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論文題目	Ecological Risk Assessment of a River on Agricultural Area in West Java Province, Indonesia and Comparison with Whole Effluent Toxicity Test (インドネシア西ジャワ地方農業地区における河川の生態リスク評価と WET 試験との比較)		
<p>(論文内容の要旨)</p> <p>This thesis comprises the ecological risk assessment in Cipeles River, West Java Province, Indonesia and its comparison to the Whole Effluent Toxicity (WET) test. The thesis was divided into 8 chapters as followed:</p> <p><b>Chapter 1 Introduction</b></p> <p>This chapter described the background, the objective, the study area, and the systematics of this thesis. The study area was Cipeles River, a tributary of Cimanuk River located in the agricultural area of Sumedang District, West Java Province, Indonesia.</p> <p><b>Chapter 2 Literature review</b></p> <p>This chapter reviewed the ecological risk assessment, Fish Embryo Toxicity (FET) test, the test that conducted in determining the toxicity level in this study, and also physicochemical toxicity such as ammonia-nitrogen and zinc to the aquatic environment. It also reviewed the organophosphorus (ORP) pesticide, its occurrence in the aquatic environment, and the toxicity level in previous studies.</p> <p><b>Chapter 3 Determination ORP Pesticides in River Water Sample using Solid Phase Extraction and Gas Chromatographic-Mass Spectrometer</b></p> <p>This chapter discussed the problem formulation of organic compounds that might emerge in the study area. The objective was to determine the ORP concentration in water samples from several sampling locations simultaneously using the SPE coupled with GC-MS. The recovery using multiple ORP standards was within the acceptable range and the coefficient of determination <math>R^2</math> of each 13 compounds <math>&gt;0.98-0.99</math>. The chlorpyrifos was detected in the water sample from upstream (St.1), city area (St.4), and downstream (St.10), at <math>1.19</math>, <math>0.49</math>, and <math>0.28\mu\text{g.L}^{-1}</math>, respectively, while terbufos and thiometon were detected on the St.1 sample at <math>0.93\mu\text{g.L}^{-1}</math> and <math>0.07\mu\text{g.L}^{-1}</math>, respectively.</p> <p><b>Chapter 4 Distribution, Source Identification, and Assessment of Heavy Metal Pollution in the Surface and Pore Waters of Cipeles River, West Java Province, Indonesia</b></p> <p>This chapter focused to determine the heavy metals in surface and pore water by ICP-MS for a basic database on pollution assessment using heavy metal pollution indices (HMPI). A spatial analysis using GIS also conducted here. The dominant heavy metals, Fe and Mn, had detected as the results of runoff from the laterite and alluvial soils in the study area. The presence of other micro concentrations of heavy metals in the city center showed the anthropogenic source of non-mining activities, such as fertilizer and pesticide usage in agriculture. As a result, the heavy metal occurrence in the pore water was <math>\text{Mn}&gt;\text{Fe}&gt;\text{Ba}&gt;\text{Co}&gt;\text{Zn}&gt;\text{Cu}&gt;\text{Pb}&gt;\text{Cr}</math> in the order of abundance, while in the surface water, <i>i.e.</i>, <math>\text{Fe}&gt;\text{Mn}&gt;\text{Zn}&gt;\text{Ba}&gt;\text{Cu}&gt;\text{Pb}&gt;\text{Co}&gt;\text{Cr}</math>. The high concentration of zinc in St.4 and St.8 samples contributed to their high HMPI.</p>			

### **Chapter 5 Lethal and Sublethal Effect on Early-life Stage of Zebrafish by Organophosphorus Phorate Exposure**

This chapter highlighted the determination of acute lethal and sublethal toxicity of ORP phorate exposure using zebrafish early-life stage toxicity test on a prolonged period. This bioassay would reduce the requirement of the sample volume, while the prolonged period 120hpf accommodates the zebrafish larvae ability to swim-up as an important stage to survive. This study revealed that increasing of phorate concentration and the exposure time ( $t_{exp}$ ) significantly different from the control of lethal rate and hatching rate. The probit analysis on lethal  $LC_{50}$  resulted  $4.54 \text{ mg.L}^{-1}$ , while the estimation of no-effect concentration was found  $7.67 \text{ }\mu\text{g.L}^{-1}$  at 30 days. The  $EC_{50}$  on hatching rate and swim-up failure was  $9.75 \text{ mg.L}^{-1}$  and  $2.14 \text{ }\mu\text{g.L}^{-1}$ , respectively. Only  $t_{exp}$  gave a significant difference to the swim-up failure rate. So, the prolonged period 120hpf was significant to be monitored as proposed. Rely on the result of a lethal effect, even  $LC_{50}$  endpoint was higher than the fish acute toxicity, this bioassay could be used as the previous screening to fish acute toxicity to support the 3Rs principle.

### **Chapter 6 Whole Effluent Toxicity (WET) Test of River Water Sample using Early-life stage Zebrafish**

The WET test to determine the lethal and sublethal effects on salinity variation and field water sample, which implemented the previous zebrafish early-life stage bioassay, was discussed in this chapter. Salinity treatment only had a significant effect on the lethal rate when above 0.17psu. Meanwhile, salinity treatment had no significant difference to the hatching rate and swim-up failure rate. This study also revealed that zebrafish embryo could survive on salinity as low as 0.04 psu. From the WET test, significant difference analysis showed on lethal effect from sample St.1, St.4, and St.7, while the hatching rate from sample St.4, St.5, and St.8. The swim-up failure rate was significantly different in the sample St.1, St.5, St.7.

