Chapter 3
Research Methodology

3.1 Assessment of Ecological Risk due to PPs in river waters

In the present study, risk quotient (RQ) has been used for assessing the ecological risk due to PPs on aquatic organisms.

3.2 Approach for Including the Effects of PPs in the River Health Assessment Framework

River health summarizes the integrity of the physio-chemical parameters of water with aquatic life. Modifying the RHI calculation framework, as given in Eq. 2.4, in the present study, a third indicator group of PPs has been added.

Accordingly, the three indicator groups included in the RHI calculation framework are as follows:

1. Dissolved Oxygen Related Parameters (DORPs): pH, EC, DO, BOD, and COD,
2. Nutrients (NTs): NH_3-N, TN, TP, and
3. Pharmaceutical Pollutants (PPs)

Among aquatic organisms, algae, macroinvertebrates, and fish represent three different trophic levels. Accordingly, in the present study, three biotic indicator groups include:

1. Algae,
2. Macroinvertebrates, and
3. Fish

3.3 Framework for calculation of River Health Index (RHI) Including the PPs

The River Health Index (RHI) calculation framework has been modified as given in Eq. 3.1 to include the effects of PPs:

$$RHI = [(DORPs \times w_1) + (NTs \times w_2) + (EPs \times w_3) + [(A \times w_4) + (MI \times w_5) + (F \times w_6)] \times 100 \text{----(Eq. 3.1)}$$

Where DORPs = Dissolved oxygen-related parameters group score, NT = Nutrient indicator group score, EPs= Pharmaceutical Pollutants group score, ABs= Algae indicator group score, MIs = Macroinvertebrate indicator group score, and F = Fish indicator group score, and w_1, w_2, w_3, w_4, w_5, and w_6 are weights assigned to these respective indicator groups.

For illustrative purposes, the DORPs group of indicators includes four parameters: DO, BOD, COD, and EC. The NTs group includes three characteristics of water: NH_3-N, TN, and TP.

Assuming justifiable weights for different groups of indicators, an RHI is calculated using (Eq. 3.1). Based on RHI, the RHI is classified into two broad categories: acceptable (RHI> 60) and poor (RHI<60). The acceptable river health condition may further be classified into three categories: good (RHI: 60-70), very good (RHI: 70-80), and excellent (RHI>80). Similarly, Poor River health conditions are subdivided into four suboptimal categories: stressed (RHI: 60-50), over-stressed (RHI: 50-40), critical (RHI: 40-20), and sick/dead (RHI<20).

The RHC has been presented as a colored circumscribed hexagon, each of whose six sectors represents the river health for one of the indicator groups selected, and its color reflects IGC at the given site. The color of the circumscribing hexagon represents the overall RHC.

3.3.1 Indicator Group Score (IGS) and Indicator Group Condition (IGC)

To evaluate the IGS of the three causative parameters (DORPs, NTs, and PPs) and three reflective biotic parameters (algae, macroinvertebrates, and fish) have been calculated by the following equation:

IGS = [∑ Scores of individual parameters / (5×No. of parameters in the group)] ×100 -- (Eq. 3.2)

The individual values of all water quality parameters have been normalized on a 0-5 scale based on acceptable concentration (score 5) and critical concentration (score 0). Further,

the arithmetic mean of normalized scores of all parameters in an indicator group has been calculated on a 0-100 scale for IGS estimation and to get the IGC of river health at a given location. IGC is presented with a color-coding scheme similar to RHC's (Table: 3.1).

Table 3.1: Color Scheme for presentation for IGC and RHC (Saxena and Singh, 2018)

Criteria	IGS or RHI	IGC or RHC	Color
Acceptable	>80	Excellent	Blue
	70-80	Very Good	Green
	60-70	Good	Yellow
Poor	50–60	Stressed	Orange
	40–50	Over Stressed	Grey
	20–40	Critical	Red
	≤20	Sick/ Dead	Black

3.3.2 Weights of Indicator Groups

Assuming physicochemical and biological parameters have a cause-and-effect relationship, 50% of the weightage is given to physicochemical parameters, such as DORPs, NTs, and PPs. In contrast, 50% of the weightage is given to biotic groups, reflecting responses to physicochemical parameters. The DORPs (w_1=0.10) are weighted lower because of their short-term presence and varying behaviour in the river environment. NTs and PPs indicators are assigned relatively higher weightage (w_2= w_3=0.20) due to their long-time presence in aquatic environments and persistent nature. Biotic indicators can be viewed as long-term integrators of river health. Algae at the lower end of the trophic level have a shorter lifespan than macroinvertebrates, followed by fish (Leigh et al., 2012). However, algae and macroinvertebrates are affected more severely due to PPs, even at lower concentrations than fish. Hence, algae and MI groups are assigned (w4=w5=0.20) rather than fish (w6=0.10).

