

Application of Advance Biosensing Technology for Monitoring of Water Contamination

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ABSTRACT

This project presents an integrated environmental monitoring system that combines multiple sensors to measure and analyze key parameters of water quality. The system incorporates pH, turbidity, temperature, and electrical conductivity (EC) sensors, interfaced with an Arduino microcontroller for data acquisition and processing.

The pH sensor measures the acidity or alkalinity of the water, providing crucial information for assessing water quality and its suitability for various applications. The turbidity sensor detects suspended particles in the water, enabling the estimation of water clarity and potential contaminants. The temperature sensor provides real-time monitoring of water temperature, which is essential for understanding the thermal characteristics and potential impacts on aquatic ecosystems. Lastly, the EC sensor measures the electrical conductivity, offering insights into the water's salinity and mineral content, a vital parameter for various environmental studies.

The Arduino microcontroller serves as the central processing unit, collecting sensor data and displaying the results in the serial monitor. The sensors are interfaced with the Arduino using appropriate communication protocols, such as analog or I2C, and their respective libraries are utilized for efficient data retrieval and interpretation.

This integrated environmental monitoring system enables continuous, real-time monitoring of multiple water quality parameters, providing valuable insights into the overall health and condition of the monitored environment. It offers a cost-effective and user-friendly solution for researchers, environmentalists, and water quality management personnel to assess water quality, identify potential issues, and make informed decisions to ensure sustainable water resource management. The system's modular design allows for flexibility and scalability, enabling additional sensors to be integrated as per specific project requirements.

1. Introduction

Water is essential to life, thus protecting it is both morally and legally right. The demand for freshwater has increased significantly as a result of rapid urbanization, industrialization, reliance on irrigated agriculture, and improving living standards, particularly as a result of the use of water for luxury spas and swimming pools [1]. The pollutants that wastewater can discharge into

the environment include inorganic and organic pollutants, heavy metals, acidity, basicity, organophosphates, pesticides, and pathogenic *Escherichia coli*. Freshwater is not only getting harder to get but also getting harder to find globally due to its poor quality. As a result, preventing toxic effluents from entering the environment is a top priority in many countries [2 and 3].

Since waterborne infections are a major public health risk, clean water is a global aim. The World Health Organization (WHO) considers water-borne diseases to be a serious health risk and states that providing "Safely managed drinking water services" is a global goal ("Drinking-water," n.d.).

In the USA alone, 76 million cases of waterborne diseases are documented annually [4]. In Bangladesh, waterborne poisoning accounts for one out of every five fatalities [5]. Therefore, the first step in enabling effective remediation of freshwater resources, the identification of threats to health and safety, and the prevention of waterborne diseases is the measurement of physical-chemical properties, contaminants (chemicals or biologicals), in water [2,6].

a number of analytical techniques based on wet chemistry, electrochemistry, gas chromatography, high performance liquid chromatography (HPLC), gas chromatography, and gas chromatography-mass spectrometry (GC-MS) can be used to assess the quality of water. Almost all of these techniques require sample pre-treatment, experienced staff, are labor-intensive, and are expensive, even if some of them (such GC and GC-MS) are highly sensitive and accurate. These techniques are also ineffective for applications that require immediate outcomes, such as monitoring emergencies that require a speedy response, in remote locations, or in developing nations. For these applications, quick and practical tests that can screen dangerous compounds are crucial. The methods stated above are also insufficient for quickly determining online water pollution.

The majority of *E. coli* strains are also safe, but some serotypes have the potential to be lethal and spread harmful diseases. Therefore, it is crucial to identify *E. coli* as a faecal indicator bacterium [7]. However, it takes between 18 and 72 hours for the findings of the standard incubation procedure to locate this indicator bacterium [8].

Because of their portability, sensitivity, and potential for automation and online use, biosensors have been proved to be useful in some circumstances [2,3]. As a result, there have been a lot more academic publications published recently on the subject of biosensors [9].

2. Biosensor Definition:

The word "biosensor" describes analytical tools that use a biological sensing component and are both novel and potent. These tools have a wide range of applications in biomedicine, environmental monitoring, defense, diagnostics, drug development, security, and food processing, among other fields. Since then, remarkable advancements have been made in both technology and applications of biosensors using cutting-edge strategies including electrochemistry, nanotechnology, and bioelectronics [10]. For quantitative biologists, biosensors serve as a foundation for understanding technical advancement in instrumentation, which includes sophisticated high-throughput machines and portable qualitative or semi-quantitative devices for nonspecialists.

3. Technical approach:

The technological techniques utilized in biosensors are based on label-free and label-based detection [10]. The label-free approach is used to detect target molecules that are not tagged or labeled [11]. It has several uses in the fields of environmental research and medical science. While the exact characteristics of label compounds are what determine the label-based detection approach for target identification. It needs a target protein that has been manufactured with a particular sensing component and has been immobilized.

Major advantage and disadvantage of biosensor are listed below[12] in table 1.

