Concentration fluctuations of pesticide residues, including four neonicotinoids, and related metabolites in a small river located near rice paddy fields

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Abstract: - It is important to investigate contamination levels of neonicotinoide insecticides and the other pesticides that may cause adverse effects, not only to human health but also to the environmental ecosystems near rivers because of the large amounts of pesticides used for the paddy rice fields. Therefore, the runoff movements of commonly used 31 pesticides (12 fungicides, 4 neonicotinoid insecticides, 8 insecticides, and 7 related metabolites) in a small river basin, which branches from a major river, located near paddy rice fields in Japan were presented in this study. The concentration of the pesticide residues found in the present study fluctuated depending on the applied periods and amounts as well as the flow of the river water. No pesticides exceeded the standard levels in environmental and drinking water set by the government of Japan. The maximum loadings of total pesticides in the river were obtained in July for fungicides, August for neonicotinoids and May for the other insecticides. The monthly loading of total pesticides exhibited unique variations. The runoff ratios of neonicotinoids from paddy fields to the river ranged from 078% of imidacloprid to 10% for dinotefuran. Results obtained in this study are important for the evaluation of the actual behavior of the insecticides and fungicides in small rivers.

Key-Words: - Pesticide residue, neonicotinoid, metabolite, river water, rice paddy field

1 Introduction

Many different pesticides have been produced and sold in the world, and then sprayed over not only commodity fields but also forests and golf fields as well as private house yards. The European Commission lists about 500 approved pesticides as well as about 900 not-approved ones [1]. Consequently, those pesticide residues contaminate foods and environmental ecosystems. Therefore, many government organizations associated with pesticides have begun to regulate their usage.

Among currently used insecticides, neonicotinoids, have recently begun to receive much attention as a major insecticide group, having adverse effects on to certain living organisms, such as honeybees [2]. Neonicotinoid insecticides are registered in over 120 countries in the world [3] and their distribution comprises 25% of whole insecticides in the world. [2] For example, the European Union (EU) has regulated the use of clothianidin (Clo), imidacloprid (limi), and thiamethoxam (Thi) as well as phenylpyrazole insecticide fipronil (Fip) because they have damaged honeybees [4].

Rice is one of the major grains cultivated in the world, particularly in Asian countries. It mainly cultivated in a paddy field. In Japan, paddy fields cover a total area of 2.38 million ha, accounting for 54.5 % of all cultivated area in FY2020. Over 400 kinds of pesticides are used for agricultural fields, including rice paddies, in Japan [5]. Large amounts of pesticides have been used for the paddy rice fields, and consequently those pesticides drain into rivers with paddy water discharges. Therefore, their contamination of the environment and ecosystem has been matters of strong concern [6]. River waters are utilized for tap-water by many countries in the world. Despite the fact that the river waters are treated intensively by cleaning plants before they are supplied as tap-water, trace or undetectable amounts of some pesticides may remain in the tap-waters. Therefore, it is very important to investigate the residue amounts of pesticides in the river waters. In particular, small rivers with cultivated areas in their basins are directly affected by the applied pesticides in the areas. These small rivers could hence be used to evaluate the pesticide runoff events from the cultivated areas [7].

One of the neonicotinoid insecticides, dinotefuran (Din) has been used heavily to exterminate stink bugs in paddy rice fields in Japan. Consequently, one report demonstrated that Din, particular, had an adverse effect on honeybees [8]. Therefore, neonicotinoid insecticide contamination in environments and ecosystems have begun to receive much attention for their possible adverse effects not only on humans but also other living organisms.

We previously reported the concentration fluctuations of some pesticides in a class A-river Shinano River [9] and related small rivers in Japan [7]. The croplands, in particular paddy rice fields, located near small river basins are readily to be contaminated by pesticides [10-14]. However, there are virtually no reports on concentration fluctuations of neonicotinoid insecticides and their relation to the volume of flowing water in small rivers braching off of class-A rivers. In the present study, the concentration fluctuations of commonly used fungicides and insecticides including neonicotinoid insecticides as well as their loadings and runoff ratios in a small river (Noshiro River) in Niigata Prefecture, Japan were investigated.

