Doctoral Dissertation

STUDY ON MANAGEMENT OF RIVER TOXICITY FROM RESIDENTIAL AREA USING MEDAKA (*Oryzias latipes*) BIOASSAY AS AN INDEX FOR AQUATIC HABITAT CONDITION

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博士論文
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STUDY ON MANAGEMENT OF RIVER TOXICITY FROM RESIDENTIAL AREA USING MEDAKA (*Oryzias latipes*) BIOASSAY AS AN INDEX FOR AQUATIC HABITAT CONDITION

（水生生物生息場指標としてのメダカを用いたバイオアッセイによる 住宅地河川の毒性管理に関する研究)

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A dissertation submitted to the Division of System Design and Engineering of Yamaguchi University in partial fulfilment of the requirement for the degree of Doctor of Engineering

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ABSTRACT

Trace chemicals such as endocrine disruptors and dioxins can cause many problems in the ecosystem, especially if released into environmental water. Previous studies have determined the acute toxicity levels of such chemicals. However, the observed concentrations of such chemicals in environmental water are usually much lower than the levels that cause acute toxicity. Furthermore, various other chemicals also exist in environmental water. Therefore, it is difficult to obtain information about the acute toxicity levels of each chemical, especially for the purpose of protecting the ecosystem.

A bioassay is an approach that can be used to obtain comprehensive information about the toxicity levels of chemicals. However, this approach is not well suited to environmental management, because it cannot be used to identify the chemicals. As an alternative, toxicity management methodologies based on bioassays, such as Whole Effluent Toxicity (WET), have recently attracted considerable attention. This method can be used to measure the toxicity of industrial wastewater without necessarily identifying the chemical.

This study had two objectives. The first objective was to demonstrate the applicability of a toxicity test using Medaka and 100-fold concentrated water and to determine the relationship between the toxicity of 100-fold concentrated water and aquatic habitat conditions. The second objective was to conduct a preliminary investigation of the relationships among chemical concentration, toxicity, and basin characteristics, which can be used to develop an approach for managing river toxicity.

On the basis of the analysis in Chapter 3, the results of the toxicity test using Medaka and 100-fold concentrated water indicated that the high levels of biodegradable organic matter (BOD) released from household wastewater also contains hydrophobic toxic matter and that the toxicity and chemical oxygen demand (COD) of seawater in industrial areas do not exhibit a clear relationship. Furthermore, the relationship between the toxicity of 100-fold concentrated water and aquatic habitat conditions was revealed; the number of clear-stream macrobenthic animals sharply decreased over an inverse if median lethal time (LT_{50}^{-1}) of 0.25 or an inverse of median effect time (ET_{50}^{-1}) of 0.5, and tolerant fish became dominant over an LT_{50}^{-1} of 0.3 or ET_{50}^{-1} of 0.5–1.0. Although this method has an advantage in that it reduces

the amount of time and sampling needed to perform toxicity tests, it also has a disadvantage in that the toxicity index required for calculating toxicity load is LT_{50}^{-1}, which is based on time and hence cannot be treated as a concentration. Therefore, in Chapter 4, the lethal dilution rate (LDR_{50}), which can be treated as a concentration, is used as a toxicity index. LDR_{50} is defined as the dilution rate at which 50% of fish survive the acute toxicity test. The equation obtained for the relationship between LDR_{50} and LT_{50}^{-1} is $y = 0.1752x$, where $y = LDR_{50}$ and $x = LT_{50}^{-1}$, with $R^2 = 0.9306$.

Chapter 5 describes a preliminary investigation of the relationships among chemical concentration, toxicity, and basin characteristics. The results suggest that the detected toxicity in residential areas is, at times, sufficiently high to affect the aquatic habitat, and therefore the toxicity should be managed. On the basis of the GC/MS analysis and cluster analysis, the toxicity tends to be highly stable even when the composition and concentration of chemicals fluctuate. Furthermore, the chemical compositions taken at sampling points that are not adjacent to commercial or industrial facilities are different from basin to basin, but almost all toxic substances present are detected in low concentrations. In contrast, sampling points adjacent to commercial or industrial facilities exhibit various differences and, at times, show higher concentrations of toxins. A model analysis shows that LDR_{50} discharged from a basin dominated by residential areas can be explained using a simple model with two parameters, k' (toxicity decrease ratio) and d_w (LDR_{50} discharged from the population without sewer coverage). The obtained k' is 0.03 km^{-1}, and d_w is 0.08. Furthermore, when a sampling point is adjacent to commercial or industrial facilities, explaining LDR_{50} using the simple model is difficult. This fact might imply that even when commercial or industrial facilities discharge specific chemicals in river basins dominated by residential areas, such chemicals will not be retained in the stream for a long duration, and chemicals discharged from residences eventually dominate the toxicity profile. These findings suggest that toxicity from residential area should be managed, and the pollution analysis procedure for sewerage designing can be applicable for toxicity management in the river the majority of which catchments are residential area.

Key words: aquatic habitat conditions, chemical concentration, LT_{50}^{-1}, ET_{50}^{-1}, LDR_{50}, residential area, toxicity

要旨

内分泌かく乱物質やダイオキシンのような微量化学物質は、特に環境水の中に排出された際に生態系の中で多くの問題を引き起こす。従来の研究では、そのような化学物質の急性毒性レベルを測定してきた。しかしながら、環境水におけるそのような化学物質の観測濃度は通常、急性毒性を引き起こすレベルよりもかなり低い。さらに、水環境の中にはさまざまな他の化学物質も存在している。それゆえ、特に生態系保全を管理目標とした場合には、個々の化学物質の急性毒性レベルを指標とすることは困難である。

一方、バイオアッセイは、化学物質の毒性レベルにおける総合的な情報を得るために使われる 1 つの手法である。しかしながら、この手法は、化学物質の特定ができないと言う理由から、環境管理にはあまり適していないとされてきた。しかし近年では、生物応答試験を用いた排水管理(WET)のようなバイオアッセイに基づいた毒性管理手法が注目を浴びている。この手法は、必ずしも化学物質を特定することなしに工業廃水の毒性管理に使用することができる。

この研究には 2 つの目的がある。1 つ目はメダカと 100 倍濃縮水を使った毒性試験の適用性を立証し、100 倍濃縮水の毒性と水生生物生息状況の関係性を特定すること。2 つ目は、住宅地河川における河川水の毒性と化学物質の挙動調査に基づき、毒性管理の考え方を示すことである。

第 3 章の分析より、住宅地河川では本毒性試験結果である 50%致死時間の逆数(LT_{50}^{-1})と BOD の間に相関がある一方、工業地帯の海水の LT_{50}^{-1} と

COD は明確な関係性を有していないことが明らかになった。さらに、生物調査と本毒性試験結果から、清流の底生生物の数が $LT_{50}^{-1}>0.25$ で急激に減少し、強耐性魚類が $LT_{50}^{-1}>0.3$ で優勢になることを示した。

本手法は、毒性試験に要する時間とサンプリング量を減すことができる利点があるが、毒性指標 LT_{50}^{-1} がすなわち時間に基づいたものであるため、濃度として扱えないという欠点もある。それゆえ、第 4 章では濃度として扱うことのできる致死希釈率(LDR_{50})を毒性指標として選び、LDR_{50} と LT_{50}^{-1} の関係性を求めた。これにより、$LDR_{50} = 0.1752\ LT_{50}^{-1}$ （$R^2 = 0.9306$）を得た。

第 5 章では、化学物質濃度、毒性と流域特性の関係性について調査している。その結果、住宅地河川で検出された毒性が水生生物に影響を及ぼすレベルに達する場合があること、それゆえその毒性は管理されるべきであることを示した。GC/MS 一斉分析による化学物質濃度分析から、化学物質の種類や濃度は同じ流域でも大きく変動するが、その毒性は比較的安定性があること、商工業施設に隣接しないサンプリング点で採取された化学組成は流域によって異なるが、ほとんどすべての毒性物質が低濃度であるのに対し、商工業施設に隣接したサンプリング点ではしばしば高い化学物質濃度が観察されることなどを示した。さらに、住宅地河川から排出される LDR_{50} は、k’（毒性減少比）と d_w (下水のない地域からの LDR_{50} 排出負荷量)を用いた下水道計画で用いられる単純なモデルによって説明できることを示し、$k'=0.03\ km^{-1}$、$d_w=0.08$ を得た。また、住宅地河川であってもサンプリング点が商工業施設に隣接している場合には、LDR_{50} を上述のモデルで説明することは困難であることから、住宅地を主体とする流域の商工業施設から排出される化

学物質は急速に分解され、最終的には住宅地から排出された化学物質により河川水の毒性が説明できると推察した。以上のように、本研究では、生物生息状況と関連づけた簡易な毒性検出手法を提案した上で、住宅地から出る毒性も工場排水同様管理されるべきであり、管理手法としては下水道設計で用いられる汚濁解析手法が援用可能であることを明らかにした。

キーワード: 水生物環境、化学濃度、LT_{50}^{-1}、ET_{50}^{-1}、LDR_{50}、住宅地、毒性

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Riyanto Haribowo

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CHAPTER 01
INTRODUCTION

1.1 General

The quality of surface water is a major factor affecting human health and ecological systems, especially around residential areas, since rivers and their tributaries passing through cities receive a multitude of contaminants released from industrial, domestic/sewage, and agricultural effluents. However, the degree to which each factor contributes to water quality is unclear (Qadir et al., 2008, Zhang Y et al., 2009). Up to date, the water and wastewater quality has been evaluated principally based on the concentration determination of a variety of individual chemicals. The individual chemical analysis, however, is impossible to give a whole evaluation of the entire toxicants in environmental samples, which contain numerous unknown contaminants and are very complex (Waite T. D., 1984).

Trace chemicals such as endocrine disruptors and dioxins can cause many problems in the ecosystem, especially if released into environmental water. Studies have already determined the acute toxicity levels of such chemicals. However, the concentrations of such chemicals in environmental water are usually much lower than those that cause acute toxicity. Furthermore, various other chemicals also exist in the water. Therefore, it is difficult to obtain information about the acute toxicity levels of each chemical with a view of protecting the ecosystem.

A bioassay is one approach that could be used to obtain comprehensive information about the toxicity levels of chemicals (Kinoshita et al., 2009). However, this approach is not well suited to environmental management because it cannot detect the chemical itself. As an alternative, toxicity management methodologies based on bioassays, such as Whole Effluent Toxicity (WET), have attracted considerable attention in recent times. This method can be used to measure the toxicity of industrial wastewater itself, without necessarily identifying the chemical. Specifically, toxicity is considered to be caused by various substances such as agricultural chemicals, detergents, and pharmaceuticals. Although many studies have focused on river water toxicity and chemical behaviours (Ichiki et al., 2009; Selvaraj

et al., 2014; Wang et al., 2011), there are not many researches which observed the behaviour of toxicity and chemicals in relation to basin characteristics in Japan.

1.2 Objectives

Purpose of this study are:

1. To demonstrate applicability of toxicity test using Medaka and 100-fold concentrated water, and show the relationship between toxicity of 100-fold concentrated water and aquatic habitat conditions.
2. Preliminarily investigate the relationship among chemical concentration, toxicity, and basin characteristics, and discuss about the approach to manage toxicity in the river.

1.3 The scope of dissertation

This dissertation comprises 6 chapters; Chapters 1 explains the background and objectives of this study. Chapters 2 present literature review on history of behavioural research, organisms used in biological indicator monitoring, international standardization for toxicity tests, the impact of the environment upon humans, and the potential risk from combinations of chemicals in the environment. Chapters 3 discuss about the relationship between toxicity and organic pollution. Furthermore, describe the survey of macrobenthic animal and fish, and discuss the relationship between toxicity and living organisms. Chapters 4 expressed the toxicity as a lethal dilution rate (LDR_{50}) which can be treated in the same way as concentration. And show the relationship with toxicity of 100-fold concentrated. In Chapters 5, investigated the river water toxicity and identified the chemical contents using GC/MS simultaneous analysis database, and preliminarily investigated the relationship between chemical concentration, toxicity, and basin characteristics; and Chapters 6 is the conclusion and future work.

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CHAPTER 02
LITERATURE REVIEW

2.1 History of monitoring and management of water resources

The basis for effective management of aquatic ecosystems and water quality due to problems of water quantity and quality is an efficient monitoring of water resources (Bae et al., 2012). The detection of disorders, such as toxicants, in the target ecosystem is the first stage in sustainable ecosystem management. In the early stages of ecosystem monitoring, sampling at the site tends to be used for evaluating environmental conditions by measuring a range of physicochemical factors, such as dissolved oxygen (DO), pH, and biochemical oxygen demand (BOD).

At present, many countries around the world use real-time monitoring systems that are sensitive to physicochemical factors to detect disturbances to aquatic ecosystems. However, physicochemical monitoring systems cannot detect all concentrations of the various chemical compounds, which have different effects on aquatic organisms and ecosystems. Thereby, biological indicator monitoring have been developed, which is based on the different response of the organism to interference. Biological indicator monitoring is used for control of water quality continuously, allows direct and continuous sensing of various pollutants or toxic conditions based on the physiology and behavior of organisms (Jeffrey and Madden, 1991; Gerhardt, 1999).

Different methods have different advantages and disadvantages. For example, when using analytical methods, information about behavioral parameters may be compressed (e.g., fractal dimension); however, local and global information cannot be simultaneously extracted from the behavioral dataset (e.g., Fourier transform). Therefore, it is important to use appropriate analytical methods extracting significant information when interpreting behavioral data. The development of behaviour monitoring methods broadly divided into 3 periods (Table 2.1).

1. Trials to observe the activity of organisms

   In the early 1900s, the behavioural research of aquatic species mainly focused on the activity of organisms (e.g., respiratory exchange). In addition, changes in the opercular rates of fish were directly observed to study the effects of environmental variation and various toxicants. Further, the gas exchange of

aquatic organisms was measured by monitoring changes in the gas content of a closed vessel of water containing various organisms, such as fish and mussels. The Regnault principle states that it is possible to determine the nature of catabolized substances using the respiratory quotient, was also applied to measure the respiratory activity of aquatic organisms. A device that could record the diurnal activity rhythms was also proposed; the tested parameters included fish vision, characteristic motion, and chemical sensitivity. Various environmental factors were also considered to elucidate fish schooling behaviour, including food supply, temperature, and chlorinated water and pH.

2. Development of behavioural observation techniques

    Various techniques were introduced to quantify the behaviour of aquatic organisms, such as visual observation, thermistor monitoring of heat conductance, use of unipolar electrodes, use of dual external electrodes, opercular wires, implementation of phototransistor systems, electromyography, ventilation volume method, and monitoring of respiratory pressure changes. Due to the increase in the need for long-term monitoring studies, the basic principles of a biological indicator monitoring for use in water-quality management were proposed in the 1970s.

3. Development of advanced techniques

    Quantitative behavioural monitoring in real time was developed with advances in computer technology as well as mathematical and computational methods in the 1980s. Quantitative image analysis allowed the behaviour of aquatic organisms to be automatically detected, including microorganisms, barnacles, and fish, as a single organism or as a group.

    The development of computer technology made it possible to record, digitize, and quantitatively analyse the swimming behaviour of individual fish (e.g., motility, turning rate, swimming depth below the surface, distance between individual fish, and habitat preference with light or dark substrata) and to quantify fish group behaviour based on geometrical parameters and activity level. In addition, biological sensors were also developed to detect specific biomolecules, such as ATP and enzymes, in parallel to the development of polymerase chain reaction techniques. The quadruple impedance conversion technique was introduced for the online bio monitoring of macro invertebrates.

Table 2.1 - The development of behaviour monitoring methods.

Period	Characteristics	References
Trials to observe the activity of organisms (1900s–1940s)	Direct observation of opercular rate changes in fish Gas exchange of aquatic organisms	Belding (1929), Ellis (1937), Jones (1947)
	▪ In a closed vessel of water	Humboldt and Provençal (1809), Henze (1910), Montuori (1913), Krogh (1916), McCleandon (1917), Keys (1930)
	▪ By using Regnault principle	Jolyet and Regnard (1877), Grehant (1886), Zuntz (1901), Bounhiol (1905), Gardner and Leetham (1914)
	▪ In a flowing water system	Winterstein (1908), Ege and Krogh (1914), Gaarder (1918), Hall (1929)
	Recording device for fish diurnal activity Fish schooling behavior	Spencer (1929a, 1929b, 1939)
	▪ Vision	Bowen (1931, 1932), Breder (1929, 1942), Breder and Gresser (1941a,1941b), Breder and Nigrelli (1935), Parr (1927, 1931), Schlaifer (1938, 1940), Spooner (1931)
	▪ Characteristic motion	Breder and Gresser (1941a), Lashley (1938)
	▪ Chemical sensitivity	Frisch (1938, 1941), Goz (1941), Hüttel (1941)
	▪ Environmental factors	Breder and Roemhild (1947), Langlois (1936a, 1936b), Breder and Nigrelli (1935),Noble and Curtis (1939), Breder andNigrelli (1935), Breder (1936), Noble and Curtis (1939), Breder and Halpern (1946)
Development of behavioral observation techniques (1950s–1980s)	Measurement of organism activity	Ermisch and Juhnke (1973), Randall and Shelton (1963), Roberts (1964), Marvin and Heath (1968), Hughes and Roberts (1970), Hughes and Saunders (1970)
	Quantitative techniques for measuring behavior	
	▪ Visual observation	Walshe (1950), Skidmore (1970), Holeton (1971), Heath (1972), Henry and Atchison (1984)
	▪ Thermistor monitoring of heat conductance	Heusner and Enright (1966)
	▪ Unipolar electrode	Shelton and Randall (1962), Randall and Shelton (1963),Marvin and Heath (1968)

Source: Bae et al (2014)

Table 2.1 – (continued)

	▪ Dual external electrodes	Roberts (1964), Sutterlin (1969), Hughes and Roberts (1970), Spoor et al. (1971), Spoor and Drummond (1972), Drummond et al. (1973)
	▪ Opercularwires	Sutterlin (1969), Saunders and Sutterlin (1971)
	▪ Phototransistor systems	Cairns et al. (1970), Porter et al. (1982)
	▪ Electromyography	Kinnamon et al. (1984)
	▪ Ventilation volume	Davis and Cameron (1970)
	▪ Respiratory pressure change	Saunders (1962), Hughes and Roberts (1970), Hughes and Saunders (1970)
	Increase in concern for long-term monitoring	
	▪ Proposal of the basic biological indicator monitoring concept	Cairns et al. (1970, 1973, 1975), Juhnke and Besch (1971)
Development of advanced techniques (1980s–present)	Behavioral analysis systems based on video images	
	▪ Computer image processing	Yachida et al. (1981), Hader and Lebert (1985), Miller et al. (1982), Spieser and Scholz (1992), Steinberg et al. (1995), Baganz et al. (1998)
	▪ Quantification of fish group behavior	Inada and Kawachi (2002), Suzuki et al. (2003), Salierno et al. (2008), Israeli and Kimmel (1996), Whitsell et al. (1997)
	Readily available computer-based systems	Godden and Graham (1983), Hoy et al. (1983), Dusenbery (1985), Ye and Bell (1991)
	Online biomonitoring based on quadruple impedance conversion technique	Gerhardt (1999, 2007), Gerhardt et al. (1998, 2006); Bisthoven et al. (2009)
	Biological sensors for detecting specific biomolecules and PCR techniques	Pomati et al. (2004), Noble and Weisberg (2005), Hawkins et al. (2005)
	Application of a wide range of computational methods for biological indicator monitoring	Little (2002), Chon et al. (2004), Park et al. (2005), Nimkerdphol and Nakagawa (2008)

Source: Bae et al (2014)

2.2 Organisms used in biological indicator monitoring

Many organisms from various trophic levels have been used for a wide range of biological indicator monitoring applications, including bacteria, algae, daphnia, macro invertebrates, and fish (Table 2.2). Technological advances enabled biological monitoring systems to become commercially available. For example, Mossel monitor was introduced in 1990 and is used to detect toxic materials based on changes in the gaping behaviour of mussels. Toximeters, which are based on the behavioural changes of Daphnia magna or Danio rerio, were introduced in 1998 and 2002, respectively. Until the 1990s, behavioural changes based on merely 1 or 2 individuals were generally recorded and quantified. However, as the importance of group behaviour as well as the battery of tests based on organisms from different trophic levels has increased, various software programs and techniques have been further developed.

Fish were the first organisms used in biological indicator monitoring in the Rhine in the early 1970s (Hendriks and Stouten, 1994). Based on van der Schalie et al. (2004), the parameters measured in biological indicator monitoring include rheotaxis, activity levels, electric organ discharges, and ventilatory patterns. In recent years, significant advances have been achieved in monitoring systems that use fish, with many commercial products being available, including Kerren Aqua-Tox-Control, bbe Fish Toximeter, and bbe ToxProtect. These systems measure the behavioral changes of swimming fish in flowing water (Mons, 2008). However, it should be noted that fish-based systems increasingly fail to detect adverse water conditions when monitoring surface water, because surface water quality has improved (Kramer, 2009).

Table 2.2 - Characteristics of biological indicator monitoring by using various groups of organism.

Biotest system	Measurement	Characteristics/advantages	Limitations	Application	Maintenance	References
Bacteria						
RODTOX (1986)[a]	Respirometer	Easy replacement of the biomass	High maintenance costs	Wastewater	Renewed every week	Kong et al. (1996), Kungolos (2005)
		Ecologically relevant	Prone to sensor fouling In a harsh environments			
Amtox (1997)	Nitrification	Provides data as a direct and continuous measure of nitrification inhibition	Temperature-sensitive			
			Affected By the presence of ammonium in analyzed water	Wastewater		Hayes et al. (1998), Woznica et al. (2010)
Microtox (1993)	Bioluminescence	Easy handling of samples	Only detects substances inhibiting bacterial cell respiration	Wastewater effluent		Somasundaram et al. (1990), Mons (2008)
			High maintenance costs			
Algae						
Algae Toximeter (1996)	Photosynthetic activity	Highly sensitive to herbicides, their by-products, and chronic toxic substances	Lag time In cultivating slow-growing algae	Herbicides	1 h per week	Mons (2008), Storey et al. (2011)
		Easy maintenance				
Daphnia						
Dynamic Daphnia test (1982)	Swimming activity	Useful for detecting accidental spills or emissions into rivers	Malfunctioning of the instrument when subject to high loads of suspended solids	Surface water monitoring	3–4 h per week	Gunatilaka and Diehl (2001), Gunatilaka et al. (2001)
		Continuous development with reliable testing, due to long-term field use	Constant temperature (20 °C)			

Source: Bae et al (2014)

(?) Indicates that the year of development could not be determined.

A Number in parenthesis represents the first use in biological indicator monitoring (i.e., paper, trademark, or registered trademark).

