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Ecological risk of pesticides in river water as determined by secular changes in species sensitivity distribution

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ABSTRACT

Pesticides applied during the rice transplanting period in paddy fields can flow out from those fields and into water environments, where they can affect ecological systems. Previous studies found the concentrations and risks of butachlor and pretilachlor to be especially high at each site investigated in 2012 and 2013 at the same points as those of this study. The present study investigates the temporal changes in concentrations and risks for these pesticides from 2015 to 2017. In addition, the ecological risk that these pesticides pose, called the "potentially affected fraction (PAF)", was assessed by species sensitivity distribution (SSD). The concentrations of the herbicides were just below the registration standards. Moreover, the changes in concentration of the herbicides over the course of the 3 y showed a significant decreasing tendency. The maximum value of the PAF was 5%, and no samples exceeded the 5% hazardous concentration. The temporal changes in PAF for the herbicides also showed a decreasing tendency during the survey period. The trend is attributed to increased compliance with pesticide usage restrictions stated in a government instruction. These results show that SSD is a useful method for visualizing risks clearly.

Keywords: Sampling; Pesticide Monitoring; River Water; Species Sensitivity Distribution; Toxicology

1. Introduction

Pesticides are essential chemicals for the stable production of crops with minimum labor. To ensure food and environmental safety, pesticides are strictly regulated at every step, from production to import, sales and use, in accordance with the laws in Japan. Even so, it is necessary to monitor the concentrations of pesticides in aquatic environments such as rivers because some pesticides wash into the aquatic environment, where they have the potential to affect aquatic ecosystems [1–5]. Pesticide concentrations in the aquatic environment fluctuate greatly; thus, it is essential to collect water quality monitoring data frequently in order to accurately assess the risks posed by pesticides that effuse or leach from agricultural areas. Past studies [2,3,6,7] have shown that pesticides are used intensively in paddy fields in Japan. Several studies [2,7] have reported that paddy herbicide and pesticide concentrations in rivers peak within two weeks after the start of the transplanting season. Since 2005, the upper limits of pesticide concentrations called "standards" in aquatic environments have been determined by risk assessments of flora and fauna according to the Agricultural Chemicals Regulation Act of Japan. Under such risk assessment, acute toxicity tests are conducted for specific species of fish, Daphnia and algae. The standard values are determined based on tests for specific items such as the

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minimum value of LC50 (lethal concentration 50) or EC50 (effective concentration 50) divided by an uncertainty factor to determine the sensitivity differences between species. However, there are not just one species inhabiting aquatic ecosystems; there are many species. Thus, risk assessment based only on standard values may not be sufficient to save entire ecosystems. Many studies have compared values measured in the environment to values given in standards in order to determine whether the measured values exceed the standards. [8,9] However, these studies have not sufficiently assessed how pesticide concentrations affect ecosystems. Nagai et al. [3] recently established a risk assessment indicator called species sensitivity distribution (SSD), which considers the overall ecosystem [10–12]. Studies have been conducted to compare the risk assessed by SSD with reference values for the risk posed by the major rice herbicides used in Japan [1,12]. This method is regarded as a very practical way of quantifying the risk. These studies have suggested that SSD can track not only temporal changes in pesticide concentrations but also temporal changes in risk posed by the pesticides. Little is known, however, about the temporal changes in risk as determined by SSD using monitoring data gathered frequently over several years.

In the present study, the temporal changes in risk from butachlor and pretilachlor as calculated by SSD for 3 y in Osaka Prefecture, Japan was investigated. Butachlor and pretilachlor were determined as target pesticides that showed relatively high concentrations in a previous study [7].

2. Material and methods

2.1. Water sampling

The Yamato River is a first-class river in Osaka Prefecture, Japan. The Ishi River and its tributaries, the Sabi River and the Asuka River in southeastern Osaka Prefecture, Japan, are part of the Yamato River system. They were selected for this study (Fig. 1). Site 1, site 2, site 3 and site 4 are at Ishikawa Bridge on the Ishi River, Enmei Bridge on the Asuka River, Otomo Bridge on the Sabi River, and Takahashi Bridge on the Ishi River, respectively. These sampling sites are close to previous research sites related to pesticide monitoring [7]. The river's watershed is predominantly an agricultural area of paddy fields [7]. The investigation was conducted from April to July in 2015, 2016 and 2017. In general, the concentrations of pesticides in river environments tend to be high from May to June. This is because May to June is the transplanting season and the period when pesticides are sprayed. The paddy fields in the basins of the Ishi River, the Sabi River and the Asuka River were transplanted during the period. Thus, samples were collected 1–3 times per week from April to July. Some samplings were canceled due to heavy rain.

