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Evaluation of Haematological Indices of *Etroplus Suratensis* and *Lutjanus Arjentimaculatus* from Kuyyali River

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ABSTRACT

Haematological parameter is the best strategy for the evaluation of the effects of poison in fish from the aquatic environment, which is showed in the blood. The present study focused on the changes evoked by industrial effluents on the haematological parameters (RBC, Hb, PCV, MCV, MCH and MCHC) of fishes, *Etroplus suratensis* and *Lutjanus arjentimaculatus.* Fishes were collected from the river Kuyyali during three seasons (summer, winter and rainy) from five different study sites (Kunduchira, Chungam, Eranjoli, Kuyyali and Koduvally) and blood was collected for the study. The statistical analysis of the data reveals that industrial effluents induced significant variations in haematological parameters of the fishes, *Etroplus suratensis* and *Lutjanus arjentimaculatus.* Significant decrease in red blood cell, haemoglobin, packed cell volume, mean corpuscular volume (MCV) mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) and increase in white blood cell (WBC) were observed as compared to control. The consequences of haematological indices shows that fishes in Kuyyali river are under strain which might be as a consequence of effect of industrial pollution.

***Key words*:** Industrial effluent, Heamatological indices, *Etroplus suratensis, Lutjanus arjentimaculatus,* Kuyyali River

Water is the most valuable of all assets in the sustenance of all living creatures on earth. Water contamination is the pollution of water bodies. This type of ecological degradation occurs when it is placed directly or indirectly discharged in water without proper treatment to remove harmful compounds. Rivers are the natural sources of freshwater. Water contamination has become a worldwide issue; it conveys a heap of dissolved and particulate matters from both normal and anthropogenic sources along with other substance. Rapid development and growth of industries in recent years has brought about the considerable expansion in effluents which, are regularly released into open land or aquatic environment causing various ecological issues. The addition of undesirable substances to the water causes changes in the physical, chemical, and biological properties of the water resulting ecological imbalance [1]. The industrial waste plays a significant role in water pollution as a hazard to oceanic plants and creatures [2-3].

Fishes are generally utilized as bio monitoring organisms in toxicological investigations as they can feature the expected risks of poisons presented in the aquatic environment [4-11]. The utilization of haematological indices in fish for valuation of effects of toxicants in environmental investigation has expanded hugely in recent years [12]. Gabriel *et al.* [13] noticed that among the cellular, biochemical, and physiological system of multicellular creatures that can be kept up with in ecotoxicology, the utilization of haematology has some particularly attractive features. Haematological measurement is a pathophysiologic model of the whole body and, as such, blood is important in the study of the composition and metabolism of fish exposed to toxins. Blood is the only substance readily available in all animals and haematological studies constitute some indices of health. Hematologic elements were chosen for the present study because the blood reflects all life processes in the body and is severe as a sign of its general condition or metabolic disorder. It is generally accepted that extreme environmental pressures cause various changes in the blood of fish.

Fishes are aquatic and poikilothermic organism. As such, the quality of the environment affects their life and performance. Fish is a significant segment of human nutrition furthermore, those from polluted sited present potential hazard to human wellbeing. It is a palatable and easily digested food which is plentiful in vitamins, calcium, phosphorous and iodine. Haematological parameters are important markers for assessing the general physiological condition of fish and can be considered as critical signals for immune response prediction, under various ecological conditions [14]. It can be considered valuable in evaluating the health of fish affected by environmental changes [15] and has been useful in monitoring adverse reactions such as biomarkers [16-17]. The present study

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deals with the study on the effect of industrial effluents on haematological parameters in fishes, *Etroplus suratensis* and *Lutjanus argentimaculatus.*

MATERIALS AND METHODS

Fishes were caught by cast net with the help of local fisherman throughout the year. The Fish was identified with morphometric characters. Blood was extracted from gills utilizing syringe and anticoagulants (ammonium oxalate, EDTA) were added and the haematological parameters such as Hb, RBC, WBC, MCV, MCH, MCHC and PCV were analyzed. All haematological parameters were verified using the standard method [18].