Table 2.2 – (continued)

Biotest system	Measurement	Characteristics/advantages	Limitations	Application	Maintenance	References
Daphnia Toximeter (1998)	Behavior change	Highly sensitive to awide range of toxicants	Cross-over swimming among daphnia	Hazardous compounds in water herbicides	4 h per week	Jeon et al. (2008), Mons (2008)
			Continuous provision of stable algal culture as a food source			
			False-positive alarms due to (adjustable) high sensitivity			
Bivalves						
Mosselmonitor (1990)	Opening/closing of valves, distance of valves	Easy maintenance (e.g., provision of food and organism replacement)	Sensitive to external vibration, resulting in the direct closure of the shells	Hazardous compounds drinking water	1 h per week	Kramer and Foekema (2001)
Dreissena Monitor (1994?)	Opening/closing of valves	Easy handling	Uses one reed switch,which reduces the resolution of the experiment	Effluent wastewater	2–5 h per week	Borcherding (2006), García-March et al. (2008)
		Easy interpretation of mussel reaction to toxicants, and reliable	Does not automatically restart after a power failure			
Fish						
Fish Toximeter (2002)	Behavior	Low maintenance level compared to that of daphnia toximeters	Necessary to specific application	Drinking water, dam monitoring, water treatment plant	1 h per week	Mons (2008)
ToxProtect™ (2004)	Swimming activity	Easy maintenance	Incapable of detecting considerably high levels of fluoroacetate	Drinking water	2 h per month	Mons (2008), Storey et al. (2011)

Source: Bae et al (2014)

(?) Indicates that the year of development could not be determined.

A Number in parenthesis represents the first use in biological indicator monitoring (i.e., paper, trademark, or registered trademark).

Table 2.2 – (continued)

Biotest system	Measurement	Characteristics/advantages	Limitations	Application	Maintenance	References
		Applicable for chlorinated drinking water				
		High sensitivity to pesticides, neurotoxins, and respiratory toxins				
Aqua-Tox-Control (?)	Swimming activity		Relatively lower sensitivity than that the other two fish devices		2 h per week	LAWA (1998)
Multi-species						
Advanced bbe Fish and Daphnia Toximeter (?)	Behavior of fish and daphnia	High sensitivity to pesticides, neurotoxins, respiratory toxins, willful or negligent damage to water systems.	Higher maintenance time and costs compared to single-species systems	Water treatment	Every week	Mons (2008)
		Integrated response to two organisms groups		Sewage		
Multispecies Freshwater Biomonitor (1994)	Measuring the changes of impedances	Measurement of different behaviors with different times and thresholds of response to chemical stress			2–5 h per week	Gerhardt (1999, 2007)
Biological Early Warning System (BEWs, 2007)	Measuring the changes of impedances	Measuring behavior strength ranging from 0 to 1				Li et al. (2007), Ren et al. (2009a, 2009b), Ren and Wang (2010), Zhang et al. (2011)

Source: Bae et al (2014)

(?) Indicates that the year of development could not be determined.

A Number in parenthesis represents the first use in biological indicator monitoring (i.e., paper, trademark, or registered trademark).

2.3 Medaka fish

Medaka is the tiny, fresh water, rice-field fish. Many scientists in Japan have used Medaka as a model animal, especially since the work of Aida in 1921 (Kinoshita et al., 2009). Since then, many Japanese scientists have tried to establish a certain kind of Medaka and to advance adding to the experimental methodologies using Medaka fish as a model. These advances have generated in the accumulation of the basic knowledge of biological Medaka, which has contributed to the invention of new biological facts in both human and other animal systems. They have helped to distinguish the functional mechanisms of various freshly invention phenomena in areas of both basic and applied research. Moreover, recent advances in Medaka genomics have provided new perception also into basic biology, ecological science, medical science and agricultural science, by comparative analyses with the substantial genomic information that now exists for the vertebrates such as humans, mice, etc.

2.3.1 Status of Medaka in toxicology

Commercialization of synthetic substance, such as industrial chemicals, pharmaceuticals and pesticides, is organized by authorized systems under the regulations of nations, and several screening methods to evaluate the toxicity of each chemical. In the terms of risk assessment for human or mammalian health, rodents are usually used in preliminary screening test. On the other side, in the term of ecological risk assessment, it indispensable to conduct several experiment with various fauna from bacteria to vertebrates. However, it is difficult to evaluate environmental influences for all species on the earth, with the consequence that some representative species covering the various fauna are selected as models for testing. Generally, in aquatic ecological evaluations bacteria and algae are used to model bacteria and phytoplankton, crustaceans represent the invertebrate model, and fish represent vertebrate model. Toxicity test using fish are performed in a lot of nations of the world. From the view of international regulations, the Organization for Economic Co-operation and Development (OECD) recommended test guidelines for chemical evaluation, and the majority guidelines using fish recommend the Japanese Medaka as one model test species. Among the fish species recommended as test model, much attention has been paid to the Medaka by many scientists and researchers for the following reasons (Kinoshita et al., 2009):

1. The lifecycles is shorter than with other species testing can be conducted within a year.
2. Fish size is smaller than other species, so the volume of test water can be reduced, such that cost of treating waste can be lessened.
3. It is easy to identify both the physiological sex type by external sex characters and genetic sex type by the detection of the male specific gene.
4. In particular, because the Medaka is a local species in east Asian countries like Japan, Korea and China, the scientist in these countries have a great deal of interest in the development of Medaka toxicity.

In the toxicology test, the potency and quantitative activity (dose effect) of the chemicals both are evaluated. Therefore, rearing and test conditions should be strictly controlled. This means that special care is required for feeding, rearing, water conditions and handling, compare to other biological experiments in developmental biology, genetics, physiology and endocrinology.

2.3.2 International standardization for toxicity tests

The objective of toxicity test is to understand the impact of substances such as industrial chemical, pharmaceutical and personal care products. These substances are essential in most human activities and they are generated for domestic consumption and also international trade. Furthermore, chemical migration happens through the influence of climatic and/or geographic conditions. For example, polluted air is carried by monsoons and wastewater is carried to others countries in international rivers. Therefore, the international regulation of toxicity test is required to regulate chemicals with a consensus between countries. Accordingly, some of testing methods have been standardized by some international organization such as the International Organization for Standardization (ISO), the European Commission and the OECD. The most typical is the chemical toxicity test guidelines standardization by the OECD.

However, some analysis methods may not be appropriate to evaluate environmental risks in some countries, even if testing protocols are strictly controlled. For example, some subarctic species such as rainbow trout are not appropriate for testing in a temperate environment. Moreover, some local species such as fathead minnows that originated in North America are not found in Asian countries, so they

are not available for environmental risk evaluation in those countries. From this, the fish chemical test guidelines are updated for some recommended species. In OECD test guidelines, the Medaka is recommended as a model for the following test:

1. Fish acute toxicity test (TG203)
2. Fish prolonged toxicity test: 14 days (TG204)
3. Fish early-life stage toxicity test (TG210)
4. Fish short-term toxicity test on embryo and sac-larvae stages (TG212)
5. Fish juvenile growth test (TG215)

2.4 The impact of the environment upon humans

The element of air, water and land may host harmful biological and chemical agents that influence the health of humans. Various kinds of communicable disease can be spread through elements of the environment by human and animal waste product. This is most clearly evidenced by the plagues of the middle ages when disease spread through rats that fed on contaminated solid and human waste and disease carried by waterborne parasites and bacteria ran rampant through the population.

It has only been in the last century that the correlation between waterborne biological agents and human disease has been proved and effective preventive measures have been taken. Through immunization and environmental control program, the major diseases transmitted via the environment have all but been eliminated in developed countries. No countries, however, is totally immune from outbreaks of environmentally transmitted disease. The transmission of viruses and protozoa has proved particularly difficult to control, and lapses on good sanitary practice have result in minor epidemics of other waterborne disease.

Other environmentally related health problems also concern the environmental engineer. The widespread use of chemicals in agriculture and industry has introduced many new compounds into the environment. Some of these compounds have been diffused in small quantities throughout the environment, while others have been concentrated at disposal sites. Such chemicals may be spread through air, water and soil as well as through the food chain, and thus pose a potential threat to all humans.

The pesticide DDT was used extensively during the mid-century decades and has been instrumental in the elimination of malaria in many parts of the world. In

addition, this pesticide was used extensively to control insect pests on food and fibre plans. Subsequent research, however, has shown that DDT is a cumulative toxin that has adversely affected many non-target species. Traces of DDT can be found in almost all living organism throughout the world – including humans. Although the use of DDT is now banned in the United States and several other countries, the chemical is still being manufactured, primarily for use in several developing countries, particularly in tropical zones where its benefit are still considered to outweigh its liabilities.

A more recent example of chemical toxins that threaten health is chemical dioxin. The formation of this chemical, the scientific name of which is 2,3,7,8-tetrachloro-dibenzoparadioxin, is an unintentional by product of manufacturing process used with some herbicides ad wood-preserving compounds. It is also formed in the production of the some disinfectants and industrial cleaning compounds. Dioxin is an extremely toxic substance, and its presence in excess of 1 ppb (part per billion) in the environmental elements becomes cause of concern.

Chemicals containing dioxin residuals have been used on widespread basis during the last few decades, and the level of this chemical in general environment is not currently known. The discovery of dioxin residuals in waste-disposal sites and in soils that were contaminated through application of the parent material has caused great concern and has resulted in expensive cleaning efforts.

2.4.1 Others concerns

Clean air and water are an aesthetic delight, yet city dwellers have all but forgotten the smell of clean air, and clear, sparkling lakes, rivers and streams are becoming increasingly rare. Littered streets and highways offend, rather than delight, and unfenced junkyards and uncontrolled dumps give further evidence of the aesthetically displeasing effect of improper solid-waste disposal technic.

As pollutants enter air, water or soil, natural processes such as dilution, biological conventions and chemical reaction convert waster material to more acceptable forms and disperse them through a larger volume. Yet those natural processes can no longer perform the clean-up alone. The treatment facilities designed by the environmental engineer are based on the principles of self-cleansing observed in nature, but the engineered processes amplify and optimize the operations observed

in nature to handler larger volumes of pollutants and to treat them more rapidly. Engineers adapt the principles of natural mechanism to engineered systems for pollution control when they construct tall stacks to disperse and dilute air pollutants, design biological treatment facilities for the removal of organics from wastewater, use chemicals to oxidize and precipitate out the iron and manganese in drinking-water supplies, or bury solid wastes in controlled landfill operations.

2.5 The potential risk from combinations of chemicals in the environment

The potential risk from combinations of chemicals in the environment has long been a concern. However, this issue has recently moved up the scientific, regulatory and political agenda, with the realisation and demonstration that man and his environment are continually exposed to a variety of chemical compounds, not singly but in combination. This has led to concerns that there must be some impact from exposure to chemical mixtures or a 'cocktail effect'. The natural environment is of course a mixture of chemicals, although the focus is often on man-made compounds - products of the 'chemical industry' - or perhaps those natural compounds which are emitted into the environment through industrial activity such as metals and mining. Indeed, the term 'mixture' is readily applied to the ecotoxicology and environmental risk assessment of chemicals. However, there are different kinds of mixtures to consider which can be classified into 4 broad categories:

1. Multi-constituent substances (e.g. defined reaction products such as isomeric mixtures) and UVCB substances - substances of unknown or variable composition, complex reaction products or biological materials - such as petroleum oils, natural dyes and essential oils.
2. Chemical formulations and preparations made by blending two or more different substances in specific proportions such as plant protection products, biocides, pharmaceuticals and other consumer products.
3. Mixtures of chemicals likely to occur due to the release of chemicals in the environment co-occurring in time and space, such as effluents or tank mixed plant protection products. Effluents may be relatively stable and continuous - such as refinery effluents - or fluctuating in concentration and chemical composition such as discharges from waste water treatment plants in urban areas.

4. Complex mixtures in the environment of unknown composition, consisting of anthropogenic discharges together with natural sources of chemicals.

Whilst the theory of mixture toxicity has received increasing attention over recent years, both in toxicology and ecotoxicology, for ecotoxicology and subsequent environmental risk assessment, the long held principles of concentration addition still seem to provide a generally reliable, albeit conservative, estimate of toxicity, with worse than additive (synergistic) effects being rare (ECETOC, 2001; Kortenkamp et al, 2009). This means we can usually predict the toxicity of mixtures, for risk assessment purposes, when either the chemical components of a mixture are known or it is characterised through summary parameters. These risk assessments tend to focus on defined mixtures, such as chemical products (petrochemical mixtures, pesticides, biocides, etc.) or perhaps on those chemicals considered likely to be released together or to co-occur in the environment. However, it is clearly more problematic to assess the potential interaction of chemicals in mixtures when not all the components are known and to determine the potential impact of all chemicals present in the environment. This can leave industry vulnerable to criticism, in particular, for not determining whether chemicals present in the environment, including those at concentrations below their respective predicted no effect concentrations (PNECs), act additively to cause an overall effect, the so-called "something from nothing" effect.

Since both the chemical industry and the water industry have stakes in ensuring good water quality, this approach may facilitate future co-operation, i.e. a wider multi-sector involvement in understanding the true impact of chemicals and the effectiveness of treatment infrastructure. To develop this retrospective approach further an ECETOC Task Force was commissioned with the following Terms of Reference:

1. Review field based approaches for assessing impacts on the aquatic environment and develop guidance on suitable methods.
2. Using case studies, identify research needs, including how methods can be implemented, what diagnostic tools are required.
3. Consider the value of retrospective assessment in assessing environmental capacity for future industrial development.

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CHAPTER 03
TOXICITY TEST USING MEDAKA (*Oryzias latipes*) AND CONCENTRATED SAMPLE WATER AS AN INDEX OF AQUATIC HABITAT CONDITION

3.1 Introduction

For more than a century in the USA, federal law has been applied to protect water resources. The most important language in that law is its powerful objective "to restore and maintain chemical, physical, and biological integrity of the Nation's waters." More recently, Australia and New Zealand's water quality guidelines (ANZEEC 1992), Australia's 2004 National Water Initiative, Japan's River Law (Tamai 2000), and the European Water Framework Directive (European Commission 2000) have also been focusing their attention on the biology of waters. As the focus on biology spreads to various new regions, demands for more effective biological monitoring (sampling the biota of a place) and biological assessment (using samples of living organisms to evaluate the biological condition or health of places) have been developed accordingly.

In the past, environmental water quality standards have an effect mainly on human health. Recently, ecological correctness has increasingly received attention since it has been recognized that trace toxic substances such as endocrine disturbing chemicals and dioxins have also adverse effects on organisms. Therefore, bioassay is re-evaluated. Bioassays can be used as assay on the toxicities from multicomponent chemicals or chemicals whose toxicity has not yet been evaluated (Sakai 2001). Although information about acute toxicity of each chemical compounds has been accumulated, sometimes concentration of these compounds in environmental water is too low to show toxicity. However, many people feel that the deterioration of aquatic life in rivers may occur even when there is no visible reason. Although water quality data such as biochemical oxygen demand (BOD) and chemical oxygen demand (COD) can be monitored, it is rather difficult to see the relationship between water quality and aquatic organisms.

A toxicity test using Medaka fish and 100-fold concentrated water had been proposed. In the previous study (Liu et al. 2007a); an efficient larval Medaka

(*Oryzias latipes*) assay has been developed. Organic toxicants were 10–100 times concentrated from 4 L of river water with disposable commercial adsorption cartridges (Liu et al. 2007a). The Japanese Medaka can be raised easily in a laboratory with limited space (Marsh et al. 2010). It has been used as a surrogate for many studies in environmental toxicology and developmental biology due its transparent chorion and its relatively large size, making it useful to facilitate observation (Chen et al. 2001). This species is assumed to have consistent reproductive capacity throughout the year (Metcalfe et al. 1999).

In this research, we demonstrate applicability of this method to various samples including seawater, and show a relationship between toxicity of 100-fold concentrated water and aquatic habitat conditions. Additionally, we give some discussion on the relationship between toxicity and organic pollution.

3.2 Materials and Methods

3.2.1 Sampling Waters

To demonstrate applicability of this method to various samples including sea water, we collect water samples from three major urban and one rural areas in Japan: “T” (“T” bay, “A” river), “O” prefecture (“O” bay, “Z” river), “N” prefecture (“Is” bay, “Tt” bay), and “Y” prefecture (“U” port, “H” river area, “K” river area, and “L” river area). Residential, commercial, and industrial sites are heavily concentrated on these regions. All the observed areas are enclosed with coastal sea and they sometimes show red and blue tide by eutrophication. The “A” River has the highest BOD value (4 mg/L) among the first-grade rivers in Japan. “Z” River has high BOD value (5 mg/L) especially at the downstream. We conducted water sampling from these areas on a fine day in September.

Figure 3.1 shows the sampling location for aquatic habitat condition. Sampling points in “Y” prefecture are classified into two categories, sea area in industrial zone and rivers in rural and residential zone. Six sampling points in “U” port are located in the midst of chemical industrial area. On the other hand, in “K” river, the upper and middle basin is mainly a hilly and an agricultural area. Some sampling points at the tributary of “K” river are the habitat of fire fry. Downstream area runs through the nearby residential area. “L” River is rather small at 8.3 km length and a basin of 18.8 km^2. The tributaries at “L” river area run through

residential area which can lead to pollution by household waste. Samplings were taken at upper stream (without effect from household effluent) and downstream (with the household effluent effect). Additionally, we get sampling at low tide and a high tide at the mouth of the river. The “H” river has an area of 30 km length and a cover area of 322.4 km^2. The tributary is famous as a habitat for fire fry. The “H” river area is the habitat of endangered *Lethenteron reissneri* and Medaka. Table 3.1 shows water quality data together with aquatic habitat sampling information of rivers.

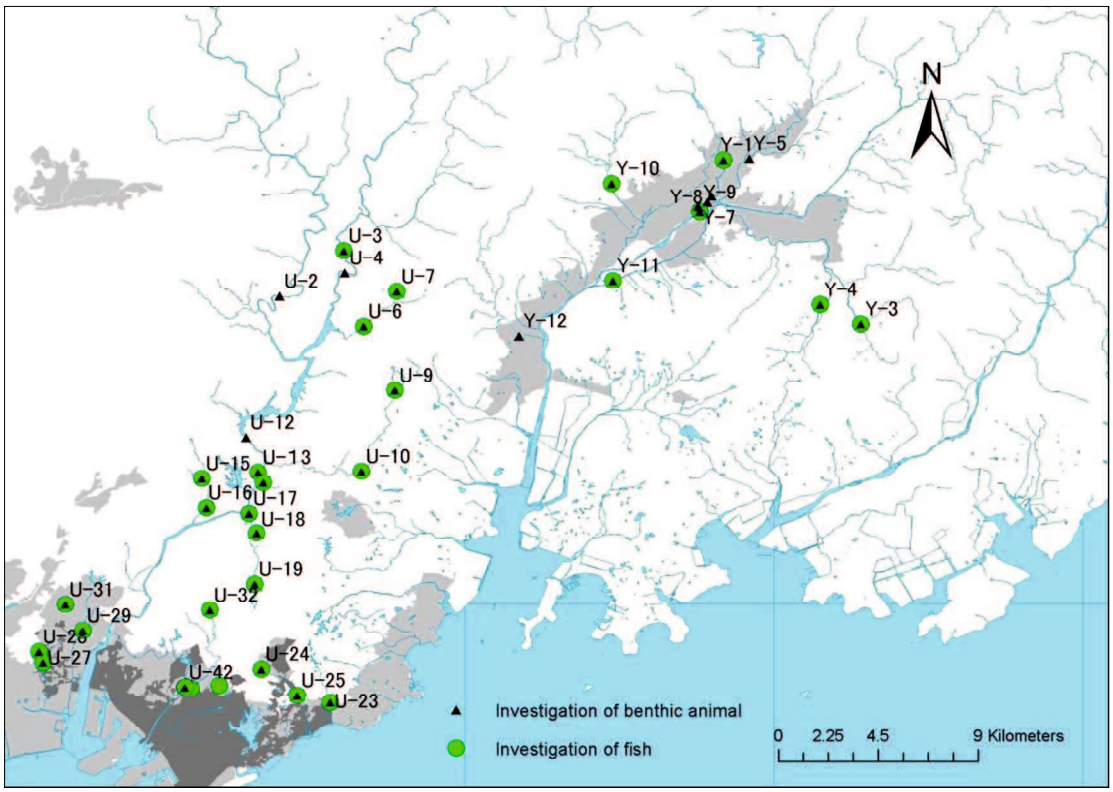

Fig. 3.1 - Sampling Location for Aquatic Habitat Condition

Table 3.1 - Water Quality Data of Rivers

Station	ASPT	IBI	Sampling date	Water temperature (°C)	Dissolved Oxygen	pH	Conductivity (μS/cm)	Turbidity (NTU)	Salinity (%)	$NO_{2,3}$-N (mg/L)	NH_4-N (mg/L)	PO_4-P (mg/L)	Ca^{2+} (mg/L)	BOD (mg/L)
U-2	a		2002-08-07	28.70	8.93	8.29	0.188	2	0.00	0.301	0.026	0.007	2.600	0.550
U-3	a	b	2002-08-07	28.20	8.77	7.86	0.145	1	0.00	0.331	0.028	0.051	1.610	0.620
U-4	a													
U-6	a	b	2004-01-14							0.328	0.018	0.015	2.380	2.944
U-7	a	b	2004-01-14							0.351	0.086	0.027	2.200	2.462
U-8			2001-12-18	7.60	10.88	7.32	0.169	29	0.00					
U-9	a	b	2004-02-03							0.292	0.013	0.020	2.250	1.829
U-10	a	b	2003-02-06	7.70	11.12	7.60	0.167	23	0.00	1.747	0.011	0.009	2.510	2.220
U-11	a	b	2002-10-10	18.30	7.35	7.65	0.830	1	0.00	0.022	0.007	0.004	0.281	1.046
U-13	a	b	2002-11-16	14.00	10.45	7.44	0.179	2	0.00	0.509	0.021	0.010	1.120	0.654
U-15	a	b	2002-10-30	15.90	9.45	7.80	0.088	4	0.00	0.151	0.099	0.006	0.159	0.636
U-16	a	b	2002-10-30	16.90	10.24	6.25	0.117	1	0.00	0.127	0.017	0.003	0.304	1.119
U-17	a	b	2002-10-06	20.50	8.95	7.63	0.175	0	0.00	0.637	0.191	0.045	0.576	0.953
U-18	a	b	2002-10-06	20.00	9.05	7.50	0.162	0	0.00	0.477	0.029	0.018	0.672	0.638
U-19	a	b	2002-10-10	19.60	10.14	8.56	0.143	0	0.00	0.474	0.025	0.023	0.655	0.777
U-21			2001-12-18	9.40	10.97	7.18	0.145	10	0.00					
U-23	a	b	2002-09-26	22.60	6.50	7.77	0.272	2	0.01	1.022	0.659	0.164	1.530	2.019
U-24	a	b	2002-10-03	26.00	8.21	8.74	0.188	1	0.00	0.674	0.026	0.045	1.120	1.621
U-25	a	b	2002-09-26	23.50	6.29	7.50	0.311	1	0.01	0.805	0.041	0.081	1.750	1.410
U-26			2001-12-10	11.20	10.05	7.91	0.221	10	0.00					
U-27	a	b	2002-12-24	11.50	8.85	7.53	0.413	3	0.01	1.233	0.712	0.103	7.060	5.740
U-28	a	b	2002-02-04	6.10	11.95	7.15	0.384	17	0.00	0.487	0.221	0.060	8.320	2.130
U-29	a	b	2002-12-24	12.70	6.79	7.52	0.417	32	0.01	1.947	3.509	0.270	6.110	8.140
U-31	a	b	2002-08-02	29.60	9.58	8.27	0.253	8	0.00	3.488	0.135	0.481	2.060	2.920

