CHAPTER THREE

RESULTS AND DISCUSSION

CHAPTER 3

RESULTS AND DISCUSSION

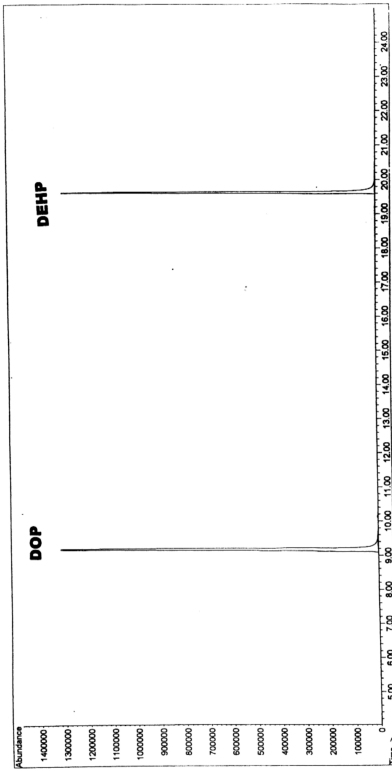
3. Optimization of instrument

The purpose of this procedure is to enable an accurate and effective analysis to produce a good resolution chromatogram. The temperature at which a chromatogram is run will effect the retention times or volume. As a consequence precise control of the volume temperature is required for adequate replication in repetitive measurements.

Temperature programming is especially helpful for complex mixture that contains both high and low retention times. The components with small retention times will come off at lower temperatures, and the retention times for the components with higher values will be reduced as the temperature rises. **[22]**

The figures below show an example of the difference in the chromatograms by using different temperatures for DOP and DEHP.

As we can see the retention times of the standards vary from one type of programming to another. What we should be interested in this preliminary run would be the maximum separation achievable for the components present in the standard/ sample. This maximum separation is called **resolution (degree** of **separation** between adjacent bands).



gure1: Temperature Programming. Hold at 150^{0}C for 5 minutes, then allow nstant movement to 200^{0}C at a rate of 5^{0}C per minute followed by 10^{0}C per inute and finally hold for 5 minutes at 250^{0}C.

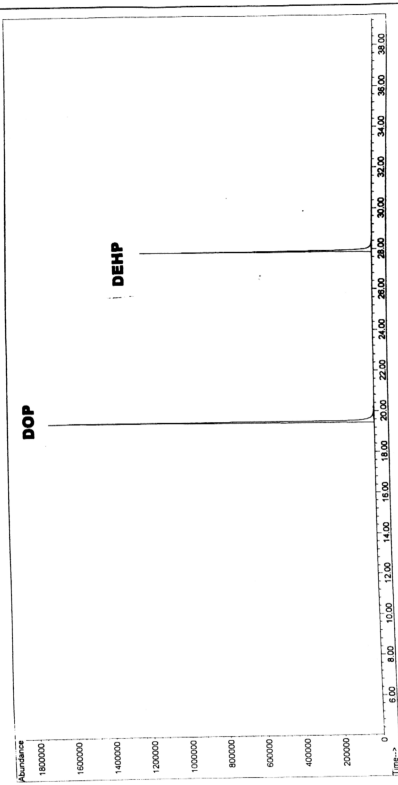


Figure2: Temperature Programming. Hold at 80^0C for 5 minutes, then allow constant movement to 250^0C at a rate of 7^0C per minute and finally hold for 5 minutes at 250^0C.