Determination of red blood corpuscles (RBC) count

RBC count was read by the development of the Neubauer crystalline counting chamber [19]. The blood was drawn over 0.5mark onto the RBC tube and instantly, Hayem's solution was sucked up to 101 marks and revolved the tube between the thumb and the forefinger so that the solution could be thoroughly mixed. (dilution: 1:200). Erythrocytes were counted in the loaded room haemocytometer and total numbers were reported at 106 mms [20].

Determination of white blood corpuscles (WBC) count

WBC count was determined [21]. The blood was drawn up to the level 0.5 in WBC pipette and instantly the diluting fluid was drawn up to the level of 101 above the bulb (the dilution fluid consist of 1.5ml of glacial acetic acid and 1 ml of aqueous gentian violet solution and made up to 100 ml with refined water). Blend the arrangement completely by shaking gently and permitted to stand for 3 min. The cleaned Neubauer counting chamber and cover glass were placed over the ruled area. Surplus solution was ousted and a drop of fluid was permitted to flow under the cover slip by holding the pipette at an angle of 40^0 and permitted to stand for 2 to 3 min. The WBC was measured at the four corner square millimetres and the number of WBC per cubic millimetre was determined.

Estimation of haemoglobin (Hb)

Hb blood levels were monitored using the cyanmethaemoglobin method [22]. Hb is changed into cyanmethaemoglobin by the addition of potassium ferricyanide (KCN) and the dye was read on a spectrophotometer at 540 nm against a reagent blank.

Determination of packed cell volume (PCV)

Packed cell volume was estimated by micro haematocrit strategy [23]. With the assistance of Pasteur pipette the heparinised blood was occupied up to the level of 100 in the haematocrit tube and centrifuged at 3000 rpm for 30min. The overall volume of the stature of the RBC's packed at the base of the haematocrit tube was recorded as packed cell volume in terms of percentage of total number of blood column carried in to the haematocrit tube.

Determination of mean corpuscular volume (MCV)

MCV determines the normal size of the blood cell in a given blood sample. MCV was assessed by the accompanying equation and expressed as femtoliter (fL).

$$MCV = Haematocrit\ (\%) \times 10 / RBC\ count$$

Determination of mean corpuscular haemoglobin (MCH)

MCH represents the average Hb content in each red blood cell. MCH is involved in the management of the HB concentration and the number of RBC. MCH was estimated by the formula given below and expressed in picogram (pg).

$$MCH = haemoglobin\ (g/dL) \times 10 / RBC\ count$$

Mean corpuscular haemoglobin concentration (MCHC)

MCHC expresses the average concentration of the haemoglobin in the red blood cells MCHC was acquired by the given formula and expressed in terms of gram percent (g%).

$$MCHC = haemoglobin\ (g/dL) \times 100/\ haemoglobin\ (\%)$$

RESULTS AND DISCUSSION

Various haematological parameters like RBC, WBC, Hb, PCV, MCV, MCH, MCHC were analyzed in the fishes, *L.argentimaculatus* and *E.suratensis* during three season. The values were presented in the form of (Table 1-6). In the present investigation a significant fluctuation in all haematological parameters were observed. A significant decrease in erythrocyte count, haemoglobin, MCV, MCH, MCHC and PCV whereas the WBC found to be increased. Changes in haematological indices in the fish *Lutjanus argentimaculatus* during rainy, winter and summer season were presented in the (Table 1-3) and in the fish *Etroplus suratensis* were presented in the (Table 4-6). Pollution of the aquatic environment through manufacturing, municipal waste and agriculture is a major concern for fish health.

Anaemia may be due to decreased red cell count, high haemoglobin, and elevated haematocrit. Singh *et al*. [24] found that *Cirrhinus mrigala* and *Catla catla* fish had decreased haemoglobin percentage, RBC count and PCV percentage significantly leading to anaemia. In the above study RBC count of fishes, *Lutjanus argentimaculatus* and *Etroplus suratensis* was decreased maximum in summer. In *Lutjanus argentimaculatus* it was 1.03±0.26 in summer and in winter it was recorded 1.73±0.26 and while in rainy season 2.23±0.26. In *Etroplus suratensis* it was 1.53±0.26 in summer and in winter it was recorded 2.03±0.26 and while in rainy season 2.40±0.16. The reduction in the number of circulating Red Blood Cells probably reflects the physiological functioning of haemopoietic system, which is viewed to be the most sensitive indicator towards environment pollutants.

