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# Haematology of freshwater Murrel (*Channa punctatus* Bloch), exposed to Phenolic industrial wastes of the Bhilai Steel plant (Chhattisgarh,India)

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**Abstract** -Haematological indices are important parameters to evaluate the general physiological status of fishes and may be considered as stress indicators for estimation of the response reactions of the fish to various environmental conditions (Docan et al., 2010). It may be considered useful in assessing the health of fish subjected to changing environmental conditions (Blaxhall, 1972 and Nair et al, 1984) and have proven useful in monitoring stress responses as bio-indicators (Bridges et al, 1976; Soivio & Oikari, 1976) The phenolic industrial wastes generated from the cock oven of steel plants and dumped in river are changed the nature of aquatic environment and pose serious threats to aquatic life. The present study describes the daily changes in the haematological parameters (TEC, TLC and % of Hb) of the Indian Murrell Channa punctatus (Bloch) upon long term exposure to high concentration of phenolic effluents of Bhilai steel plant. Commercial available phenol was also taken in Same concentration for comparison. During experiment over all % increase in TEC was found, highest augmentation in TLC and % changes in Hb was occurred.

**Introduction-:** The rapid industrial growth throughout the world particularly due to alarming rise in human population has been responsible for tremendous amount of environmental pollution. In developing countries, industrial effluents are indiscriminately discharged into aquatic ecosystems and even into adjoining fields without any pretreatment thus creating serious problems to the non target organisms. Discharging of effluents into freshwater systems depletes the dissolved oxygen content causing heavy mortality in fish by interfering with respiratory metabolism (David and Ray, 1966; Hingoroni et al., 1979).

Fishes are aquatic and poikilothermic animals. Hence, their existence and performance is dominated by the quality of their environment. While, conditions in large bodies of water are relatively stable, a greater magnitude of environmental change exists in smaller bodies of water, particularly in the presence of man -made stressors. The Bhilai Steel Plant is an integrated steel plant situated 30 kilometers (west) of Raipur, the capital of Chhattisgarh. Besides the major marketable product that is good quality steel, it also produces important by products, such as, Coal tar, Naphthalene and Benzol, which is further rectified to Benzene, Toluene and Xylene. Effluents generated in the three main units are further dumped into the two local rivers, the Kharoon and the Shivnath through various channels. The present work on the effect of phenolic industrial wastes on food fishes is a preliminary one. Sinha (PhD Thesis, 1999) studied the physico- chemical characteristics of the Bhilai steel plant effluent and reported it to be rusty brown in color and with a pungent phenolic odour. Besides other parameters, such as, total alkalinity, chloride ions, total dissolved solids (TDS), chloride ions and sulphates etc. were also found to be far above normal levels. Although the Bhilai Steel Plant happens to be an important industry of Chhattisgarh, no studies however, have yet been done on the effect of its phenolic wastes on humans and animals, particularly food fishes. Hence the present work is an attempt to understand the impact of a stressful phenolic environment on the food fish *Channa punctatus.*An industrial effluent is a complex of stressors and studies on their effect on organisms must be rather complicated. Thus, a reductionist approach has been employed taking into consideration only one important constituent for comparison. The coke oven effluent arises from a place where important byproducts are generated in the form of coal tar and benzene. Hence, Phenol has also been taken as the environmental stressor along with the phenolic effluent.

