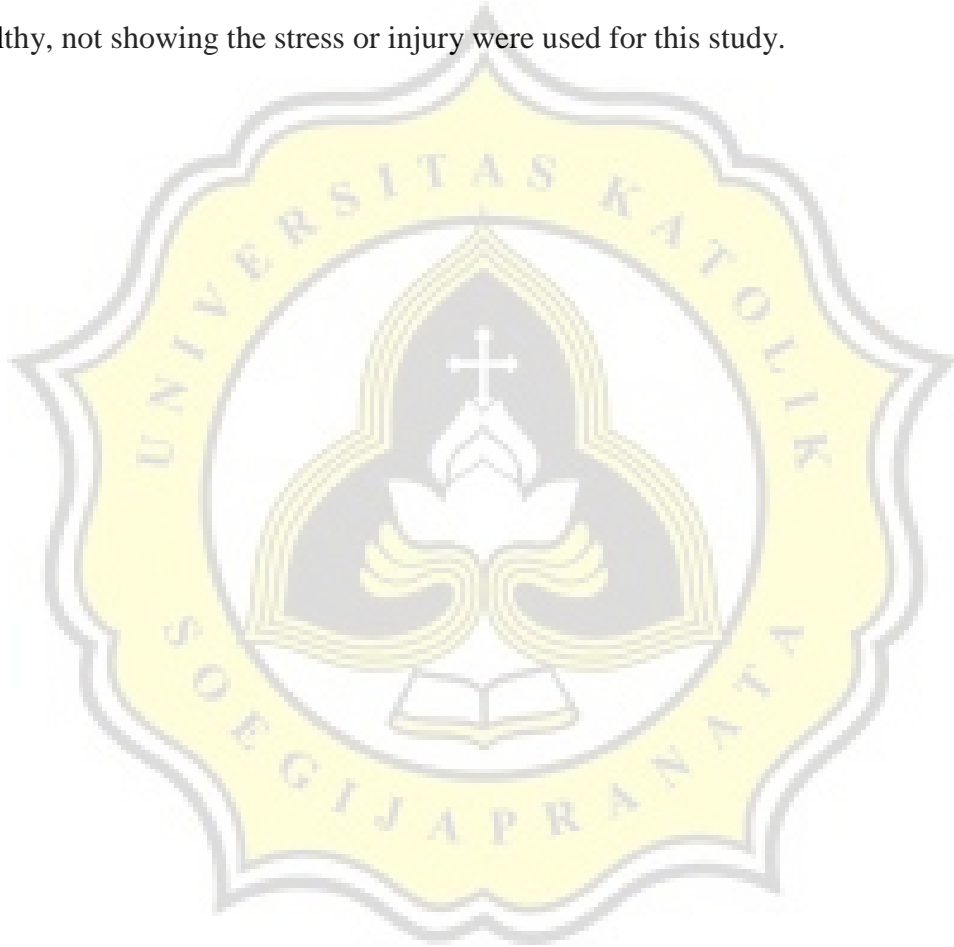


7. APPENDICES

Appendix 1. Fish Acclimation Condition

The tanks kept at 25 of salinity, 23-24.6°C, 6.5-8.0 mg/L of dissolved oxygen (DO), a pH of $7,9 \pm 0.04$, and with a photoperiod of 12 hours light and 12 hours dark. The salinity reflected *in situ* condition. Before the tests were started, the fish had two weeks to acclimate to the laboratory water condition, and they fed twice a day. Fish that categorized as healthy, not showing the stress or injury were used for this study.



Appendix 2. Determination of 96 LC50 Values

Milkfish was exposed to metal contaminants, copper chloride ($\text{CuCl}_2 \cdot \text{H}_2\text{O}$: Merck) and cadmium sulfate ($3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$: Merck). Five levels of concentrations for Cu were 0 mg/L, 6.25 mg/L, 12.5 mg/L, 25 mg/L, 50 mg/L, and 100 mg/L. While the concentration for Cd were 0 mg/L, 3.13 mg/L, 6.25 mg/L, 12.5 mg/L, 25 mg/L, and 50 mg/L. Each concentration was dissolved in 8 liters tank, contains 10 milkfish, and each concentration had three replicates. Thus, the total milkfish that needed for each concentration is 30 fish.