Table 3.1 - (continued)

Station	ASPT	IBI	Sampling date	Water temperature (°C)	Dissolved Oxygen	pH	Conductivity (µS/cm)	Turbidity (NTU)	Salinity (%)	$NO_{2,3}$-N (mg/L)	NH_4-N (mg/L)	PO_4-P (mg/L)	Ca^{2+} (mg/L)	BOD (mg/L)
U-32	a	b	2002-09-10	23.60	7.80	6.23	0.108	2	0.00	0.324	0.012	0.017	0.207	0.558
U-33			2002-02-01	8.30	11.42	7.91	0.304	7	0.00	0.580	0.085	0.017	1.300	1.830
U-34			2002-02-01	8.20	11.20	7.88	0.305	6	0.00	0.580	0.085	0.017	1.300	1.830
U-35	b		2002-02-14	8.40	13.97	9.36	0.281	8	0.00					
U-38	b		2002-02-14	9.10	11.15	7.79	0.336	3	0.00					
U-39			2002-02-14	7.80	11.51	7.85	0.253	4	0.01					
U-40	b		2001-12-10	11.80	9.41	7.63	0.306	9	0.01					
U-41	b		2002-01-03	4.40	12.35	7.30	0.241	2	0.01					
U-42	a	b	2002-09-10	25.90	5.72	6.29	0.271	5	0.01	1.239	0.158	0.033	0.756	2.432
Y-1	a	b	2003-02-11	9.10	10.80	7.38	0.075	14	0.00	0.452	0.010	0.021	0.591	1.430
Y-2	a		2003-02-11	9.90	9.85	7.38	0.094	82	0.00	0.697	0.069	0.027	0.713	1.890
Y-3	a	b	2003-02-04	8.70	10.08	7.21	0.108	4	0.00	0.738	0.050	0.030	0.687	6.480
Y-4	a	b	2003-02-09	11.20	10.65	7.14	0.116	8	0.00	0.289	0.008	0.013	0.839	1.580
Y-5	a		2002-01-17	11.20	10.92	7.33	0.141	23	0.00	0.833	0.037	0.031	0.092	1.870
Y-6	a		2003-02-09	9.80	10.70	7.49	0.087	55	0.00	0.977	0.022	0.033	0.922	3.010
Y-7	a		2002-01-17	11.40	8.82	7.97	0.140	6	0.00	0.662	0.018	0.035	0.099	2.370
Y-8	a	b	2003-10-21							0.879	0.027	0.057	1.400	0.888
Y-9	a		2002-01-17	11.40	10.96	7.52	0.142	5	0.00	0.807	0.025	0.035	0.968	2.120
Y-10	a	b	2003-08-25							0.554	0.012	0.034	0.921	0.656
Y-11	a	b	2002-02-19	7.40	14.25	7.37	0.186	10	0.00	1.213	0.037	0.082	1.170	3.420
Y-12	a		2003-08-25							0.340	0.028	0.016	1.090	0.690

The most important purpose of this study is to show the relationship between toxicity and aquatic habitat condition. We conducted a survey on aquatic habitat condition at river areas ("H", "K", and "L" river areas) which are mostly residential areas and excluding three major urban areas in Japan ("A", "Z" and "N").

3.2.2 Pre-concentration of Organic Toxicants and Medaka (*Oryzias Latipes*) Acute Toxicity Test

Organic toxicants were concentrated from river waters with solid-phase extraction using disposable commercial Sep-Pak® Plus PS-2 cartridges. This cartridge was selected due to the porous styrene-divinyl benzene copolymer PS-2, with a surface area of 660 m^2/g and an average particle diameter of 80 μm. It is applicable for a wide range of pH (1–13) and it has tens to hundreds times higher absorbable volume than the conventional adsorbent of reverse phase C-18 (Ishii et al. 2000; Nakamura et al. 2001).

Filtrated with 1-μm glass filter, the sample was loaded into precondition Sep-Pak® Plus PS-2 by a glass syringe pump at 10 ml/min. Two cartridges were set in series and 5 L filtration was loaded with 10 ml/min flow rate. Hydrophobic organic matters are adsorbed at PS-2 and desorbed by 10 ml acetone from each Sep-Pak® Plus PS-2 cartridges. Air was injected into the cartridge with a syringe to drive out the space water. The 20 ml eluate acetone solution was evaporated to 200 μl under the purge of nitrogen gas. This is diluted with activated carbon treatment water to 50 ml and separated by 25 and 25 ml. Ten Medaka fish are exposed to each of these.

In the toxicity test, every 10 individuals of 48–72 h post-hatch age larvae were exposed for 48 h to 25 ml of each test solution in a glass Petri dish of 90 mm diameter and 40 mm depth. Experiment condition is 25±1°C and light irradiation time is 16 h/day. Used as control sample was 25 ml of active carbon treatment water. No water ventilation and food were supplied. The number of death and disorder of Medaka are counted at 1, 2, 3, 6, 12, 24, and 48 h. If the death rate of the samples exceeds 10 %, the whole experiment is considered invalid. The method of 100-fold concentrated 48-h test is used as a screening; and once toxicity is found, tests using lower concentrations are repeated until no toxicity is found (Liu et al. 2007b). In this research, the procedure is modified to obtain result quickly but as quantitatively as possible. Conducted alone is the 100-fold concentrated 48-h test, and it disclosed

toxicity that is the inverse of median effect time and median lethal time (ET_{50}^{-1}, LT_{50}^{-1}).

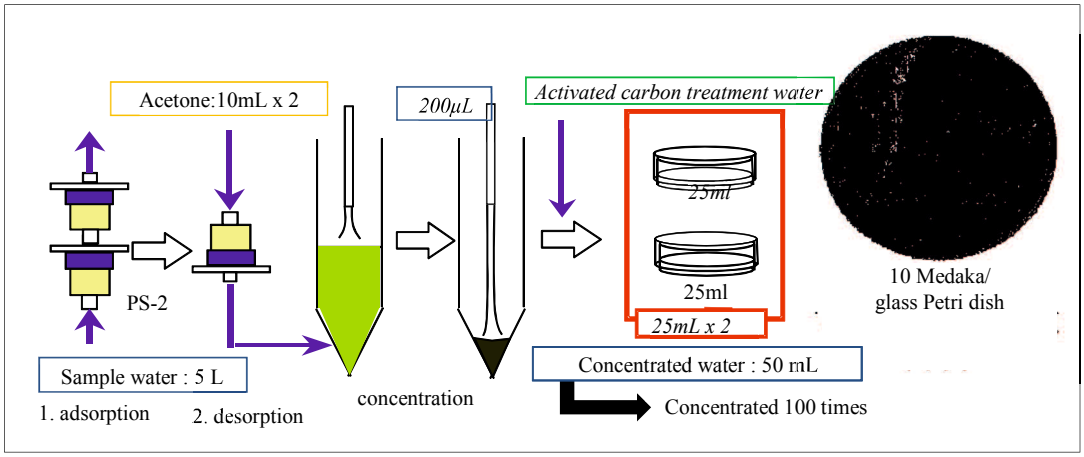

Fig. 3.2 - Material and Method of Sampling Water

3.2.3 Gas Chromatograph/Mass Spectrometer (GC/MS) simultaneous analysis database

A Shimadzu GC-2010 gas chromatograph (Kyoto, Japan) coupled with a Shimadzu QP2010 mass spectrometer was used for GC/MS analysis. The gas chromatograph was fitted with fused silica capillary column J&W DB-5 ms (30 mm×0.25 mm i.d., 0.25 μm film thickness). The following oven temperature was initiated at 40°C, increased at the rate of 8°C/min to 310°C. The carier gas was helium at a constant flow of 40 cm/s. Injector, interface, and ion source were kept at 250, 300, and 200°C, respectively. Splitting ratio was 20:1. Electron impact mass spectra were taken at 70 eV. Scan at 0.2 scans/s from 33 m/z to 600 amu. GC/MS simultaneous analysis database can identify and quantify 942 chemical compounds altogether without standard substance. The source of toxicity could be analyzed by using this system (Kadokami et al. 2005).

For the sampling water, 1 L sample water is passed into Sep-Pak® Plus PS-2 cartridges, and organic matters are desorbed by 10 ml acetone. Nitrogen purge evaporates all the acetone, and moderate amount of hexane is added. Sodium sulfate is appended to get rid of moisture; after that, sodium sulfate is removed. Hexane is evaporated to 1 ml.

3.2.4 Aquatic Habitat Condition

Since the rivers run mainly in rural and residential area, we can assume that there is no metal or other industrial pollution. Sampling points are chosen from fish-abundant area where the depth is shallower than the knee and flow is calm.

Average score per taxon (ASPT) column in Table 3.1 shows macrobenthic animals sampling points in “K”, “L”, and “H” river areas in “Y” prefecture. The surveys were conducted on February, May, July, and October, a total of four times. Macrobenthic animals were collected from four points in each spots. Placed at each point is 25×25 cm quadrate. We revealed that the taxonomic group exists and that it is a division of a family.

Data of survey on macrobenthic animal (Diamond and Daley, 1999) are organized based on ASPT method. ASPT is the average of scores found at a sampling point. The score is runs from 1 to 10. A lower score indicates a more tolerant family group. However, a higher score indicates a better environment. ASPT is a water quality index reflecting aquatic habitat conditions. Organic pollution of the river and aquatic environment can be evaluated by ASPT. ASPT has been said to have a correlation with pollution index or diversity index (Kumiko et al. 1993).

The Index of Biotic Integrity (IBI) column in Table 3.1 shows investigation areas for survey on fish in “K”, “L”, and “H” river. The surveys were conducted from July to October. Fishes were caught with hand and casting nets (18 or 24 mm). Casting net is targeted at shellfish, and the survey is conducted every 15 min by four persons. We identified the species of the fishes we caught, took pictures of them, and counted their numbers. The mode of life for each species will be shown. In this study, the fish habitat condition of each observed location was evaluated by IBI firstly proposed by Karr (1981). The original IBI (Karr 1981) is based on the observation of 12 items. However, in this study, we used 10 items shown in Table 3.2 as proposed by Koizumi (1997).

3.2.5 Statistical Analysis

Relationships between ET_{50}^{-1} and BOD or COD were analyzed quantitatively with Spearman rank correlation test model. This analysis is used to determine the relationship between influence variables (x variable) to the affected variable (y variable). The relationship between toxicity and ASPT were analyzed with Pearson

product moment correlation coefficient and the relationship between toxicity and aquatic habitat conditions were analyzed using partial regression coefficient method.

Table 3.2 - Concepts and Items of IBI Modified by Koizumi (1997)

Concept	Item
A. Variety of species	1. Number of native species
	2. Number of natatorial species
	3. Number of demersal species
B. Tolerance of species	4. Existence of weakly species
	5. Ratio of tolerance fishes (%)
C. Exotic species	6. Ratio of exotic fishes
D. Health of fishes	7. Ratio of disorderly, deformed and injured
E. Ecological condition	8. Ratio of incent-eating fish
	9. Ratio of plant-eating fish
F. Productivity of fishes	10. Number of fishes

3.3 Results and Discussion

3.3.1 Toxicity Test Using Medaka Fish and Concentrated Water

Figure 3.3 shows results of the toxicity test using Medaka fish and concentrated water. The different levels of toxicity were detected in seawater from the industrial zones of “T”, “N”, “O”, and “Y” prefecture. At “H” river area, toxicity cannot be detected. In rivers, high toxicity appeared at urban districts without sewerage.

From the analysis using Spearman coefficient, the relationship between toxicity and BOD coefficient values was obtained at 0.313, with a value of z=1.715. It can be decided that there is a relationship between toxicity and BOD. It means that high BOD household wastewater also contains hydrophobic toxic matters (Figure 3.4). Meanwhile, for the relationship between toxicity and COD, the results of analysis of the coefficient values was obtained at 0.277, with n=12 and α=0.05; it can be concluded that there is no relationship between them. It is believed that seawater in industrial area does not show clear relationship between toxicity and COD (as shown in Fig. 3.5).

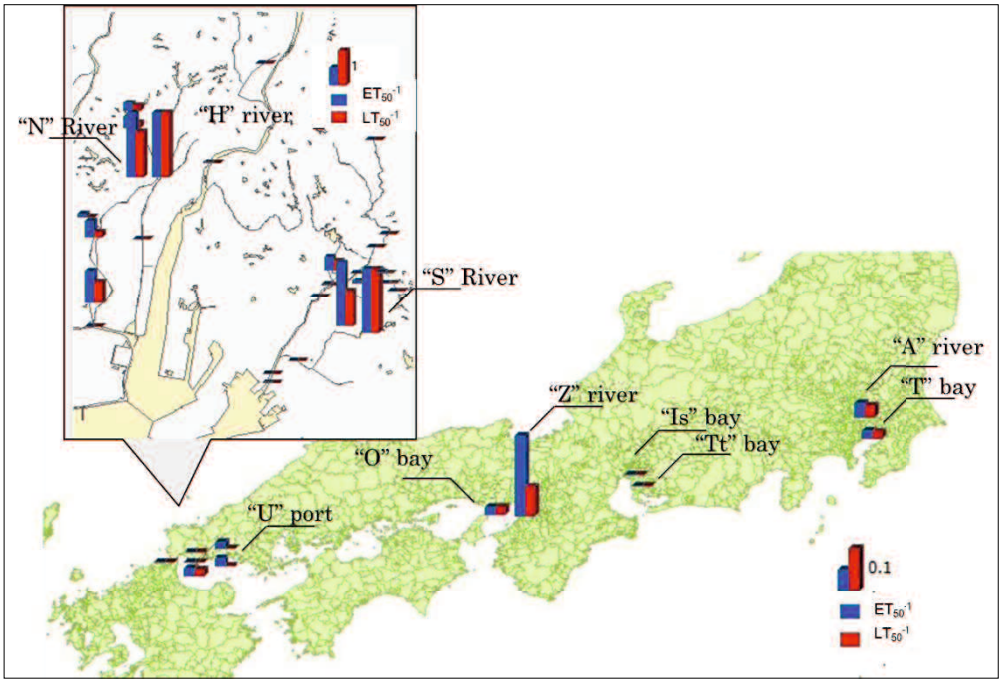

Fig. 3.3 - Result of toxicity test

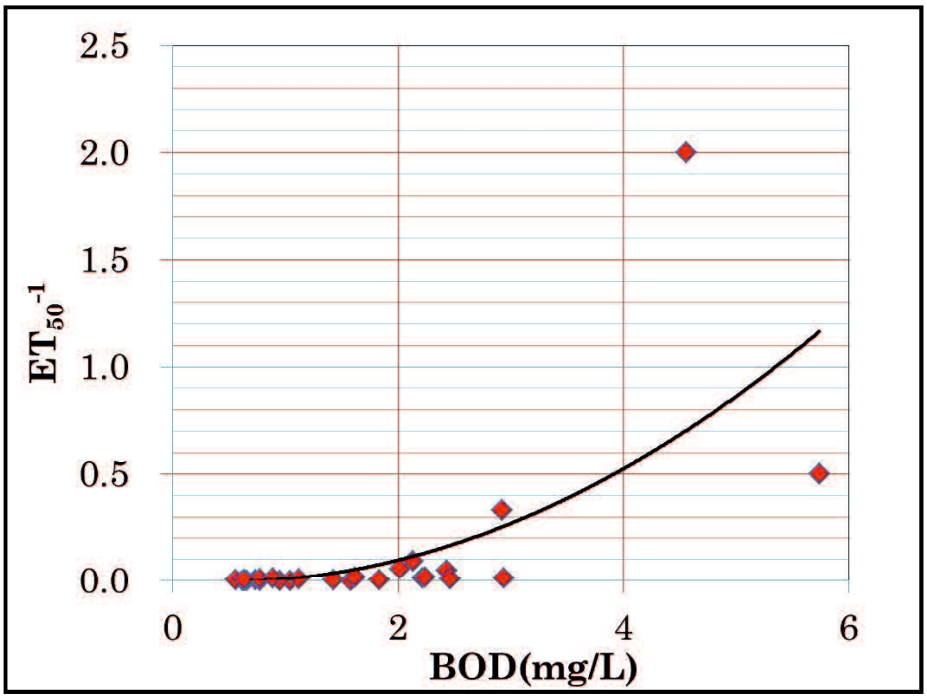

Fig. 3.4 - The relationship between ET_{50}^{-1} and BOD

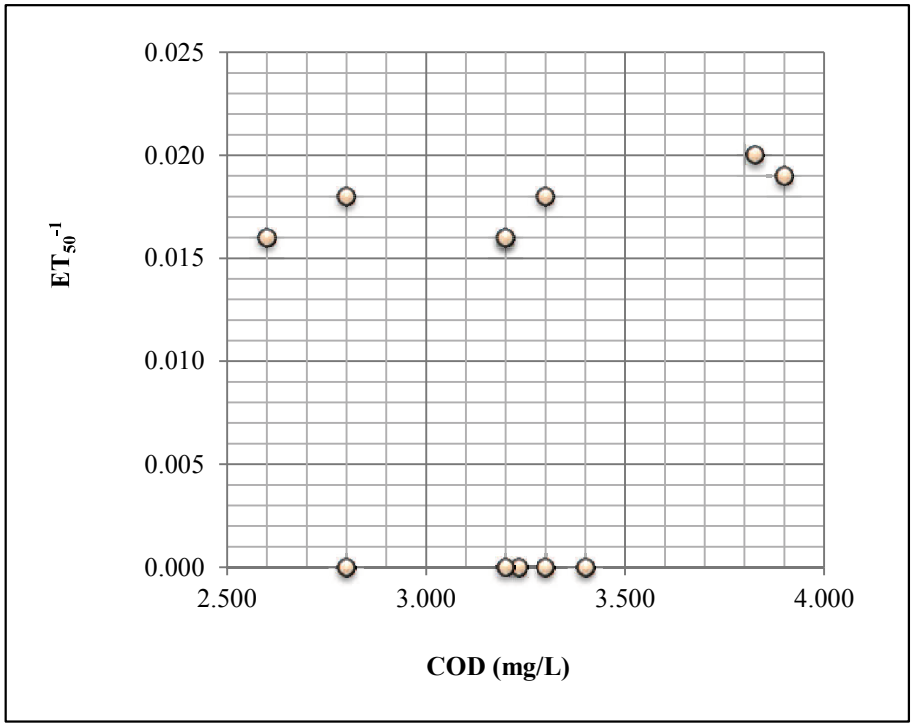

Fig. 3.5 - The relationship between ET_{50}^{-1} and COD

3.3.2 The Result of Analysis Using GC/MS

Four samples with high toxicity (from "Z" river, "O" bay, "S" river at "M" river area, and "N" river at "K" river area) were analyzed using GC/MS simultaneous analysis database. Table 3.3 shows the result of toxicity test. Table 3.4 shows the concentration and acute toxicity of the detected chemicals. Table 3.5 shows the concentration of compounds which contain high toxicity. The concentration of the detected chemicals and group of compounds are the values after 100-fold concentration. The acute toxicity is 96 h-LC_{50} and adult Medaka is used. The method conforms to the Organization for Economic Cooperation and Development test guide line 203 "fish, acute toxicity test". Since the "Z" River is connected to "O" bay, most chemical compounds detected at "O" bay are also contained in the sample taken from "Z" River. The highest toxicity is detected at "S" River. In Table 3.4, a number of detected compounds and the sum of the concentration of all compounds are mostly high values at "S" River. Furthermore, Table 3.5 shows that high concentrate *phthalic acid ester* is detected at "S" River. *Phenol* has higher concentration in the "Z" River sample than the other samples. It is considered that individual samples have other source of toxicity.

Table 3.3 - Result of Toxicity Test

	"Z" River	"O" Bay	"S" River	"N" River
ET_{50}^{-1}	0.202	0.020	2.000	2.000
LT_{50}^{-1}	0.075	0.020	2.000	0.939

Table 3.4 - Concentration of Acute Toxicity and Detected Chemicals

Name	Acute Toxicity (ppm)	"Z" River (ppm)	"O" Bay (ppm)	"S" River (ppm)	"N" River (ppm)	Blank (ppm)
Naphthalene				0.003	0.002	
3-4-Methylphenol	14	0.017		0.046		
2,4-Dimethylphenol	16	0.002				
4-Methyl-2,6-di-t-butylphenol	1.1	0.002				
4-tert-Octylphenol	0.36	0.004				
Nonylphenol	0.24	0.250				
2,5-Dichlorophenol		0.003				
2,4,5-Trichlorophenol	1.5	0.005				
Triclosan	0.67	0.013				
Dimethyl phthalate		0.009	0.003	0.007	0.003	0.00071
Diethyl phthalate		0.008	0.004	0.004	0.006	0.00136
Diisobutyl phthalate	3	0.022	0.007	0.020	0.033	0.00259
Di-n-butyl phthalate	2.8	0.104	0.039	0.085	0.168	0.01824
Butyl benzyl phtalate	1.08	0.026	0.003			
Bis(2-ethylhexyl) phthalate	75	0.229	0.075	2.216	1.673	0.29662
Stearic acid methyl ester		0.003				
2-Ethyl-1-hexanol		0.018		0.011		
Phenylethyl alcohol		0.005		0.009		
Ethanol, 2-phenoxy-		0.017	0.006	0.643		
Di (2-thylhexyl) adipate	50	0.010				
Aniline	27	0.009				
2-Methylaniline		0.001				
Quinoline		0.005	0.002			
Formamide, N-cyclohexyl-		0.011				
Tris(2-chloroethyl) phosphate		0.058	0.011		0.014	
Diethyltoluamide		0.018	0.002		0.005	
Crotamiton		0.388	0.056	0.108	0.141	

Table 3.5 - Concentration of Group of Compounds

	"Z" River	"O" Bay	"S" River	"N" River
ET_{50}^{-1}	0.20	0.02	2.00	0.94
Phthalic Acid Ester	0.00	0.00	2.33	1.90
Phenol	0.00	0.00	0.05	0.00
Sum of Conc. Off all compounds	2.15	0.45	4.26	3.16
Number of detected compounds	51	31	37	30

3.3.3 Relationship between Toxicity and Macrobenthic Animal

Table 3.6 shows the existence of macrobenthic animal for each spot and score for each family. We found 28 families from 38 investigated spots. At U-29 of investigation points, there were no families; and the highest toxicity was detected. Some areas that have high score, for example Y-5 or U-3, have lower toxicity. From the calculations using the Pearson product–moment correlation coefficient, obtained correlation coefficient between toxicity and ASPT are -0.773 (ET_{50}^{-1}) and -0.742 (LT_{50}^{-1}) at 1 % level of significance with a high negative correlation.