At each site, 1 L water samples were collected in pre-cleaned amber glass bottles. Water temperature, pH, electrical conductivity, dissolved oxygen, turbidity and flow velocity were measured onsite.

2.2. Analytical procedure

The analytical procedure for obtaining pesticide samples from the sampled water was the same as in a previous study [7]. The sampled water was filtered through 0.7 μm glass fiber filters (GF/F, Whatman, UK). Five hundred milliliters of the filtrate was passed through an Oasis HLB Plus cartridge (225 mg/cartridge, Waters, USA) at a flow rate of 10 mL/min after the cartridge had been conditioned with 10 mL of methanol and 10 mL of ultra-pure water. The cartridge was then centrifuged for 10 min at 3,000 rpm. The components retained in the cartridge were eluted with 15 mL of methanol at a flow rate of 1 mL/min. The eluted components were then evaporated to approximately 0.5 mL with a rotary evaporator (Buchi, Switzerland) and dried under

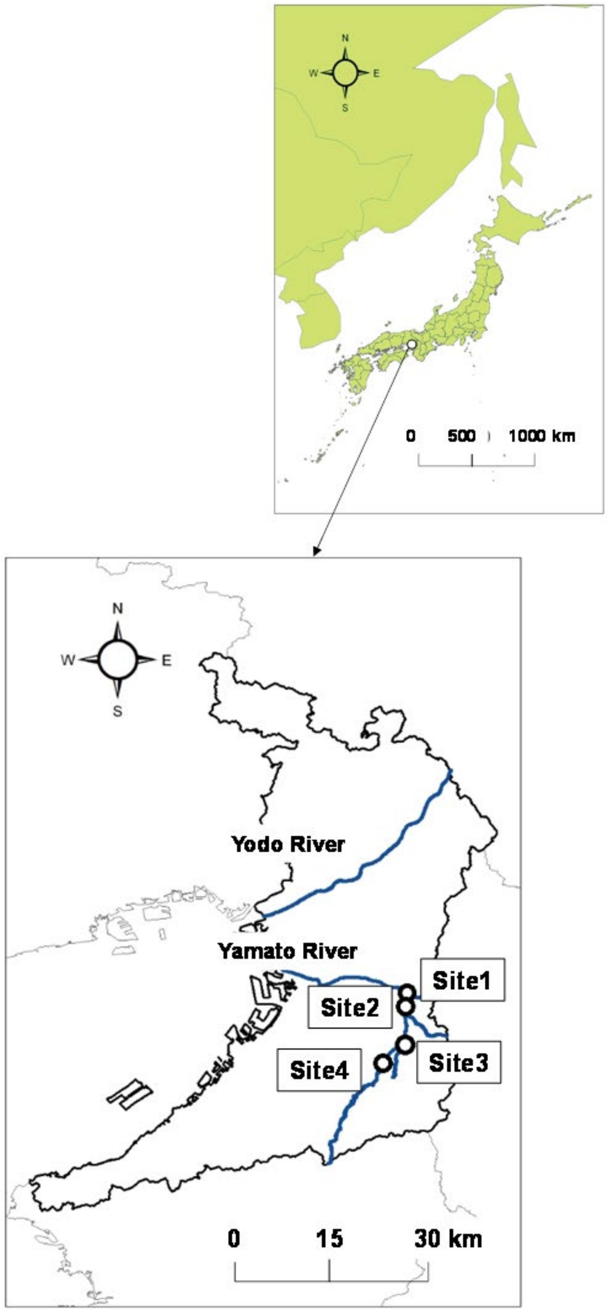

Fig. 1. Map of the sampling site. The blue lines show rivers.

a nitrogen stream. The residue was then dissolved in 1 mL of 0.1% PEG acetone for gas chromatography with tandem mass spectrometric detection (GC-MS/MS) analysis. Table 1 shows the GC-MS/MS conditions. The standards and solvents were purchased from Wako Pure Chemical Industries (Osaka, Japan).