Phenol and phenolic compounds are hydroxy-derivatives of aromatic hydrocarbons and are formed during the decomposition of organic materials under natural conditions. They are an important constituent of coal tar. The major portion of the phenol present in the environment is of anthropogenic origin. And they are ubiquitous pollutants which come to the natural water resources from the effluents of a variety of industries such as cool refineries, phenol manufacturing, pharmaceuticals and industries of resin paint, dying, textile wood, petrochemical, pulp mill, etc Consequently, aquatic organisms including fish are subjected to these pollutants (Shalaby et al,2007). They are known to induce genotoxic, carcinogenic, immunotoxic, haematological and physiological effects (Jagetia and Aruna, 1997; Tsutsui et al, 1997; Taysse et al, 1995; Abo-Hegab et al, 1990; Assem et al, 1992) and have a high bioaccumulation rate along the food chain due to its lipophilicity. Besides, Phenol intoxication tests have been found to induce considerable unfavorable changes in the blood and dystrophic and necrobiotic lesions in tissues of fish leading to dysfunction of both haemopoietic and reproductive processes (Wlasow et al, 2010). Penetration of the ecosystems occurs as a result of drainage off the municipal or industrial sewage to surface waters. Mukherjee *et al.* (1990) reported that they are commonly found in the marine habitat and in fish tissues and induces toxic effects for fish health. Angus (1986) studied phenol tolerance in populations of mosquito fish *Gambusia affinis* from industrially polluted and non polluted waters and reported that the median lethal concentration (LC50) of phenol was significantly higher for mosquito fish from a stream that received coke-treatment wastewater from a steel plant than for the other two populations. Especially at low phenol concentrations, most mortality occurred during the first few hours of exposure, indicating that some individuals were much more susceptible to phenol than others.

Sub lethal bioassay studies on the effect of phenol on tropical fresh water fishes are limited (Razani et al,1986 a). Nair and Sherrif (1998) studied the acute toxicity of phenol and long term effects on food consumption and growth of juvenile *Labeo rohita* (Ham.) under tropical conditions. Fishes exposed to 5-10 mg/l phenol showed significantly lower mean wet weight gain, specific growth rate, and food conservation efficiency. According to them the maximum allowable toxicant concentration for juvenile fishes was 3.16 mg/l.

## Materials and method

**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(ref) .The analysis report of the effluent along with tap water is represented in table-1

Live, healthy *Channa punctatus* in the same size range were collected from local streams and acclimatized under normal laboratory conditions for 15 days. Live healthy fishes after acclimatization were divided into 7 groups of 16 fishes each. Exposure of fishes to the 10, 20, and 30% of the toxicants (Phenol and Effluent) was done for duration of 4weeks along with tap water as control. Feeding of fishes and aeration of the tanks were done uniformly throughout the experiment. Blood samples were collected separately from 4 live fishes belonging to each group at weekly intervals by severing the caudal peduncle.

**Blood Analysis-** The samples collected were analyzed for various haematological parameters, such as, Total Erythrocyte count (TEC) and Total Leukocyte count (TLC) in Cells/ cu.mm and Hemoglobin (gm/dl) according to the method of Dacie & Lewis, 1984. The results obtained were analyzed statistically by Anova (Two way with replication) using MS Excel.

The overall effects of phenol and phenolic effluents on the haematology 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 exposure of *Channa punctatus* to different concentrations of phenol and phenolic effluent are shown in Tables 2- 7. Percent rise in haematological parameters of exposed fishes vis-avis control have been represented in Figures1-6. The following abbreviations have been used throughout- Control (CON), Phenol (P), Effluent (E), Concentrations used (%v/v) - 10(P10/E10), 20(P20/E20), 30(P30/E30).

**Total Erythrocyte Count (Cells/ cu.mm)-** Effect of concentrations (10, 20 ,30%) of Phenol (Figure 1)shows a gradual % rise above normal in TEC, Observations made on the effect of high concentrations of Phenol and effluent on TEC (million cells/cu.mm).The overall percent increase [Table-2] in TEC compared from control was found to be highest (172.04%) in the first week in P10, followed by 192.8, 173.05 and 133.72 % changes respectively, in the following weeks. An initial hike of 89.17% followed by a raise of 200.08,210.76,158.63% respectively, in the following weeks was also observed in case of P20 and 74.59,114.07,147.14 & 95.88% in P30 (Fig.1and 2).

**TOTAL LEUCOCYTE COUNT (TLC)**

A highly significant augmentation in TLC was observed upon exposure to higher concentrations (20 and 30%)of Phenol and effluent .Overall ,percent changes in TLC in response to higher concentrations (20 & 30%) of the toxicants were found to be far higher than control and more so in winter (Table 3 and Figures 3,4).

**HAEMOGLOBIN (G/DL)**

The overall effect was a highly significant effect of various concentrations of Phenol and effluent on percent change. A percent hike of 27.28, 32.59, 31.89 and 22.28 was observed in weeks 1-4 in P10 .P20 showed a % hike of 34.41, 44.76, 49.71 and 39.42, while P30 a hike of 32.39, 38.65, 42.16 and 33.94%.

