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Research Paper

Hematological changes in the Indian Murrel (*Channa punctatus* Bloch) in response to phenolic industrial wastes of the Bhilai Steel plant (Chhattisgarh, India)

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Abstract - *Hematological parameters may be considered useful as health indicators of fish during changing environmental conditions. Indiscriminate dumping of phenolic industrial wastes generated from the Coke ovens of steel plants into rivers pose serious threats to aquatic life. The present study describes the daily changes in the hematological parameters (TEC, TLC and gm % Hb) of the Indian murrel Channa punctatus (Bloch) upon short term exposure to low and high concentrations of phenolic effluent of the Bhilai Steel Plant, Chhattisgarh (INDIA). Commercially available Phenol in the same concentration has been taken as a reference for comparison. Fishes adapted to environmental change by significant augmentation in their TEC, TLC and Hb(g/dl) up to 120 hours followed by a tendency to resume back normal conditions thereafter.*

Keywords: Bhilai Steel Plant, Phenolic industrial wastes, Coke ovens, Hematology, *Channa punctatus* (Bloch) etc.

Introduction

The onset of the industrial revolution has led to massive exploitation of a vast amount of our natural resources within a period of a few hundred years at unimaginable rates. Large amounts of wastes generated are being dumped straight in to the environment seriously damaging its natural processes. Fishes are aquatic and poikilothermic animals and perform best under a narrow range of optimum environmental conditions. A slight diversion from these, results in *stress* leading thereby to disease [1]. Hematological parameters may be considered useful in assessing the health of fish subjected to changing environmental conditions [2,3] and have proven useful in monitoring stress responses as bio-indicators [4,12].

The Bhilai Steel Plant is an integrated steel plant situated 30 kilometers (west) of Raipur, the capital of the state of Chhattisgarh (INDIA). Besides the major marketable product which is good quality steel, it also produces important by products, such as, Coal tar, Naphthalene and Benzol. Coke which is used as a fuel and reducing agent in the blast furnace is obtained from the conversion of coal in the coke ovens. The effluent thus generated, has a strong phenolic odour and contains a high amount of phenol, besides the presence of other toxic substances [13]. Primarily, this phenolic wastewater is being used for irrigation purposes by the local villagers, since its use causes the onset of skin rashes in humans and cattle. After travelling an approximate distance of 15 kilometers, it is further dumped into the river Kharoon which caters to the needs of Raipur, the capital of Chhattisgarh and adjoining cities. The soil texture of the embankment of the channel also appears to be oily black. Inevitably, all these factors raise questions on the toxic effects of the effluent on the aquatic life dwelling in the river.

Phenol has been reported to lead to threatening environmental conditions due to its lipophilic nature [14], genotoxic[15],carcinogenic [16] and immunotoxic[17]properties. Some phenolic compounds, such as, Polycyclic Aromatic Hydrocarbons and Polychlorinated biphenyls are Endocrine disrupters [18]-[22] and adversely affect fish metabolism[23][24]. Most importantly, they are reported to have cytotoxic effects on fish [25] and human [26] erythrocytes. Large uptake of phenol above lethal concentrations by fish erythrocytes [27]and severe changes in erythrocyte membrane

properties in humans due to Chlorophenol exposure [28] have also been reported .Thus the present work on the hematological changes in the Indian Murrel (*Channa punctatus* Bloch) in response to phenolic industrial wastes of the Bhilai Steel plant (Chhattisgarh,India) has been undertaken.

Material and Methods

Collection and analysis of effluent

The effluent discharged by the coke oven of the Bhilai Steel Plant was collected in small closed bottles from the origin point of the effluent channel at Purena (Bhilai 3) and analyzed for its physico-chemical characteristics by standard methods [29]. The analysis report of the effluent along with tap water is presented in Table 1.

Short term exposure

Live, healthy *Channa punctatus* in the same size range were collected from local streams and acclimatized under normal laboratory conditions for 15 days. For the exposure of fishes, two concentration ranges viz., low (0.01, 0.015, 0.02 and 0.025%) and high (10, 20 and 30%) of the whole phenolic effluent were taken after dilution with normal tap water. Commercially available Phenol was taken in similar concentrations for comparison. Normal tap water served as control.