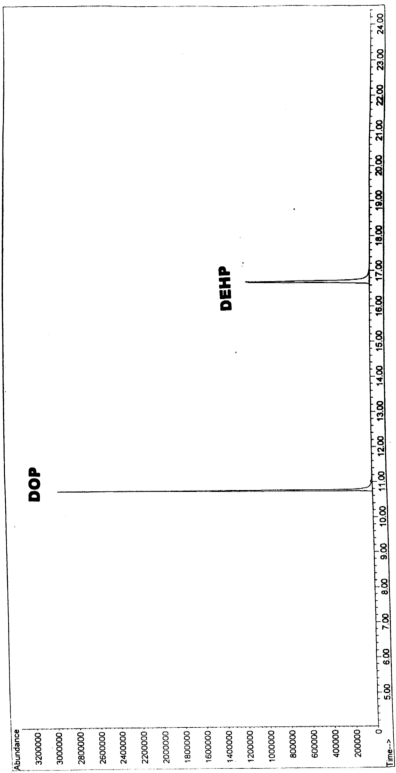


Figure3: Temperature Programming. Hold at 80^0C for 6 minutes, then allow constant movement to 200^0C at a rate of 35^0C per minute followed by 5^0C per minute and finally hold for 5 minutes at 250^0C.

3.1. Identification with GC

As there were no reference chromatograms for PEs using BP – 5 capillary column, a preliminary run was conducted on the samples.

From the preliminary run, which was done, on the samples it was discovered that only two very visible peaks appeared in the chromatogram. Their retention times were correlated with those of DOP and DEHP standards, thus the whole identification process was focused on these two samples, as no other phthalates appeared in the subsequent chromatograms of samples from different locations.

Elution of DOP was at 9.19 minutes and that of DEHP was at 19.91 minutes.

Identification and conformation of PEs compounds were done using the GC-MSD (mass selective detector). A Hewlett Packard 6890 series GC system was connected to a HP 6890 series mass selective detector. Comparison of a peak in the standard / sample was achieved with corresponding its mass spectra to a library search (i.e. the Wiley database). This mass spectrum is based on the fact that most compounds / PEs in this case have distinct fragmentation pattern.
Upon electron impact, most PEs have high abundance fragment ion at m / z 149 except for DMP which is at 163 and BEHA at 129. The fragment ions, which were analyzed for DOP and DEHP were 149, 167 and 279.

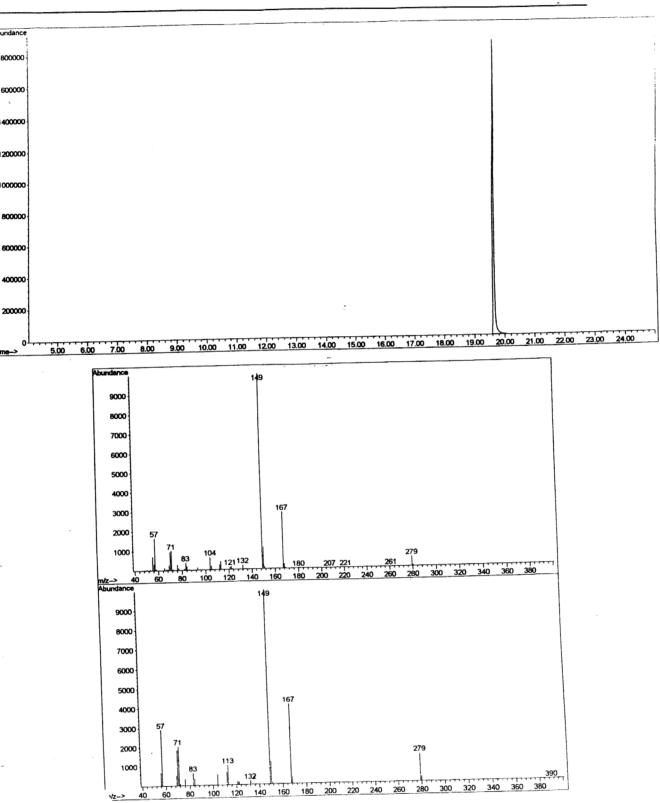
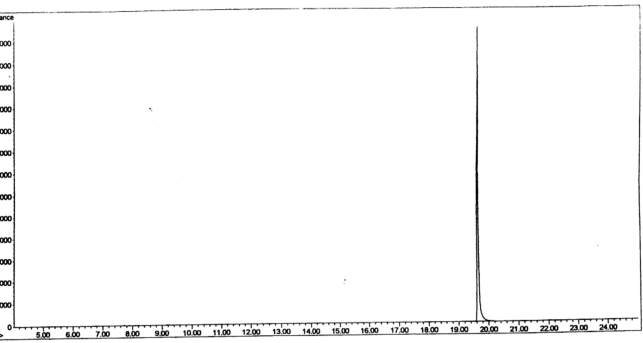


