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Biodegradation of Chlorothalonil, Fenobucarb and Methidathion in Nakdong River, South Korea

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Abstract: The study was to evaluate the biodegradability of three pesticides in river water, Korea. The biodegradation tests for three pesticides, chlorothalonil, fenobucarb and methidathion, were performed on two rivers, Nakdong River and Kumho River, in Korea according to the modified river die-away method. The biodegradations for fenobucarb and methidathion were rapid in polluted river (Kumho River) and slow in clean river (Nakdong River). The reason for the difference might be due to the acclimatization of microbial community to chemical contaminants in the river for a long time. Biodegradation of chlorothalonil exhibited the highest rate compared to those of fenobucarb and methidathion. Chlorothalonil, fenobucarb and methidathion are unlikely to persist in aquatic environment long enough to be a serious groundwater or drinking water problem. Further studies are needed to clarify biodegradation of these pesticides by bacterial specie.

Key words: Biodegradability, chlorothalonil, fenobucarb, methidathion, Nakdong river.

Introduction

Pesticides are widely used in agriculture fields all over the world although it may hazard to environment. However, the safety of pesticide has become one of the serious world public health concerns. Indeed, health risk assessments of pesticides have been conducted by many international organizations, including the EPA, WHO, OECD and UNEP (Van Leeuwen and Hermens, 1995) as well as individual researchers (Sharma et al., 2014; Sharma et al., 2014; Margoum, 2014).

Several studies focused on biodegradation on various chemicals (Gupta et al., 2006; Shin et al., 2008; Kim et al., 2010; Kumar and Mohanty, 2012). Few studies which focused on biodegradation on pesticides were found. However, the increasing amount of pesticides released in the aquatic environment and their ecological impact require the development of evaluation methods for pesticides in order to study their persistence in the environment. Biodegradability of pesticides is a very important criterion in ecotoxicological risk assessment. Several authors have already reported methods for biodegradability of pesticides for river water in order to predict the fate of pesticides in water bodies (Watson, 1977; Fushiwaki and Urano, 1988; Nishihara et al., 1997, Nawaz, 2011).

Recently, the contamination of drinking water is becoming a serious problem in Korea since river water is the main drinking water source in the country. The river water in Korea is usually contaminated by

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industrial wastes, accidental spills, leakage from waste disposal sites and pesticides from farmland (Chung et al., 1997; Min, 2014). Little, however, is known for the biodegradation of pesticides in river water of Korea and researches on this issue have not been widely carried out in Korea. Therefore, there is an urgent need to evaluate the biodegradability of pesticides in river water of Korea.

Three pesticides have been selected because they have not been sufficiently investigated while they are very commonly used in agriculture (Montgomery, 1996). Chlorothalonil (2, 4, 5, 6-tetrachloroisophthalonitrile) is an organochlorine pesticide, a nonsystemic foliar fungicide used against many plant pathogens affecting many agricultural crops, including vegetables and fruits. Fenobucarb (2-sec-butylphenyl methyl carbamate) is a carbamate insecticide, which is used for sucking insects, bugs and weevils on rice. Methidathion (S-2, 3-dihydro-5-methoxy-2-oxo-1, 3, 4-thiadiazol-3-ylmethyl *O*, *O*-dimethyl phosphorodithioate) is an organophosphorous pesticide, a nonsystemic insecticide to control a wide range of sucking and leaf-eating insects. The aim of this study was to evaluate the biodegradability of those three pesticides in river water of Korea.

Materials and Methods

The study is focused on Nakdong River, longest river in Korea – about 510 km and covers about 23,384 km^2 area, especially the point where Kumho River, the second biggest of Nakdong River branch, merges with Nakdong River. Figure 1 shows the study area.

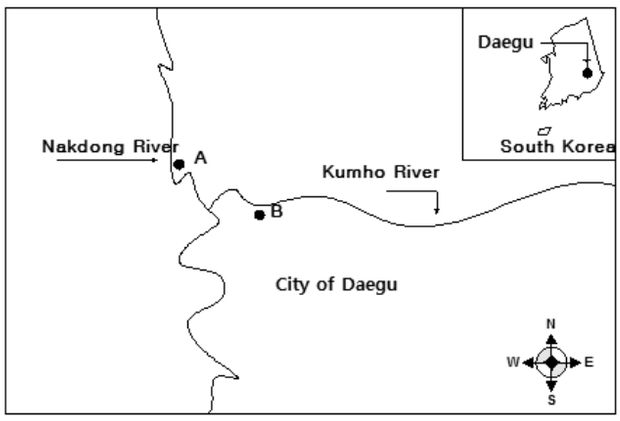

Figure 1: Study area and sampling locations.