The fishes were divided into 16 groups consisting of 28 fishes each. Nine of the groups were individually exposed to Tap water (Control) and low concentrations (0.01, 0.015, 0.02 and 0.025%) of Phenol and Effluent in 20 liters glass aquaria. The remaining 7 groups were similarly exposed to the higher concentration ranges (10, 20, and 30%) of the two toxicants along with tap water as control. Feeding of fishes and aeration of the tanks were done uniformly throughout the experiment.

Collection of blood samples from live fishes

Blood samples were collected separately from 4 live fishes from each group at 24,48,72,96,120,144 &168 hours by severing the caudal peduncle.

Blood Analysis

The samples collected were analyzed for the Total Erythrocyte count(TEC) and Total Leukocyte count(TLC) in Cells/ cu.mm and Hemoglobin(g/dl) [30].The results obtained were analyzed statistically by ANOVA(two way with replication) using MS Excel.

The whole experiment was repeated twice in summer (set1 and 2) and twice in winter (set1 and 2) and similar results obtained each time. The overall effects of phenol and phenolic effluent on the hematology of *C.punctatus* are presented below as mean values of the above mentioned 4 sets.

Results and Discussion

Observations made on the TEC, TLC and g/dl Hb after short term exposure of *Channa punctatus* to low and high concentrations of phenol and phenolic effluent are shown in Tables 2- 7. Percent rise in hematological parameters of exposed fishes vis-avis control have been represented in Figures1- 12.

The following abbreviations have been used throughout- Control (CON), Phenol (P), Effluent (E), Concentrations used (%v/v) -Low - 0.01(P1/E1), 0.015(P2/E2), 0.02(P3/E3), 0.025(P4/E4), High- 10(P10/E10), 20(P20/E20), 30(P30/E30).

Effect of low concentrations (0.01, 0.015, 0.02, 0.025 %)

(a) Total Erythrocyte Count (Cells/ cu.mm)

Effect of low concentrations(0.01,0.015,0.02,0.025%) of Phenol (Figure 1)shows a gradual % rise above normal in TEC, being maximum at 96 hours(26.02,29.07,37.64 and 37.84) and followed by a plateau at 120 hours. Thereafter, TEC in P1,P2 and P3 gradually declined to 19.55, 24.87, 31.62 percent above normal. In P4 however, TEC rose by 46.70% up to 144 hours and declined thereafter. All changes were found highly significant by ANOVA ($F= 16.938$, $P<0.0001$). A similar significant change ($F=12.356$;$P<0.0001$) was also observed in case of whole effluent .TEC rose gradually above normal upto 96 hours, with a maximum of 24.52,32.25 ,39.86 ,41.45 % respectively, in all 4 concentrations (E1,E2,E3 and E4). Plateau was reached at 120 hours, which declined to 13.82, 17.77, and 22.14% above normal at168 hours. Similar to phenol, in E4, TEC rose to 53.87% at 144 hours and declined thereafter (Figure 2).

(b) Total Leucocyte count (cells/cu.mm)

TLC was observed to rise gradually up to 96 hours in Phenol (P1, P2, P3 and P4), respectively being 19.21, 26.32, 24.18, 34.51 percent above normal and declining thereafter to 9.7, 18.36, 18.3 and 20.93 % above normal in 168 hours (Figure 3). All results were found to be highly significant ($F=118.865$, $P<0.0001$). Similarly, upon exposure to effluent, TLC rose significantly above normal by 25.79,24.66,23.77,35.26 percent , respectively in all four concentrations,E1,E2, E3 and E4 ($F=105.689$,$P<0.0001$) up to 96 hours and declined thereafter to 9.73,20.77,18.82 and 26.6% above normal in 168 hours (Figure 4).

