Simulating Water Toxicity Tests Using Phosphorescent Stickers and a Light Sensor

By Meenakshi Pozhamkandath Sundaran (32714)

1. INTRODUCTION

Ensuring good water quality is important for both environmental protection and human health. One widely accepted technique to detect water toxicity is the bioluminescence inhibition assay, which uses naturally glowing bacteria like `Vibrio fischeri`. These bacteria emit light as part of their natural metabolism. If a toxic substance is present in the water, it can reduce or stop the bacteria from producing light. By measuring this decrease in light, we can determine the level of toxicity in the water. This method is recognized by the International Organization for Standardization (ISO) as a reliable standard for toxicity testing.

In research laboratories, the measurement of bioluminescence is usually done using large and specialized machines. One example is the Tristar Multimode Reader(fig1), which is used in our university. This machine is very efficient but also expensive. The goal of this project is to create our own measurement system that can record light levels in a similar way.

The bacteria used in this test, `Vibrio fischeri` (fig 2), is a special type of marine bacteria that naturally produces light. However, it is quite expensive and hard to grow in large amounts. For the project, we would need a high concentration of these bacteria to get strong luminescence, which is a limitation. Because of this, the experiment is simulating with the glowing effect using phosphorescent stickers (also known as glow-in-the-dark stickers) as a substitute.





Fig1: Tristar multimode reader(own image) Fig2: Vibrio fischeri vial (own image)

2. MATERIALS

TSL2591 (Light sensor)

For this project, the TSL2591 (Fig 3) high dynamic range digital light sensor was used to measure light intensity within the dark box. It has a precise lux measurements over a wide range (up to 88,000 lux), making it ideal for detecting even small changes in simulated bioluminescence.



Fig 3: TSL2591 (own image)

Adafruit QT Py ESP32-S2 microcontroller

The Adafruit QT Py ESP32-S2 is a small compact microcontroller featuring the ESP32-S2 chip. The microcontroller enables real-time data acquisition from the TSL2591 light sensor through its integrated I²C interface. Its built-in Wi-Fi capability allows wireless transmission of sensor data, facilitating remote monitoring of bioluminescence signals in the toxicity assay. It supports CircuitPython and Arduino, making it ideal for the project.

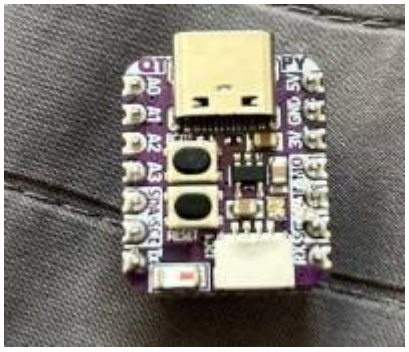


Fig 4: QT Py ESP32-S2 (own image)

Phosphorescent stickers

Phosphorescent stickers (fig 5) are glow-in-the-dark materials that emit light after being charged by an external light source. In this project, they simulate the bioluminescence of `Vibrio fischeri` bacteria, allowing light sensor testing without using live bacteria.



Fig 5: phosphoroscent stickers(own image)

Jumper wires Jumper wires(fig 6) are used to make temporary electrical connections between the microcontroller and sensors.



Fig 6: jumper wires (own image)

USB C-to-C cable

A USB C-to-C cable(fig 7) is used to connect the microcontroller to a laptop for power supply and data communication.



Fig 7: USB C-to-C cable(own image)

Flashlight A flashlight (fig 8) was used to provide light flash since the TSL2591 sensor does not have a built-in LED. It helps charge the phosphorescent stickers to simulate bioluminescence for testing.



Fig 8: Flash light (own image)

3. METHODS

3.1 Hardware Setup and Connections

The TSL2591 digital light sensor was connected to the Adafruit QT Py ESP32-S2 microcontroller using jumper wires. Specifically, the sensor's SCL pin was connected to the microcontroller's SCL pin, and the sensor's SDA pin was connected to the microcontroller's SDA pin. The sensor's VCC pin was connected to the 3.3 V output of the microcontroller to power the sensor, while the GND pin was connected to the common ground. Because the TSL2591's built-in pull-up resistors were insufficient for stable communication, two external 10 K Ohm; resistors were added as pull-up resistors on the SCL and SDA lines. Due to this issue with the pull-up resistors, the STEMMA QT connector and cables could not be used, so manual jumper wires were employed for direct connections. The entire setup

was powered via a USB C-to-C cable connected from the microcontroller to a laptop, providing both power and programming interface.

