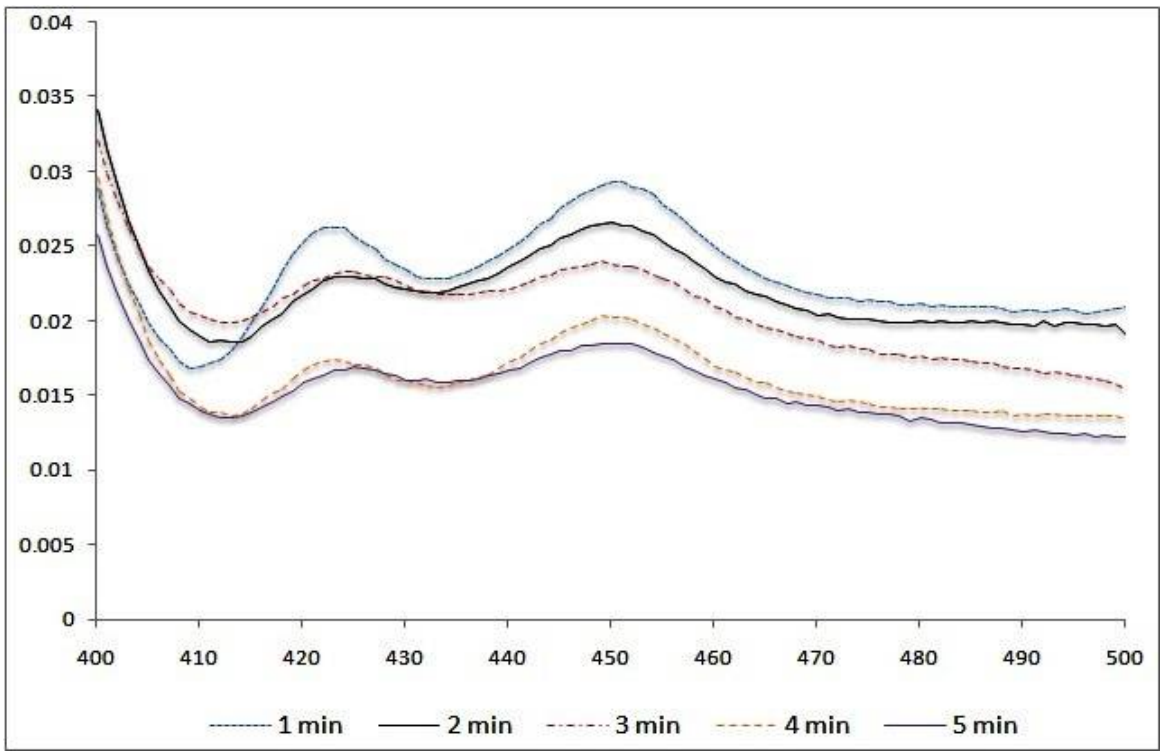
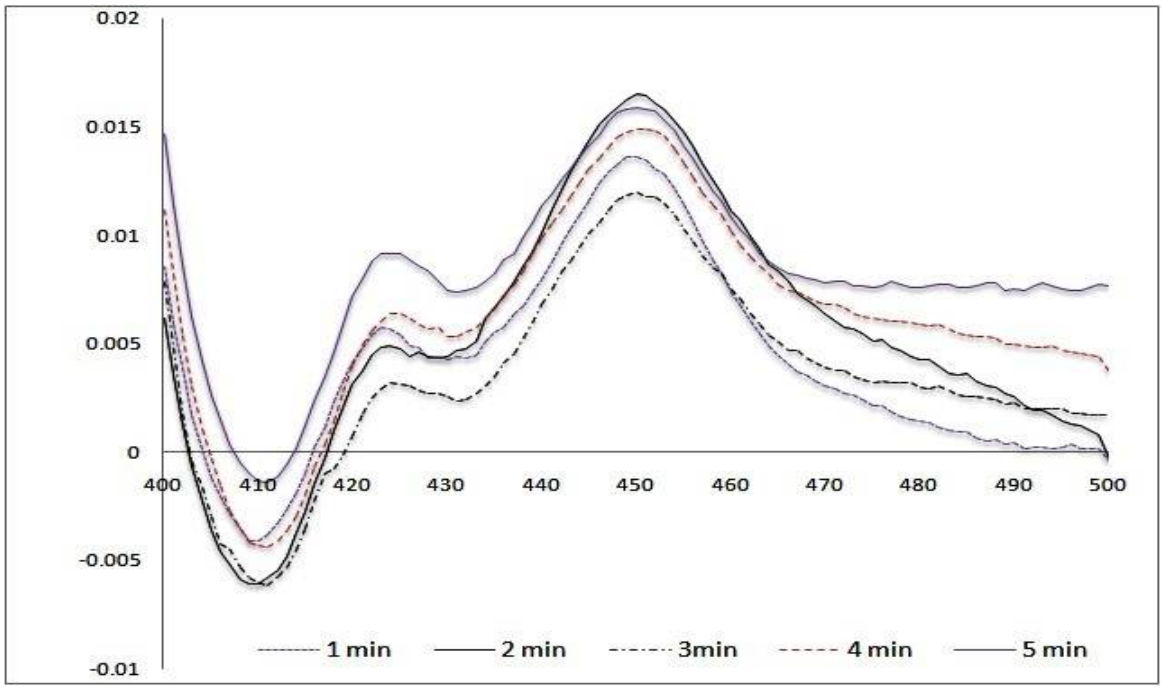
5. RESULTS

5.1. Spectral analysis of liver microsomes of *C. punctatus, H. fossilis* and *C. batrachus*

The carbon monoxide difference spectra of dithionite reduced liver microsomes of *C. punctatus, H. fossilis* and *C. batrachus* studied at 1 minute interval over a period of 5 minutes showed the maximum absorbance at 1 to 2 minutes and then a gradual decrease in absorbance was seen with the increase in time interval (Fig. 5.1, 5.2 and 5.3). Two soret peaks were seen in the dithionite reduced spectra of liver microsomes in all the 3 control and experimental fish, *C. punctatus, H. fossilis* and *C. batrachus.* These peaks consisted of the characteristic absorbance at around 450 nm for the reduced CYP 450-CO complex and the other of lesser magnitude at around 420-425 nm. Both the control and experimental groups displayed similar patterns of soret peak.

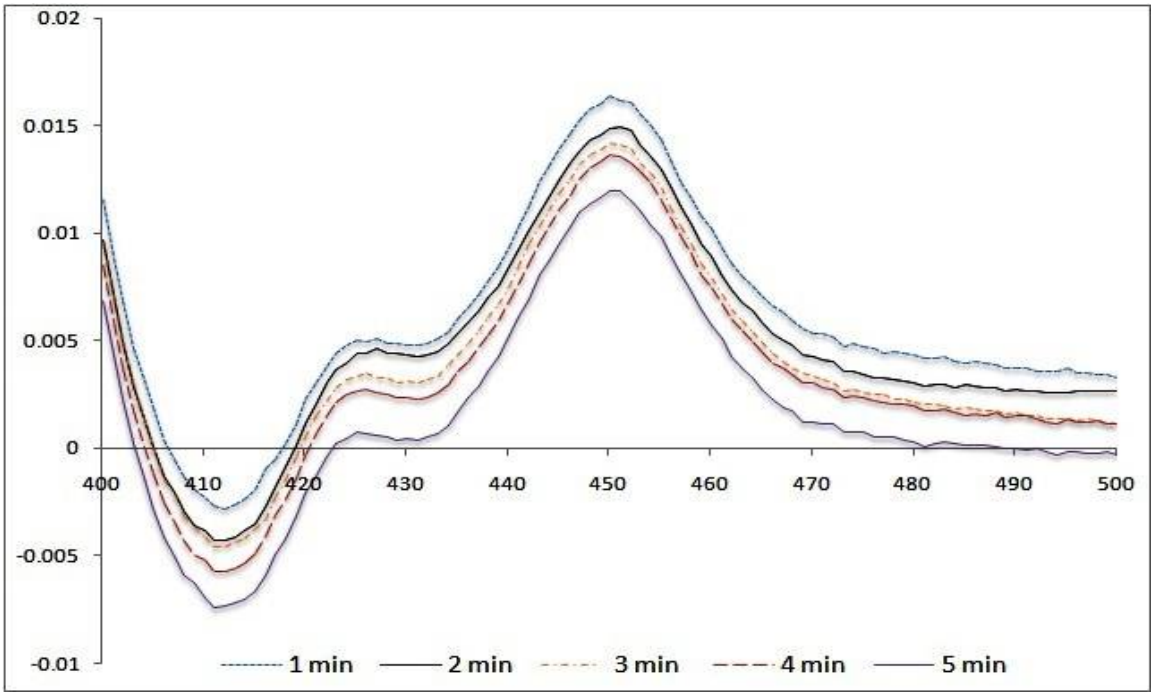


(a)

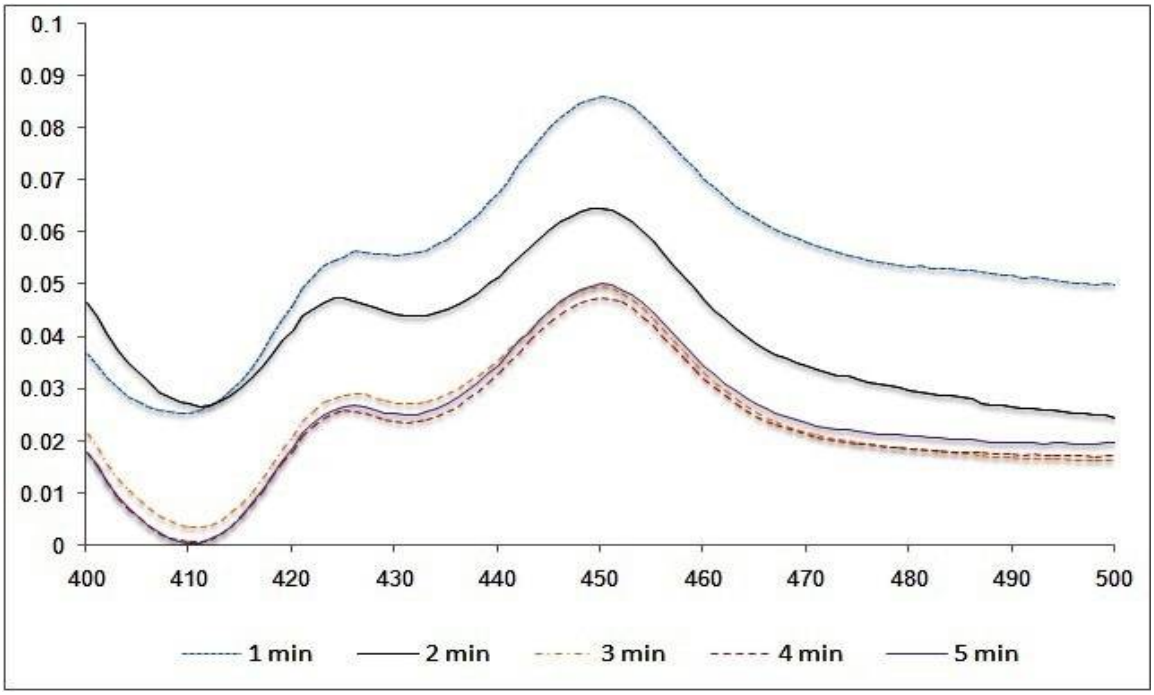


(b)

Figure 5.1. Carbon monoxide difference spectra of dithionite reduced liver microsomes of (a) control, and (b) experimental *C. punctatus* at 1 min interval over a period of 5 min.

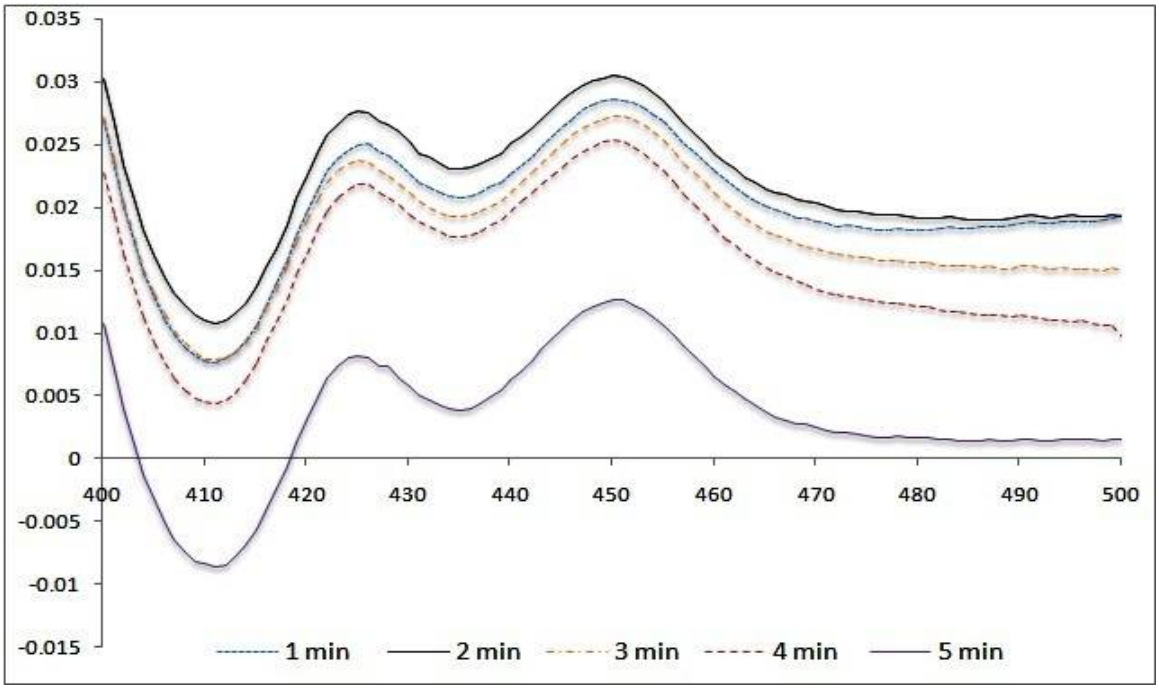


(a)

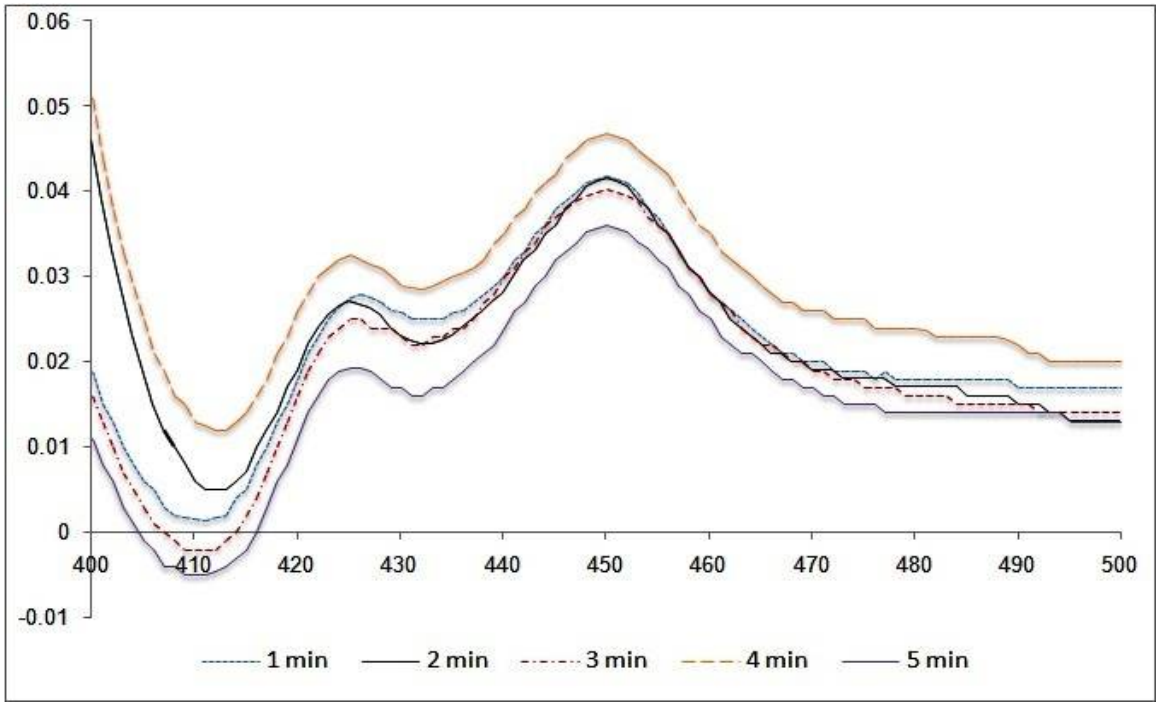


(b)

Figure 5.2. Carbon monoxide difference spectra of dithionite reduced liver microsomes of (a) control, and (b) experimental *H. fossilis* at 1 min interval over a period of 5 min.



(a)



(b)

Figure 5.3. Carbon monoxide difference spectra of dithionite reduced liver microsomes of (a) control, and (b) experimental *C. batrachus* at 1 min interval over a period of 5 min.

5.2. Multiple forms of cytochrome P450 family in liver of *Heteropneustes fossilis* treated with mammalian specific inducers of cytochrome P450

Spectral analysis: Carbon monoxide difference spectra upon dithionite reduction followed by bubbling of CO, showed the presence of cytochrome P450 in hepatic microsomal fractions of control and fish treated with naphthalene, phenobarbitone, deflazacort and acetone (Fig. 5.4). The peak absorbance of the control fish showed a maximum peak at 450 nm and the fish treated with naphthalene, phenobarbitone, acetone and deflazacort showed peaks at 451 nm, 452 nm, 450 nm and 449 nm respectively (Fig. 5.4 and Table 5.1).

CYP 450 content: In the control fish, cytochrome P450 **(**CYP 450) content was 0.214±0.102 nmole/mg protein and in experimental fish treated with naphthalene, phenobarbitone, acetone and deflazacort, the CYP 450 content were 0.330±0.089, 0.378±0.083, 0.428±0.011 and 0.440±0.096 nmole/mg protein respectively (Table 5.1). All the experimental fish displayed a significant difference [naphthalene and phenobarbitone ($p<0.05$); acetone and deflazacort ($p< 0.01$)] when compared to the control fish.

EROD activity: EROD (ethoxyresorufin O-deethylase) activity in control fish was 17.989±12.034 pmole resorufin formed/mg protein/min while in the experimental fish treated with naphthalene, phenobarbitone, acetone and deflazacort was 66.274±23.059, 41.053±9.506, 58.591±23.376 and 38.982±7.446 pmole resorufin formed/mg protein/min respectively though only naphthalene treated fish displayed a significant induction ($p<0.05$) in the activity (Table 5.2).

N,N-DMA activity: No significant difference were observed in N,N-dimethylaniline (N, N-DMA) demethylase activity between the control and the experimental groups. The N,N-DMA activity in control fish was 0.054±0.045 nmole formaldehyde formed/mg protein/min and in experimental fish treated with naphthalene, phenobarbitone, acetone and deflazacort, the N,N-DMA activity was 0.052±0.048, 0.060±0.013, 0.036±0.005 and 0.059±0.004 nmole formaldehyde formed/mg protein/min respectively (Table 5.2).

AH activity: In the control fish, aniline hydroxylase (AH) activity was 0.037±0.028 nmole p-aminophenol formed/mg protein/min and in the fish treated with naphthalene, phenobarbitone, acetone and deflazacort were 0.020±0.005, 0.015±0.002, 0.277±0.008 and 0.053±0.012 nmole p-aminophenol formed/mg protein/min respectively. Only acetone treated fish showed significant induction ($p<0.01$) as compared to control fish in AH activity. The

fish treated with naphthalene and phenobarbitone showed down regulation in AH activity as compared to control fish but was not significant (Table 5.2).

ERND activity: Erythromycin N-demethylase (ERND) activity in control fish was 0.898±0.062 nmole formaldehyde formed/mg protein/min whereas in naphthalene, phenobarbitone, acetone and deflazacort were 0.815±0.714, 0.506±0.103, 0.127±0.022 and 3.129±0.545 nmole formaldehyde formed/mg protein/min respectively. Only deflazacort (synthetic corticosteroid) treated fish showed significant elevation ($p<0.01$) in the activity while the other treated groups showed down regulation in the activity than that of the control group with significant difference seen in phenobarbitone ($p<0.05$) and acetone ($p<0.01$) treated groups (Table 5.2).

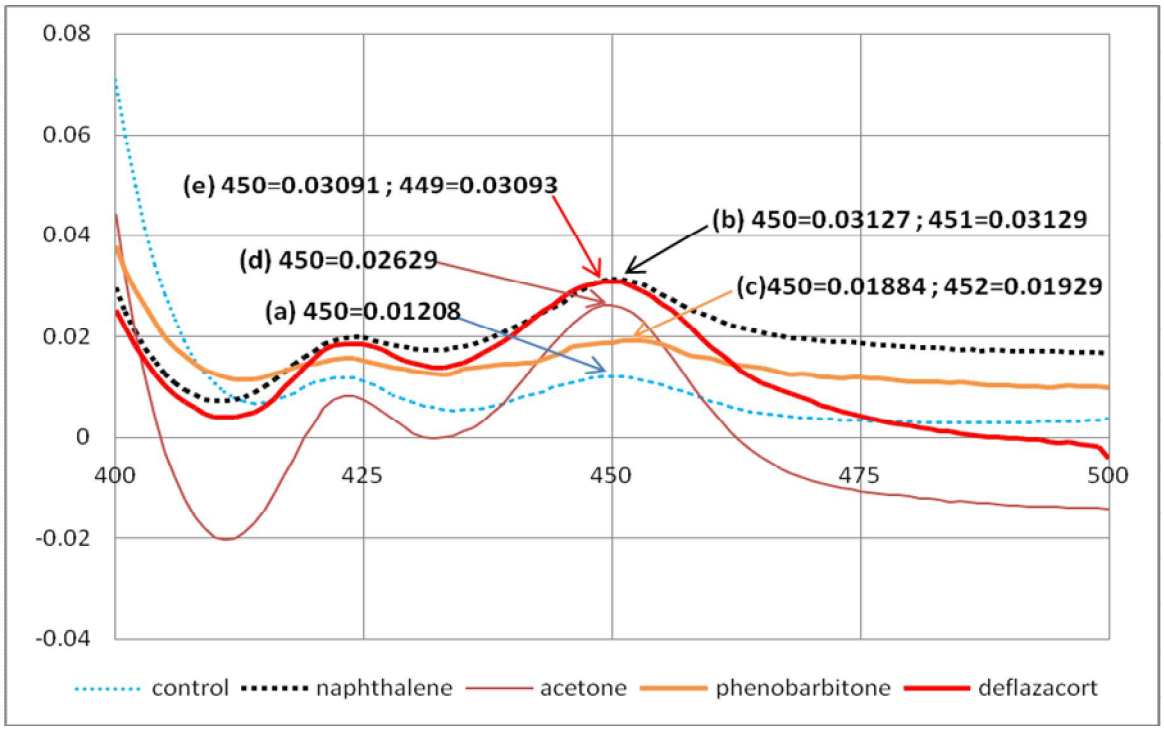


Figure 5.4. Carbon monoxide difference spectra of dithionite reduced liver microsomes of (a) control *Heteropneustes fossilis* and fish treated with different inducers (b) naphthalene, (c) phenobarbitone, (d) acetone, and (e) deflazacort.

Table 5.1. CYP 450 content and absorption maxima of control fish, *H. fossilis* and fish administered with different inducers.

INDUCER/dose	**CYP 450 CONTENT (nmole/mg protein)**	**Absorption maxima**
CONTROL VEHICLE (Water/Sesame oil)	0.214 ±0.102 (n=3)	450 nm
NAPTHELENE (20 mg/kg body weight)	0.330 ±0.089 (n=4)*	451 nm
PHENOBARBITAL (20 mg/kg body weight)	0.378±0.083 (n=3)*	452 nm
ACETONE (2 ml/kg body weight)	0.428 ±0.011 (n=3)**	450 nm
DEFLAZACORT (20 mg/kg body weight)	0.440 ±0.096 (n=3)**	449 nm

Values are the mean of (n) number of experiments ± SD. Significantly different from control * ($p \leq 0.05$), ** ($p \leq 0.01$)

Table 5.2. EROD, N, N-dimethylaniline demethylase (N,N-DMA), aniline hydroxylase (AH) and erythromycin N-demethylase (ERND) activities in *H. fossilis* treated with different inducers.