(c) Haemoglobin(g/dl)

All 4 concentrations of Phenol (P1,P2,P3,P4 respectively) significantly affected g/dl hemoglobin ($F=15.934$,$P<0.0001$) by causing a gradual % rise of 13.04,14.58 and 18.30 upto 96 hours,followed by a plateau(14.34,14.52,19.24,18.60) upto 120 hours ,thereafter rising upto 144 hours in P1 and P4 and declining at 168 hours (Figure 5).Effect of effluent **(**E1,E2,E3,E4 respectively), showed a gradual % rise 12.34,16.19,20.66,20.43 up to 96 hours followed by a decline in E2 and E3.In E1 and E4 gm% Hb rose upto 144 hours and declined thereafter (Fig 6). All results were found significant at $F=12.258$, $P<0.0001$.

Effect of high concentration(10, 20 & 30%) on

(a) Total Erythrocyte Count (Cells/ cu.mm)

Figure 7 shows a gradual % rise from 2.05 to 14.89 above normal in TEC upto 120 hours followed by a decline to 8.48 in P10.Effect of P20 on the other hand,shows an abrupt rise in TEC from 10.82% above normal at 24 hours to 19.09% at 48 hours followed by a plateau upto 72 hours followed by a hike to 33.73% at 168 hours.At P30 % rise declines from 19.53 at 72 hours to 9.73 at 96 hours followed by a % rise upto 24.67 above normal at 168 hours.All results were found significant at F=10.53,P<0.0001.Figure 1 on the other hand shows a decline of 4.42 % below normal at 48 hours, followed by an abrupt hike of 7.93 % above normal at 72 hours rising gradually to 13.25% at 168 hours at E10.Effect of E20 shows an abrupt % hike of 27.52 above normal at 48 hours followed by a decline to 18.10 and 17.19 at 96 and 120 hours and rising subsequently to 35.03 % above normal at 168 hours. At E30 TEC rises up to 72 hours (23.70) declining thereafter upto 120 hours (10.62) followed by a hike to 29.48% above normal at 168 hours. All results varied significantly at F=9.24, P<0.0001.

(b) Total Leucocyte Count (Cells/ cu.mm)

Effect of P10,P20 and P30, respectively, show a gradual % rise in TLC from 22.28,33.41 ,32.75 in 24 hours, being maximum at 120 hours (58.65,79.15,87.67) and declining (36.77,57.82,72.95) thereafter up to 168 hours(Figure 9). Variations were highly significant at F=68.65, P<0.0001.A gradual % rise in TLC from 22.48,27.50,32.65 at 24 hours of exposure to 50.63,72.00,100.83 at 168 hours seen in case of all 3 concentrations of effluent(Figure 10).% rise in TLC was found to increase with concentration of effluent. Variations were highly significant at F=793.45, P<0.0.0001.

(c) Haemoglobin (g/dl)

Effect of P10 shows a gradual % rise in Hb from (-0.91) below normal in 48 hours to 6.45 above normal in 120 hours, followed by decline (-0.24) below normal in 144 hours (Figure 11). While, P20 shows a gradual % rise upto14.73 in 168 hours, P30 shows rises up to 9.02 in 72 hours followed by a decline (5.03, 5.41) in 96 and 120 hours respectively, rising subsequently upto 9.70 in 168 hours. Variations were significant at F=9.29, P<0.0001.Figure 12 shows an initial % rise upto (2.73, 10.72 and 10.84) in 72 hours followed by decline to 1.77,8.02,4.98% in 120 hours and rising subsequently to 4.20,15.08,12.11% in 168 hours. Variations were significant at F=9.22, P<0.0001.

Summarizing, results obtained show that the overall effect of phenol and phenolic effluent leads to a marked % elevation above normal up to 120 hours, followed by a decline thereafter in TEC,TLC and g/dl Hb upon short term exposure to low concentrations (0.01,0.015,0.02 and 0.025%) of phenol and whole effluent. Percent rise was found to increase with increasing concentrations. In all cases, the fishes showed a tendency of resuming back their normal TEC levels from the augmented values, may be as a consequence of adaptive response to low concentrations of the effluent. This property however, was found to be lost at higher concentrations (P20 and P30).The time required for this resumption back to normal increased with increasing concentration. The increase in the number of circulating RBC in the present work is comparable to observations made by [31-34]. It may be opined that increased RBC count probably reflects hypoxic stress exposure of the fish resulting in secondary polycythaemia.