In response to effluent, E10 showed a % hike of 37.47, 36.82, 40.37 &31.04, E20 (41.98, 52.50, 59.23 & 47.64% and E30 (44.47, 50.15, 50.58 and 42.33 (Table 4 and Figures V-5,6).

Summarizing, results obtained show that the overall effect of phenol and phenolic effluent leads to a marked % elevation above normal up to 4 week ,followed by a decline thereafter in TEC,TLC and g/dl Hb upon exposure to different concentrations (10, 20, 30%) of phenol and whole effluent. Percent rise was found to increase with increasing concentrations.

The overall percent increase in TEC compared from control was found to be highest in the first week followed by declines in the following weeks. The increase in the number of circulating RBC in the present work is comparable to observations made by Gautam and Gupta (1989) and Zaki et al (2010) who reported the impact of phenol on the hematological profile of fish polycythemia accompanied by elevated hemoglobin level. Similar findings were reported by Mckim et al *(*1970), Hilmy et al (1979) and Taylor et al *(*1985) who recorded polycythemia in *Rasy barb*. Thus, it is beyond doubt that increased RBC count in the present work reflects hypoxic stress exposure of the fish resulting in secondary polycythaemia. We agree with Zaki et al (2010) that the increased RBC count may be due to stimulation of erythropoietin by elevated demands for $O_2$ or $CO_2$ transport as a result of destruction of gill membranes causing faulty gaseous exchange. A stressful phenolic environment becomes further additive to hypoxia leading to hyper excitability and high muscular activity in the fishes. We also agree with Erslev (1977) that the release of large number of erythrocytes is appropriate and part of compensatory effect to minimize a threatening tissue hypoxia which improves the oxygen carrying capacity of blood. Thus, it is beyond doubt that increased RBC count in the present work reflects hypoxic stress exposure of the fish resulting in secondary polycythaemia. We agree with Zaki et al (2010) that the increased RBC count may be due to stimulation of erythropoietin by elevated demands for $O_2$ or $CO_2$ transport as a result of destruction of gill membranes causing faulty gaseous exchange. A stressful phenolic environment becomes further additive to hypoxia leading to hyper excitability and high muscular activity in the fishes. We also agree with Erslev (1977) that the release of large number of erythrocytes is appropriate and part of compensatory effect to minimize a threatening tissue hypoxia which improves the oxygen carrying capacity of blood. Long term exposures also showed highly significant augmentations in TLC.

A highly significant effect of various concentrations of Phenol and effluent on percent change was also visible during long term exposure to toxicants. The increase Hb content could be explained as a process where the body tries to replace the oxidized denatured Hb. the duration of exposure also significantly affected the percent change in TLC. The effects of concentration and duration were found to be independent of each other. Long term exposures also showed highly significant augmentations in TLC. The white blood cells in fish respond to various stressors including infections and chemical irritants (Christensen and Faindt-Poeschi , 1978). Thus increasing or decreasing numbers of white blood cells are a normal reaction on the exposure of toxicants (Kori-Siakpere etal, 2006). We agree with Gabriel et al, 2009 that the increase in WBC (leukocytosis) may have resulted from the excitation of defense mechanism of the fish to counter the effect of the toxicant.

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. 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, 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. We 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 (USEPA, NRWQC,1950).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 (Kirk and Othmer,1982;Neff,2002).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 of effluent water (Bhilai steel plant) obtained from origin point of Purena channel vis avis Tap water and national recommended water quality criteria for fresh water organisms according to USEPA**

| Parameters | Purena | Tap Water | NRWQC |
|---|---|---|---|
| pH | 9.23±0.46 | 7.20±0.21 | 9.00 |
| Temp(°C) | 32.00±8.12 | 30.50±0.71 | |
| Color | Black | Color less | |
| Smell | Phenolic | Odorless | |
| DO | 8.70±0.97 | 6.05±0.13 | |
| BOD | 122.92±34.12 | 37.00±9.27 | |
| Alkalinity | 211.41 | -- | 20.00 |
| Chloride | 263.00±30.87 | 138.93±14.55 | 230.00 |
| Nitrate | 4.46±0.81 | 0.85±0.07 | 10.00 |
| Nitrite | 5.73±0.92 | 2.97±0.16 | |