2 Materials and Method

2.1 Chemicals and Reagents

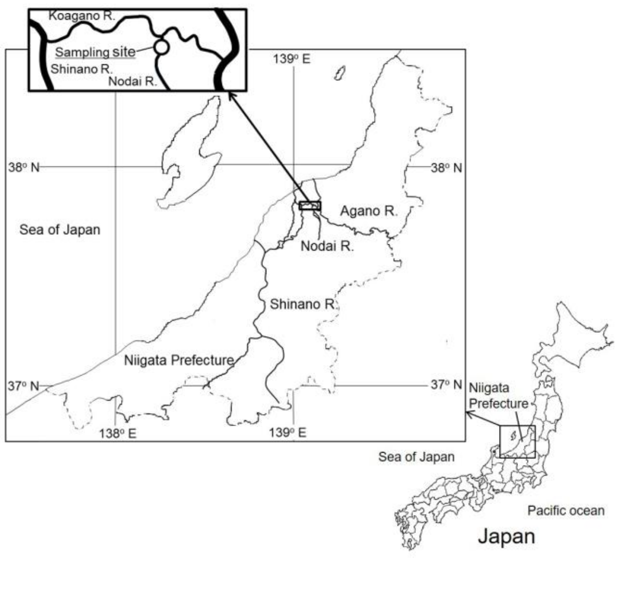

Fig. 1: The locations where residues of pesticides and metabolites were collected

Standard pesticides and their metabolites were bought from Wako Pure Chemical Industries (Osaka, Japan) and Kanto Chemicals (Tokyo, Japan). A mixture of their standard solutions was prepared in methanol (1 ng/mL). HPLC internal standard was prepared by dissolving diuron-d_6, from Dr. Fhrenstorfer GmbHinternal (Augsburg, Germany), in methanol (100 mg/mL).

2.2 Sample Collections

Figure 1 shows the sample collection location. It is at the Ohshima Bridge, which is 150 m upstream from the confluence of Nodai River and Koagano River (37º, 49', 55" North latitude; 139º, 7', 26" East longitude). Water samples were collected once on February 28, 2011 and every two weeks from April 4, 2011 to September 26, 2011. The eleven samples were collected and stored at 4 ºC in the dark and analyzed as soon as possible.

2.3 Sample Preparations

Samples were measured by a previously reported method [6,15,16]. Briefly, a sample (500 mL) was filtered using a glass fiber filter (Whatman plc., Kent, UK). After the pH of the filtrate was adjusted to 3 with formic acid, the sample was eluted through a cartridge packed with Oasis HLB Plus (Waters, Milford, MA). Subsequently, the cartridge was washed with 5 mL purified water. The pesticides trapped in the cartridge were eluted with 8 mL acetone. After the acetone was removed from the eluate with a purified nitrogen stream, 100 μL of diuron-d_6 (100 ng/mL) was added as an HPLC internal standard. The volume of the sample was adjusted to exactly 1 mL with methanol. The

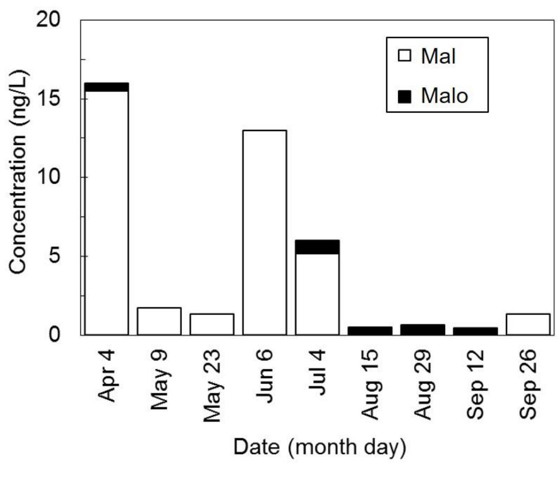

Fig. 2: The times and concentrations of Mal and/or Malo detected

Table 1: Concentration of pesticides found in the water samples collected from the Nodai River along with their experimental recovery efficiency, limit of detection, limit of quantitation, and frequency