Enzyme Activity	**EROD activity**	**N,N-DMA activity**	**AH activity**	**ERND activity**
INDUCER/dose	(pmole resorufin formed/mg protein/min)	(nmole formaldehyde formed/mg protein/min)	(nmole p-aminophenol formed/mg protein/min)	(nmole formaldehyde formed /mg protein/min)
CONTROL VEHICLE (Water/Sesame oil)	17.989±12.034 (n=3)	0.054±0.045 (n=3)	0.037±0.028 (n=3)	0.898±0.062 (n=3)
NAPTHELENE (20 mg/kg body weight)	66.274±23.059* (n=4)	0.052±0.048 (n=4)	0.020±0.005 (n=4)	0.815±0.714 (n=4)
PHENOBARBITONE (20 mg/kg body weight)	41.053±9.506 (n=3)	0.060±0.013 (n=3)	0.015±0.002 (n=3)	0.506±0.103* (n=3)
ACETONE (2 ml/kg body weight)	58.591±23.376 (n=3)	0.036±0.005 (n=3)	0.277±0.008** (n=3)	0.127±0.022** (n=3)
DEFLAZACORT (20 mg/kg body weight)	38.982±7.446 (n=3)	0.059±0.004 (n=3)	0.053±0.012 (n=3)	3.129±0.545** (n=3)

Values are the mean of (n) number of experiments ± SD. Significantly different from control * ($p \leq 0.05$), ** ($p \leq 0.01$)

5.3. Treatment of the fish, *C. punctatus*, *H. fossilis* and *C. batrachus* with CYP1A specific inducer β-naphthoflavone

Liver somatic index (LSI) and Microsomal protein content: Table 5.3 presents data for LSI and liver microsomal protein content of control and experimental fish, *C. punctatus*, *H. fossilis* and *C. batrachus* treated with β- naphthoflavone. *C. punctatus* (1.066±0.098) showed the highest basal value for LSI followed by *H. fossilis* (0.988±0.140) and *C. batrachus* (0.912±0.155). Though LSI values were higher in all the 3 treated fish, only *C. batrachus* showed significantly ($p<0.05$) higher LSI value (1.219±0.076) while *C. punctatus* (1.095±0.024) and *H. fossilis* (1.192±0.296) showed no significant differences in the LSI value in comparison to their respective control group.

The microsomal protein content in control fish was highest in *H. fossilis* (3.324±0.577 mg/gm liver) followed by *C. batrachus* (3.284±0.719 mg/gm liver) and *C. punctatus* (2.875±0.612 mg/gm liver). Exposed *C. punctatus* showed higher liver microsomal protein content (3.353±3.355 mg/gm liver) than the control group but was not significant. In contrast, *H. fossilis* with 4.639±1.282 mg/gm liver ($p<0.05$) and *C. batrachus* with 5.073±0.450 mg/gm liver ($p<0.01$) showed a significant difference when compared with their respective control group.

Table 5.3. Liver somatic index (LSI) and protein content of *C. punctatus*, *H. fossilis* and *C. batrachus* administered with β-naphthoflavone (n= 4).

Fish	Control	Treated
Liver somatic index [LSI %]		
C. punctatus	1.066±0.098	1.095±0.024
H. fossilis	0.988±0.140	1.192±0.296
C. batrachus	0.912±0.155	1.219±0.076*
Microsomal protein content (mg/gm)		
C. punctatus	2.875±0.612	3.353±1.402
H. fossilis	3.324±0.577	4.639±1.282*
C. batrachus	3.284±0.719	5.073±0.450**

Values are the means ± sd. Significantly different from control * ($p \leq 0.05$), **($p \leq 0.01$)

CYP 450 content, EROD, N,N-DMA, AH and ERND activities: CYP 450 content, EROD, N, N-dimethylaniline demethylase (N,N-DMA), aniline hydroxylase (AH) and erythromycin N-demethylase (ERND) activities of control and β-naphthoflavone administered fish are illustrated in Table 5.4. The CYP 450 content of control fish was higher in *H. fossilis* (0.313±0.061 nmole/mg protein) followed by *C. batrachus* (0.296±0.071 nmole/mg protein) and *C. punctatus* (0.280±0.054 nmole/mg protein). The CYP 450 content in all the treated fish increased significantly in comparison to that of their respective control group [*C. punctatus*, 0.432±0.097 ($p<0.05$); *H. fossilis*, 0.509±0.069 ($p<0.001$); *C. batrachus*, 0.466±0.118 nmole/mg protein ($p<0.05$)].

EROD activity was positively detected in all the fish species studied and was significantly ($p<0.001$) elevated in all the treated fish in comparison to their respective control group. The basal EROD activity in *C. punctatus*, *H. fossilis* and *C. batrachus* were 50.498±7.197, 44.685±3.340 and 55.631±1.275 pmole resorufin formed/mg protein/min respectively. The highest elevation was observed in *H. fossilis* with 5.7 fold increase (255.415±9.615 pmole resorufin formed/mg protein/min) followed by *C. punctatus* with 3.8 fold increase (194.811±7.961 pmole resorufin formed/mg protein/min) and *C. batrachus* with 3.3 fold increase (185.252±22.159 pmole resorufin formed/mg protein/min).

N,N-DMA activities in control fish, *C. punctatus*, *H. fossilis* and *C. batrachus* were 0.100±0.007, 0.129±0.050 and 0.207±0.042 nmole formaldehyde formed/mg protein/min respectively. The treatment of fish to β-naphthoflavone had no effect on N,N-DMA activities in all the 3 fish although *H. fossilis* showed a slight increase (0.169±0.033 nmole formaldehyde formed/mg protein/min) and *C. punctatus* a slight decrease (0.077±0.140 nmole formaldehyde formed/mg protein/min) in the activity but was not significant. The N,N-DMA activity in treated *C. batrachus* (0.201±0.035 nmole formaldehyde formed/mg protein/min) was homogeneous with respect to the control.

The AH activities in β-naphthoflavone treated fish were homogeneous with respect to the control. AH activities in control fish, *C. punctatus*, *H. fossilis* and *C. batrachus* were 0.109±0.003, 0.169±0.061 and 0.215±0.054 nmole p-aminophenol formed/mg protein/min and in β-naphthoflavone treated fish were 0.103±0.022, 0.166±0.010 and 0.221±0.035 nmole p-aminophenol formed/mg protein/min respectively.

ERND activity was positively detected in all the fish species and control fishes were homogeneous with respect to the activity. ERND activities in control fish, *C. punctatus*, *H.*

fossilis and *C. batrachus* were 0.812±0.005, 0.743±0.168 and 0.782±0.272 nmole formaldehyde formed/mg protein/min respectively. ERND activity in experimental fish was significantly elevated ($p<0.05$) only in *H. fossilis* (1.026±0.078 nmole formaldehyde formed/mg protein/min) while *C. punctatus* (1.156±0.321 nmole formaldehyde formed/mg protein/min) and *C. batrachus* (1.117±0.265 nmole formaldehyde formed/mg protein/min) though revealed an increase in the enzyme activity, no significant difference was seen in comparison to their respective control group.

Table 5.4. CYP 450 content, EROD, N, N-dimethylaniline demethylase (N,N-DMA), aniline hydroxylase (AH) and erythromycin N-demethylase (ERND) activities of *C. punctatus*, *H. fossilis* and *C. batrachus* administered with β-naphthoflavone (n= 4).

Fish	Control	Treated
CYP 450 content (nmole/mg protein)		
C. punctatus	0.280±0.054	0.432±0.097*
H. fossilis	0.313±0.061	0.509±0.069***
C. batrachus	0.296±0.071	0.466±0.118*
EROD activity (pmole resorufin formed/mg protein/min)		
C. punctatus	50.498±7.197	194.811±7.961***
H. fossilis	44.685±3.340	255.415±9.651***
C. batrachus	55.631±1.275	185.252±22.159***
N,N-DMA activity (nmole formaldehyde formed/mg protein/min)		
C. punctatus	0.100±0.007	0.077±0.140
H. fossilis	0.129±0.050	0.169±0.033
C. batrachus	0.207±0.042	0.201±0.035
AH activity (nmole p-aminophenol formed/mg protein/min)		
C. punctatus	0.109±0.003	0.103±0.022
H. fossilis	0.169±0.061	0.166±0.010
C. batrachus	0.215±0.054	0.221±0.035
ERND activity (nmole formaldehyde formed/mg protein/min)		
C. punctatus	0.812±0.005	1.156±0.321
H. fossilis	0.743±0.168	1.026±0.078*
C. batrachus	0.782±0.272	1.117±0.265

Values are the mean of (n) number of experiments ± SD. Significantly different from control * ($p \leq 0.05$), *** ($p \leq 0.001$)

5.4. Assessment of LC_{50} value in fish, *C. punctatus*, *H. fossilis* and *C. batrachus*

The details of the pesticide concentration, fish mortality and LC_{50} values are presented in Tables 5.5, 5.6 and 5.7. The tested concentration of the pesticide, cypermethrin (Ripcord 10% EC), ethion (ethion 50 % EC) and dicofol (COLONEL S 18.5 % EC) varied between the fish, *C. punctatus*, *H. fossilis* and *C. batrachus*. All the applied pesticide belonged to a different class. Cypermethrin belonged to pyrethroid, ethion belonged to organophosphate and dicofol belonged to organochlorine class of pesticide.

The calculated LC_{50} values of the pesticide, cypermethrin in *C. punctatus*, *H. fossilis* and *C. batrachus* were 19.9 µg/L, 3.7 µg/L and 5.6 µg/L respectively. *H. fossilis* was observed to be highly susceptible towards cypermethrin followed by *C. batrachus* and *C. punctatus* (Table 5.5). The fish *C. punctatus*, *H. fossilis* and *C. batrachus* were administered with 6.6,1.2 and 1.9 µg/L of cypermethrin (1/3 of LC_{50} value) for a period of 5, 10 and 15 days.

The calculated LC_{50} values of the pesticide, ethion in *C. punctatus*, *H. fossilis* and *C. batrachus* were 43.9 µg/L, 54.8 µg/L and 48.7 µg/L respectively. *C. punctatus* was observed to be highly susceptible towards cypermethrin followed by *C. batrachus* and *H. fossilis* (Table 5.6). The fish *C. punctatus*, *H. fossilis* and *C. batrachus* were administered with 14.5, 18.1 and 16.1 µg/L of ethion (1/3 of LC_{50} value) for a period of 5, 10 and 15 days.

The calculated LC_{50} values of the pesticide, dicofol in *C. punctatus*, *H. fossilis* and *C. batrachus* were 45.8 µg/L, 36.1 µg/L and 51.8 µg/L respectively. *H. fossilis* was observed to be highly susceptible towards dicofol followed by *C. punctatus* and *C. batrachus* (Table 5.7). The fish *C. punctatus*, *H. fossilis* and *C. batrachus* were administered with 15.2, 12.1 and 17.2 µg/L of dicofol (1/3 of LC_{50} value) for a period of 5, 10 and 15 days.

Table 5.5. Cumulative mortalities and LC_{50} values of the pesticide, cypermethrin for 3 different air breathing fish, *C. punctatus*, *H. fossilis* and *C. batrachus*.

Dose (µg/L)	***C. punctatus* (N/10)**	**Dose (µg/L)**	***H. fossilis* (N/10)**	**Dose (µg/L)**	***C. batrachus* (N/10)**
18	1/10	2	1/10	4	2/10
19	3/10	3	3/10	5	3/10
20	6/10	4	5/10	6	6/10
21	8/10	5	7/10	7	8/10
22	9/10	6	9/10	8	9/10
LC_{50}	**19.9 µg/L**	**LC_{50}**	**3.7 µg/L**	**LC_{50}**	**5.6 µg/L**

N/10 = number of dead fish/ total number of fish

LC50 = calculated concentration (Dose) required to kill 50% of the fish (Calculated from relationship between probits of percentage mortalities and the logs of pesticide concentration applied)

Table 5.6. Cumulative mortalities and LC_{50} values of the pesticide, ethion for 3 different air breathing fish, *C. punctatus*, *H. fossilis* and *C. batrachus*.

Dose (µg/L)	***C. punctatus* (N/10)**	**Dose (µg/L)**	***H. fossilis* (N/10)**	**Dose (µg/L)**	***C. batrachus* (N/10)**
42	2/10	53	2/10	46	1/10
43	4/10	54	3/10	47	3/10
44	5/10	55	6/10	48	4/10
45	6/10	56	7/10	49	7/10
46	8/10	57	9/10	50	9/10
LC_{50}	**43.9 µg/L**	**LC_{50}**	**54.8 µg/L**	**LC_{50}**	**48.7 µg/L**

N/10 = number of dead fish/ total number of fish

LC50 = calculated concentration (Dose) required to kill 50% of the fish (Calculated from relationship between probits of percentage mortalities and the logs of pesticide concentration applied)

Table 5.7. Cumulative mortalities and LC_{50} values of the pesticide, dicofol for 3 different air breathing fish, *C. punctatus*, *H. fossilis* and *C. batrachus*.

Dose (µg/L)	***C. punctatus* (N/10)**	**Dose (µg/L)**	***H. fossilis* (N/10)**	**Dose (µg/L)**	***C. batrachus* (N/10)**
44	2/10	34	1/10	50	2/10
45	4/10	35	3/10	51	4/10
46	5/10	36	5/10	52	6/10
47	7/10	37	7/10	53	8/10
48	9/10	38	8/10	54	9/10
LC_{50}	**45.8 µg/L**	**LC_{50}**	**36.1 µg/L**	**LC_{50}**	**51.8 µg/L**

N/10 = number of dead fish/ total number of fish

LC50 = calculated concentration (Dose) required to kill 50% of the fish (Calculated from relationship between probits of percentage mortalities and the logs of pesticide concentration applied)

5.5. Assessment of cytochrome P450 in fish, *C. punctatus*, *H. fossilis* and *C. batrachus* administered with cypermethrin

Liver somatic index (LSI): Table 5.8 presents the LSI values for all the control and cypermethrin treated fish, *C. punctatus*, *H. fossilis* and *C. batrachus*. The LSI values of all the 3 control groups of fish displayed a homogeneous range. The LSI values in control fish, *C. punctatus*, *H. fossilis* and *C. batrachus* were 0.914±0.113, 0.997±0.173 and 0.976±0.162 respectively. All the treated fish displayed an elevated LSI values. The LSI values in *C. punctatus* for 5, 10 and 15 days treated groups were 0.985±0.096, 1.009±0.120 and 1.042±0.109 respectively. The LSI values in *H. fossilis* for 5, 10 and 15 days treated groups were 1.181±0.337, 1.214±0.280 and 1.245±0.144, and in *C. batrachus* were 1.090±0.070, 1.126±0.088 and 1.167±0.222 respectively. The significant difference ($p<0.05$) was shown by only 15 days treated group of *C. punctatus* and *H. fossilis* while *C. batrachus* showed no significant difference in any treated groups in comparison to their respective control group.

Microsomal protein content: Table 5.9 illustrates the microsomal protein content in control and cypermethrin treated fish. The microsomal protein content in control fish, *C. punctatus* was 2.796±0.397 mg/gm liver and in 5, 10 and 15 days treated groups were 3.622±0.725, 3.765±0.707 and 4.125±0.669 mg/gm liver respectively. The values were significantly different [5 and 10 days ($p<0.05$); 15 days ($p<0.01$)] from the control. Among *H. fossilis*, the microsomal protein content for control fish was 3.147±0.657 mg/gm liver and in 5, 10 and 15 days treated groups were 3.803±0.729, 4.566±0.709 and 4.981±0.633 mg/gm liver respectively. Although 5 days treated group showed an elevation in microsomal protein content, no significant difference was seen but 10 days and 15 days treated groups displayed a significant difference ($p<0.01$) in comparison to the control. In *C. batrachus*, the microsomal protein content for control, 5, 10 and 15 days treated groups were 3.068±0.537, 4.112±0.281, 4.262±0.148 and 4.994±0.385 mg/gm liver respectively. All the treated groups displayed a significant difference [5 and 10 days ($p<0.05$); 15 days ($p<0.01$)] in comparison to the control group. The 15 days treated group was observed to have higher protein content than 5 days and 10 days treated group.

CYP 450 content: Table 5.10 depicts the CYP 450 content in all 3 fish species for control and cypermethrin treated groups. In *C. punctatus*, the CYP 450 content in control group was

0.276±0.089 nmole/mg protein and in 5, 10 and 15 days treated groups were 0.359±0.020, 0.382±0.042 and 0.425±0.053 nmole/mg protein respectively. The CYP 450 content in control group of *H. fossilis* was 0.325±0.129 nmole/mg protein and in 5, 10 and 15 days treated groups were 0.404±0.083, 0.495±0.082 and 0.523±0.234 nmole/mg protein respectively. In *C. batrachus*, the CYP 450 content in control group was 0.296±0.082 nmole/mg protein and in 5, 10 and 15 days treated groups were 0.382±0.034, 0.461±0.091 and 0.484±0.131 nmole/mg protein respectively. The 10 days ($p<0.05$) and 15 days ($p<0.01$) treated groups displayed significant differences in all the fish studied compared to their respective control group. The 5 days treated group showed only a marginal increase in the CYP 450 content (Table 5.10).

EROD activity: Table 5.11 illustrates EROD activity from liver microsomes in control and cypermethrin treated fish. EROD activities in control fish, *C. punctatus* was 35.424±15.553 pmole resorufin formed/mg protein/min and in 5, 10 and 15 days treated groups were 71.119±17.685, 81.570±26.395 and 99.341±13.451 pmole resorufin formed/mg protein/min respectively. In *H. fossilis*, EROD activities in control, 5, 10 and 15 days treated groups were 42.935±11.097, 79.397±9.555, 94.886±16.453 and 97.516±15.619 pmole resorufin formed/mg protein/min respectively. In *C. batrachus*, EROD activities in control group was 37.891±14.398 pmole resorufin formed/mg protein/min and in 5, 10 and 15 days treated groups were 75.249±17.273, 99.284±14.351 and 123.934±29.316 pmole resorufin formed/mg protein/min respectively. Of the different treated groups, the 15 days treated groups revealed the highest elevation in EROD activity than 10 days and 5 days treated groups in all the 3 fish studied. Metabolism of 7-ER was higher in all the cypermethrin treated groups of 3 fish, *C. punctatus*, *H. fossilis* and *C. batrachus* displaying a significant difference ($p<0.001$) compared with their respective control group (Table 5.11).

N,N-DMA activity: Table 5.12 represents N, N-dimethylaniline demethylase (N,N-DMA) activity in hepatic microsomes in *C. punctatus*, *H. fossilis* and *C. batrachus* exposed to cypermethrin. In *C. punctatus*, 5 days (0.108±0.042 nmole formaldehyde formed/mg protein/min) and 10 days (0.116±0.052 nmole formaldehyde formed/mg protein/min) treated group showed no significant increase in N,N-DMA activity. The activity was significantly induced only in 15 days treated group (0.186±0.082 nmole formaldehyde formed/mg

protein/min) when compared to the control (0.091±0.044 nmole formaldehyde formed/mg protein/min). *H. fossilis*, on the other hand, revealed a significant increase in all the treated groups. The mean value increased from 0.135±0.056 nmole formaldehyde formed/mg protein/min in control to 0.229±0.084 nmole formaldehyde formed/mg protein/min after 5 days ($p<0.05$), 0.285±0.111 nmole formaldehyde formed/mg protein/min after 10 days ($p<0.001$) and 0.217±0.051 nmole formaldehyde formed/mg protein/min after 15 days ($p<0.05$). *C. batrachus* displayed a similar trend of response to that of *C. punctatus* with only 15 days treated group (0.338±0.147 nmole formaldehyde formed/mg protein/min) showing a significant difference ($p<0.05$) in activity. No significant difference was observed in 5 days (0.330±0.085 nmole formaldehyde formed/mg protein/min) and 10 days (0.331±0.138 nmole formaldehyde formed/mg protein/min) treated groups in comparison to that of the control group, 0.210±0.072 nmole formaldehyde formed/mg protein/min (Table 5.12).

AH activity: Table 5.13 illustrates aniline hydroxylase (AH) activity of hepatic microsomes in *C. punctatus*, *H. fossilis* and *C. batrachus* exposed to cypermethrin. *C. punctatus* showed no significant variation in the treated groups in AH activity. The AH activity in control, 5, 10 and 15 days treated groups were 0.119±0.046, 0.114±0.043, 0.110±0.056 and 0.170±0.061 nmole p-aminophenol formed/mg protein/min respectively. *H. fossilis*, on the other hand, displayed a significant difference ($p<0.05$) only in 15 days treated group (0.318±0.152 nmole p-aminophenol formed/mg protein/min). No elevation was observed in 5 days treated group (0.183±0.075 nmole p-aminophenol formed/mg protein/min) while a slight increase in 10 days treated group (0.274±0.094 nmole p-aminophenol formed/mg protein/min) was observed in comparison to the control (0.188±0.066 nmole p-aminophenol formed/mg protein/min). *C. batrachus* displayed elevated activity with a significant difference ($p<0.01$) in 10 days (0.395±0.059 nmole p-aminophenol formed/mg protein/min) and 15 days (0.387±0.143 nmole p-aminophenol formed/mg protein/min) treated groups in comparison to the control group (0.224±0.055 nmole p-aminophenol formed/mg protein/min). The 5 days treated group (0.248±0.093 nmole p-aminophenol formed/mg protein/min) displayed no change in the activity.

ERND activity: Table 5.14 illustrates erythromycin N-demethylase (ERND) activity of hepatic microsomes in *C. punctatus*, *H. fossilis* and *C. batrachus* exposed to cypermethrin.

Control *C. punctatus* (0.847±0.220 nmole formaldehyde formed/mg protein/min), *H. fossilis* (0.836±0.277 nmole formaldehyde formed/mg protein/min) and *C. batrachus* (0.886±0.223 nmole formaldehyde formed/mg protein/min) scored a value which was almost homogeneous with respect to the activity. All the fish exposed to cypermethrin showed down regulation in the enzyme activity. In *C. punctatus*, the ERND activities for 5, 10 and 15 days were 0.674±0.163, 0.561±0.139 and 0.611±0.139 nmole formaldehyde formed/mg protein/min respectively. In *H. fossilis*, the activities for 5, 10 and 15 days were 0.578±0.107, 0.564±0.201 and 0.504±0.142 nmole formaldehyde formed/mg protein/min respectively and in *C. batrachus* the activities for 5, 10 and 15 days were 0.637±0.149, 0.606±0.108 and 0.676±0.136 nmole formaldehyde formed/mg protein/min respectively. In *C. punctatus* and *H. fossilis* only 10 and 15 days treated groups resulted in significant difference ($p<0.05$) whereas *C. batrachus* revealed a significant difference in all the treated groups (5, 10 and 15 days) with respect to their control group (Table 5.14).

Table 5.8. Liver somatic index (LSI) of *C. punctatus*, *H. fossilis* and *C. batrachus* exposed to cypermethrin (n= 6).

Liver Somatic Index [LSI]			
	C. punctatus	***H. fossilis***	***C. batrachus***
Control	0.914±0.113	0.997±0.173	0.976±0.162
5 days	0.985±0.096	1.181±0.337	1.090±0.070
10 days	1.009±0.120	1.214±0.280	1.126±0.088
15 days	1.042±0.109*	1.245±0.144*	1.167±0.222
F value	1.924	2.637	1.849
P value	0.155	0.070	0.177

Values are the means ± SD. Means were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Significantly different * ($p<0.05$)

Table 5.9. Microsomal protein content of *C. punctatus*, *H. fossilis* and *C. batrachus* exposed to cypermethrin (n= 6).

Microsomal protein content (mg/gm liver)			
	C. punctatus	***H. fossilis***	***C. batrachus***
Control	2.796±0.397	3.147±0.657	3.068±0.537
5 days	3.622±0.725*	3.803±0.729	4.112±0.281*
10 days	3.765±0.707*	4.566±0.709**	4.262±0.148*
15 days	4.125±0.669**	4.981±0.633**	4.994±0.385**
F value	3.329	7.759	16.600
P value	0.039	0.022	0.008

Values are the means ± SD. Means were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Significantly different *($p<0.05$), ** ($p<0.01$)

Table 5.10. CYP 450 content of *C. punctatus*, *H. fossilis* and *C. batrachus* exposed to cypermethrin (n= 6).

