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METHOD OF QUANTITATION FOR PARAQUAT HERBICIDE AND MONITORING OF ITS LEVELS IN SELECTED MALAYSIAN RIVERS

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ABSTRACT : Residual levels of paraquat in two major Malaysian rivers was quantified using solid phase extraction (SPE) cartridges and high performance liquid chromatography (HPLC). The assay method provided adequate sensitivity, specificity, linearity, accuracy, and precision. Inter-day precision and percentage inaccuracy were between -2.4 % and 0.9%, Intra-day precision and percentage inaccuracy were between -11.2% and 8.6% and average recovery was between 81%-98%. Limit of quantitation (LOQ) and limit of detection (LOD) for this method was 0.6 ng/ml and 0.3 ng/ml respectively. Paraquat was detected in two Malaysian rivers mainly in the range of ppb. The highest concentration of paraquat was observed in Pertang (1.83 ng/ml) and Klang (2.29 ng/ml) rivers.

KEYWORDS : Paraquat; parts per million, HPLC, solid phase extraction

INTRODUCTION

Synthetic pesticides along with several other environmental agents are the major focus of endocrine-disrupting chemical (EDC) research. Paraquat (1,1'-dimethyl-4,4'-bipyridilium dichloride) is a synthetic herbicide widely used in pre and post emergent weed control. Paraquat ranks tops in the agrochemical list of herbicides used in Malaysia (Star, Oct. 1 2002). About 10 million litres of paraquat herbicide is used yearly in the country (Star, Oct. 1 2002). For the general population, the main source of exposure to pesticides with endocrine effects is from food and water intake (Colbrn *et al.,* 1993). Paraquat is known to be strongly bound to soil sediments. However, some studies show evidence of soil run off, erosion and leaching following heavy rain fall (Wauchope *et al.,* 1992; Herbicide handbook 1994). In this study, a simple analytical method was developed and validated to quantify low concentrations of paraquat (1,1'-dimethyl-4,4'-bipyridilium dichloride salt) in selected Malaysian river water using solid phase extraction (SPE) and high performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Sample collection

Water samples were obtained from Selangor and Cameron Highland rivers, which are the two major drinking water sources for West Malaysia. Most of Malaysia's agricultural areas are situated close to the Selangor and Cameron Highland river areas. Agricultural activities around the sectors include, vegetable farms, oil palm and rubber plantation farms. Sampling was done for a period of four months which represent the peak periods for farm erosion and coverage of leaching activity following heavy rainfall. Rainfall data for Selangor and Cameron Highland areas shows that for the last 36 years, the highest annual and daily rainfall records in Malaysia occurs between August and November of each year (Malaysian metrological service). Sampling was carried out by Alam Sekitar Malaysia Sdn Bhd for three to four months (August-November, 2005). Water samples collected were stored in the cold room in the Department of Pharmacology, Faculty of Medicine, University of Malaya, Malaysia and maintained at 4°C. Extraction of paraquat residue was completed within 7 days following sample arrival.

Apparatus

HPLC analysis was carried out on a Shimadzu high perfomance liquid chromatography mass spectrometry (LC-10 AT) consisting of a system controller SCL10A P (Shimadzu), column oven (model CTO 10A, Shimadzu), UV detector (model SPD-10A, Shimadzu). Isocratic pumping system, constant flow (Waters 6000A HPLC pump) was used. HPLC Conditions include: semi-micro column C18, (Shimadzu,1.5 , 33 mm x 4.6mm); Column Temperature 30.0° C; Flow Rate1.1 mL/min, Injection Volume: 200 L; UV Detector Settings: Wavelength

Range: 210 - 370 nm; Sample Rate: 1 scan/sec. Wavelength Step: 1 nm; Integration Time: 1 sec; Run Time: 5.0 min. Wavelengths: Paraquat - 257 nm; Retention time window : 3.6-3.8 minutes.

Reagents

Deionized water; methanol (HPLC grade); orthophosphoric acid (85% (W/V), HPLC grade); diethylamine; concentrated sulfuric acid; sodium hydroxide; concentrated hydrochloric acid; cetyl trimethyl ammonium bromide; sodium thiosulfate; aexanesulfonic acid sodium salt; 1-heptanesulfonic acid sodium salt; and pure paraquat dichloride salt (analytical grade) as a reference. Reagents were ordered from Aldrich Chemical, and were HPLC compliant.