3.3.3 Normalization Scheme for Water Quality Parameters

Table 3.2 presents the normalization scheme on a 0-5 scale for IGS calculation based on acceptable and target concentration values for DORPs and NTs indicator group water quality parameters such as EC, DO, BOD, COD, NH_3-N, TN, and TP.

Table 3.2: Normalization scheme on 0-5 scale for DORPs and nutrient related parameters

(5 for within Target Value, 0 for beyond critical threshold value)

S.No.	Indicator Groups	Parameters included	Normalized Score (0-5 scale)					
			5	4	3	2	1	0
1.	Dissolved Oxygen Related Parameters (DORPs)	i. EC (µmhos/cm)	≤400	400-750	750-1000	1000-1250	1250-1500	>1500
		ii. DO (mg/L)	≥7	6-7	5-6	4-5	3-4	<3
		iii. BOD (mg/L)	≤3	3.0-4.0	4.0-5.0	5.0-6.5	6.5-8	>8
		iv. COD (mg/L)	≤30	30-40	40-50	50-65	65-80	>80
2.	Nutrients (NTs)	i. NH_3-N (mg/L)	≤0.3	0.3-0.6	0.6-0.9	0.9-1.2	1.2-1.5	>1.5
		ii. TN (mg/L)	≤0.5	0.5-0.8	0.8-1.2	1.2-1.6	1.6-2.0	>2
		iii. TP (mg/L)	≤0.1	0.1-0.15	0.15-0.2	0.2-0.25	0.25-0.3	>0.3

The study used ten pharmaceuticals above the PNEC to calculate IGS and RHC. A normalized score on a 0-5 scale has been established; the normalization is based on PNEC (5) as the acceptable concentration and RC_T as a critical concentration (0). For each rivers with PPs presence above the PNEC, The normalized score is assigned based on the reported concentration of PPs. Table 3.3 gives a scoring scheme for pharmaceutical pollutants under the EPs category.

Table 3.3: Normalization scheme on a 0-5 scale for the effect of PPs based on MEC and RC_T

S.No.	Pharmaceutical Compounds	Biotic Indicator Groups	Normalized Score (0-5 scale)					
			0 (RQ_h>10)	1 (10> RQ_h≥ 8)	2 (8>RQ_h≥ 5)	3 (5 >RQ_h≥ 3)	4 (3 >RQ_h≥1)	5 (RQ_h < 1)
			MEC ranges (µg/L)					
i.	Azithromycin	Algae	>1.8	1.79-1.44	1.43-0.9	0.89-0.54	0.53-0.18	<0.18
		MI	>4.4	4.39-3.52	3.51-2.2	2.1-1.32	1.31-0.44	<0.44
		Fish	>4600	4599-3680	3679-2300	2299-1380	1379-460	<460
ii	Acetaminophen	Algae	>130	130-103	104-64	65-38	39-12	<13
		MI	>92	92-73.5	73.6-45.9	46-27.5	27.6-9.2	<9.2
		Fish	>380	380-303.9	304-189.9	190-113.9	114-38	<38
iii	Amoxicillin	Algae	>50	50-39.9	40-24.9	25-14.9	15-5	<5
		MI	>1827	1827-1461	1461.6-913	913.5-548	548.1-182.7	<182.70
		Fish	NA	NA	NA	NA	NA	NA
iv	Caffeine	Algae	>1.5	1.49-1.2	1.99-0.75	0.74-0.45	0.44-0.15	<0.15
		MI	>1820	1819-1456	1455-910	909-546	545-182	<182
		Fish	>875	874-700	699-437.5	437-262.5	262-88	<88
v	Diclofenac	Algae	>2	1.9-1.6	1.5-1	0.9-0.6	0.59-0.2	<0.2
		MI	>200	199-160	159-100	99-60	59-20	<20
		Fish	>0.5	0.49-0.4	0.39-0.25	0.24-0.15	0.14-0.05	<0.05
vi	Naproxen	Algae	>318	317-254.4	254-159	158-95.4	95-31.8	<31.8
		MI	>26.2	26-20.96	20-13.1	13-7.86	7.7-2.62	<2.62
		Fish	>1152	1151-921.6	921-576	575-345.6	345-115.2	<115.2
vii	Norfloxacin	Algae	>16	15.9-12.8	12-8	7.9-4.8	4.7-1.6	<1.6
		MI	>120	119-96	95-60	59-36	35-12	<12
		Fish	>14	13.9-11.2	11-7	6.9-4.2	4-1.4	<1.4
viii	Ofloxacin	Algae	>50	50-39.9	40-24.9	25-14.9	15-5	<5
		MI	>317.5	317.5-253.9	254-158.74	158.75-95.24	95.25-31.75	<31.75
		Fish	>1010	1010-807.9	808-504.9	505-302.9	303-101	<101
ix	Sulfamethoxazole	Algae	>2.7	2.69-2.16	2.1-1.35	1.34-0.81	0.80-0.27	<0.27
		MI	>250	249-200	199-125	124-75	74-25	<25
		Fish	>5600	5599-4480	4479-2800	2799-1680	1679-560	<560
x	Triclosan	Algae	>0.14	0.14-0.111	0.112-0.069	0.07-0.041	0.042-0.014	<0.014
		MI	>3.9	3.9-3.11	3.12-1.94	1.95-1.16	1.17-0.39	<0.39
		Fish	>2.6	2.6-2.07	2.08-1.2	1.3-0.77	0.78-0.26	<0.26

