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RESEARCH ARTICLE

MONITORING OF HEAVY METAL TOXICITY IN WATER SAMPLES USING BIOLUMINESCENT ASSAY

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ABSTRACT

Environmental and biodiversity risks were greatly increased by toxic heavy metal pollution, The various water samples environmental with a history of heavy metal toxicity resulting from human activities or natural origin released in the environment. Ensure representative sampling from various depths and locations within the fields. Their widespread distribution in the environment as a result of their numerous industrial, household, agricultural, medical, and technical applications has sparked worries about their possible consequences on the environment and public health. Bioluminescent bacteria are thought to be an essential and effective tool. The luminous bacteria for Waste water, Seawater, Tap water and monitoring of different toxicants was to be isolated, optimized, and applied. The Microtox assay is frequently employed as the initial screening method in a test battery to identify microbial toxicity. From various source it is reported that a heavy metal toxicity monitoring efficacy of *Vibrio harveyi* has the ability to detect pollution in distinct environmental and various water conditions. *It is anticipated that the Vibrio* sp. Could be succeed in toxicity detection at different environmental and various salinity conditions. The history of bacterial bioluminescence discoveries and its present uses in environmental research will be reviewed, with a focus on the Microassay and luminometer methods.

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INTRODUCTION

One of the most significant renewable natural resources is water. It is necessary for all living organisms to survive. But this valuable resource is negatively impacted by human activity. Point and non-point sources are the two main categories of sources that can contaminate water. While non-point sources of pollution, such as urban wastes and runoff from agricultural fields, have varying quantities and origins of contaminants that pollute both surface and groundwater, point sources of pollution are direct, definite, and distinguishable sources, such as factory pipes that extend into the water or industrial effluent. As a result, there are two categories of water pollutants: organic and inorganic. The most prevalent kinds of organic pollutants are herbicides, insecticides, heavy metals, and phosphates found in agricultural drainage systems and industrial effluents. Inorganic pollutants include nitrates, phosphates, and volatile organic compounds (Howaida Hassan*, et al,* 2022). Despite being naturally occurring elements present in all of the earth's crust, most environmental contamination and human exposure to heavy metals are caused by human activities like mining, smelting, industrial production, and the use of metals and metal-containing compounds in agriculture and domestic settings. In addition, metal corrosion, air deposition, soil erosion, heavy metal leaching, sediment re-suspension, and metal evaporation from water resources into soil and ground water can all contaminate the environment.

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It has also been noted that weathering and volcanic eruptions are examples of natural events that greatly contribute to the pollution caused by heavy metals (Paul. B. Tchounwou, *et al,* 2014). Consequently, biological sensors, markers, or detectors have received a lot of interest. Bacteria, nematodes, cladocerans, fish, amphiphods, algae, plants, and cultured cell lines are among the trophic levels of organisms that have been exploited. When identifying hazardous substances, toxicological animal-based approaches can be costly, time-consuming, need high sample quantities, and cause ethical dilemmas. Battery testing needs to incorporate in vitro techniques, which are frequently employed for chemical screening and rating, in order to properly assess danger. For the past few years, the advantages of employing quick, sensitive, repeatable, and affordable bacterial tests for toxicity evaluation and screening have been highlighted in every study (Stefano Girotti*, et al*, 2007). One of the known fascinating glowing creatures on this tiny blue planet earth is bioluminescent bacteria. Numerous studies from diverse contexts have examined the distribution and diversity of these bacteria in a variety of marine a biotic and biotic samples. Several physiochemical factors, in addition to structural and core lux genes, regulate these bacteria's brightness. Quorum sensing is a phenomenon involving chemical signaling in which blue-green luminescence is produced by core genes luxCDABE in response to luminous bacteria releasing a high concentration of quorum sensing molecules into the growth media. All known cases of bacterial bioluminescence have emission spectra in the 450–490 nm region. Currently, research is focused on isolating bacteria that produce high luminescence in order to create quick bioassays based on luminescence that can monitor different contaminants in a range of biological and environmental samples

(Chatragadda Ramesh, *et al,* 2021). Another benefit that bioluminescent bacteria (BLB) can provide is an easy way to document the effects that are made on a living thing. Because it is quick and inexpensive, a bioluminescence (BL) inhibition assay is frequently selected as the initial screening technique in a test battery. The BLB test procedure is typically straightforward, particularly when used with extracts or aqueous materials. As a result, expensive chemical analyses of every sample are no longer required; instead, they will only be carried out in the event that the bioluminescent sensor raises an alarm. Comparing this bioluminescence assay to other bacterial assays like nitrification inhibition, respirometry, ATP luminescence, and enzyme inhibition, researchers have found that *Vibrio harveyi* , the first and most widely used luminescent strain, is the most sensitive strain across a broad range of chemicals. Furthermore, BL assays exhibit a strong connection with bioassays for toxicity, such as those conducted on fish, algae, and crustaceance (Stefano Girotti*, et al*, 2007).