2.3. SSD and determination of targets

Various organisms inhabit aquatic ecosystems such as rivers, and the toxicity of pesticides differs depending on the target organism. However, it is impossible, in practice, to conduct toxicity tests on all of them. The sensitivity of many species to chemicals is empirically known to fit a log-normal distribution and can be expressed as a cumulative probability distribution. The SSD statistically expresses such sensitivity differences among species [12]. To calculate the SSD, at least five toxicity data are needed to create a minimum data set. Nagai et al. [3] suggested *Daphnia magna*, *Gammaridea* species, *Paratya improvisa*, *Chironomidae* species, and *Cheumatopshyche brevilineata* as a standard data set for calculating SSD for insecticides. In addition, at least five kinds of primary producers, such as algae, and a water plant data set for the SSD of herbicides are needed. The database provided by the National Institute for Agro-Environmental Sciences includes 476, 984, 592, and 169 records for primary producers, aquatic arthropods, vertebrates, and others, respectively [12]. This database was used in the present study. Using the SSD, it will be possible to predict the potentially affected fraction (PAF) from the concentration of pesticides in the environment with certain toxicity data. The SSD curve shows that the higher the pesticide concentration, the higher the proportion of affected species. The proportion is classified into the four categories of non-detection (<0.1%), low risk (0.1–5%), medium risk (5%–50%), and high risk (>50%).

In Europe and the United States, the concentration corresponding to the 5th percentile value of SSD (the concentration at which 5% of species are affected) is expressed as HC5 (5% hazardous concentration), which is considered the upper limit of the non-effect concentration. This is based on the assumption that if 95% or more species could be protected, there would be no significant impact on species diversity. The advantages of using SSD to assess the risk to ecosystems are (1) quantitative risk assessment, (2) the targeting of a wide range of organisms, and (3) ease of use for risk prediction. In this study, based on the results of a previous study [12], the single risk for each pesticide and the combined risk calculated by combining two target pesticides were assessed. In the calculation, a single risk is expressed as PAF and combined risk is expressed as msPAF (multi-substance PAF).

Among the pesticides detected in a previous study [7] at the same sampling point, the concentration of bromobutide was the highest, followed by the concentrations of butachlor and then pretilachlor. In contrast, the risks of butachlor and pretilachlor calculated by SSD were higher than those of other pesticides, including bromobutide. Therefore, to attempt to assess the ecological risk of herbicides used in paddy fields, butachlor and pretilachlor were chosen as the target pesticides. These are paddy herbicides used at the early stage of rice planting.

3. Results and discussion

Figs. 2–5 show the temporal changes in the concentration and the PAF of butachlor and pretilachlor over the 3 y of study. At site 3, the highest concentrations of butachlor in 2015, 2016 and 2017 were 2.8, 0.70, and 0.37 μg/L, respectively, and those of pretilachlor in 2015 and 2017 were 2.2 and 0.37 μg/L, respectively. In 2016, the highest concentration of pretilachlor was 0.33 μg/L at site 2.

The pesticide concentrations were observed to peak from early to mid-June at all sites (Figs. 2–5). This period was about one or two weeks after the transplanting period (late May to early June) for paddy rice in the study area. This tendency coincided with a previous report [7]. In 2015, 2016 and 2017, the maximum concentration was 2.8 μg/L for butachlor and 2.2 μg/L for pretilachlor. The concentrations of herbicides in river water were lower than the registration standards for agricultural chemicals established by the Ministry of Environment, Japan, which are 3.1 and 2.9 μg/L for butachlor and pretilachlor, respectively. In addition, the peak times for the 3 y were constant: the beginning of June. This implies that the factor controlling the highest peaks was not meteorological, but was an agrochemical input to the paddy fields. In fact, the peak times do not correlate with meteorological factors such as precipitation, river flow rate or air temperature.