Preparation of solutions and standards

SPE conditioning solvents

Conditioning solution A and B was used together with methanol and deionized water to condition the MFC18 cartridges in the sequence shown in Section 2.8. Conditioning solution A: 0.5 g of cetyl trimethyl ammonium bromide and 5 ml of concentrated ammonium hydroxide were dissolved in 500 ml of deionized water and diluted to 1000 ml in volumetric flask. Conditioning solution B: 10.0 g of 1-hexanesulfonic acid, sodium salt and 10 ml of concentrated ammonium hydroxide were dissolved in 250 ml of deionized water and diluted to 500 ml in volumetric flask.

SPE cartridge eluting solution & Cartridge conditioning

13.5 ml of orthophosphoric acid and 10.3 ml of diethyl amine were dissolved in 500 ml of deionised water and diluted to 1000 ml in a volumetric flask. 3.75 g of 1-hexanesulfonic acid was dissolved in 15 ml of the cartridge eluting solution and diluted to 25 ml in a volumetric flask with the disk eluting solution. SPE MFC18 extraction cartridges were placed on the cartridge extraction system manifold. 5 ml of deionized water, followed by 5 ml of methanol, 5 ml of deionized water, 5 ml of conditioning solution A, 5 ml of deionized water, 10 ml of methanol, 5 ml of deionized water, 20 ml conditioning solution B. Conditioning solution B was retained in the MFC18 cartridge to keep it activated. The flow rate through the cartridge was maintained at 10 ml/min.

Mobile phase

The mobile phase to elute paraquat was prepared by adding the following to 500 ml of deionized water: 13.5 ml of orthophosphoric acid; 10.3 ml of diethyl amine; 3.0 g of 1-hexanesulfonic acid sodium salt. Contents was mixed and diluted with deionized water to a final volume of 1

litre. 3.75 g of 1-hexanesulfonic acid was dissolved in 15 ml of the SPE cartridge eluting solution and diluted to 25 ml in a volumetric flask with the cartridge eluting solution.

Paraquat standard solution (1 mg/ml) & Laboratory fortified blanks (LFBs)

100 mg of dried paraquat salt was placed into a 100 ml polypropylene volumetric flask. Approximately 50 ml of deionized water was added, vortex mixed and diluted to the mark with deionized water to achieve a standard solution of 1 mg/ml. Laboratory fortified blanks (LFBs) were prepared from the following stock standards containing paraquat concentrations of 2000, 1600, 1000, 750, 500, 300, 200, 150, 110, 60, and 30 ng/ml. 500 l of each of the above standards were separately spiked into 100 ml parts of deionized water using a 1000 ml micropipette, to achieve LFB's with the following paraquat concentrations; 10.0, 8.0, 5.0, 3.75, 2.5, 1.5, 1.0, 0.75, 0.55, 0.3, and 0.15 ng/ml

Laboratory reagent blanks (LRB) & paraquat retention time

Analysis were carried out on clean blank samples (deionized water) and on aqueous sample of pure paraquat standard. Using chromatographic conditions described (in Section 2.2), 200 l of blank reagent water and 200 l of aqueous solution of pure paraquat standard were injected into the HPLC injector port and analyzed. The LRB produced no peak (Figure 1) at the retention time window of paraquat.

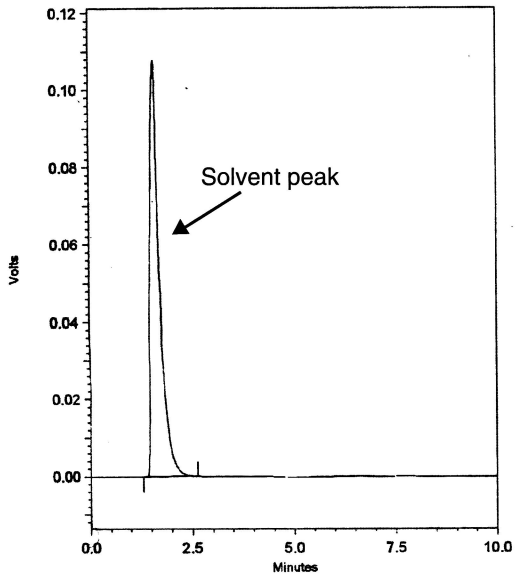

Figure 1. *Chromatogram of LRB showing no paraquat peak at the retention time window of 3.56- 3.78 minutes.*

