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Evaluation of Toxicities of Herbicides Using Short-Term Chronic Tests of Alga, Daphnid and Fish

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1. Introduction

Agriculture plays a significant role around the world in order to achieve sustainable development, as it is the major source of food supply, and plays an important role in building the social structure of rural areas (Oerke et al., 2004; Cooper et al., 2007; Damalas et al., 2009; Wilson et al., 2001). Depending on the region/country, these aspects weigh differently. Japan's agricultural scenario, although not very massive, is sometimes termed as an outstanding contributing sector to the country's economy (1.5% of GDP) (Ministry of Agriculture, Forestry and Fisheries, 2010). It is characterized by high production and secured subsidies, owing to its socio-cultural background and environmental characteristics.

Japan, an archipelago of many islands with a northeast-southwest arc, stretches for approximately 2,400 km through the western Pacific Ocean. The country has a total land area of 377,887 square km. About 70-80% of the country is mountainous, leaving 46,280 square km available for profitable cultivation. Several agricultural crops are highly associated with rich social history of Japan. Similar to the other Asian countries, paddy is one of the major and oldest cultivated crops in Japan. Paddy cultivation in Japan is most favored because of its relatively wet climate, which prevails all round the year with intermittent rains and associated freshwater supplies. Approximately, 54.3% (2,516,000 ha) of total cultivable land in the country is used for paddy cultivation (Fig. 1) (Ministry of Agriculture, Forestry and Fisheries, 2011). Unfortunately, several factors, like ever-growing population, intense industrialization, and their associated demands for space, have reduced the agriculturally cultivable lands. This has not only resulted in the total land shrinkage, but the average percentage of agriculturist has also gone down drastically. For instance, in the year 2005, 3,353,000 people were engaged in agriculture but by 2011 it had declined to 2,606,000 people (▼22.3%) (Ministry of Agriculture, Forestry and Fisheries, 2005, 2010).

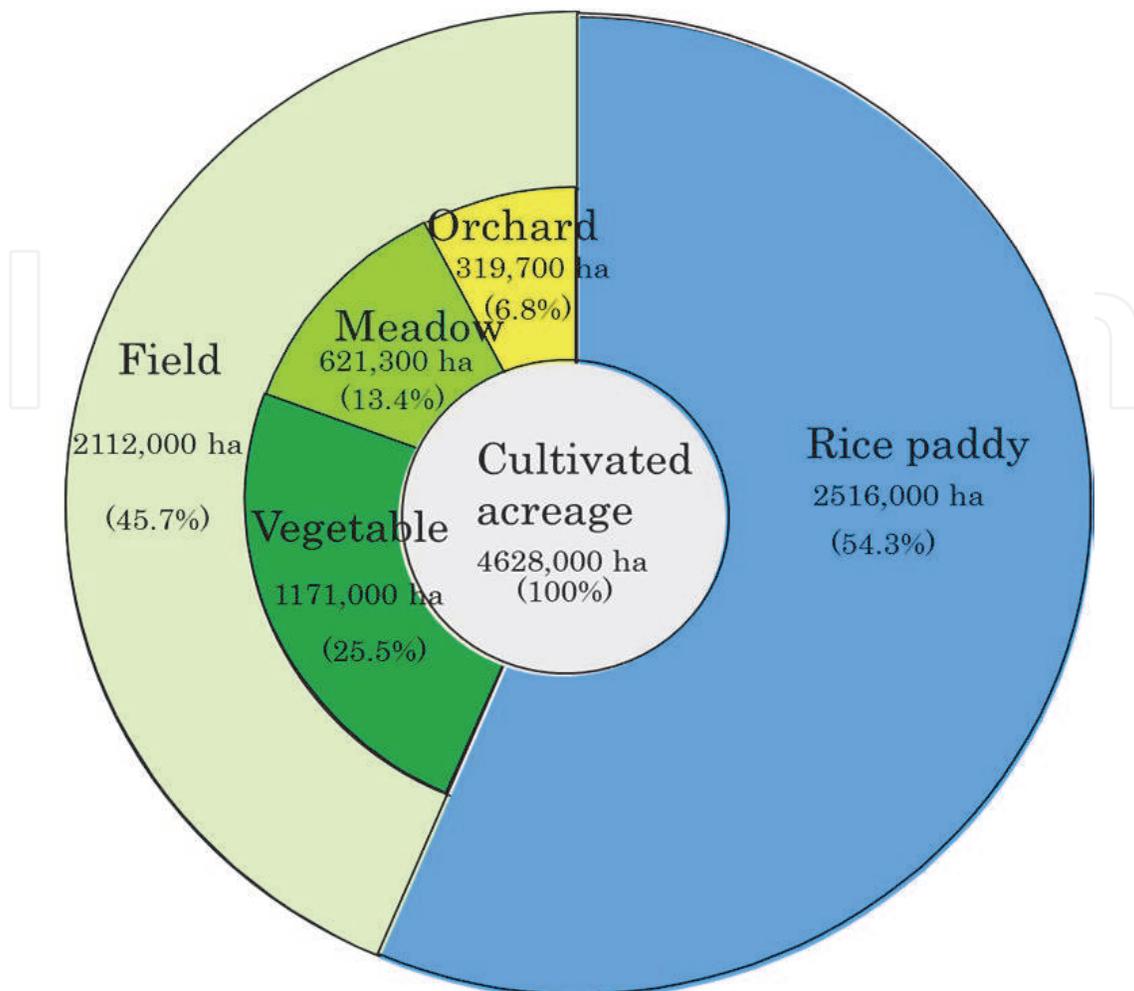


Fig. 1. Ratio of area according to the arable land.