Table 1
Operating conditions of GC-MS/MS

GC		7890A (Agilent, USA)
	Injection mode	Splitless
	Injection volume	1 μL
	Injection temperature	250°C
	Column	DB-5ms (30 m, 0.25 mm, 0.25 μm)
	Column flow rate	1.0 mL/min
	Column oven temperature	50°C (1 min) – 25°C/min–10°C/min – 300°C (10 min)
MS/MS		7000B (Agilent, USA)
	MS method	MRM
	Detector temperature	280°C

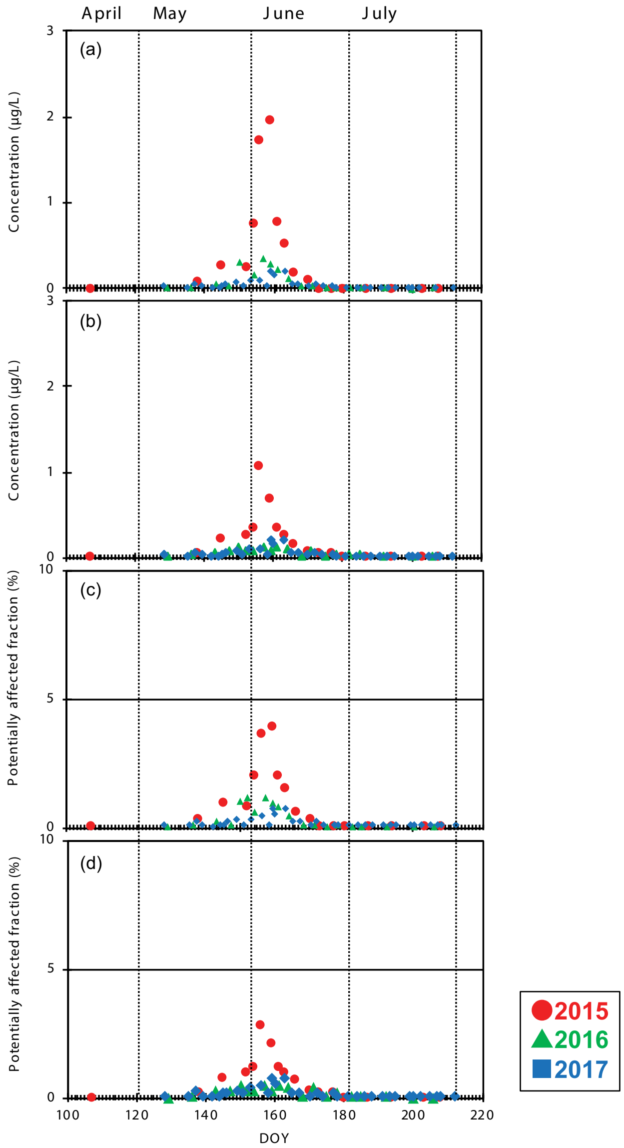

Fig. 2. Temporal changes in (a) butachlor, (b) pretilachlor, (c) PAF of butachlor, and (d) PAF of pretilachlor at site 1, DOY: day of the year.