Advantage of biosensor	Disadvantage of biosensor
1. They are able to measure nonpolar molecules, which are insensitive to normal measuring methods.	1. Due to denaturation of biological material, heat sterilization is not possible.
2. Because of the immobilized system they utilize, biosensors are specific.	2. The natural properties of the molecule, which are susceptible to denaturalization under environmental conditions, determine the stability of biological material (such as an enzyme, cell, antibody, tissue, etc). (pH, temperature or ions)
3. Biosensors allow for continuous and quick control.	3. Some chemicals that are able to diffuse through the membrane can make the cells in the biosensor inebriated.
4. Quick response time, usually under a minute	
5-Low cost.	

4. METHODOLOGY

This framework makes use of four designed sensors (temperature, turbidity, pH, and conductivity).

System designe:

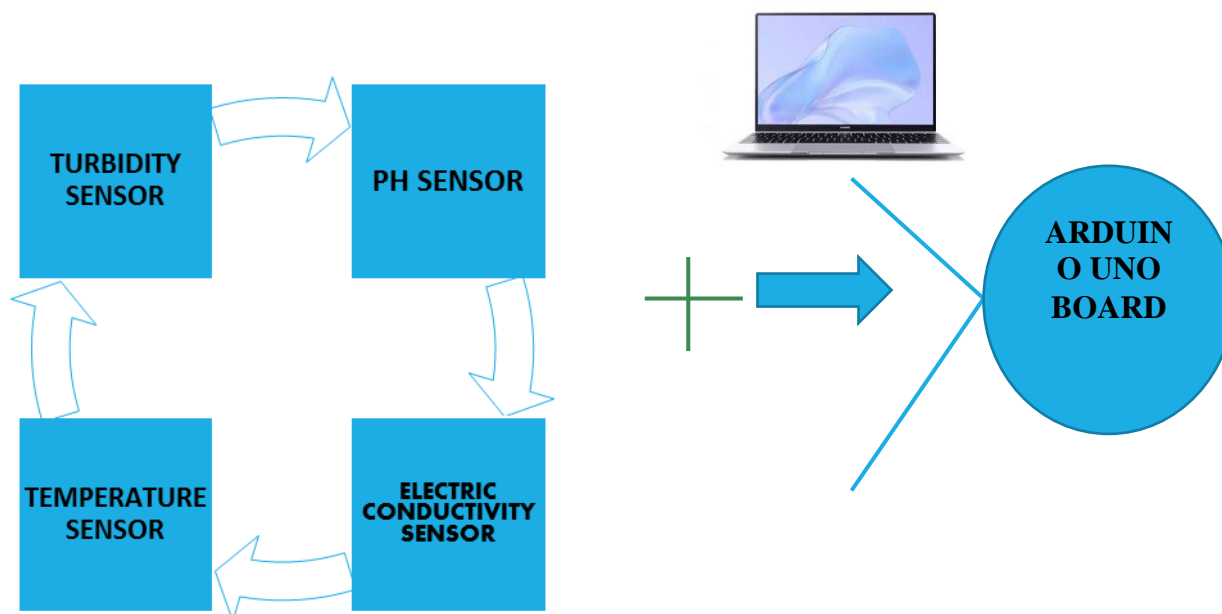


Figure.1 Interfacing various sensors with the Arduino Uno

4.1. Components

4.1.1. pH Sensor:

The pH sensor is a device used to measure the acidity or alkalinity of a solution, specifically water in this case. It provides a quantitative measurement of the hydrogen ion concentration in the water, which is an essential parameter for assessing water quality. The pH sensor typically consists of a glass electrode that produces a voltage proportional to the pH value of the solution.

The Arduino microcontroller reads the analog output of the pH sensor and converts it into pH values using appropriate calibration and mapping techniques.

3.1.2. Turbidity Sensor:

A turbidity sensor is employed to measure the level of suspended particles or turbidity in water. It detects the scattering or absorption of light caused by these particles in the water column. The sensor utilizes a light source and a detector to analyze the amount of light passing through the water. Higher turbidity levels indicate a higher concentration of suspended solids, which can affect water quality and clarity. The Arduino reads the output of the turbidity sensor and interprets it to provide turbidity values or an indication of water clarity.

4.1.3. Temperature Sensor:

A temperature sensor is used to measure the temperature of the water being monitored. It provides accurate and real-time data on water temperature, which is crucial for various environmental applications. The sensor can be based on various technologies, such as a thermistor or a digital temperature sensor. The Arduino reads the temperature sensor's output and converts it into a temperature value in degrees Celsius or Fahrenheit, enabling the monitoring of thermal variations and their potential effects on aquatic ecosystems.

4.1.4. Electrical Conductivity (EC) Sensor:

An EC sensor measures the electrical conductivity of a solution, which provides information about the concentration of dissolved salts or ions in the water. It is commonly used to assess the salinity or mineral content of the water, which has implications for agricultural, ecological, and water quality studies. The sensor employs two or more electrodes to measure the electrical conductivity, and the Arduino reads the sensor's output and converts it into EC values in units such as microsiemens per centimeter ($\mu\text{S}/\text{cm}$).

The Arduino microcontroller serves as the central component that interfaces with these sensors, collects the sensor data, and processes it. It uses appropriate communication protocols (such as analog or I2C) to communicate with the sensors. The collected data can be displayed in the serial monitor or further utilized for data logging, analysis, or integration into a broader environmental monitoring system.

