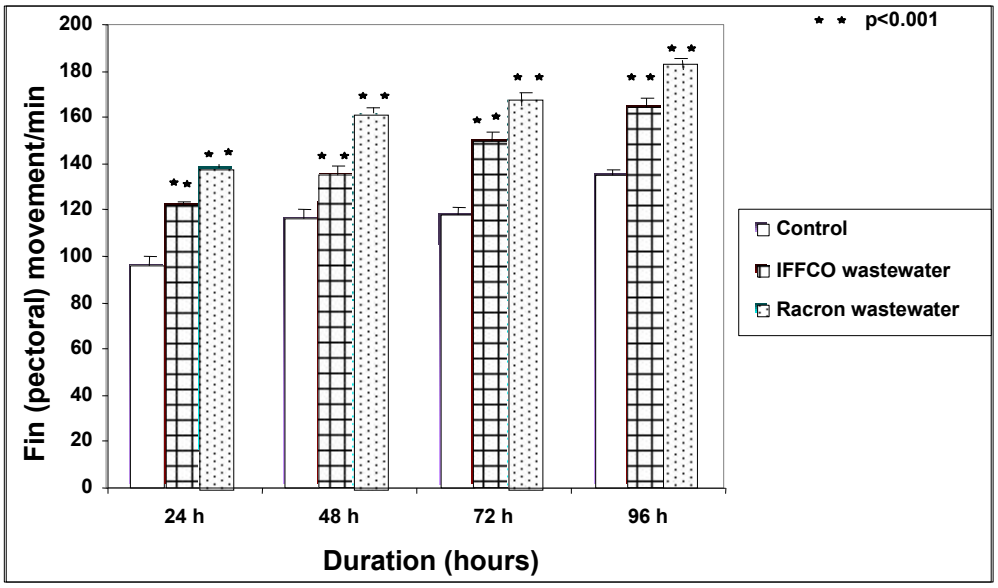

Fig. 2a. Behavior changes: Fin movement of *Cyprinus carpio* after wastewater exposure

It indicates that the exposed fish showed the behavioral changes due to the stress caused by pollutants (low DO; high EC, BOD, COD, SO_4, phosphate and traces of heavy metal concentrations) in wastewater which may also signifies respiratory impairment, an outcome of the impact of the wastewater on the gills of fish. Similar findings were also reported by Adewoye *et al.* [19] and Manson [20]. The rate of fin movement was found highest in Racron wastewater exposed fishes than IFFCO wastewater due to high pH, EC and Pb in Racron wastewater. The high pH have a role in increasing the metals reactivity and adsorption by exposed fishes affecting fish gills due to Pb toxicity [21].

The space between gills and operculum wastewater was found to be highest in the wastewater exposed fish than the control due to the presence of stress causing by stressors such as low DO, high EC, BOD, COD and heavy metals concentration in wastewater [22], leading to an increase in ventilation volume which may cause higher risk of parasitism under polluted aquatic environment [23]. In case where the fish 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 indicating the impact of low DO and high heavy metals (Fe, Mn, Ni, Pb and Zn) mixture concentration, with special concern to Fe metal known for damaging fishes respiratory organs [9,24,25]. Apart from the common changes, some specific changes were also observed in the wastewaters exposed fishes, such as excessive excretion followed by IFFCO wastewater. The

I. Increase space between gills and operculum of *C. carpio*:

Control< Racron wastewater < IFFCO wastewater

II. Excretion order of *C. carpio*:

Control< IFFCO wastewater< Racron wastewater

III. Increase surface activity of *C. carpio*:

Racron wastewater < Control< IFFCO wastewater

Fig. 2b. Behavior changes in *Cyprinus carpio* after wastewater exposure

excessive excretion by fish indicates the presence and role of heavy metals like Pb in wastewaters, affecting fish organs: liver, kidneys, spleen and digestive tract [21]. Similar findings were reported by Luckey et al. [26]. The high concentration of heavy metals in wastewater may lead to metals bioaccumulation in the skin, gill, muscles, liver, kidney and also affects fish biomolecules which may leads into behaviour changes due to oxidative stress, reduction in carbohydrates, lipid and protein profile making fish vulnerable to diseases [27,28,29]. Increase surface activity of the fish 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 and increased metals concentration [30,31].

4. CONCLUSION

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

ETHICAL ISSUE

There is no ethical issues regarding this experiment as no mortality was found among the wastewater exposed fish and fish species used for experiment were used by following standard protocol and previous researches.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/23915