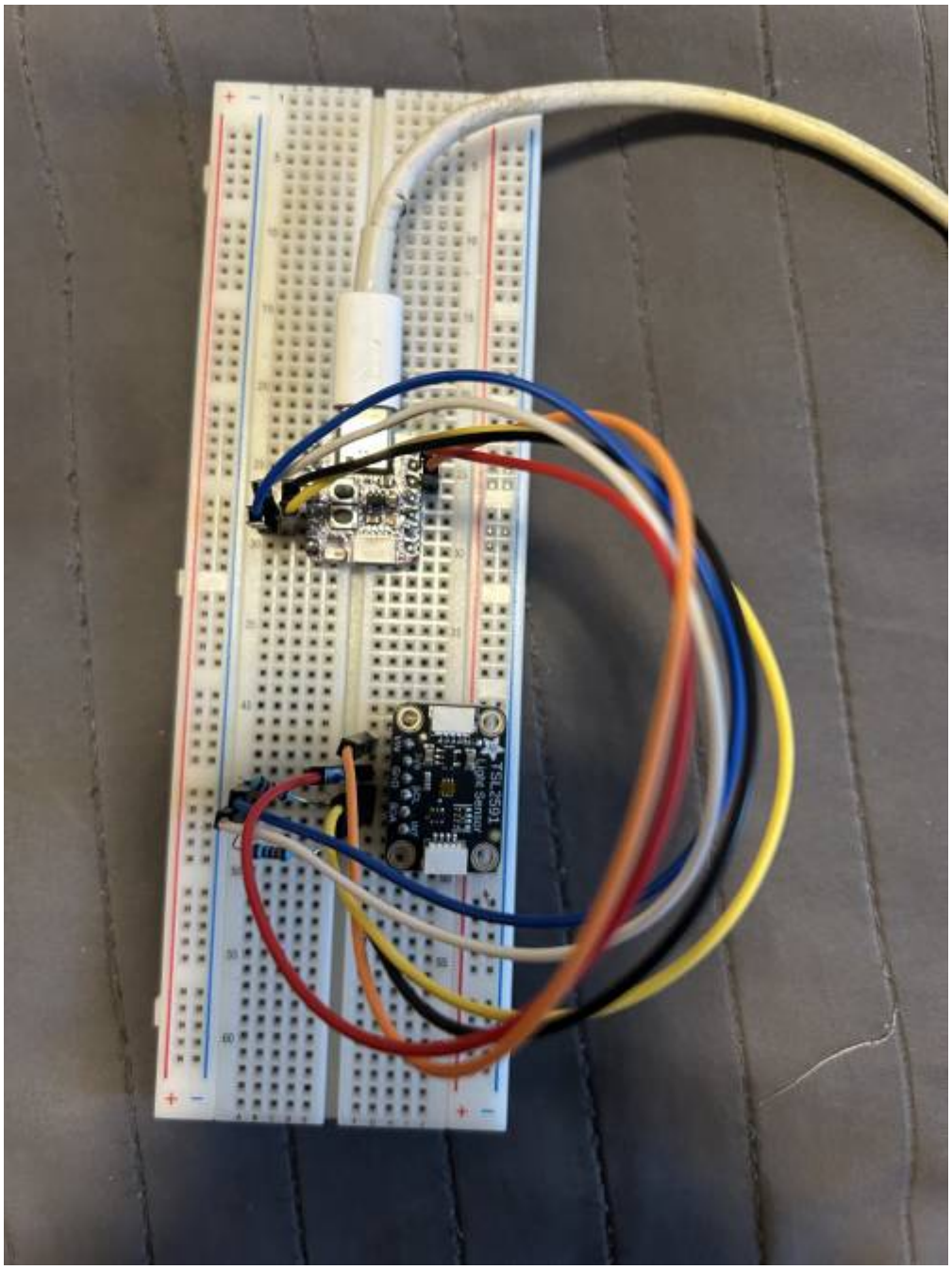


Fig 9: Hardware connections (own image)

3.2 Dark box preparation

A light-proof dark box was prepared by placing one cardboard box inside a larger one to enhance light isolation and minimize external ambient light interference. A custom plastic holder was DIY-made and glued onto the lid of the inner cardboard box to securely hold the phosphorescent sticker in place. This setup allowed the sticker to simulate the bioluminescence emitted by `Vibrio fischeri` bacteria within a controlled environment.