Pesticide	Abbr.	Recovery (%)	LOD [b] (ng/L)	LOQ [c] (ng/L)	Fre. [d] (%)	Concentration (ng/L) Min	Max	(month)	Mean
Fungicides									
Azoxystrobin	Azo	88 ± 3.1	0.4	1.4	67	0.7	1.9	(Aug.)	1.1
Edifenphos	Edi	100 ± 5.7	0.3	1.0	7	0.7	0.7	(Aug.)	0.7
Ferimzone	Fer	88 ± 5.1	1	4	20	2	7	(Aug.)	5
Flutolanil	Flu	96 ± 3.0	1	4	0	<1			
Furametpyr	Fur	91 ± 5.0	0.5	1.7	13	0.9	34	(Aug.)	17
Iprobenphos	Ipr	89 ± 19	0.5	1.7	93	0.6	2.9	(Aug.)	1.5
Isoprothiolane	Iso	99 ± 5.9	0.4	1.4	93	1.1	58	(Aug.)	9.3
(E)-Metominostrobin	Met-E	97 ± 5.8	0.3	1.0	93	0.8	250	(Jul.)	28
(Z)-Metominostrobin	Met-Z	98 ± 5.5	0.7	2.4	27	0.8	17	(Jul.)	5.8
Pyroquilon	Pyr	100 ± 9.8	0.3	1.0	87	0.5	64	(Jun.)	22
Tiadinil	Tia	100 ± 3.5	9	30	13	32	32	(May)	32
Tricyclazole	Tri	100 ± 5.8	0.5	1.7	93	1.4	190	(Aug.)	27
Neonicotinoid insecticide									
Clothianidin	Clo	76 ± 7.4	1	4	47	3	10	(May)	6
Dinotefuran	Din	92 ± 5.7	3	10	93	6	410	(Aug.)	50
Imidacloprid	Imi	78 ± 5.7	1	4	73	2	9	(Aug.)	5
Thiamethoxam	Thi	98 ± 8.2	1	4	67	1	8	(Aug.)	4
The other insecticides		±							
Diazinon	Dia	75 ± 8.8	0.3	1.0	93	0.8	4.5	(Jun.)	2.1
Fenobucarb	Feb	99 ± 5.6	2	7	7	4	4.2	(Jul.)	4.2
Fenthion	Fet	93 ± 6.0	4	14	0	<4			
Fipronil	Fip	99 ± 4.7	10	34	40	31	34	(May)	33
Malathion	Mal	98 ± 5.3	0.6	2.0	40	1.3	16	(Apr.)	6.4
Methidathion	Met	77 ± 2.0	8	27	13	450	490	(May)	470
Phenthoate	Phe	97 ± 14	2	7	20	7	58	(Jun.)	32
Tebufenozide	Teb	89 ± 4.2	6	20	0	<6			
Metabolites									
Diazoxon	Diao	120 ± 6.3	1	4	0	<1			
Fenthoxon	Feo	120 ± 12	0.3	1.0	0	<0.3			
Fenthoxon sulfone	Feos	85 ± 3.1	0.8	2.7	0	<0.8			
Fenthoxon sulfoxide	Feoso	100 ± 6.3	0.9	3.0	0	<0.9			
Fenthion sulfone	Fes	110 ± 5.8	10	34	0	<10			
Fenthion sulfoxide	Feso	100 ± 5.2	0.8	2.7	67	1.1	2.1	(Aug.)	1.6
Malaoxon	Malo	93 ± 5.7	0.4	1.4	33	0.5	0.8	(Jul.)	0.6

[a] Mean ± standard deviation (n = 3)
[b] Limit of detection
[c] Limit of quantitation
[d] Frequency: Number of times detected at more than MDL divided by number of sampling times

methanol solution (1 μL) was analyzed for pesticides by an HPLC/MS with an internal standard.

2.4 Instrumental

Agilent 1200 HPLC interfaced to Agilent 6460 Triple Quad LC/MS/MS (Agilent Technologies Inc., Santa Clara, CA) and equipped with an Agilent Zorbaz SB C18 column (50 mm x 2.1 mm i.d., particle size, 1.8 μm) was used for analysis of the pesticides of interest. Mobile phase A was water (containing 0.1% formic acid) and mobile phase B was CH_3CN. The gradient mode was initially set at A/B ratio of 95/5, then linearly increased to 20/80 for 20 min and held for 2 min. The flow rate was 0.2 mL/min. MS/MS was operated under positive/negative ESI and capillary voltage at 3,500 V.