Japanese agricultural farmers have very small land holding, unlike their western counterparts (Fig. 2) (Ministry of Agriculture, Forestry and Fisheries, 2011). Although the cultivable area in Japan is approximately 3,632,000 ha, the average management cultivated area per management constitution (farming family) is about 2.2 ha (in Hokkaido: 23.5 ha and other prefectures: 1.6 ha). This statistics therefore shows that the farmers are left with no other choice, other than use pesticides and fertilizers, in order to harvest the maximum yield from the available land resource. According to OECD (Organisation for Economic Co-operation and Development), Japan uses maximum pesticide (1.5 tons/km²) followed by Korea (1.29 tons/km²) Netherlands (1.06 tons/km²), Belgium (0.92 tons/km²) (OECD, 2002).

Majority of the Japanese farming communities (70% or 1,740,000 farmers) are engaged in paddy culture (Ministry of Agriculture, Forestry and Fisheries, 2011), thereby making its culture methods a monopoly amongst the farmers. This leads to a situation where the farmers are at a liberty to freely choose the pesticide as well as its dosage and application schedule. These practices result in the release of many different kinds of herbicides and insecticides (pesticides) in the environment during a stipulated period of time (Japan Plant Protection Association, 2010; Añasco et al., 2010; Sudo et al., 2002). Therefore, it is extremely difficult to analyze the effect and fate of each pesticide being used in a particular agricultural field as a whole.

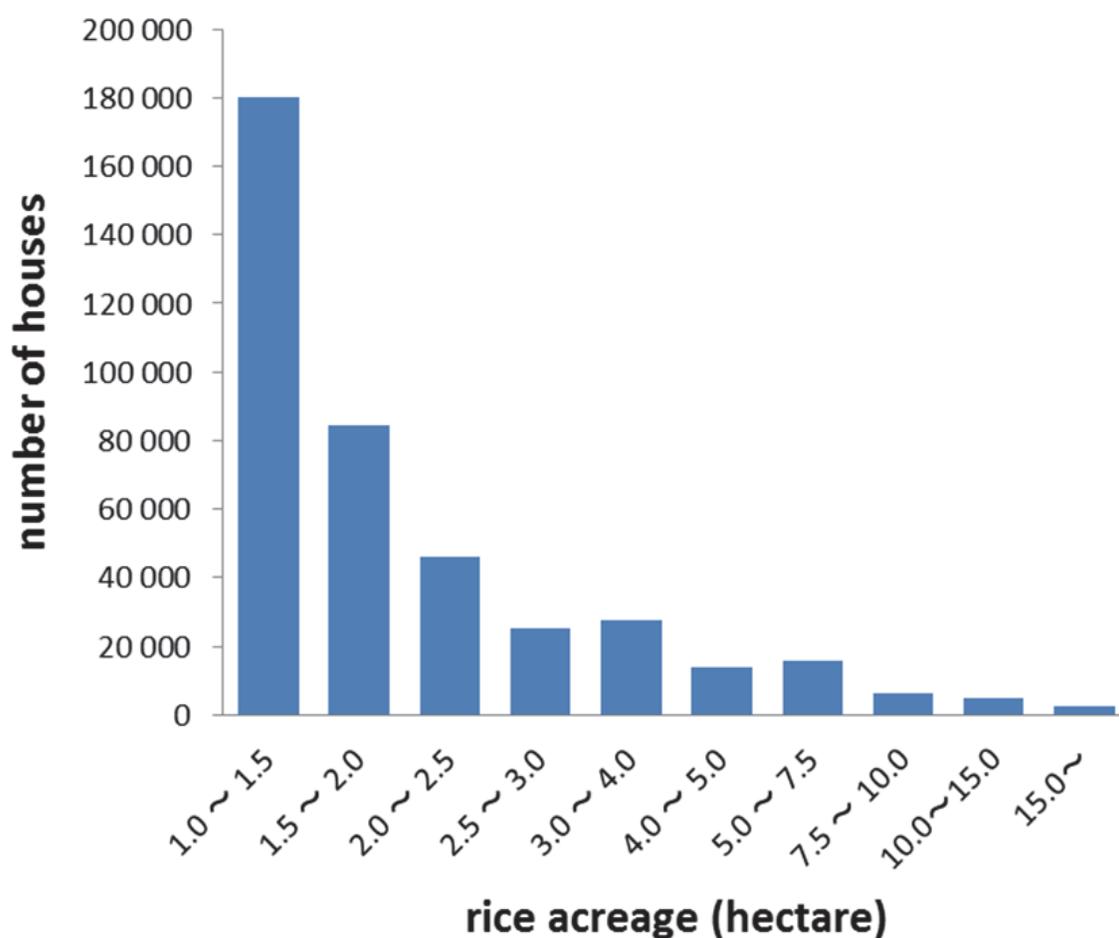


Fig. 2. Area under cultivation in Japan.

The mainstay of the Japanese agriculture scenario is the wet-rice farming. Therefore, the effect of these abundantly used herbicides in the paddy farming and on the aquatic environment cannot be ignored. The herbicides spread through the water, logged in the field, which later diffuse into the soil, thereby giving it a weeding effect. Therefore, Japanese paddy culturists maintain several days (preferably one week) of long static water environment after the application of herbicide in the field (Vu, 2006). Such conventional practice not only increases the weeding effect but also prevents the outflow of herbicides to a large extent. Despite such practices, traces of herbicides are detected in rivers and wetlands (Tanabe et al., 2001; Sudo et al., 2002; Nakano et al., 2004; Numaba et al., 2006; Miyashita et al., 2009; Tsuda et al., 2011). In order to reduce such environmental contamination, farmers may need to pay more attention regarding their use especially the amount of pesticide per square meter to be used.