Sample extraction & Sample analysis

Water samples were passed entirely through a 0.45 m nylon membrane filter and collected in 100 ml plastic volumetric flask. The pH of the samples were measured and adjusted to between the range of 7.0 and 9.0 using 10% NaOH or 10% HCl. Conditioned MFC18 cartridge was placed on a solid phase extraction vacuum manifold. The 100-ml plastic volumetric flask reservoir was attached to the MFC18 cartridge with a plastic adapter. 100 ml of a clean river water sample was transferred into the reservoir. Vacuum pump was maintained at 6 ml/min. The sample was filtered through and the column was washed with 5 ml of HPLC grade methanol. The sample cartridge was vacuum dried for one additional minute. Finally the vacuum was released and sample waste and methanol was discarded. 4.9 ml of the eluting solution was added to the sample cartridge and the vacuum was turned on and adjusted to a flow rate of 2 ml/min. Paraquat extract was collected in a 5 ml graduated plastic test tube. The extracted paraquat was fortified with 100 l of the ion-pair concentrate and volume adjusted to the mark with cartridge eluting solution with vortex mixing. Extracts were analysed using the HPLC conditions described. Quantitation and data deduction were consistent with that performed in the method validation. The width of the retention time window (Figure 2) used to make identification was based upon measurements of actual retention time variations of working standards. Concentration of paraquat in samples from Cameron Highland and Selangor river basins are shown in Tables 3 and 4 respectively.

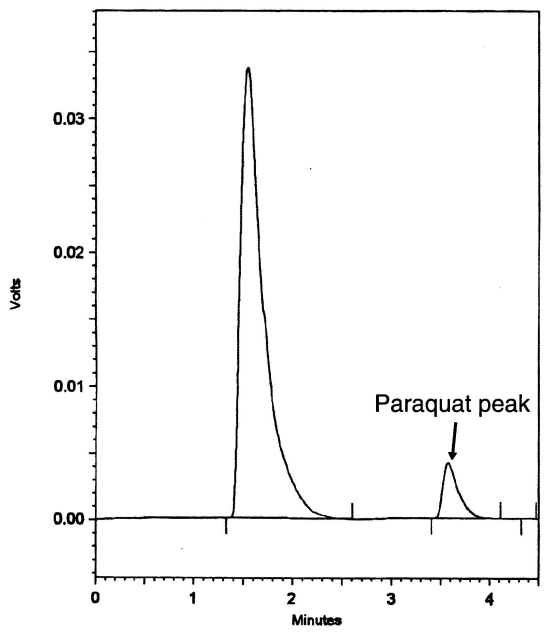

Figure 2. *Chromatogram showing peak area for paraquat dichloride at retention time of 3.56- 3.78 minutes*

Table 3. Concentration of paraquat in samples from Selangor river basins for (Aug-Nov, 2005).