3.3 Sticker activation

Since the phosphorescent stickers require excitation to emit light, a flashlight was used to briefly illuminate the sticker for one second. This 'charging' process causes the sticker to glow in the dark, mimicking the natural bioluminescence of bacteria, and then gradually losing its luminescence over time.



Fig 9: experimental setup (own image)

3.4 Microcontroller Power and Programming

Required libraries, including those from the Bundle for Version 9.x, such as `adafruit_tsl2591.mpy`

and `adafruit_bus_device`, were downloaded from https://circuitpython.org/libraries and copied to the board's lib folder to enable sensor communication and Wi-Fi functionality. The program was written and edited using the Mu Editor and saved as `code.py` on the microcontroller. This script reads light intensity data from the TSL2591 sensor and attempts wireless transmission via the built-in Wi-Fi. However, since the microcontroller supports only 2.4 GHz Wi-Fi and the home network operates on 5 GHz, wireless transmission was not functional.

Below is a screenshot of the program used in this project:

code.py

```
import time
import board
import busio
import adafruit_tsl2591

i2c = busio.I2C(board.SCL, board.SDA)
sensor = adafruit_tsl2591.TSL2591(i2c)

print("Starting light measurement. Get ready...")
time.sleep(10)  # Time to flash sticker

start_time = time.monotonic()
duration = 60  # seconds

while (time.monotonic() - start_time) < duration:
    elapsed = time.monotonic() - start_time
    lux = sensor.lux
    print(f"Time: {elapsed:.2f}s, Lux: {lux:.2f}")
    time.sleep(1)

print("Measurement complete.")
```

Fig 10: screenshot of the code(own image)

3.5 Data Acquisition

Once the phosphorescent sticker was glowing, the TSL2591 sensor measured the light intensity inside the dark box. A phosphorescent sticker was first used to simulate normal bioluminescence. To simulate the presence of a toxic substance, a transparent light brown plastic cover was placed over the sticker, mimicking how toxins reduce bacterial luminescence. The microcontroller continuously read the light intensity data, and the decay curve was analyzed to observe changes in luminescence over time.

4. RESULTS

Light intensity readings were collected over time under two different conditions: one representing a non-toxic environment and the other simulating a toxic environment. The TSL2591 light sensor, connected to the Adafruit QT Py microcontroller, recorded lux values at one-second intervals using CircuitPython via the Mu Editor.

Recorded sensor readings of non-toxic environment:

CircuitPython REPL

```
Starting light measurement. Get ready...
Time: 0.00s, Lux: 2.89
Time: 1.00s, Lux: 2.57
Time: 2.01s, Lux: 2.24
Time: 3.01s, Lux: 2.08
Time: 4.01s, Lux: 1.91
Time: 5.01s, Lux: 1.91
Time: 6.02s, Lux: 1.75
Time: 7.02s, Lux: 1.59
Time: 8.02s, Lux: 1.42
Time: 9.03s, Lux: 1.42
Time: 10.03s, Lux: 1.42
Time: 11.03s, Lux: 1.53
Time: 12.04s, Lux: 1.53
Time: 13.04s, Lux: 1.53
Time: 14.04s, Lux: 1.36
Time: 15.04s, Lux: 1.36
Time: 16.05s, Lux: 1.36
Time: 17.05s, Lux: 1.36
Time: 18.05s, Lux: 1.20
Time: 19.05s, Lux: 1.20
Time: 20.06s, Lux: 1.20
Time: 21.06s, Lux: 1.20
Time: 22.06s, Lux: 1.20
Time: 23.07s, Lux: 1.20
Time: 24.07s, Lux: 1.20
Time: 25.07s, Lux: 1.04
Time: 26.08s, Lux: 1.04
Time: 27.08s, Lux: 1.04
Time: 28.08s, Lux: 1.04
Time: 29.08s, Lux: 1.04
Time: 30.09s, Lux: 1.04
Time: 31.09s, Lux: 1.04
Time: 32.09s, Lux: 1.04
Time: 33.10s, Lux: 1.04
Time: 34.10s, Lux: 1.04
Time: 35.10s, Lux: 1.04
Time: 36.10s, Lux: 0.87
Time: 37.11s, Lux: 0.87
Time: 38.11s, Lux: 0.87
Time: 39.11s, Lux: 0.87
Time: 40.12s, Lux: 0.87
Time: 41.12s, Lux: 0.87
```

```
Time: 39.11s, Lux: 0.87
Time: 40.12s, Lux: 0.87
Time: 41.12s, Lux: 0.87
Time: 42.12s, Lux: 0.87
Time: 43.12s, Lux: 0.87
Time: 44.13s, Lux: 0.87
Time: 45.13s, Lux: 0.87
Time: 46.13s, Lux: 0.87
Time: 47.14s, Lux: 0.87
Time: 48.14s, Lux: 0.87
Time: 49.14s, Lux: 0.87
Time: 50.14s, Lux: 0.71
Time: 51.15s, Lux: 0.71
Time: 52.15s, Lux: 0.71
Time: 53.15s, Lux: 0.55
Time: 54.16s, Lux: 0.55
Time: 55.16s, Lux: 0.55
Time: 56.16s, Lux: 0.55
Time: 57.16s, Lux: 0.39
Time: 58.17s, Lux: 0.39
Time: 59.17s, Lux: 0.39
Measurement complete.

Code done running.

Press any key to enter the REPL. Use CTRL-D to reload.
```