As a statutory measure, Japanese government has established the application criteria and examination for different pesticides in order to reduce the residual concentration in the crops, soil and water and hence minimize the aquatic pollution (Nagayama, 2010). Few years back, in 2006, only 799 pesticides had been listed for their residual effect in food (Akiyama et al., 2005; Maitani, 2005, 2007; Saito, 2007; Hirahara et al., 2007). However, the criterion about remaining in environment did not exist. As for the pesticide registration

reservation criterion about prevention of toxic damage to the fisheries animals and plants, the standard value was established only in consideration of the acute toxicity of the carp about a pesticide used conventionally in a rice field. But, by criterion revision of March, 2003, the criterion is set in consideration of effect for a fish, a crustacean, algae and the forecast concentration in public waters and postpones the pesticide registration of the criterion that it is inadequate. However, a major problem concerning this registration is the test organism and negligence about environmental exposure levels. For example, till now, the degree of adversity is calculated based on several acute toxicity tests, like LC50 at 96h for fish, EC50 (effective concentration 50) at 48h for water flea swimming inhibition and EC50 at 72h for green algae growth inhibition (Wei et al., 2008; Kim et al., 2008). But few chronic tests are being conducted to address the environmental health in the long term (Sakai, 2002; Kang et al., 2009; Marques et al., 2011). Information about the usage of herbicides and the levels of herbicides in the environment must be monitored to calculate the possible adverse effect of these pesticides on our ecosystem.

Although herbicides have a relatively shorter half-life their application window is more important. Majority of aquatic insects, fish, and amphibians in Japan, spawns/hatch in early spring, the time when weedcides are sprayed in field. This makes the newborns most vulnerable and more prone to death. The bioassay using the aquatic species is effective in predicting the effect of herbicides on the ecosystem. Therefore, it is necessary to carry out the herbicidal short-term chronic toxicity examinations (or sub-chronic toxicity examinations) as well as acute toxicity tests using the aquatic species (Matthews, 2006). It is also important to perform the chronic toxicity evaluations on the plural endpoints like breeding and/or growth of the aquatic species. In this regard, since April 2005, the Ministry of the Environment has initiated research to re-evaluate the predicted concentration of pesticides in the public waters, along with their toxic concentration for fish, crustacean and algae, in order to prevent the adverse effects of pesticides on wildlife and ecosystem. However, information about chronic toxicity tests using fish, crustacean and alga, which might help in eco-toxicological assessment of different herbicides, is incredibly little in Japan. Therefore, we have carried out bioassays for chronic toxicity to check the ecological effect of pesticides and also investigated the agriculture drainage sample, collected from several Japanese environments (Ministry of the Environment, 2006, 2007, 2008, 2009, 2010). In this present investigation, we collected water samples directly from rice field drainage just after 7-14 days of herbicide application and performed chemical analysis by GC/MS (Gas chromatography-mass spectroscopy). We also carried out short term chronic toxicity tests i.e. Algal growth inhibition test (OECD TG201; OECD test guideline No.201, 2002), Daphnia reproduction test (Canada Ministry of the Environment: Test of Reproduction and Survival Using the Cladoceran, *Ceriodaphnia dubia*) (Environment Canada, 2007; EPA Biological Test Method, 2007), and Fish short-term toxicity test on embryo and sac-fry stages (OECD TG212, 1998) using an alga (*Pseudokirchneriella subcapitata*), a crustacean (*Ceriodaphnia dubia*), and fish (*Danio rerio*) respectively, for the 10 major kinds of herbicide formulation.

2. Research methodologies

2.1 Sampling

Present investigation was carried out in 14 sampling points of 9 prefectures of Japan from 2009 to 2010 (Ministry of the Environment, 2009, 2010). The field samplings were conducted

during May and June, generally after 1-2 weeks from herbicide application, from paddy field drainage system. Three water samples were collected in glass bottles, and then these samples were filtrated with a glass fiber of 0.7 μm and kept at 4°C till further analysis.

In order to keep correct information about dispersion period, respective owner/ farmer was interviewed. However, the effect from target outskirts farmhouse and the weather conjugation was not controlled. To gain some idea about the risk factors associated with uncertainties, water from one sampling station was sampled every week during one month from a day of the herbicide dispersal.

2.2 Chemicals

Dimethametryn (CAS: 22936-75-0), pretilachlor (CAS: 51218-49-6), bromobutide (CAS: 74712-19-9), carbetamide (CAS: 16118-49-3), bendiocarb (CAS: 22781-23-3), triazine (CAS: 101-05-3), cyanazine (CAS: 21725-46-2), simetryn (CAS: 1014-70-6), esprocarb (CAS: 85785-20-2), and mefenacet (CAS: 73250-68-7) were purchased from Wako Pure Chemicals Industries, Ltd (Osaka, Japan). Chemicals were dissolved at a concentration of 100ppm in the test medium for 48 h at room temperature. Chemicals with low solubility were dissolved in the test water at concentration of aqueous solubility (100% solution) for 48 h at room temperature, and filtrated with 1.2 μm glass fiber.

2.3 Analysis of chemical composition by GC-MS

The water samples were subjected to Sep-pacC18 matrix solid-phase dispersion clean up followed by simultaneous analysis of 917 different kinds of herbicides/pesticides using gas chromatography-mass spectrometry-choice ion monitoring system (GC-MS-SIM; Shimazu QP-2010, Kyoto, Japan). Seven substances (naphthalene-d8, acenaphthene-d10, phenanthrene-d10, chrysene-d12, perylene-d12, 4-chlorotoluene-d4, 1,4-dichlorobenzene-d4) were used as internal standard. In addition, liquid chromatograph mass spectrometry (LC/MS) was applied to analyse a pesticide which we failed to characterized by GC-MS (Kadokami et al., 2005).