Figure 3: The fragment ions for DOP 149, 167 and 279.



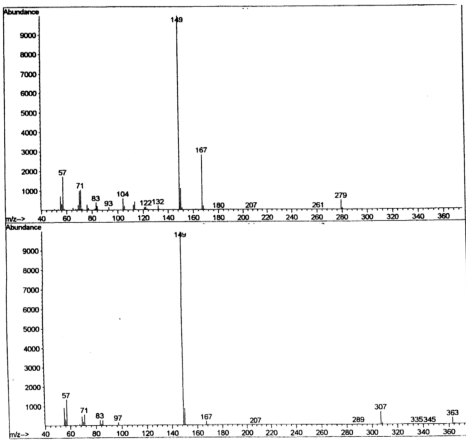


Figure 4: The fragment ions for DEHP149, 167 and 279.

3.2. Calibration Result

An external standard method was used. By injecting different concentration of standard solution, a linear calibration curve of response versus amount of standard was obtained from the computer as shown in the following pages.

The standards of the following concentrations, 5ppm, 10ppm, 20ppm, 30ppm and 40ppm, were prepared for both DEHP and DOP:

The calibration graphs are in the following pages.

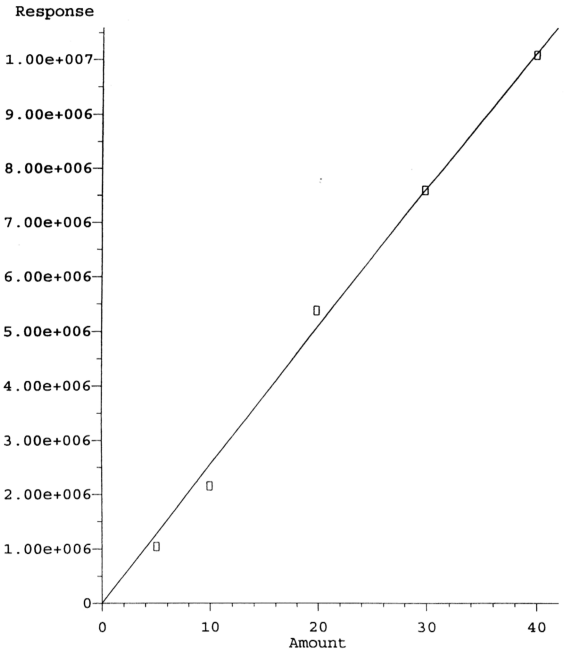


Figure 5: Calibration graph for DOP

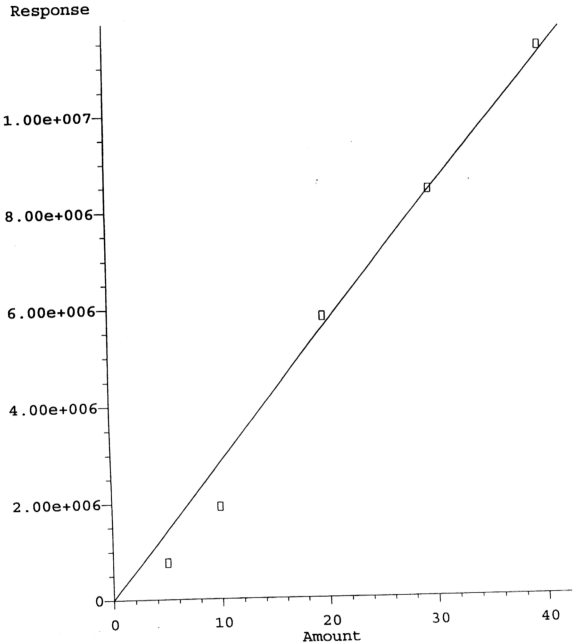


Figure 6: Calibration graph for DEHP

3.3. Sampling

4 sampling stations along the Klang River were chosen for this analysis. The locations are described in the table below.

Station	Location	Remarks
1	Jambatan Kota at Klang Town	Looked relatively clean but there were some plastic containers (mineral water bottles) floating around
2	Bridge along the LDP near Puchong	Clean
3	River near Guinness Malaysia	Muddy
4	Bridge opposite Citibank	Brownish in color but not dirty.

Table 3: Brief description on sampling sites along the Klang River

The sampling record is tabulated below for further justifications.

Station	Time	Weather		Water pH	River condition
		Sampling day	Day before		
1	1pm	Clear	Clear	7.8	Slow flow
2	2.30pm	Clear	Drizzle	7.2	Slow flow
3	3pm	Clear	Drizzle	6.9	Slow flow
4	4.30pm	Clear	Clear	6.8	Slow flow

Table 4: Sampling record on the 3rd of December 2001.

3.4. Clean – up Method