Macrobenthic animals were classified into three groups with scores of 10–8, 7-5, and 4-1. Maximum catch number for score 10-8 is 12. Maximum catch number for score 7-5 is 5. The maximum catch number of score 4-1 is 5.

Figures 3.6, 3.7, and 3.8 show the relationship between toxicity and the number of family groups for each category. Incidentally, not only water quality but also physical environment reflects habitat condition for benthic animal. Therefore, it is almost impossible to have a functional relation between toxicity and aquatic habitat condition. In Fig. 3.9, a comprehensive line is drawn since toxicity limits the maximum number of family groups that may exist. All groups show inverse proportion about toxicity and number of families. Especially, higher-score group show more rapid decrease of comprehensive line. On score 10-8 group, the ratio of clear stream benthic animal sharply decreased over 0.25 of LT_{50}^{-1} or 0.5 of ET_{50}^{-1}. Tolerant fish become dominant over 0.3 of LT_{50}^{-1} or 0.5-1.0 of ET_{50}^{-1}. As an overall result, the ratio of clear stream benthic animal sharply decreased over 0.25 of LT_{50}^{-1} or 0.5 of ET_{50}^{-1}. The relationship between toxicity and benthic animal habitat condition is shown in Fig. 3.9.

Table 3.6 - Existence of Macrobenthic Animal for Each Spots

Name of Family	score	Investigation point																	
		U-2	U-3	U-4	U-6	U-7	U-9	U-10	U-11	U-12	U-13	U-15	U-16	U-17	U-18	U-19	U-23	U-24	U-25
Ephemerellidae	9	●	●	●	●	●		●	●		●	●		●			●	●	●
Heptageniidae	9	●	●	●	●	●		●	●	●	●	●					●	●	●
Ephemeridae	9	●	●	●							●								
Leptophlebiidae	9	●	●	●	●	●	●	●	●	●	●	●		●	●	●	●	●	●
Neuroptera	8	●	●	●		●					●	●		●					
Potamanthidae	8	●	●	●				●	●									●	
Baetidae	6	●	●	●	●	●	●	●	●	●	●	●		●	●	●	●	●	●
Plecoptera	9	●	●	●								●							
Nemouridae	6		●	●		●													
Annulipalpia	7	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Integripalpia	10	●	●	●				●			●					●			
Lepidostomatidae	9																		
Leptoceridae	8																		
Rhyacophilidae	9																		
Glossosomatidae	9	●	●	●		●					●	●						●	
Corydalidae	9	●	●																
Gomphidae	7	●	●	●	●		●			●	●		●					●	
Psephenidae	8	●	●	●	●	●	●		●	●	●	●		●		●	●	●	
Lampyridae	6						●		●			●							
Corixidae	2					●			●					●			●	●	●
Chironomidae	1												●				●		●
Hirudinea	2	●	●	●	●	●	●	●				●		●	●		●	●	●
Oligochaeta	1	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Dugesiidae	7							●	●										
Tipulidae	8		●				●		●						●	●	●	●	
Gammaridae	9								●									●	
Lymnaeidae	3					●	●					●					●	●	
Corbiculidae	5	●		●		●	●	●		●	●	●			●	●	●		
ET50^{-1}		0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.06	0.02	0.02
LT50^{-1}		0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
ASPT score		7.35	7.44	7.18	6.44	6.00	5.64	6.64	6.85	6.50	7.46	6.50	4.00	5.78	5.43	6.75	5.38	6.47	5.11

Table 3.6 - (continued)

Name of Family	score	Investigation point																	
		U-27	U-28	U-29	U-31	U-32	U-42	Y-1	Y-2	Y-3	Y-4	Y-5	Y-6	Y-7	Y-8	Y-9	Y-10	Y-11	Y-12
Ephemerellidae	9					●					●	●					●	●	
Heptageniidae	9				●	●	●	●		●	●	●					●		●
Ephemeridae	9											●							
Leptophlebiidae	9				●	●	●	●		●		●	●	●			●		●
Neuroptera	8																		
Potamanthidae	8											●							
Baetidae	6				●	●	●	●	●	●	●	●		●			●	●	●
Plecoptera	9							●		●						●	●		●
Nemouridae	6																●		
Annulipalpia	7	●			●	●	●	●	●	●		●	●			●	●	●	●
Integripalpia	10					●						●							
Lepidostomatidae	9											●					●		
Leptoceridae	8											●					●		
Rhyacophilidae	9							●		●		●	●				●		●
Glossosomatidae	9					●											●	●	
Corydalidae	9																		
Gomphidae	7							●	●	●									●
Psephenidae	8	●				●		●	●	●		●	●			●	●	●	●
Lampyridae	6							●		●							●		
Corixidae	2	●	●		●	●	●		●	●									
Chironomidae	1					●	●		●					●		●	●	●	●
Hirudinea	2	●	●			●	●			●		●		●				●	●
Oligochaeta	1	●	●		●	●	●	●	●	●			●	●		●	●	●	●
Dugesiidae	7					●				●									
Tipulidae	8					●				●		●					●		
Gammaridae	9					●													
Lymnaeidae	3	●	●		●									●					
Corbiculidae	5		●		●	●		●			●	●			●	●	●		
$ET50^{-1}$		0.50	0.09	2.00	0.22	0.01	0.05	0.01	0.00	0.17	0.00	0.00	0.01	0.01	0.01	0.00	0.00	0.00	0.01
$LT50^{-1}$		0.17	0.00	1.43	0.14	0.00	0.03	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
ASPT score		4.00	2.50	0.00	5.25	6.38	4.63	6.91	4.57	6.43	7.25	7.73	6.80	3.67	5.00	5.17	7.00	5.38	6.18

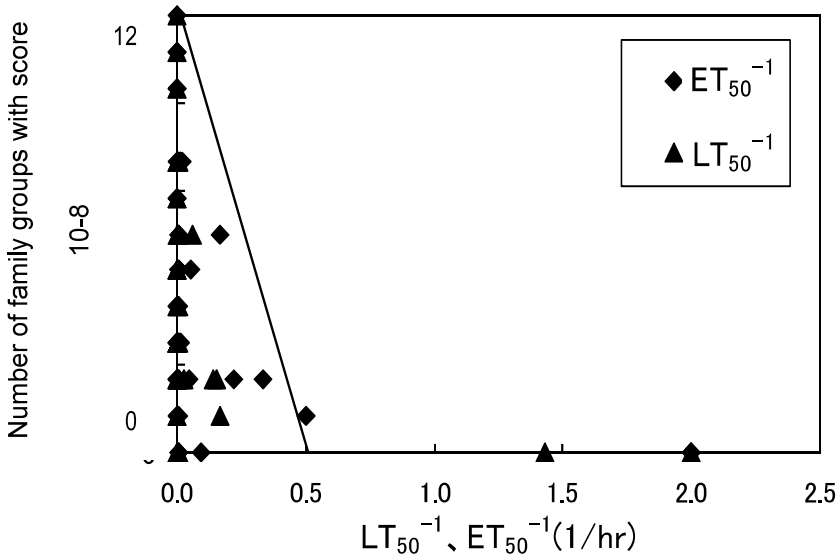

Fig. 3.6 - Relationship between Toxicity and Number of Family Groups with a Score 10–8

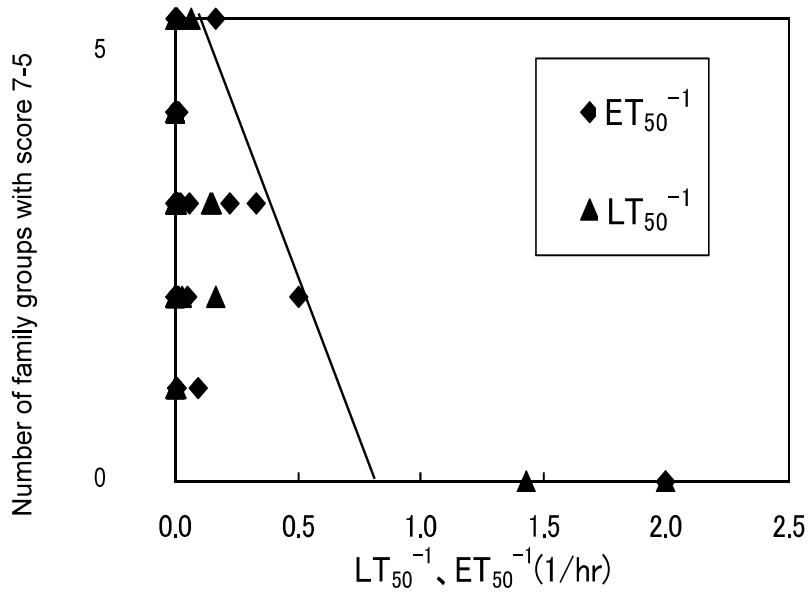

Fig. 3.7 - Relationship between Toxicity and Number of Family Groups with a Score 7–5

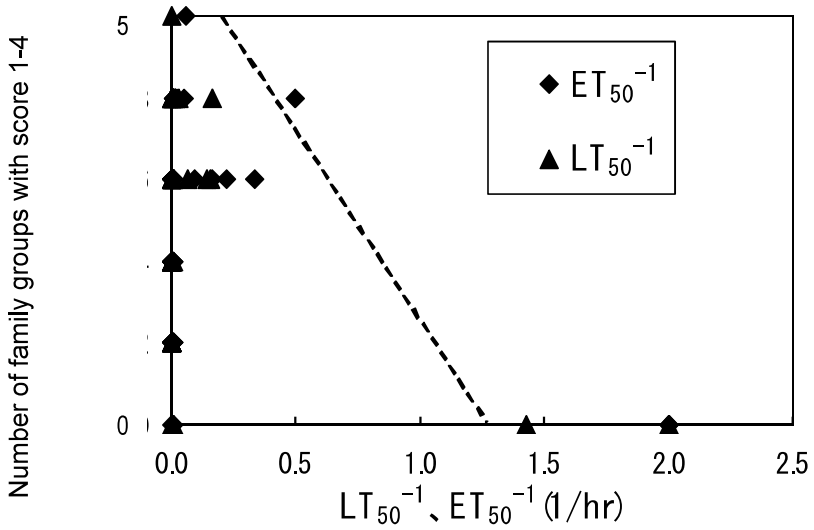

Fig. 3.8 - Relationship between Toxicity and Number of Family Groups with a Score 1–4

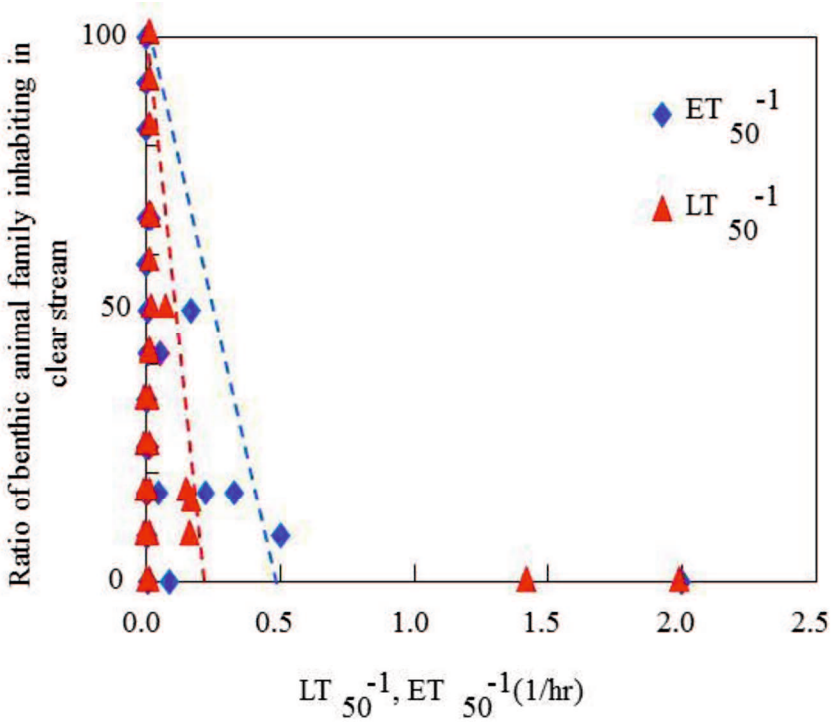

Fig. 3.9 - Relationship between Toxicity and Benthic Animal Habitat Condition

3.3.4 Relationship between toxicity and fish habitat condition

Table 3.7 shows the number of pieces for each spot and the mode of life for each species-native or exotic, natatorial or demersal-tolerance and food habitat composition for modes of life is also shown. Each point has a different character.

Nipponocypris temminckii has 537 species, which is the highest, with the highest number at U-11 by 114 species. At point U-31, Rhinogobius sp. amounted to 118 species, which is the highest total number of species that is equal to 209 species.

From the calculations using multiple regression analysis, with equation being $LT_{50}^{-1}=(\Sigma a_i \times item_i)$, the results that ratio of tolerant species has strong correlation with toxicity was obtained. Stronger toxicity increases the ratio of tolerant species. From the analysis also, it was found that IBI is influenced by environmental physical change and not only water quality. Some other factors that are influenced were the ratio of herbivore species and ratio of natatorial species, which inhabits weak species. Pearson product–moment correlation coefficient obtained correlation coefficient between toxicity and IBI is -0.155 (ET_{50}^{-1}) and -0.190 (LT_{50}^{-1}) with 1 % level of significance and has a low or no correlation between toxicity and IBI.

Toxicity (LT_{50}^{-1}) has a strong correlation with the ratio of tolerant species. It was considered that there is relationship between toxicity and tolerant or intolerant species. The maximum number of the caught tolerant species is five. The maximum number of the caught intolerant species is 12. The maximum number of the caught tolerant fishes is 73. The maximum number of the caught intolerant fishes is 201. Figures 3.10, 3.11, 3.12, and 3.13 shows the relationship between toxicity and the number of tolerant species or fishes. Stronger toxicity produces the increasing number of tolerant species or fishes. Figure 3.14 shows the relationship between toxicity and ratio of intolerant fish. Tolerant fish become dominant over 0.3 of LT_{50}^{-1} or 0.5-1.0 of ET_{50}^{-1}.

Table 3.7 - The Number of Pieces and Life Mode of Each Species for Each Spot

Name of species	Mode of life				Investigation point														
	Nv or Ex	Nat or Dem	Tolerance	Food habit	U-3	U-6	U-7	U-9	U-10	U-11	U-13	U-15	U-16	U-17	U-18	U-19	U-23	U-24	U-25	U-27
Tanakia limbata	Nv	Nat	Weak	Herbivore								2	13			11	9			4
Plecoglossus altivelis altivelis	Nv	Nat		Herbivore	1															
Squalidus gracilis gracilis	Nv	Nat		Insect	2	1			1		1		3							
Zacco platypus	Nv	Nat		Omnivore		4			12		3		7	3	3	6			9	1
Coreoperca kawamebari	Nv	Nat		Insect							2					6				
Pseudogobio esocinus	Nv	Dem		Insect	5	5			6	2	2	2	3	1	3	1				
Nipponocypris temminckii	Nv	Nat		Insect	9	29	41	21	27	114		43	2	49	3	35	11			
Pelteobagrus nudiceps	Nv	Dem		Insect	1															
Carassius langsdorfii	Nv	Nat	Tolerant	Omnivore			1		2			1	1		1		5	2		
Cyprinus carpio	Nv	Nat	Tolerant	Omnivore							1									2
Crayfish	Ex	Dem	Tolerant	Omnivore													1			1
Geothelphusa dehaani	Nv	Dem		Omnivore												1				
Palaemon paucidens	Nv	Dem		Omnivore	9	20	1	2					3		11		4	33	12	2
Hemibarbus longirostris	Nv	Dem		Insect		2							3							
matsubarai	Nv	Dem		Omnivore						1		2			4	1				
Rhodeus ocellatus ocellatus	Ex	Dem	Weak	Herbivore																
Phoxinus oxycephalus jouyi	Nv	Nat		Omnivore																
Gnathopogon elongatus	Nv	Nat		Omnivore	1					1										
Misgurnus anguillicaudatus	Nv	Dem	Tolerant	Omnivore				5							1			3	1	
Odontobutis obscura	Nv	Dem		Carnivore		2	2	16	8	7		4	7		11	5	2	9	21	1
Caridina multidentata	Nv	Dem		Omnivore	13	1	1					9	14	8		18			2	1
T. brevispinis	Nv	Dem		Omnivore	2						1									
Micropterus salmoides	Ex	Nat	Tolerant	Carnivore																
Lepomis macrochirus	Ex	Nat	Tolerant	Omnivore												1				
Mugil cephalus	Nv	Nat		Herbivore																6
Pungtungia herzi	Nv	Nat		Insect	3	3			7		4	9	12	1	8	5				
Oryzias latipes	Nv	Nat	Tolerant	Herbivore						2			1				5			21
Eriocheir japonica	Nv	Dem		Omnivore					1		2			1		2	6	9	4	5
Tanakia lanceolata	Nv	Nat	Weak	Omnivore																
Rhinogobius sp.	Nv	Dem		Insect	15	3				8	4			16		5	3	8	6	
Ischikauia steenackeri	Ex	Nat	Tolerant	Herbivore																
fry	Nv	Nat				23	2				8	2		36	10		1	6		2
					61	93	48	44	64	135	28	74	69	115	55	97	47	70	55	46

Nv=Native; Ex=Exotic; Nat=Natatorial; Dem=Demersial

Table 3.7 - (continued)

Name of species	Mode of life																		Σ
	Nv or ex	Nat or Dem	Tolerance	Food habit	U-28	U-29	U-31	U-32	U-35	U-38	U-40	U-41	U-42	Y-1	Y-3	Y-4	Y-8	Y-10	Y-11	
Tanakia limbata	Nv	Nat	Weak	Herbivore	38						36									39
Plecoglossus altivelis altivelis	Nv	Nat		Herbivore									1							1
Squalidus gracilis gracilis	Nv	Nat		Insect												4				8
Zacco platypus	Nv	Nat		Omnivore	2	18						8	7	1	12	1		2	6	48
Coreoperca kawamebari	Nv	Nat		Insect										1			1			8
Pseudogobio esocinus	Nv	Dem		Insect		1				1				1		4	1		2	30
Nipponocypris temminckii	Nv	Nat		Insect				19						10	53	19		49	3	384
Pelteobagrus nudiceps	Nv	Dem		Insect		1														1
Carassius langsdorfii	Nv	Nat	Tolerant	Omnivore		62			4		1		10			7				13
Cyprinus carpio	Nv	Nat	Tolerant	Omnivore	7						2		2							3
Crayfish	Ex	Dem	Tolerant	Omnivore	7						1						1			2
Geothelphusa dehaani	Nv	Dem		Omnivore										2		2				1
Palaemon paucidens	Nv	Dem		Omnivore	2	2	83				7					11			3	97
Hemibarbus longirostris	Nv	Dem		Insect																5
matsubarai	Nv	Dem		Omnivore												1				8
Rhodeus ocellatus ocellatus	Ex	Dem	Weak	Herbivore								1								0
Phoxinus oxycephalus jouyi	Nv	Nat		Omnivore				2										3		0
Gnathopogon elongatus	Nv	Nat		Omnivore															2	2
Misgurnus anguillicaudatus	Nv	Dem	Tolerant	Omnivore		2	7													10
Odontobutis obscura	Nv	Dem		Carnivore	2	1		3	18	2	7			1	5	2	1	2	16	95
Caridina multidentata	Nv	Dem		Omnivore	2													7	28	67
T. brevispinis	Nv	Dem		Omnivore																3
Micropterus salmoides	Ex	Nat	Tolerant	Carnivore															1	0
Lepomis macrochirus	Ex	Nat	Tolerant	Omnivore	2	9					1	1	1							1
Mugil cephalus	Nv	Nat		Herbivore		5														6
Pungtungia herzi	Nv	Nat		Insect											1	1	2	2	4	52
Oryzias latipes	Nv	Nat	Tolerant	Herbivore			1		16	13	15									29
Eriocheir japonica	Nv	Dem		Omnivore	2				4											30
Tanakia lanceolata	Nv	Nat	Weak	Omnivore																0
Rhinogobius sp.	Nv	Dem		Insect		4	118	6			1		1	9	13	7	1	12	7	68
Ischikauia steenackeri	Ex	Nat	Tolerant	Herbivore																0
fry	Nv	Nat			1					2				4				6		90
					65	105	209	30	42	18	71	10	22	29	84	59	7	83	72	