The results were analyzed according to "GC/MS Database Software Ver.2 for Environmental Simultaneous Analysis" which was developed by Shimazu Corporation and Kitakyusyu Municipal University and was purchased from Shimazu Corporation (Tokyo, Japan).

2.4 Short-term chronic toxicity tests

2.4.1 Fish toxicity test

Fishtoxicity test was performed using zebrafish (*Danio rerio*) according to the OECD test guideline No. 212 (Fish short-term toxicity test on embryo and sac-fry stages). Zebrafish were obtained from National Institute for Environmental Studies, Japan. Eggs were collected within 4h of fertilization and distributed at 20 eggs/test jar (80 ml glass cup). Four replicates of each control and exposure group were incubated in 50 ml of filtered water samples and test medium respectively for 9 days. 100% media replacement was carried out every 2 days. The water samples were diluted (0-80%) using test water when required. The experiment was conducted at $26 \pm 1^\circ\text{C}$ (water temperature) and photoperiod of 16h : 8h (light : dark). The hatchability and survivability were recorded daily.

2.4.2 Daphnia toxicity test

Ceriodaphnia dubia has been acclimatized for several years in NIES, Japan to breed at hardness of 70 mg/l (similar to average hardness of Japanese water). We used water flea subacute toxicity test method "Test of Reproduction and Survival using the Cladoceran *C. dubia*" (Environment Canada, 2007). Changes (decreases) in the number of offspring per female in the definite period (7-8 days) upon exposure were used as an index of the effect. A fixed quantity of green unicellular alga (*Chlorella vulgaris*, Chlorella Industry Co., Ltd., Tokyo, Japan) and YCT (yeast, Cerophyll and trout chow) (Marinco Bioassay Laboratory, Inc., FL, USA) were fed every day. The cultures were performed at a water temperature of $26 \pm 1^\circ\text{C}$ under 16:8h of light and dark cycle. Homogenous populations of female offspring born within 24 h from mature individuals (age >7 days) were used for a test. *C. dubia* was exposed in semi-static condition for 7-8 days with three times water exchange per week. The filtered water was subjected to 2 fold serial dilution to form 6-7 different concentrations and 10 replicates/ concentration were tested for this study. The environmental water sample were examined without pH adjustment in the case of 6.5-8.5. Those falling out of range were adjusted with HCl or NaOH 1N. The lethality of the parent and the number of the neonates were counted every day and documented. When more than 60% of control population had laid their third batch of neonates, the test was terminated and added up number of offspring in litter size/ mother.

2.4.3 Green alga growth inhibition test

A stock (NIES -35) of Green unicellular alga *Pseudokirchneriella subcapitata* (former name *Selenastrum capricornutum* Printz) was used for the test. OECD-TG201 test that has been used in the Japanese "Act on the Evaluation of Chemical Substances and Regulation of Their Manufacture" was used to measure the algal growth inhibition. A test was conducted using a standard medium of the OECD. The filtered water samples were sterilized with 0.22 μm filter prior to incorporation in the medium. pH was also measured and adjusted to the range of 7.5-8.5 with NaOH or HCl.

The tests were conducted using following conditions: the initial cell density, 5×10^3 cells/ml; test temperature, 23 ± 2 degrees Celsius; the light intensity of the flask surface neighborhood, 60-120 $\mu\text{mol}/\text{m}^2/\text{s}$; pH, 7.8-8.0; and 72 h concussion culture (100 rpm). The test concentration sets 5 steps or more in dilution ratio 2 or less, because these concentrations are necessary to detect NOEC (No Observed Effect Concentration)/LOEC (Lowest Observed Effect Concentration). The cell numbers were estimated using a particle counter for all test containers at 24 h interval. In case of abnormal cell count, the cells were critically assessed under microscope.

2.4.4 Statistics

All the data were analysed by one-way ANOVA (Analysis of Variance) at 95% confidence limit. Dunnett's test was performed to calculate the significance between different groups. When data showed nonequivalent dispersibility, an analysis of variance was carried out if dispersibility was satisfied by log transformation. When equal dispersibility was not satisfied by the variable conversion, statistical test independent on distribution (e.g. Kruskal-Wallis or Dunn's test) was carried out. We also analyzed the statistical significance using the software JMP (Ver. 6.0.3, SAS Institute, Inc. North Carolina, USA).

3. The ecological effect of the pesticide

3.1 The bioassay using the environmental samples

Because the environmental water is already contaminated with a large number of herbicides, newly introduced pesticides may have little ecological effect; on the other hand, some may even become moderately to deleteriously toxic due to the factors such as the composition effect between pesticides and/or humic substances in the environment. Therefore, low detection level and relatively small ecological toxicity of herbicides does not always imply an ecologically undisturbed environment. Hence, further collection of data to understand the effects of herbicides on the environment.

For two years, from 2009 through 2010, chemical analysis and short-term chronic bioassays using an alga, a water flea, and a fish were performed with the agricultural drainage water collected from 14 different spots (A-N) of Japan.

The result of the chronic toxicity test in each species is shown in Table 1. The algal test showed evidence of effect in 9 spots. Of these, 3 spots, D, L, and N, showed TU (Toxicity Units) value as 10 or more. TU here is shown as 100/NOEC, and is directly proportional to ecological outcome. That is, if a value of TU is larger, it signifies that ecological effect is larger. The effect on water flea was observed in two spots, and only spot D had TU greater than 10; Spot D showed strong effects in both alga and water flea tests. The effect on hatching, survival rate of fish was checked as well, but no significant toxicity was found in any spots examined.