Sampling Site No	River	Number of detection/ Paraquat concentration in ppb (ng/ml)			
		August	September	October	November
IK01	Klang	ND	ND	< LOQ	ND
IK02	Klang	ND	ND	< LOQ	ND
IK03	Klang	ND	ND	< LOQ	ND
IK04	Klang	ND	ND	< LOQ	ND
IK05	Klang	ND	ND	ND	ND
IK06	Klang	ND	ND	ND	ND
IK07	Klang	ND	ND	< LOQ	ND
IK08	Klang	ND	ND	< LOQ	ND
IK09	Klang	ND	ND	< LOQ	ND
IK10	Klang	ND	ND	< LOQ	ND
IK11	Damansara	ND	ND	< LOQ	ND
IK12	Damansara	ND	ND	< LOQ	ND
IK13	Damansara	ND	ND	< LOQ	ND
IK14	Klang	ND	ND	< LOQ	ND
IK15	Kuyoh	ND	ND	ND	ND
IK16	Gombak	ND	ND	< LOQ	ND
IK17	Gombak	2.29	ND	ND	ND
IK18	Gombak	< LOQ	ND	ND	ND
IK19	Batu	< LOQ	ND	ND	ND
IK20	Batu	ND	ND	ND	ND
IK21	Keroh	ND	ND	ND	ND
IK22	Jinjang	ND	ND	ND	ND
IK23	Ampang	< LOQ	ND	ND	ND
IK24	Gombak	ND	ND	ND	ND
ISRO6	Kerling	ND	ND	ND	ND
ISRO5	Selangor	ND	ND	ND	ND
ISRO4	Selangor	ND	ND	ND	ND
ISRO3	Batang Kali	ND	ND	ND	ND
ISRO8	Serendah	ND	ND	ND	ND
ISRO7	Kancing	ND	ND	ND	ND
ISRO9	Sembah	ND	ND	ND	ND
ISRO2	Selangor	ND	< LOQ	ND	ND
ISRO2	Sepang	ND	ND	ND	ND
IS01	Sepang	ND	ND	ND	ND
IS02	Sepang	ND	< LOQ	ND	ND
IS03	Sepang	ND	ND	ND	ND
1L04	Langat	ND	ND	ND	ND
IL17	Balk	ND	ND	< LOQ	ND
ITO1	Tengi	ND	ND	ND	ND
IB01	Buluh	ND	ND	< LOQ	ND
IB02	Buluh	ND	ND	< LOQ	ND
IB03	Buluh	ND	ND	ND	ND
IB04	Buluh	ND	ND	< LOQ	ND
IB05	Buluh	ND	ND	< LOQ	ND

*ND- Not Detected/Not Applicable; * < LOQ- Present-below Limit of quantitation

Table 4. Concentration of paraquat in samples from Cameron Highland river (Aug-Nov, 2005).

Sampling Site No	River	Number of detection/ Paraquat concentration in ppb (ng/ml)			
		August	September	October	November
UMCX-01	Tajam	ND	ND	< LOQ	< LOQ
UMCX-02	Ikan	ND	1.32	< LOQ	< LOQ
UMCX-03	Telom	ND	ND	< LOQ	< LOQ
UMCX-04	Terla	< LOQ	1.63	< LOQ	< LOQ
UMCX-05	Pertang	ND	1.83	< LOQ	< LOQ
UMCX-06	Telom	ND	ND	< LOQ	< LOQ
UMCX-07	Tringkap	ND	< LOQ	ND	ND
UMCX-08	Bertam	ND	< LOQ	ND	ND
UMCX-09	Habu	ND	ND	< LOQ	ND
UMCX-10	Ulung	< LOQ	ND	< LOQ	ND
UMCX-11	Ulung	ND	< LOQ	ND	ND
UMCX-12	Lenggok	ND	ND	< LOQ	< LOQ
UMCX-13	Bertam	ND	ND	< LOQ	< LOQ
UMCX-14	Ringlet	ND	< LOQ	< LOQ	ND
UMCX-15	Bertam	ND	ND	< LOQ	ND
UMCX-16	Bertam	ND	< LOQ	ND	ND
UMCX-17	Bertam	ND	< LOQ	< LOQ	ND
UMCX-18	Bertam	ND	ND	< LOQ	ND
UMCX-19	Bertam	ND	ND	< LOQ	ND
UMCX-20	Bertam	ND	< LOQ	ND	ND

*ND- Not Detected/Not Applicable; * < LOQ- Present-below Limit of quantitation

Formula and calculations

Recovery = Recovery calculations were performed by comparing the results for extracted samples with the unextracted reference quality control standards that represent 100 % recovery.

$$\text{Percentage absolute recovery} = \frac{\text{Peak area of extracted sample}}{\text{Peak area of unextracted sample}} \times 100\%$$

$$\text{Accuracy} = \frac{\text{Expected concentration - predicted concentration}}{\text{Expected concentration}} \times 100\%$$

Concentration of paraquat in the sample = The concentration of paraquat injected was calculated from the peak area using the calibration curves

$$\text{Concentration of paraquat in ng/ml (X)} = \frac{C - Y}{M}$$

Where : X = Concentration of paraquat in the sample (ng/ml)
Y = Peak area from chromatogram
M = Slope or gradient value
C = Y- intercept value

$$\text{Coefficient of variation (CV\%)} = \frac{\text{Standard deviation}}{\text{Mean}} \times 100\%$$

Precautions

Paraquat binds to glassware (Hodgeson *et al.,* 1992; Worobey, 1987) hence the use of glassware was minimized in the extraction. Paraquat is sensitive to light (US EPA, 1987; Worobey, 1987). Water samples were collected and stored in amber coloured plastic bottles, and analysis was conducted in a dimly lit area.