Code

```
// Libraries for I2C communication and the sensors
#include <Wire.h>
#include <Adafruit_Sensor.h>
#include <Adafruit_TSL2561_U.h>
#include <Adafruit_BMP280.h>
// Define the analog pins for the pH and EC sensors
const int pH_pin = A0;
const int EC_pin = A1;
// Create instances of the turbidity, temperature, and EC sensor objects
Adafruit_TSL2561_Unified tsl=
Adafruit_TSL2561_Unified(TSL2561_ADDR_FLOAT, 12345);
Adafruit_BMP280 bmp;
```

```
void setup() {
// Initialize serial communication
Serial.begin(9600);
// Initialize the I2C connection
Wire.begin();
// Initialize the turbidity sensor
if (!tsl.begin()) {
Serial.println("TSL2561 sensor not found!");
while (1);
}
// Configure the turbidity sensor settings (if required)
// Refer to the sensor documentation for specific configuration options
// Enable the turbidity sensor
tsl.enableAutoRange(true);
tsl.setIntegrationTime(TSL2561_INTEGRATIONTIME_13MS);
// Initialize the temperature sensor
if (!bmp.begin()) {
Serial.println("BMP280 sensor not found!");
while (1);
}
void loop() {
// Read the analog value from the pH sensor
int pH_value = analogRead(pH_pin);
// Convert the analog value to pH
float pH = map(pH_value, 0, 1023, 0, 14); // Assuming pH range is 0-14
// Print the pH value to the serial monitor
Serial.print("pH: ");
Serial.println(pH);
// Read the turbidity sensor data
sensors_event_t event;
tsl.getEvent(&event);
// Print the turbidity value to the serial monitor
Serial.print("Turbidity: ");
Serial.println(event.light);
// Read the temperature sensor data
float temperature = bmp.readTemperature();
```

```
// Print the temperature value to the serial monitor
Serial.print("Temperature: ");
Serial.print(temperature);
Serial.println(" °C");
// Read the analog value from the EC sensor
int EC_value = analogRead(EC_pin);
// Convert the analog value to EC
// Adjust the mapping formula according to the specific characteristics of your EC sensor
float EC = map(EC_value, 0, 1023, 0, 1000); // Assuming EC range is 0-1000  $\mu$ S/cm
// Print the EC value to the serial monitor
Serial.print("EC: ");
Serial.print(EC);
Serial.println("  $\mu$ S/cm");
delay(1000); // Delay for stability (adjust as needed)
}
```

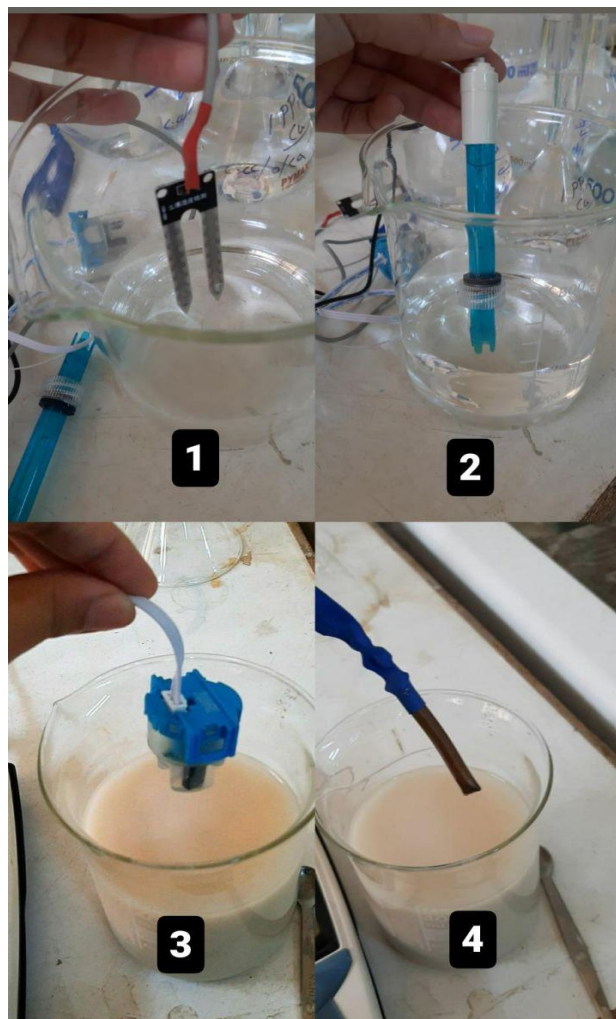


Figure.2: (1) electrical conductivity sensor, (2)ph sensor, (3)turbidity sensor, (4)temperature sensor.