	NOEC (%)*			TU (=100/NOEC)		
	<i>P. subcapitata</i>	<i>C. dubia</i>	<i>D. rerio</i>	<i>P. subcapitata</i>	<i>C. dubia</i>	<i>D. rerio</i>
A	25	100	No data	4	-	No data
B	12.5	100		8	-	
C	50	100		2	-	
D	6.25	6.25		16	16	
E	25	100		4	-	
F	25	100		4	-	
G	25	100		4	-	
H	80	80	80	-	-	-
I	80	80	80	-	-	-
J	80	80	80	-	-	-
K	80	80	80	-	-	-
L	2.5	80	80	40	-	-
M	80	80	80	-	-	-
N	2.5	40	80	40	2.5	-
N (late 1 month)	80	80	80	-	-	-

* Sample water is considered to be 100 percent and shows the concentration of NOEC at percentage when it was diluted with unaffected water (e.g. culture water).

Table 1. Results of chronic toxicity test using the environmental sample.

3.2 Short-term chronic toxicity tests using candidate herbicides

Ten candidate herbicides, which were detected in the water samples, are typical herbicides used in Japan. Short-term chronic toxicity tests using alga, water flea and fish were carried out. As a note, it is important to collect data on each chemical substance accumulated in the environment before conducting mixed exposure of herbicides.

The results of the chronic toxicity tests in each species are shown in Table 2. In this study, the strongest effect was obtained in the alga test. Toxic effects on the alga were found in pretilachlor, cyanazine and simetryn at 1-10 mg/L.

The effects of herbicides on water flea were weaker than on alga, with the only exception of bendiocarb, which showed adverse effect on daphnid at 10 times lower concentrations compared to an alga. However, the effects of herbicides were generally found in order of alga > water flea > fish. In addition, bromobutide showed no adverse effects in all species tested.

	NOEC (mg/L)		
	<i>P. subcapitata</i>	<i>C. dubia</i>	<i>D. rerio</i>
Dimethametryn	0.005	0.63	2.5
Pretilachlor	0.001	1.3	1.5
Bromobutide	1	1.5	3
Carbetamide	0.063	2	10
Bendiocarb	0.078	0.006	12.5
Triazine	0.21	0.31	100<
Cyanazine	0.005	3.85	20.7
Simetryn	0.004	5.59	2.4
Esprocarb	0.028	0.27	0.37
Mefenacet	0.026	0.45	0.84

Table 2. Chronic toxicity tests of herbicides using the aquatic species.

3.3 Concentrations of herbicides in water samples in Japan

Simultaneous analysis of the GC-MS of the water samples from the bioassay was carried out. Ninety-two different herbicides were detected in water samples in this study, equivalent to approximately one-tenth of 917 kinds of analyzable pesticides in this analytical method. Actual concentrations of 10 typical herbicides are shown in Table 3a,b and Fig 3.

Bromobutide was detected at high concentration in each spot. Dimethametryn and pretilachlor were also detected from more than half of the samples. In addition, other herbicides were also detected at high concentration at spots D, G, K and N. At point N, water samples were collected again one month later, and were checked for the decrement of pesticides in the environment. Results showed a significant decrease in concentration of bromobutide, below detection limit value of other substances.

According to the resultant data, the herbicide including Bromobutide seemed to disappear immediately by disintegration or proliferation in the environment.

2009	MEC ($\mu\text{g/L}$)						
	A	B	C	D	E	F	G
Dimethametryn	0.65	0.71	0.64	3.33	0.11	0.17	0.17
Pretilachlor	0.40	0.56	0.13	1.66	5.98	-	3.23
Bromobutide	7.78	0.94	2.99	38.60	7.27	5.28	81.40
Carbetamide	0.85	-	-	1.35	-	-	3.56
Bendiocarb	0.16	-	0.26	5.68	-	-	-
Triazine	-	-	-	-	-	-	-
Cyanazine	-	-	-	-	-	-	-
Simetryn	-	-	-	-	-	-	-
Esprocarb	0.27	0.32	-	-	0.11	-	1.85
Mefenacet	0.92	-	1.89	-	1.32	0.21	0.65

-,not detected in the field.

(a)

2010	MEC ($\mu\text{g/L}$)							
	H	I	J	K	L	M	N	N (30d after)
Dimetha- metryn	0.05	0.04	0.13	0.03	-	-	0.03	-
Pretilachlor	-	-	-	-	-	-	-	-
Bromobutide	0.35	0.55	47.29	54.84	0.54	0.18	53.88	0.13
Carbetamide	-	-	0.83	1.66	-	-	1.64	-
Bendiocarb	-	-	-	-	-	-	-	-
Triazine	-	-	-	-	-	-	-	-
Cyanazine	-	-	-	-	-	-	-	-
Simetryn	0.27	0.17	-	-	-	-	-	-
Esprocarb	-	-	-	-	-	-	-	-
Mefenacet	-	-	-	-	-	-	0.03	-

-,not detected in the field.

(b)

Table 3. Actual concentrations of herbicides in the water samples by the simultaneous analysis (MEC; Measured Environmental Concentrations) in 2009 (a) and 2010 (b).