Chlorothalonil (97% purity), fenobucarb (99% purity) and methidathion (99% purity) were obtained from Kyung Nong Corporation in Korea and used without further purification. River water was collected from two different locations, sampling site A (Nakdong River) and sampling site B (Kumho River), as shown in Figure 1. Water analysis was followed as per Standard Methods (APHA-AWWA-WPCF, 2005). Water temperature, pH, total numbers of viable bacteria, DO, BOD, SS, ABS, NH_3-N and NO_3-N were measured. Bacterial counting was performed using agar plates purchased from Difco Co. Ltd. containing meat extract 5 g, peptone 10 g, sodium chloride 5 g and agar 15 g in one litre of distilled water. The plates were incubated at 25° for three days before counting colonies.

The biodegradation tests were performed according to the modified river die-away method developed by Kondo called the Handai method (Kondo, 1983). Three pesticides were dissolved in dimethylsulfoxide and prepared to 100 mg/L, respectively. This solution (0.1 ml) was added to the mixture of 4.9 ml of river water sample and 5 ml of 0.2% autoclaved polypepton solution of pH 7 in sterilized glass test tubes (20 mm × 180 mm). This process resulted in the initial concentration being adjusted to 1 µg/ml. Autoclaved river water samples were used as a control. These test tubes were shaken in the dark at 120 rpm and 25°C for seven days. Bacterial cell number was also measured after 0, 1, 2, 3, 5 and 7 days.

To evaluate the concentration of each pesticide, 5 ml of test water were collected and extracted with 5 ml of ethyl ether/hexane (4/1, v/v). Extraction with ethyl ether+hexane was repeated and all extracts were combined and passed through glass column with anhydrous sodium sulphate. The eluate was dried in a rotary evaporator under vacuum at 40 °C and dissolved in 2 ml hexane and analyzed by using a gas chromatography equipped with electron capture detector (GC-ECD) and flame photometric detector (GC-FPD). Chlorothalonil was determined by GC-ECD. Fenobucarb was converted to its N-trifluoroacetyl derivatives with trifluoroacetic anhydride and was determined by GC-ECD. Methidathion was determined by GC-FPD.

The above GC analysis was performed with fused silica capillary column (DB-17, 1 µm thickness, 0.53 mm ID, 30 m length, J&W Scientifics). Nitrogen was used as carrier gas at a flow of 1 kg/cm^2. The temperature of oven, injector and detector were 220, 240 and 270 °C for chlorothalonil; 220, 240 and 270°C for fenobucarb and 270, 280 and 300°C for methidathion, respectively. Average recoveries (n = 3) of chlorothalonil, fenobucarb and methidathion were 97, 93 and 98% at a spiked level of 1 µg/ml. Quantitation was carried out by means of external standard method.

Results and Discussion

Results of water sample analysis, averages from three samples, are shown in Table 1. As shown in Table 1, Kumho River (B) is more polluted than Nakdong River (A) since it runs through agriculture fields and one of major cities in Korea, City of Daegu – 2.5 million population. The initial colony forming unit (CFU) ranged from 2.7×10^3 to 3.7×10^3 at site A and from 5.1×10^4 to 6.5×10^4 at site B. The initial CFU at site B was more than ten times higher than that of site A.

Tests for biodegradation rate of pesticide were performed to investigate the impact on river waters. Biodegradation is expressed as the percentage of the remaining pesticide after cultivation with river water. Figure 2 shows the biodegradation rates of chlorothalonil, fenobucarb and methidathion in river water.

The biodegradation rates of chlorothalonil at sites A and B were both 100% for 24 hours; that of fenobucarb at sites A and B 27% and 40% for seven days; and that of methidathion at sites A and B 23% and 36% for seven days, respectively. The biodegradation rates of fenobucarb and methidathion at site A were higher than those at site B which means that the biodegradation was rapid in polluted river (B) and slow in clean river (A). The reason for the difference between sites A and B might be due to the acclimatization of microbial community to chemical contaminants in the river for a long time. After 0, 1, 2, 3, 5 and 7 days of incubation, the increase of CFU count in water samples of site A was not found while the number of bacteria increased ten times more than the initial CFU for water samples at site B. It shows that the biodegradation rates for these pesticides are dependent on the initial CFU.