This procedure ensured a quantitative analysis of river water was possible without the interference of accompanying components owing to this rigorous clean-up procedure. Generally river water should contain large amounts of dissolved and suspended organic materials. These high levels of co-extracted organic compounds such as polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and pesticides present a considerable challenge to precise and accurate determination of PEs in river water. Therefore a very selective and efficient extraction clean-up procedure is necessary to produce final extracts of sufficient quality for reliable GC-MSD determination. Normally impurities show up on the chromatogram as noise, these can affect the identification process of the components. The following two figures (Figure 7 and Figure 8) indicate the importance of a clean-up procedure.

3.5. Recovery Study

Based on the recovery rate obtained it was found a small part of the PEs were not recovered. The loss could be due to adsorption of the PEs to the glass wall of the apparatus used and degradation process during solvent evaporation and pre-concentration. A study found that PEs would reversibly adsorb to glass. **[23]**

Recovery of PEs from 1-liter water sample spiked with 2ppm standard solution was carried out. Based on the computer output the recovery was rate was determined.

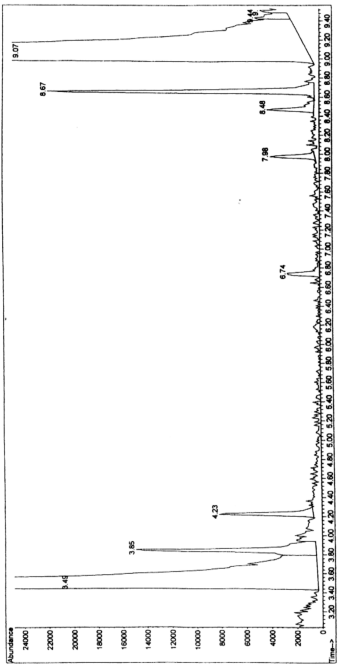


Figure 7: Sample from station 1 (without clean-up procedure).

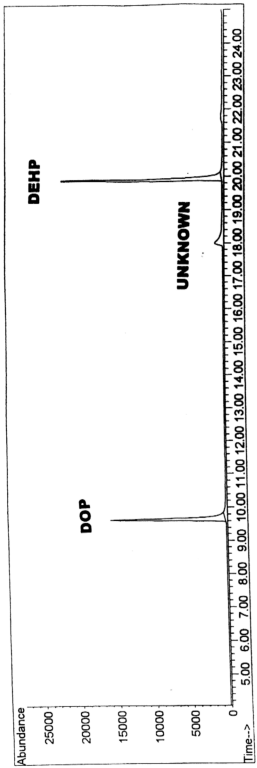


Figure 8: Sample from station 1 (with clean-up procedure).