| | | | |
|---|---|---|---|
| Sulphates | 402.75±44.23 | 153.50±36.27 | |
| Phenol | 6.81±1.35 | 0.48±0.46 | 10.00 |
| Ca | 108.00±18.97 | 75.20±5.34 | |
| Mg | 39.50±13.96 | 26.00±4.55 | |
| Hardness | 500.77±43.76 | 345.25±67.95 | |
| TDS | 765.02±76.53 | 349.75±28.11 | 250.00 |
| TSS | 218.50±79.72 | 255.25±103.88 | |
| TS | 870.77±60.86 | 433.25±13.50 | |

**Table 2 TEC (million cells /cu.mm) of *C.punctatus* in response to long term exposure to high concentration of Phenol and Effluent (Overall effect)Mean and SD**

| Treatment | Duration of Exposure(weeks) | | | |
|---|---|---|---|---|
| Overall effect | W1 | W2 | W3 | W4 |
| CON | 4.57±0.41 | 4.79±0.49 | 4.82±0.49 | 5.05±0.4 |
| P10 | 12.45±13.76 | 14.04±15.25 | 13.18±13.14 | 11.82±12.28 |
| P20 | 8.66±3.16 | 14.39±9.02 | 15±8.62 | 13.08±8.37 |
| P30 | 7.99±2.14 | 10.27±3.43 | 11.93±4.33 | 9.9±3.63 |
| E10 | 12.94±12.27 | 14.13±12.15 | 13.01±9.93 | 11.79±8.97 |

| | | | | |
|---|---|---|---|---|
| E20 | 10.36±3.78 | 16.23±9.32 | 22.88±12.46 | 15.26±9.7 |
| E30 | 9.86±2.56 | 12.72±3.3 | 13.11±2.87 | 11.1±2.59 |

**Table 3 Overall percent increase in TEC in response to phenol and effluent vis-à-vis control**

| Treatment | Duration of Exposure(weeks) | | | |
|---|---|---|---|---|
| Overall effect | W1 | W2 | W3 | W4 |
| P10 | 172.04 | 192.80 | 173.05 | 133.72 |
| P20 | 89.17 | 200.08 | 210.76 | 158.63 |
| P30 | 74.59 | 114.07 | 147.14 | 95.88 |
| E10 | 194.46 | 169.53 | 133.19 | 133.19 |
| E20 | 126.35 | 238.39 | 373.99 | 201.90 |
| E30 | 115.49 | 165.27 | 171.73 | 119.57 |

**Table 4 .TLC (Thousand cells/cu.mm) of *C.punctatus* in response to short term exposure to low concentration of Phenol and Effluent (Overall effect)Mean values and SD**

| Treatment | Duration of Exposure(weeks) | | | |
|---|---|---|---|---|
| Overall effect | W1 | W2 | W3 | W4 |
| CON | 87.34±1.56 | 103.65±1.90 | 85.42±1.53 | 83.39±1.49 |

| | | | | |
|---|---|---|---|---|
| P10 | 221.20±2.45 | 265.29±2.93 | 255.50±2.91 | 223.47±2.67 |
| P20 | 121.49±1.43 | 476.26±7.75 | 154.24±1.76 | 144.24±1.64 |
| P30 | 894.11±1.02 | 1463.80±1.62 | 1685.23±1.87 | 1549.77±1.73 |
| E10 | 203.66±2.23 | 258.95±2.83 | 295.17±3.22 | 258.06±2.82 |
| E20 | 843.97±1.25 | 102.06±1.47 | 116.79±1.64 | 100.26±1.47 |
| E30 | 762.77±1.14 | 872.08±1.29 | 973.91±1.44 | 893.60±1.36 |