An augmentation in TLC up to 120 hours followed by decline thereafter was visible. % rise was found to be proportional to increasing concentration of phenol .In case of whole effluent, on the contrary, TLC went on increasing gradually up to 168 hours. WBC are phagocytic and scavenger cells. The increase in WBC count or leucocytosis in treated fishes probably reflects increased demand for WBC for the removal of cellular debris at a faster rate. The increase in concentration of the stressor probably puts a greater demand for phagocytic cells.

Similar disturbances were also seen in case of g/dl hemoglobin.It is agreeable with [34-35] that the increase in Hb content reflects an adaptive response by fishes attempting to increase oxygen transport in the face of hypoxic stress.

Stress means the sum of all the physiological responses by which an animal tries to maintain or reestablish a normal metabolism in the face of physical and chemical forces [36].A series of morphological, biochemical and physiological changes occur as a result of stress and constitutes the General Adaptation Syndrome (GAS) [1]. Results obtained in this case clearly depict that the fishes exposed to low concentrations of whole effluent showed a tendency to adapt to changing environmental condition by a highly significant augmentation in TEC, TLC and g/dl hemoglobin up to 120 hours, followed by a decline thereafter at 168 hours. On the contrary, the tendency to resume back to normal conditions, although visible in the lowermost level (10%) of high concentration of whole effluent, disturbances (rise followed by decline and subsequent rise) in cell counts proportional to the concentration of effluent were visible in fishes exposed to higher(20%,30%) concentrations. Thus, it may be speculated that higher concentrations of whole effluent may be leading the fishes to a stage of exhaustion when adaptation to stress has been lost due to severity of the stressor. The present authors strongly opine that although fishes generally try to adapt to stressful conditions of a phenolic environment in short term exposures, long term exposures could be harmful to these aquatic beings even in very low concentrations. According to the National Recommended Water Quality Criteria phenol has been listed as a priority pollutant with an organoleptic effect criterion of 300µg/l [37]. It is very toxic to fish and has a unique quality of tainting the taste of fish, if present in marine environments at 0.1-1.0 ppm[38-39].Hence, dumping of this phenolic wastewater of the Purena Channel into

the river Kharoon may have deleterious consequences and must be taken care off.

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Table 1.Physico-chemical characteristics (Mean values of winter & summer set1 and 2) of coke oven whole effluent (Bhilai Steel Plant) obtained from origin point of Purena channel vis avis Tap water and National recommended water quality criteria for freshwater organisms according to USEPA.

Physicochemical parameters	Purena Nala	Tap Water	NRWQC
pH	9.23±0.46	7.20±0.21	9.00
Temp(°C)	32.00±8.12	30.50±0.71	-
Colour	Black	Colorless	-
Smell	Phenolic	Odorless	-
DO (mg/l)	8.75±0.91	5.46±1.18	-
BOD(mg/l)	122.92±34.12	37.00±9.27	-
Alkalinity(mg/l)	211.41	53.14	20.00
chloride(mg/l)	263.00±30.87	145.24±8.88	230.00
Nitrate(mg/l)	4.46±0.81	0.85±0.07	10.00
Nitrite(mg/l)	5.73±0.92	2.97±0.16	-
Sulphates(mg/l)	402.75±44.23	164.67±35.00	-
Phenol(mg/l)	6.81±1.35	0.48±0.46	10.00
Ca(mg/l)	108.00±18.97	76.27±6.00	-
Mg(mg/l)	39.50±13.96	28.00±2.65	-
Hardness(mg/l)	525.89±92.44	360.33±74.57	-
TDS(mg/l)	807.65±95.08	358.00±27.87	250.00
TSS(mg/l)	218.50±79.72	245.25±123.82	-
TS(mg/l)	870.75±60.85	436.00±15.10	-

Table 2. Effect of low concentration phenol and effluent on TEC (Cells x10^4/cu.mm) of *Channa punctatus* (mean values ± SD).