The table below indicates the concentrations DEHP and DOP from 1-liter water spiked with 2ppm standard.

Station	DOP (ppm)		DEHP (ppm)	
	1	2	1	2
1	2.2	2.2	6.8	6.7
2	3.2	3.0	3.2	3.4
3	2.4	2.5	2.5	2.7
4	12.7	12.6	13.7	13.9

Table 5: Concentrations DEHP and DOP from 1-liter water spiked with 2ppm standard.

The table below indicates the concentrations DEHP and DOP from 1-liter water un-spiked.

Station	DOP (ppm)		DEHP (ppm)	
	1	2	1	2
1	0.8	1.1	5.1	5.2
2	1.4	1.3	1.5	1.4
3	0.7	0.6	0.8	0.8
4	11.3	11.2	17.1	17.0

Table 6: Concentrations DEHP and DOP from 1-liter water un- spiked.

The table below indicates the concentrations percentages of DEHP and DOP recovered from 1-liter water.

Station	DOP (ppm)	DEHP (ppm)
	%	%
1	62.5	80
2	87.5	92.5
3	90	83.5
4	70	77.5
Average recovery	77.5	83.4

Table 8: Concentrations percentages of DEHP and DOP recovered from 1-liter water.

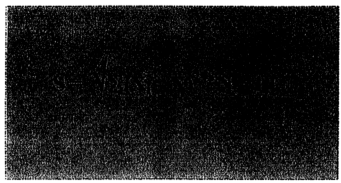
In the spike-in recovery study, the recovery obtained is considered to be low (Table 8). Apart from degradation process caused by high temperature condition and microbial activity, hydrolysis of PEs in an acidic or basic environment also led to the low recovery rates. In a related study **[24]**, fluvic acids, which are present in anaerobically digested waste, can complex with the phthalates. These complexes cannot be extracted with organic solvent; this could lead to the low recovery rate obtained. Another contributing factor to the low recovery would be the attachment of PEs to the glass wall of the separator funnel.

The mean recovery of DOP was 77.5% and of DEHP was 83.4%. Based on the results, the liquid-liquid extraction has some inherent weakness. Extra precaution should be taken to minimize the loss of the PEs caused by adsorption to the glass surfaces. A good alternative would to glassware with smaller surface area or a larger sample size.

3.6. Errors and precision

Analytical studies are subjected to various errors, which affect the precision as well as the accuracy of the results, and in return it will influence the validity and the reliability of any decision and assumption made based on those results. Therefore, it is necessary to be able to determine the magnitude and orientation of these errors. This implies that the magnitude of errors must be determined and controlled within a limit so that the validity and reliability of the results will not be jeopardized.

The standard deviation, S, caused by both the instrument and the procedures are calculated using the equation below.



X_i = value of sample i

X = average value of all samples

N = total number of samples

The source of instrumental error could be contributed by the injection technique employed. The volume of injection was small ~ 1 µl. This problem can be become significant when high concentration of compounds injected is high.

Water sample from station 1 was randomly chosen to determine the deviation caused by instrumental and procedure sources, as shown in the following table.

Station	DOP				DEHP			
	1	2	Average	S	1	2	Average	S
1	1.4	1.1	1.25	0.21	1.7	1.5	1.6	0.14
2	1.8	1.7	1.75	0.07	1.7	2	1.85	0.21
3	1.7	1.9	1.8	0.14	1.7	1.6	1.65	0.07
4	1.4	1.4	1.4	0	1.5	1.6	1.55	0.07

Table 9: Standard deviation of phthalates in water samples

The standard deviation values are generally, very small. Generally, the smaller the standard deviation value, more acceptable the analytical value. A factor, which can affect the standard deviation values, could be due to the inconsistency in the instrument's condition, which may have caused variation, observed.