RESULTS AND DISCUSSION

Results from validation performance

A series of intra-day and inter-day (Tables 1 and 2) method validation tests were performed to test the method's reliability, specificity, precision, accuracy, linearity, recovery and limit of detection and quantitation of paraquat dichloride in river water. Standard and calibration curves for paraquat are shown on Figures 5 and 6 respectively.

Table 1. Summary of the calibration curve equations obtained from six replicates

Studied compound	Mean ± SD(n=6)		
Paraquat dichloride	Slope of calibration curve (m)	Y-axis intercept (c)	Coefficient correlation (R2)
	4479.85 ± 162.85	506.25 ± 218.06	0.9991± 0.00

Table 2. Summary of precision, accuracy and recoveries of spiked paraquat samples obtained from five replicates at low (0.55 ng/ml), median (3.75 ng/ml) and high (8 ng/ml) concentrations.

Studied compound	LOQ	Intra day data			Inter day data		
Paraquat dichloride	0.3 ng/ml	0.55 ng/ml	3.75 ng/ml	8 ng/ml	0.55 ng/ml	3.75 ng/ml	8 ng/ml
Mean ± SD (% bias)		0.61 ± 0.04 (-11.24 ± 0.51)	3.42 ± 0.07 (-8.60 ± 1.97)	7.51 ± 0.21 (6.04 ± 2.60)	0.56 ± 0.03 (-2.80 ± 5.58)	3.84 ± 0.08 (-2.40 ± 1.97)	7.92 ± 0.32 (0.95 ± 3.97)
Percentage recovery (%)		81.03 ± 10.39	85.21 ± 6.61	90.07 ± 3.50	83.40 ± 9.43	94.22 ± 4.46	92.09 ± 4.25