Treatment	Duration of exposure (hours)						
	24	48	72	96	120	144	168
CON	50.31±3.20	53.66±2.30	54.82±1.37	55.00±1.74	54.73±7.02	53.16±1.95	55.41±5.89
P1	50.80±5.18	56.69±6.27	61.81±7.18	69.31±9.21	70.49±5.12	71.25±5.17	69.19±5.54
P2	52.18±6.05	57.88±6.31	63.30±7.17	70.99±9.33	71.68±5.12	68.44±4.39	66.24±11.42
P3	55.00±8.49	61.13±8.93	66.75±11.36	75.69±14.81	76.44±9.68	73.19±9.19	72.93±18.55
P4	57.55±9.14	64.68±9.84	69.68±10.98	75.81±13.48	75.68±10.61	77.99±8.21	78.18±13.74
E1	50.75±1.89	56.38±2.39	62.30±3.18	68.49±3.85	70.06±2.86	69.18±6.59	63.06±3.00
E2	52.56±2.73	58.55±3.91	65.05±5.78	72.74±7.85	71.31±4.14	69.00±2.25	65.25±7.69
E3	55.44±4.33	62.44±6.46	69.63±7.31	77.80±11.21	76.94±5.23	70.25±5.68	67.68±11.92
E4	57.05±3.63	63.81±4.29	70.31±8.50	76.93±11.32	77.94±6.76	81.80±0.73	77.18±6.89

Table 3. Effect of low concentration of phenol and effluent on TLC (Cells x10^2/cu.mm) of *Channa punctatus* (mean values ± SD).

Treatment	Duration of exposure (hours)						
	24	48	72	96	120	144	168
CON	94.68±1.37	97.06±7.28	98.06±1.80	99.25±8.94	100.62±2.88	99.06±3.10	102.43±1.80
P1	96.31±2.84	99.81±2.29	107.12±2.93	118.31±7.00	118.68±0.64	113.59±1.98	112.37±6.94
P2	105.31±1.06	111.93±0.71	119.31±1.56	125.37±2.08	124.9±6.03	119.62±6.37	121.25±2.2
P3	100.62±3.8	106.03±3.88	114.37±4.33	123.25±8.09	123±1.13	116.56±0.64	121.18±9.45
P4	111±2.24	118±0.6	125±2.2	134±5.78	129±4.13	123±0.7	124±9.39
E1	96.53±3.2	101.53±2.68	110.59±2.13	124.84±2.68	120.25±3.02	116.8±1.86	112.4±8.58
E2	108.65±5.55	114.65±2.89	121.84±0.97	123.72±12.85	126.12±4.33	123±8.09	123.71±1.33
E3	101.93±3.95	108.5±4.89	115.12±5.72	122.84±8.96	124.9±2.57	119.9±1.73	121.71±6.52
E4	115.5±9.05	120.15±6.66	127.9±2.62	134.25±4.5	134.5±5.43	127.25±8.49	130±3.6

Table 4. Effect of low concentration of phenol and effluent on Hb (g/dl) of *Channa punctatus* (mean values ± SD).

Treatment	Duration of exposure(hours)						
	24	48	72	96	120	144	168
CON	10.58±0.37	10.91±0.24	11.02±0.19	11.04±0.23	11.02±0.13	10.92±0.17	11.11±0.08
P1	10.63±0.56	11.22±0.68	11.73±0.78	12.48±0.97	12.60±0.54	12.68±0.49	12.47±0.59
P2	10.76±0.66	11.34±0.69	11.88±0.79	12.65±0.99	12.62±0.35	12.39±0.48	12.18±1.18
P3	11.05±0.90	11.66±0.95	12.22±1.20	13.06±1.47	13.14±1.03	12.87±0.96	12.76±1.75
P4	11.31±0.95	12.01±1.04	12.49±1.17	13.06±1.25	13.07±0.98	13.31±0.80	13.24±1.12
E1	10.63±0.25	11.19±0.30	11.78±0.38	12.40±0.45	12.49±0.24	12.49±0.63	11.86±0.38
E2	10.81±0.33	11.41±0.45	12.06±0.64	12.83±0.84	12.68±0.46	12.45±0.25	12.09±0.80
E3	11.10±0.49	11.79±0.71	12.51±0.79	13.32±1.18	13.24±0.57	12.60±0.59	12.34±1.22
E4	11.34±0.34	11.97±0.43	12.64±0.88	13.29±1.13	13.39±0.71	13.70±0.25	13.32±0.69

