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Assessment of Selected Herbicides and Chelating Agents in Water Using Gas Chromatography-Electron Capture Detector (GC-ECD)

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ABSTRACT

Water contamination by herbicides and chelating agents is increasing mainly due to the increasing agricultural activities. Water contamination by these compounds has become a concern due to their adverse effects to the environment and humans. Seven sampling sites of water sources in Selangor and Johor were chosen for the study. Contamination level of Mecoprop (MCCP), Nitrilotriacetic acid (NTA) and Ethylenediaminetetraacetic acid (EDTA) in these water body areas was determined by using Gas Chromatography-Electron Capture Detector (GC-ECD). Our results indicated that water samples of Sungai Melot in Selangor showed the highest presence of EDTA. MCCP was detected at a high level at Sungai Sarang Buaya, Johor while NTA showed similar level of concentration at three different sites, Ladang 10, Ladang Sayur and Mardi, Selangor

INTRODUCTION

Rivers are an important source of life. They provide water to human as well as animals and other organisms and irrigation in agricultural field. Nowadays rivers have been used for drainage and points for discharge of domestic waste. Water pollution by herbicides and pesticides has become a concern as they are widely used for agricultural activities around the world [1]. Most of the pesticide compounds diffuse into the environment via surface run-off following diffuse contamination and normal spraying in agricultural fields [2].

In Asia, the amount of herbicides used has increased dramatically. It has been reported that 47% of pesticides have been applied in paddy activities in Philippines [3]. About 80% of pesticides used in Bangladesh and Vietnam for agriculture were applied in paddy field [4]. Nigeria is one of the countries that have high pesticides dependent for economical crop management as its tropical climates enhance the harmful insects'survivability, disease and weeds to grow [5]. In Malaysia about 20% of pesticides were used for agricultural activities. Selangor River was detected with few compounds of pesticides even though the usage of certain pesticides has been banned in Malaysia [6].

Pesticides and herbicides are compounds that are used as defoliant, desiccant or plant regulator [7] and intended specifically for destroying, repelling, preventing or mitigating any pest. They are grouped based on their chemical structure and target pest [5]. Their high production in amount and continuous usage has made them "pseudo-persistent" substances to environment, especially in water bodies [8]. The most common pesticides used and detected

in water are triazine herbicide and organophosphorus insecticide [9].

Mecoprop (MCPP), $C_{10}H_{11}ClO_3$ is one of the common compounds of herbicide. Mecoprop belongs to phenoxy of herbicide family and it is usually combined with other herbicides [10]. It targets broadleaf plants, which typically kills plants that grow in turf and lawn and farmers have used MCPP to kill weeds in cereal crops [11]. The chemical relatives of MCPP, 2, 4-dichlorophenoxyacetic acid and 2, 4, 5-trichlorophenoxyacetic acid imitate the hormones of plant growth, auxins and cause the shoot of the plant to grow in distorted manner and elongated form and eventually die. The National Institute for Occupational Safety and Health, USA has labeled MCPP as a mutagen compound since MCPP causes the inhibition of deoxyribonucleic acid (DNA) synthesis in mice [12], blood clotting in platelet, liver and kidney damage to rats, effects on reproduction and function of immune system in animal [13].

Besides pesticide molecules, another group of contaminant known as chelating agents also contributes to the water pollution. Chelating agents such asethylenediaminetetraacetic (EDTA) acid and nitrilotriacetic acid are mostly detected flowing from agricultural field as well as housing estate (NTA) [14]. They are chemical compounds that have ability to form complexes with metal ions or other substrates. The chelating agents that form complexes with many metal cations will increase the solubility of metal ion in aqueous solution [15].

EDTA is thermodynamically more stable once it chelates with metal ions. Because EDTA is one of the cheapest multipurpose chelating agents [16] it has become one of the compounds used massively in industrial applications and household usage. In shampoo lotions and liquid soaps, EDTA prevents the formation of hard-water soap curds [17]. In industrial field, EDTA is widely used in photochemicals industries, agricultural activities, pharmaceutical, paper manufacturing, galvanizing and in textiles field. EDTA has been reported to solubilize radioactive metals, which in turn increases their mobility in environment [18]. A study has shown that photosynthesis was inhibited at low concentration of unchelated EDTA [19]. Like EDTA, NTA is a chelating agent formed as a polyamino carboxylic acid, $C_6H_9NO_6$ and is available as sodium salt. It has the ability to mobilize nickel compound from heart, kidney, brain and liver in rats [20, 21]. NTA is anticipated to be carcinogenic as shown in animals that had been exposed to NTA [21].