Low haemoglobin can reduce the ability of the fish to improve its functions to meet special needs such as seeking food and escape [25]. In *Lutjanus argentimaculatus* minimum amount of haemoglobin recorded in summer season (1.8±0.13), while in winter and rainy season it was recorded 2.8±0.28 and 3.10±0.28. In *Etroplus suratensis* minimum amount of haemoglobin recorded in summer season (2.4±0.13), while in winter and rainy season it was recorded 3.1±0.23 and 3.9±0.23. Witeska and Kosciuk [26] also reported that the heavy metals such as Hg, Cd, Cr, Cu, Zn, As, Ni and Pb present in the effluent alter the haemoglobin levels by reducing their affinity towards oxygen binding capacity rendering the erythrocytes more delicate and penetrable and causing in cell swelling, distortion and damage. Reduction in haemoglobin concentration indicates confined capacity of fish to give adequate oxygen to the tissues and this outcomes in decrease of physical activity.

Increased WBC count in *Lutjanus argentimaculatus* and *Etroplus suratensis* was recorded in summer season. In *Lutjanus argentimaculatus* it was 6200±322.07 in summer while in winter and rainy season it was recorded 5400±297.07 and 3500±197.07. In *Etroplus suratensis* it was 7400 ± 436.07 while in winter and rainy season it was recorded 5100 ± 109.23 and 2900 ± 135.45. Elevated white blood cell counts specify damage caused by infection of body tissues, severe physical stress, and as well leukaemia.

In *Lutjanus argentimaculatus* minimum amount of PCV recorded in summer season (06.7±0.32), while in winter and rainy season it was recorded 08.3±0.42 and 09.0 ± 0.42. In *Etroplus suratensis* minimum amount of PCV recorded in summer season (06.0 ± 0.32), while in winter and rainy season it was recorded 06.5 ± 0.42 and 07.0 ± 0.52. The reduction in PCV % may be due to the bioaccumulation of the toxicant in the body.

MCV, MCH and MCHC are the components of full blood count and they are additionally called as red cell markers. It implies that they express the size and haemoglobin content of erythrocytes. In *Lutjanus argentimaculatus* lowest value of MCV is in summer (8.16±0.45), while in winter and rainy season it was recorded 07.4 ± 0.42 and 10.4 ± 0.52. In *Etroplus suratensis* lowest value of MCV is in summer (4.6 ± 0.32), while in winter and rainy season it was recorded 8.5 ± 0.42 and 10.04 ± 0.72. The decline in MCV with low haemoglobin content demonstrates that the red blood cells have contracted, either because of hypoxia or microcytic anaemia.

In *Lutjanus argentimaculatus* lowest value of MCH is in summer (2.3±0.18), while in winter and rainy season it was noticed 3.4±0.24 and 09.3±0.47. In *Etroplus suratensis* lowest value of MCH is in summer (04.2±0.29), while in winter and rainy season it was calculated 06.3±0.36 and 07.5±0.64. The variation in the MCH in the current examination, clearly shows that the level of haemoglobin in the red blood cells were much lower and thus indicate anaemic condition.

In *Lutjanus argentimaculatus* lowest value of MCHC is in summer (6.2±0.41), while in winter and rainy season it was noticed 8.00±0.45 and 09.4±0.46. In *Etroplus suratensis* lowest value of MCHC is in summer (03.6±0.23), while in winter and rainy season it was calculated 04.3±0.32 and 07.0±0.71. The critical decline in the MCHC is most likely a sign of red blood cell swelling and /or to a reduction in haemoglobin synthesis.

Table 1 Haematological changes in blood of the fresh water fish, *Lutjanus argentimaculatus* at different sampling stations of Kuyyali river during rainy season