CYP 450 content (nmole/mg protein)			
	C. punctatus	***H. fossilis***	***C. batrachus***
Control	0.276±0.089	0.325±0.129	0.296±0.082
5 days	0.359±0.020	0.404±0.083	0.382±0.034
10 days	0.382±0.042*	0.495±0.082*	0.461±0.091**
15 days	0.425±0.053**	0.523±0.234**	0.484±0.131**
F value	6.190	3.491	5.730
P value	0.003	0.029	0.007

Values are the means ± SD. Means were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Significantly different * ($p<0.05$), ** ($p<0.01$)

Table 5.11. EROD (ethoxyresorufin O-deethylase) activity of hepatic microsomes in *C. punctatus, H. fossilis* and *C. batrachus* exposed to cypermethrin (n= 6).

EROD activity (pmole resorufin formed/mg protein/min)			
	C. punctatus	***H. fossilis***	***C. batrachus***
Control	35.424±15.553	42.935±11.097	37.891±14.398
5 days	71.119±17.685**	79.397±9.555***	75.249±17.273**
10 days	81.570±26.395***	94.886±16.453***	99.284±14.351***
15 days	99.341±13.451***	97.516±15.619***	123.934±29.316***
F value	17.032	27.455	29.461
P value	0.000	0.000	0.000

Values are the means ± SD. Means were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Significantly different ** ($p<0.01$), *** ($p<0.001$)

Table 5.12. N, N-dimethylaniline demethylase (N,N-DMA) activity of hepatic microsomes in *C. punctatus, H. fossilis* and *C. batrachus* exposed to cypermethrin (n= 6).

N,N-DMA demethylase activity (nmole formaldehyde formed/mg protein/min)			
	C. punctatus	***H. fossilis***	***C. batrachus***
Control	0.091±0.044	0.135±0.056	0.210±0.072
5 days	0.108±0.042	0.229±0.084*	0.330±0.085
10 days	0.116±0.052	0.285±0.111***	0.331±0.138
15 days	0.186±0.082*	0.217±0.051*	0.338±0.147*
F value	3.301	6.586	2.438
P value	0.039	0.002	0.098

Values are the means ± SD. Means were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Significantly different * ($p<0.05$), *** ($p<0.001$)

Table 5.13. Aniline hydroxylase (AH) activity of hepatic microsomes in *C. punctatus*, *H. fossilis* and *C. batrachus* exposed to cypermethrin (n= 6).

Aniline hydroxylase activity (nmole p-aminophenol formed/mg protein/min)			
	C. punctatus	***H. fossilis***	***C. batrachus***
Control	0.119±0.046	0.188±0.066	0.224±0.055
5 days	0.114±0.043	0.183±0.075	0.248±0.093
10 days	0.110±0.056	0.274±0.094	0.395±0.059**
15 days	0.170±0.061	0.318±0.152*	0.387±0.143**
F value	1.326	3.454	6.279
P value	0.291	0.031	0.004

Values are the means ± SD. Means were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Significantly different * ($p<0.05$), ** ($p<0.01$)

Table 5.14. Erythromycin N-demethylase (ERND) activity of hepatic microsomes in *C. punctatus*, *H. fossilis* and *C. batrachus* exposed to cypermethrin (n= 6).

Erythromycin N-demethylase activity (nmole formaldehyde formed/mg protein/min)			
	C. punctatus	***H. fossilis***	***C. batrachus***
Control	0.847±0.220	0.836±0.277	0.886±0.223
5 days	0.674±0.163	0.578±0.107	0.637±0.149*
10 days	0.561±0.139**	0.564±0.201*	0.606±0.108**
15 days	0.611±0.139*	0.504±0.142**	0.676±0.136*
F value	3.632	3.507	3.870
P value	0.030	0.035	0.025

Values are the means ± SD. Means were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Significantly different * ($p<0.05$), ** ($p<0.01$)

5.6. Assessment of cytochrome P450 in fish, *C. punctatus*, *H. fossilis* and *C. batrachus* administered with ethion

Liver somatic index (LSI): Table 5.15 presents the LSI values of all the 3 fish species exposed to ethion. All of the treated fish displayed elevated LSI values. In *C. punctatus*, the significant difference ($p<0.01$) was shown by 10 days (1.075±0.085) and 15 days (1.155±0.122) treated groups in comparison to the control group (0.927±0.312). No significant difference was observed in 5 days exposure (1.051±0.044). *H. fossilis* and *C. batrachus* showed no significant elevation in LSI values in any of the treated groups. LSI values in *H. fossilis* for control, 5, 10 and 15 days exposed groups were 0.968±0.283, 1.026±0.119, 1.057±0.156 and 1.073±0.120 and in *C. batrachus* were 0.992±0.237, 1.033±0.053, 1.045±0.024 and 1.068±0.040 respectively. 15 days treated group showed the maximum increase in LSI value compared to 5 days and 10 days treated group in all the fish studied.

Microsomal protein content: Table 5.16 illustrates the microsomal protein content of *C. punctatus, H. fossilis* and *C. batrachus* exposed to ethion. All the ethion treated fish species displayed an increase in the microsomal protein content but only 15 days treated group showed a significant difference when compared with their respective control group. The microsomal protein content for control, 5, 10 and 15 days exposed groups in *C. punctatus* were 2.988±0.361, 3.272±0.763, 3.393±0.853 and 4.055±0.236 ($p<0.05$) mg/gm liver respectively. Among *H. fossilis*, the microsomal protein content in control, 5, 10 and 15 days exposed groups were 3.081±0.792, 3.435±0.192, 3.605±0.441 and 4.136±0.403 ($p<0.01$) mg/gm liver and in *C. batrachus*, the microsomal protein content for control, 5, 10 and 15 days exposed groups were 3.239±0.891, 3.447±0.062, 3.571±0.111 and 4.110±0.563 ($p<0.05$) mg/gm liver respectively.

CYP 450 content: Table 5.17 represents the CYP 450 content of all 3 fish species for control and ethion treated group. All the treated groups (5, 10 and 15 days) displayed an elevated level in CYP 450 content when compared with their respective control group. In *C. punctatus*, the mean value increased from 0.291±0.065 in control to 0.319±0.021 after 5 days, 0.417±0.187 after 10 days ($p<0.05$) and 0.550±0.120 nmole/mg protein after 15 days ($p<0.001$) of exposure. In *H. fossilis*, the mean value increased from 0.352±0.112 in control

to 0.438±0.074 after 5 days, 0.487±0.179 after 10 days and 0.599±0.159 nmole/mg protein after 15 days ($p<0.001$) of exposure. In *C. batrachus*, the mean value increased from 0.316±0.098 in control to 0.377±0.065 after 5 days, 0.424±0.133 after 10 days ($p<0.05$) and 0.542±0.084 nmole/mg protein after 15 days ($p<0.001$) of exposure.

EROD activity: Table 5.18 represents EROD activities from liver microsomes by using 7-ethoxyresorufin as a substrate in control and ethion treated fish. In the case of *C. punctatus*, EROD activity for the control group was 37.812±12.293 pmole resorufin formed/mg protein/min. The 5 days treated group displayed the highest induction (99.867±19.907 pmole resorufin formed/mg protein/min) while in 10 days and 15 days, the values were 88.593±11.527 and 92.195±34.060 pmole resorufin formed/mg protein/min respectively. The EROD activity in *H. fossilis* for control, 5, 10 and 15 days exposed groups were 40.153±13.381, 58.915±14.793, 67.624±21.636 and 86.944±21.720 pmole resorufin formed/mg protein/min respectively. In *C. batrachus*, the mean value increased from 42.101±16.173 in control to 74.925±16.197 after 5 days, 111.933±20.631 after 10 days and 104.447±24.162 pmole resorufin formed/mg protein/min after 15 days of exposure. All the treated groups of 3 fish species displayed a significant difference ($p<0.001$) when compared with their respective control group.

N,N-DMA activity: Table 5.19 displays the N, N-dimethylaniline demethylase (N,N-DMA) activity from hepatic microsomes of control and ethion treated fish, *C. punctatus*, *H. fossilis* and *C. batrachus*. N,N-DMA activities in *C. punctatus* for control, 5, 10 and 15 days treated groups were 0.094±0.059, 0.092±0.026, 0.098±0.010 and 0.120±0.020 nmole formaldehyde formed/mg protein/min respectively. *C. punctatus* hardly revealed any variation in N,N-DMA activity and only showed a marginal increase in 15 days treated group. *H. fossilis* revealed significant increase in 5 days (0.212±0.070 nmole formaldehyde formed/mg protein/min; $p<0.05$) and 10 days (0.249±0.077 nmole formaldehyde formed/mg protein/min; $p<0.001$) treated groups when compared to the control (0.148±0.086 nmole/mg protein/min), while the 15 days treated group (0.189±0.054 nmole formaldehyde formed/mg protein/min) displayed only a marginal increase in the activity. In *C. batrachus*, the N,N-DMA activity was significantly ($p<0.05$) induced only in the 5 days treated group (0.312±0.064 nmole formaldehyde formed/mg protein/min) in comparison to that of control group (0.208±0.095

nmole formaldehyde formed/mg protein/min) while 10 days (0.209±0.041 nmole formaldehyde formed/mg protein/min) and 15 days (0.273±0.075 nmole formaldehyde formed/mg protein/min) treated groups displayed a negligible response.

AH activity: Table 5.20 displays aniline hydroxylase (AH) activity of control and ethion treated fish. *C. punctatus* showed a significant increase ($p<0.05$) only in 10 days treated group (0.177±0.045 nmole p-aminophenol formed/mg protein/min). 5 days (0.086±0.046 nmole p-aminophenol formed/mg protein/min) and 15 days (0.121±0.050 nmole p-aminophenol formed/mg protein/min) treated groups displayed a negligible response in AH activity compared to the control group (0.109±0.052 nmole p-aminophenol formed/mg protein/min). *H. fossilis*, on the other hand, displayed a significant difference [10 days ($p<0.001$); 5 and 15 days ($p<0.001$)] in all the treated groups with respect to its control group. The AH activity in control, 5, 10 and 15 days treated groups were 0.197±0.086, 0.380±0.133, 0.316±0.076 and 0.455±0.078 nmole p-aminophenol formed/mg protein/min respectively. *C. batrachus* showed a negligible response in AH activity in all the treated groups. The AH activity in control, 5, 10 and 15 days treated groups were 0.214±0.075, 0.248±0.108, 0.172±0.032 and 0.219±0.064 nmole p-aminophenol formed/mg protein/min respectively. The 5 days treated group revealed a slight increase and 10 days a slight decrease, whereas 15 days treated group showed negligible response when compared to its control group.

ERND activity: Table 5.21 displays erythromycin N-demethylase activity (ERND) of hepatic microsomes in *C. punctatus*, *H. fossilis* and *C. batrachus* exposed to ethion. The ERND activity in control *C. punctatus* was 0.827±0.420 nmole formaldehyde formed/mg protein/min. The 5 days treated group (1.642±0.612 nmole formaldehyde formed/mg protein/min; $p<0.01$) reflected the highest induction. The significant increase in ERND activity were also noticed in 10 days (1.297±0.053 nmole/mg protein/min; $p<0.05$) and 15 days (1.585±0.309 nmole formaldehyde formed/mg protein/min; $p<0.01$) treated groups. In *H. fossilis*, the ERND activity varied significantly. The ERND activity in control was 0.878±0.327 and in 5, 10 and 15 days treated group were 1.743± 0.570 ($p<0.05$), 3.978±1.067 ($p<0.001$) and 3.630±1.058 ($p<0.001$) nmole formaldehyde formed/mg protein/min respectively. In *C. batrachus*, the ERND activity in control, 5, 10 and 15 days treated group were 0.836±0.291, 1.073±0.334, 1.314±0.210 and 1.579±0.520 nmole

formaldehyde formed/mg protein/min respectively. The activity differed significantly ($p<0.05$) only after 15 days of exposure in comparison to its control group.

Table 5.15. Liver somatic index (LSI) of *C. punctatus*, *H. fossilis* and *C. batrachus* exposed to ethion (n= 6).

Liver Somatic Index [LSI]			
	C. punctatus	***H. fossilis***	***C. batrachus***
Control	0.927±0.312	0.968±0.283	0.992±0.237
5 days	1.051±0.044	1.026±0.119	1.033±0.053
10 days	1.075±0.085**	1.057±0.156	1.045±0.024
15 days	1.155±0.122**	1.073±0.120	1.068±0.040
F value	6.173	0.401	0.672
P value	0.004	0.754	.580

Values are the means ± SD. Means were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Significantly different ** ($p<0.01$)

Table 5.16. Microsomal protein content of *C. punctatus*, *H. fossilis* and *C. batrachus* exposed to ethion (n= 6).

Microsomal protein content (mg/gm liver)			
	C. punctatus	***H. fossilis***	***C. batrachus***
Control	2.988±0.361	3.081±0.792	3.239±0.891
5 days	3.272±0.763	3.435±0.192	3.447±0.062
10 days	3.393±0.853	3.605±0.441	3.571±0.111
15 days	4.055±0.236*	4.136±0.403**	4.110±0.563*
F value	2.501	4.396	2.943
P value	0.108	0.021	0.070

Values are the means ± SD. Means were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Significantly different * ($p<0.05$), ** ($p<0.01$)

Table 5.17. CYP 450 content of *C. punctatus*, *H. fossilis* and *C. batrachus* exposed to ethion (n= 6).

CYP 450 content (nmole/mg protein)			
	C. punctatus	***H. fossilis***	***C. batrachus***
Control	0.291±0.065	0.352±0.112	0.316±0.098
5 days	0.319±0.021	0.438±0.074	0.377±0.065
10 days	0.417±0.187*	0.487±0.179	0.424±0.133*
15 days	0.550±0.120***	0.599±0.159***	0.542±0.084***
F value	5.416	5.858	7.182
P value	0.007	0.004	0.002

Values are the means ± SD. Means were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Significantly different * ($p<0.05$), *** ($p<0.001$).

Table 5.18. EROD (ethoxyresorufin O-deethylase) activity of hepatic microsomes in *C. punctatus, H. fossilis* and *C. batrachus* exposed to ethion (n= 6).

	EROD activity (pmole resorufin formed/mg protein/min)		
	C. punctatus	***H. fossilis***	***C. batrachus***
Control	37.812±12.293	40.153±13.381	42.101±16.173
5 days	99.867±19.907***	58.915±14.793	74.925±16.197**
10 days	88.593±11.527***	67.624±21.636**	111.933±20.631***
15 days	92.195±34.060***	86.944±21.720***	104.447±24.162***
F value	15.322	8.938	29.610
P value	0.000	0.000	0.000

Values are the means ± SD. Means were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Significantly different ** ($p<0.01$), *** ($p<0.001$)

Table 5.19. N, N-dimethylaniline demethylase (N,N-DMA) activity of hepatic microsomes in *C. punctatus, H. fossilis* and *C. batrachus* exposed to ethion (n= 6).

	N,N-DMA demethylase activity (nmole formaldehyde formed/mg protein/min)		
	C. punctatus	***H. fossilis***	***C. batrachus***
Control	0.094±0.059	0.148±0.086	0.208±0.095
5 days	0.092±0.026	0.212±0.070*	0.312±0.064*
10 days	0.098±0.010	0.249±0.077***	0.209±0.041
15 days	0.120±0.020	0.189±0.054	0.273±0.075
F value	0.471	5.102	2.315
P value	0.706	0.006	0.115

Values are the means ± SD. Means were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Significantly different * ($p<0.05$), *** ($p<0.001$)

Table 5.20. Aniline hydroxylase (AH) activity of hepatic microsomes in *C. punctatus*, *H. fossilis* and *C. batrachus* exposed to ethion (n= 6).

Aniline hydroxylase activity (nmole p-aminophenol formed/mg protein/min)			
	C. punctatus	***H. fossilis***	***C. batrachus***
Control	0.109±0.052	0.197±0.086	0.214±0.075
5 days	0.086±0.046	0.380±0.133***	0.248±0.108
10 days	0.177±0.045*	0.316±0.076**	0.172±0.032
15 days	0.121±0.050	0.455±0.078***	0.219±0.064
F value	2.768	17.656	1.146
P value	0.067	0.000	0.358

Values are the means ± SD. Means were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Significantly different * ($p<0.05$), ** ($p<0.01$), *** ($p<0.001$)

Table 5.21. Erythromycin N-demethylase (ERND) activity of hepatic microsomes in *C. punctatus*, *H. fossilis* and *C. batrachus* exposed to ethion (n= 6).

Erythromycin N-demethylase activity (nmole formaldehyde formed/mg protein/min)			
	C. punctatus	***H. fossilis***	***C. batrachus***
Control	0.827±0.420	0.878±0.327	0.836±0.291
5 days	1.642±0.612**	1.743±0.570*	1.073±0.334
10 days	1.297±0.053*	3.978±1.067***	1.314±0.210
15 days	1.585±0.309**	3.630±1.058***	1.579±0.520*
F value	7.474	25.462	4.382
P value	0.003	0.000	0.020

Values are the means ± SD. Means were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Significantly different * ($p<0.05$), ** ($p<0.01$), *** ($p<0.001$).

5.7. Assessment of cytochrome P450 in fish, *C. punctatus*, *H. fossilis* and *C. batrachus* administered with dicofol

Liver somatic index (LSI): Table 5.22 shows the LSI values of control and dicofol treated fish, *C. punctatus*, *H. fossilis* and *C. batrachus*. In *C. punctatus*, the mean value of LSI increased significantly from 0.936±0.349 in control to 1.079±0.186 ($p<0.01$) after 5 days, 1.161±0.126 ($p<0.05$) after 10 days and 1.177±0.140 ($p<0.01$) after 15 days of exposure. In *H. fossilis*, the 10 days (1.171±0.091; $p<0.05$) and 15 days (1.202±0.085; $p<0.01$) treated group revealed a significant increase in LSI value. The 5 days treated group (1.110±0.194) only showed a marginal increase in comparison to the control (0.964±0.316). In *C. batrachus*, the mean value increased significantly ($p<0.01$) from 0.982±0.296 in control to 1.531±0.488 after 5 days of exposure. Thereafter, the LSI value decreased to 1.410±0.284 ($p<0.01$) after 10 days and further to 1.263±0.049 ($p<0.05$) after 15 days of exposure.

Microsomal protein content: Table 5.23 illustrates the microsomal protein content in control and dicofol treated fish, *C. punctatus*, *H. fossilis* and *C. batrachus*. In *C. punctatus*, the microsomal protein content in control, 5, 10 and 15 days were 3.096±0.417, 3.352±0.961, 3.948±0.479 ($p<0.05$) and 4.264±0.656 ($p<0.01$) mg/gm liver respectively. In *H. fossilis*, the microsomal protein content in control, 5, 10 and 15 days were 3.281±0.693, 3.774±0.898, 3.986±0.422 ($p<0.05$) and 4.205±0.450 ($p<0.01$) mg/gm liver respectively. *C. batrachus* revealed a significant difference ($p<0.05$) only in 15 days treated group (4.380±1.128 mg/gm liver) with respect to its control group (2.996±0.732 mg/gm liver). The microsomal protein content in 5 and 10 days treated groups were 3.533±1.083 and 3.885±0.380 mg/gm liver respectively.

CYP 450 content: Table 5.24 illustrates the CYP 450 content in control and dicofol treated fish, *C. punctatus*, *H. fossilis* and *C. batrachus*. In *C. punctatus*, no significant difference was seen between control (0.288±0.091 nmole/mg protein) and 5 days treated group (0.318±0.085 nmole/mg protein) while the 10 days (0.401±0.107 nmole/mg protein; $p<0.05$) and 15 days (0.524±0.031 nmole/mg protein; $p<0.001$) treated groups displayed a significant difference. *H. fossilis* revealed a significant difference ($p<0.001$) only in 15 days treated group (0.713±0.397 nmole/mg protein) in comparison to its control group (0.338±0.157 nmole/mg protein). The CYP 450 content in 5 days and 10 days treated groups were 0.433±0.072 and

0.547±0.306 nmole/mg protein respectively. In *C. batrachus*, the CYP 450 content varied significantly ($p<0.001$) in all the treated groups compared to the control group (0.306±0.098 nmole/mg protein). The CYP 450 content in 5, 10 and 15 days treated groups were 0.534±0.111, 0.613±0.105 and 0.705±0.069 nmole/mg protein respectively.

EROD activity: Table 5.25 represents the EROD activities from liver microsomes in both the control and dicofol treated groups of *C. punctatus*, *H. fossilis* and *C. batrachus*. The entire dicofol treated groups of 3 fish species varied significantly ($p<0.001$) compared to their respective control group. In *C. punctatus*, the mean value increased from 39.142±14.964 pmole resorufin formed/mg protein/min in control to 128.861±29.076 pmole resorufin formed/mg protein/min after 5 days, then a drastic increase was seen at 10 days (173.690±30.021 pmole resorufin formed/mg protein/min), thereafter, the activity decreased slightly after 15 days of exposure (135.096±26.195 pmole resorufin formed/mg protein/min). On the other hand, the 15 days treated groups of *C. batrachus* and *H. fossilis* revealed the highest elevation in EROD activity. In *H. fossilis*, EROD activity in control, 5, 10 and 15 days treated groups were 41.621±13.147, 99.236±8.225, 115.037±11.420 and 153.767±18.840 pmole resorufin formed/mg protein/min respectively. In *C. batrachus*, EROD activity in control, 5, 10 and 15 days treated groups were 44.573±16.246, 130.736±5.375, 140.843±14.073 and 156.055±8.652 pmole resorufin formed/mg protein/min respectively.

N,N-DMA activity: Table 5.26 displays the N, N-dimethylaniline demethylase (N,N-DMA) activity from hepatic microsomes in *C. punctatus*, *H. fossilis* and *C. batrachus* exposed to dicofol. In *C. punctatus* and *H. fossilis*, only the 15 days treated group displayed a significant induction ($p<0.001$) in comparison to the control. The N,N-DMA activities in *C. punctatus* for control, 5, 10 and 15 days treated group were 0.102±0.052, 0.105±0.013, 0.119±0.011 and 0.291±0.091 nmole formaldehyde formed/mg protein/min respectively. In *H. fossilis*, the N,N-DMA activities in control, 5, 10 and 15 days treated group were 0.141±0.074, 0.150±0.016, 0.175±0.028 and 0.267±0.063 nmole formaldehyde formed/mg protein/min respectively. *C. batrachus* displayed a slight decrease in the activity with no significant difference at 5 days (0.142±0.055 nmole formaldehyde formed/mg protein/min) and 10 days (0.156±0.067 nmole formaldehyde formed/mg protein/min) treated groups compared to its

control group (0.221±0.095 nmole formaldehyde formed/mg protein/min). The activity recovered and increased to 0.250±0.053 nmole formaldehyde formed/mg protein/min after 15 days of exposure but was not significant.

AH activity: Table 5.27 displays aniline hydroxylase (AH) activities of control and dicofol treated fish species from hepatic microsomes. In *C. punctatus*, the activity decreased from 0.116±0.051 nmole p-aminophenol formed/mg protein/min in control to 0.098±0.022 after 5 days, 0.079±0.019 after 10 days and 0.081±0.034 nmole p-aminophenol formed/mg protein/min after 15 days of exposure but was not significant. *H. fossilis*, on the other hand, displayed a significant increase ($p<0.001$) in all the treated groups. The AH activities in control, 5, 10 and 15 days treated groups were 0.197±0.082, 0.583±0.172, 0.491±0.087 and 0.726±0.230 nmole p-aminophenol formed/mg protein/min respectively. *C. batrachus* displayed a decrease in AH activity in 5 days treated group (0.169±0.031 nmole p-aminophenol formed/mg protein/min), then recovered after 10 days (0.241±0.118 nmole p-aminophenol formed/mg protein/min) and 15 days (0.212±0.064 nmole p-aminophenol formed/mg protein/min) when compared to the control group (0.219±0.079 nmole p-aminophenol formed/mg protein/min). No significant difference was observed in any of the treated groups.

ERND activity: Table 5.28 displays erythromycin N-demethylase (ERND) activity of hepatic microsomes in control and dicofol treated *C. punctatus*, *H. fossilis* and *C. batrachus*. The ERND activity of *C. punctatus* in control, 5, 10 and 15 days treated groups were 0.858±0.412, 2.183±0.213 ($p<0.05$), 5.889±0.523 ($p<0.001$) and 9.566±1.807 ($p<0.001$) nmole formaldehyde formed/mg protein/min respectively. In *H. fossilis*, only 5 days treated group (3.156±1.410 nmole formaldehyde formed/mg protein/min) showed a significant difference ($p<0.001$) in comparison to its control group (0.844±0.327 nmole formaldehyde formed/mg protein/min). The ERND activities in 10 days and 15 days treated groups were 1.234±0.423 and 1.278±0.260 nmole formaldehyde formed/mg protein/min respectively. *C. batrachus*, on the other hand, revealed highest induction (2.168±0.392 nmole formaldehyde formed/mg protein/min) and a significant difference ($p<0.001$) only in 15 days treated group with respect to its control group (0.878±0.298 nmole formaldehyde formed/mg protein/min). The 5 days treated group showed a marginal increase (1.171±0.121 nmole formaldehyde

formed/mg protein/min) in the activity, thereafter, it decreased in 10 days (0.913±0.142 nmole formaldehyde formed/mg protein/min) of exposure.