Nv=Native; Ex=Exotic; Nat=Natatorial; Dem=Demersial

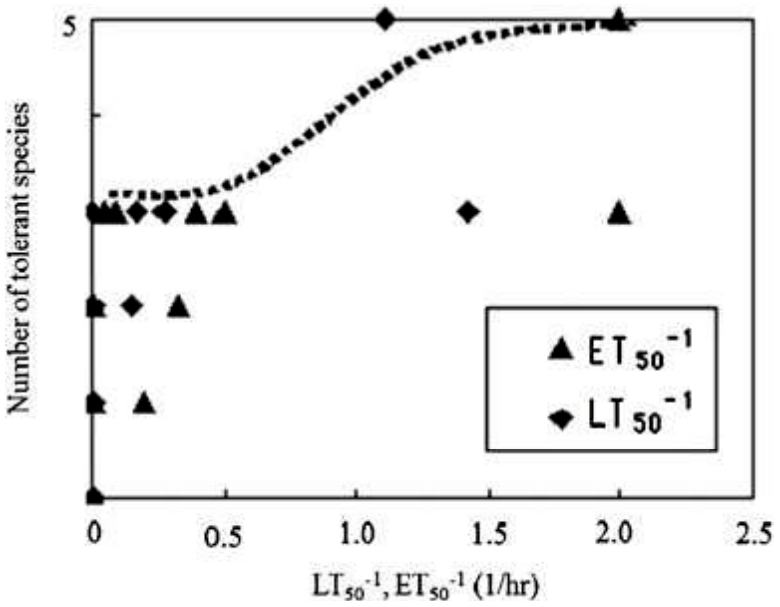

Fig. 3.10 - Relationship between Toxicity and Number of Tolerant Species

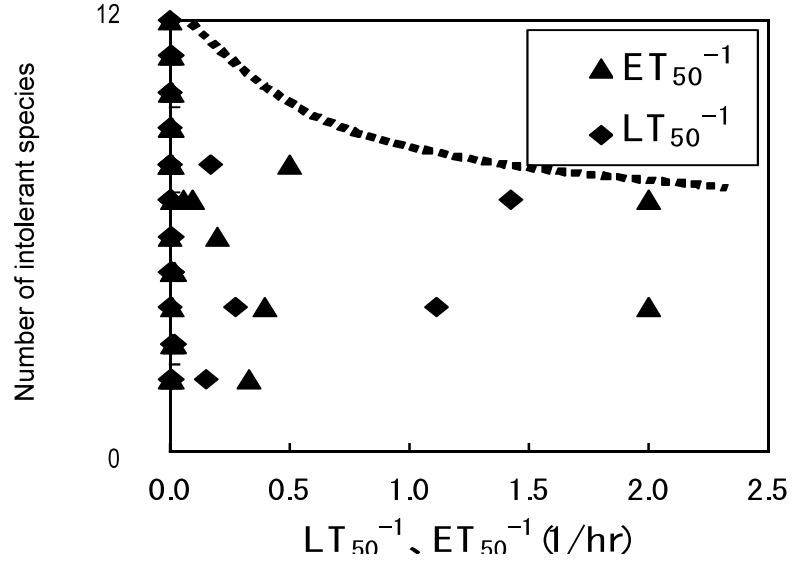

Fig. 3.11 - Relationship between Toxicity and Number of Intolerant Species

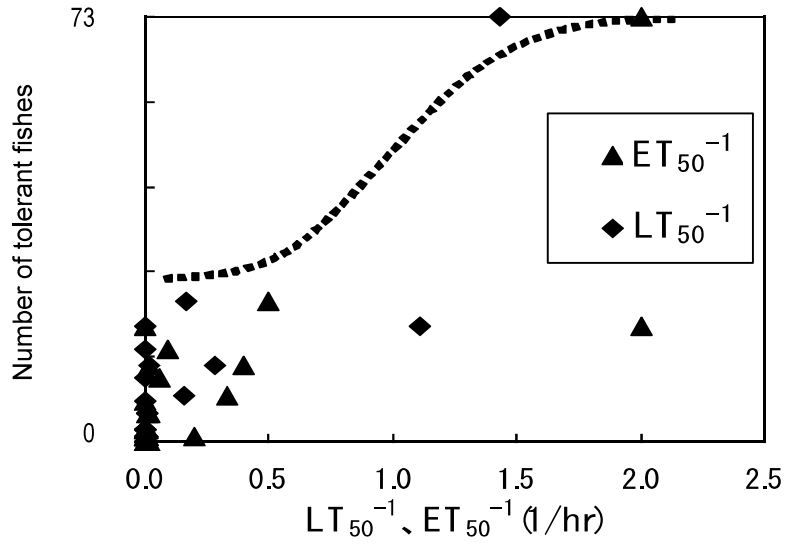

Fig. 3.12 - Relationship between Toxicity and Number of Tolerant Fishes

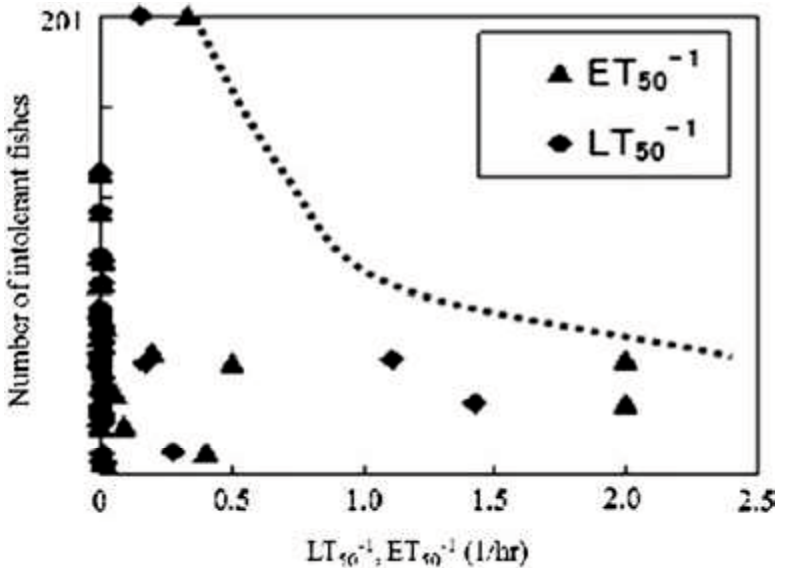

Fig. 3.13 - Relationship between Toxicity and Number of Intolerant Fishes

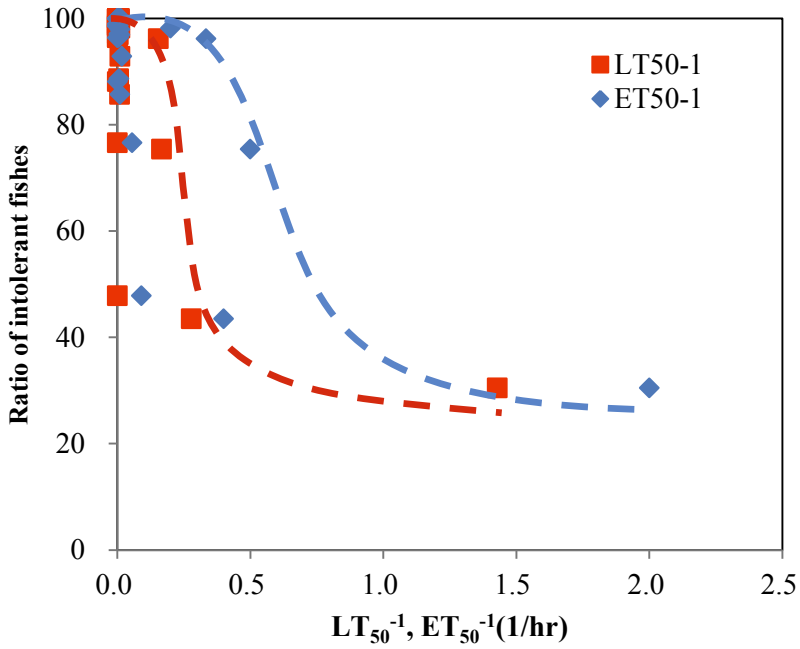

Fig. 3.14 - Relationship between Toxicity and Ratio of Intolerant Fishes

3.4 Conclusion

1. We propose a semi quantitative quick toxicity test using Medaka fish and 100-fold concentrated water. ET_{50}^{-1} and LT_{50}^{-1} are used instead of EC_{50}^{-1} and LC_{50}^{-1}. Therefore, we can reduce the time required to conduct toxicity test.
2. The test revealed various levels of toxicity in the rivers and seas in Japan. We have verified the applicability of this method in various samples including seawater.
3. It shows that high BOD of household wastewater also contains hydrophobic toxic matters, and the seawater in industrial area does not show clear relationship between toxicity and COD.
4. Ratio of clear stream benthic animal sharply decreased over 0.25 of LT_{50}^{-1} or 0.5 of ET_{50}^{-1}. Tolerant fish become dominant over 0.3 of LT_{50}^{-1} or 0.5–1.0 of ET_{50}^{-1}. These signify that the toxicity test using Medaka fish and 100-fold concentrated water has a relationship with aquatic habitat condition.

3.5 References

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CHAPTER 04
STUDY ON RELATIONSHIP BETWEEN AN ORDINAL SCALE TOXICITY INDEX LT_{50}^{-1} AND A RATIO SCALE TOXICITY INDEX LDR_{50} IN RIVER BASINS

4.1 Introduction

There are many toxicity test methods which have been recommended by ISO, OECD, USEPA, and other international or national standard organizations. Most of the methods were established to measure the toxicity of pure single chemical, but not for unknown environmental water samples with complex components (ECETOC, 1993). However, even if the toxicity of environmental sample is tested, there is no guidance on how to evaluate the water quality in terms of protection of aquatic living organisms.

One effective way for assessing the aquatic safety of water samples is to expose them to aquatic organisms directly, a method called bioassay (Wei et al, 2006). Fish as secondary or advanced consumer in aquatic food chain, is popularly selected as toxicity test species in scientific researches and environmental management (Zha et al 2005). The Ministry of Environment of Japan collected ecotoxicity data and compared the sensitivities of Japanese Medaka (Oryzias latipes) with other six fish species recommended by OECD, and the results indicated that the sensitivity of Japanese Medaka was equal to or a little higher than others surveyed fish species (MOE of Japan, 2002, 2003).

Toxicity test using Medaka early fry and 100-fold concentrated water were proposed to obtain result quickly and as quantitatively as possible. Conducted only 100-fold concentrated and 48-hours test and it disclosed toxicity that is the inverse of median effect time and median lethal time (ET_{50}^{-1}, LT_{50}^{-1}). ET_{50}^{-1} and LT_{50}^{-1} are used instead of EC_{50}^{-1} and LC_{50}^{-1} scale (Yamashita et al, 2012). Although this method had an advantage in reducing the amount of time and sampling needed to perform toxicity tests, it also had a disadvantage that it cannot be handled as concentration. In this research, we needed an index which can be treated in the same way as concentration.

From this reason, we expressed the toxicity as a lethal dilution rate (LDR_{50}). LDR_{50} is the inverse of lethal concentration rate (LCR_{50}) which Liu et al. (2006) proposed, and defined as the dilution rate at which 50% of fish survive the acute toxicity test. There, in this research, will be discussed about the relationship between an ordinal scale toxicity index LT_{50}^{-1} to a ratio scale toxicity index LDR_{50} and show an index for calculating toxicity of unknown concentrations of toxic compounds in the same characteristic area, which can subsequently be used to estimate toxic effects in organisms at any time of exposure for any level of concern.

4.2 Materials and Methods

4.2.1 Study Area

During June – December 2012, water samples were collected from three rivers in Japan which have majority catchment area is residential area. Data taken between 9AM -12PM with the assumption that household waste was released. First area was located in "M" river (Figure 4.1), 9 point Samples were taken from this river. The river function is to accommodate the flow of rain water and household waste from the area around the river. The second area is located in "Y" river (Figure 4.2). Flood risk has been increased because the middle zone of the basin has been urbanized rapidly in recent three decades. The third area was chosen from "Z" river (Figure 4.3), where residential, commercial and industrial sites are heavily concentrated on these regions.

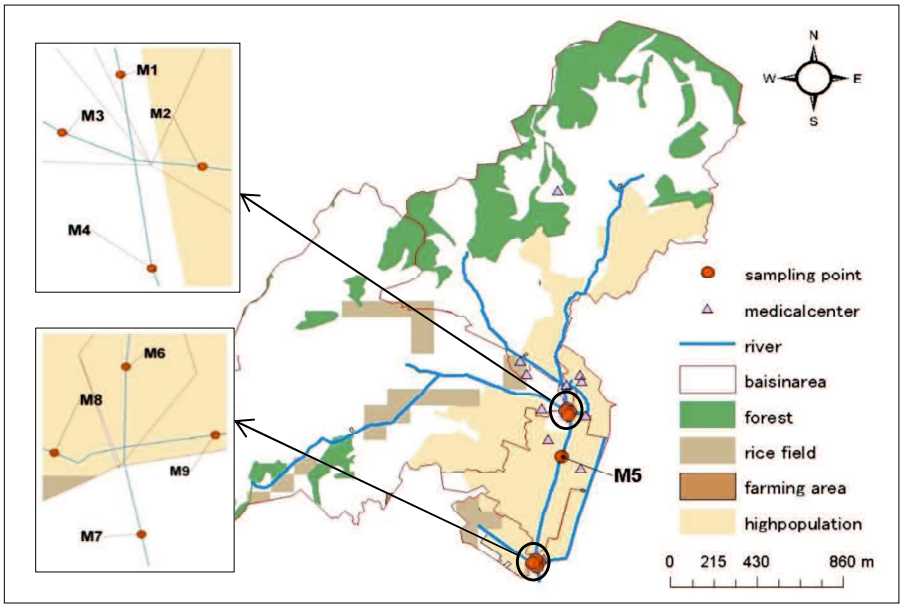

Fig. 4.1 - Basin Areas of Rivers "M"

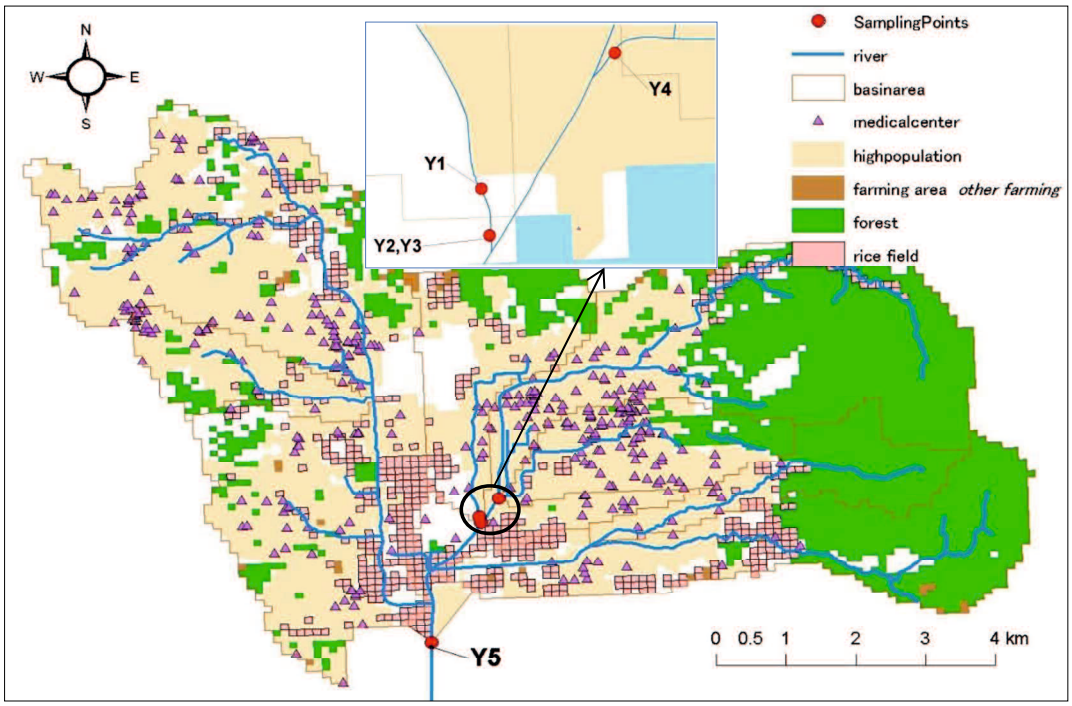

Fig. 4.2 - Basin Areas of Rivers “Y”

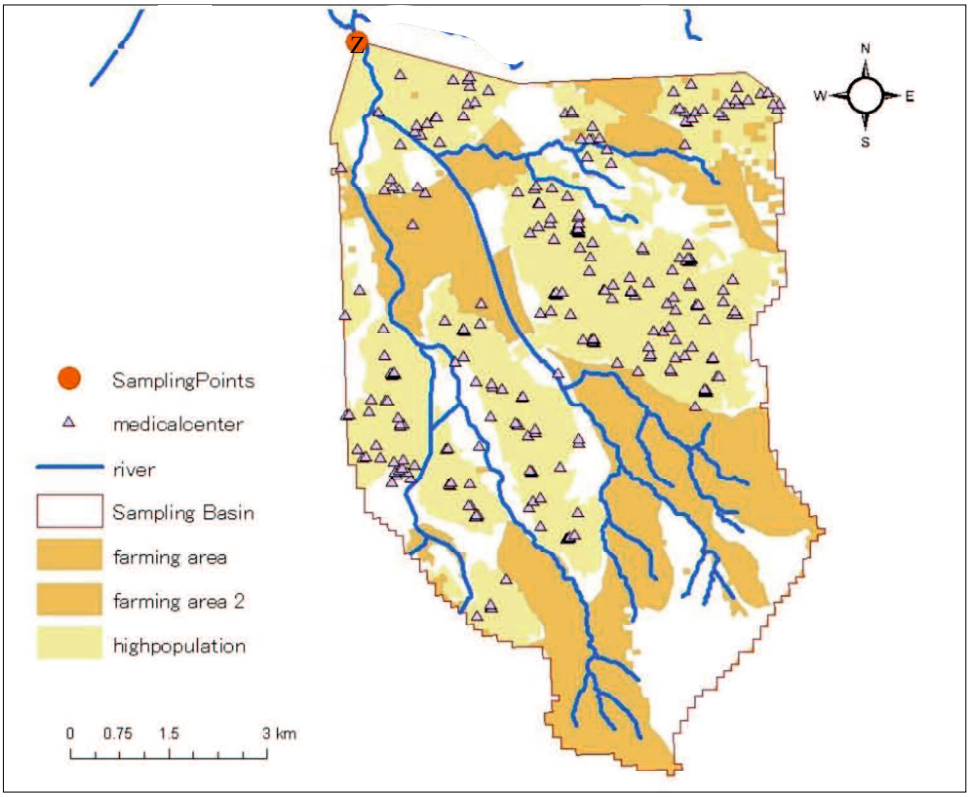

Fig. 4.3 - Basin Areas of Rivers “Z”

4.2.2 Acute Toxicity Test

For LT_{50}^{-1} acute toxicity test is based on Yamashita (2012). Figure 4.4 showed processes to concentrate sample water for LDR_{50} analysis. 10 L of river water was filtered with 1-μm glass filter. Two set of Sep-Pak® Plus PS-2 cartridges were set in series (Ishii et al, 2000). Hydrophobic organic matter was adsorbed at 10 ml/min for each 5 L sampling water, and desorbed from each cartridge in 10 ml of acetone. Air was injected into the cartridge with a syringe to drive out the space water. 40 ml acetone solution will be generated, 36 ml will be used to acute toxicity test and 4 ml will be used for analysis of GC/MS. A 36-ml volume of acetone solution was evaporated to 200 μl under a purge of nitrogen gas. The acetone solution was diluted to 90 ml with carbon treatment water. In toxicity test based on Liu et al. (2007), organic toxicants were 10, 20, 50 and 100-fold concentrated from the sample. The lethal effect was observed by exposing every ten individuals of 48-72 h old larval Medaka to 25 mL of each solution for 48 h. When there was a striking difference in test results between the two solutions, the test was considered a failure. Toxicity analysis was calculated using the Probit method (Yamashita et al, 2012).

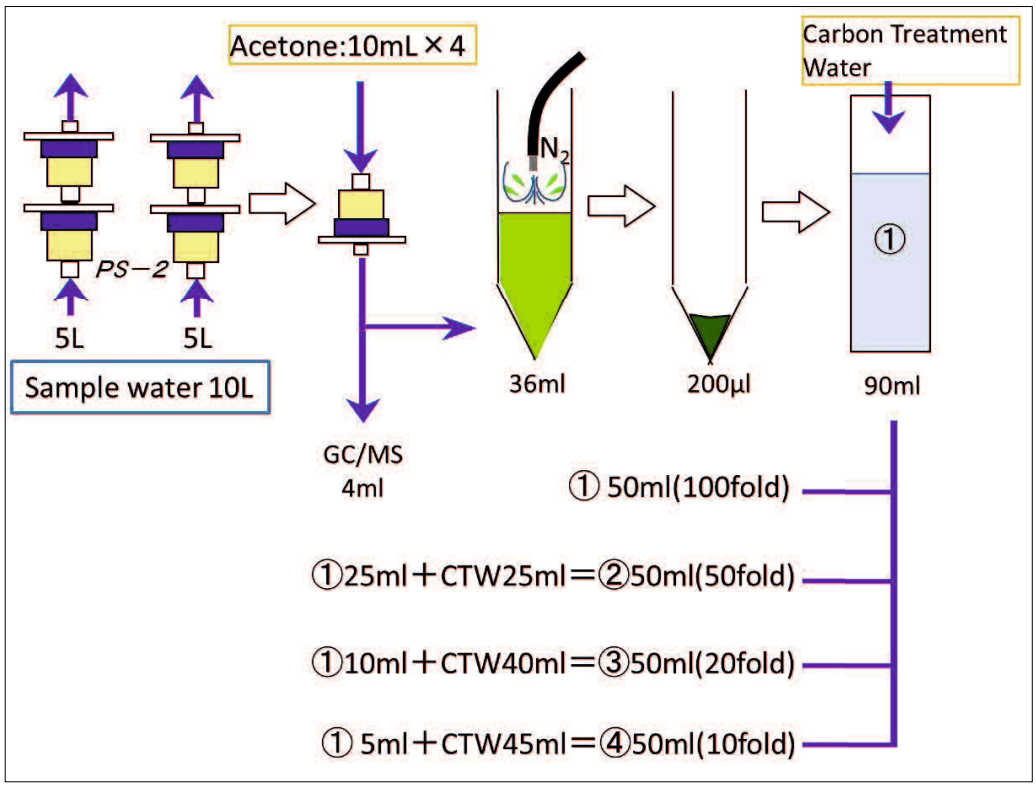

Fig. 4.4 – Process to Concentrate Sample Water

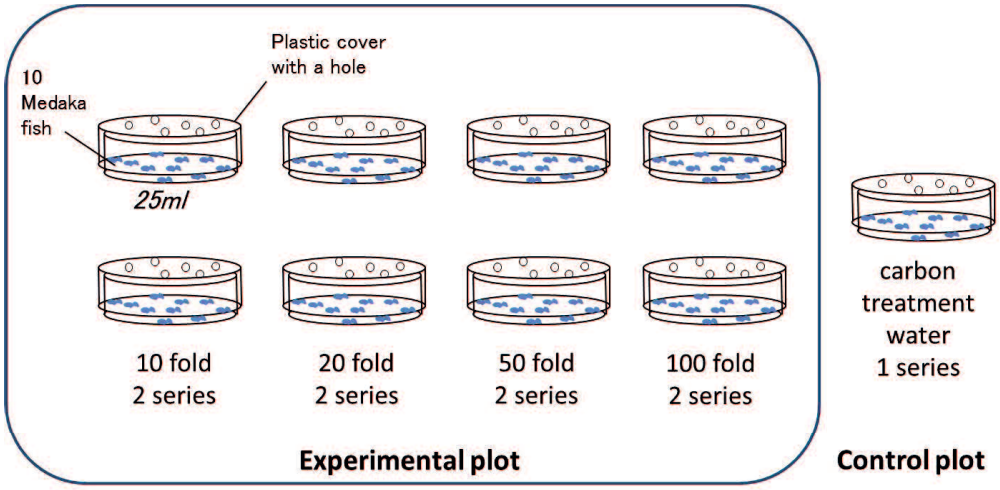

Fig. 4.5 – Plot of toxicity test for 10, 20, 50 and 100-fold concentrated

Toxicity analysis was calculated using the Probit method (H. Yamashita et al, 2012). Death rate approximated the following expression (1); LDR_{50} is defined as the inverse of μ value. Higher value of LDR_{50} indicates more toxicity.

$$f(x) = \frac{1}{\sigma\sqrt{2\pi}} \exp\left(-\frac{(x.\mu)^2}{2\sigma^2} \right) \quad (1)$$

Here σ = standard deviation of lethal dilution, x = lethal dilution, and μ = average of lethal dilution

4.3 Results and Discussion

4.3.1 Relationship between LT_{50}^{-1} and LDR_{50}

Table 4.1 showed result of LT_{50}^{-1} and LDR_{50} of the sampling water. The reliable range for LT_{50}^{-1} value is between 0.02-2.0. Whereas, the reliable range for LDR_{50} value is between 0.01-0.2. From various concentrations of toxicity test (10, 20, 50 and 100 fold), not all of them yielded LT_{50}^{-1} or LDR_{50} value, this is because of the content of toxic condition of the river is very low or its toxic content is very toxic high. With the assumption that by using 2-3 grade of LT_{50}^{-1} value can represent the relationship between LT_{50}^{-1} and LDR_{50}, for optimum results in determining the relationship of that, at least there are should be obtained minimum 2-3 grade of LT_{50}^{-1} value in each sample taken.