3 Results and Discussions

Table 1 shows the analytical results of water samples collected from Nodai River along with their experimental recovery efficiency and limit of detection (LOD). The experimental recovery efficiency of the pesticides found ranged from 120 ±

12 % (metabolite Feo) to 75 ± 8.8 % (Dia). Over 90% recoveries were obtained from the majority of the pesticides and their metabolites found. These results were almost comparable to the reported values [9,14,17]. The limit of detection (LOD) ranged from 0.3 ng/L (Edi, Met-E, Pyt, and metabolite Feo) to 10 ng/L (Fip and metabolite Fes). These values are sufficient to discuss the analytical results.

The 31 pesticides of interest in the present study included 12 fungicides, 12 insecticides, and 7 metabolites. Among these 31 pesticides, 23 pesticides were detected. They were 11 fungicides except Flu, 10 insecticides except Fet and Teb, and 2 metabolites.

Among the neonicotinoid insecticides, Din was found in the highest level. This may be due to the aerial spray of Din with an unmanned helicopter during August [14]. The maximum levels of the other 3 neonicotinoid insecticides (Clo, Imi, and Thi) were significantly lower than that of Din (approximately 2.5% of Din). One of the non-neonicotinoid insecticides, Fip, was recovered at a higher level than those three neonicotinoid insecticides (Clo, Imi, and Thi), whereas its max value was only 8.3% of that of Din.

The metabolite of Dia (max, 4.5 ng/L), Diao, was not detected. On the other hand, Fet was not detected but its metabolite Feso (max, 2.1 ng/L) was detected. Mal was detected in 6 samples and detected together with Malo in 2 samples (from April 4 and July 4). Either Mal or Malo was found in the rest of the samples.

Figure 2 shows concentrations of Mal/Malo found in the samples collected on the different dates. Both Mal and Malo were found in the samples collected on April 4 and July 4. Only Mal was found in the samples collected on May 9, 23, June 6, and September 26. On the other hand, only Malo was detected in the samples collected on August 15, 20, and September 12, suggesting that sprayed Mal might have degraded into Malo and then blew into the river. Amounts of a metabolite higher than those of its original pesticide have been reported. For example, arbofuran (Feso) was found in greater quantities than carbosulfan in the Sakura River, Ibaraki Prefecture [5]. Also, the same phenomenon was reported in the case of isofenpos oxon and tolchlofos methyl oxon in the Shinano River [9].

The levels of pesticides detected ranged from 0.5 ng/L (Pyr) to 490 ng/L (Met) in the present study (Table 1). Among the pesticides regulated by the ministries in Japan [18,19,20], no pesticides studied in the present study exceeded their standard levels, suggesting that there should not be adverse effects on human health caused by these residues. However, Dia and Feb exceeded the predicted no-effect concentration (PNEC) set by the Japan Ministry of Environment [21], suggesting that they could have adverse effects on some aquatic animals.

The maximum levels of Azo, Fur, Iso, Pyr, and Din were equal to the those found in Kotsuki River, Kagoshima Prefecture, which is located in the southernmost part of Kyushu [22]. The level of Met found in the present study was approximately 10 times that found in Tsurumi River, Yokohama City [23]. On the other hand, the other pesticides found in the present study were within the low and medium levels found in the same river [23,24], The levels of three neonicotinoid insecticides (Clo, Imi, and Thi) were 4 to 400 folds less than those found in a river near Sidney, Australia [25].

The levels of other pesticides found in the present study were compared with those of previously reported cases. Clo and Thi were found at the same level as in a river in Osaka Prefecture, Japan [17]. Dia was found at the same level as in surface water in the US (Presque Isle, Maine, Parma, Idaho and Hancock, Wisconsin) [26]. Feb, Imi, and Met were found at the same level as in a river in Yokohama City [23,27]. Tri was found at lower levels than in the Shinkawa River, Niigata Prefecture [7]. Ipr was found at lower levels than in Sakura River, Ibaragi Prefecture (max, 853 ng/L) [6] and in Tsurumi River, Yokohama City (max, 2,400 ng/L) [24]. Mal was found at lower levels than in Kotsuki River, Kagoshima Prefecture (max, 297 ng/L) [22];Tsurumi River (max, 70 ng/L) [23]and (max, 1,300 ng/L) [24].