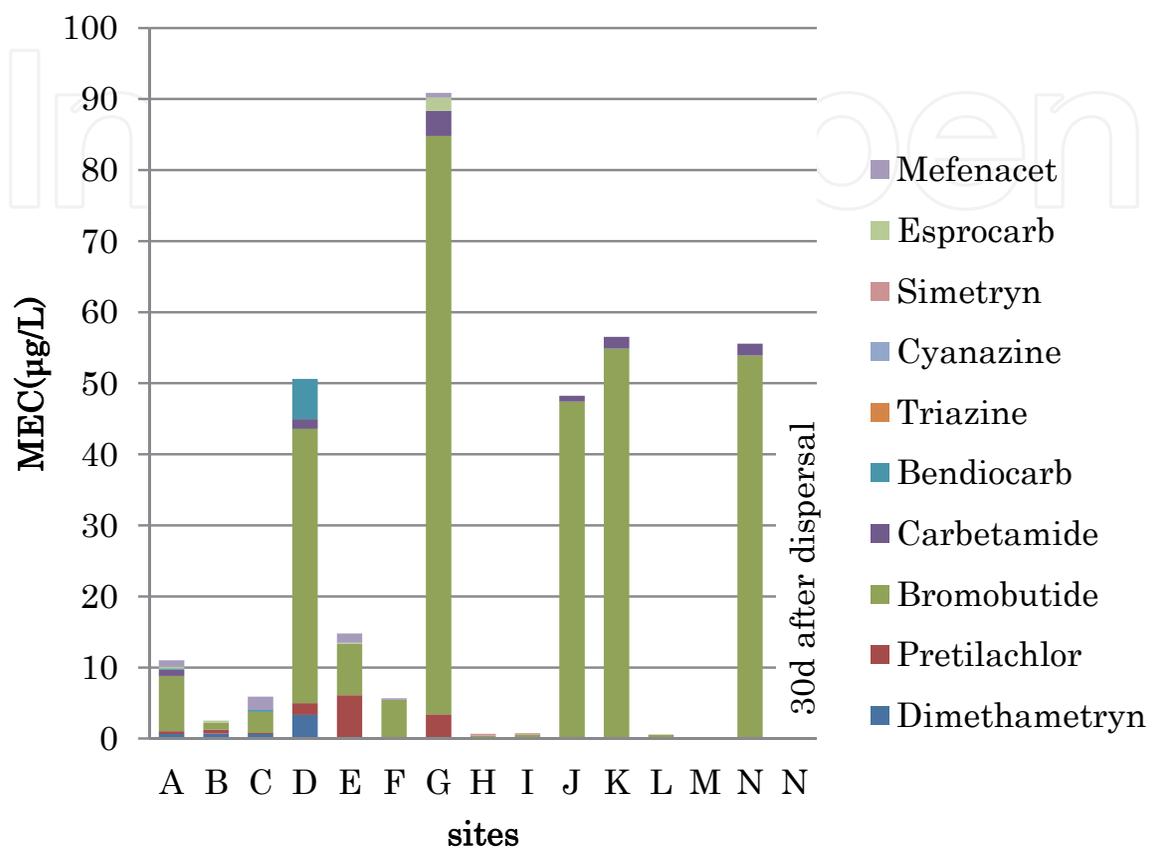


Fig. 3. Actual concentrations of herbicides in each sampling spot.

3.4 The risk evaluation of each spot based on the field measurements

Applying ecological toxicity data of 10 herbicides, a risk evaluation based on the MEC of each spot was performed. MEC/NOEC was calculated with the species that showed lowest NOEC for each target substance used in the present study (shown in Table 4 and Fig 4).

When the MEC/NOEC ratios of each herbicide are simply tallied, the total sums exceeded 1 in three spots (D, E and G). Yet from the results of Table 1, ecological effects at nine spots, A, B, C, D, E, F, G, L and N, are reported to be present. From the results of Table 4, at least at spots D, E, and G the ecological effect of the pesticide, which was measured in this study, is suspected to be present. Because Σ (MEC/NOEC) measured in this exposure was less than 1 in eleven spots of A, B, C, F, H, I, J, K, L, M and N, it is suggested that the observed effect may be attributed to a wholly different chemical substance, perhaps a herbicide that is unaccounted for in Table 4, or a non-pesticide chemical.

2009	MEC/NOEC						
	A	B	C	D	E	F	G
Dimetha-metryn	0.129	0.143	0.128	0.666	0.023	0.034	0.034
Pretilachlor	0.398	0.558	0.133	1.660	5.980	-	3.230
Bromobutide	0.008	0.001	0.003	0.039	0.007	0.005	0.081
Carbetamide	0.014	-	-	0.022	-	-	0.057
Bendiocarb	0.025	-	0.042	0.902	-	-	-
Triazine	-	-	-	-	-	-	-
Cyanazine	-	-	-	-	-	-	-
Simetryn	-	-	-	-	-	-	-
Esprocarb	0.010	0.013	-	-	0.004	-	0.066
Mefenacet	0.036	-	0.073	-	0.051	0.008	0.025
Σ (MEC/NOEC)	0.619	0.714	0.378	3.288	6.065	0.047	3.493

(a)

2010	MEC/NOEC							
	H	I	J	K	L	M	N	N (30d after)
Dimetha-metryn	0.01	0.008	0.026	0.006	-	-	0.006	-
Pretilachlor	-	-	-	-	-	-	-	-
Bromobutide	0.0004	0.0006	0.0473	0.0548	0.0005	0.0002	0.0539	0.0001
Carbetamide	-	-	0.013	0.027	-	-	0.026	-
Bendiocarb	-	-	-	-	-	-	-	-
Triazine	-	-	-	-	-	-	-	-
Cyanazine	-	-	-	-	-	-	-	-
Simetryn	0.075	0.047	-	-	-	-	-	-
Esprocarb	-	-	-	-	-	-	-	-
Mefenacet	-	-	-	-	-	-	0.001	-
Σ (MEC/NOEC)	0.085	0.056	0.087	0.087	0.001	0.0002	0.087	0.0001

(b)

Table 4. A risk evaluation based on the measurement value (MEC/NOEC) in 2009 (a) and 2010 (b).