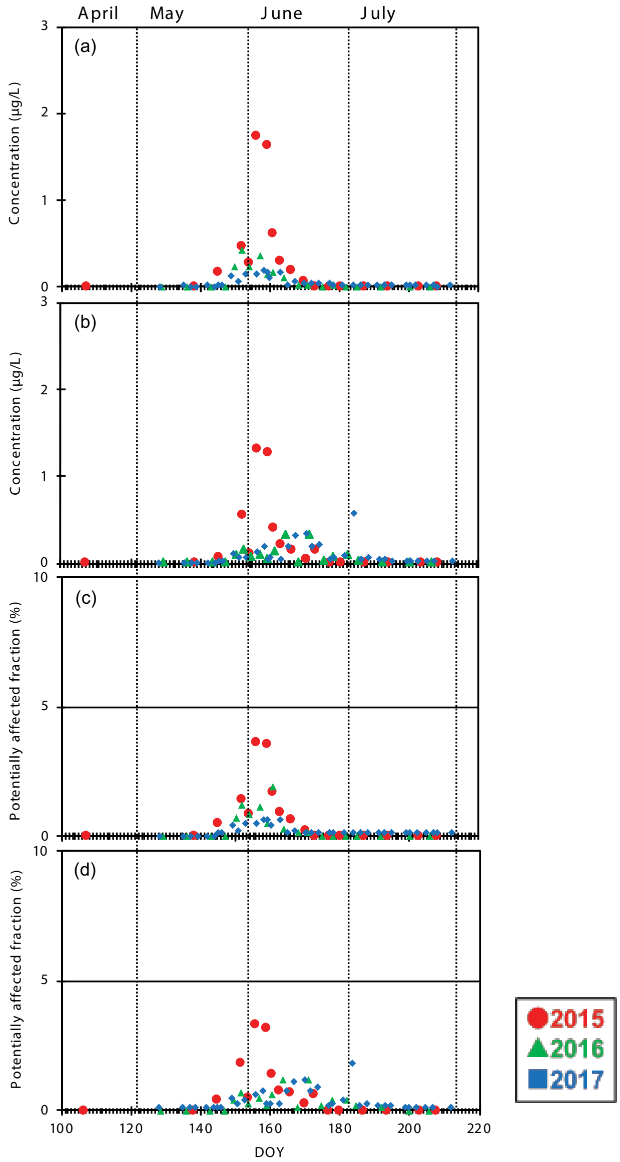

Fig. 3. Temporal changes in (a) butachlor, (b) pretilachlor, (c) PAF of butachlor, and (d) PAF of pretilachlor at site 2, DOY: day of the year.

The concentration peaks of the two pesticides consistently decreased over the 3 y at all sites. To address the decreasing tendency, the pesticide shipment volumes were investigated. Those of butachlor in 2015, 2016 and 2017 were 33.95, 39.95, and 32.75 t, respectively (Pesticides Handbook 2016, 2017 and 2018) and those of pretilachlor in 2015, 2016, and 2017 were 46.05, 43.95, and 40.20 t, respectively [13–15]. The trend of the shipment volume for pretilachlor showed a slight reduction; thus, there could be a decreasing trend for pretilachlor concentration. In contrast, the shipment volume for butachlor was nearly constant. Therefore, the decreased concentration of butachlor in the rivers should be attributable to factors other than shipment volume. Nevertheless, the temporal changes in concentration do not correlate with the precipitation amount, flow rate, flow volume, or turbidity (data not shown). The results indicate that the significant peaks of the pesticide concentrations were unaffected by dilution from river water or by soil runoff, which can adsorb pesticides. The Japanese government has been promoting the proper use of pesticides. They have continued their 2012 instruction that water containing pesticides should be kept from overflowing rice paddies by being retained in the fields for at least 7 d after the day of pesticide application. For example, it has been observed that an outlet connecting a paddy field to a gutter leading to a river was blocked by boards and sandbags to keep the water in the paddy field (Fig. 6). This was an effort by farmers to follow the instruction of the government. In general, it has been taking a long time to spread this instruction to farmers. Thus, the decreasing trend of the pesticide concentration might be due to improvements in paddy field water management.