Figure 3 shows the concentration variation of 4 neonicotinoid insecticides (Clo, Din, Imi, and Thi) and Fip. Din and Imi were detected during early April and late September. The concentration of Din (410 ng/L) was the greatest in the sample obtained on August 15. The maximum concentration was lower than reported results of 10,000 ng/L in the

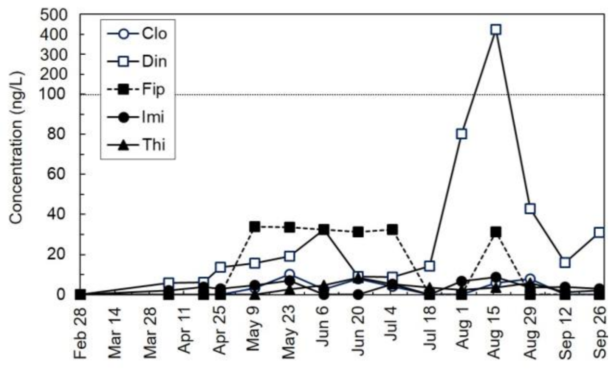

Fig. 3: Concentration fluctuations of neonicotinoid insecticides (Clo, Din, Imi, and Thi) and Fip

Washinoki-Odori River, Niigata, Prefectur [14] and 1900 ng/L in the Sabigawa River, Osaka Prefecture [29]. The maximum concentration of Imi (9 ng/L) was lower than that of 500 ng/L in the Kanzaki River, Osaka Prefecture [29] and 25.9 ng/L in the Iowa River [30]. Clo was detected during early May and late August and its concentration (10 ng/L) was the greatest on May 15. The value was lower than the maximum concentration of 350 ng/L in the Taisho River, Osaka Prefecture [29], and was comparable to 13.3 ng/L in the Iowa River[30]. Thi was detected during early May and late September and its concentration (8 ng/L) was the highest on August 15, which was comparable to 8.23 ng/L in the Iowa River[30].

Fip was found in relatively high levels during early May and early July and then not detected thereafter until after mid-August. The maximum concentration of 34 ng/L was twice the reported values of 14 ng/L in Yamato River, Osaka Prefecture [29] and 40 times that (0.92 ng/L) in the River Elbe[28].

Figure 4 shows the concentration variation of 6 pesticides. These are pesticides that have been considered ones likely to cause some adverse effects on the ecosystem. They were detected at relatively high levels in the present study. Fungicides Iso, Met-E, Pyr, and Tri were detected during early April and late September. Their maximum levels were 5.8 ng/L for Iso and 190 ng/L for Tri, respectively, in mid-August; 250 ng/L for Met-E in mid-July; and 64 ng/L for Pyr in mid-June. The peak time when insecticide Met was detected was in May and the peak for Phe was in early May, early Jun, and mid-July.

Various pesticides are applied in paddy fields, which comprises most of the cultivated field area of the river basin, during May and August. Most of Din was sprayed by unmanned helicopter early in August on the paddy fields in the basin of the river, which caused the increase of Din concentration in August.

The maximum concentrations of the pesticides found were Mal in April; Tia, Clo, Fip, and Met in May; Dia, phe, and Thi in Jun; and Met-E, Met-Z, Pyr, and Feb in July. In August, maximum amounts of Azo, Edi, Fer, Ipr, Iso, Tri, Din, and Imi were detected. Among them, the concentrations of Ipr and Imi increased in May and Jun. Edi, Fer, Fur, Feb, and Met were detected only during a relatively short period of time, whereas Ipr, Iso, Met-E, Pyr, Tri, Dia, Din, Imi, and Thi were found over a long period from April to September. Regarding neonicotinoids, their concentration fluctuations of were different from those in rivers in Osaka Prefecture [17]. Fip applied to rice seeds was reported as transferring to paddy water after seedling transplanting to paddy field [31]. The concentration of Fip seemed to depend on the applied time and drainage status of water from paddy field after application. The maximum levels of metabolites Malo and Feso were found in July and in August, respectively.