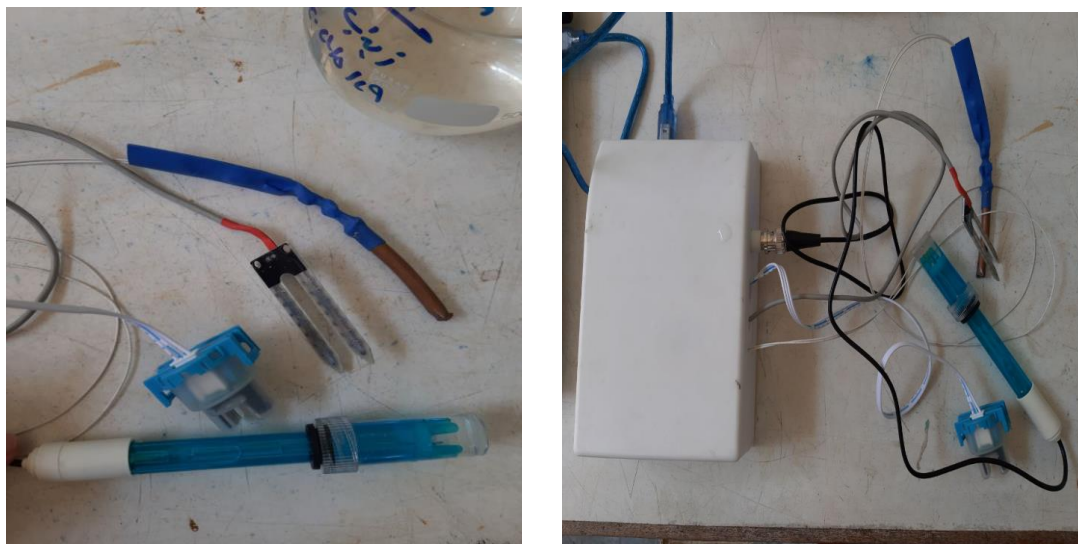


Figure.3 appearance of device from the outside

5. Calibration of the sensors

5.1. pH sensor

The pH sensor was calibrated in three solutions buffers with values of 4, 7 and 10. The pH sensor was immersed in these solutions and adjusted on the basis of the values of these buffers .where the results of this sensor were +- 0.2.

5.2. Calibration of turbidity sensor

For calibrate turbidity sensor need two matters which is Hydrazine sulfate ($N_2H_6SO_4$) and Hexamethylenetetramine ($C_6H_{12}N_4$) for each one provide 10 grams.

10 grams of Hexamethylenetetramine ($C_6H_{12}N_4$) is weighed and 1 gram of Hydrazine sulfate ($N_2H_6SO_4$) weighed too.

After that, each weighed substance separately dissolved in 100 ml of distilled water. Take 5 ml of each mixture (the substance dissolved in distilled water) then it is mixed with each other. Leave the two mixtures for a whole day to be completely homogenized. After this day measure the turbidity for them. It should appear 4000 NTU. Dilutions were made for this quantity by 1000 and 500 NTU.



Figure.4 Hexamethylenetetramine and Hydrazine sulfate matters

6. Arduino part

The most important part is Arduino drum type (Arduino uno Rev 3) which all sensors are connected to it.

Arduino is an open source electronic platform. Arduino contains more than one communication port and input and output ports through which devices are connected to the board. Information from these devices is transferred to the microcontroller board, which processes the data that comes through it.

First, download and install the IDE, then search the Internet for the code that interests and need it according to the device connected to the Arduino and upload it to the workspace. Then connect the corresponding wires with the devices in the special study here, and with presence of a program that interacts with these devices, the final results appear. All this at the lowest economic cost, Arduino is a simple way to implement useful interactive projects

For Ph sensor (brand DF robot) the connection to the analog A0 input of the Arduino board was through this circuit board

For turbidity sensor (brand DF robot) the connection to the analog A1 input of the Arduino board was through this circuit board

For ORP sensor (brand DF robot) the connection to the analog A5 input of the Arduino board was through this circuit board

For Temperature sensor the connection to the analog A3 input of the Arduino board was through this circuit board

For electric conductivity sensor the connection to the analog A2 input of the Arduino board was through this circuit board