Fig 11 :screen shot of sensor readings(own image)

Recorded sensor readings of the toxic environment:

CircuitPython REPL

```
Starting light measurement. Get ready...
Time: 0.00s, Lux: 0.39
Time: 1.00s, Lux: 0.39
Time: 2.01s, Lux: 0.39
Time: 3.01s, Lux: 0.39
Time: 4.01s, Lux: 0.39
Time: 5.01s, Lux: 0.39
Time: 6.02s, Lux: 0.39
Time: 7.02s, Lux: 0.39
Time: 8.02s, Lux: 0.22
Time: 9.02s, Lux: 0.22
Time: 10.03s, Lux: 0.22
Time: 11.03s, Lux: 0.22
Time: 12.03s, Lux: 0.22
Time: 13.04s, Lux: 0.22
Time: 14.04s, Lux: 0.22
Time: 15.04s, Lux: 0.22
Time: 16.05s, Lux: 0.22
Time: 17.05s, Lux: 0.22
Time: 18.05s, Lux: 0.22
Time: 19.05s, Lux: 0.22
Time: 20.06s, Lux: 0.22
Time: 21.06s, Lux: 0.22
Time: 22.06s, Lux: 0.22
Time: 23.06s, Lux: 0.22
Time: 24.07s, Lux: 0.22
Time: 25.07s, Lux: 0.22
Time: 26.07s, Lux: 0.22
Time: 27.08s, Lux: 0.22
Time: 28.08s, Lux: 0.22
Time: 29.08s, Lux: 0.22
Time: 30.09s, Lux: 0.22
Time: 31.09s, Lux: 0.22
Time: 32.09s, Lux: 0.22
Time: 33.09s, Lux: 0.22
Time: 34.10s, Lux: 0.22
Time: 35.10s, Lux: 0.22
Time: 36.10s, Lux: 0.22
Time: 37.11s, Lux: 0.22
Time: 38.11s, Lux: 0.22
Time: 39.11s, Lux: 0.22
Time: 40.11s, Lux: 0.22
Time: 41.12s, Lux: 0.22
```

```
Time: 42.12s, Lux: 0.22
Time: 43.12s, Lux: 0.22
Time: 44.13s, Lux: 0.22
Time: 45.13s, Lux: 0.22
Time: 46.13s, Lux: 0.22
Time: 47.14s, Lux: 0.22
Time: 48.14s, Lux: 0.22
Time: 49.14s, Lux: 0.22
Time: 50.14s, Lux: 0.22
Time: 51.15s, Lux: 0.22
Time: 52.15s, Lux: 0.22
Time: 53.15s, Lux: 0.22
Time: 54.16s, Lux: 0.22
Time: 55.16s, Lux: 0.22
Time: 56.16s, Lux: 0.16
Time: 57.16s, Lux: 0.00
Time: 58.17s, Lux: 0.00
Time: 59.17s, Lux: 0.00
Measurement complete.

Code done running.

Press any key to enter the REPL. Use CTRL-D to reload.
```

Fig 12: screenshot of sensor reading(own image)

4.1 Decay Curve

A decay curve was plotted using the recorded values, with time (in seconds) on the x-axis and light intensity (in lux) on the y-axis. Both samples exhibited a decrease in light intensity over time. However, the decay in the toxic condition was significantly steeper than in the non-toxic condition.