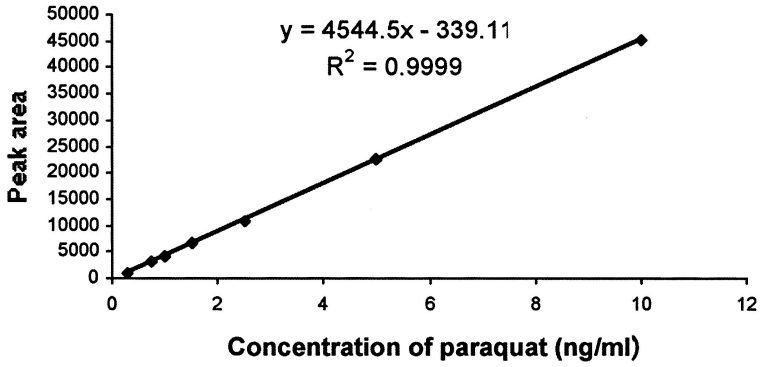

Figure 5. *Paraquat standard curve*

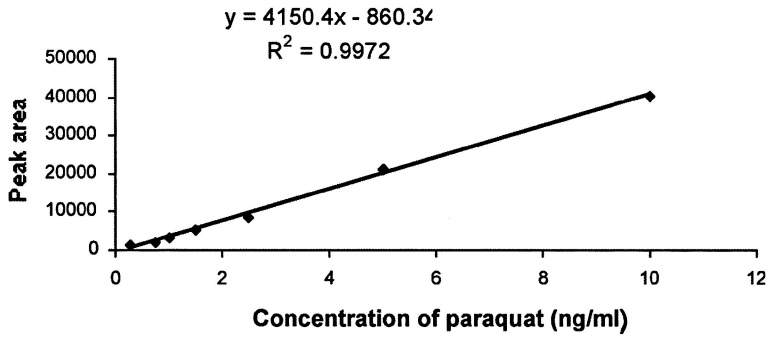

Figure 6. *Paraquat calibration curve*

Discussion

Results show that paraquat dichloride herbicide residue was detected in Selangor and Cameron Highland rivers. Out of a total of 80 Cameron Highland river samples analyzed, 44% of the samples were found to test positive for paraquat herbicide contamination. Frequency of paraquat detection was highest for the months of September and October. The mean range of the herbicide detected in 10 stations, of the 20 sampling sites, was between 0.3-0.71 ng/ml. Pertang (site 5) and Terla (site 4) river basins recorded the highest mean concentration (0.71 ng/ml and 0.54 ng/ml respectively) of paraquat detected. The highest residual levels were recorded for the month of September, for Pertang (site 5), 1.83 ng/ml; Terla (site 4), 1.63 ng/ml; Ikan (site 2), 1.32 ng/ml; and Bertam (site 16), 0.97 ng/ml. From the 20 sites sampled, Bertam (site 8) was found to be free of paraquat contamination. From a total of 62 Selangor river water samples analyzed, 47% of the samples were found to test positive for paraquat herbicide contamination. Frequency of paraquat detection was highest for the months of October and August 2005. The range of paraquat detected in 14 stations, of the 44 river sampling sites covered were between 0.3-2.29 ng/ml. Klang river basins (site IK17 and site IK19) recorded the highest paraquat concentration (2.29 ng/ml and 0.36 ng/ml respectively) for the months monitored. The highest contaminant levels were recorded for the month of August at site IK17, with concentration of 2.29 ng/ml. Other contaminated sites include Sungai Buloh (site IB04), 0.35 ng/ml and Sungai Buloh (site IB02) 0.34 ng/ml. Although paraquat is known to be strongly bound to soil sediments, when applied on soil for agricultural purposes followed by heavy rain fall, paraquat herbicide is transported to the aquatic environment via soil run off, erosion and leaching (Wachope, 1992; Herbicide Handbook 1994). Paraquat has also been found in surface water systems associated with soil particles carried by erosion (US EPA, 1987). Cameron Highland and Selangor rivers and their tributaries are surrounded by agricultural land use sectors. Heavy rainfall following paraquat pre-harvest and post-harvest spray application may have resulted in soil runoff and leaching of paraquat herbicide into these rivers.

CONCLUSION

The method used in this study is economically suitable to analyze water samples within a short period and is more sensitive than previous HPLC attempts to quantify residual amounts of paraquat in water. The highest concentration of paraquat detected in two rivers, Pertang (1.83 ng/ml) and Klang (2.29 ng/ml) (Figures 3 and 4) levels were within the established threshold limit of 0.02 ng/ml water contaminant levels enforceable (US EPA, 1987), results confirm that despite the Malaysian government's ban on the use of all forms of paraquat in the agrochemical sector, paraquat herbicide appear to be still in use. This method could be adopted by the relevant regulatory authorities to perform regular monitoring of all Malaysian rivers to ensure that local farmers are complying with the ban.