Table 5.22. Liver somatic index (LSI) of *C. punctatus*, *H. fossilis* and *C. batrachus* exposed to dicofol (n= 6).

Liver somatic index [LSI %]			
	C. punctatus	***H. fossilis***	***C. batrachus***
Control	0.936±0.349	0.964±0.316	0.982±0.296
5 days	1.079±0.186*	1.110±0.194	1.531±0.488**
10 days	1.161±0.126**	1.171±0.091*	1.410±0.284**
15 days	1.177±0.140**	1.202±0.085**	1.263±0.049*
F value	6.714	3.447	6.555
P value	0.002	0.030	0.003

Values are the means ± SD. Means were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Significantly different * ($p<0.05$), ** ($p<0.01$)

Table 5.23. Microsomal protein content of *C. punctatus*, *H. fossilis* and *C. batrachus* exposed to dicofol (n= 6).

Microsomal protein content (mg/gm liver)			
	C. punctatus	***H. fossilis***	***C. batrachus***
Control	3.096±0.417	3.281±0.693	2.996±0.732
5 days	3.352±0.961	3.774±0.898	3.533±1.083
10 days	3.948±0.479*	3.986±0.422*	3.885±0.380
15 days	4.264±0.656**	4.205±0.450*	4.380±1.128*
F value	4.708	2.951	1.746
P value	0.014	0.059	0.203

Values are the means ± SD. Means were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Significantly different * ($p<0.05$), ** ($p<0.01$)

Table 5.24. CYP 450 content of *C. punctatus*, *H. fossilis* and *C. batrachus* exposed to dicofol (n= 6).

CYP 450 content (nmole/mg protein)			
	C. punctatus	***H. fossilis***	***C. batrachus***
Control	0.288±0.091	0.338±0.157	0.306±0.098
5 days	0.318±0.085	0.433±0.072	0.534±0.111***
10 days	0.401±0.107*	0.547±0.306	0.613±0.105***
15 days	0.524±0.031***	0.713±0.397***	0.705±0.069***
F value	12.992	4.551	29.980
P value	0.000	0.010	0.000

Values are the means ± SD. Means were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Significantly different * ($p<0.05$), *** ($p<0.001$)

Table 5.25. EROD (ethoxyresorufin O-deethylase) activity of hepatic microsomes in *C. punctatus, H. fossilis* and *C. batrachus* exposed to dicofol (n= 6).

	EROD activity (pmole resorufin formed/mg protein/min)		
	C. punctatus	***H. fossilis***	***C. batrachus***
Control	39.142±14.964	41.621±13.147	44.573±16.246
5 days	128.861±29.076***	99.236±8.225***	130.736±5.375***
10 days	173.690±30.021***	115.037±11.420***	140.843±14.073***
15 days	135.096±26.195***	153.767±18.840***	156.055±8.652***
F value	48.939	88.325	195.437
P value	0.000	0.000	0.000

Values are the means ± SD. Means were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Significantly different *** ($p<0.001$).

Table 5.26. N, N-dimethylaniline demethylase (N,N-DMA) activity of hepatic microsomes in *C. punctatus, H. fossilis* and *C. batrachus* exposed to dicofol (n= 6).

	N,N-DMA demethylase activity (nmole formaldehyde formed/mg protein/min)		
	C. punctatus	***H. fossilis***	***C. batrachus***
Control	0.102±0.052	0.141±0.074	0.221±0.095
5 days	0.105±0.013	0.150±0.016	0.142±0.055
10 days	0.119±0.011	0.175±0.028	0.156±0.067
15 days	0.291±0.091***	0.267±0.063***	0.250±0.053
F value	17.732	7.160	2.603
P value	0.004	0.008	0.082

Values are the means ± SD. Means were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Significantly different *** ($p<0.001$)

Table 5.27. Aniline hydroxylase (AH) activity of hepatic microsomes in *C. punctatus*, *H. fossilis* and *C. batrachus* exposed to dicofol (n= 6).

Aniline hydroxylase activity (nmole p-aminophenol formed/mg protein/min)			
	C. punctatus	***H. fossilis***	***C. batrachus***
Control	0.116±0.051	0.197±0.082	0.219±0.079
5 days	0.098±0.022	0.583±0.172***	0.169±0.031
10 days	0.079±0.019	0.491±0.087***	0.241±0.118
15 days	0.081±0.034	0.726±0.230***	0.212±0.064
F value	2.009	28.615	0.831
P value	0.138	0.000	0.493

Values are the means ± SD. Means were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Significantly different *** ($p<0.001$)

Table 5.28. Erythromycin N-demethylase (ERND) activity of hepatic microsomes in *C. punctatus*, *H. fossilis* and *C. batrachus* exposed to dicofol (n= 6).

Erythromycin N-demethylase activity (nmole formaldehyde formed/mg protein/min)			
	C. punctatus	***H. fossilis***	***C. batrachus***
Control	0.858±0.412	0.844±0.327	0.878±0.298
5 days	2.183±0.213*	3.156±1.410***	1.171±0.121
10 days	5.889±0.523***	1.234±0.423	0.913±0.142
15 days	9.566±1.807***	1.278±0.260	2.168±0.392***
F value	103.195	11.735	27.492
P value	0.000	0.000	0.000

Values are the means ± SD. Means were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Significantly different * ($p<0.05$), *** ($p<0.001$)

5.8. Assessment of *in vitro* kinetics of hepatic phase I biotransformation reactions in 3 air breathing teleost fish, *C. punctatus, H. fossilis* and *C. batrachus*

Results for Vmax and Km values of EROD, N,N-DMA, AH and ERND kinetics are presented in Tables 5.29, 5.31, 5.33 and 5.35 respectively. Vmax/Km ratios are depicted in Tables 5.30, 5.32, 5.34 and 5.36. Representative plots of enzyme kinetic data obtained from *in vitro* metabolism of various compounds by CYP 450 system and fitted in the Michaelis-Menten kinetics model is presented in Figure 5.5.

5.8.1. 7-Ethoxyresorufin O-deethylation (EROD) kinetics

Vmax and Km values of EROD reaction in 3 fish, *C. punctatus*, *H. fossilis* and *C. batrachus* are illustrated in Table 5.29. In *C. punctatus*, EROD Vmax values in control, cypermethrin, ethion and dicofol exposed groups were 42.785±4.560, 114.517±13.222, 109.952±8.493 and 161.665±12.252 pmole resorufin formed/mg protein/min respectively. In *H. fossilis*, EROD Vmax values in control, cypermethrin, ethion and dicofol exposed groups were 49.685±3.835, 111.190±10.412, 98.632±7.103 and 160.332±11.976 pmole resorufin formed/mg protein/min respectively. Similarly, in *C.batrachus*, EROD Vmax value in control, cypermethrin, ethion and dicofol exposed groups were 40.490±6.359, 147.740±13.296, 122.107±10.756 and 180.770±12.185 pmole resorufin formed/mg protein/min respectively. The entire experimental groups of all the fish studied displayed a significant difference ($p<0.001$) in Vmax value when compared to their respective control with the highest induction seen in dicofol exposure.

In *C. punctatus*, Km values in control, cypermethrin, ethion and dicofol exposed groups were 0.421±0.035, 0.479±0.020, 0.715±0.073 and 0.494±0.080 µM respectively. Only ethion exposed group showed a statistically significant difference ($p<0.001$) from the control. *H. fossilis* displayed lower Km value with a significant difference in all the treated groups when compared with the control (0.731±0.021 µM). The Km values in cypermethrin, ethion and dicofol treated groups were 0.507±0.017 ($p<0.001$), 0.477±0.013 ($p<0.001$) and 0.633±0.017 ($p<0.05$) µM respectively. In *C. batrachus*, all the pesticide treated groups showed a significant difference ($p<0.001$) from the control (0.427±0.019 µM). The Km values in cypermethrin, ethion and dicofol treated groups were 0.629±0.043, 0.669±0.023 and 0.630±0.017 µM respectively. The highest Km value was recorded for *C. punctatus* treated with ethion (0.715 µM) and lowest for ethion treated *H. fossilis* (0.477 µM) (Table 5.29).

Intrinsic clearance expressed as Vmax/Km ratio for 3 fish, *C. punctatus*, *H. fossilis* and *C. batrachus* are shown in Table 5.30. In case of EROD activity in *C. punctatus*, Vmax/Km ratio varied significantly when compared to the control. Vmax/Km ratio in control, cypermethrin, ethion and dicofol were 102.416±24.325, 238.687±52.658 ($p<0.01$), 154.074±35.321 and 337.881±62.318 ($p<0.001$) respectively. In *H. fossilis*, Vmax/Km ratio in control, cypermethrin, ethion and dicofol were 68.056±12.534, 218.709±48.153, 206.580±39.452 and 252.870±42.158 respectively.The values were significantly different ($p<0.001$) from the control. In *C.batrachus*, Vmax/Km ratio in control was 94.469±18.348 and in cypermethrin, ethion and dicofol exposed groups were 236.519±34.572 ($p<0.001$), 183.097±31.257 ($p<0.05$) and 287.371±52.876 ($p<0.001$) respectively. In all the 3 fish species, the highest Vmax/Km ratio was seen in dicofol exposure followed by cypermethrin and ethion (Table 5.30).

Table 5.29. Michaelis-Menten parameters for 7-ethoxyresorufin O-deethylase activity using 7-ethoxyresorufin as a substrate in hepatic microsomal suspensions prepared from control and treated fish (n= 4).

Subject of Study		Vmax (pmole resorufin formed/mg protein/min)	Km (µM)
C. punctatus	Control	42.785±4.560	0.421±0.035
	Cypermethrin	114.517±13.222***	0.479±0.020
	Ethion	109.952±8.493***	0.715±0.073***
	Dicofol	161.665±12.252***	0.494±0.080
H. fossilis	Control	49.685±3.835	0.731±0.021
	Cypermethrin	111.190±10.412***	0.507±0.017***
	Ethion	98.632±7.103***	0.477±0.013***
	Dicofol	160.332±11.976***	0.633±0.017*
C. batrachus	Control	40.490±6.359	0.427±0.019
	Cypermethrin	147.740±13.296***	0.629±0.043***
	Ethion	122.107±10.756***	0.669±0.023***
	Dicofol	180.770±12.185***	0.630±0.017***

Values are the means ± SD. Means were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Significantly different * ($p<0.05$), *** ($p<0.001$)

Table 5.30. Comparison of microsomal intrinsic clearance values analyzed by enzyme kinetic method: CI_{int} = Vmax/Km for selected substrate 7-ethoxyresorufin between control and treated *C. punctatus*, *H. fossilis* and *C. batrachus* (n=4).

	Intrinsic clearance		
	C. punctatus	***H. fossilis***	***C. batrachus***
Control	102.416±24.325	68.056±12.534	94.469±18.348
Cypermethrin	238.687±52.658**	218.709±48.153***	236.519±34.572***
Ethion	154.074±35.321	206.580±39.452***	183.097±31.257*
Dicofol	337.881±62.318***	252.870±42.158***	287.371±52.876***

Values are the means ± SD. Means were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Significantly different * ($p<0.05$), ** ($p<0.01$), *** ($p<0.001$)

5.8.2. N, N-Dimethylaniline demethylation (N,N-DMA) kinetics

N, N-Dimethylaniline demethylase kinetic parameters for 3 fish examined are presented in Table 5.31. In *C. punctatus*, the Vmax values varied significantly in all the experimental groups when compared to the control group. Vmax values in control, cypermethrin, ethion and dicofol exposed groups were 0.099±0.012, 0.198±0.016 ($p<0.01$), 0.144±0.018 ($p<0.05$) and 0.305±0.019 ($p<0.001$) nmole formaldehyde formed/mg protein/min respectively. In *H. fossilis*, the entire experimental groups showed a significant difference ($p<0.001$) when compared to the control (0.138±0.019 nmole formaldehyde formed/mg protein/min). Vmax values in cypermethrin, ethion and dicofol exposed groups were 0.289±0.020, 0.224±0.024 and 0.278±0.011 nmole formaldehyde formed/mg protein/min respectively. In *C.batrachus*, the Vmax value with a significant difference was seen in cypermethrin (0.357±0.021 nmole formaldehyde formed/mg protein/min; $p<0.001$) and ethion (0.279±0.019 nmole formaldehyde formed/mg protein/min; $p<0.01$) exposure with respect to its control group (0.222±0.015 nmole formaldehyde formed/mg protein/min). The Vmax value in dicofol exposed group was 0.255±0.018 nmole formaldehyde formed/mg protein/min.

All the tested fish species displayed a mixed response for the Km value. *C. punctatus* displayed the highest basal Km value (0.285±0.021 mM) followed by *C. batrachus* (0.244±0.018 mM) and *H. fossilis* (0.182±0.013 mM). The entire experimental groups in *C. punctatus* displayed a lower Km value with a significant difference compared to its control group. The Km value in *C. punctatus* for cypermethrin, ethion and dicofol exposure was 0.212±0.018 ($p<0.05$), 0.165±0.033 ($p<0.01$) and 0.203±0.016 ($p<0.05$) mM respectively. In *H. fossilis* a significant difference was observed in cypermethrin (0.156±0.014 mM; $p<0.05$) and dicofol exposure (0.294±0.020 mM; $p<0.001$). The Km value was homogeneous in ethion (0.186±0.012 mM) exposed group with respect to its control. Dicofol exposed group displayed a higher Km value while cypermethrin and ethion exposed groups displayed a lower Km value. In *C. batrachus*, the Km values were almost homogeneous in all the experimental groups compared to the control (0.244±0.023 mM). Km values for cypermethrin, ethion and dicofol exposure were 0.225±0.018, 0.216±0.015 and 0.236±0.020 mM respectively (Table 5.31).

Intrinsic clearance expressed as Vmax/Km ratios in case of N,N-DMA for 3 fish, *C. punctatus, H. fossilis* and *C. batrachus* are presented in Table 5.32. In *C. punctatus* all the experimental groups displayed a higher Vmax/Km ratio with a significant difference ($p<0.001$) compared to its control group (0.350±0.059). The Vmax/Km ratios in

cypermethrin, ethion and dicofol exposure were 0.936 ±0.218, 0.884±0.246 and 1.511±0.464 respectively. Both *H. fossilis* and *C. batrachus* displayed a significant difference ($p<0.001$) in cypermethrin and ethion exposed groups. The Vmax/Km ratio in control, cypermethrin, ethion and dicofol exposure in *H. fossilis* were 0.758±0.229, 1.861±0.418, 1.207±0.425 and 0.946±0.324 respectively and in *C. batrachus*, the Vmax/Km ratio were 0.911±0.314, 1.592±0.498, 1.299±0.512 and 1.083±0.435 respectively (Table 5.32).

Table 5.31. Michaelis-Menten parameters for N,N-DMA demethylase activity using N, N-dimethylaniline as a substrate in hepatic microsomal suspensions prepared from control and treated fish (n= 4).

Subject of Study		Vmax (nmole formaldehyde formed/mg protein/min)	Km (mM)
	Control	0.099±0.012	0.285±0.021
	Cypermethrin	0.198±0.016**	0.212±0.018*
C. punctatus	Ethion	0.144±0.018*	0.165±0.033**
	Dicofol	0.305±0.019***	0.203±0.016*
	Control	0.138±0.019	0.182±0.013
	Cypermethrin	0.289±0.020***	0.156±0.014*
H. fossilis	Ethion	0.224±0.024***	0.186±0.012
	Dicofol	0.278±0.011***	0.294±0.020***
	Control	0.222±0.015	0.244±0.023
	Cypermethrin	0.357±0.021***	0.225±0.018
C. batrachus	Ethion	0.279±0.019**	0.216±0.015
	Dicofol	0.255±0.018	0.236±0.020

Values are the means ± SD. Means were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Significantly different * ($p<0.05$), ** ($p<0.01$), *** ($p<0.001$)

Table 5.32. Comparison of microsomal intrinsic clearance values analyzed by enzyme kinetic method: CI_{int} = Vmax/Km for selected substrate N, N-dimethylaniline between control and treated *C. punctatus*, *H. fossilis* and *C. batrachus* (n=4).

	Intrinsic clearance		
	C. punctatus	***H. fossilis***	***C. batrachus***
Control	0.350±0.059	0.758±0.229	0.911±0.314
Cypermethrin	0.936 ±0.218***	1.861±0.418***	1.592±0.498***
Ethion	0.884±0.246***	1.207±0.425***	1.299±0.512***
Dicofol	1.511±0.464***	0.946±0.324	1.083±0.435

Values are the means ± SD. Means were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Significantly different *** ($p<0.001$)

5.8.3. Aniline hydroxylation (AH) kinetics

Table 5.33 illustrates Vmax and Km values of AH reaction in 3 fish, *C. punctatus*, *H. fossilis* and *C. batrachus*. In *C. punctatus*, cypermethrin and ethion exposed groups displayed a significant difference in Vmax value compared to the control. Vmax value for AH activity in control was 0.122±0.015 nmole p-aminophenol formed/mg protein/min, and in cypermethrin, ethion and dicofol were 0.181±0.010 ($p<0.001$), 0.149±0.015 ($p<0.05$) and 0.107±0.011 nmole p-aminophenol formed/mg protein/min respectively. *H. fossilis* showed a significant difference ($p<0.001$) in all the exposed groups compared to control (0.189±0.019 nmole p-aminophenol formed/mg protein/min). The Vmax values in cypermethrin, ethion and dicofol were 0.315±0.021, 0.446±0.033 and 0.720±0.027 nmole p-aminophenol formed/mg protein/min respectively. In *C. batrachus*, only cypermethrin (0.345±0.029 nmole p-aminophenol formed/mg protein/min) exposed group displayed a significant increase ($p<0.001$) in Vmax value when compared to the control group (0.229±0.014 nmole p-aminophenol formed/mg protein/min) while ethion (0.215±0.019 nmole p-aminophenol formed/mg protein/min) and dicofol (0.210±0.017 nmole p-aminophenol formed/mg protein/min) exposed groups showed reduced activity with no significant difference.

In *C. punctatus*, the Km values in control, cypermethrin, ethion and dicofol exposure were 1.056±0.159, 1.068±0.090, 1.120±0.102 and 1.102±0.125 mM respectively. In *H. fossilis*, ethion (1.133±0.033 mM; $p<0.05$) and dicofol (0.842±0.045 mM; $p<0.01$) exposed groups displayed a lower Km value with a significant difference in comparison to the control (1.834±0.120 mM). The cypermethrin exposed group (1.781±0.164 mM) did not show any difference with the control. In *C. batrachus*, only cypermethrin (1.588±0.087 mM) exposed group with a lower Km value displayed a significant difference ($p<0.01$) with respect to its control group (2.170±0.130 mM). The Km values in ethion and dicofol exposed groups were 2.286±0.179 mM and 2.346±0.150 mM respectively (Table 5.33).

Intrinsic clearance expressed as Vmax/Km ratios for 3 fish, *C. punctatus*, *H. fossilis* and *C. batrachus* in relation to aniline hydroxylation are presented in Table 5.34. In *C. punctatus*, Vmax/Km ratio revealed a significant difference only in cypermethrin exposure (0.170±0.053), showing it to be catalytically efficient ($p<0.01$) in comparison to the control (0.116±0.023). The Vmax/Km ratio in ethion and dicofol exposed groups were 0.133±0.025 and 0.098±0.015 respectively. In *H. fossilis*, Vmax/Km ratio in control was 0.103±0.014 and in cypermethrin, ethion and dicofol exposure were 0.179±0.046, 0.394±0.086 and 0.857±0.123 respectively. The dicofol exposed group ($p<0.001$) was seen to be the most

catalytically efficient of all followed by ethion ($p<0.001$) while cypermethrin exposed group showed a negligible variation with respect to the control. In *C. batrachus*, only cypermethrin exposed group (0.217±0.065) was seen to be catalytically efficient ($p<0.001$) in comparison to the control (0.106±0.017) while the ethion (0.094±0.014) and dicofol (0.090±0.012) exposed groups showed lower Vmax/Km ratio with negligible variation (Table 5.34).

Table 5.33. Michaelis-Menten parameters for aniline hydroxylase activity using aniline as a substrate in hepatic microsomal suspensions prepared from control and treated fish (n= 4).

Subject of Study		Vmax (nmole p-aminophenol formed/mg protein/min)	Km (mM)
	Control	0.122±0.015	1.056±0.159
	Cypermethrin	0.181±0.010*	1.068±0.090
C. punctatus	Ethion	0.149±0.015*	1.120±0.102
	Dicofol	0.107±0.011	1.102±0.125
	Control	0.189±0.019	1.834±0.120
	Cypermethrin	0.315±0.021***	1.781±0.164
H. fossilis	Ethion	0.446±0.033***	1.133±0.033*
	Dicofol	0.720±0.027***	0.842±0.045**
	Control	0.229±0.014	2.170±0.130
	Cypermethrin	0.345±0.029***	1.588±0.087**
C. batrachus	Ethion	0.215±0.019	2.286±0.179
	Dicofol	0.210±0.017	2.346±0.150

Values are the means ± SD. Means were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Significantly different * ($p<0.05$), ** ($p<0.01$), *** ($p<0.001$)

Table 5.34. Comparison of microsomal intrinsic clearance values analyzed by enzyme kinetic method: CI_{int} = Vmax/Km for selected substrate aniline between control and treated *C. punctatus*, *H. fossilis* and *C. batrachus* (n=4).

	Intrinsic clearance		
	C. punctatus	***H. fossilis***	***C. batrachus***
Control	0.116±0.023	0.103±0.014	0.106±0.017
Cypermethrin	0.170±0.053***	0.179±0.046	0.217±0.065***
Ethion	0.133±0.025	0.394±0.086***	0.094±0.014
Dicofol	0.098±0.015	0.857±0.123***	0.090±0.012

Values are the means ± SD. Means were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Significantly different *** ($p<0.001$)

5.8.4. Erythromycin N-demethylation (ERND) kinetics

Erythromycin N-demethylase kinetics parameters are shown in Table 5.35 for 3 fish, *C. punctatus*, *H. fossilis* and *C. batrachus* examined in this study. All the control groups showed a homogeneous Vmax value. In *C. punctatus*, the Vmax value for control was 0.896±0.071 nmole formaldehyde formed/mg protein/min and in cypermethrin, ethion and dicofol exposed groups, the Vmax values were 0.817±0.027, 1.578±0.096 and 5.633±0.203 nmole formaldehyde formed/mg protein/min respectively. Dicofol ($p<0.001$) and ethion ($p<0.01$) exposed group displayed a significant difference while cypermethrin displayed a lower value with a negligible difference in comparison to the control. In both *H. fossilis* and *C. batrachus*, the Vmax values varied significantly with a significant increase seen in ethion and dicofol exposure and a significant reduction in cypermethrin exposed group. In *H. fossilis*, the Vmax values for control, cypermethrin, ethion and dicofol exposed groups were 0.872±0.033, 0.615±0.021 ($p<0.05$), 3.817±0.118 ($p<0.001$) and 1.648±0.066 ($p<0.01$) nmole formaldehyde formed/mg protein/min respectively. In *C. batrachus*, the Vmax values for control, cypermethrin, ethion and dicofol exposed groups were 0.842±0.023, 0.717±0.018 ($p<0.05$), 1.713±0.060 ($p<0.01$) and 2.201±0.033 ($p<0.001$) nmole formaldehyde formed/mg protein/min respectively.

C. punctatus exposed to cypermethrin (0.233±0.016 mM) displayed a significant elevation ($p<0.001$) while ethion exposure (0.041±0.015 mM) displayed a significant reduction ($p<0.001$) in Km values when compared to control (0.105±0.014 mM). The dicofol exposure (0.084±0.012 mM) displayed a lower Km value with negligible variation. In *H. fossilis* only cypermethrin exposure (0.210±0.016 mM) displayed a significant difference ($p<0.01$) in Km value compared to its respective control group (0.167±0.019 mM). The ethion exposure (0.184±0.018 mM) displayed a slightly higher and dicofol exposure (0.146±0.013 mM) a slightly lower Km values. On the other hand, *C. batrachus* showed a homogeneous value for Km in all the experimental groups along with the control. The Km values in control, cypermethrin, ethion and dicofol were 0.137±0.015, 0.146±0.010, 0.145±0.018 and 0.142±0.017 mM respectively. The basal Km value was highest in *H. fossilis* followed by *C. batrachus* and *C. punctatus* (Table 5.35).