"M" river with a total sample of 9 points, only 8 units of LT_{50}^{-1} values was obtained. For M4, M5, and M7, LT_{50}^{-1} values could be obtained only on the condition of 100 fold. As for the M2, M6 and M8 no LT_{50}^{-1} values was obtained , because the toxic conditions was extremely low for M6 and M8, and otherwise for M2 have very high toxic. For M9 with high toxic conditions, LT_{50}^{-1} values were obtained at 10 and 20 fold. In the "Y" River, from 5 points taken, at Y3 the LT_{50}^{-1} values were obtained as much as 3 units and consecutively as much as 2 point of LT_{50}^{-1} values for Y4 and 1 point of LT_{50}^{-1} values for Y1, Y2 and Y5. As for the "Z" river because of the low toxic conditions can only 1 point of LT_{50}^{-1} value could be obtained.

Table 4.1 - Result of LT_{50}^{-1} and LDR_{50} of the sampling water

Sampling point	LDR_{50}	LT_{50}^{-1}			
		(10 fold)	(20 fold)	(50 fold)	(100 fold)
M1	0.07	0.019	**0.06**	**0.29**	>2.00
M2	>0.20	>2.00	>2.00	>2.00	>2.00
M3	0.09	0.01	**0.38**	>2.00	>2.00
M4	0.02	0.01	0.017	0.01	**0.33**
M5	0.02	0.01	0.017	0.018	**0.10**
M6	0.01	0.01	0.01	0.017	0.01
M7	0.01	0.01	0.01	0.01	**0.05**
M8	0.01	0.01	0.01	0.01	0.019
M9	>0.20	**0.61**	**1.12**	>2.00	>2.00
Y1	0.02	0.01	0.01	0.01	**0.06**
Y2	0.02	0.01	0.01	0.01	**0.05**
Y3	0.05	0.01	**0.022**	**0.04**	**0.09**
Y4	0.02	0.01	0.01	**0.02**	**0.09**
Y5	0.01	0.016	0.01	0.01	**0.022**
Z	0.01	0.01	0.01	0.01	**0.07**
Nonylphenol	0.03	0.01	0.01	**0.43**	>2.00
Triclosan	0.11	0.01	**0.33**	>2.00	>2.00

LT_{50}^{-1} values which can use for analysis written with bold characters

Based on the assumption that by using 2-3 grade of LT_{50}^{-1} value can represent the relationship between LT_{50}^{-1} and LDR_{50} and the maximum value of LDR_{50} from each sampling point is 0.20, equation obtained for the relationship between LDR_{50} and LT_{50}^{-1} is $y = 0.1752x$ with $R^2 = 0.9306$ (Fig. 4.7). It shows a significant value of R^2, but with a small sample, it might be difficult to obtain statistical evidence of strong relation (Berthouex and Brown, 1994). Furthermore, for better accuracy a more profound study is required. In future work, more data will be collected and identify

the land use areas for each catchment still require to obtain accurate results. However, with an accurate equation result, it can be used for calculating toxicity of unknown concentrations of toxic compounds; which can subsequently be used to estimate toxic effects in organisms at any time of exposure for any level of concern.

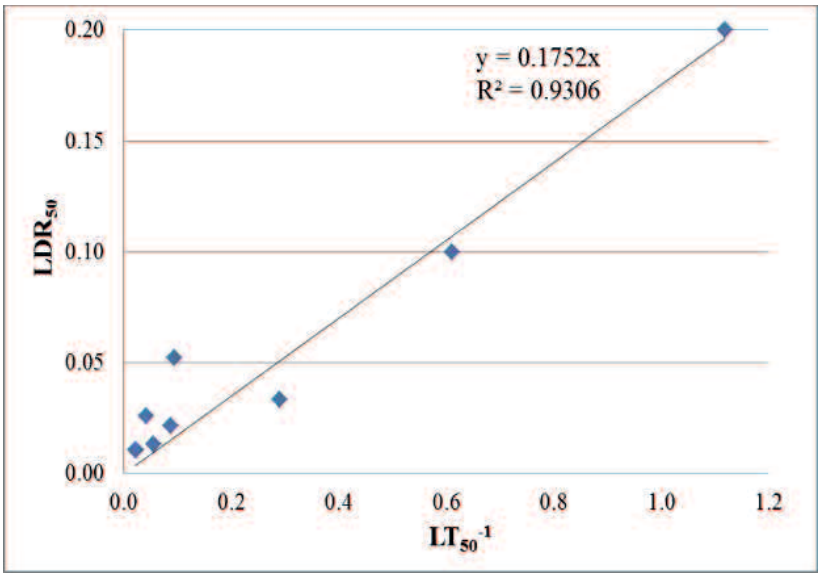

Fig. 4.6 - Relationship between LT_{50}^{-1} and LDR_{50} using sampling point that have more than one LT_{50}^{-1} value

4.4 Conclusions

Equation obtained for the relationship between LDR_{50} and LT_{50}^{-1} is y = 0.1752x with $R^2 = 0.9306$. It shows a significant value of R^2, but with a small sample, it might be difficult to obtain statistical evidence of strong relation. Furthermore, for better accuracy more data will be collected and identify the land use areas for each catchment still require to obtain accurate results.

4.5 References

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CHAPTER 05
BEHAVIOUR OF CHEMICAL CONCENTRATION AND TOXICITY IN RIVER BASINS DOMINATED BY RESIDENTIAL AREAS

5.1 Introduction

Trace chemicals such as endocrine disruptors and dioxins can cause many problems in the ecosystem, especially if released into environmental water. Studies have already determined the acute toxicity levels of such chemicals. However, the concentrations of such chemicals in environmental water are usually much lower than those that cause acute toxicity. Furthermore, various other chemicals also exist in the water. Therefore, it is difficult to obtain information on protecting the ecosystem from the acute toxicity levels of each chemical.

A bioassay is one approach that could be used to obtain comprehensive information about the toxicity levels of chemicals. However, this approach had been thought not to be well suited to environmental management because it cannot detect the chemical itself. As an alternative, toxicity management methodologies based on bioassays, such as Whole Effluent Toxicity (WET), have attracted considerable attention in recent times. This method can be used to measure the toxicity of industrial wastewater itself, without necessarily identifying the chemical (Tonkes et al., 1999).

Separately, the authors have shown that a toxicity test using 100-fold concentrated river water and the Medaka early fly could be used to detect acute toxicity in river (Yamashita et al., 2012). The detected toxicity tended to be higher under higher BOD concentration even when there were no industries. This might imply that the toxicity comes from household wastewater. Furthermore, the authors showed the relationship between the toxicity and aquatic animal habitation. For example, the ratio of clear stream benthic animals sharply decreased in river waters in which 50% of the Medaka early fly died within 4 h, and tolerant fish became dominant in waters in which 50% of the Medaka early fly died within 3.3 h. This

result shows that toxicity, which is not negligible for ecosystem conservation, comes not only from industry but also from diffused pollutant source such as residence.

Specifically, toxicity is considered to be caused by various substances such as agricultural chemicals, detergents, and pharmaceuticals. Although many studies have focused on river water toxicity and chemical behaviors (Ichiki et al., 2009; Wang et al., 2011), there are not many researches which observed the behavior of toxicity and chemicals in relation to basin characteristics in Japan. In this study, we investigated the river water toxicity in three basins dominated by residential area and identified the chemical contents using gas chromatography/mass spectrometry (GC/MS) simultaneous analysis database. Based on our obtained results, we preliminarily investigated the relationship among chemical concentration, toxicity, and basin characteristics.

5.2 Materials and Methods

5.2.1 Study Area

Samples were taken from rivers in Japan wherein the majority of catchment areas were within residential areas. From three rivers in Japan, a total of fifteen grab samples were collected from June–December 2012, and six composite samples were collected from August–January 2014. A maximum volume of 10 L of water was collected at each site using stainless steel buckets rinsed with site water prior to collection.

The first sample area was located in river “M,” shown in Figure 5.1. River “M” is 2.4 km long and accommodated the flow of rain water and household waste from the area around a larger river channel. A residential area was upstream from this river. Five sampling sites along the river (M1, M4, M5, M6, and M7) were selected from upstream and downstream reaches, and four sampling sites were selected from the tributary (M2 and M3 in the upstream area and M8 and M9 in down-stream area). The second sample area was located in river “Y”. The river basin is wide and the pollution risk was increased because the middle zone of the basin has rapidly urbanized over the past three decades (Figure 5.2). The third sample area was located in river “Z”. The basin of this river includes one of the most densely populated cities in Japan (Figure 5.3).

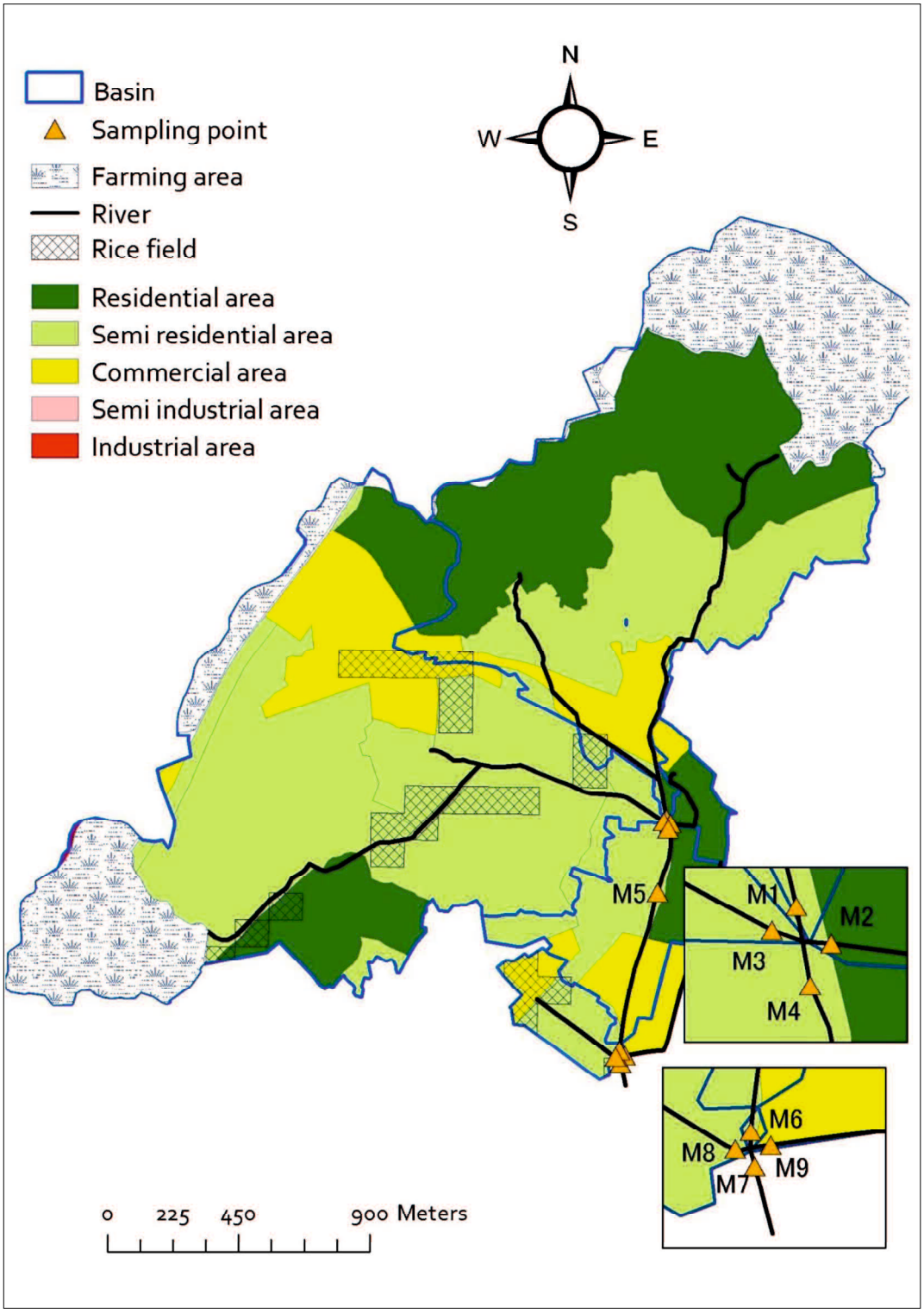

Fig. 5.1 - Basin Areas of Rivers “M”

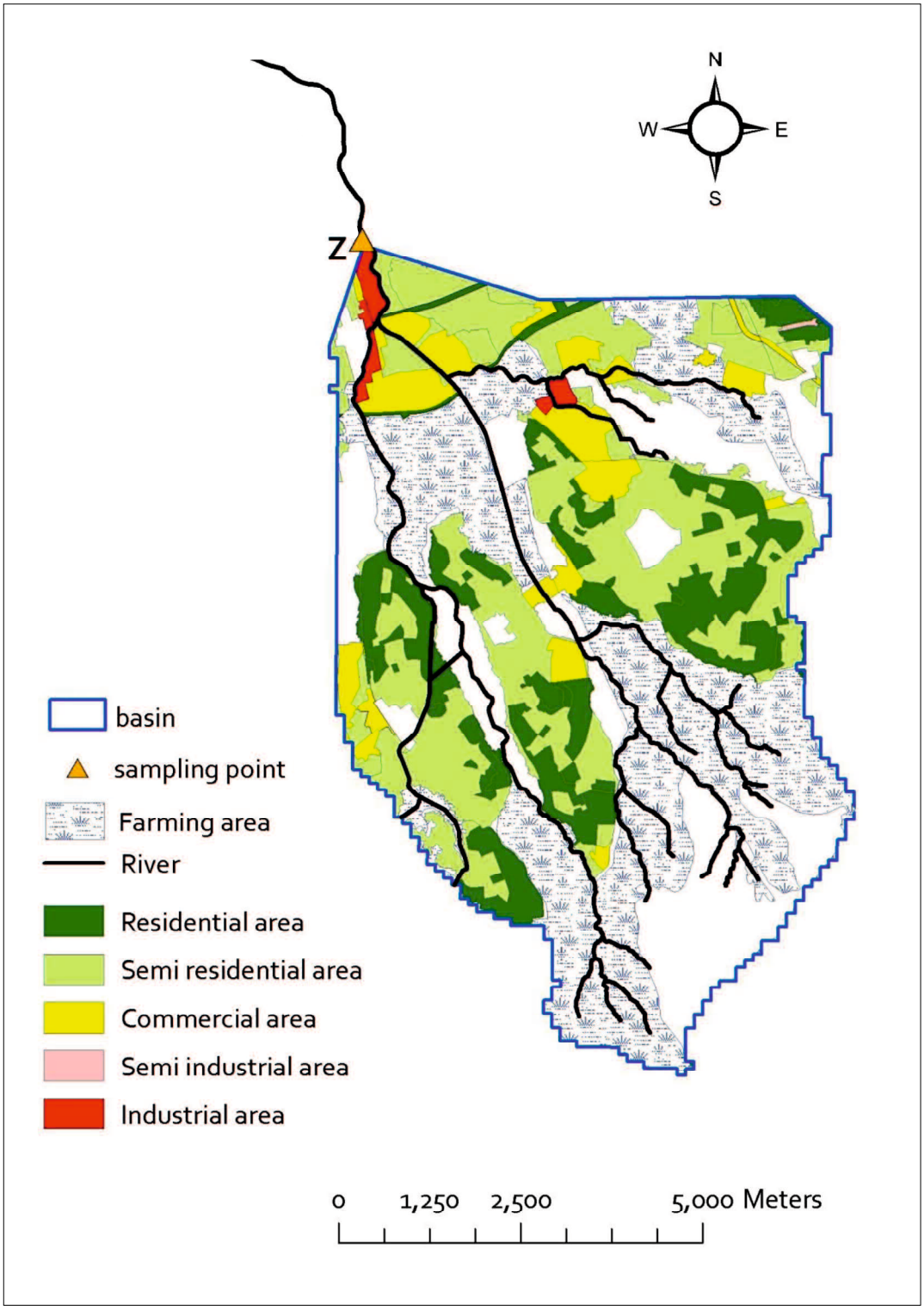

Fig. 5.2 - Basin Areas of Rivers “Z”

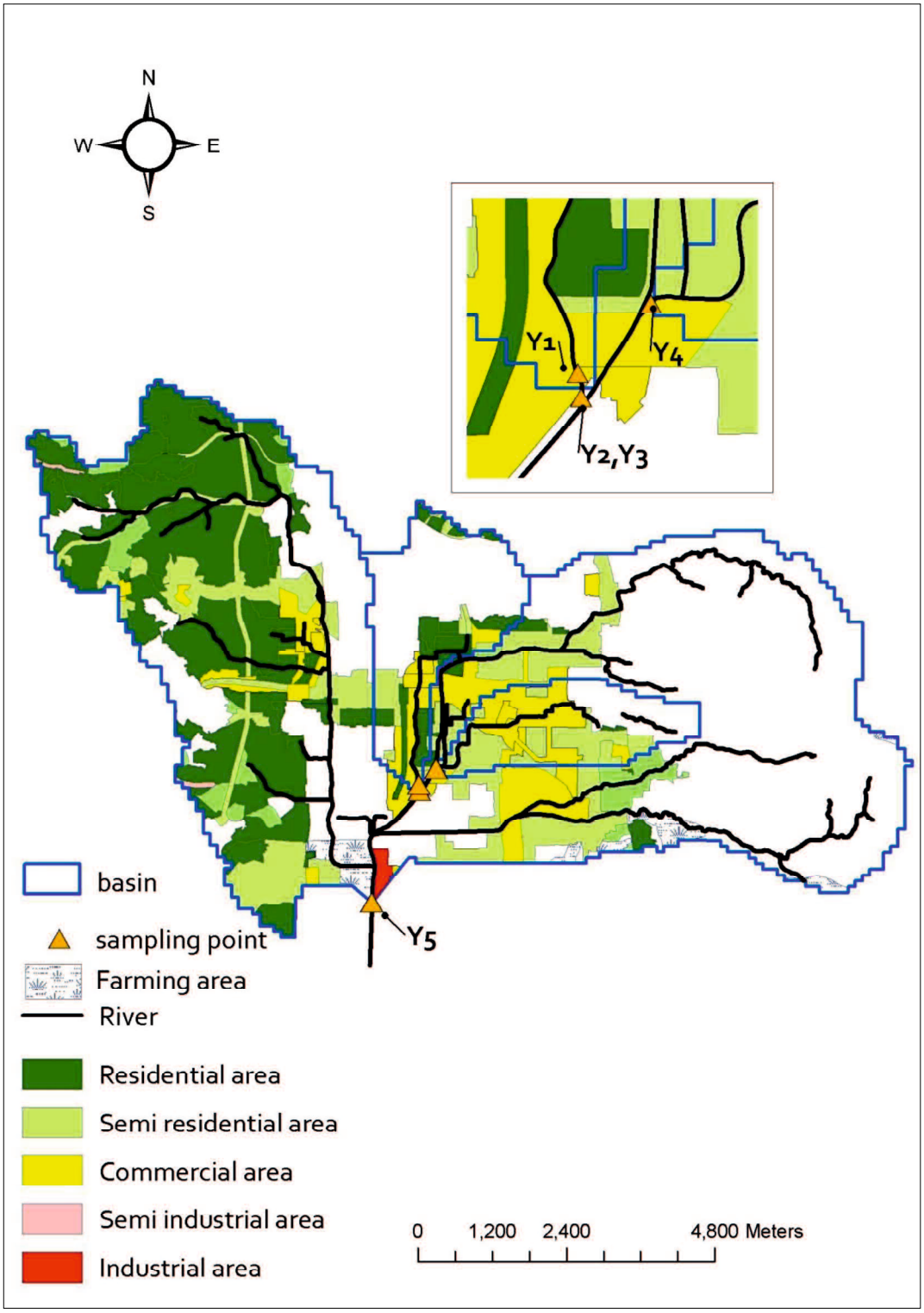

Fig. 5.3 - Basin Areas of Rivers “Y”

Increasingly complex human social activities continue to produce and consume wide chemical concentration ranges. It is very important to know the parameters that can affect to the water quality. Table 5.1 showed the spatial data for each river basin which used to analyse the process of toxicity flowing out from a basin.