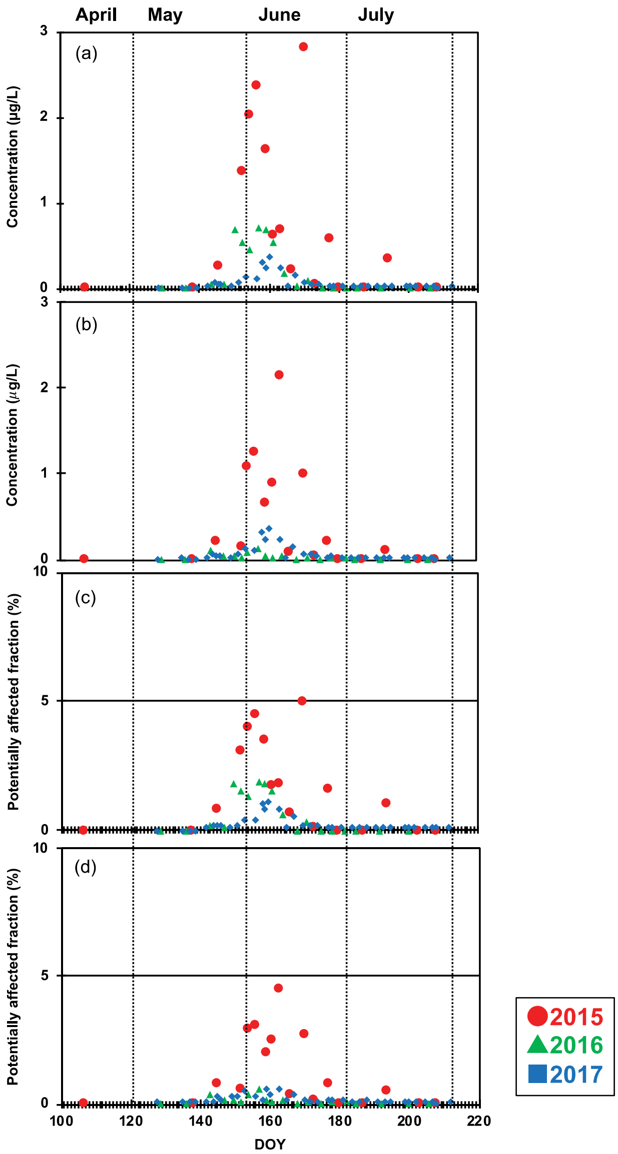

Fig. 4. Temporal changes in (a) butachlor, (b) pretilachlor, (c) PAF of butachlor, and (d) PAF of pretilachlor at site 3, DOY: day of the year.

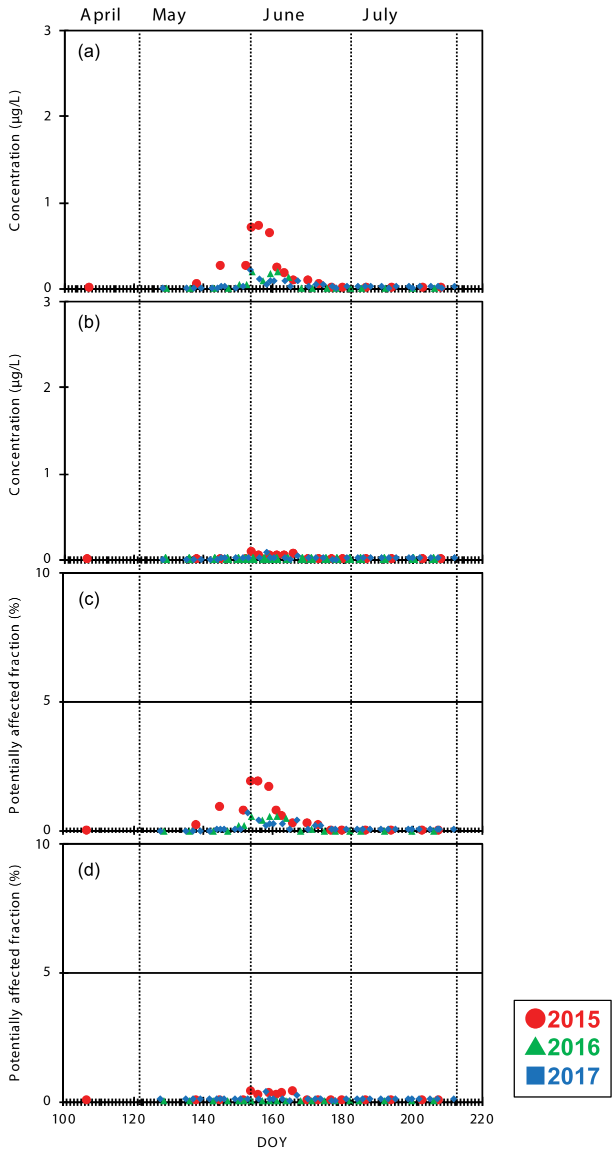

Fig. 5. Temporal changes in (a) butachlor, (b) pretilachlor, (c) PAF of butachlor, and (d) PAF of pretilachlor at site 4, DOY: day of the year.