Parameters	S_1	S_2	S_3	S_4	S_5
Total RBC count (millions / cu mm)	4.62 ± 0.30	2.23± 0.26	4.08 ± 0.31	4.12 ± 0.15	4.08 ± 0.31
't' value		2.67*	0.63n	0.26nss	0.63n
Percent (%)		-51.7	-11.6	-10.8	-11.6
Total WBC count (cells / cu mm)	1300 ± 109.18	3500 ± 197.07	3100 ± 201.13	1800 ± 145.12	2100 ± 169.13
't' value		281.23**	331.58**	250.52**	275.38**
Percent (%)		+169.2	+138.4	+38.4	+61.5
Blood haemoglobin level (gms/dl)	5.0± 0.35	3.10 ± 0.28	3.9 ± 0.23	4.4 ± 0.36	4.1 ± 0.33
't' value		2.34*	2.1*	1.95*	1.68ns
Percent (%)		-38	-22	-12	-18
PCV count (%)	14.7 ± 0.79	09.0 ± 0.42	13.4 ±0.62	14.4 ±0.75	13.5 ± 0.59
't' value		3.29ns	0.85ns	0.25ns	0.63ns
Percent (%)		-38.7	-8.8	-2.04	-8.1
MCV (cu mm)	18.0 ± 0.79	10.4 ± 0.52	11.4±0.62	16.0±0.75	13.5 ± 0.59
't' value		7.29**	6.25**	1.85*	4.63**
Percent (%)		-42.2	-36.6	-11.1	-25
MCH (pg)	11.9±0.56	09.3±0.47	09.5±0.45	10.5±0.53	10.3±0.52
't' value		7.6**	2.89*	0.45ns	0.34ns
Percent (%)		-21.8	-20.1	-11.7	-13.4
MCHC (%)	11.7±0.54	09.4±0.46	09.7±0.47	10.6±0.58	10.0±0.51
't' value		1.78*	1.89*	0.67ns	0.54ns
Percent (%)		-19.6	-17.09	-9.4	-14.5

Table 2 Haematological changes in blood of the fresh water fish, *Lutjanus argentimaculatus* at different sampling stations of Kuyyali river during rainy season

Parameters	S_1	S_2	S_3	S_4	S_5
Total RBC count (millions / cu mm)	3.62 ± 0.10	1.73 ± 0.26	2.43 ± 0.15	2.93 ± 0.23	2.62± 0.11
't' value		1.64ns	1.26*	0.68ns	1.03ns
Percent (%)		-52.2	-32.8	-19.06	-27.6
Total WBC count (cells / cu mm)	3400 ± 163.18	5400 ± 297.07	4600 ± 388.13	4200± 327.12	4300 ± 311.13
't' value		381.23**	451.58**	460.52**	485.38**
Percent (%)		+58.8	+35.2	+23.5	+26.4
Blood haemoglobin level (gms/dl)	4.6 ± 0.35	2.8 ± 0.28	2.9 ± 0.13	3.6 ± 0.26	3.2 ± 0.13
't' value		1.84*	2.1*	0.95ns	0.68ns
Percent (%)		-39.1	-36.9	-21.7	-30.4
PCV count (%)	13.2 ± 0.69	08.3±0.42	08.4 ± 0.42	11.8±0.75	9.5 ± 0.49
't' value		4.25**	4.29**	1.85*	3.63*
Percent (%)		-37.1	-36.3	-10.6	-28
MCV (cu mm)	17.1 ± 0.89	07.4 ± 0.42	9.3 ± 0.49	15.6±0.75	09.4±0.42
't' value		9.29**	7.63**	1.85*	7.25**
Percent (%)		-56.7	-45.6	-8.7	-45.02
MCH (pg)	09.4±0.47	3.4±0.24	06.56±0.39	8.56±0.47	07.5±0.43
't' value		5.69**	2.78*	0.65ns	1.59*
Percent (%)		-63.8	-30.2	-8.9	-20.2
MCHC (%)	10.8±0.53	8.00±0.45	8.6±0.44	9.3±0.48	9.0±0.47
't' value		1.89*	1.76*	1.5ns	1.8*
Percent (%)		-25.9	-20.3	-13.8	-16.6

Table 3 Haematological changes in blood of the fresh water fish, *Lutjanus argentimaculatus* at different sampling stations of Kuyyali river during rainy season