Intrinsic clearance (Vmax/Km) in case of erythromycin N-demethylation for 3 fishes, *C. punctatus*, *H. fossilis* and *C. batrachus* are presented in Table 5.36. The Vmax/Km ratio in *C. punctatus* for control, cypermethrin, ethion and dicofol were 8.533±1.258, 3.523±0.823, 42.968±7.238 and 67.979±11.687 respectively. The Vmax/Km ratio in *H. fossilis* for control,

cypermethrin, ethion and dicofol were 5.278±1.671, 2.947±0.762, 20.913±4.687 and 11.283±1.897 respectively, and in *C. batrachus*, the Vmax/Km ratio were 6.205±1.982, 4.953±1.245, 11.942±2.634 and 15.636±5.179 respectively. Vmax/Km ratio indicated that the dicofol and ethion exposed groups were observed to be catalytically efficient ($p<0.001$) while the cypermethrin exposed group showed a lower Vmax/Km ratio with no significant difference.

Table 5.35. Michaelis-Menten parameters for erythromycin N-demethylase activity using erythromycin as a substrate in hepatic microsomal suspensions prepared from control and treated fish (n= 4).

Subject of Study		Vmax (nmole formaldehyde formed/mg protein/min)	Km (mM)
	Control	0.896±0.071	0.105±0.014
	Cypermethrin	0.817±0.027	0.233±0.016***
C. punctatus	Ethion	1.578±0.096**	0.041±0.015***
	Dicofol	5.633±0.203***	0.084±0.012
	Control	0.872±0.033	0.167±0.019
	Cypermethrin	0.615±0.021*	0.210±0.016**
H. fossilis	Ethion	3.817±0.118***	0.184±0.018
	Dicofol	1.648±0.066**	0.146±0.013
	Control	0.842±0.023	0.137±0.015
	Cypermethrin	0.717±0.018*	0.146±0.010
C. batrachus	Ethion	1.713±0.060**	0.145±0.018
	Dicofol	2.201±0.033***	0.142±0.017

Values are the means ± SD. Means were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Significantly different * ($p<0.05$), ** ($p<0.01$), *** ($p<0.001$)

Table 5.36. Comparison of microsomal intrinsic clearance values analyzed by enzyme kinetic method: CI_{int} = Vmax/Km for selected substrate erythromycin between control and treated *C. punctatus*, *H. fossilis* and *C. batrachus* (n=4).

	Intrinsic clearance		
	C. punctatus	***H. fossilis***	***C. batrachus***
Control	8.533±1.258	5.278±1.671	6.205±1.982
Cypermethrin	3.523±0.823	2.947±0.762	4.953±1.245
Ethion	42.968±7.238***	20.913±4.687***	11.942±2.634***
Dicofol	67.979±11.687***	11.283±1.897***	15.636±5.179***

Values are the means ± SD. Means were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Significantly different *** ($p<0.001$)

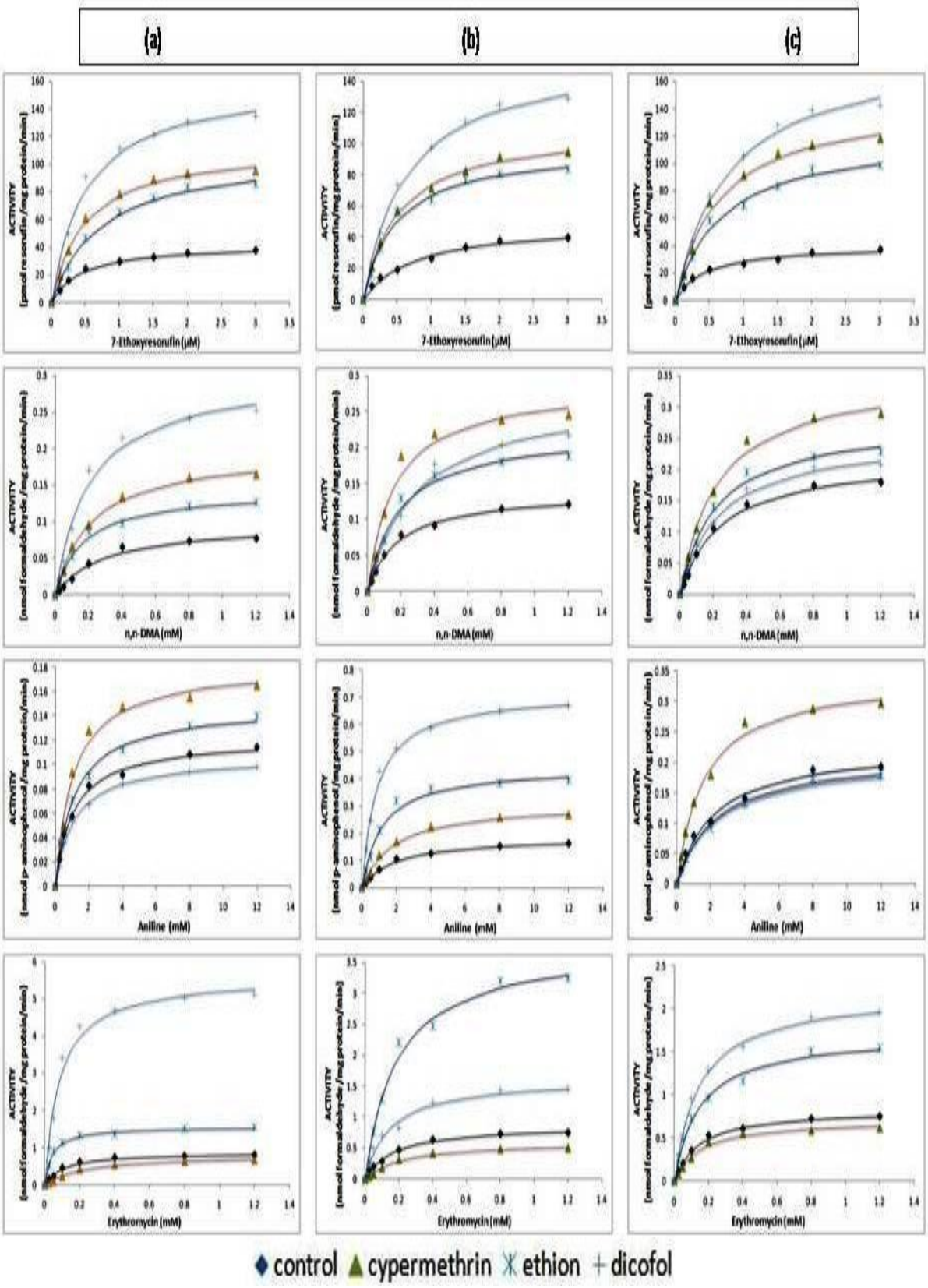


Figure 5.5. Michaelis-Menten plots for EROD, N,N-DMA, AH and ERND activities obtained from *in vitro* metabolism by CYP 450 system: (a) *C. punctatus*, (b) *H. fossilis*, and (c) *C.batrachus*.

5.9. Assessment of CYP450 from hepatic tissue in fish collected from local areas near the tea plantation in Chopra (Balarampur and Katagaon), Kalaghaj (Dangra Dangri and Ariagoan), Sonapur, Magurmari, Lotchka, Changa and Deomani

The fish, *Channa punctatus*, *Channa striatus*, *Channa gachua*, *Heteropneustes fossilis* and *Clarias batrachus* were collected from different sites, though all the species were not found in every sites. The fish, *C. punctatus* and *H. fossilis* were collected from all the sites except Changa and Deomani, while *C. batrachus* were not found in Balarampur (Chopra), Dangra Dangri and Ariagoan in Kalaghaj, Changa and Deomani. *C. striatus* was found only in Katagaon (Chopra) and *C. gachua* was found only at sites, Changa and Deomani. N,N-DMA, AH and ERND activities for *C. gachua* could not be performed due to lack of enzyme quantity. Fishes were collected during November, 2012 to June, 2013. During the survey and collection of fishes, pesticide bottles and packets were also found near the pond sites in Balarampur, Katagoan, and Ariagoan (Fig. 5.6).

Figure 5.6. Pesticide bottles and packets found near the pond sites from where fish were caught: (a) Balarampur, Chopra (b) Katagoan, Chopra and (c) Ariagoan, Kalaghaj.

Liver somatic index (LSI): Table 5.37 shows LSI values of fishes collected from different sites. Among *C. punctatus*, the fish collected from Magurmari (1.16±0.39) displayed a significant difference ($p<0.05$) in LSI value in comparison to the fish collected from Dangra Dangri (0.92±0.31). The LSI values in *C. punctatus* collected from Balarampur, Katagaon, Ariagoan, Sonapur and Lotchka were 0.98±0.34, 0.972, 0.95±0.46, 1.09±0.40 and 1.08±0.32 respectively. The LSI value of *C. striatus* collected from Katagaon was 0.970 and the LSI values of *C. gachua* collected from Changa and Deomani were 1.02±0.41 and 1.04±0.53 respectively (Table 5.37).

The LSI values in *H. fossilis* collected from all the sites displayed a homogeneous range. The LSI values in *H. fossilis* collected from Balarampur, Katagaon, Dangra Dangri, Ariagoan, Sonapur, Magurmari and Lotchka were 1.26±0.42, 1.285, 1.07±0.41, 1.114, 1.23±0.52 1.20±0.40 and 1.18±0.43 respectively (Table 5.37).

In the case of *C. batrachus*, the fish collected from Lotchka (1.42±0.42) displayed a significant difference ($p<0.05$) in LSI value compared to fish collected from Magurmari (1.18±0.41).The LSI values in fish caught from Katagaon and Sonapur were 1.360 and 1.202 respectively (Table 5.37).

CYP 450 content: CYP 450 content in hepatic microsomes of fishes collected from different sites has been tabulated in Table 5.38. Among *C. punctatus*, fish brought from Balarampur (0.56±0.21 nmole/mg protein), Magurmari (0.48±0.22 nmole/mg protein) and Lotchka (0.58±0.28 nmole/mg protein) displayed a significant difference ($p<0.05$) in comparison to fish collected from Dangra Dangri (0.34±0.12 nmole/mg protein) and Ariagoan (0.32±0.09 nmole/mg protein). The CYP 450 content in fish collected from Katagoan and Sonapur were 0.711 and 0.42±0.18 nmole/mg protein respectively. Overall, *C. punctatus* collected from Katagaon showed the highest and the fish from Ariagoan showed the lowest CYP 450 content. Among *C. gachua*, the fish caught from Changa showed higher CYP 450 content (0.28±0.08 nmole/mg protein) than that caught from Deomani (0.19±0.07 nmole/mg protein). The CYP 450 content of *C. striatus* caught only in Katagaon was 0.400 nmole/mg protein (Table 5.38).

The CYP 450 content in *H. fossilis* collected from Balarampur (0.66±0.24 nmole/mg protein), Magurmari (0.84±0.31 nmole/mg protein) and Lotchka (0.74±0.33 nmole/mg protein) displayed a significant difference ($p<0.05$) compared to fish caught from Dangra Dangri

(0.25±0.08 nmole/mg protein) and Sonapur (0.42±0.12 nmole/mg protein). The CYP 450 content in fish collected from Katagoan and Ariagoan were 0.710 and 0.325 nmole/mg protein respectively. *H. fossilis* collected from Magurmari showed the highest and the fish from Dangra Dangri showed the lowest CYP 450 content (Table 5.38).

No statistical significance could be found among *C. batrachus*. CYP 450 content in *C. batrachus* brought from Katagaon, Sonapur, Magurmari and Lotchka were 1.066, 0.557, 0.57±0.28 and 0.54±0.22 nmole/mg protein respectively. Overall *C. batrachus* brought from Katagaon showed the highest CYP 450 content and the fish from Lotchka showed the lowest (Table 5.38).

EROD activity: Table 5.39 shows the EROD activity in all the species of fish collected from different sites. All the fishes displayed a variable range of EROD activity. Among *C. punctatus,* the fish brought from Sonapur (163±42 pmole resorufin formed/mg protein/min) and Magurmari (160±45 pmole resorufin formed/mg protein/min) displayed a significant difference ($p<0.05$) compared to fish brought from Dangra Dangri (107±34 pmole resorufin formed/mg protein/min) and Ariagoan (100±24 pmole resorufin formed/mg protein/min). The EROD activity in *C. punctatus* brought from Balarampur, Katagoan and Lotchka were 154±32, 153 and 147±28 pmole resorufin formed/mg protein/min respectively. *C. striatus* collected from Katagoan showed an EROD activity of 155 pmole resorufin formed/mg protein/min. Among *C. gachua*, the fish collected from Changa (83±28 pmole resorufin formed/mg protein/min) displayed a significant difference ($p<0.05$) from the fish collected from Deomani (22±12 pmole resorufin formed/mg protein/min) (Table 5.39).

Among *H. fossilis*, the fish collected from Sonapur (157±46 pmole resorufin formed/mg protein/min) and Magurmari (167±64 pmole resorufin formed/mg protein/min) displayed a significant difference ($p<0.05$) from the fish collected from Dangra Dangri (106±59 pmole resorufin formed/mg protein/min). The EROD activity in *H. fossilis* collected from Balarampur, Katagoan, Ariagoan and Lotchka were 132±48, 127, 95 and 146±51 pmole resorufin formed/mg protein/min respectively. Overall the fish collected from Magurmari showed the highest EROD activity and the fish collected from Ariagoan the lowest (Table 5.39).

No statistical significance was seen between *C. batrachus* in EROD activity. The fish brought from Magurmari (187±52 pmole resorufin formed/mg protein/min) displayed the highest

activity followed by Sonapur (167 pmole resorufin formed/mg protein/min), Katagoan (153 pmole resorufin formed/mg protein/min) and Lotchka (150±56 pmole resorufin formed/mg protein/min) (Table 5.39).

N,N-DMA activity: N, N-dimethylaniline demethylase (N,N-DMA) activity in air breathing teleost fish, *C. punctatus*, *C. striatus*, *H. fossilis* and *C. batrachus* brought from different sites have been presented in Table 5.40. The N,N-DMA activity in fish, *C. punctatus* brought from Dangra Dangri (0.64±0.31 nmole formaldehyde formed/mg protein/min) and Ariagoan (0.73±0.49 nmole formaldehyde formed/mg protein/min) showed a significant difference ($p<0.05$) than that collected from other sites. The N,N-DMA activity in fish, *C. punctatus* collected from Balarampur, Katagoan, Sonapur, Magurmari and Lotchka were 0.21±0.11, 0.282, 0.17±0.12, 0.27±0.13 and 0.21±0.10 nmole formaldehyde formed/mg protein/min respectively. *C. striatus* collected from Katagoan showed 0.137 nmole formaldehyde formed/mg protein/min of N,N-DMA activity (Table 5.40).

Among *H. fossilis*, the fish collected from Dangra Dangri (0.89±0.43 nmole formaldehyde formed/mg protein/min) and Magurmari (0.36±0.16 nmole formaldehyde formed/mg protein/min) displayed a significant difference ($p<0.05$) in N,N-DMA activity compared to fish collected from all other sites. A significant difference ($p<0.05$) in N,N-DMA activity was also seen between the fish collected from Dangra Dangri and Magurmari. The N,N-DMA activity in fish, *H. fossilis* collected from Balarampur, Katagoan, Ariagoan, Sonapur and Lotchka were 0.20±0.11, 0.272, 0.756, 0.16±0.09 and 0.31±0.17 nmole formaldehyde formed/mg protein/min respectively (Table 5.40).

No statistical significance was observed among *C. batrachus* caught from various site. The highest N,N-DMA activity was observed in fish collected from Lotchka (0.32±0.16 nmole formaldehyde formed/mg protein/min) followed by Magurmari (0.31±0.14 nmole formaldehyde formed/mg protein/min), Sonapur (0.295 nmole formaldehyde formed/mg protein/min) and Katagoan (0.198 nmole formaldehyde formed/mg protein/min) (Table 5.40).

AH activity: Aniline hydroxylase (AH) activity in air breathing teleost fish, *C. punctatus, C. striatus*, *H. fossilis* and *C. batrachus* collected from different sites have been presented in Table 5.41. Among *C. punctatus*, the fish collected from Sonapur (0.19±0.09 nmole p-aminophenol formed/mg protein/min) and Magurmari (0.18±0.10 nmole p-aminophenol

formed/mg protein/min) displayed a significant difference ($p<0.05$) in AH activity compared to fish collected from other sites. The AH activity in *C. punctatus* collected from Balarampur, Katagoan, Dangra Dangri, Ariagoan and Lotchka were 0.12±0.05, 0.137, 0.09±0.02, 0.08±0.03 and 0.12±0.06 nmole p-aminophenol formed/mg protein/min respectively. Overall, *C. punctatus* collected from Sonapur showed the highest and Ariagoan showed the lowest AH activity. The AH activity in *C. striatus* collected from Katagoan was 0.119 nmole p-aminophenol formed/mg protein/min (Table 5.41).

Among *H. fossilis*, the fish collected from Balarampur (0.34±0.12 nmole p-aminophenol formed/mg protein/min) displayed a significant difference ($p<0.05$) in AH activity than the fish collected from Lotchka (0.20±0.09 nmole p-aminophenol formed/mg protein/min). The AH activity in fish collected from Katagoan, Dangra Dangri, Ariagoan, Sonapur and Magurmari were 0.409, 0.24±0.09, 0.236, 0.25±0.11 and 0.21±0.14 nmole p-aminophenol formed/mg protein/min respectively (Table 5.41).

On the other hand, *C. batrachus* displayed homogeneous activity from all the sampling sites with no significant difference. The AH activity in fish collected from Katagoan, Sonapur, Magurmari and Lotchka were 0.341, 0.327, 0.30±0.12, 0.33±0.18 nmole p-aminophenol formed/mg protein/min respectively (Table 5.41).

ERND activity: Erythromycin N-demethylase (ERND) activity in air breathing teleost fish, *C. punctatus*, *C. striatus*, *H. fossilis* and *C. batrachus* collected from different sites have been presented in Table 5.42. Among *C. punctatus*, the fish brought from Dangra Dangri (2.58±0.91 nmole formaldehyde formed/mg protein/min), Ariagoan (2.18±0.98 nmole formaldehyde formed/mg protein/min), Sonapur (3.03±1.25 nmole formaldehyde formed/mg protein/min) and Magurmari (2.09±1.21 nmole formaldehyde formed/mg protein/min) displayed a significant difference ($p<0.05$) in ERND activity when compared to fish collected from Balarampur (0.98±0.42 nmole formaldehyde formed/mg protein/min). The fish collected from Lotchka and Katagoan showed 1.69±0.99 and 0.866 nmole formaldehyde formed/mg protein/min of ERND activity. The ERND activity of *C. striatus* collected from Katagoan was 1.097 nmole formaldehyde formed/mg protein/min (Table 5.42).

Among *H. fossilis*, the fish collected from Dangra Dangri (3.55±1.58 nmole formaldehyde formed/mg protein/min), Magurmari (4.13±1.52 nmole formaldehyde formed/mg protein/min) and Lotchka (3.14±1.32 nmole formaldehyde formed/mg protein/min) displayed

a significant difference ($p<0.05$) from Balarampur (1.37±0.85 nmole formaldehyde formed/mg protein/min) and Sonapur (1.69±0.86 nmole formaldehyde formed/mg protein/min). The ERND acivity of *H. fossilis* collected from Katagoan and Ariagoan were 1.402 and 4.080 nmole formaldehyde formed/mg protein/min respectively (Table 5.42).

No significant difference was observed in *C. batrachus* caught from different sites. The highest ERND activity was observed in fish caught from Magurmari (3.21±1.43 nmole formaldehyde formed/mg protein/min) followed by Sonapur (2.917 nmole formaldehyde formed/mg protein/min), Lotchka (2.34±1.02 nmole formaldehyde formed/mg protein/min) and Katagoan (0.944 nmole formaldehyde formed/mg protein/min) (Table 5.42).

Table 5.37. Liver somatic index (LSI) in air breathing teleost fish, *C. punctatus*, *C. striatus*, *C. gachua*, *H. fossilis* and *C. batrachus* brought from different sites. Values are the mean of (n) number of experiments.

Liver Somatic Index [LSI%]					
Sampling site	***Channa punctatus***	***Channa striatus***	***Channa gachua***	***Heteropneustes fossilis***	***Clarias batrachus***
Balarampur	0.98±0.34 [ab] (2)	-	-	1.26±0.42[a] (2)	-
Katagaon	0.972 (1)	0.970 (1)	-	1.285 (1)	1.360 (1)
Dangra Dangri	0.92±0.31[a] (3)	-	-	1.07±0.41[a] (2)	-
Ariagoan	0.95±0.46 [ab] (2)	-	-	1.114 (1)	-
Sonapur	1.09±0.40 [ab] (2)	-	-	1.23±0.52 [a] (2)	1.202 (1)
Magurmari	1.16±0.39[b] (4)	-	-	1.20±0.40 [a] (3)	1.18±0.41[a] (3)
Lotchka	1.08±0.32[ab] (2)	-	-	1.18±0.43 [a] (2)	1.42±0.42[b] (2)
Changa	-	-	1.02±0.41[a] (2)	-	-
Deomani	-	-	1.04±0.53[a] (2)	-	-

Values are the means ± sd. Means were compared using Tukey HSD.

Values with different superscripts in a column are significantly different at $p<0.05$

Table 5.38. CYP 450 content in air breathing teleost fish, *C. punctatus*, *C. striatus*, *C. gachua*, *H. fossilis* and *C. batrachus* brought from different sites. Values are the mean of (n) number of experiments.

CYP 450 content (nmole/mg protein)					
Sampling site	***Channa punctatus***	***Channa striatus***	***Channa gachua***	***Heteropneustes fossilis***	***Clarias batrachus***
Balarampur	0.56±0.21 [b] (2)	-	-	0.66±0.24 [b] (2)	-
Katagaon	0.711 (1)	0.400 (1)	-	0.710 (1)	1.066 (1)
Dangra Dangri	0.34±0.12 [a] (3)	-	-	0.25±0.08 [a] (2)	-
Ariagoan,	0.32±0.09 [a] (2)	-	-	0.325 (1)	-
Sonapur	0.42±0.18 [ab] (2)	-	-	0.42±0.12 [a] (2)	0.557(1)
Magurmari	0.48±0.22 [b] (4)	-	-	0.84±0.31 [b] (3)	0.57±0.28 [a] (3)
Lotchka	0.58±0.28 [b] (2)	-	-	0.74±0.33 [b] (2)	0.54±0.22 [a] (2)
Changa	-	-	0.28±0.08 [a] (2)	-	-
Deomani	-	-	0.19±0.07 [a] (2)	-	-

Values are the means ± sd. Means were compared using Tukey HSD.

Values with different superscripts in a column are significantly different at $p<0.05$

Table 5.39. EROD activities in air breathing teleost fish, *C. punctatus*, *C. striatus*, *C. gachua*, *H. fossilis* and *C. batrachus* brought from different sites. Values are the mean of (n) number of experiments.

EROD activity (pmole resorufin formed/mg protein/min)					
Sampling site	***Channa punctatus***	***Channa striatus***	***Channa gachua***	***Heteropneustes fossilis***	***Clarias batrachus***
Balarampur	154±32 ^{ab} (2)	-	-	132±48 ^{a} (2)	-
Katagaon	153 (1)	155 (1)	-	127 (1)	153(1)
Dangra Dangri	107±34 ^{a} (3)	-	-	106±59^{a} (2)	-
Ariagoan,	100±24 ^{a} (2)	-	-	95 (1)	-
Sonapur	163±42 ^{b} (2)	-	-	157±46 ^{b} (2)	167 (1)
Magurmari	160±45 ^{b} (4)	-	-	167±64 ^{b} (3)	187±52 ^{a} (3)
Lotchka	147±28 ^{ab} (2)	-	-	146±51^{ab} (2)	150±56 ^{a} (2)
Changa	-	-	83±28 ^{b} (2)	-	-
Deomani	-	-	22±12 ^{a} (2)	-	-

Values are the means ± sd. Means were compared using Tukey HSD.