**Table 5 Overall percent increase in TLC in response to phenol and effluent(LTE)**

| Treatment | Duration of Exposure(weeks) | | | |
|---|---|---|---|---|
| Overall | W1 | W2 | W3 | W4 |
| P10 | 153.26 | 155.96 | 199.13 | 167.99 |
| P20 | 1332.88 | 4494.69 | 1707.91 | 1628.35 |
| P30 | 923.70 | 1312.29 | 1872.96 | 1758.50 |
| E10 | 133.18 | 149.83 | 245.57 | 209.47 |
| E20 | 866.29 | 892.85 | 1266.01 | 1100.73 |
| E30 | 773.33 | 741.40 | 1040.20 | 971.62 |

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**Table 6 Hb(g/dl) of *C.punctatus* in response to long term exposure to high concentration of Phenol and Effluent (Overall effect)Mean and SD**

| Treatment | Duration of Exposure(weeks) | | | |
|---|---|---|---|---|
| LTE Overall effect | W1 | W2 | W3 | W4 |
| CON | 10.18±0.41 | 10.44±0.45 | 10.42±0.52 | 10.66±0.42 |
| P10 | 12.96±3.60 | 13.84±3.81 | 13.74±3.18 | 13.03±3.40 |
| P20 | 13.68±2.30 | 15.11±2.36 | 15.60±1.59 | 14.86±1.87 |
| P30 | 13.48± | 14.47± | 14.81± | 14.28± |
| E10 | 13.99±3.29 | 14.28±3.70 | 14.63±1.97 | 13.97±2.25 |
| E20 | 14.45±1.64 | 15.92±1.34 | 16.59±1.63 | 15.74±1.26 |
| E30 | 14.71±0.84 | 15.67±0.51 | 15.69±0.83 | 15.17±1.31 |

**Table 7 Overall percent increase in Hb in response to phenol and effluent**

| Treatment | Duration of Exposure(weeks) | | | |
|---|---|---|---|---|
| LTE overall | W1 | W2 | W3 | W4 |
| P10 | 27.28 | 32.59 | 31.89 | 22.28 |
| P20 | 34.41 | 44.76 | 49.71 | 39.42 |
| P30 | 32.39 | 38.65 | 42.16 | 33.94 |

| | | | | |
|---|---|---|---|---|
| E10 | 37.47 | 36.82 | 40.37 | 31.04 |
| E20 | 41.98 | 52.50 | 59.23 | 47.64 |
| E30 | 44.47 | 50.15 | 50.58 | 42.33 |