Table 5. Acute LC₅₀ Determination for Copper Chloride Contaminant

Contaminant	Concentration(mg/L)	Tank	Content	Replicates	Sub-Total Fish
$\text{CuCl}_2 \cdot \text{H}_2\text{O}$	0	1	10 fish	3	30 fish
	6.25	1	10 fish	3	30 fish
	12.5	1	10 fish	3	30 fish
	25	1	10 fish	3	30 fish
	50	1	10 fish	3	30 fish
	100	1	10 fish	3	30 fish
Total fish needed for Cu contaminant					180 fish

Table 6. Acute LC₅₀ Determination for Cadmium Sulfate Contaminant

Contaminant	Concentration(mg/L)	Tank	Content	Replicates	Sub-Total Fish
$3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$	0	1	10 fish	3	30 fish
	3.13	1	10 fish	3	30 fish
	6.25	1	10 fish	3	30 fish
	12.5	1	10 fish	3	30 fish
	25	1	10 fish	3	30 fish
	50	1	10 fish	3	30 fish
Total fish needed for Cu contaminant					180 fish

Appendix 3. Amount of Initial and Re-dose Concentrations for LC₅₀ Values

The amount of either Cu or Cd contaminant has been calculated to establish the initial and re-dose concentration that can be seen in the table below.

Table 7. Amount of Cu to Achieve Initial and Re-dosing Concentration for LC₅₀ Value

Amount of Cu that added after water renewal to maintain the initial concentration

Contaminant	Initial Concentration (mg/L)	Amount of Metal for Initial Concentration (g)	Amount of Metal for Re-dose Concentration (g)
CuCl ₂ .H ₂ O	0	0	0
	6.25	0.136	0.064
	12.5	0.272	0.136
	25	0.536	0.272
	50	1.072	0.536
	100	2.288	1.072

Table 8. Amount of Cd to Achieve Initial and Re-dosing Concentration for LC₅₀ Value

Amount of Cd that added after water renewal to maintain the initial concentration

Contaminant	Initial Concentration (mg/L)	Amount of Metal for Initial Concentration (mg/L)	Amount of Metal for Re-dose Concentration (mg/L)
3CdSO ₄ .8H ₂ O	0	0	0
	3.13	0.056	0.032
	6.25	0.112	0.056
	12.5	0.232	0.112
	25	0.456	0.232
	50	1.152	0.456

Appendix 4. Amount of Initial and Re-dose Concentrations for Sub Lethal Concentration

The amount of either Cu or Cd contaminant has been calculated to establish the initial and re-dose concentration that can be seen in the table below.

Table 9. Amount of Cu to Achieve Initial and Re-dosing Concentration for Sub-Lethal Concentration

Contaminant	Initial Concentration (mg/L)	Amount of Metal for Initial Concentration (g)	Amount of Metal for Re-dose Concentration (g)
CuCl ₂ .H ₂ O	0	0	0
	1/20of LC ₅₀	0.024	0.018
	1/10of LC ₅₀	0.036	0.027
	1/5of LC ₅₀	0.084	0.063

Table 10. Amount of Cd to Achieve Initial and Re-dosing Concentration for Sub-Lethal Concentration

Contaminant	Initial Concentration (mg/L)	Amount of Metal for Initial Concentration (g)	Amount of Metal for Re-dose Concentration (g)
3CdSO ₄ .8H ₂ O	0	0	0
	1/20of LC ₅₀	0.036	0.027
	1/10of LC ₅₀	0.072	0.054
	1/5of LC ₅₀	0.132	0.099