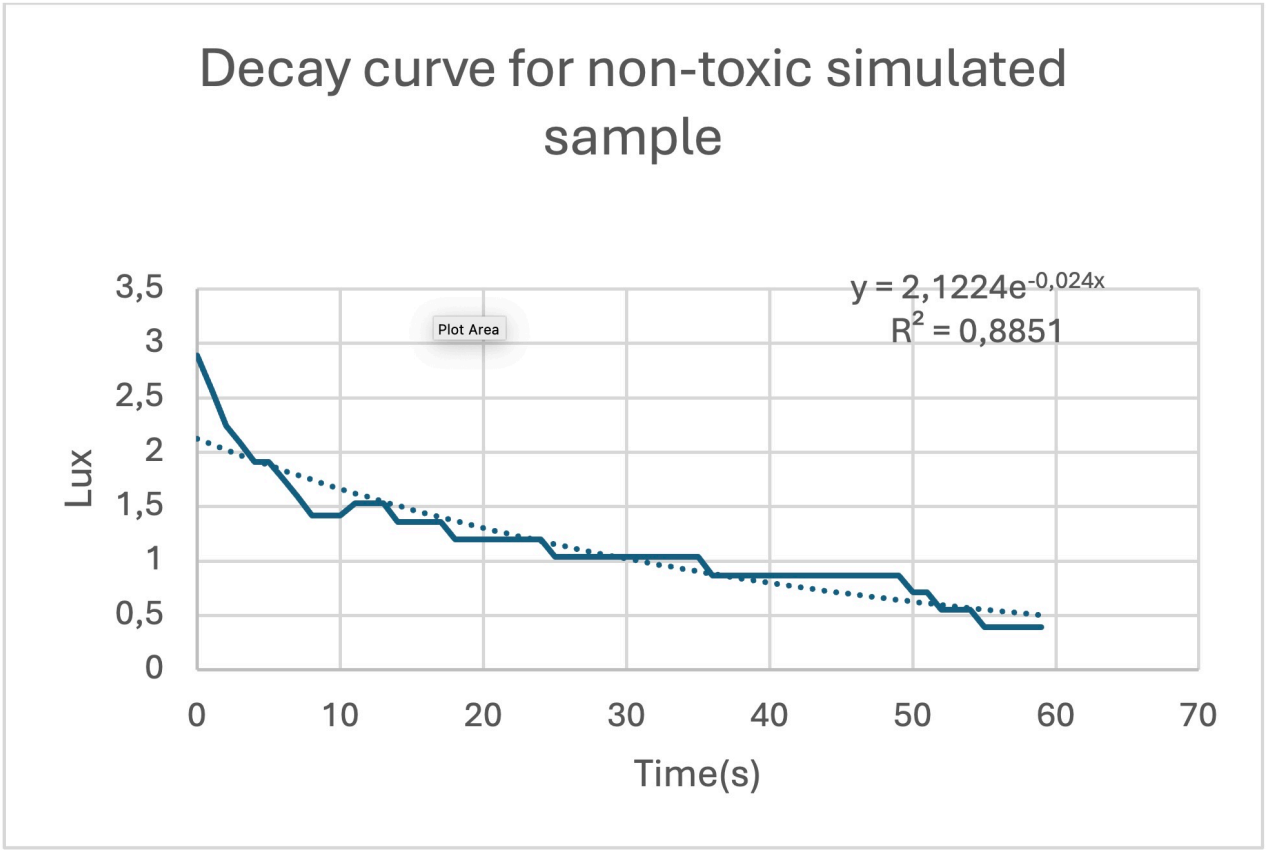


Fig 13: Decay curve (own image)