Table 1: Analysis of water quality of two water samples

Sampling stations	*A*	*B*
Water temperature (°C)	24.2	24.7
pH	7.0	7.7
Standard plate counts (CFU ml^{-1})	3.2×10^3	5.8×10^4
DO (mg/l)	7.4	8.0
BOD (mg/l)	1.5	5.1
SS (mg/l)	15.4	43.5
ABS (mg/l)	0.04	0.07
NH_3-N (mg/l)	0.07	0.52
NO_3-N (mg/l)	1.64	3.67

A: Nakdong River B: Kumho River

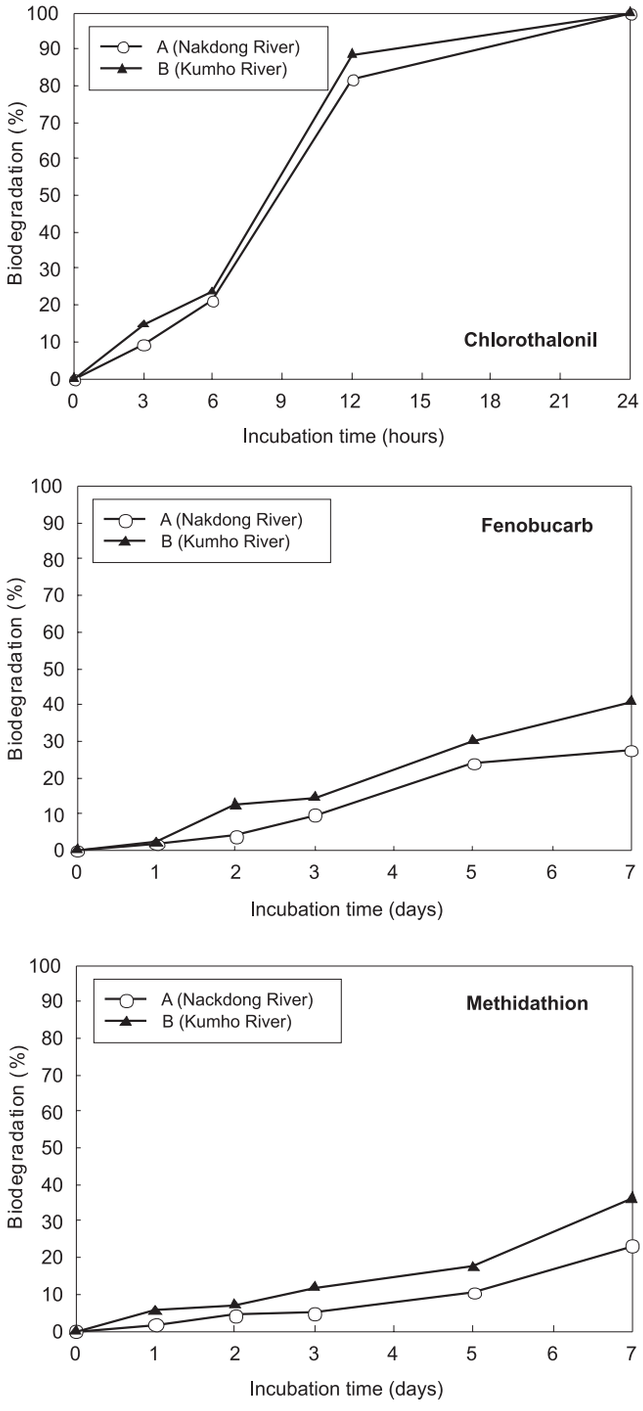

Figure 2: Biodegradation of chlorothalonil, fenobucarb and methidathion in two water samples.

Also, biodegradation of chlorothalonil exhibited the highest rate compared to those of fenobucarb and methidathion. Generally, chlorothalonil is stable to UV light in aqueous media and in crystalline state, and stable in acidic and alkaline aqueous solutions; slow

hydrolysis (Tomlin, 1994). These results suggested that chlorothalonil was mainly degraded by biodegradation in the aquatic environment. In addition, fenobucarb is stable to sunlight, but hydrolysed by acidic and alkaline aqueous solutions (half-life >28 days at pH 2, 2.06 days at pH 10; Tomlin, 1994). Therefore, it was thought that hydrolysis and biodegradation might influence the degradation of fenobucarb in aquatic environment.

The biodegradation rate of methidathion was similar to fenobucarb. Methidathion was relatively stable to hydrolysis in neutral and slightly acidic media, and rapidly hydrolysed in alkaline and acid media (half-life 37 days at pH 5, 48 days at pH 7, 13 days at pH 9 and 30 min at pH 13; Tomlin, 1994). Also, methidathion is rapidly degraded in soil and water by chemical and photolytic processes (half life 3~18 days; Tomlin, 1994). From these results, it was thought that various processes might influence the degradation of methidathion in aquatic environment. Therefore, chlorothalonil, fenobucarb and methidathion are unlikely to persist in aquatic environment long enough to be a serious groundwater or drinking water problem. Further studies are needed to clarify biodegradation of these pesticides by bacterial specie.

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