A few methods have been reported for determination of herbicide residues in water [22]. However, the compounds to be analyzed are basically too complex and diluted, hence making the extraction and analysis process difficult [22, 23]. Liquid Liquid Extraction (LLE) is the most common method of extraction due to its low cost and simplicity. Solid Phase Extraction (SPE) is another method in which complex matrices are used to adsorb and extract the analytes by applying small volume of organic solvent [24]. Solid Phase Micro Extraction (SPME) is also one of the most preferred extraction techniques to analyze herbicides contamination in water as it extracts the organic compounds from matrix, followed by desorption process of retained substances into an analytic instrument [25].

Recently, Gas Chromatography (GC) technique has gained popularity in detecting contaminating herbicides from environment. One of them is Gas Chromatography-Electron Capture Detector (GC-ECD). GC-ECD gives high selectivity and low level of herbicide detection. Moreover, this analytical method is easier to acquire [22]. Therefore the present study was carried out to study contamination level of selected Mecoprop, Nitrilotriacetic acid and Ethylenediaminetetraacetic acid in selected water in Malaysia and to validate methods in running Gas Chromatography- Electron capture Detector (GC-ECD) for detecting selected herbicide compounds and chelating agents.

MATERIALS AND METHODS

Study areas and water sampling

Water samples were obtained from various locations in Johor and Selangor, including Universiti Putra Malaysia (Table 1). Sampling areas were selected based on the existent of agricultural activities and housing estates. The collected samples from a depth of 1-2 m were filled into 1 L container fitted with screw cap. Bottle and cap liner are washed and rinse with methylene chloride or acetone. The containers were fitted with foil to protect samples from light. On site measurements of temperature and pH were done on collected water samples. Sample containers were kept at 4 °C or placed in ice and protected from light during compositing and right after the collection until extraction process.

Table 1. Source of water samples collected for this study.

Sites	Locations	Descriptions
Sungai Melot	2.833766 N; 101.679721 E	Palm oil plantation and housing estate near to this site.
Mardi, Serdang	2.5906.5 N; 101.4119.3 E	A few agricultural activities situated near this site.
Ladang 2, UPM	3.007134 N; 101702924 E	This site has few plantation activities of vegetables.
Ladang Sayur, UPM	2.990313 N; 101.70737 E	Vegetable plantations are situated near to this site.
Ladang 10, UPM	2.989783 N; 101.71284 E	Few agriculture activities are situated near to this site.
Sungai Sarang Buaya	1.89089 N; 102.768278 E	There are huge paddy field and few housing estates around this site.
Parit Kepala Baba	1.890288 N; 102768633 E	Housing estate and palm oil plantation near to this site.

Mecoprop stock preparation

10 mg of pure materials were weighed. The materials were dissolved in acetone and diluted into 10 mL of volumetric flask. The solutions were transferred into screw cap bottles and protected from light exposure. The standard solution of MCPP was stored in 4°C prior to analysis.

Ethylenediaminetetraacetic acid (EDTA) and Nitrilotriacetic acid (NTA) stocks preparation

10 mg of pure EDTA and NTA were weighed. The materials were dissolved in distilled water and diluted into 10 mL of volumetric flask. The solutions were transferred into screw cap bottles and protected from light exposure. The standard solution of each chelating agents was stored in 4°C prior to analysis.

Liquid - liquid Extraction for MCPP compound

Two portions of 500 mL of water samples were transferred into 1 L of glass-separating funnel. The pH of sample was adjusted to < 11 by using sodium hydroxide (NaOH). The water sample was extracted three times with 30 mL of dichloromethane. The sample was extracted by shaking for 2 min with periodic venting in order to release the pressure. The separatory funnel was clamped for 10 min for separation process. The organic phase was combined into a 250 mL beaker and the pH was adjusted to < 2 by using sulfuric acid (H_2SO_4). The combined of organic phase was decanted into 250 mL round bottom flask and concentrated using rotary evaporator at 40°C. The sample was then reconstituted with dichloromethane up to 5 mL and transferred into 2 mL vial.

Schaffner-Giger method for EDTA and NTA compounds extraction

100 mL of water samples were evaporated using rotary evaporator to dryness. The sample was then acidified with 5 mL of 50% (v/v) of formic acid and then was evaporated to dryness again. After that, the solution of 10% of acetyl chloride/propanol was added and heated for 1 h at 90°C in order to form propyl-ester which then will be extracted with chloroform. The chloroform solution was the evaporated to dryness. Then it was re-dissolved in toluene solution for further analyzed by GC-ECD.