Table 5. Effect of high concentration of phenol and effluent on TEC (Cells x10^5/cu.mm) of *Channa punctatus* (mean values ± SD).

Treatment	Duration of exposure (hours)						
	24	48	72	96	120	144	168
CON	52.16±5.41	51.86±6.34	55.06±4.23	55.94±1.48	53.93±0.45	53.16±2.44	53.53±5.20
P10	53.23±5.77	53.53±7.64	59.66±8.24	61.93±6.17	61.96±5.77	58.30±6.21	58.07±9.49
P20	57.80±9.86	61.76±13.61	65.41±11.12	67.49±9.40	66.39±7.80	69.34±11.32	71.59±12.74
P30	58.08±8.48	62.43±9.47	65.82±11.80	61.39±8.36	60.58±8.08	63.28±10.78	66.74±13.23
E10	52.99±8.66	49.57±10.76	59.43±8.96	58.09±10.24	56.80±11.03	56.19±7.57	60.63±8.73
E20	54.66±9.60	66.14±14.03	68.46±11.25	66.07±14.44	63.20±13.74	67.73±15.47	72.28±15.64
E30	59.31±10.16	62.84±10.13	68.11±10.92	64.80±11.53	59.66±9.20	64.01±10.53	69.31±11.62

Table 6. Effect of high concentration of phenol and effluent on TLC (Cells x10^2/cu.mm) of *Channa punctatus* (mean values ± SD).

Treatment	Duration of exposure (hours)						
	24	48	72	96	120	144	168
CON	94.09±1.18	93.00±6.31	97.28±1.56	95.37±7.75	99.37±2.50	97.71±2.68	101.65±1.56
P10	115.06±6.16	121.03±9.22	138.31±24.75	146.37±31.25	157.65±31.88	146.75±11.70	139.03±9.57
P20	125.53±14.39	138.71±19.95	150.65±25.23	164.40±32.30	178.03±40.83	167.87±20.34	160.43±5.21
P30	124.90±6.80	130.81±17.97	150.03±8.92	165.43±13.83	186.50±24.81	180.59±7.98	175.81±19.92
E10	115.25±5.5	119.18±5.18	125.25±2.31	132.87±5.61	141.81±5.86	147.78±9.01	153.12±4.57
E20	119.96±4.29	130.06±1.95	137.78±3.04	147±4.06	156.93±6.80.	165.65±7.69	174.84±4.77
E30	124.81±2.78	138.81±5.01	150.75±3.76	162.5±7.92	176.93±3.54	192.03±5.32	204.15±11.39

Table 7. Effect of high concentration of phenol and effluent on Hb (g/dl) of *Channa punctatus* (mean values ± SD).

Treatment	Duration of exposure (hours)						
	24	48	72	96	120	144	168
CON	10.88±0.39	11.03±0.25	11.20±0.19	11.16±0.15	11.06±0.16	10.95±0.13	11.16±0.08
P10	10.91±0.57	10.92±0.74	11.52±0.81	11.77±0.59	11.77±0.72	10.92±1.21	11.44±0.91
P20	11.39±0.93	11.67±1.40	12.02±1.15	12.29±0.93	12.22±0.75	12.53±1.09	12.80±1.12
P30	11.39±0.81	11.81±0.93	12.21±1.13	11.73±0.82	11.66±0.78	11.87±1.05	12.24±1.30
E10	10.91±0.82	11.03±0.87	11.51±0.88	11.37±0.99	11.25±1.10	11.20±0.73	11.62±0.87
E20	11.57±1.21	12.24±1.35	12.40±1.12	12.17±1.42	11.95±1.25	12.34±1.53	12.84±1.52
E30	11.50±1.01	11.89±0.99	12.42±1.08	12.06±1.11	11.61±0.84	11.96±1.04	12.51±1.14