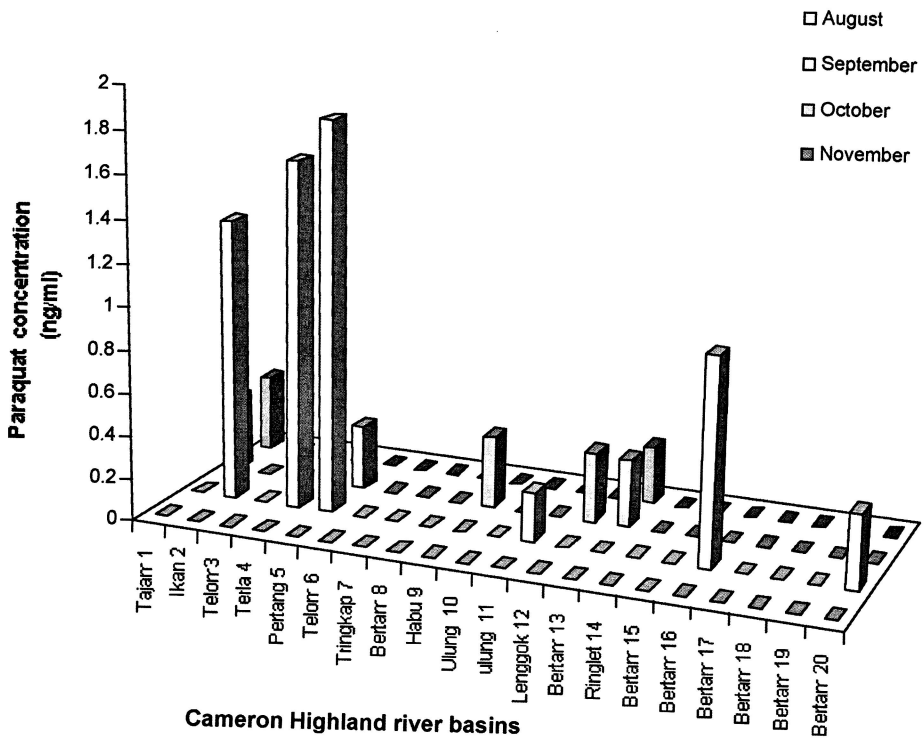

Figure 3. *Concentration of paraquat in Cameron Highland river basins for (August to November, 2005)*

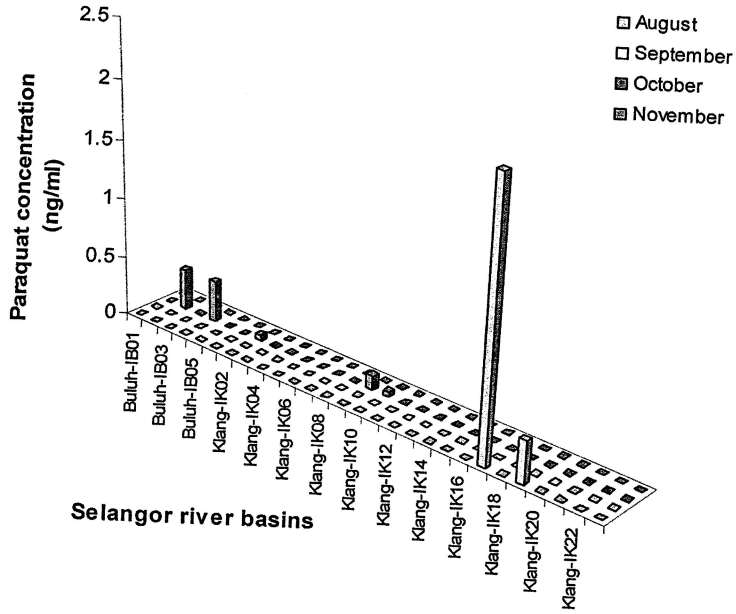

Figure 4. *Concentration of paraquat in Selangor river basins (August to November, 2005)*

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REFERENCES

Colborn F.S., Vom Saal S.F. and Soto A.M. (1993) Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ. Health Perspec.; 101: pp378-384.

Hodgeson J.W., Bashe W.J. and Eichelberger J.W. Method 549. (1992). Determination of diquat and paraqut in drinking water by liquid-solid extraction and High Performance Liquid Chromatography with ultraviolet detection, Supplement II, U.S. EPA/600/R-92/129.

Malaysia Crop Care and Public Health Association (MCPA) (2002) "Paraquat is safe to use in agriculture" the Star newspaper, October 1.

Malaysian Meteorological Service (Bahagian Kajiiklim, Perkhidmatan Kajicuaca) Ibu Pejabat Kajicuaca, Jalan Sultan, 46667 Petaling Jaya, Selangor, Malaysia .

U.S. Environmental Protection Agency. Health Advisory Draft Report (1987): Paraquat. Office of Drinking Water, Washington, DC. 10 - 112.

Wauchope R.D., Butler T.M., Hornsby A.G., Augustijn Beckers P.W.M. and Burt J.P. (1992) Pesticide properties database for environmental decision making. Rev. Environ. Contam. Toxicol; 123: 1 - 157, 10-12.

Weed Science Society of America. (1994) Herbicide Handbook, 7th Edition. Champaign IL. 10-59.

Worobey, B. L., (1987) Analytical method for the simultaneous determination of diquat and paraquat residues in potatoes by High Pressure Liquid Chromatography, Pestic. Sci 18(4), 245.