Table 5.1 - Spatial Data for Each River Basin

Date	Sampl-ing Point	LDR_{50}	Temp. (°C)	Catchment Area (km^2)	Populati-on (people)	Farmland Area (km^2)	Residenti-al area (km^2)	Commercial area (km^2)	Industri-al area (km^2)	Sewer Population (people)
2012-12-14	M1	0.067	10.9	1.75	3374	0.01	0.71	0.05	0.00	1
2012-12-14	M2	>0.2	10.9	0.06	348	0.00	0.05	0.01	0.00	0
2012-12-14	M3	0.086	10.7	1.99	4915	0.17	1.39	0.25	0.00	154
2012-10-31	M4	0.019	17.1	3.81	8289	0.18	2.85	0.52	0.00	249
2012-10-31	M5	0.020	17.1	3.88	8864	0.18	2.96	0.52	0.00	916
2012-06-16	M6	0.011	23.1	4.08	9439	0.18	2.96	0.52	0.00	1026
2012-06-16	M7	0.014	23.3	4.21	10108	0.21	3.00	0.52	0.00	1654
2012-06-16	M8	0.019	23.6	0.09	629	0.03	0.04	0.02	0.00	600
2012-06-16	M9	>0.2	23.6	0.04	44	0.00	0.01	0.03	0.00	28
2012-11-23	Y1	0.016	12.0	6.52	20799	0.63	2.06	1.05	0.00	17411
2012-11-23	Y2	0.016	12.0	6.52	20799	0.63	1.83	0.80	0.00	17411
2012-09-23	Y3	0.039	22.8	6.52	20799	0.63	1.83	0.80	0.00	17411
2012-09-23	Y4	0.022	22.7	4.40	24312	0.08	1.61	1.47	0.00	20352
2013-08-20	Y4c1	0.011	30.9	4.40	24312	0.08	1.61	1.47	0.00	20352
2013-10-21	Y4c2	0.010	20.2	4.40	24312	0.08	1.61	1.47	0.00	20352
2014-01-08	Y4c3	0.016	6.5	4.40	24312	0.08	1.61	1.47	0.00	20352
2012-09-23	Y5	0.011	22.7	69.90	246123	6.80	28.20	5.75	0.17	206025
2013-08-20	Y5c1	0.011	31.2	69.90	246123	6.80	28.20	5.75	0.17	206025
2013-10-21	Y5c2	0.010	19.2	69.90	246123	6.80	28.20	5.75	0.17	206025
2014-01-08	Y5c3	0.000	6.5	69.90	246123	6.80	28.20	5.75	0.17	206025
2012-09-22	Z	0.019	28.2	59.47	205872	11.82	22.90	4.02	0.17	183604

5.2.2 Acute Toxicity Test

Yamashita et al. (2012), proposed a toxicity index which expressed using inverse of median lethal time (LT_{50}^{-1}). Although it had an advantage in obtaining semi-quantitative index using smaller number of test fish, LT_{50}^{-1} also had a disadvantage that it cannot be handled as concentration. In this research, we needed an index which can be treated in the same way as concentration. From this reason, we expressed the toxicity as a lethal dilution rate (LDR_{50}). LDR_{50} is the inverse of lethal concentration rate (LCR_{50}) which Liu et al. (2006) proposed, and defined as the dilution ratio at which 50% of fish survive the acute toxicity test.

In this study, 10 L of river water was filtered with 1-μm glass filter. Two set of Sep-Pak® Plus PS-2 cartridges were set in series (Figure 5.4). Hydrophobic organic matter was adsorbed at 10 ml/min for each 5 L sampling water, and desorbed from each cartridge in 10 ml of acetone. Air was injected into the cartridge with a syringe to drive out the space water. 40 ml acetone solution will be generated, 36 ml

will be used to acute toxicity test and 4 ml will be used for analysis of GC/MS. A 36-ml volume of acetone solution was evaporated to 200 µl under a purge of nitrogen gas. The acetone solution was diluted to 50 ml with carbon treatment water and then separated into two 25-ml portions. In toxicity test based on Liu et al. (2007), organic toxicants were 10, 20, 50 and 100-fold concentrated from the sample. The lethal effect was observed by exposing every ten individuals of 48–72 h old larval medaka to 25 mL of each solution for 48 h. When there was a striking difference in test results between the two solutions, the test was considered a failure. Toxicity analysis was calculated using the Probit method (H. Yamashita et al, 2012). Death rate approximated the following expression (1); LDR_{50} is defined as the inverse of µ value. Higher value of LDR_{50} indicates more toxicity.

$$f(x) = \frac{1}{\sigma\sqrt{2\pi}} \exp\left(-\frac{(x.\mu)^2}{2\sigma^2} \right) \quad (1)$$

Here σ = standard deviation of lethal dilution, x = lethal dilution, and µ = average of lethal dilution

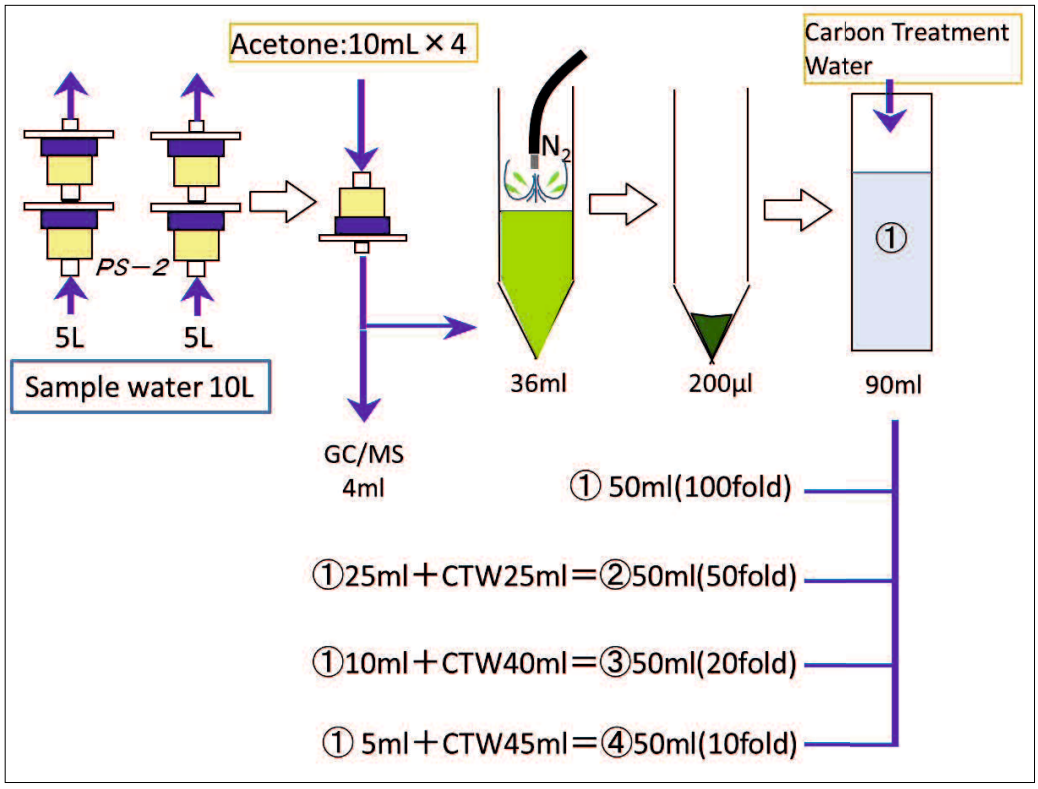

Fig. 5.4 – Process to Concentrate Sample Water

5.2.3 Gas Chromatograph/Mass Spectrometer (GC/MS) Analysis

A nitrogen purge evaporated the 4 ml acetone solution, and a moderate amount of hexane was added. Sodium sulphate was applied to remove moisture and was then removed. Hexane was evaporated to 1 ml. A Shimadzu GC-2010 gas chromatograph (Kyoto, Japan) coupled with a Shimadzu QP-2010 mass spectrometer was used for GC/MS analysis. The GC/MS simultaneous analysis database can identify and quantify 942 chemical compounds without the use of a standard substance. (Kadokami et al. 2005).

5.2.4 Cluster Analysis

In order to investigate the similarity of chemical substances among water sampling sites, cluster analysis was employed. When the degree of dispersion of the data is unknown, it is difficult to determine the clustering method theoretically, and a trial and error is needed. In this study, Ward's method, group average linkage method, centroid method, and complete linkage method were used for clustering method, and Euclidean distance and squared Euclidean distance were used for subjects' distance in statistical analysis software SPSS. In the following chapter, we use the results obtained using Ward's method with Euclidean distance and squared Euclidean distance which showed rather clear grouping results.

5.2.5 Formulation of Toxicity runoff process

The decay of non-conservative substances is frequently modeled as a first-order reaction; that is assumed that rate of substance is proportional to the amount of substance that is present. The decomposition rate is calculating based on the first order kinetics reaction

$$C_t = C_0 . e^{-kt} \quad (1)$$

Here, C_t = concentration remaining at time t, C_0 = initial concentration, k = ratio of pollutant decrease, and t = time elapse. In practice, estimating the flow time of rivers is sometimes difficult. Therefore, time elapse was replaced with flow-down distance (Sekine et al, 1991).

$$C = C_0 \times e^{-k' x} \tag{2}$$

We assume that the maximum flow-down distance was proportional to the square root of the basin area. Since LDR_{50} can be treated as a concentration, then concentration at the exit of basin becomes:

$$C = \frac{C_0}{\sqrt{B}} \int_0^{\sqrt{B}} e^{-k' x} dx \tag{3}$$

Then equation (3) becomes:

$$C = \frac{C_0}{k' \times \sqrt{B}} \times \left(1 - e^{-k' \sqrt{B}}\right) \tag{4}$$

Here, C = LDR_{50} in a basin outlet (-), B = basin area (km^2), k' = ratio of toxicity decrease (km^{-1}).

We assume that factors such as population, farming, industrial activity, and sewerage conditions can contribute to discharge of toxic substances, and that C_0 is expressed by a linear combination of these elements as follows:

$$C_0 = \sum_{i=1}^{n} d_i \times F_i \tag{5}$$

Here, d_i = unit loading ratio (-), F_i = Percentage of frame values (-), i = spatial category. In this research, i = {F, C, I, S, W} where F represents Farmland area, C represents commercial area, I represents Industrial area, S represents Sewer Population, and W represents Without Sewer Population. For example, FW represents the ratio of people not covered by sewer, and d_w represents LDR_{50} discharged from the people not covered by sewer.

Since the minimum winter temperature is the critical design temperature, consequently the toxicity runoff process also affected by temperature. Furthermore, the pollutant decrease ratio can be estimated at any other temperature by using:

$$k' = k'' \times \theta^{T-20} \tag{6}$$

Here, k" = ratio of toxicity decrease (km^{-1}), T = temperature (°C). The values of θ for the domestic sewage generally used in 1.035 (Arceivala et al, 2009).
Combining Eqs.4, 5, and 6, C is expressed as follows:

$$C = \frac{\sum d_i \times F_i}{k'' \times 1.035^{T-20} \times \sqrt{B}} \times \left(1 - e^{-k'' \times 1.035^{T-20} \times \sqrt{B}}\right) \tag{7}$$

Since values of B, F_i, T, and C are known through our surveys, we should be able to determine k' and di by minimizing the square error between calculated and observed C using nonlinear optimization technique, if the model represents toxicity runoff process properly. For optimization, we employ Generalized Reduced Gradient Nonlinear Solving Method implemented in Solver add-in of Microsoft Excel 2010.

5.3 Results and Discussion

5.3.1 Detected Toxicity

Table 1 includes LDR_{50} results. Almost all samples have some toxicity. M2 and M9 show very high toxicity and they over-scaled. M1 and M3 also show rather high toxicity. In our previous research (Yamashita et al. 2011), we concluded that ratio of clear stream benthic animal sharply decreased over 0.25 of LT_{50}^{-1}, and tolerant fish become dominant over 0.3 of LT_{50}^{-1}. In this research, on the way to calculate LDR_{50}, we can obtain LT_{50}^{-1} too. LT_{50}^{-1} of M2, M9, M1, and M3 are all over 2.0. Thus, toxicity from residential area sometimes have high enough toxicity to affect the aquatic habitat, and it should be managed.

5.3.2 Chemical Concentration Present and Grouping based on Cluster Analysis

Figure 5.5 and 5.6 shows results of the cluster analysis using the Ward's method with Euclidean distance and squared Euclidean distance. A distance shows the similarity of chemical substances among water sampling sites; smaller distance means stronger similarity. We chose distance of 15 for classification threshold because it showed rather clear grouping results. By synthesizing the results of the two cluster analyses, we determine seven groups. The sites included in Group 1 were Z, M3, Y1, M1 Y3, Y4, and Y5. Euclidian distances placed M2 and M4 in the same cluster, while squared Euclidean distances excluded them. However, since M2 and M4 still included together in the same cluster, they were placed in Group 2. M5 was included in Group 3, and M6 was included in Group 4, since Euclidian distances placed M5 and M6 in the same group, meanwhile squared Euclidean distance separated M5 from M6. M7 and M8 were included in Group 5, M9 was included in Group 6, and Y2 was included in Group 7.

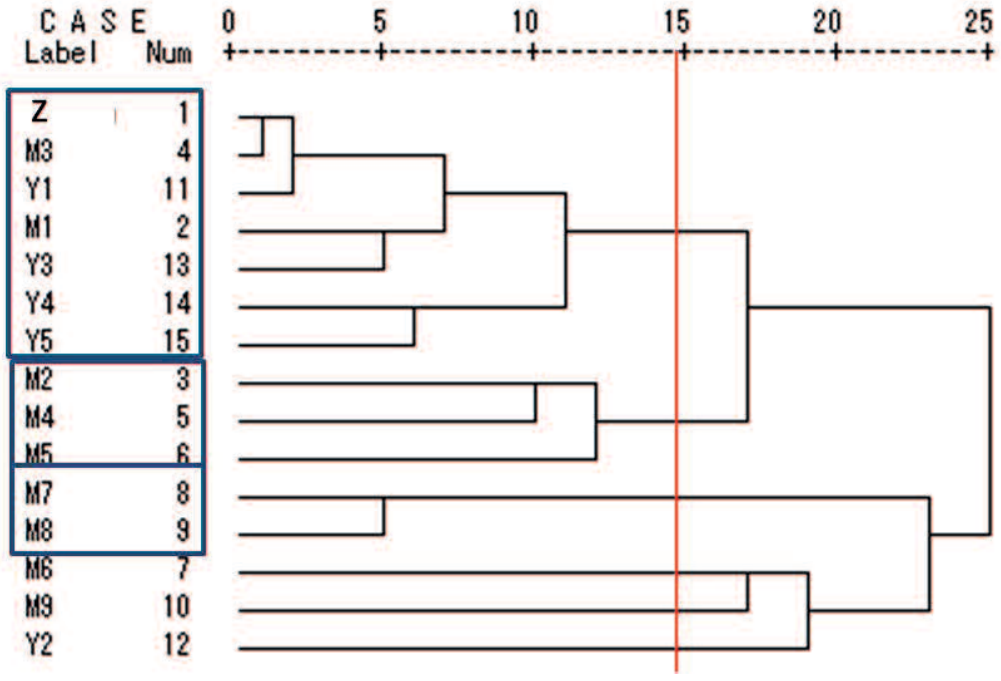

Fig. 5.5 - Classification based on the Ward's method using Euclidian distances

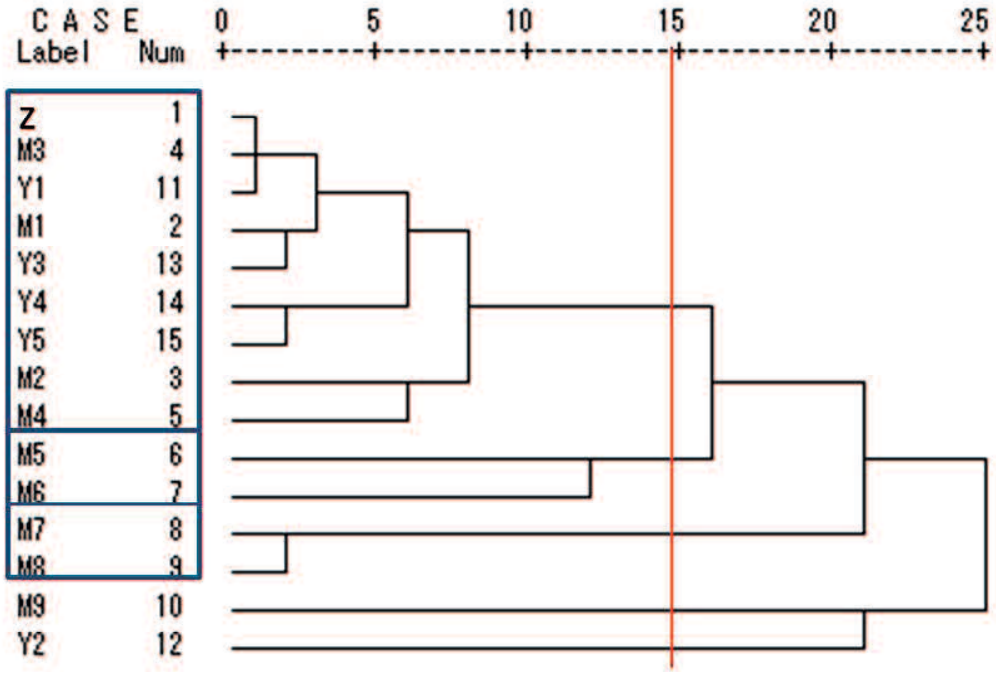

Fig. 5.6 - Classification based on squared Euclidean distance

Table 5.2 shows GC/MS analysis result grouped based on the result of cluster analysis. In Group 1, chemical composition is different from basin to basin, and almost all substances are in low concentrations. High toxicity not always show high chemical concentrations. For example, compared with other sample points, M3 had highest toxicity value as much as 0.086, but all the chemical concentration showed low concentration. There are several reasons for this inconsistency such as difficulty of detecting all chemicals, synergistic effect of chemicals, etc. Thus it is difficult to prove apparent relationship between the toxicity and chemical concentrations using the methods we employed in this research.

In Group 2 (M2 and M4), various chemicals presented high concentrations. For example, high concentrations of 2-phenoxyethanol (found in hair care products and perfumes) appeared in M2 and also M4. Sampling point of M2 is surrounded by commercial facilities, while M4 is located in the downstream from M2 and also directly adjacent to commercial facilities. These facts are likely to be the reason of high chemical concentrations. The conditions in Group 2 also similar with the condition in Group 4 and 7, when Sampling point adjacent to commercial or industrial facilities various chemicals presented high concentrations.

M5 was included in Group 3 and collected on the same day with M4, specifically to determine the decrease ratio. On many chemicals, concentration increased from M4 to M5, or several chemicals were undetectable in M4 but appeared in M5. Composition and concentration of chemicals were fluctuating too. However, toxicity tends to be more stable even when composition and concentration of chemicals fluctuate.

M6 was included in Group 4 and located downstream from M5. Since M6 is located nearby commercial areas, it might become sources of high concentration. M7 and M8 were included in Group 5, and both sites had low chemical concentrations like Group 1 except bromobutide (herbicide). Stream from M6, M8, and M9 were mixed and flew down to M7. Therefore, herbicide used in a farming area of small M8 basin was also detected at M7. It showed that, especially in small basins, high chemical concentrations can be detected as the result of irregular events such as the spraying herbicides.

M9 which differed from the other sampling points, was included in Group 6. M9 was unique in that it contained mostly commercial area and had high levels of chemical concentrations and toxicity. Although we cannot see a clear characteristic of Group1 in land use composition, it can be said that the sampling points of Group 1 are not adjacent to commercial and/or industrial facilities.

Table 5.2 - GC/MS Analysis Results based on the Cluster Analysis (mg/L)

Name	Group	1							2		3	4	5		6	7
	Toxicity	Z	M1	M3	Y1	Y3	Y4	Y5	M2	M4	M5	M6	M7	M8	M9	Y2
Hexachloropropylene																2.06
Pentamethylbenzene							0.03								1.87	
1,2-Dichlorobenzene	A 96hr-LC_{50} = 3.8mg/L							0.01								
1,2,3-Trichlorobenzene										0.02						
7,12-Dimethylbenz(a)anthracene											2.86					
Triphenylmethane															2.14	
Longifolene								0.10						0.08		
Dicyclopentadiene				0.24												
1,2,5,6,9,10-Hexabromocyclododecane									2.48							
Dibenzylether														0.09	2.88	
Bis(2-chloroethyl)ether								0.02								
Anthraquinone				0.16												
3- & 4-tert-Butylphenol															2.85	
2,4-Dimethylphenol	A 96hr-LC_{50} = 16mg/L												0.01			
4-n-Hexylphenol								0.01								
2-tert-Butyl-4-methoxyphenol															3.48	
2-tert-Butylphenol														0.01		
2-Methoxyphenol		0.19												0.04		
Phenol, 2,6-dimethoxy-														0.20		
Nonylphenol	B 96hr-LC_{50}=0.24mg/L						0.17						0.16		2.55	1.99
4-Bromophenol										0.01						
2-Chlorophenol							0.04									
Dimethyl phthalate			0.09				0.45					2.74		0.02	2.75	2.99
Diethyl phthalate			0.10				0.18		2.44	0.28	2.36				2.64	
Diisobutyl phthalate	A 96hr-LC_{50}=3.0mg/L		0.18				0.27			0.33	2.53		0.10			2.28
Di-n-butyl phthalate	A 96hr-LC_{50}=2.8mg/L	0.23	0.72		1.52		0.97	0.45	2.38		2.40	2.45	0.54	0.45	2.41	2.37
Bis(2-ethylhexyl)phthalate	A 96hr-LC_{50}=75mg/L		3.63			2.54	1.77		2.45	1.44	2.39	2.45	0.37		2.40	2.48
Dicyclohexyl phthalate	A 96hr-LC_{50}= >2.00mg/L						0.02				2.59	2.45				2.48
Methyl dodecanoate										0.03	2.50					2.51
Methyl hexanoate							1.11			0.07	2.02	2.84	0.03		2.40	
Methyl octanoate										0.06			0.03			
Methyl heptadecanoate														0.05		
Stearic acid methyl ester															2.37	
1-Acetoxy-2-methoxyethane				0.13												
2-Heptanol												1.65	0.02			
Octanol									2.66					0.05		
Ethanol, 2-phenoxy-				0.27	0.65				2.80	3.70	2.37					
2-Butoxyethanol	A 96hr-LC_{50} = >100mg/L						0.80	0.37		0.06			0.37	0.33	2.25	
Benzyl alcohol										0.10						
Butanoic acid, butyl ester										0.04						
Phenylethyl alcohol			0.07													
alpha-Terpineol α			0.15													
2-Heptanol					0.21		0.41			0.16	2.37	2.35		0.20	2.49	
Isosafrole			0.06													
2-Methyl-2,4-pentandiol	A 96hr-LC_{50}=> 100mg/L				0.18						2.07	2.81	0.12		2.06	2.85
Di(2-ethylhexyl)adipate	A 96hr-LC50= >50mg/L							0.02								
2-Cyclohexen-1-one											1.57					
3-Hexanol, 4-ethyl-													0.01			
Bis(2-ethylhexyl) sebacate													0.03			
n-Butylacrylate	A 96hr-LC_{50} = 2.4mg/L						0.05									
3-Methoxy-1-butyl acetate							0.31	0.25							2.41	
Coprostanol							0.39									
1-Nonanol	A 96hr-LC_{50}=3.2mg/L										2.45	1.96				
2-Ethyl-1-hexanol															2.51	
Cholesterol			0.89												2.62	
1,3-Dichloro-2-propanol															2.12	
1,1,1-Trichloro-2-methyl-2-propanol	A 96hr-LC_{50}= >50mg/L							0.24								
Aniline	A 96hr-LC_{50}=27mg/L				0.00							2.34	0.12			
2-Methylaniline	A 96hr-LC_{50} = 150mg/L												0.04			
3-Toluidine												1.81				
Benzidine			0.50													4.61
N-Phenyl-2-naphthylamine			0.02													
N,N-Dimethylaniline								0.03								
Phenacetin														0.03		
3,5-Dimethylaniline	A 96hr-LC_{50} = 34mg/L													0.02		
Quinoline							0.14									
4-Nitrotoluene																1.56
1-Nitronaphthalene																2.85
3-Nitroaniline				0.15												
2-Amino-4,6-dinitrotoluene						2.36										
Nitrobenzene										0.02						
4-Nitrophenanthrene												1.72				
2-Nitronaphthalene			0.08													
9-Nitrophenanthrene															1.44	
N-Nitrosomorpholine										0.06						
Cyclohexanamine, N-cyclohexyl-															4.81	
4-Nitrophenol													0.03			
e-Caprolactam						1.56										
Urea, N,N-diethyl-																1.68
Diburylamine											1.46	2.30	0.16			2.46

A = Medaka; B = Persistent toxicity test organisms (fish); C = Rainbow trout; D = Bluegill
Concentration in sample water is mg/L, In environmental water is μg / L.