The pesticide loading is more suitable than the pesticide concentration to evaluate the pesticide fluctuation in a river and the runoff from paddy fields because the concentration is affected by the flow of the river water [9,14,32]. Figure 5 shows the monthly loading of total pesticides of interest in the present study. The values were calculated according to a previously reported method [9]. The values are two weeks' worth of river flow rate times the concentration of each pesticide. The total pesticide loading was the highest in May (16 kg) when most applications of insecticides other than neonicotinoids were conducted. The highest total loadings of fungicides, neonicotinoids and the other insecticides and metabolites during April and September were 5.5

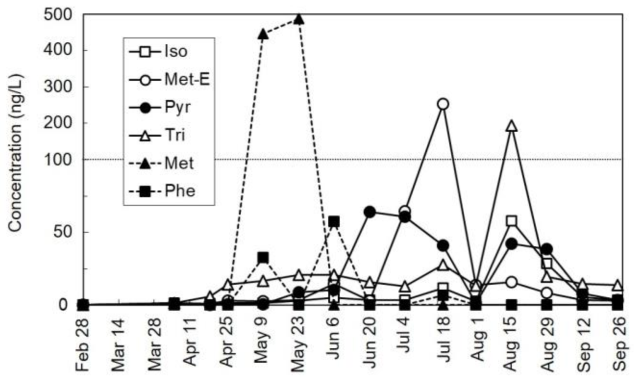

Fig. 4: Concentration fluctuations of fungicides (Iso, Met-E, Pyr, and Tri) and insecticides (Met and Phe)

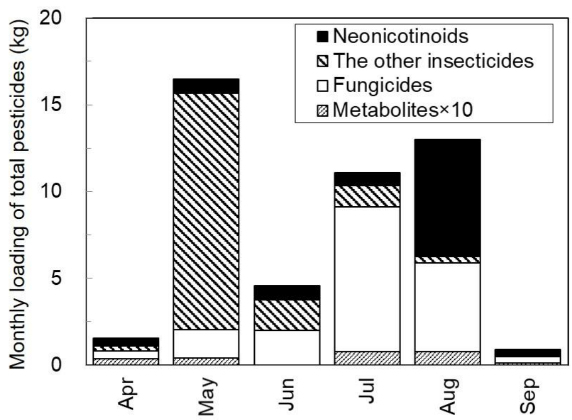

Fig. 5: Monthly total loading fluctuations of fungicides insecticides and metabolites

kg (Met-E), 8.2 kg (Din), 12.2 kg (Met) and 0.2 kg (Feso), resprctivery. The monthly loading of total pesticides exhibited unique variations.

The maximum value of fungicides was obtained in July (8.4 kg), followed by August (5.1 kg). The ratios of fungicides in July and August were 47% and 28% of the total fungicide loading, respectively. The major fungicides were Met-E of 4.7 kg and Pyr of 2.0 kg in July, and Tri of 2.4 kg in August.

Insecticides were detected in the greatest amounts in May, followed by August. The level of neonicotinoids was the highest in August, when it was 95% of the total loading. As mentioned above, 87% of the total loading neonicotinoids was Din, which was sprayed over the paddies by an unmanned helicopter in August [14]. The total loadings of neonicotinoid pesticides were 0.56 kg from Clo, 8.2 kg from Din, 0.68 kg from Imi, and 0.54 kg from Thi. In addition, a total loading of Fip was 2.8 kg, which was the next highest level to Din. All pesticides flowed down from spring to fall, indicating that they had been used for relatively long time-period.

The total loading of metabolites was the greatest in July and August. In the case of insecticides, they were the highest in May and reduced in Jun and July, whereas they increased in August. The pesticides found in the highest amounts during May and Jun were Tia (fungicide), Clo, Fip, Mal, Met, Phe, and Thi (insecticides). The other pesticides were found in the greatest amounts during July and August. Mal flowed into the river mainly during April and July and its value was the highest in Jun. On the other hand, its metabolite Malo flowed into the river in April and during July and September and was found in the highest in July. This may due to degradation of Mal to Malo over the time progress.