The volume of injection, which determines the accuracy of the procedure, can be improved through auto injection technique. In this technique reproducible injection volume are easily obtained, thus, minimizing the standard deviation, which in return betters the precision and accuracy.

3.7. Comparison of residue level of PEs in river water samples

STATION	DOP (ppm)	DEHP (ppm)	TOTAL (ppm)
1	1.25	1.6	2.85
2	1.75	1.85	3.6
3	1.8	1.65	3.45
4	1.4	1.55	2.95

Table 10: Comparison of residue level of PEs in river water samples

As we can see from table 10, total PE, which was detected from each station, were quite low. Based on the information in the table the highest levels of PEs were from station 2 (Bridge on the LDP), followed by station 3 (Next to Guinness Malaysia). Relatively lower levels were from station 1 (Jambatan Kota) and 4 (Bridge next to Citibank).

The levels of PEs detected in the river gives us an idea on the extent of pollution of the river water due to industrial waste.

But based on the results we cannot say conclusively that the PE contaminants actually originated from industrial areas, as their levels are quite well distributed along the Klang River.

The PEs could also be due to household and commercial waste, this statement can be supported by the fact that, the sampling areas they have the highest population densities in Malaysia i.e. The Klang Valley.

The higher levels of DEHP and DOP show their wide usage and great persistency against degradation, as compared other PEs which were unable to be detected.

The next few pages show the chromatogram obtains from the respective station. The peaks (without mention), which appear, are believed to that of PEs, but due to the non-availability of appropriate standards identification and quantification couldn't be carried out.