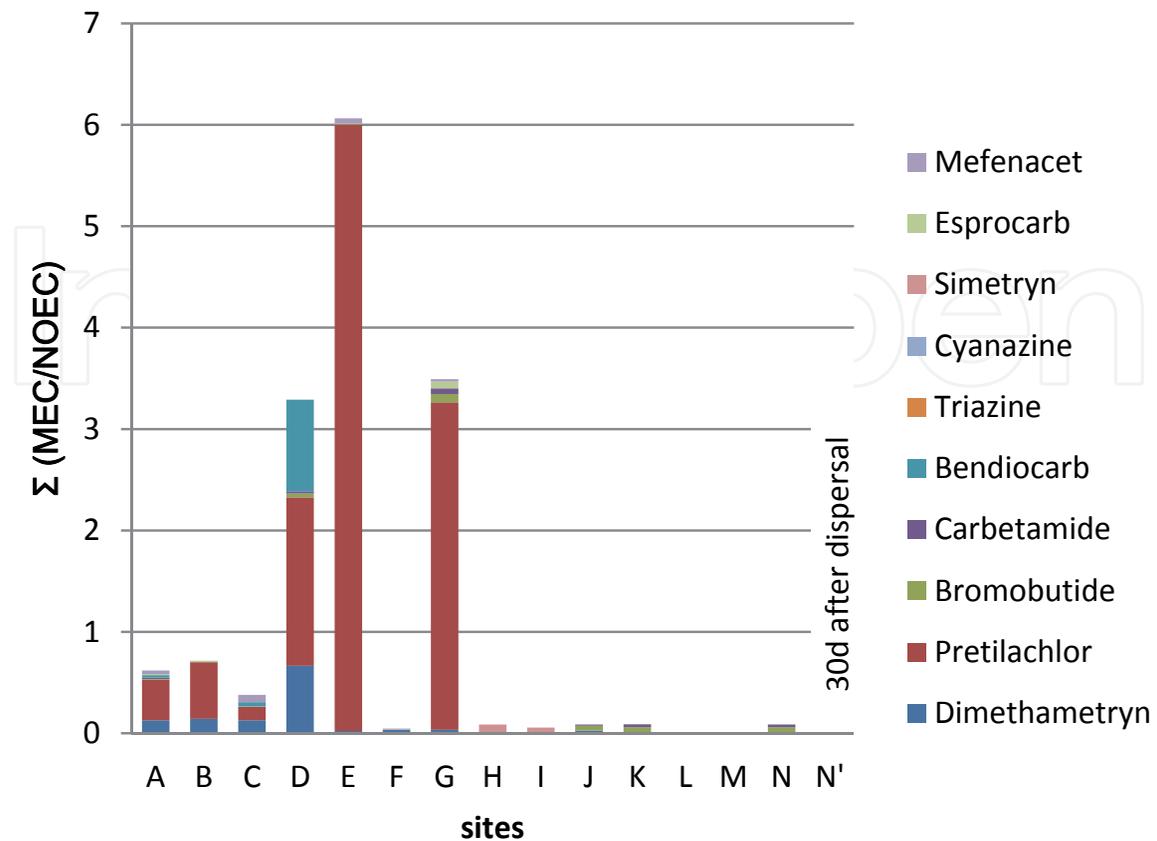


Fig. 4. Σ (MEC/ NOEC) of each sampling spot.

Three aquatic species were used for toxicity tests using dimethametryn, pretilachlor, cyanazine and simetryn, all of which showed growth inhibition of alga even at concentrations lower than 10 $\mu\text{g/L}$. The aquatic species most strongly affected by these herbicides was the alga in the present study. Separately, in bendiocarb the highest toxicity was encountered in the crustacean, decreasing the number of offspring at 12.5 $\mu\text{g/L}$ and was 10 times more sensitive compared to alga. *Daphnia* had the highest sensitivity to bendiocarb. In summary, 100-1000 times differences in toxicity of various herbicides were encountered. The fish were far less sensitive to toxicity of herbicides than alga, similarly or less sensitive than daphnid in this test. Though fish were shown to be less sensitive, the pesticide dispersion period in Japanese farm occurs during same time as spawning and/or hatching period in wildlife. Therefore, accumulation of toxicity data including fish is needed to perform a more detailed evaluation of ecological risk of herbicides. In addition, accumulation of chronic data of herbicides using the aquatic species is also needed to protect wildlife and the ecosystem.

4. A green alga and a blue-green alga

Relying solely upon green alga for risk evaluation and analysis of herbicide effect is not only insufficient for proper analysis, but may also lead to bias and error. For example, the effect of a chemical substance on germination and rooting cannot be evaluated because the green alga is a unicellular organism. Furthermore, different toxicity for various organism species

has been reported in some herbicides (Suárez-Serrano et al., 2010; Roubeix et al., 2011; Pereira et al., 2009). In other words, a herbicide may have a selective property; imposing no effect on growth of agricultural crops, yet able to effectively inhibit weeds growth e.g., the ineffective to rice and effective to wild millet. *Lemna* sp. Growth Inhibition Test (OECD TG221, 2006) can be used in addition to green alga toxicity test; however, herbicides toxicity data using duckweed are limited at present.

Blue-green alga (*Synechococcus leopoliensis*) has been used as a test species in addition to the green alga (*P. subcapitata*), and compared for herbicide toxicity. Because the blue-green alga is also a single cell organism, it can only serve as a biological reference to show species specific difference (Kaur et al., 2002; Vaishampayan et al., 1984; Lehmann-Kirk et al., 1979). Differences in toxicity effect between the green alga and blue-green alga using eight kinds of pesticides are shown in Figure 5.