Parameters	S_1	S_2	S_3	S_4	S_5
Total RBC count (millions / cu mm)	2.46 ± 0.15	1.03 ± 0.26	1.19 ± 0.15	2.14 ± 0.23	2.03± 0.11
't' value		1.64ns	1.26ns	0.68ns	0.63ns
Percent (%)		-58.1	-51.6	-13	-17.4
Total WBC count (cells / cu mm)	4400 ± 246.18	6200 ± 322.07	6100 ± 346.13	5200± 297.12	5800± 311.13
't' value		451.23**	478.58**	370.52**	495.38**
Percent (%)		+40.9	+38.6	+18.1	+31.8
Blood haemoglobin level (gms/dl)	3.8 ± 0.25	1.8 ± 0.13	2.4 ± 0.18	3.0 ± 0.26	2.7 ± 0.13
't' value		2.1*	1.34ns	0.8ns	1.68ns
Percent (%)		-52.6	-36.8	-21.05	-28.9
PCV count (%)	11.6 ± 0.59	06.7±0.32	08.1 ± 0.32	10.7±0.55	8.8± 0.49
't' value		4.25**	2.29*	0.85ns	2.63*
Percent (%)		-42.2	-30.1	7.7	-24.1
MCV (cu mm)	11.4 ± 0.59	8.16±0.45	8.2± 0.42	9.8 ± 0.49	8.4±0.42
't' value		2.85*	2.29*	7.63**	2.25*
Percent (%)		-28.4	-28	-14.03	-26.3
MCH (pg)	6.6±0.34	2.3±0.18	05.31±0.38	6.1±0.37	5.9±0.39
't' value		4.3**	1.3ns	0.5ns	0.89ns
Percent (%)		-65.1	-19.5	-7.5	-10.6
MCHC (%)	7.15±0.43	6.2±0.41	6.3±0.35	7.06±0.48	7.06±0.48
't' value		0.76ns	0.65ns	0.67ns	0.67ns
Percent (%)		-13.2	-11.8	-1.2	-1.2

Table 4 Haematological changes in blood of the fresh water fish, *Etroplus suratensis* at different sampling stations of Kuyyali river during rainy season

Parameters	S_1	S_2	S_3	S_4	S_5
Total RBC count (millions / cu mm)	3.93 ± 0.20	2.40 ± 0.16	2.63 ± 0.15	3.83 ± 0.13	3.16 ± 0.11
't' value		1.64ns	1.26ns	0.68ns	0.63ns
Percent (%)		-38.93	-33.07	-2.54	-19.5
Total WBC count (cells / cu mm)	2100 ± 104.21	2900 ± 135.45	2600 ± 125.03	2600 ± 126.34	2500 ± 118.96
't' value		224.18**	241.58**	239.52**	253.38**
Percent (%)		+38.09	+23.8	+23.8	+19.04
Blood haemoglobin level (gms/dl)	5.6 ± 0.35	3.9 ± 0.23	4.5 ± 0.38	5.4 ± 0.36	4.9 ± 0.33
't' value		2.7*	1.1ns	0.95ns	1.8*
Percent (%)		-30.35	-19.6	-3.57	-12.5
PCV count (%)	12.4 ± 0.69	07.0 ± 0.52	10.2 ±0.82	11.6 ±0.75	11.0 ± 0.59
't' value		9.4**	2.25*	0.85ns	0.63ns
Percent (%)		-43.5	-17.7	-6.45	-11.29
MCV (cu mm)	19.1 ± 0.89	10.04 ± 0.72	10.41±0.62	12.4 ±0.75	10.9 ± 0.79
't' value		9.29**	9.25**	7.42**	9.63**
Percent (%)		-47.4	-45.4	-7.35	-42.9
MCH (pg)	10.1±0.79	07.5±0.64	08.5±0.67	09.6±0.83	09.5±0.72
't' value		2.74*	1.89*	0.79ns	0.86ns
Percent (%)		-25.7	-15.8	-4.95	-5.94
MCHC (%)	17±0.84	07.0±0.71	07.3±0.65	09.9±0.79	08.5±0.73
't' value		10.0**	9.79**	7.65**	8.58**
Percent (%)		-58.8	-57.05	-41.7	-50

Table 5 Haematological changes in blood of the fresh water fish, *Etroplus suratensis* at different sampling stations of kuyyali river during winter season