Thus, based on risk conditions posed by the presence of PPs on aquatic organisms and calculated RHI, the Ecological Risk Condition (ERC) and River Health Condition (RHC) have been defined and depicted as given in Table 3.4.

Table 3.4: Color depiction of Ecological Risk Condition (ERC) and River Health Condition (RHC) based on Risk Quotient (RQ) and River Health Index (RHI)

RQ<1 and RQ_h> 1-10	Level of Ecological Risk	Ecological Risk Condition (ERC) (based on RQ and RQ_h)	Tool Used	RHI Score	RHC	Color	Category
0	Low Risk	No- Risk	RHI Calculation Framework	>80	Excellent	Blue	Acceptable
0.01-0.10		Negligible		70-80	Very Good	Green	
0.10- 1.0		Endurable		60-70	Good	Yellow	
1-3	High Risk	Moderately Stressed		50–60	Stressed	Orange	Poor
3-5		Significantly Stressed		40–50	Overstressed	Grey	
5-8		Critically Stressed		30–40	Critical	Red	
8-10		Severely Stressed		20-30	Sick	Brown	
>10		Impaired Condition		≤20	Dead		

Table 3.5 gives an overall comparative view of changes in the River Health Assessment framework developed earlier by Leigh et al. (2012), Saxena and Singh (2020), and the present study.

Table 3.5: Schematic flow model for the application of IGS in developing River Health Index.

	Source: Leigh et al. (2012)					Source: Saxena and Singh (2020)					Present Study					
Indicator Group	**P&C**	**NT**	**A**	**MI**	**F**	**OEB**	**NT**	**A**	**MI**	**F**	**DORP**	**NT**	**EPs**	**AB**	**MI**	**F**
Weightage	0.14 (w_1)	0.14 (w_2)	0.20 (w_3)	0.26 (w_4)	0.26 (w_5)	0.15 (w_1)	0.15 (w_2)	0.20 (w_3)	0.25 (w_4)	0.25 (w_5)	0.10 (w_1)	0.20 (w_2)	0.20 (w_3)	0.20 (w_4)	0.20 (w_5)	0.10 (w_6)
Parameters Considered	DO BOD_5 CODMn EC Phenols	TN TP NH_4	ABI2, ABP	MS, MBMWP, MEPTS MBP	FN, FS, FBL, FBP	EC DO BOD COD EC	NH_3-N TN TP	APPI	MSW MBMWP	FS FSW	EC, DO, BOD, COD	NH_3-N, TN TP	CAFN, DIC, SMZ	APPI, FC	MSW MBMWP	FS FSW
Normalization Scale of Parameters	0 to 1	0 to 1	0 to 1	0 to 1	0 to 1	0 to 5	0 to 5	0 to 5	0 to 5	0 to 5	0 to 5	0 to 5	0 to 5	0 to 5	0 to 5	0 to 5
Nomenclature used	Ecosystem Health Score Scale: 0-1.0	Ecosystem health score: >0.8: Excellent > 0.6 ≤ 0.8: Good ≤ 0.6: Fair ≤ 0.4: Poor < 0.2: Critical				River Health Index (RHI) Scale: 0-100		River health condition classification: **Acceptable**: >80: Excellent 70-80: Very Good 60-70: Good **Poor**: 50-60: Stressed 40-50: Over-Stressed 20-40: Critical ≤20: Sick/Dead			River Health Index (RHI): 0-100		River health condition classification: **Acceptable**: >80: Excellent 70-80: Very Good 60-70: Good **Poor**: 50-60: Stressed 40-50: Over-Stressed 20-40: Critical ≤20: Sick/Dead			