Values with different superscripts in a column are significantly different at $p<0.05$

Table 5.40. N, N-dimethylaniline demethylase (N,N-DMA) activities fish, *C. punctatus*, *C. striatus*, *C. gachua*, *H. fossilis* and *C. batrachus* brought from different sites. Values are the mean of (n) number of experiments.

N,N-DMA demethylase activity (nmole formaldehyde formed/mg protein/min)					
Sampling site	***Channa punctatus***	***Channa striatus***	***Channa gachua***	***Heteropneustes fossilis***	***Clarias batrachus***
Balarampur	0.21±0.11^{a} (2)	-	-	0.20±0.11 ^{a} (2)	-
Katagaon	0.282 (1)	0.137(1)	-	0.272 (1)	0.198 (1)
Dangra Dangri	0.64±0.31^{b} (3)	-	-	0.89±0.43 ^{c} (2)	-
Ariagoan,	0.73±0.49 ^{b} (2)	-	-	0.756 (1)	-
Sonapur	0.17±0.12 ^{a} (2)	-	-	0.16±0.09 ^{a} (2)	0.295 (1)
Magurmari	0.27±0.13 ^{a} (4)	-	-	0.36±0.16 ^{b} (3)	0.31±0.14 ^{a} (3)
Lotchka	0.21±0.10 ^{a} (2)	-	-	0.31±0.19 ^{a} (2)	0.32±0.16 ^{a} (2)
Changa	-	-	-	-	-
Deomani	-	-	-	-	-

Values are the means ± sd. Means were compared using Tukey HSD.

Values with different superscripts in a column are significantly different at $p<0.05$

Table 5.41. Aniline hydroxylase (AH) activities in air breathing teleost fish, *C. punctatus*, *C. striatus*, *C. gachua*, *H. fossilis* and *C. batrachus* brought from different sites. Values are the mean of (n) number of experiments.

Aniline hydroxylase activity (nmole p-aminophenol formed/mg protein/min)					
Sampling site	***Channa punctatus***	***Channa striatus***	***Channa gachua***	***Heteropneustes fossilis***	***Clarias batrachus***
Balarampur	0.12±0.05 [a] (2)	-	-	0.34±0.12 [b] (2)	-
Katagaon	0.137 (1)	0.119 (1)	-	0.409 (1)	0.341 (1)
Dangra Dangri	0.09±0.02 [a] (3)	-	-	0.24±0.10 [ab] (2)	-
Ariagoan,	0.08±0.03 [a] (2)	-	-	0.236 (1)	-
Sonapur	0.19±0.09 [b] (2)	-	-	0.25±0.11 [ab] (2)	0.327 (1)
Magurmari	0.18±0.10 [b] (4)	-	-	0.21±0.14 [ab] (3)	0.30±0.12[a] (3)
Lotchka	0.12±0.06 [a] (2)	-	-	0.20±0.09 [a] (2)	0.33±0.18[a] (2)
Changa	-	-	-	-	-
Deomani	-	-	-	-	-

Values are the means ± sd. Means were compared using Tukey HSD.

Values with different superscripts in a column are significantly different at $p<0.05$

Table 5.42. Erythromycin N-demethylase (ERND) activities in air breathing teleost fish, *C. punctatus*, *C. striatus*, *C. gachua*, *H. fossilis* and *C. batrachus* brought from different sites. Values are the mean of (n) number of experiments.

Erythromycin N-demethylase activity (nmole formaldehyde formed/mg protein/min)					
Sampling site	***Channa punctatus***	***Channa striatus***	***Channa gachua***	***Heteropneustes fossilis***	***Clarias batrachus***
Balarampur	0.98±0.42 [a] (2)	-	-	1.37±0.85 [a] (2)	-
Katagaon	0.866 (1)	1.097 (1)	-	1.402 (1)	0.944 (1)
Dangra Dangri	2.58±0.91 [b] (3)	-	-	3.55±1.58 [b] (2)	-
Ariagoan,	2.18±0.98 [b] (2)	-	-	4.080 (1)	-
Sonapur	3.03±1.25 [b] (2)	-	-	1.69±0.86 [a] (2)	2.917 (1)
Magurmari	2.09±1.21 [b] (4)	-	-	4.13±1.52 [b] (3)	3.21±1.43[a] (3)
Lotchka	1.69±0.99 [ab] (2)	-	-	3.14±1.32 [b] (2)	2.34±1.02[a] (2)
Changa	-	-	-	-	-
Deomani	-	-	-	-	-

Values are the means ± sd. Means were compared using Tukey HSD.

Values with different superscripts in a column are significantly different at $p<0.05$

5.10. SDS-PAGE and Heme staining

Electrophoresis of hepatic microsomal preparations from 3 fish species, *C. punctatus*, *H. fossilis* and *C. batrachus* were stained for heme-related peroxidase activity to estimate the relative molecular mass of the CYP 450 enzyme. Following heme staining by N,N,N,N-tetramethyl-p-phenylenediamine (TMPD), proteins in the vicinity of molecular mass 50-56 kDa were stained in each of the 3 fish species along with other bands at around 20-29 kDa as shown by densitometric curve (Fig. 5.7 a, b and c; left panel). Similar enhancement was observed in SDS-PAGE gels as evidenced by coomassie brilliant blue (CBB) staining (Fig. 5.7 a, b and c; left panel).

The SDS electrophoretic profile of microsomal proteins from control and pesticide treated fish species, *C. punctatus*, *H. fossilis* and *C. batrachus* are shown in figure 5.8, 5.9 and 5.10 a, b and c. In *C. punctatus*, the molecular mass 53-54 kDa protein increased in intensity when treated with all 3 pesticides, cypermethrin, ethion and dicofol but was most pronounced in the dicofol treated (53.4 kDa) followed by the ethion (54 kDa) and cypermethrin (53.5 kDa) treated groups (Fig. 5.8 a, b and c) as evidenced by densitometric curve.

In *H. fossilis*, a protein band of approximately 52-53 kDa exhibited higher intensity in the pesticide treated fish in comparison to that of control fish. The highest intensity was seen in cypermethrin (52.6 kDa) followed by ethion (52.4 kDa) and dicofol (52.7 kDa) treated fish population (Fig. 5.9 a, b and c).

In microsomal preparation from control and pesticide treated *C. batrachus*, bands were visualized in molecular mass 50-52 kDa with increased intensity in all pesticide treated groups. The highest intensity in band was seen in dicofol (50.6 kDa) followed by ethion (50.4 kDa) and cypermethrin (51.5 kDa) treated groups (Fig. 5.10 a, b and c).

Many other bands in the different molecular mass range were also seen to have higher intensities in microsomes of treated fish species but were not verified as they were not relevant to the present study.

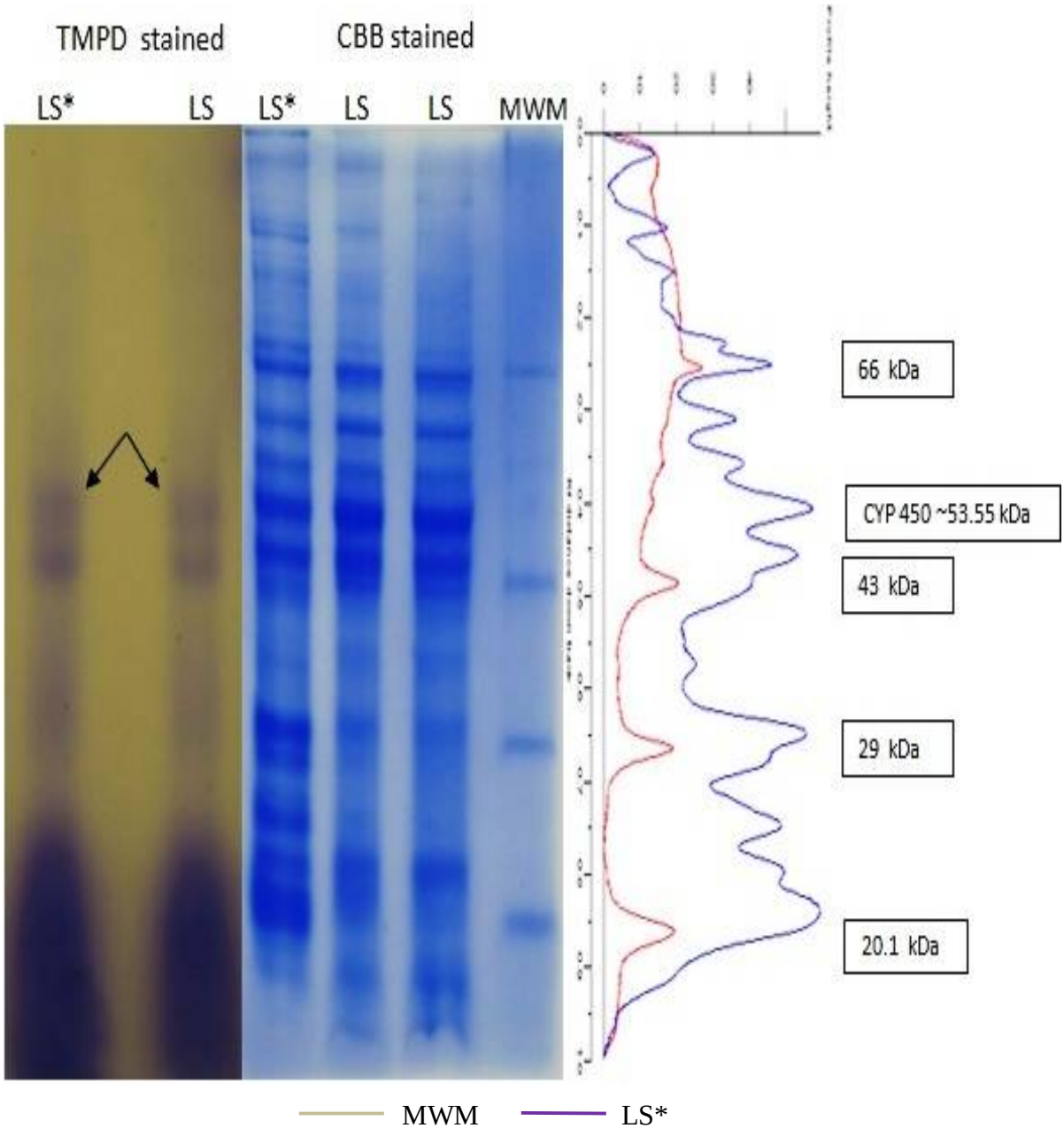


Figure 5.7a. Differentially stained SDS-PAGE protein gels showing the analysis of CYP 450 enzymes from hepatic microsomes in *C. punctatus.* Left panel: Protein gel developed with TMPD/hydrogen peroxide stain specific for heme. Right panel: Protein gel stained with Coomassie dye. A faint band corresponding to free heme at around ~53.55 kDa can be seen as indicated by the arrow.

MWM= Molecular weight marker; LS = liver sample

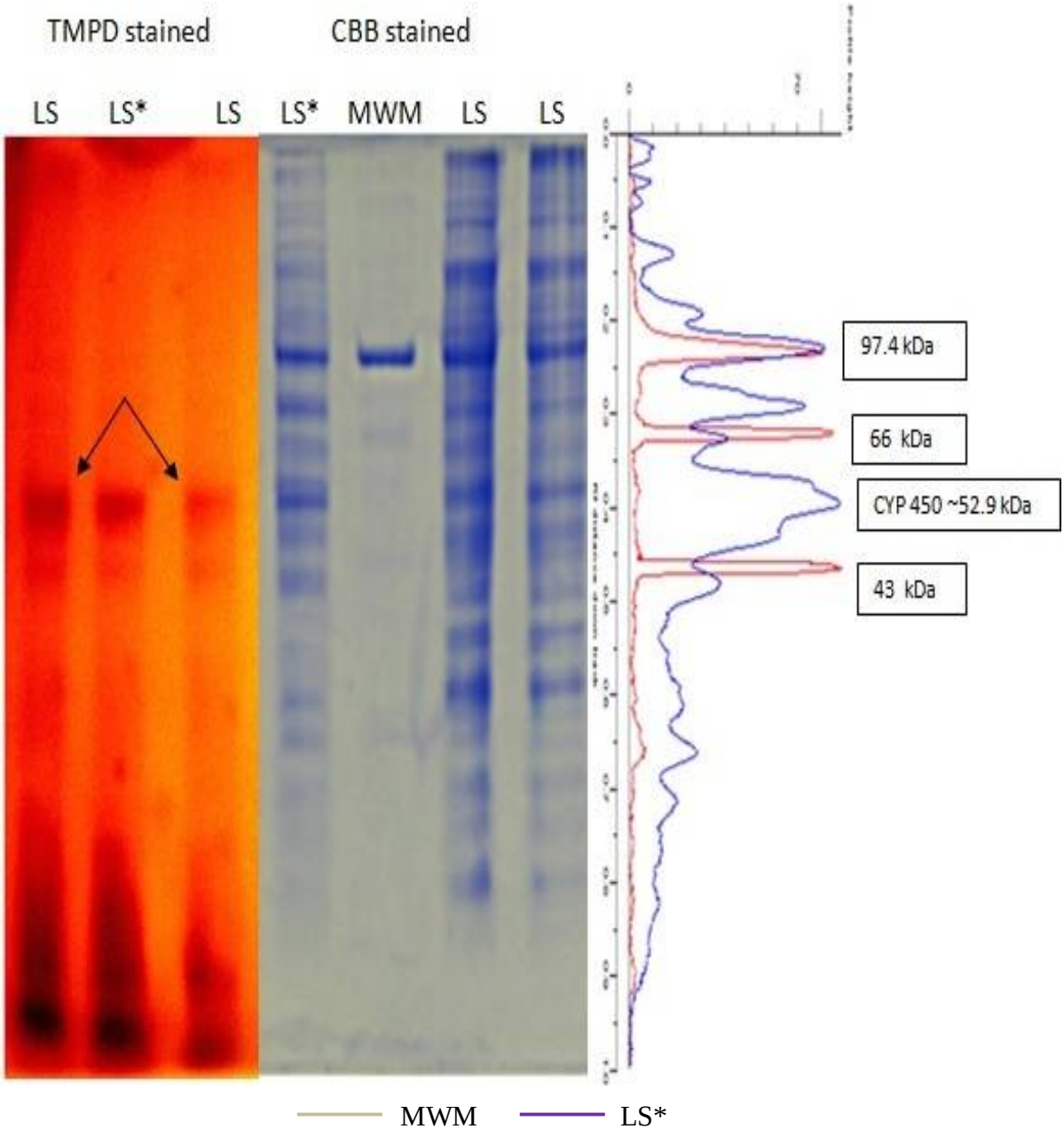


Figure 5.7b. Differentially stained SDS-PAGE protein gels showing the analysis of CYP 450 enzymes from hepatic microsomes in *H. fossilis*. Left panel: Protein gel developed with TMPD/hydrogen peroxide stain specific for heme. Right panel: Protein gel stained with Coomassie dye. A faint band corresponding to free heme at around ~52.9 kDa can be seen as indicated by the arrow.

MWM= Molecular weight marker; LS = liver sample

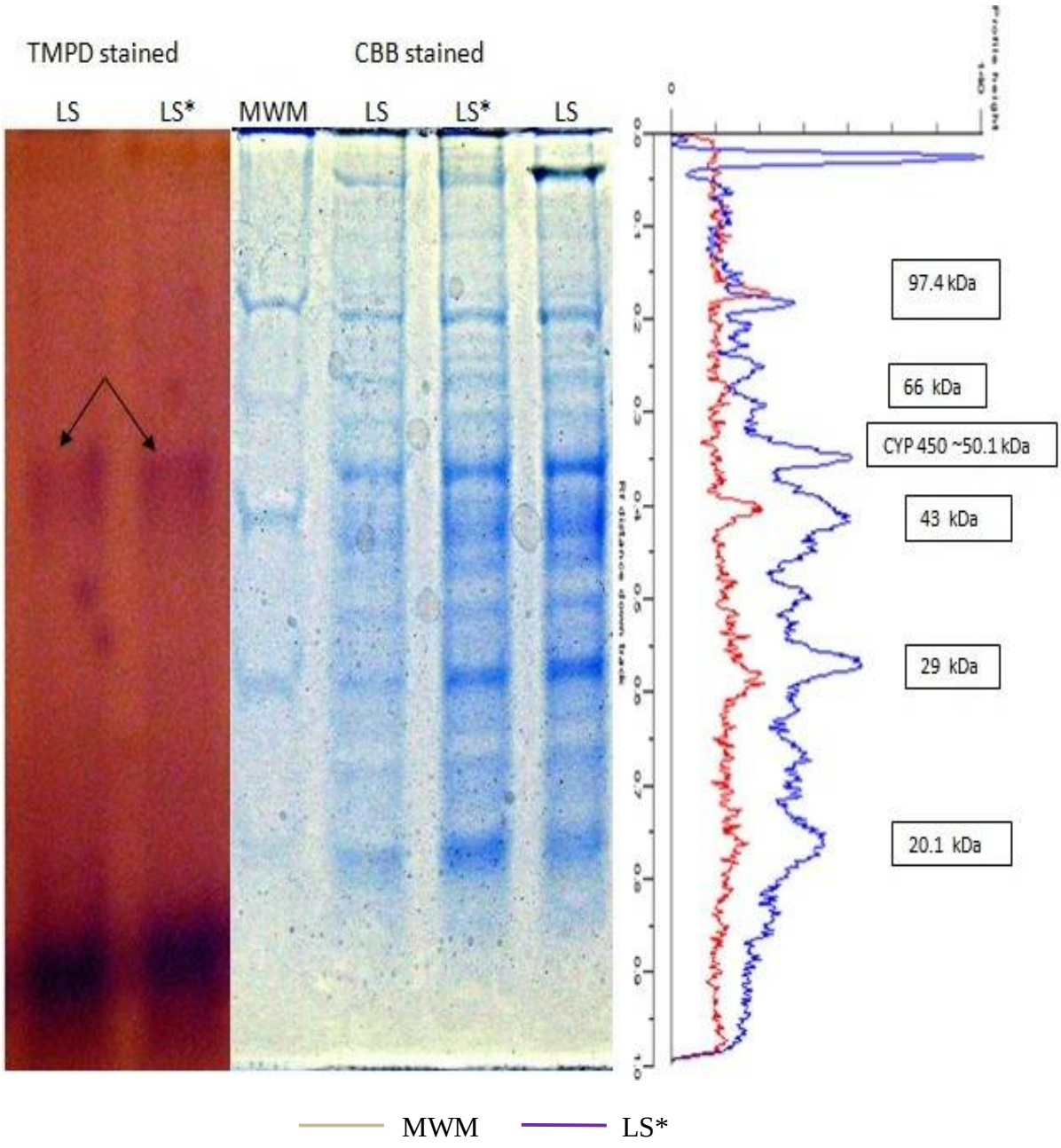


Figure 5.7c. Differentially stained SDS-PAGE protein gels showing the analysis of CYP 450 enzymes from hepatic microsomes in *C. batrachus*. Left panel: Protein gel developed with TMPD/hydrogen peroxide stain specific for heme. Right panel: Protein gel stained with Coomassie dye. A faint band corresponding to free heme can be seen at around ~50.1 kDa as indicated by the arrow.

MWM= Molecular weight marker; LS = liver sample

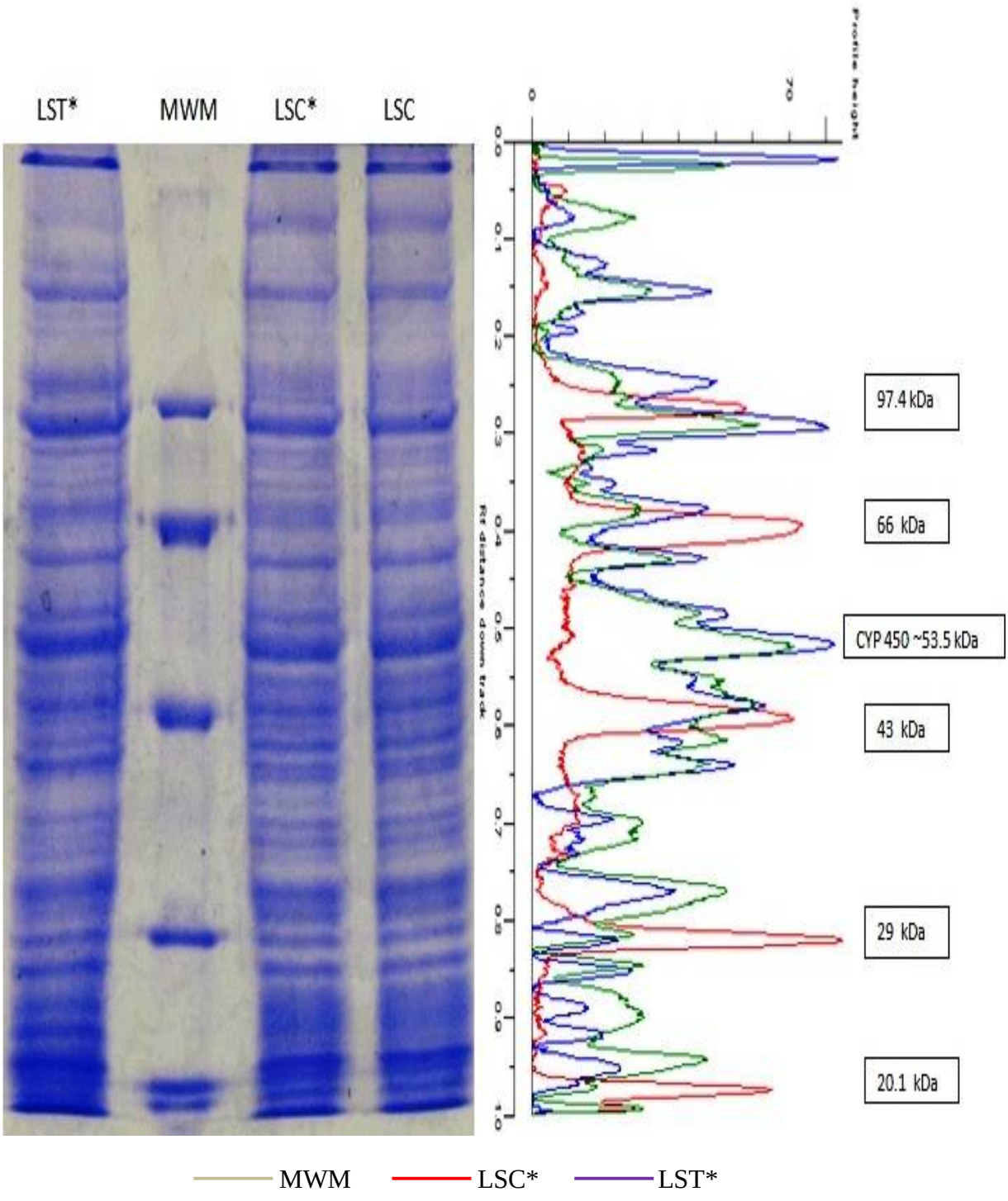


Figure 5.8a. 10% SDS-PAGE of the microsomal fraction of liver of cypermethrin treated and control fish, *C. punctatus* stained with 0.025% CBB. The documentation was done using Spectronics ImageAide software, version 3.06.04. When the gel was analysed using gel documentation software, the protein bands in the region of ~53.5 kDa was visualized.