Analysis with Gas Chromatography Electron Capture Detector

Agilent 7890 Gas Chromatograph with programmable splitless/split injector was used for compound separation. The instrumental conditions used in study were summarized in Table 2. Recovery test of selected herbicide and chelating agents were carried out at three different concentrations (0.25 µg/mL, 2 µg/mL and 5 µg/mL) in order to test the efficiency of the methodology process for different groups of tested compounds.

Table 2. Analytical conditions of GC-ECD analysis.

Injector module	Split/splitless
Injection mode	Splitless
Sample volume	1 mL
Analytical column	SGE Analytical Science BPX5: 30 m x 0.25 mm x 0.25 µm
Carrier gas	Nitrogen
Flow rate	3 mL/min
Oven program	50°C , 4 min hold, 8°C/min to 270°C, 5 min hold
ECD detector temperature	300°C

Standard curve of selected compounds

Calibration of MCPP, EDTA and NTA was carried out at four different concentration levels (25 µg/mL, 50 µg/mL, 75 µg/mL and 100 µg/mL). They were plotted based on squared correlation coefficient, R^2 (data not shown).

RESULTS AND DISCUSSION

Method validation

Validation analysis of method was carried out to monitor the efficiency of extraction process and its reproducibility. In this study the method for MCPP,EDTA and NTA determination using GC-ECD was validated through recoveries parameter of these three selected compounds. Distilled water was used to carry out extraction method in order to quantify MCPP herbicide. The water sample was added (spiking) with three known concentrations of MCPP (0.25µg/mL, 2µg/mL and 5µg/mL). The differences in the result obtained at the end of analysis are called recovery [26]. Method validation for EDTA and NTA was same with MCPP, which was the known concentration of each compound that were spiked into distilled water. Quantification process before being analysed by GC-ECD was carried out with same methodology as was used for water sample at seven sites. Three fortification levels were selected (0.25µg/mL, 2µg/mL and 5µg/mL). The recovery of MCPP, EDTA and NTA for each fortification level was summarized in Table 3.

Table 3. Recovery concentration and percentage of compounds in distilled water at three different fortification levels

Compound	Fortification level (µg/ml)	Recovery Concentration (µg/ml)	Recovery percentage Concentration in Distilled Water (%)
MCPP	0.25	0.23±0.0070	92±2.8284
	2	1.8±0.2121	90±10.6066
	5	2.8±0.2616	56±5.2325
NTA	0.25	0.2±0.1414	80±56.5685
	2	1.6±0.0707	80±3.5355
	5	4±0.1414	80±2.8284
EDTA	0.25	0.21±0.0141	84±5.6568
	2	1.3±0.3535	65±17.6777
	5	3.5±0.7071	70±14.1421

It is stated the recoveries within 70-110% are considered to be satisfactory [27]. Our results showed average recoveries within acceptable range for all three compounds, except for NTA at 0.25 µg/mL, EDTA at 2 and 5µg/mL and MCPP at 5 µg/mL which shows value less than 70%.

Analysis of compounds in water

The concentration of these 3 compounds were analysed using GC-ECD as shown in fig. 1, 2 and 3 from seven sampling sites, only six water samples (Ladang 10, Ladang 2, Sungai Melot, Sungai Sarang Buaya, Parit Kepala Baba and Mardi) contained Mecoprop compound with concentration ranging from 2.4352- 186.8739 µg/mL.

Based on **Fig.** 1, Sungai Sarang Buaya recorded the highest amount of MCPP as compared to the other sites, with concentration of 186.8739 µg/mL. The second highest MCPP concentration was detected from Parit Kepala Baba sample (171.9310µg/mL) while water sample from Ladang Sayur showed no detections of MCPP. Sungai Sarang Buaya has high agricultural activities especially rice farming along its river banks. Therefore, the herbicides applied by farmers might drained to the water source from the area of paddy fields. Sprayed pesticides tend to leach to the water bodies due to the high concentration of the compound being used [28]. It is interesting to note that a guideline from World Health Organization (WHO), 2004 has stated concentration of MCPP in drinking water must not exceed 0.01 µg/mL.

EDTA shows higher concentration in seven sampling sites compared to NTA. The highest EDTA concentration was recorded at 105.7941 µg/mL from water samples of Sungai Melot while 0.57 µg/ml of NTA was detected in Ladang Sayur (**Fig.** 2 and 3**).** The high concentration of EDTA at Sungai Melot could be due to the discharge from a nearby palm oil plantation, housing estate and the present of disposal site. EDTA might be leached especially from the disposal site as the size of the area is massive and near to water bodies.