The pesticide concentrations were higher at site 3 than at the other sites. According to maps of site 1, site 2, site 3 and site 4 shown in a previous study [7], there are vast agricultural areas on both sides of the Sabi River, including at site 3. For land in the basin upstream of site 3, 35% falls into the "farmland" land use category, and most of this is paddy field. In contrast, "paddy field" accounts for a much lower percentage of land use in the river basin of site 2, even though the percentage of land categorized as "farmland" is higher (41%). The shares of farmland in the river basins of sites 1 and 2 are 15% and 6%, respectively. Thus, the variation in river basin conditions could be a reason for the differences in pesticide concentrations among the three rivers.

The PAF of the pesticides showed the same decreasing tendency for that 3 y. The maximum value of PAF was 5.0%, which is close to HC5, at site 3 on 19 June 2015. The high concentrations and high PAF values of butachlor may be attributable to the incomplete blockage of the flow of pesticide-containing water from paddy fields into rivers. Another potential reason for the high concentration could be that farmers with paddy fields in the watershed would spray butachlor at about the same time. The spraying of butachlor onto the paddy fields at the same time could produce such a high-concentration event. If the paddy fields retained large amounts of water from heavy rainfall, it could make it easy for water to leak from the paddy fields. However, heavy rainfall was not observed before the high-concentration day. Thus, heavy rainfall was rejected as a candidate for the high concentration. As mentioned above, the shipment volume of butachlor for the 3 y was constant, and the peak time did not correlate with meteorological factors. Therefore, if the farmers had not followed

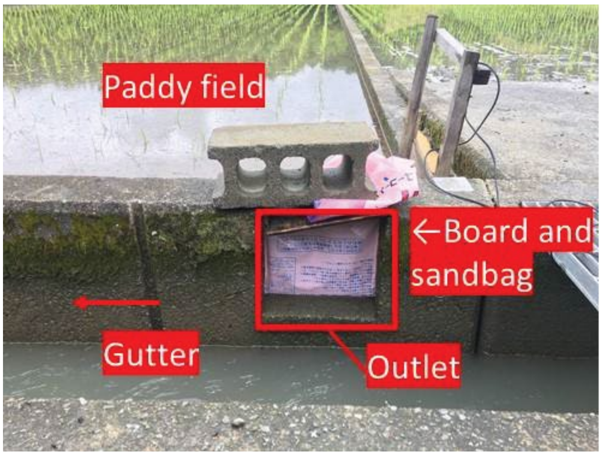

Fig. 6. The blocking of water from a paddy filed to an outlet.

the instructions of the government, there would have been the risk of exceeding the standard limit for the aquatic environment from legal pesticide use. No sample exceeded HC5. In addition, the msPAF calculated for butachlor and pretilachlor concentrations did not exceed HC5 on any date, nor at any site. Although both pretilachlor and butachlor are herbicides that inhibit the formation of very long-chain fatty acids in weeds, butachlor is becoming a substitute for pretilachlor due to its less hazardous properties. The trend of reduction in the concentration of pretilachlor with an increase in the concentration of butachlor in the rivers could be attributed to this. However, since the PAF of butachlor is lower than that of pretilachlor, the risk to the aquatic environment could be lower. The SSD method can numerically visualize the risks and precisely assess the effects of pesticides on the aquatic environment, rather than merely monitoring their concentrations. Therefore, to assess the risk posed to aquatic organisms, it is essential to monitor pesticide concentrations and to use the SSD method to evaluate the risk posed by those chemicals. There is, however, a limit to the number of chemicals on the list of pesticides that can be evaluated by the SSD method. To examine the risk to the aquatic environment for more pesticides, a complete SSD list is required. To assess the risk to the overall ecology from paddy rice agriculture, the SSD should be calculated for herbicides and insecticides.

4. Conclusions

The temporal changes of butachlor and pretilachlor concentrations at four sites on rivers in Osaka Prefecture, Japan were investigated. The actual risks posed by the pesticides were determined by SSD calculation. This study has made several contributions to the existing literature. First, a significant tendency for pesticide concentrations in Osaka Prefecture to have decreased from 2015 to 2017 was observed. Second, we investigated whether the main reason for this decreasing tendency could be greater compliance with regulations to prevent pesticide runoff from paddy fields. Overall, the ability of SSD to monitor the actual risk of pesticides was revealed. It was confirmed that appropriate pesticide usage has worked as an efficient way of protecting the environment.

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