Figure 2

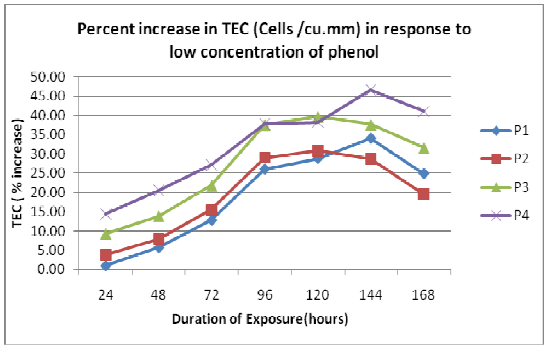


Figure 3

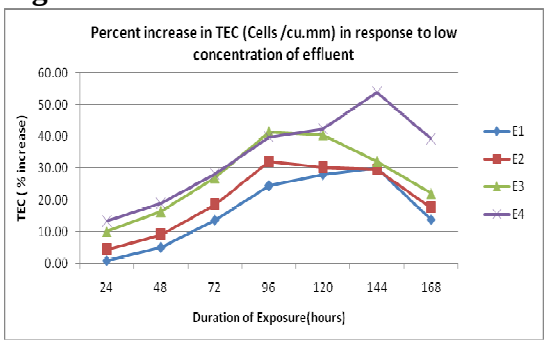
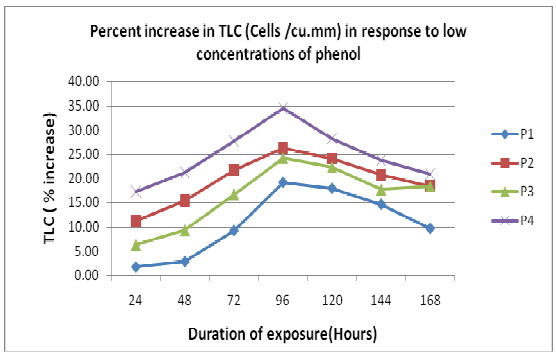


Figure 3



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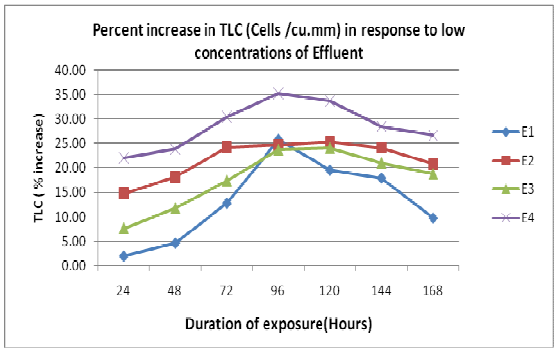

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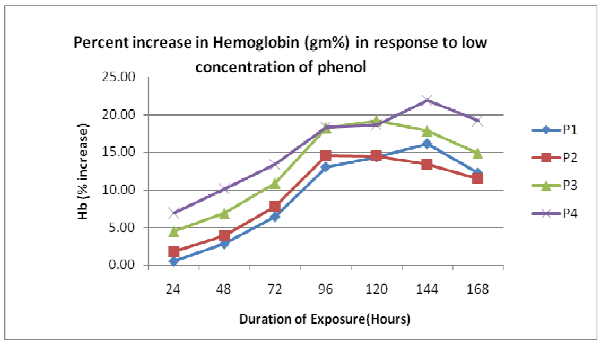