Table 5.2 – (continued)

Name	Group	1							2		3	4	5			6	7
	Toxicity	Z	M1	M3	Y1	Y3	Y4	Y5	M2	M4	M5	M6	M7	M8	M9	Y2	
2-(Methylthio)-benzothiazol							0.24										
Diphenyldisulfide						2.23											
Tributyl phosphate	A 96hr-LC_{50}=14mg/L									0.09							2.95
Tris(2-ethylhexyl) phosphate	A 96hr-LC_{50}=40mg/L						0.13										2.52
L-Menthol									2.50								
Nicotine	A 48hr-LC_{50}=10mg/L								2.07								
Aspirin								3.60								1.88	1.89
Ethenzamide				0.30													
Diethyltoluamide							0.74			0.10			0.21	0.27	2.55		4.27
Crotamiton				0.24	0.34				2.91	1.38	2.64		0.67	0.78	2.48		3.15
Caffeine				0.28				0.29			2.96		0.52				2.76
Cypermethrin 1												1.61					
Azinphos-methyl							0.33										
Allethrin 1	C 96hr-LC_{50}=0.019mg/L									0.11							
Fenobucarb										0.01							
Methamidophos				0.27						5.67	2.57						2.98
Chlorethoxyfos											1.70						
Pyridaben	C 96hr-LC_{50}=1.1mg/L													0.06			1.92
Methiocarb										0.03				0.08			
Bendiocarb											2.40						
Mevinphos 1								0.25		0.16							
Cyhalothrin 2	C 96hr-LC_{50}=0.00054mg/L												0.20				
Dicrotophos													0.05				
Dimethoate	A 48hr-LC_{50}=>40mg/L												0.05				
Dialifos													0.07				
Pyrethrin 4										2.51			0.02		2.77		
Pyriproxyfen														0.01			
Terbufos				0.17													
Methoprene						1.78											
Chlorfenvinphos E							0.15										
Ethiofencarb	A 48hr-LC_{50}=4.8mg/L																1.56
Thiocyclam	A 24hr-LC_{50}=0.25mg/L												0.07				1.62
Trichlorfon								1.17					0.50				
Tetramethrin-2							0.14										
Tribenuron-methyl												2.42					
Oxabetrinil										0.08							2.26
Terbacil	A 48hr-LC_{50}=40mg/L			0.20													
Bromobutide	A 48hr-LC_{50}=10mg/L							0.01				2.12	11.53	7.25	2.40		
Dimethametryn	A 24hrLC_{50}=3.2 mg/L											2.62					
Carbetamide													0.24	0.10			
Prometryn	A 48hr-LC_{50}=4.3mg/L				1.05												2.50
Esprocarb	A 96h-LC_{50}=1.3mg/L										1.57				1.76		
Metribuzin DADK													0.04				
Propham														0.03			
Pretilachlor	C 96h-LC_{50}=0.9mg/L														1.99		
Butachlor	A 96hr-LC_{50}=280 µg/L											2.43					
Ethalfluralin											1.73						
Pyriminobac-methyl Z												2.23	0.33	0.19			
Norflurazon												2.09					
EPTC																	1.93
Bensulide							1.12										2.00
Simazine (CAT)																	1.36
Terbutryn																	2.52
Triclopyr	D 96hr-LC_{50}=48mg/L	0.18													2.32		
Desmedipham								0.18									
Dimethenamid								0.02									
Difenzoquat metilsulfate								0.38									
Pebulate							0.10										
Amino-chlornitrofen																	1.81
MCPA-thioethyl (Phenothiol)	A 48hr-LC_{50}=8.4mg/L						0.16										
Etobenzanid															1.53		
Bitertanol	C 96hr-LC_{50}=2.2mg/L													0.09			
Hexaconazole	C 96hr-LC50= 3.4mg/L												0.10	0.15			
Hymexazol							4.77	4.65				4.82	1.75		2.17		
Propamocarb															2.68		
Triadimenol 2							2.46					1.94			2.62		
TCMTB												2.37					
Cyproconazole							0.25										
Tricyclazole	A 48hr-LC_{50}=9.5mg/L											2.13					
Pyraclostrobin												1.59					
Trichlamid																	1.64
Fluquinconazole						1.68											
Triadimenol 1									2.20								
Thifluzamide														0.05			
Trifloxystrobin																	1.68
Isoprothiolane	A 96hr-LC_{50} = 9.0mg/L							0.02									
Triadimefon	D 96hr-LC_{50}=11mg/L									0.03	1.60						
Clofentezine	D 96hr-LC_{50}=>0.25mg/L														1.76		
Spirodiclofen							1.11								3.27		2.08
Dicofol-deg										0.06	1.70						

A = Medaka; B = Persistent toxicity test organisms (fish); C = Rainbow trout; D = Bluegill
Concentration in sample water is mg/L, In environmental water is µg / L.

From Table 5.3 it can be seen the logarithmic ratio of the concentration detected and the median lethal concentration value. A logarithmic scale is a measurement scale that uses the logarithm of a physical quantity instead of the quantity itself. From the calculations, the value for 100 concentrations is equal to -2. If the value is greater than -2, it has less than 100-concentration, which means that it has higher toxicity. The median lethal concentration values were obtained from literature review.

Table 5.3 - The Logarithmic Ratio of the Concentration Detected and the Median Lethal Concentration

No	Group			1							2		3	4	5		6	7
	Name	Description	Toxicity*	Z	M1	M3	Y1	Y3	Y4	Y5	M2	M4	M5	M6	M7	M8	M9	Y2
1	1,2-Dichlorobenzene	Using for the synthesis of agrochemicals	Medaka 96hr-LC_{50} = 3.8mg/L							-5.5								
2	2,4-Dimethylphenol	Use for feedstock or commercial products for industry and agriculture.	Medaka 96hr-LC_{50} = 16mg/L												-6.5			
3	Nonylphenol	Using to commercially important detergents	Persistent toxicity test organisms (fish) LC_{50} = 0.24mg/L						-3.1						-3.2		-2.0	-2.1
4	Diisobutyl phthalate	Use in nitro cellulose plastic, nail polish, explosive material, lacquer manufacturing	Medaka 96hr-LC_{50} = 3.0mg/L		-4.2				-4.0			-4.0	-3.1		-4.5			-3.1
5	Di-n-butyl phthalate	Use as an additive to adhesives or printing inks and substance in cosmetics	Medaka 96hr-LC_{50} = 2.8mg/L	-4.1	-3.6		-3.3		-3.5	-3.8	-3.1		-3.1	-3.1	-3.7	-3.8	-3.1	-3.1
6	Bis(2-ethylhexyl) phthalate	The dominant applications are for plastics, especially polyvinyl chloride (PVC)	Medaka 96hr-LC_{50} = 75mg/L		-4.3			-4.5	-4.6		-4.5	-4.7	-4.5	-4.5	-5.3		-4.5	-4.5
7	Dicyclohexyl phthalate	DCHP is used to stabilize some rubbers, resins and polymers	Medaka 96hr-LC_{50} = >2.00mg/L						-5.0				-2.9	-2.9				-2.9
8	2-Butoxyethanol	Solvent in paints and surface coatings.	Medaka 96hr-LC_{50} = >100mg/L						-5.1	-5.4		-6.2			-5.4	-5.5	-4.6	
9	2-Methyl-2,4-pentandiol	To control the flow properties of industrial products	Medaka 96hr-LC_{50} => 100mg/L				-5.7						-4.7	-4.6	-5.9		-4.7	-4.5
10	Di(2-ethylhexyl) adipate	DEHA is use as a functional hydraulic fluid.	Medaka 96hr-LC_{50} = >50mg/L							-6.3								
11	n-Butylacrylate	Use in the production of coatings and inks, adhesives, sealants, textiles, plastics and elastomers.	Medaka 96hr-LC_{50} = 2.4mg/L						-4.7									
12	1-Nonanol	The primary use for manufacture of artificial lemon oil.	Medaka 96hr-LC_{50} = 3.2mg/L										-3.1	-3.2				
13	1,1,1-Trichloro-2-methyl-2-propanol	Commonly used for cosmetic and pharmaceutical products	Medaka 96hr-LC_{50} = >50mg/L							-5.3								

* National Institute of Technology and Evaluation (NITE)

Table 5.3 - (continued)

No	Group			1							2		3	4	5			6	7
	Name	Description	Toxicity*	Z	M1	M3	Y1	Y3	Y4	Y5	M2	M4	M5	M6	M7	M8	M9	Y2
14	Aniline	Used as a chemical intermediate for the dye, agricultural, polymer, and rubber industries	Medaka 96hr-LC_{50} = 27mg/L				-6.8							-4.0	-5.3			
15	2-Methylaniline	Use for manufacture of dyes, pharmaceuticals, and pesticides.	Medaka 96hr-LC_{50} = 150mg/L												-6.5			
16	3,5-Dimethylaniline	Use for production of pesticides, dyes, and other chemicals	Medaka 96hr-LC_{50} = 34mg/L													-6.2		
17	Tributyl phosphate	Use as a solvent in inks, synthetic resins, gums, adhesives, herbicide and fungicide concentrates.	Medaka 96hr-LC_{50} = 14mg/L									-5.2						-3.7
18	Tris(2-ethylhexyl) phosphate	Used as a plasticizer, fire retardant and solvent.	Medaka 96hr-LC_{50} = 40mg/L						-5.5									-4.2
19	Nicotine	Used as a stimulant of a drug and tabaco industries	Medaka 48hr-LC_{50} = 10mg/L								-3.7							
20	Allethrin 1	Used in public health against mosquitoes, houseflies and cockroaches.	Rainbow trout 96hr-LC_{50} = 0.019mg/L									-2.2						
21	Pyridaben	Use for pesticide	Rainbow trout 96hr-LC_{50} = 1.1mg/L													-4.3		-2.8
22	Cyhalothrin 2	Use for Insecticide	Rainbow trout 96hr-LC_{50} = 0.00054mg/L												-0.4			
23	Dimethoate	Widely used organophosphate insecticide	Medaka 48hr-LC_{50} = >40mg/L												-5.9			
24	Ethiofencarb	Use for insecticide.	Medaka 48hr-LC_{50} = 4.8mg/L															-3.5
25	Thiocyclam	Use for insecticide	Medaka 24hr-LC_{50} = 0.25mg/L												-3.6			-2.2
26	Terbacil	Use to control annual and perennial grasses and broad-leaf weeds in agricultural fields	Medaka 48hr-LC_{50} = 40mg/L			-5.3												
27	Bromobutide	Use as a herbicide	Medaka 48hr-LC_{50} = 10mg/L							-5.9				-3.7	-2.9	-3.1	-3.6	
28	Dimethametryn	Use to control annual and perennial grasses and broad-leaf weeds in agricultural fields	Medaka 24hrLC_{50} = 3.2 mg/L											-3.1				
29	Prometryn	Used as herbicide	Medaka 48hr-LC_{50} = 4.3mg/L				-3.6											-3.2
30	Esprocarb	Use as herbicide	Medaka 96h-LC_{50} = 1.3mg/L										-2.9				-2.9	

* National Institute of Technology and Evaluation (NITE)

Table 5.3 - (continued)

No	Group			1							2		3	4	5		6	7
	Name	Description	Toxicity*	Z	M1	M3	Y1	Y3	Y4	Y5	M2	M4	M5	M6	M7	M8	M9	Y2
31	Pretilachlor	Use as pre-emergence herbicide in transplanted rice fields	Rainbow trout 96h-LC_{50} = 0.9mg/L														-2.7	
32	Butachlor	Use as herbicide	Medaka 96hr-LC_{50} = 280 µg/L											-2.1				
33	Alachlor	Use as herbicide	Bluegill 96hr-LC_{50} = 2.8mg/L															
34	Triclopyr	Use as herbicide	Bluegill 96hr-LC_{50} = 48mg/L	-5.9													-4.8	
35	MCPA-thioethyl (Phenothiol)	Use as herbicide	Medaka 48hr-LC_{50} = 8.4mg/L						-4.7									
36	Bitertanol	Use as a fungicide	Rainbow trout 96hr-LC_{50} = 2.2mg/L													-4.4		
37	Hexaconazole	Use for the control of many fungi	Rainbow trout 96hr-LC_{50} = 3.4mg/L												-4.5	-4.3		
38	Tricyclazole	Use as a fungicide	Medaka 48hr-LC_{50} = 9.5mg/L											-3.6				
39	Isoprothiolane	Isoprothiolane is one of fungicides	Medaka 96hr-LC_{50} = 9.0mg/L							-5.6								
40	Triadimefon	Use as a fungicide	Bluegill 96hr-LC_{50} = 11mg/L									-5.5	-3.8					
41	Clofentezine	Use for the residual control of mites in plant protection	Bluegill 96hr-LC_{50} = >0.25mg/L														-2.2	

* National Institute of Technology and Evaluation (NITE)

5.3.3 Outflow Mechanism of Toxicity in River Basin based on Land Use Parameter

Figure 5.7 and 5.8 shows the relationship between observed and calculated toxicity runoff. Although several trial and error to determine k and d_i in equation (7) using data from all sampling points, the observed and calculated C did not show clear relationship (Figure 5.7). From analysis, unit loading ratio value from farmland area, commercial area, industrial area, and sewer population show 0 value that means LDR_{50} did not released from that area. Then, by using data from Group 1 sampling points and set $d_i = 0$ other than dw, it could get rather linear relationship between observed and calculated C (Figure 5.8). This means LDR_{50} discharged from Group 1 basin can be explained using equation (7) with only two parameters, k' and dw. The obtained k' is 0.03/km, and di is 0.08. As shown in Figure 5.8, sampling points not included in Group 1 did not fit with the equation (7). When a sampling point is adjacent to commercial and/or industrial facilities, simple model like equation (2) becomes difficult to explain LDR_{50}. This fact might imply that in river basins dominated by residential areas, even when commercial and/or industrial facilities

discharge specific chemicals, they would not stay very long in the stream, and eventually chemicals discharged from residencies becomes majority.

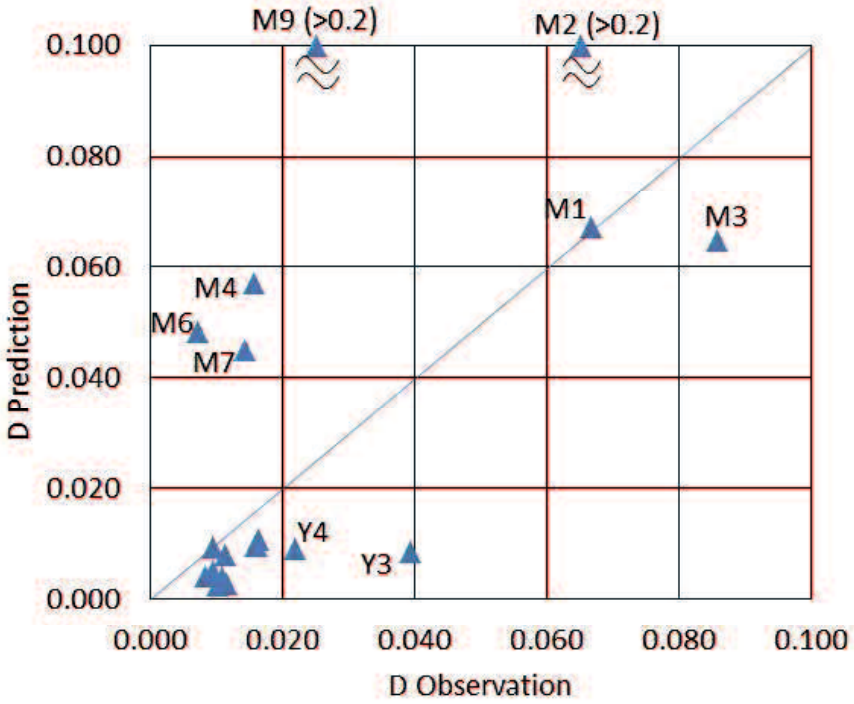

Fig. 5.7 – Relationship between Toxicity Load of Observation and Prediction Using Data From All Sampling Points

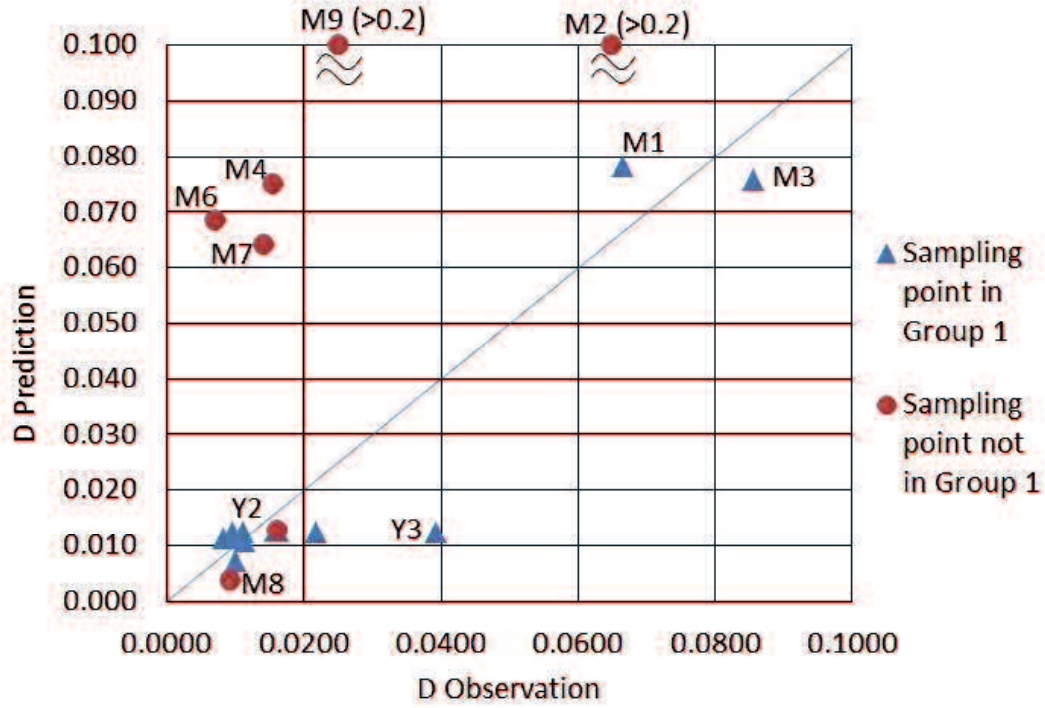

Fig. 5.8 – Relationship between Toxicity Load of Observation and Prediction Using Data From Group 1

5.4 Conclusions

We investigated the river water toxicity in three basins dominated by residential area and identified the chemical contents using GC/MS simultaneous analysis database.

1. From detected toxicity, we conclude that toxicity from residential area sometimes have high enough toxicity to affect the aquatic habitat, and it should be managed
2. From GC/MS analysis and cluster analysis:
    a. High toxicity not always shows high chemical concentrations. It is difficult to prove apparent relationship between the toxicity and chemical concentrations using the methods we employed in this research.
    b. Even when composition and concentration of chemicals are fluctuating, toxicity tends to be more stable.
    c. Chemical compositions, taken at the sampling points not adjacent to commercial and/or industrial facilities, are different from basin to basin, but almost all substances are in low concentrations. Otherwise, if taken adjacent to those facilities, has various differences and sometimes shows higher concentrations.
3. From model analysis:
    a. LDR_{50} discharged from a basin dominated by residential areas can be explained using a simple model with two parameters, k' (toxicity decrease ratio) and d_w (LDR_{50} discharged from the people not covered by sewer). The obtained k' is 0.03/km, and d_w is 0.08.
    b. When a sampling point is adjacent to commercial and/or industrial facilities, the simple model becomes difficult to explain LDR_{50}.
    c. Sometimes sampling point which is adjacent to commercial or industrial facilities also fit with this model equation, this fact might imply that in river basins dominated by residential areas, even when commercial or industrial facilities discharge specific chemicals, they won't stay very long in the stream, and eventually chemicals discharged from residencies becomes majority.

5.5 References

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CHAPTER 06
CONCLUSION

6.1 Conclusions

1. Toxicity by using Medaka fish and 100-fold concentrated water could be used to detect toxicity level which affects aquatic habitat condition. Furthermore, the ratio of clear stream macrobenthic animal sharply decreased over 0.25 of LT_{50}^{-1} or 0.5 of ET_{50}^{-1} and tolerant fish become dominant over 0.3 of LT_{50}^{-1} or 0.5 - 1.0 of ET_{50}^{-1}.
2. a. Detected toxicity from residential area sometimes has high enough toxicity to affect the aquatic habitat, and it should be managed.
   b. Toxicity discharged from a basin dominated by residential areas can be explained using a simple model with two parameters, k' (toxicity decrease ratio) and d_w (LDR_{50} discharged from the people not covered by sewer). The obtained k' is 0.03/km, and d_w is 0.08.
   c. These findings suggest that toxicity from residential area should be managed, and the pollution analysis procedure for sewerage designing can be applicable for toxicity management in the river the majority of which catchments are residential area.

6.2 Future Work

1. Due to the limitation in the number of the data, relationship between LT_{50}^{-1} and LDR_{50} is not clear. Therefore, more data should be collected pertaining to more research work to obtain better results for relationship between LT_{50}^{-1} and LDR_{50}.
2. These studies only focus on the rivers in Japan the basins of which are dominated by residential areas. Therefore, more research work in this area should be carried out, considering different land use areas with respect to Japan as well as other countries. Eventually, this will help in getting a wider perspective to understand the situation. Thus leading to holistic approach for better management of the rivers.