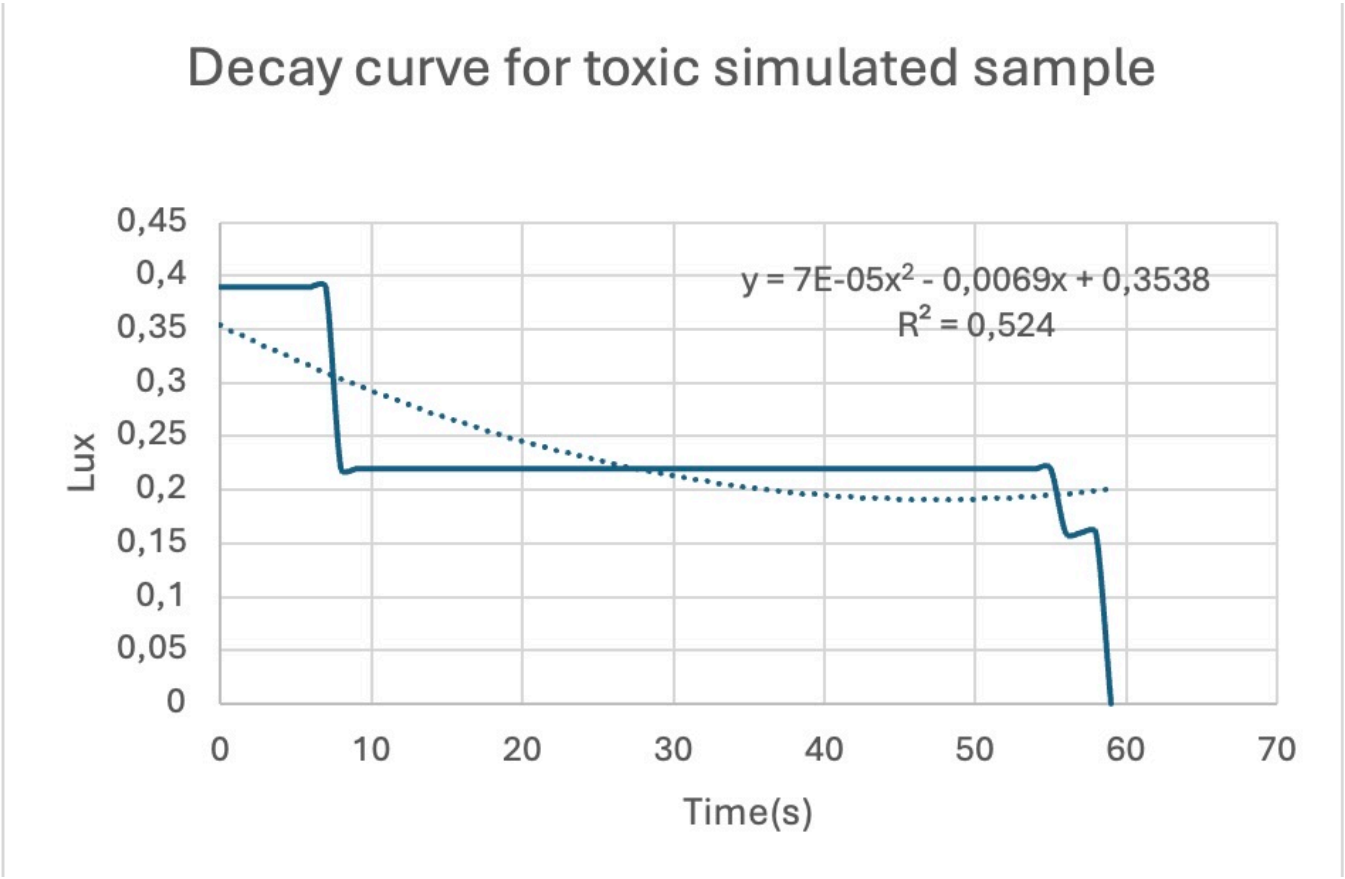


Fig 14: Decay curve (own image)

5. DISCUSSION

The system, built with an Adafruit QT Py and TSL2591 sensor, successfully measured differences in light intensity between toxic and non-toxic samples. The dark box reduced outside light, improving the accuracy of the readings. Future improvements could include better wireless support and testing with real bioluminescent bacteria.

6. REFERENCES

* Adafruit QT Py ESP32-S2 Guide: CircuitPython

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