MWM= Molecular weight marker; LSC= liver sample control; LST = liver sample treated

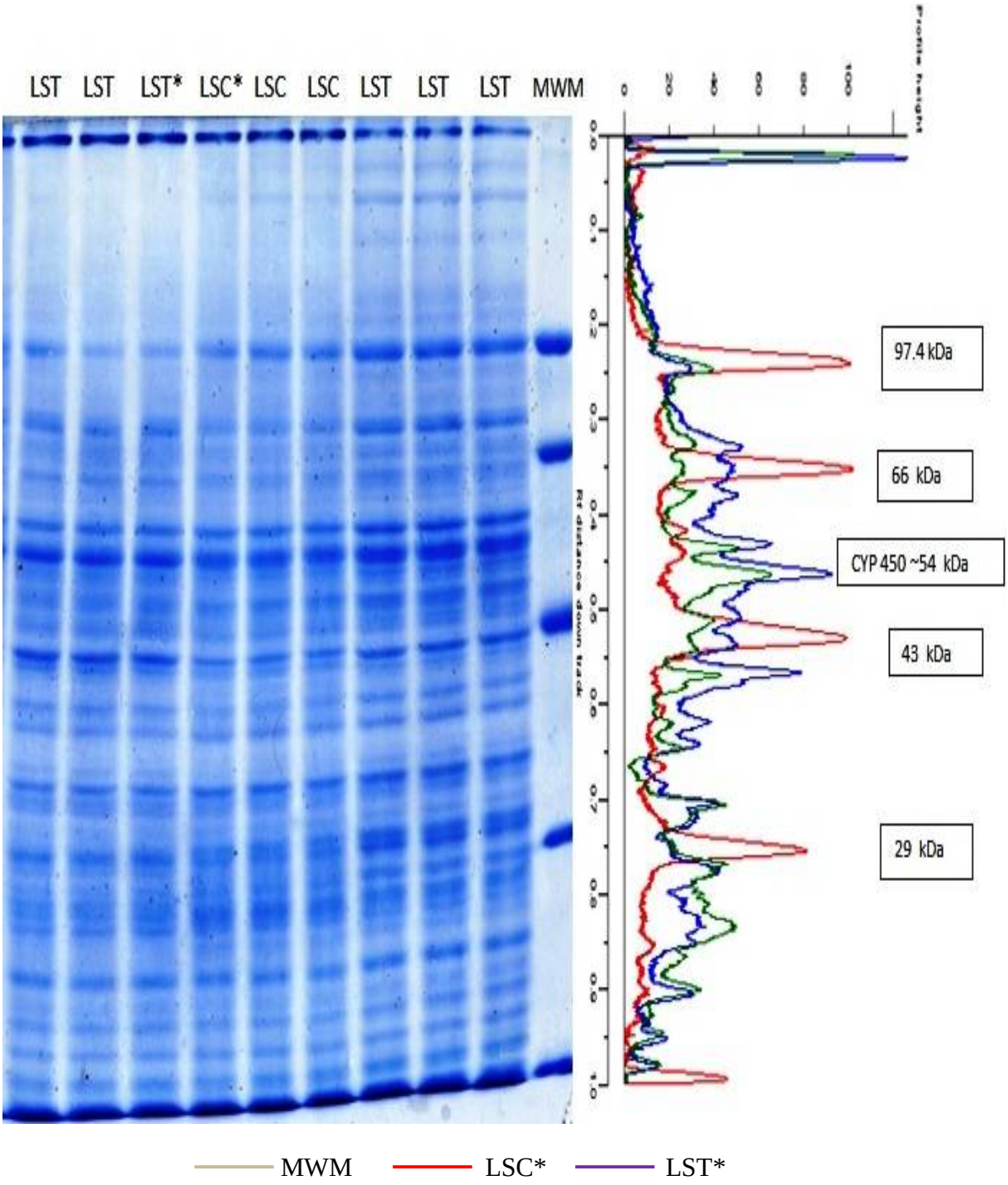


Figure 5.8b. 10% SDS-PAGE of the microsomal fraction of liver of ethion treated and control fish, *C. punctatus* stained with 0.025% CBB. The documentation was done using Spectronics ImageAide software, version 3.06.04. When the gel was analysed using gel documentation software, the protein bands in the region of ~54 kDa was visualized.

MWM= Molecular weight marker; LSC= liver sample control; LST = liver sample treated

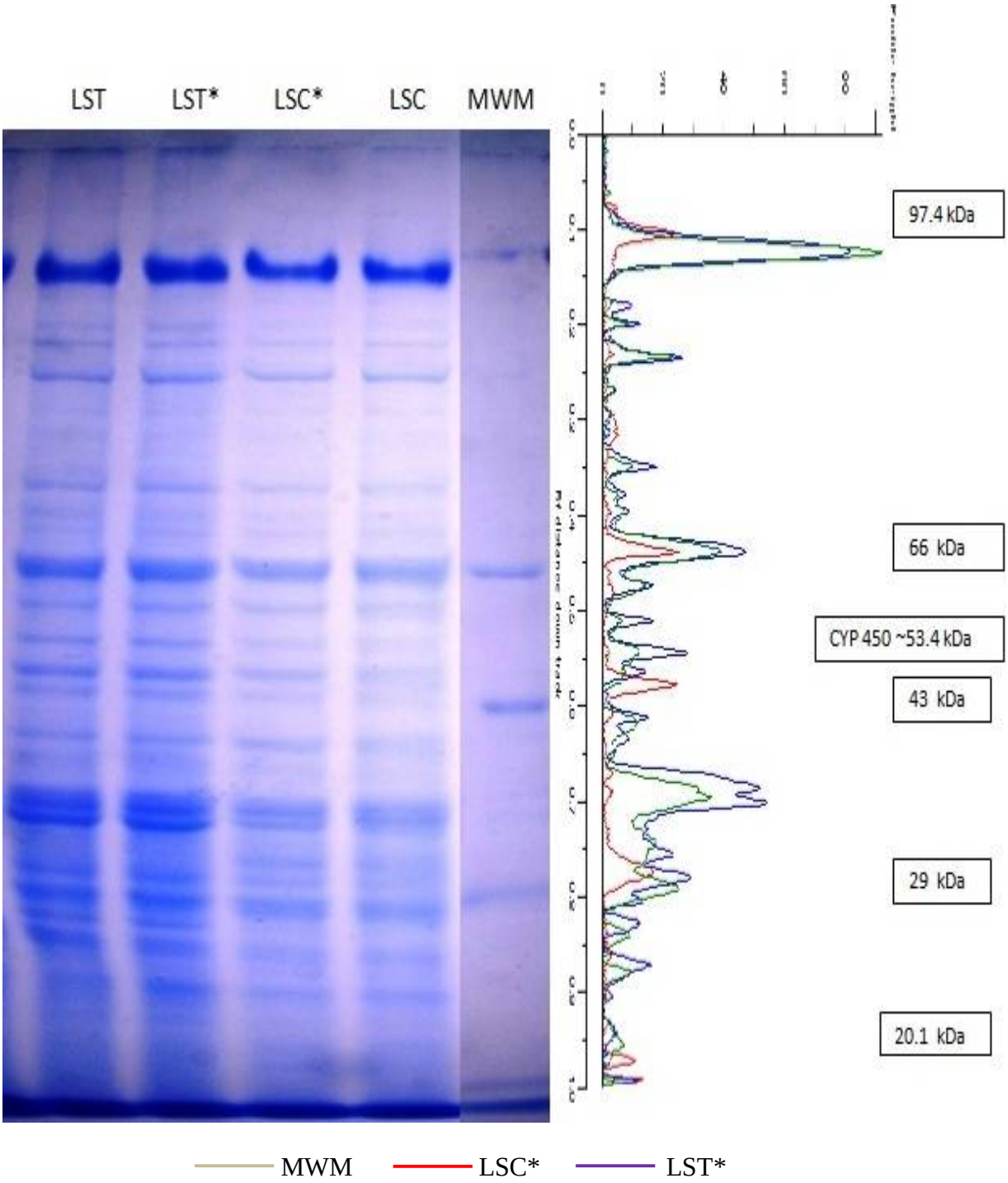


Figure 5.8c. 10% SDS-PAGE of the microsomal fraction of liver of dicofol treated and control fish, *C. punctatus* stained with 0.025% CBB. The documentation was done using Spectronics ImageAide software, version 3.06.04. When the gel was analysed using gel documentation software, the protein bands in the region of ~53.4 kDa was visualized.

MWM= Molecular weight marker; LSC= liver sample control; LST = liver sample treated

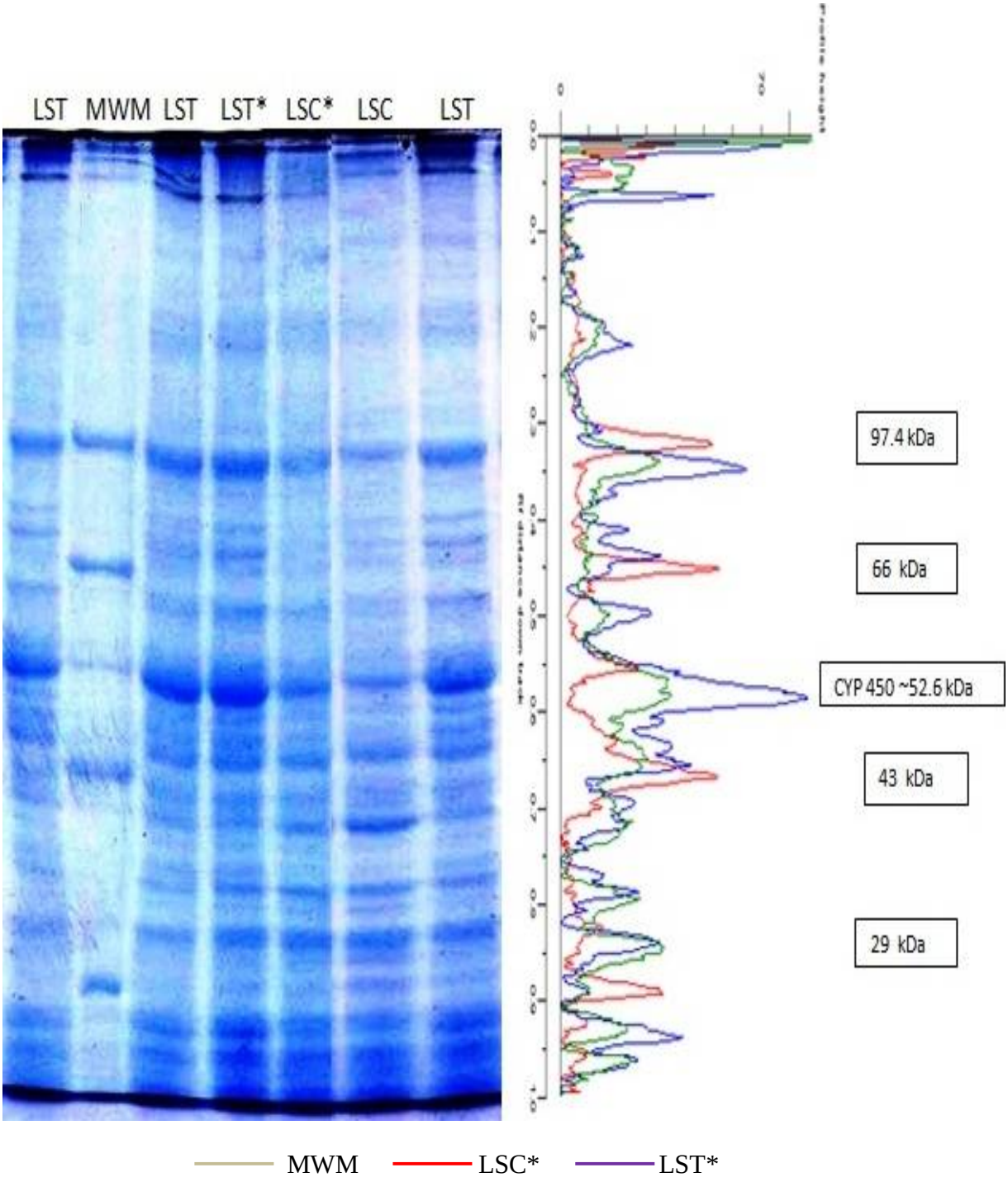


Figure 5.9a. 10% SDS-PAGE of the microsomal fraction of liver of cypermethrin treated and control fish, *H. fossilis* stained with 0.025% CBB. The documentation was done using Spectronics ImageAide software, version 3.06.04. When the gel was analysed using gel documentation software, the protein bands in the region of ~52.6 kDa was visualized.

MWM= Molecular weight marker; LSC= liver sample control; LST = liver sample treated

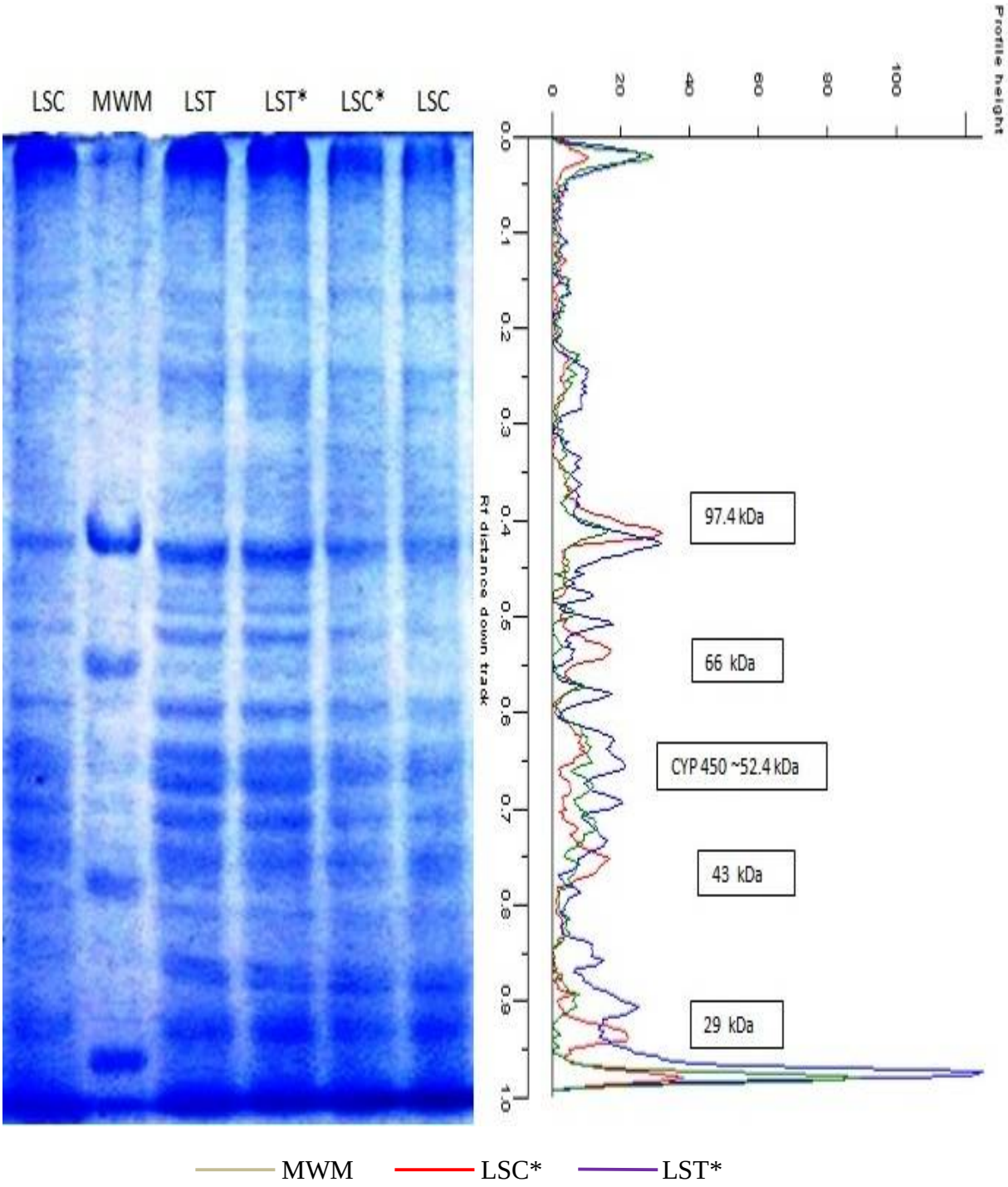


Figure 5.9b. 10% SDS-PAGE of the microsomal fraction of liver of ethion treated and control fish, *H. fossilis* stained with 0.025% CBB. The documentation was done using Spectronics ImageAide software, version 3.06.04. When the gel was analysed using gel documentation software, the protein bands in the region of ~52.4 kDa were visualized.

MWM= Molecular weight marker; LSC= liver sample control; LST = liver sample treated

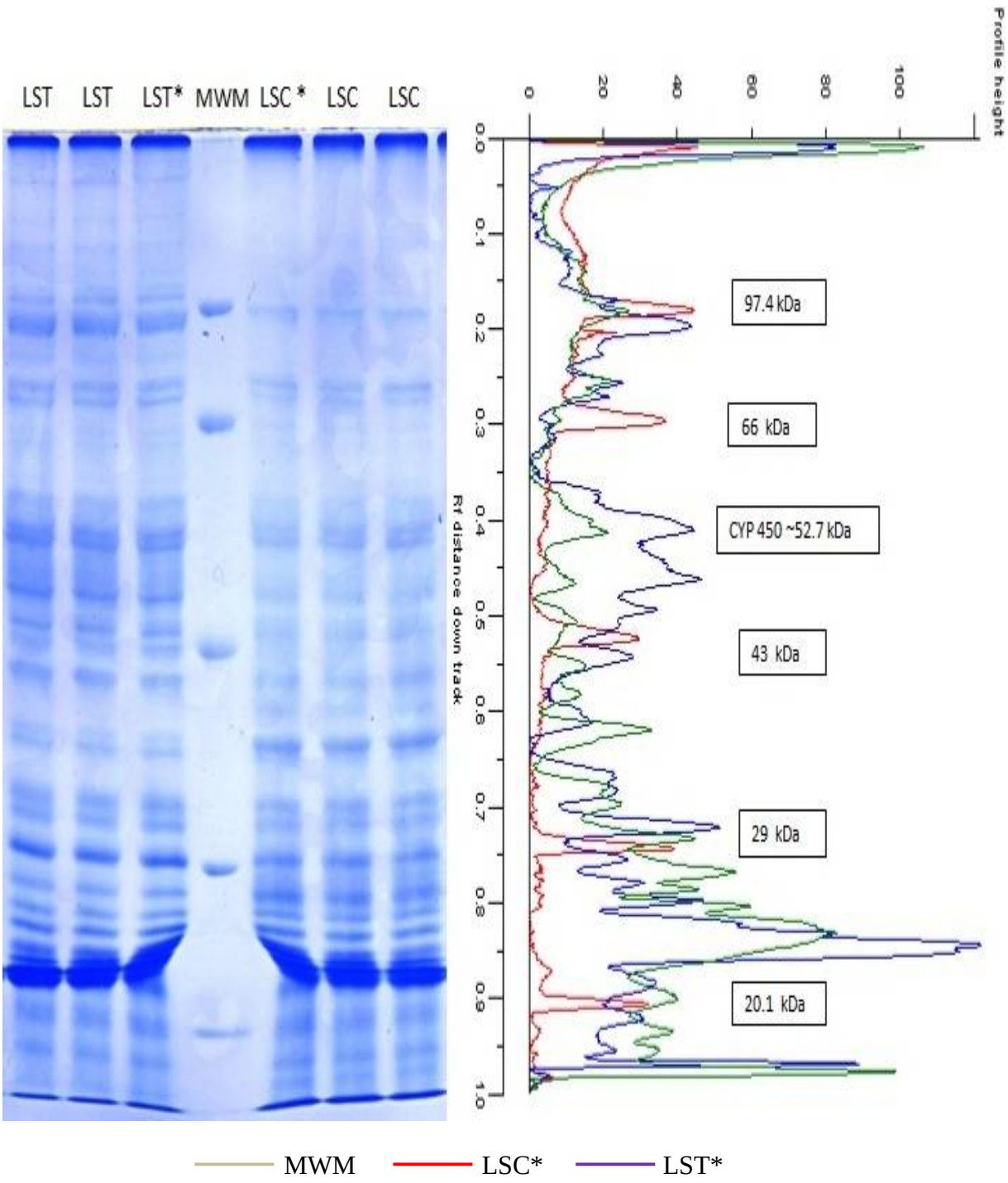


Figure 5.9c. 10% SDS-PAGE of the microsomal fraction of liver of dicofol treated and control fish, *H. fossilis* stained with 0.025% CBB. The documentation was done using Spectronics ImageAide software, version 3.06.04. When the gel was analysed using gel documentation software, the protein bands in the region of ~52.7 kDa were visualized.

MWM= Molecular weight marker; LSC= liver sample control; LST = liver sample treated

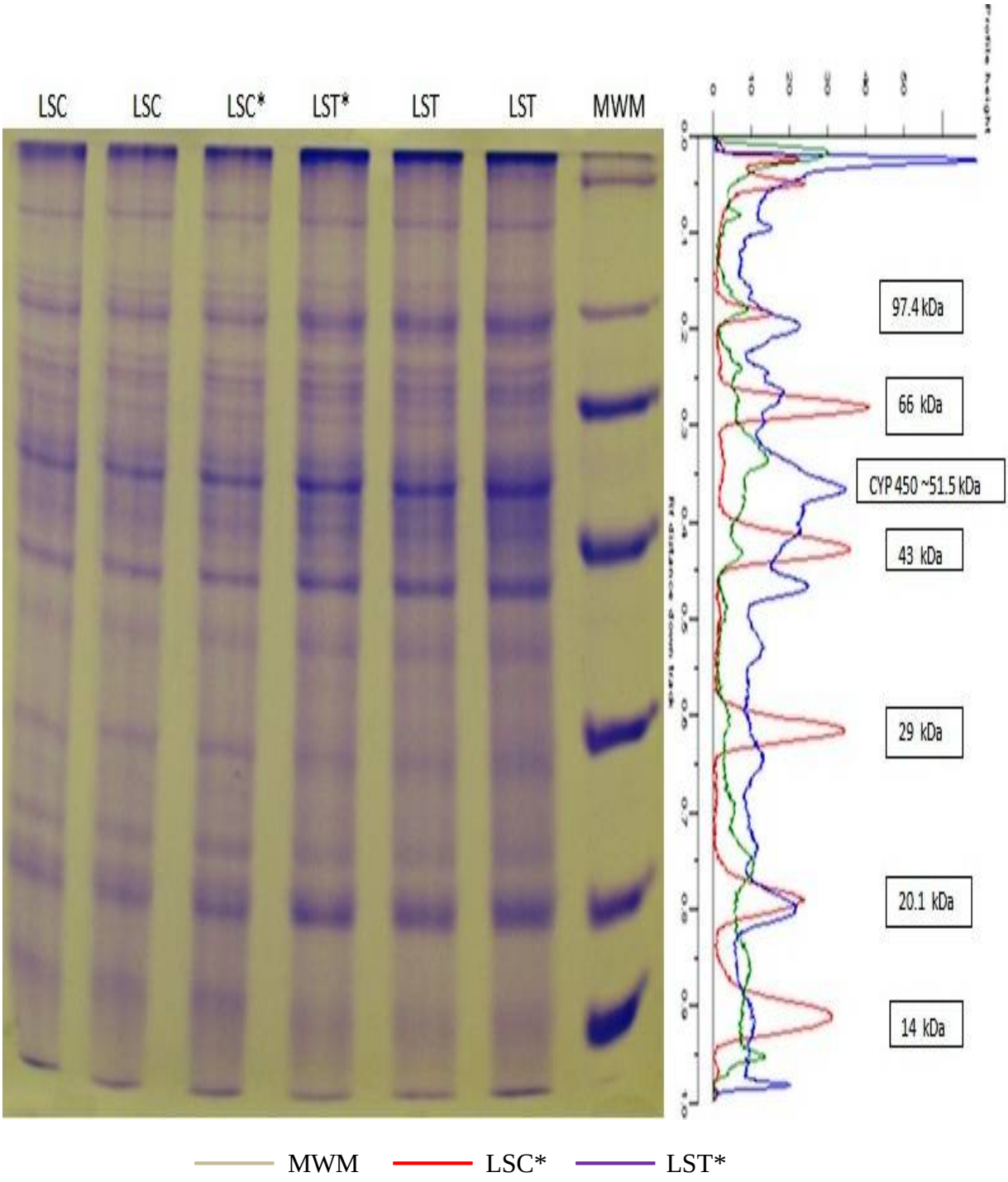


Figure 5.10a. 10% SDS-PAGE of the microsomal fraction of liver of cypermethrin treated and control fish, *C. batrachus* stained with 0.025% CBB. The documentation was done using Spectronics ImageAide software, version 3.06.04. When the gel was analysed using gel documentation software, the protein bands in the region of ~51.5 kDa were visualized.