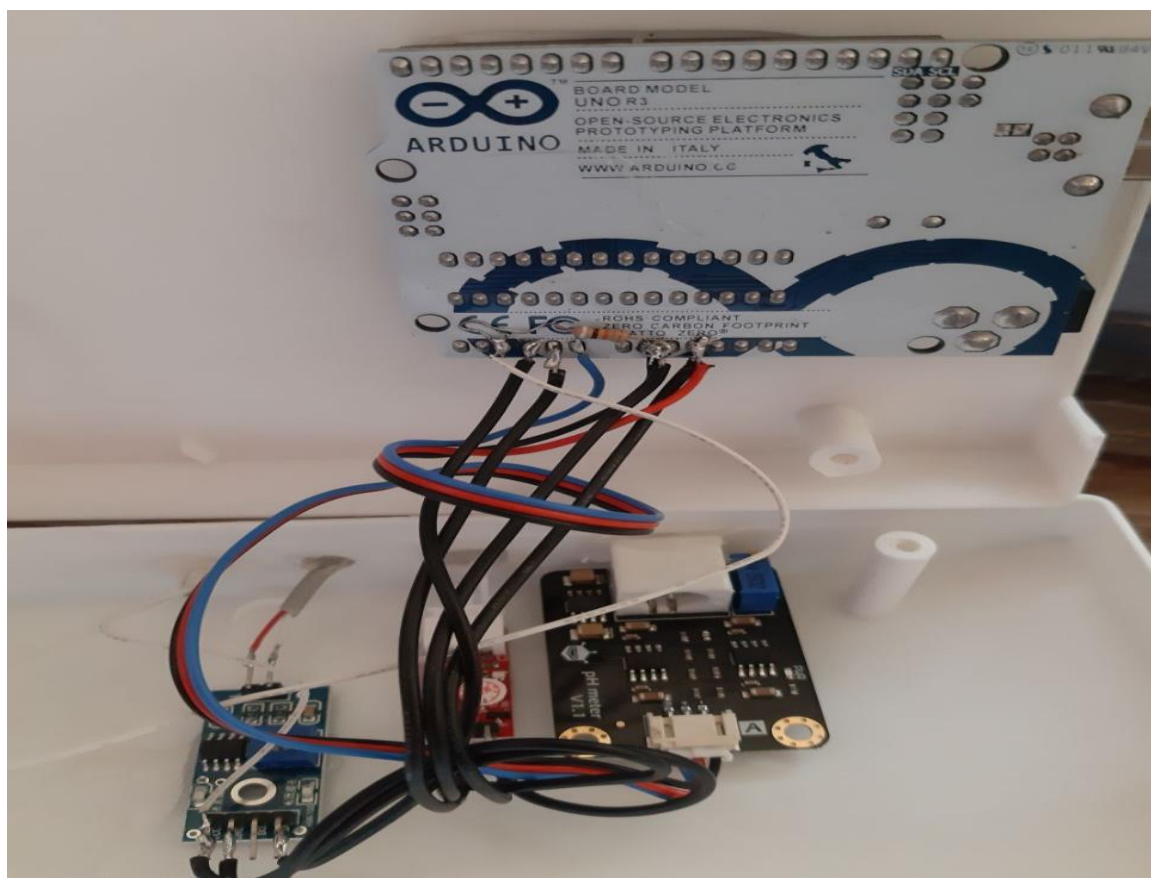


Figure.5 internal electrical connection

6.1 Displaying the result :

The results are shown by the computer in Excel program (PLX-DAQ for Excel "Version 2" by Net^Devil) in form of tables, the result of each sensor appears in a separate column from the other, it allows for easy comparison and analysis of the data. This layout ensures that the data is not mixed and that each sensor's measurements remain distinct, making it simple to identify trends and patterns, and the results of the sensors are not mixed, this is the best and most accurate way in which the results are shown in an instantaneous manner, as shown below:

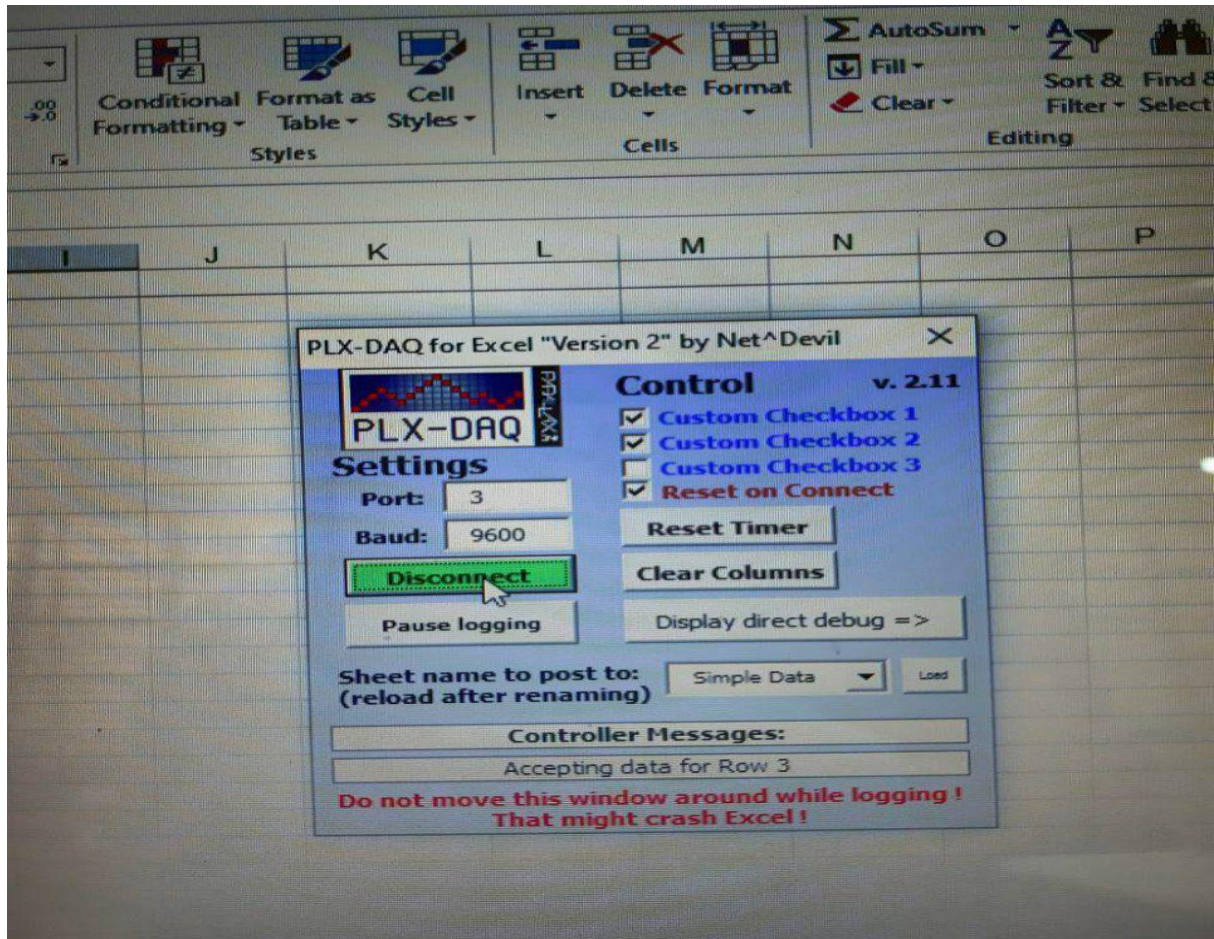


Figure.6 The program interface

7. Tests

After making the part related to sensors and after programming them, connecting them to the Arduino, a manufactured sample was made contaminated with Escherichia coli bacteria, and several dilutions done for it and its turbidity and ph were measured to ensure the response of the sensors and the validity of the results

A/Isolation of E.coli

Escherichia coli isolates were provided by microbiological laboratory / Al-Mustansiriayah University / College of Science. The plate containing the bacterial isolate was received ready by the laboratory The bacteria were cultured on a macConkey agar plate as shown below :



Figure.7 Escherichia coli isolates prepared by microbiological laboratory

B/ cultural growth of E. coli bacteria

The culture media is prepared for the purpose of growth and reproduction of bacteria, where 3.5 grams of nutrient agar were taken and dissolved in 125 ml of distilled water after sterilizing the conical flask and all the tools in the convection oven. Dissolve the nutrient agar in water using heat with continuous stirring.



Figure.8 until dissolve the nutrient agar

After making sure that the medium is completely dissolved, leave it to cool, then we pour it into Petri dishes. Here, 4 Petri dishes were used, and the nutrient medium was distributed over them. Leave it until it completely solidifies in the dishes. After that, come to the stage of transferring the bacteria from the isolated medium to the media. The feeder, where the transfer takes place using the flame sterilized loop, and the bacteria are transferred by it and placed in the dishes in different forms as shown in the pictures below. After that, the dishes are left for 24 hours to allow the bacteria to grow and multiply.