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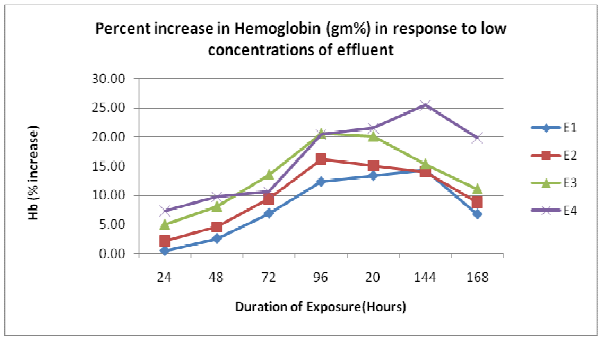

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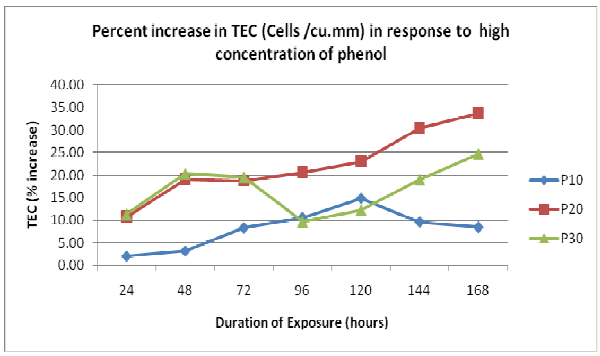

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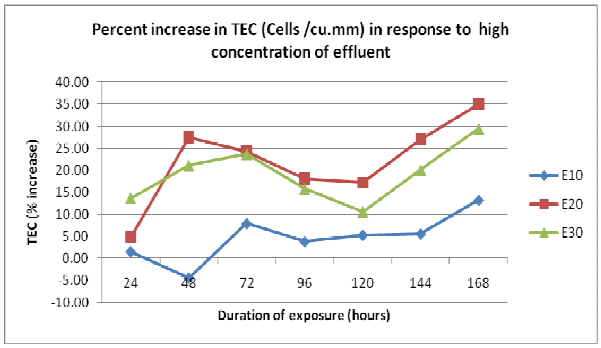

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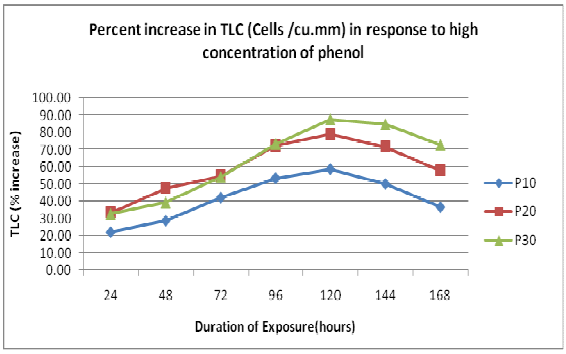
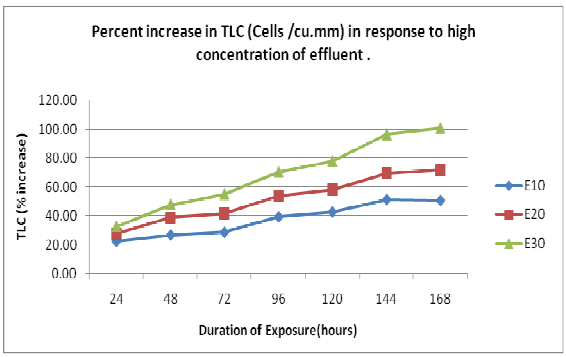


Figure 10



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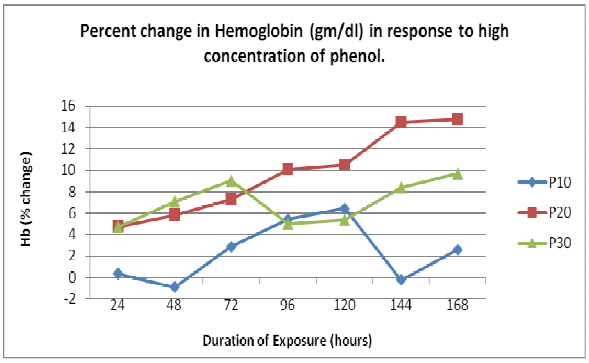

Figure 12