### **Chapter 7 Ecological Risk Assessment (ERA) Estimation**

The ERA calculation especially on detected ORP in the surface water sample was discussed in this chapter. Regarding the risk quotient (RQ) on three trophic levels of aquatic organisms, the ORPs detected in water samples put concern not only the acute lethal-sublethal effect to the local aquatic organisms that have sensitivity as early-life stage *Lepomis m.* but also the chronic effect on the lower trophic level, such as daphnids. At St.1, the RQs resulted by chlorpyrifos, *i.e.*, on daphnid (1.45) and on adult *Lepomis m.* (0.699); by terbufos, *i.e.*, on daphnid (2.33), on adult *Lepomis m.* (0.518), and on juvenile *Lepomis m.* (1.211). At St.4, the RQ by chlorpyrifos on daphnid is 0.595. The concern also addressed to St.7 because of ammonia (RQ = 1.69), meanwhile to St. 4 (RQ = 2.04) and St.8 (RQ = 0.76) because of zinc.

### **Chapter 8 General Conclusion and Future Recommendation**

This chapter summarized the conclusions of the various findings and their implications. The ERA estimation on concerning the physicochemical parameter and ORP pesticide had significant correlation with the WET test result. The recommendations for further research also discussed in this chapter.

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(論文審査の結果の要旨)

本論文は、インドネシア西ジャワ地方農業地区における Cipeles 川を対象として、重金属や農薬成分による生態リスク評価を行った結果と、ゼブラフィッシュを用いた WET (全排水毒性) 試験結果との比較研究を行った成果をまとめたものであり、得られた主な成果は以下のとおりである。

1. Cipeles 川で 13 種の有機リン農薬成分を分析した結果、chlorpyrifos が上流の St.1 地点で  $1.19 \mu\text{gL}^{-1}$ 、中流都市部の St.4 地点で  $0.49 \mu\text{gL}^{-1}$ 、下流の St.10 地点で  $0.28 \mu\text{gL}^{-1}$  の濃度で検出された。Terbufos と thiometon は St.1 地点のみで  $0.93 \mu\text{gL}^{-1}$  と  $0.07 \mu\text{gL}^{-1}$  の濃度で検出された。また、重金属についても、亜鉛濃度が St.4 地点と下流の St.8 地点で基準値を超える  $469 \mu\text{gL}^{-1}$  と  $176 \mu\text{gL}^{-1}$  の濃度で検出されたことなどから、Cipeles 川の生態リスクが無視できないレベルであることを示した。
2. 生態リスクの評価指標である RQ 値によって Cipeles 川の状況を評価した場合、St.1 地点での chlorpyrifos について、ミジンコに対し 1.45、成体の Lepomis m. に対し 0.699 であり、terbufos については、ミジンコに対し 2.33、成体の Lepomis m. に対し 0.518、幼体の Lepomis m. に対し 1.21 であるなど、生態リスクが懸念されるレベルであった。また、中流の St.7 地点ではアンモニアの RQ 値が 1.69 であり、St.4 地点と St.8 地点では亜鉛の RQ 値が懸念されるレベルであることを示した。
3. WET 試験としては、受精後 120 時間ゼブラフィッシュ胚バイオアッセイを、河川水曝露による致死性および亜致死性効果を決定するために実施した。なお、受精卵の培養液は塩分濃度 0.22psu なので、河川水曝露によって塩分濃度が変化することの影響を考慮するために、試験水の塩分濃度変化が致死性および亜致死性効果に及ぼす影響も実験により解析した。その結果、塩分濃度 0.17psu 以上で致死率に対してのみ有意な影響が見られたが、孵化率や稚魚の浮上不成功率には有意な差は見られなかった。したがって、河川水曝露における塩分濃度の影響は考慮する必要がないことがわかった。河川水曝露においては、St.1, St.4, St.7 地点のサンプルは致死率に対し有意な差を示したが、孵化率に対しては St.4, St.5, St.8 地点のサンプルが、稚魚の浮上不成功率に対しては St.1, St.5, St.7 地点が有意な影響を示した。よって、WET 試験結果は生態リスク評価において懸念された水質指標と良い相関を示すことを明らかにした。

以上の結果は、インドネシア西ジャワ地方における河川の生態リスクレベルを示す貴重なデータであり、また、ゼブラフィッシュを用いた WET 試験の応用に大きく貢献するものであって、学術上、實際上寄与するところが少なくない。よって、本論文は博士 (工学) の学位論文として価値あるものと認める。また、令和 2 年 8 月 24 日、論文内容とそれに関連した事項について試問を行って、申請者が博士後期課程学位取得基準を満たしていることを確認し、合格と認めた。なお、本論文は、京都大学学位規程第 14 条第 2 項に該当するものと判断し、公表に際しては、令和 3 年 9 月 30 日までの間、当該論文の全文に代えてその内容を要約したものとすることを認める。

要旨公開可能日： 年 月 日以降