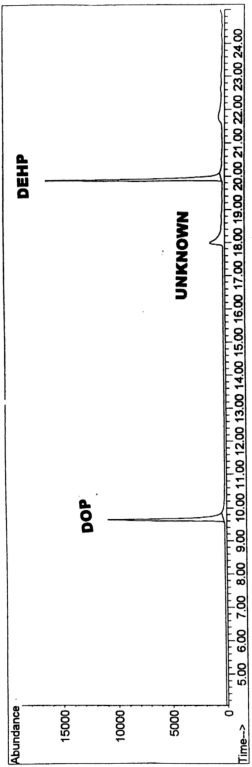


Figure 9: Chromatogram from station 1

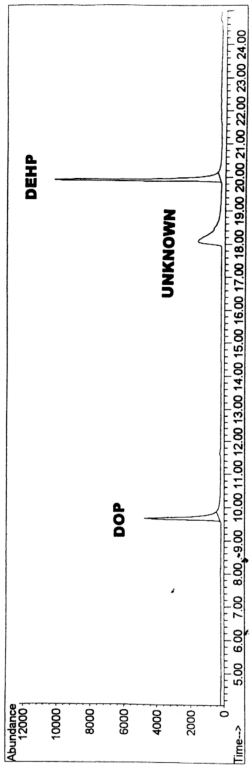


Figure 10: Chromatogram from station 2

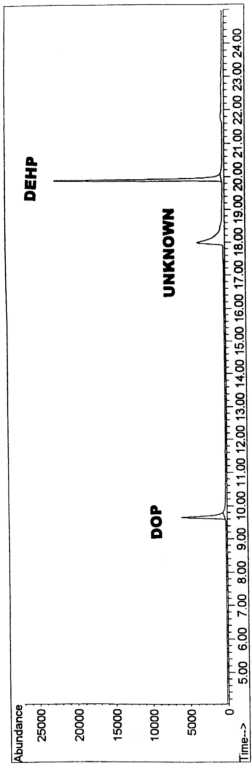


Figure 11: Chromatogram from station 3

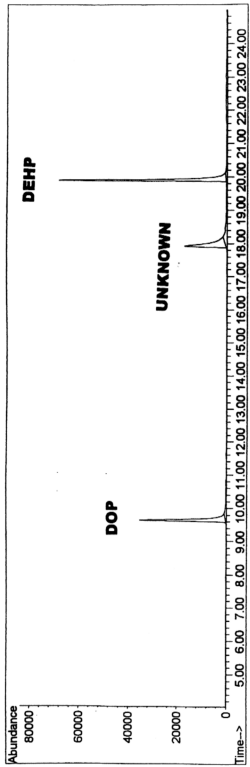


Figure 12: Chromatogram from station 4

3.7. Evaluation of the results

From table 10, the residue levels of PEs in the river water, PEs of lower molecular weight such as DMP and DEP were not detected, these indicates their low persistency and ease of adsorption to other particle not to mention degradation.

Meanwhile, for the 2 isomers DIBP and DBP, which are different with respect to the configuration of the side-chain although they share the same molecular weight. Their long side-chains could lead to their degradation due to biological activities.

DOP and DEHP were not found in very high amounts in any of the stations, although they are more widely used in industries. They are chiefly used as plasticizers with synthetic polymers, such as polyvinyl chloride (PVC) **[25]**. Their other uses include building and construction products, automobile and home furnishings, food covering and even medical products.

Due to their high octanol/water partition coefficients (log Kow) DEHP and DOP are assumed to have a high bioaccumulation potential. Literature results apparently show evidence of high concentrations in biota. The ubiquitous presence of DEHP and DOP in the environment and the alleged persistence in biodegradation tests has raised concern about this group of substances.

However, recent results did not only demonstrate that phthalates are readily biodegradable but also that they bioaccumulation to very much lesser degree than anticipated.

Results of these recent biodegradation studies are summarized in Fig. The inserted box indicates the 10 - day window, which describes a period of ten days, during which a minimum biodegradation rate has to be

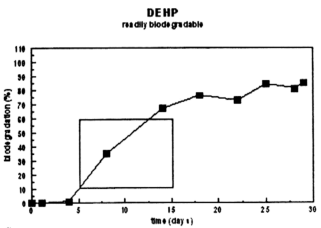


Figure 13: DEHP biodegradability

Biodegradation pattern of DEHP in a test system for ready biodegradability

Recently bioaccumulation studies were performed employing a novel labeling technique. In this case, the hydrogen atoms of the aromatic ring of the phthalic acid are replaced by deuterium. The following esterification leads to a ring deuterated phthalate with the same chemical properties as the normal ester except for a slightly higher molecular mass. **[26].**

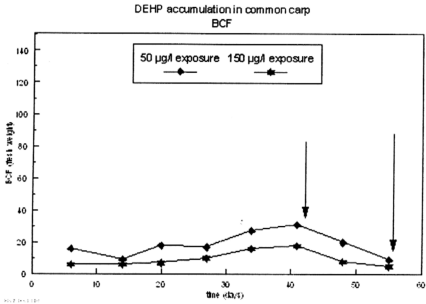


Figure 14: DEHP accumulation in common carp

As we can see from the above graph, DEHP concentration in the fish drops dramatically, leading to a fast decline of DEHP within the organism. Here too, the measured value is much lower than the theoretical one. Generally, bioaccumulation and biomagnifications across a food chain can be neglected for phthalate esters.

The conditions of the sampling site with respect to waste (plastics etc.), river flow-rates and the weather contribute quite significantly to levels of the PEs.

It was found that the levels of the PEs did not show any significant trends, although the PEs could also be due to household and commercial waste, this statement can be supported by the fact that, the sampling areas they have the highest population densities in Malaysia i.e. The Klang Valley.

Al-Omran et. al.[26] has found that the adsorption of PEs is generally dependent on the presence of salt and the degree of adsorption depends on the characteristics of the particulate. The adsorption of PEs is influenced by the chemical composition of the particulate and is more closely correlated with their lipid content.