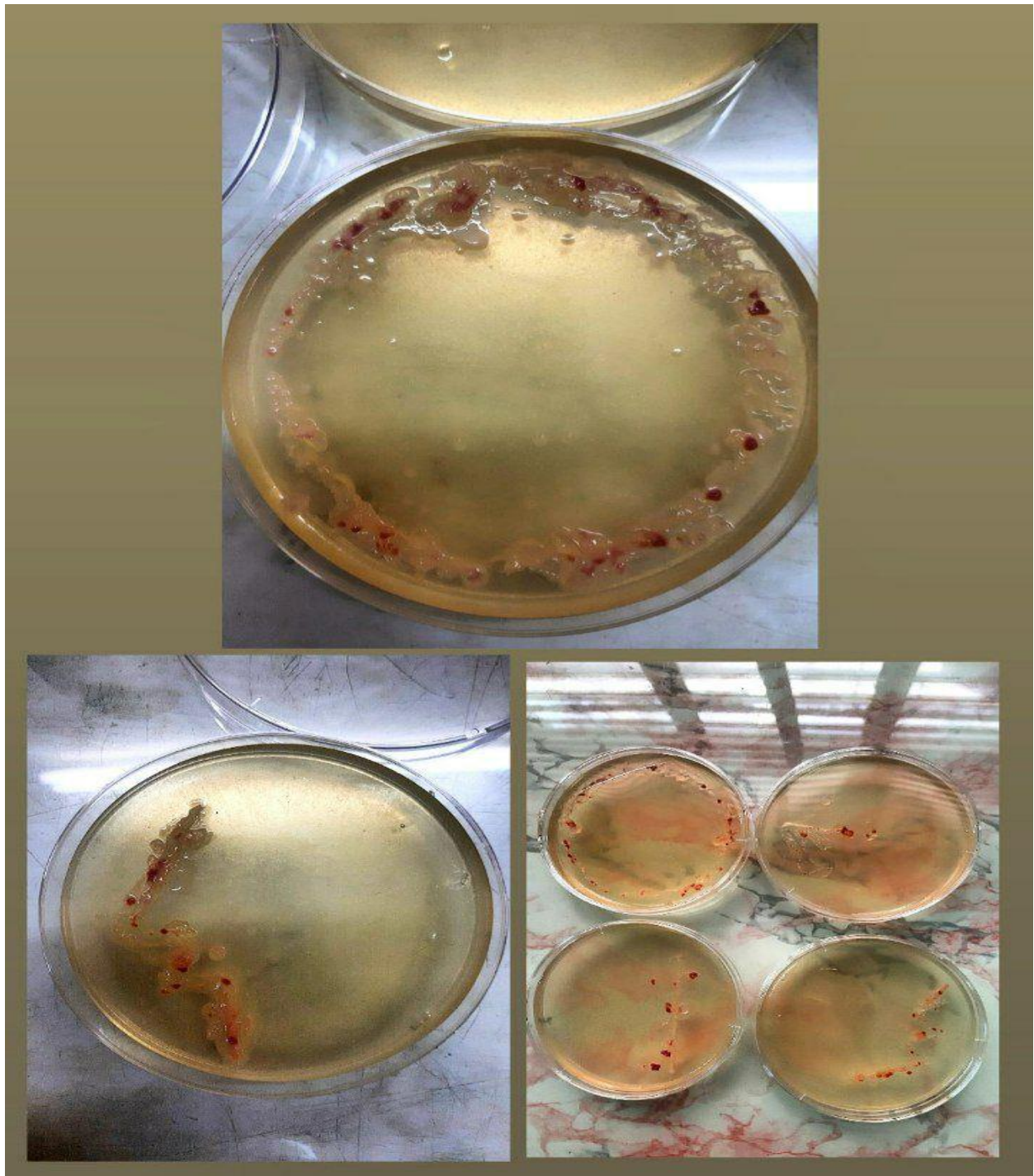


Figure.9 Growth and culturing of *Escherichia coli* bacteria after incubation for 24 hours.

C/ Preparation of liquid nutrient broth medium

After the bacteria multiplied, the liquid nutrient broth medium was prepared, where 1.625 grams of liquid broth was taken and dissolved in 125 ml distilled water. The dissolution takes place by means of heat with the use of continuous stirring inside the conical flask, and after the complete dissolution of the broth material, it is left to cool completely. Then the broth is poured into well sterilized four test tubes size 30 ml as shown below:

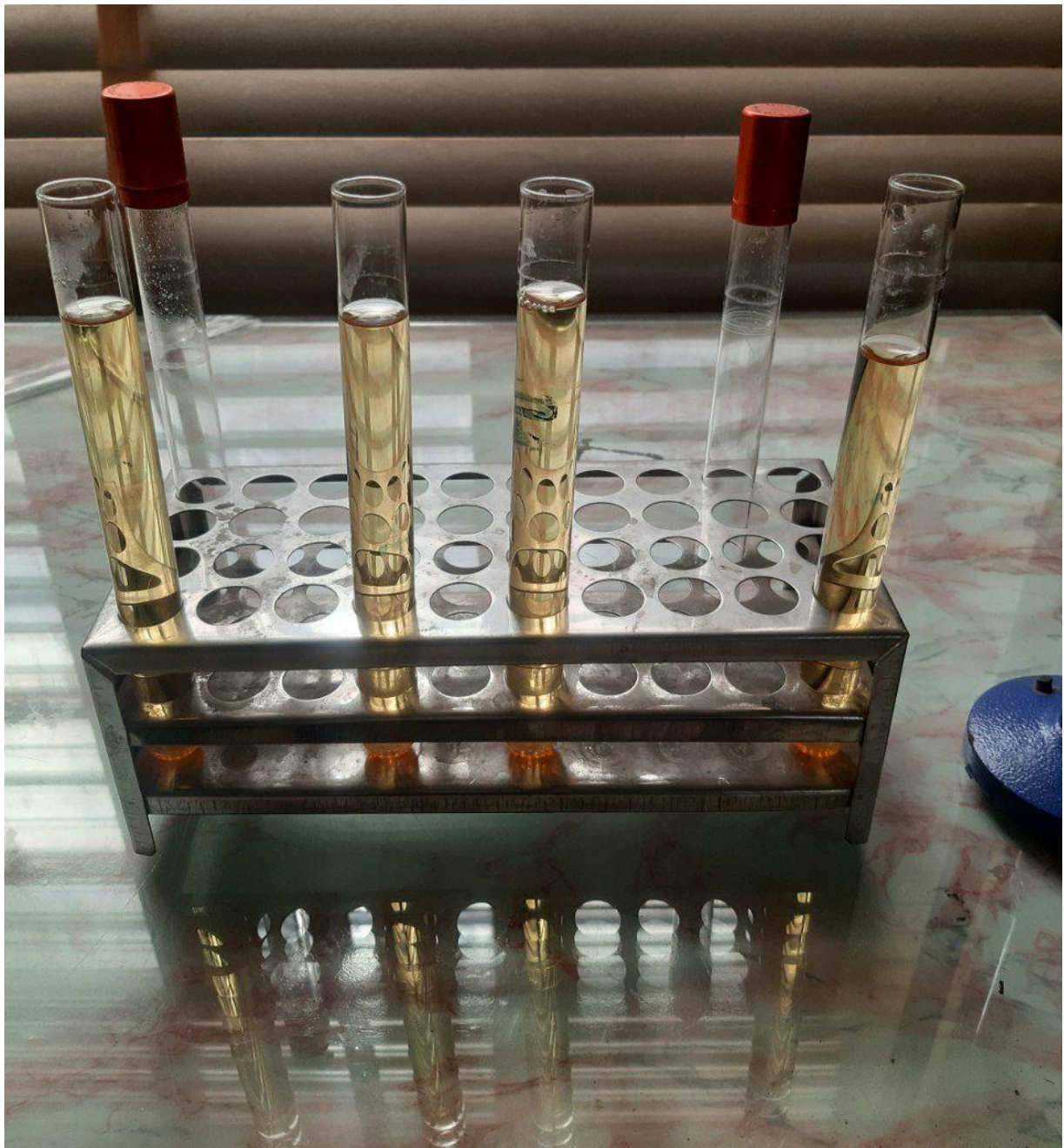


Figure.10 liquid nutrient broth

D/ e. coli transport to liquid nutrient broth

Using the sterile loop, the bacteria are transferred from the culture media to the tube that contains the liquid broth. Different amounts of bacteria are transferred to each tube size (30 ml), where one loop of bacteria was taken and placed in the first tube, 2 loops were placed in the second tube, and 3 loops of bacteria were placed in the second tube. It was placed in the third tube, and 4 loops of bacteria were placed in the fourth tube. Thus, the concentration of bacteria became different in each test tube in order to be measured by sensors. The bacteria are left in the nutritious broth for two days. After that, the turbidity was measured by the turbidity sensor for each tube separately, and the pH was measured by a ph sensor for them as well.



Figure.11 Escherichia coli in nutrient liquid broth after two days of incubation.

Turbidity and pH readings were recorded for each glass tube content, and the effectiveness, efficiency, and speed of their response to changes in the percentage of pollutants were seen.

8. Results

Table.2 Esherchia coli bacteria test

E. Coli bacteria	DILUTIONS		
	1:10	1:100	1:1000
TURBIDITY	2.255	2.141	2.04
PH	6.23	6.414	6.90

A series of dilutions was made for Escherichia coli bacteria placed in distilled water, and their turbidity and pH were measured, and it was shown through table.2 that with an increase in the percentage of the pollutant (bacteria) in the water, the turbidity value increased, as at the ratio 1:10 in which the volume of water is 10 ml and the volume of bacteria is 1 ml, the highest value of turbidity was shown, and this gives an indication of the validity of the sensor response. As the percentage of the pollutant is directly proportional to the value of the turbidity, as it increases with its increase and decreases with the decrease in the presence of the pollutant. And at the ratio of 1:1000, where one millimeter was placed in a volume of a liter of water 1000 ml, and in it the percentage of bacteria was small relative to the water in which the value of the turbidity appeared less than the previous one

As for the pH value, the pH value is inversely proportional to the presence of pollutants. In the above table, at the highest bacteria percentage, which is 1:10, the pH sensor value showed us the lowest value, and at the lowest bacteria percentage, which is 1:1000, the pH value showed the highest value.

Table.3 Laboratory devices values

E. Coli bacteria	DILUTIONS		
	1:10	1:100	1:1000
TURBIDITY	2.55	2.403	2.3
PH	6.02	6.2	6.7

In table.3 the same measurements were made, but with laboratory equipment for turbidity and pH, to find out if there was a difference in the percentage of results between the local sensors that were made in this study and the expensive laboratory equipment.

The results measured in the laboratory devices showed that the percentage difference in the turbidity device was not large, ranging approximately 0.3 Ntu. The difference in the ph device showed that the difference was approximately 0.2.

9. Conclusion:

In conclusion, the integrated environmental monitoring system developed using Arduino and multiple sensors provides valuable insights into water quality parameters. The program successfully measures and monitors pH, turbidity, temperature, and electrical conductivity (EC) in real-time.

The pH measurement helps assess the acidity or alkalinity of the water, crucial for determining its suitability for various applications. The turbidity measurement provides information about the clarity of the water and the presence of suspended particles, aiding in the detection of potential contaminants. Monitoring temperature enables the assessment of thermal variations, which can impact aquatic ecosystems. The EC measurement offers insights into the salinity and mineral content of the water, assisting in evaluating its usability for specific purposes.

By continuously collecting data and displaying the results in the serial monitor, the program allows for ongoing monitoring and analysis of water quality. Trends, patterns, or deviations from expected values can be observed and used to identify changes in water conditions. Such information is essential for environmentalists, researchers, and water quality management personnel to make informed decisions regarding resource management, pollution control, and ecosystem preservation.

The versatility of the Arduino platform and the integration of multiple sensors provide a cost-effective and user-friendly solution for environmental monitoring. The modular design allows for scalability and the potential addition of other sensors to address specific project requirements. The program's ease of use and data accessibility make it a valuable tool in various fields, including environmental research, water resource management, and ecological conservation.

Overall, the integrated environmental monitoring system demonstrates the potential for leveraging technology to monitor and understand key water quality parameters. It empowers stakeholders to proactively address water-related challenges, ensure sustainable water resource management, and contribute to the preservation and protection of our natural ecosystems.

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