# LABORATORY OF FOREST PRODUCTS CHEMISTRY FACULTY OF FORESTRY MULAWARAMAN UNIVERSITY



## STANDARD OPERATING PROCEDURE

	Date:
Toxicity Testing Methods	Create by:
	File:

### **1. OBJECTIVE**

To provide guidelines for conducting toxicity testing.

#### 2. PRINCIPLE

Toxicity testing is performed to determine the toxicity level of a sample on a living organism using the Brine Shrimp Lethality Test (BSLT) method.

#### **3. REOUIRED EOUIPMENT**

Laboratory coat, glasses, clean cloth/tissue.

Title:

#### **3. MATERIALS AND EQUIPMENT**

Material	EQUIPMENT
Sea water	Vial bottles
Plant sample extract	Droper pipette
Brine shrimp eggs (Artemia salina)	500 ml beaker
Ethanol Solvent	Air circulation
	Analytical scales
	Micropipettes & Tips

#### **4. PROCEDURE**

#### **4.1. HATCHING THE BRINE SHRIMP**

4.1.1 Pour seawater into a glass beaker;

- 4.1.2 Put enough shrimp eggs into sea water;
- 4.1.3 Connect the circulating water with the beaker;
- 4.1.4 Shrimp will be ready for testing after 2 days of hatching.

#### 4.2. PREPARATION OF STOCK SOLUTION AND SAMPLE CONCENTRATION

4.2.1 For stock solution, weigh a 20 mg sample of the extract;

4.2.2 Dissolve the extract in 2 ml of ethanol to achieve a stock solution concentration of 10,000 ppm;

4.2.3 Transfer 500 µl of the stock solution into vial bottles, performing this step in triplicate. During testing, adding sea water up to 5 ml will yield a sample concentration of 1,000 ppm. Concentration can be adjusted as needed;

4.2.4 Evaporate the ethanol in the vial bottles, allowing them to sit for 1-2 days until the solvent completely evaporates.

#### 4.3. TOXICITY TESTING

4.3.1 Dissolve the dried sample with sufficient seawater until the sample dissolves;

4.3.2 Put 10 brine shrimp nauplii that have hatched into each sample;

4.3.3 Add sea water until the volume reaches 5 ml.;

4.3.4 Place the vials containing samples and nauplii in a well-lit area for 24 hours;

4.3.5 Count dead and live nauplii the next day;

4.3.6 Calculate the mortality percentage (toxicity) using the formula:

%Mortality = 
$$\frac{A}{B} \times 100\%$$

Where:  $A = \sum dead nauplii$ 

 $B = \Sigma$  initial nauplii

4.3.7 Clean the work area and equipment when you have finished testing

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