Rainfall and the contaminant concentrations in the sampling sites

Water is one of the medium responsible in draining the contaminating compounds from soil. Rainfall and snowmelt runoff were the main contributors to the loading of herbicide to water [29]. The compounds are affected by rainfall in two ways, either the bonds are breaking with the soil and then dissolved in water or transport the contaminated soil through soil abrasion [28]. In this study we hypothesize the herbicides and chelating agents were drained from the area of their application to water bodies mainly through water runoff. **Fig.** 4 shows the distribution of rainfall in March 2015 in Selangor and Johor. During this time the amount of rainfall was high, ranging between 150 mm and 200 mm.

Heavy rain contributes to the rate of water runoff in soil. This situation will cause the draining of herbicide and chelating agents to water bodies. Therefore, the high concentration of MCPP detected at Sungai Sarang Buaya, Johor is due to herbicide and chelating agents leaching to the river by water runoff during heavy rain.

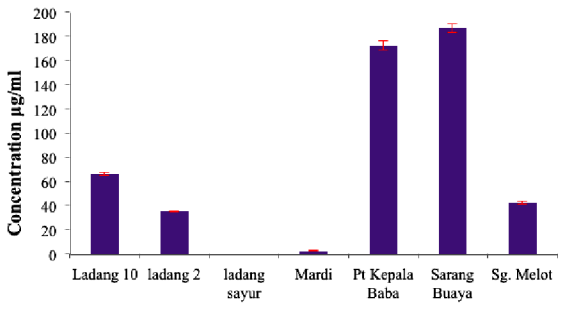

Fig. 1. Concentration of MCPP detected from different water sources. Vertical bar indicates error bar.

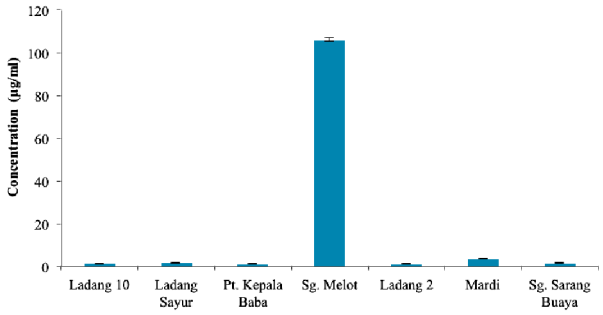

Fig. 2. Concentration of EDTA detected from different water sources. Vertical bar indicates error bar.

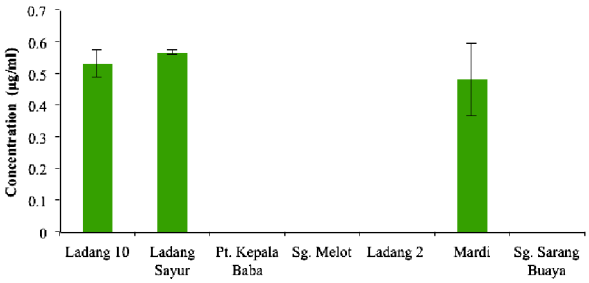

Fig. 3. Concentration of NTA detected from different water sources. Vertical bar indicates error bar.

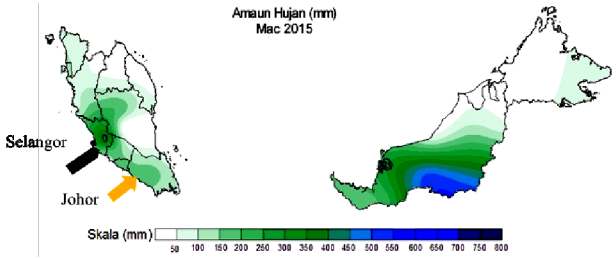

Fig. 4. The distribution of rainfall in Malaysia in March 2015 (lifted from Malaysian Meteorological Department http://www.met.gov.my)

CONCLUSION

We successfully analyzed selected herbicides and chelating agents at different concentrations using gas chromatography equipped with electron capture detector (GC-ECD). Therefore, GC-ECD could become an alternative method in measuring compounds of herbicides and chelating agents from environment. Our result also shows that MCPP compound was high in Sungai Sarang Buaya and this could lead to serious health issue if no corrective action is taken. However, the concentrations of NTA and EDTA in all seven sites were recorded at low level. Further studies on other compounds of herbicides and chelating agents using GC-ECD and other analytical methods at different water sources are needed to monitor contamination level compounds for health and safety reasons. Moreover, continuous monitoring in each water bodies is needed to prevent the deterioration in quality of water by irresponsible people.

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