Parameters	S_1	S_2	S_3	S_4	S_5
Total RBC count (millions / cu mm)	2.93 ± 0.18	2.03 ± 0.26	2.04 ± 0.15	2.84 ± 0.13	2.43± 0.11
't' value		0.64ns	0.26ns	0.68ns	0.63ns
Percent (%)		-30.7	-30.3	-3.07	-17.06
Total WBC count (cells / cu mm)	2900 ± 113.54	5100 ± 109.23	4100 ± 121.13	3200 ± 119.78	4000 ± 153.13
't' value		261.23**	271.58**	290.52**	265.38**
Percent (%)		+75.8	+41.3	+10.3	+37.9
Blood haemoglobin level (gms/dl)	4.4 ± 0.35	3.1 ± 0.23	3.7 ± 0.28	4.1 ± 0.36	4.0 ± 0.23
't' value		1.1ns	1.34ns	0.95ns	0.68ns
Percent (%)		-29.5	-15.9	-6.81	-9.09
PCV count (%)	12.0 ± 0.69	06.5 ± 0.42	6.1 ±0.42	10.6 ±0.55	9.5 ± 0.49
't' value		5.29**	5.25**	2.85*	3.63*
Percent (%)		-45.8	-49.16	-11.66	-20.83
MCV (cu mm)	11.4 ± 0.59	8.5 ± 0.42	9.6±0.42	11.0±0.75	9.61 ± 0.49

't' value		2.29*	2.25*	0.85ns	2.63*
Percent (%)		-25.4	-15.78	-3.5	-15.7
MCH (pg)	08.7±0.48	06.3±0.36	07.5±0.41	08.4±0.46	07.6±0.47
't' value		2.4*	1.2ns	0.3ns	1.1ns
Percent (%)		-27.58	-13.79	-3.4	-12.6
MCHC (%)	11.0±0.53	04.3±0.32	05.3±0.45	09.0±0.48	06.9±0.38
't' value		6.57**	5.87**	2.01*	5.25**
Percent (%)		-60.9	-51.8	-18.18	-37.2

Table 6 Haematological changes in blood of the fresh water fish, *Etroplus suratensis* at different sampling stations of kuyyali river during summer season

Parameters	S_1	S_2	S_3	S_4	S_5
Total RBC count (millions / cu mm)	2.04 ± 0.10	1.53 ± 0.26	1.56 ± 0.15	2.03 ± 0.23	1.82± 0.11
't' value		1.64ns	1.26ns	0.68ns	1.63ns
Percent (%)		-25.1	-23.5	-0.49	-10.78
Total WBC count (cells / cu mm)	3800 ± 120.18	7400 ± 436.07	6200 ± 378.13	4200± 289.12	6000± 351.13
't' value		611.23**	692.58**	712.52**	675.38**
Percent (%)		+94.7	+63.1	+10.5	+57.8
Blood haemoglobin level (gms/dl)	3.51 ± 0.25	2.4 ± 0.13	2.6 ± 0.18	3.5 ± 0.26	2.9 ± 0.23
't' value		1.15ns	0.34ns	0.95ns	1.45ns
Percent (%)		-31.6	-25.9	-0.28	-17.3
PCV count (%)	11.5 ± 0.59	06.0 ± 0.32	06.1±0.32	67.0±4.65	06.4 ± 0.49
't' value		5.29**	5.25**	56.85**	5.63**
Percent (%)		-47.8	-46.9	-39.1	-44.3
MCV (cu mm)	10.4 ± 0.59	4.6 ± 0.32	07.1±0.32	09.4±0.45	07.4 ± 0.39
't' value		6.29**	3.3*	1.0ns	3.63*
Percent (%)		-55.7	-31.7	-9.61	-28.8
MCH (pg)	07.5±0.41	04.2±0.29	06.3±0.36	06.9±0.36	06.8±0.35
't' value		3.3*	1.2ns	0.65ns	0.63ns
Percent (%)		-44	-16.1	-8	-9.3
MCHC (%)	10.5±0.56	03.6±0.23	04.2±0.32	08.7±0.46	06.0±0.37
't' value		6.8**	5.6**	1.89*	3.5*
Percent (%)		-65.7	-60	-17.1	-42.8

CONCLUSION

The present study reveals that due to the influence of industrial effluent the amount of RBC, Hb, MCV, MCH, PCV and MCHC have been decreased in blood of fishes, *L. argentimaculatus* and *E. suratensis* and the amount of WBC has been increased as an immunological defence to survive against the harmful substance in the effluent.

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