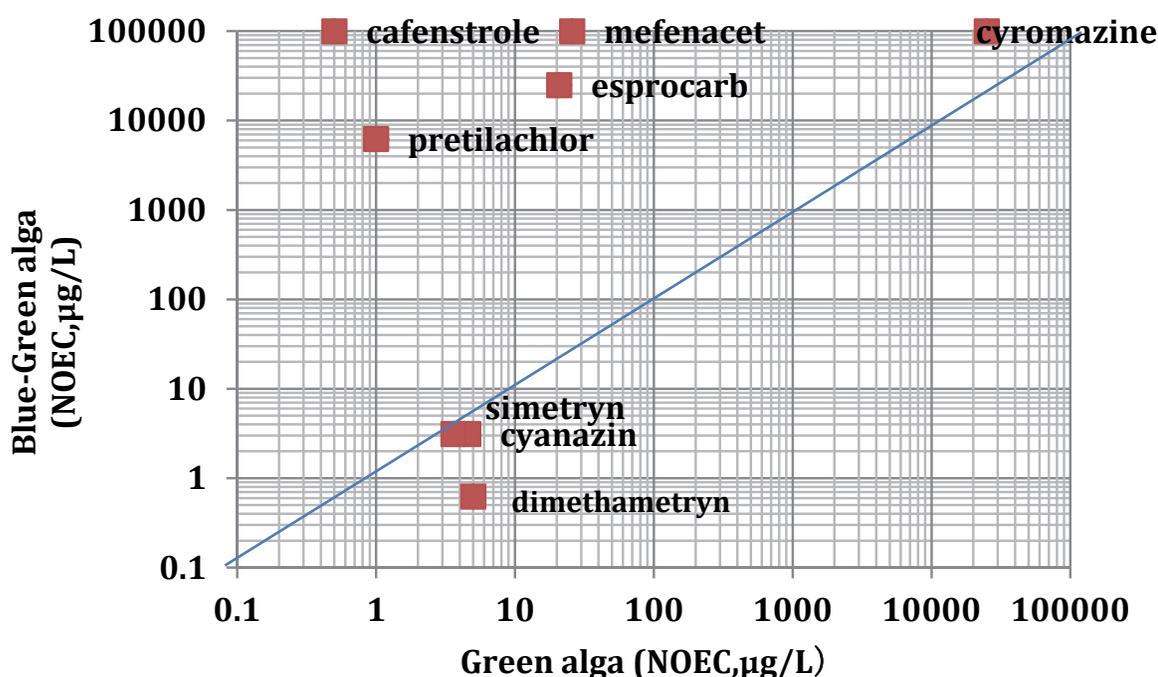


Fig. 5. Comparison of herbicide toxicity using green alga and blue-green alga.

Correlation of herbicide toxicity was hardly shown between green alga and blue-green alga (Fig. 3). However, the green alga and blue-green alga displayed approximately similar sensitivity to simetryn, cyanazin, and cyromazine. The green alga showed susceptible sensitivity in the toxicity other than dimethametryn. The green alga has been commonly used for ecological risk evaluation of chemicals including herbicides; however, it is also necessary to accumulate the test data using multicellular plants such as floating weeds in the future.

5. Conclusions

Fate of herbicides after their release into the environment is extremely difficult to grasp precisely. Regarding the adverse effects of herbicides on the environment (water, soil and

air contamination from leaching, runoff, and spray drift, as well as the detrimental effects on wildlife, fish, plants, and other non-target organisms), the well being of resulting environmental state depends on the toxicity of the herbicides themselves (Monaco et al., 2002; Eleftherihorinos, 2008). Detailed information will be needed concerning measurements of exposure levels of herbicides during their application, the dosage applied, the adsorption on soil colloids, the weather conditions prevailing after application, and pesticide persistence in the environment.

As for the risk assessment of the impact of herbicides on the environment, a simple and precise process does not exist (Commission of the European Communities, 1991; EPA, 2009; FAO, 2002; Abrantes et al., 2009). Various examples point to multivariate ecological effect based on various environments, and the ecological risk changes on a case-by-case basis. Hence, we need to instead depend upon data gained through exposure periods and exposure levels, toxicity and the durability of applied herbicides, as well as taking in account the local environmental characteristics for proper risk evaluation of herbicides.

It has been recognized, however, that an impact on the environment of herbicides included in the agriculture drainage could be estimated to some extent by performing short-term chronic toxicity tests (Cantelli-Forti et al., 1993). The ecological toxicity tests may detect the effect of not only herbicides but also the chemical substances used for daily life and sewage effluents. For consideration of environmental risk of chemicals in general, synergistic effects with herbicides and other substances should be detected. The monitoring of the environmental water using the aquatic species will become an important index for the chemical safety and control of environmental chemicals including herbicides.

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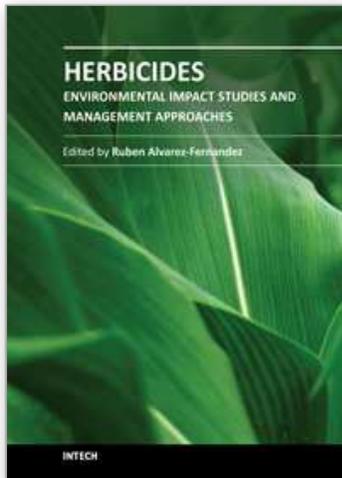
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Weeds severely affect crop quality and yield. Therefore, successful farming relies on their control by coordinated management approaches. Among these, chemical herbicides are of key importance. Their development and commercialization began in the 1940's and they allowed for a qualitative increase in crop yield and quality when it was most needed. This book blends review chapters with scientific studies, creating an overview of some the current trends in the field of herbicides. Included are environmental studies on their toxicity and impact on natural populations, methods to reduce herbicide inputs and therefore overall non-target toxicity, and the use of bioherbicides as natural alternatives.

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