MWM= Molecular weight marker; LSC= liver sample control; LST = liver sample treated

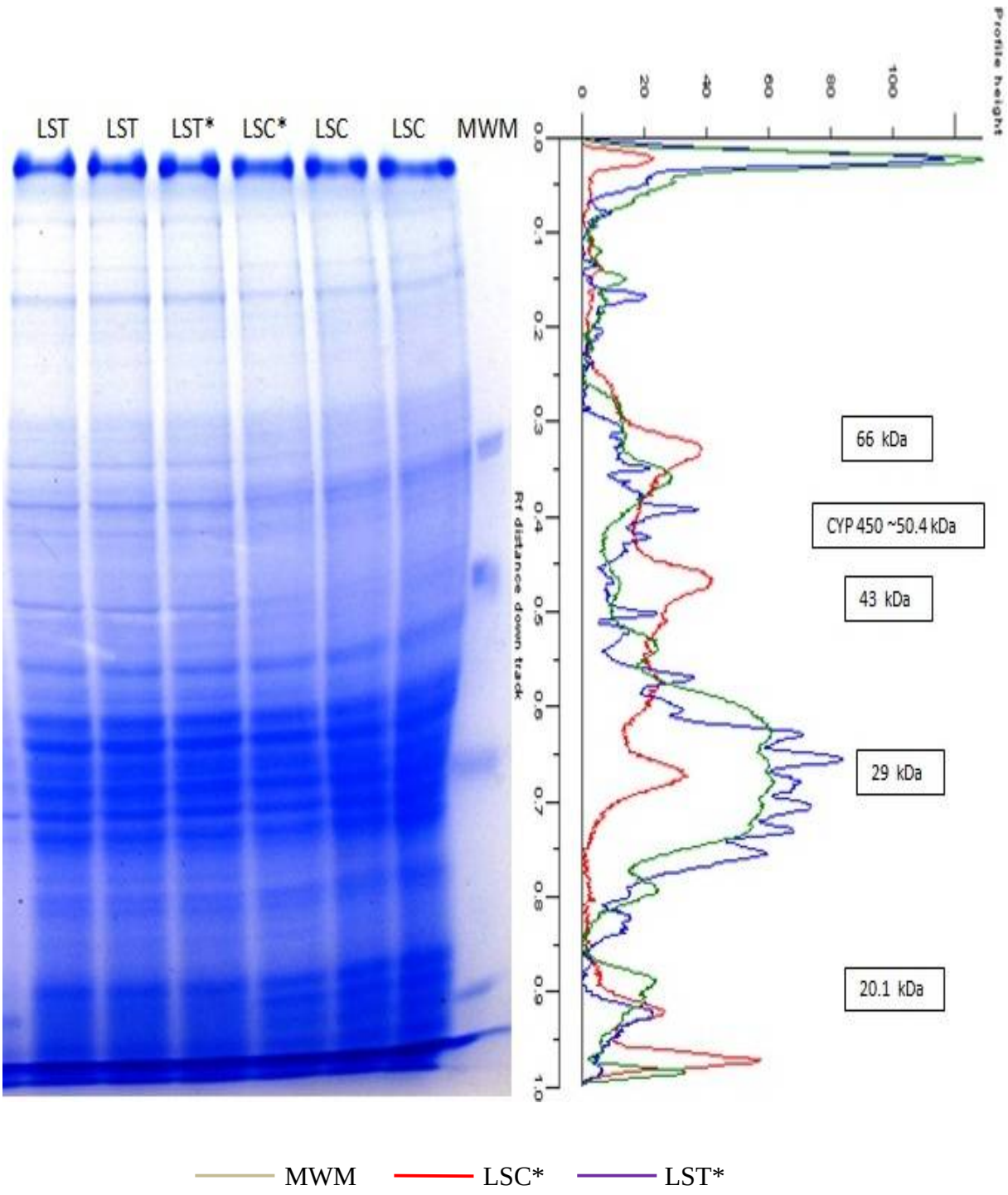


Figure 5.10b. 10% SDS-PAGE of the microsomal fraction of liver of ethion treated and control fish, *C. batrachus* stained with 0.025% CBB. The documentation was done using Spectronics ImageAide software, version 3.06.04. When the gel was analysed using gel documentation software, the protein bands in the region of ~50.4 kDa were visualized.

MWM= Molecular weight marker; LSC= liver sample control; LST = liver sample treated

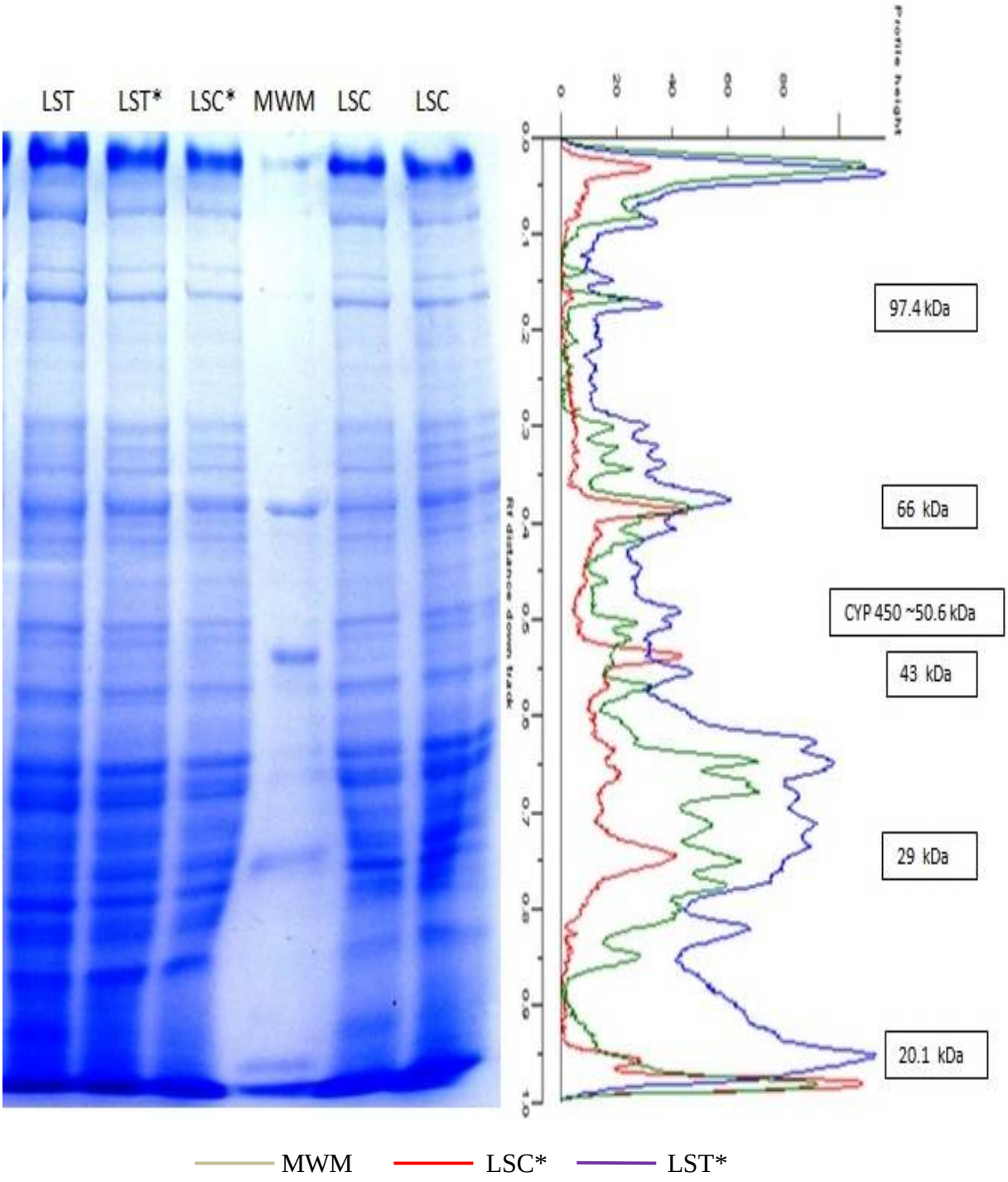


Figure 5.10c. 10% SDS-PAGE of the microsomal fraction of liver of dicofol treated and control fish, *C. batrachus* stained with 0.025% CBB. The documentation was done using Spectronics ImageAide software, version 3.06.04. When the gel was analysed using gel documentation software, the protein bands in the region of ~ 50.6 kDa were visualized.

MWM= Molecular weight marker; LSC= liver sample control; LST = liver sample treated

5.11. Nucleotide sequence analysis of genomic CYP1A and phylogenetic analysis of 3 fish species, *C. punctatus, H. fossilis* and *C. batrachus*

The CYP1A genes of 3 fish species, *C. punctatus, H. fossilis* and *C. batrachus* were successfully amplified by using the primers presented in Table 4.3 in materials and methods.

In *C. punctatus*, the 3 sequences of CYP1A genomic DNA were amplified with 3 different primers and were submitted to the GenBank. The received accession number of the sequences were KP203843 (1041 bp), KP231221 (1498 bp) and KP271996 (1108 bp). These 3 sequences of genomic DNA were joined together to make it a single genomic DNA. Sequence analysis showed that the obtained sequence contained CYP1A structural gene of approximately 2511 bp in length for *C. punctatus* (Fig.5.11). In both *H. fossilis* and *C. batrachus*, a single primer was used to amplify CYP1A genomic DNA. Sequence analysis showed 534 bp for *H. fossilis* (Fig. 5.12) and 509 bp for *C. batrachus* (Fig. 5.13). All the gene sequences were successfully deposited in the GenBank/NCBI data bank except the gene of *H. fossilis* which was deposited in GenBank/EMBL. The accession numbers of CYP1A genes received from GenBank were KP282054 for *C. punctatus*, LN736019 for *H. fossilis* and KP336485 for *C. batrachus*.

Comparison of genomic DNA of *C. punctatus* with CYP1A cDNA sequence of *C. punctatus* obtained from NCBI (EU930319) identified 6 exons and 5 introns. The nucleotide size of 6 exons were 720, 127, 90, 124, 87 and 298 bp respectively with 389 bp of untranslated sequence in 3' region while the nucleotide size of 5 introns were 124, 102, 106, 123 and 221 bp respectively (Table 5.43). cDNA of the CYP1A gene obtained from NCBI had 1566 bp of the coding region but in the genomic DNA of the present study initial 120 bp were not amplified and only 1446 bp sequence were obtained coding 481 amino acid.

The amplified sequences of *H. fossilis* (534 bp) and *C. batrachus* (509 bp) showed highest similarity/homogeneity with cDNA sequences of *Peltobagrus fulvidraco* (yellow catfish). When comparing their genomic DNA with CYP1A cDNA sequence of *P. fulvidraco* obtained from NCBI, 2 exons were identified for each fish species with a nucleotide size of 381 and 57 bp for *H. fossilis* coding 146 amino acids, and 366 and 46 bp for *C. batrachus* coding 137 amino acids. Both the species had 1 intron with *H. fossilis* having nucleotide size of 94 bp and *C. batrachus* having nucleotide size of 97 bp respectively (Table 5.43). All the introns begin with the sequence GT and end with AG.

5.11.1. Comparison of amino acid sequences

Table 5.44 shows the percent identities of deduced amino acid sequences of *C. punctatus*, *H. fossilis* and *C. batrachus* CYP1A between and with the other fish CYP1A genes employed in the present study. The highest identity was 93% between *C. punctatus* and *C. maculata* and between *H. fossilis* and *C. batrachus*. Closely followed was the 86% and 85% identity of *H. fossilis* and *C. batrachus* with *P. fulvidraco*.

5.11.2. Phylogenetic analysis

The phylogenetic tree based on the amino acid sequences were used to assess the relationship of CYP1A of *C. punctatus*, *H. fossilis* and *C. batrachus* with those of other fish species belonging to order Cypriniformes, Salmoniformes, Perciformes and Siluriformes. Figure 5.14 clearly shows *C. punctatus* and *C. maculata* CYP1A and *H. fossilis* and *C. batrachus* CYP1A to be more closely related to each other than to other fish CYP1A. *H. fossilis* and *C. batrachus* CYP1A was more closely related with *P. fulvidraco* (yellow catfish). Comparing the orders, the order Siluriformes (*Ancistrus sp.*, *Peltobagrus fulvidraco*, *H. fossilis* and *C. batrachus*) was closely related to order Cypriniformes (*Catla catla* and *Cyprinus carpio*). The order Perciformes (*Stenotomus chrysops*, *Oreochromis niloticus*, *Channa maculate* and *C. punctatus*) was closely related to Salmoniformes (*Oncorhynchus mykiss*).

>*Channa* (2511 bp)

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>gi|794541524|gb|KP282054.1| Channa punctata isolate CHANNA cytochrome p450
CYP1A (cyp1A) gene, partial cds

        1 GGGCTTTGTC GTCTCCCTGG CCCAAAGCCC TTGCCTCTCA TCGGGAATGT GCTGGAGCTT
       61 CGCCACAAAC CTTATCAGAG TCTCACTGCT ATGAGCAAGC GTTATGGTCA TGTCTTCCAG
      121 ATCCACATTG GCACACGTCC TGTGGTTGTG TTGAGTGGCA GTGAGACGGT TCGTCAGGCT
      181 CTCATCAAGC AAGGGGAAGA GTTCGCAGGC AGACCTGACT TGTACAGCTT TCAATTCATC
      241 AATGACGGAA AAAGTCTGGC TTTCAGTACA GATCAGTCTG GTGTCTGGCG TGCTCGCAGA
      301 AAGCTGGCTT ACAGTGCCCT GCGCTCCTTT TCCAACCTGG AGAGCAAGAA CTCAGAGTAC
      361 TCCTGTGTTC TAGAAGAACA CGTCAGTAAA GAGGCAGAGT ATCTAATCAA ACGACTCTGC
      421 ACTGTCATGA AGGCAGACGG CAGCTTCGAC CCCGTCCGTC ACATTGTCGT CTCTGTGGCA
      481 AATGTCATCT GCGGAATTTG CTTTGGCCGA CGCTACAGCC ACGATGACCA GGAGCTGCTC
      541 AGCTTAGTGA CCCTTGCTGA TGACTTTAAC CAGGTGGCGG GAAGTGGGAA CCCTGCTGAC
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 601 TTCATCCCCA TTCTCCAGTA TCTGCCTAGC AGAAACATGA AGAATTTTAT GGACCTCAAT
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 721 gtacaccgca aactaaatca ctcatgaaat gagttcacgt tttctcaaag ccataatttc
 781 cttattattt ttgtgtgtat ttgtatgtac gtatgtgatt gtgattgttc ttaacttttt
 841 tcagGACAAC ATCCGTGATA TCACAGACTC CCTCATTGAT CACTGCGAGG ACAGGAAGCT
 901 GGATGAGAAC TTCAATGTTC AGGTGTCAGA TGAGAAGATT GTGGGAATTG TCAATGACCT
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1021 gtctgcctaa agaaaacaaa agtgccctca cataattaac atctcccttt cagGTTTTGA
1081 CACTGTCACC ACTGCATTGT CATGGTCAGT GATGTACATG GTGGCTTACC CAGAGATACA
1141 AGAGAGGCTT TATGATGAGC TGAgtaagta cactgatttt ggattttaca gttctattgc
1201 aaaaccactg aaggaatgtg gactaaaatc caaaaaaacg catactgagc atggatgact
1261 ttttttcagA GGTCAATGTG GGTCTGGAGC GGAGCCCGCG TCTCTCTGAT AAACCCAATT
1321 TACCATTTCT GGAGGCCTTC ATCCTGGAGA TGTTGCGGCA CTCTTCATTC CTGCCCTTTA
1381 CTATCCCACA CTGgtaaggt tcaactcaaa aagggtgaaa atagagctgt taagtgcata
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1801 atttcctttc tctacccctc tcagTGAGCT GTGGAAAGAT CCATTTTCCT TCAACCCAGA
1861 CCGCTTCTTG AGCGCTGATA GCACTGAGGT CAACAAGGTG GAAGGGGAGA AGGTAGTGGC
1921 TTTCGGCCTA GGAAAGCGGC GCTGCATCGG CGAGGTCATT GCACGAAATG AAGTCTACCT
1981 CTTCTTGGCA ATTCTTATCC AGAAGCTAGA GTTCCACCAA ATGCCTGGGG TACCACTGGA
2041 CATGACGCCA CAATATGGTC TCACAATGAA ACACAAACCC TGCCACCTGA GAGCCACAAT
2101 GCGAGCAATC AATGAGCAGT GAAACTATTT ATATATTTAC TGTATTCAGA ATGAATTACT
2161 CAACAGTTGA TAATTGTTCA CTCAAGAACT GTAAGGTTCA GTGAAACAAG CATCTTTCTC
2221 AGAATGTACG GCAATGGTTG CCAAATCTGA TACATAGAGC AAATGGATTT GAAGCAAATA
2281 GGTGAAATTT GCTTGCTTGC TTGCAGACTG TCAGATATTT CTGGGTTTGT AAGATGAGGG
2341 GTTCCTCTAT ATGATCGATG CGTCTTGAGG AAGAATAAGC AGATACTTGG TTTTCCTGCT
2401 GTGTTTTTTT GCTGTGCTGG AAACGTAACT GTTCTTATGT AGAAGTTGTA TAGCACACAA
2461 ACTATGTTGC TTCAATCAAC TTTGGGACAC ATTATGTTTT ACTGGATATG C
```

Figure 5.11. Nucleotide sequences (2511 bp) of *C. punctatus* genomic cytochrome CYP1A. The nucleotides are represented in uppercase letters for the exons and in lowercase letters for the introns. The nucleotide marked by yellow colour represents 389 bp of untranslated sequence in 3' region.

H. fossilis (534 bp)

>gi|748762338|emb|LN736019.1| Heteropneustes fossilis partial cyp4501a gene for Cytochrome P450 1A

```
  1 TCGAGGGTGA GAGTTCTGAG TATTCCTGTG CCCTGGAGGA ACACATCAGC AAAGAGGGCC
 61 TGTACCTGAT CGAGAGGCTG CACAGTGTTA TGAAGGCCAA TGGTGGATTC GACCCGTTCC
121 GTCACATTGT GGTGTCTGTG ACAAACGTGA TCTGTGGCAT GTGCTTTGGC CGACGCTACA
181 GCCACGACGA CCACGAGCTG TTAAGCCTGG TGAACTTAAG CGAAGAGTTC AACCAAGTGG
241 TGGGCAGCGG AAACCCGGCC GACTTCATTC CCTTCCTGCG CCTCCTGCCC AGCACGAGCA
301 TGAATAAATT CCTGGCCATC AACCAGCGGT TTAACGTGTT CATGCAGAAG CTGGTCAGAG
361 AGCATTACGA GACATTCAAT AAGgttcgtg cacagtgtat acaaatttca tgacgagatt
421 ctgtcaaaat tcaggatgct cacactgtct tgtatcttct gatttttgt tttttagGAC
481 AACATCCGTG ATATCACTGA CTCTCTCATC GATCACTGTG AGGACAGGAA GCTG
```

Figure 5.12. Nucleotide sequences (534 bp) of *H. fossilis* genomic cytochrome CYP1A. The nucleotides are represented in uppercase letters for the exons and in lowercase letters for the introns.

C. batrachus (509 bp)

>gi|794541545|gb|KP336485.1| Clarias batrachus isolate HM514 cytochrome p450 CYP1A (cyp1A) gene, partial cds

```
  1 GAGTACTCCT GCGCCCTGGA GGAACACATC AGCAAGGAAG GCCTGTACCT GATTGAGAGG
 61 CTGCACAGTG TTATGAAGGC CAGCGGCGGA TTCGACCCGT TCAGTCACAT TGTTGTGACT
121 GTCACGAACG TGATCTGCGG CATGTGCTTT GGTCGCCGCT ACAGCCACGA TGACCGGGAA
181 CTGTTAAGCC TGGTGAACTT AAGCGAAGAG TTCAACCAAG TGGTGGGCAG CGGAAACCCG
241 GCTGACTTCA TTCCCTTCCT GCGCCTCCTG CCCAGCACGA GCATGAAGAA ATTCCTGGCC
301 ATCAACGAGC GCTTTAACGT GTTCATGCAG AGGCTGGTCA AAGAGCATTA CGAGACATAC
361 AATAAGgttc gtgcacactt tgatcaagtg tatccgaata gcgtgatgag attccatcaa
421 aaattagcat gttcacgctt tgtgtttttt tctttccctc cagGACAACA TTCGTGATAT
481 CACAGACTCT CTCATCGATC ACTGTGAGG
```

Figure 5.13. Nucleotide sequences (509 bp) of *C. batrachus* genomic cytochrome CYP1A. The nucleotides are represented in uppercase letters for the exons and in lowercase letters for the introns.

Table 5.43. Comparison of CYP1A exons and introns length (bp).

C. punctatus		*H. fossilis*		*C. batrachus*	
Exons	**Introns**	**Exons**	**Introns**	**Exons**	**Introns**
720 bp	124 bp	381 bp	94 bp	366 bp	97 bp
127 bp	102 bp	57 bp		46 bp	
90 bp	106 bp				
124 bp	123 bp				
87 bp	221 bp				
298 bp					

Table 5.44. Percent identities of deduced amino acid sequences of fish CYP1A gene subfamilies.

	Cyprinus carpio	**Rainbow trout**	***Channa maculate***	**Scup**	**Nile tilapia**	***P. fulvidraco***	***Ancistrus sp.***	***Channa punctatus***	***H. fossilis***	***Clarias batrachus***
Catla catla	92	77	76	76	73	78	76	75	82	80
Cyprinus carpio		80	77	79	74	79	78	76	81	78
Rainbow trout			81	82	74	75	76	78	75	75
Channa maculate				83	77	76	76	93	75	74
Scup					79	77	76	81	76	74
Nile tilapia						71	70	78	73	72
Peltobagrus fulvidraco							80	76	86	85
Ancistrus sp.								74	76	74
Channa punctatus									74	73
Heteropneustes fossilis										93

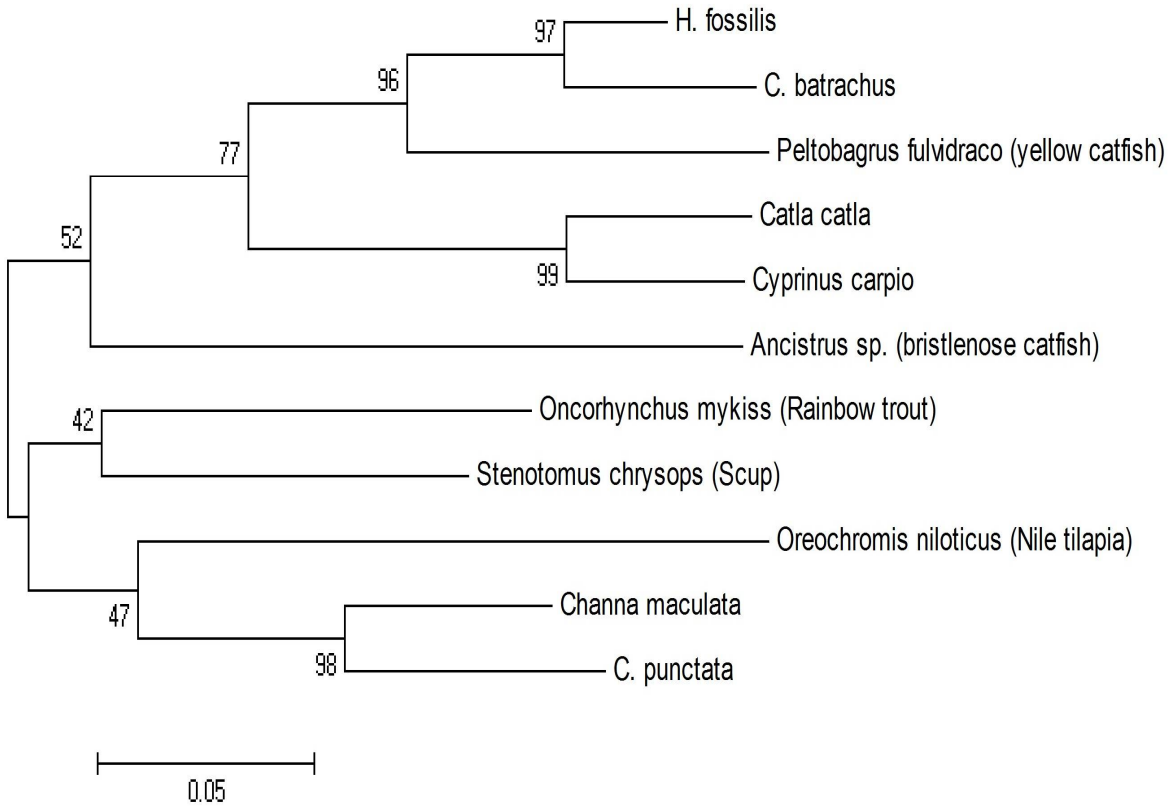

Figure 5.14. Phylogenetic tree of CYP1A genes constructed by the neighbour joining method using the amino acid sequences. A number at each branch and the length of the stem indicate bootstrap value. Evolutionary analyses were conducted in MEGA6.