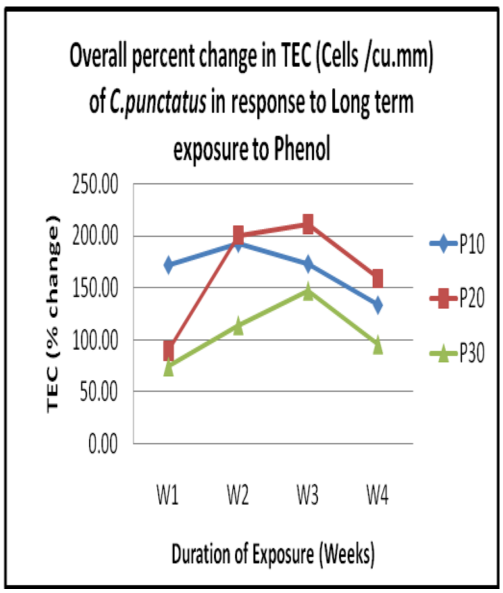


Fig -1

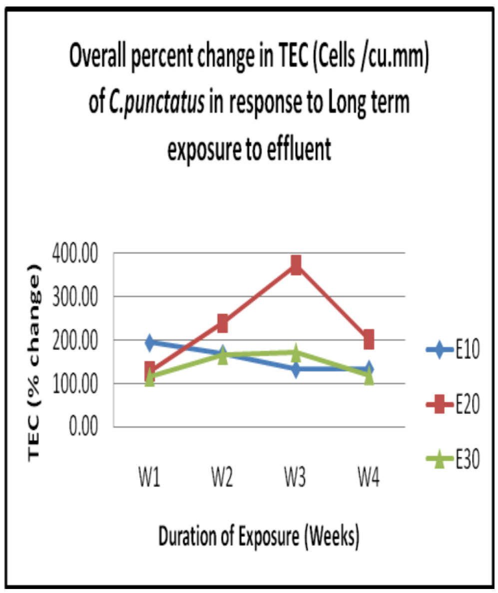


fig -2

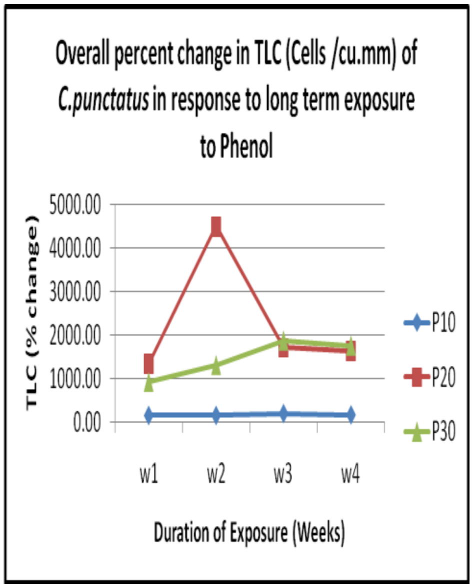


Fig -3

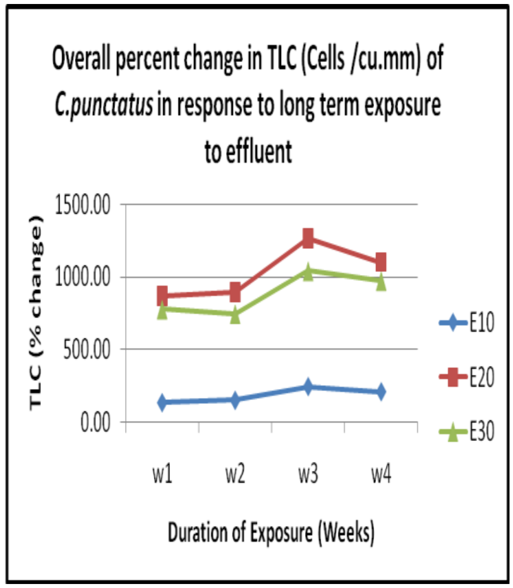


fig -4

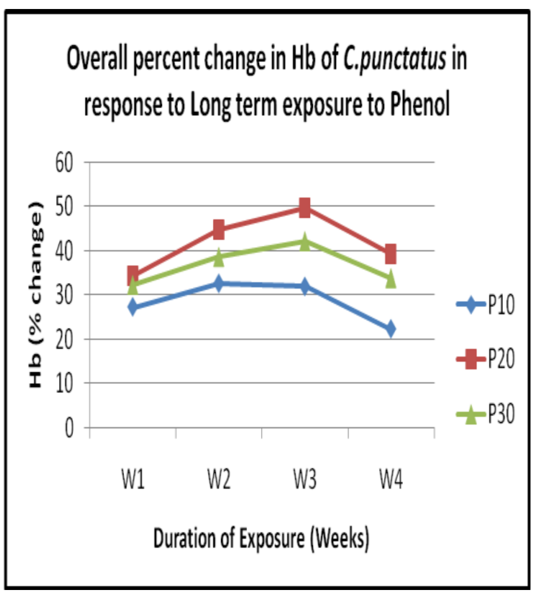


Fig -5

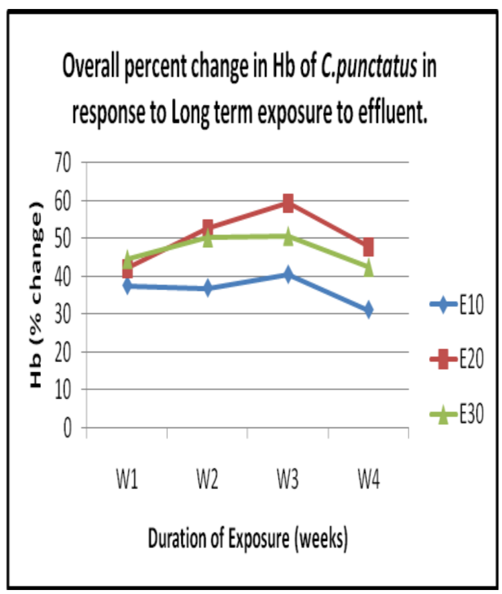


Fig - 6