Among the pesticides flowing into the Nodai River, the ones with known runoff amount of its basin were calculated their runoff ratio (%). The runoff ratio is a ratio of total loading of a pesticide in runoff water amount [14,32,33]. In the case of Met, total loading value of Met-E and Met-Z was used. The results indicated that the runoff ratios of pesticides ranged from 0.28% (Dia) to 12% (Met-E). In the case of fungicides, the runoff ratio was 2.1% for Azo, 0.48% for Fer, 12% for Met-E 1.3% for Pyr, and 7.6% for Tri. In the case of insecticides, it was 0.28% for Dia, 4.8% for Fip, 6.1% for Met, and 2.2% for Phe. The runoff ratios of neonicotinoids were 2.2% for Clo, 10% for Din, 078% for Imi, and 6.3% for Thi. The ratio of Din was a quarter of 38% in the Washinoki-Odori River, Niigata Prefecture[14]. Since there have been few reports on runoff ratios in rivers for the other neonicotinoids, the values in this study are important for further investigation on their behaviors in river. In addition, the results suggest that drainage improvement would be recommended to decrease runoff of applied pesticides.

It was expected that the higher the water solubility or lower the n-octanol/water distribution coefficient (log P) the higher the runoff amount would be. However, no appreciable relationship between pesticide runoff amount and water solubility or log P was observed. The runoff ratios of Dia and Imi, which were sprayed over farmland, were relatively lower than those of pesticides sprayed over paddy fields. This may be due to fact that the pesticides sprayed over paddy fields readily flowed into the river when paddy water was drained into it. Runoff amounts of pesticides varied among different reports, suggesting that the runoff values are not only depended on the physical natures of specific pesticides but also on various external factors, such as spraying areas, methods, and the weather. In the case of fungicide Tri, the runoff ratio value (4.5%) of Nodai River was within the range reported (0.06 – 9.4%) from Shinkawa River, Niigata Prefecture [33]. The runoff ratio of Din (10%) from Nodai River was consistent with the one reported in the Washinoki-Odori River, Niigata Prefecture [14]. These runoff events appeared to be caused by the drainage of the paddy water in the river basin. The pesticide concentration remained at high level in paddy waters for one or two weeks after application [11,14]. Therefore, the results suggest that the paddy water should keep in the paddy field for the period.

4 Conclusion

The concentration fluctuations, the loadings and the runoff ratios of commonly used fungicides and insecticides including neonicotinoid insecticides were investigated in a small river (Noshiro River) flowing through rice paddy fields in Niigata Prefecture, Japan. The residues of the investigated pesticides found ranged from 0.8 ng/L of Malo to 490 ng/L of Met. Din was found in the highest level (410 ng/L at maxim) among the four investigated neonicotinoid insecticides. This may be due to the aerial spray of Din with an unmanned helicopter during August. The maximum levels of the other 3 neonicotinoid insecticides and Fip were significantly lower than that of Din. No pesticides studied in the present study exceeded the standard levels in environmental and drinking water set by the government of Japan. The highest total loadings of fungicides, neonicotinoids and the other insecticides and metabolites during April and September were 5.5 kg (Met-E), 8.2 kg (Din), 12.2 kg (Met) and 0.2 kg

(Feso), resprctivery. The maximum loadings of total pesticides were obtained in July for fungicides, August for neonicotinoids and May for the other insecticides. The runoff ratios of some pesticides from paddy fields ranged from 0.28% of Dia to 12% of Met-E, and the ratios of neonicotinoids were 2.2% for Clo, 10% for Din, 078% for Imi, and 6.3% for Thi.

The results of the present study provided the data under certain field conditions in Japan, suggesting that pesticide concentrations in small rivers located in agricultural areas are influenced by the pesticides used in its basin. Moreover, in this study, the concentrations of some pesticides exceeded the PNEC, and this is a concern for potential ecological impacts. The analysis approach used in the present study is an effectual measure for the fluctuations of pesticide residues in river. However, more frequent samplings and determinations will be required for the residue fluctuations. Further studies are needed to evaluate the fluctuations in concentration and loading of neonicotinoid insecticides under different field conditions in the Nodai River and another small rivers.

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