Studies on Bioluminescence: Many believe that bacteria were among the first bioluminescent systems in nature. Both land and aquatic habitats are home to bioluminescent microorganisms worldwide. In actuality, raw seafood and any tissue or debris seen on beaches are easy sources of these viruses. Darwin extensively recorded this amazing phenomenon—the vivid oceans that emerge from these microbes—in his writings. There are places on the planet where you can see these oceans. (Kalpna katiyar and Aditya Srivastava, *et al,* 2021). The three genera of bioluminescent bacteria—*Vibrio, Photobacterium*, and *Xenorhabdus*— are the only ones that comprise the class Gamma *Proteobacteria*. Of them, *Xenorhabdus* lives on land, whereas *Vibrio* and *Photobacterium* are primarily found in aquatic environments. There is constant discovery of new bioluminescent bacterial strains. One amazing thing about bacterial bioluminescence is that all of these systems share the same biochemistry, meaning they all depend on oxygen, myristic aldehyde, and NADH in addition to flavin mononucleotide (FML) (Aditya Srivastava and Kalpna Katiyar *et al,* 2021). Luciferase is an enzyme needed for bioluminescence, which is chemiluminescence. A number of processes would be involved in bioluminescence, according to early research in the clarification of the mechanism behind bacterial bioluminescence. At first, it was suggested that luciferase was decreased using a single molecule of reduced flavin mononucleotide (FMNH2). A year later, two decreased flavin molecules were discovered to be involved, as opposed to only one, changing these conclusions (Stefano Girotti*, et al*., 2007). It was proposed by a different study group that one FMNH2 molecule reacted with oxygen during bioluminescence to generate a highly reactive organic peroxide, while the other molecule combined with an aldehyde molecule to form an aldehyde-FMNH2 complex. Though it was challenging to reconcile this with its spectral requirements, these reactions were thought to account for the energetics. As of right now, scientists know that the blue-green light emission of bioluminescence, which is emitted by bacteria like *Vibrio harveyi* and *Photobacterium phosphoreum*, results from the reaction of molecular oxygen with FMNH2 and a long-chain aldehyde, which produces FMN, water, and a matching fatty acid. The long-chain aldehyde and FMNH2 undergo a mixed function oxidation that is catalyzed by the enzyme luciferase. When FMNH2 is coupled to the enzyme, it is shielded from autooxidation, so the reaction is extremely selective for it. (Elida Nora Ferri, et al, 2007).

Light + H2O + R-COOH + FMNH2 + O2 + R-CHO à FMN

The energy produced more than meets the need of 60 Kcal mol-1 for light emission. Nevertheless, this is an energy- intensive procedure. Assuming 100% efficiency for the reaction, Hastings and Nealson calculated that light emission translates into an energy expenditure of roughly six ATP molecules for each photon. This explains why bioluminescent creatures only express energy when it is physiologically required and save it otherwise (Elida Nora Ferri, et al, 2007). McElroy and Green were the ones who initially isolated the luciferase enzyme. Eventually, several researchers came to the conclusion that luciferase's molecular weight fell between 58 and 76 KDa. According to more recent research, all bacterial luciferases are ~80 KDa hetero dimers with two subunits that are roughly 40 and 35 KDa, respectively. There are two types of common methods used to monitoring the heacy metals toxicity in water samples Atomic Absorption Spectroscopy (AAS) & Microtox assay. These are used to identify and isolated bilominescence assay in various invertebrate samples. In Microtox assay is used to identify the present of different types of heavy metals toxicity in water samples by using bioluminescence assay. Whether the heavy metals are present to samples the luminescence intensity and density are reduced. If its present they produced light (Vania da Silva Nunes-Halldorson, et al, 2003).

Toxicology bioassays are mostly based on the degree of their shining intensity, either in the short or long term, by analyzing changes in their viability and growth rate. The luciferase enzyme, which is essential for the reduction of flavin mononucleotide (FMN) to FMNH2, which then combines with oxygen to form 4a-peroxy-flavin, may be the general process for bioluminescence. The fatty aldehyde is oxidized by this intermediate component to a stable complex called luciferase- hydroxyflavin, which breaks down gradually and emits blue-green light. The transcription of lux-operon, or the luminescence genes, is the genetic mechanism that governs *V. harveyi* bioluminescence. The luxL gene and luxM are the two primary transcription components of the lux-operon. Surprisingly, *Vibrio harveyi* was the first and most commonly utilized bioluminescent bacteria for this purpose; it is much more sensitive to a wide variety of substances than other bacterial strains. The invertebrate samples like fish, squids are used to isolate the *Vibrio haryei* (Vania da Silva Nunes-Halldorson, *et al,* 2003). This review deals with the applications of bioluminescent bacteria to the environmental analyses. Organisms from different trophic levels have been used bacteria as a assay for detection of heavy metals toxicity. By various projects undertaken, the main objectives are to isolate, optimize and apply luminescent bacteria for toxicity monitoring of various toxicants in Waste water, Seawater, Tap water, Surface and Ground water. The sector of water more than 700,000 analyses per year. Heavy metals like (Cd, Ld, Mercury). First and most employed luminescent strain, luciferase enzyme as the most sensitive, across a wide range of chemicals. A bioluminescence (BL) inhibition assay, is often chosen as the first screening method in a test battery, based on speed and cost considerations then it is used to detect microbial toxicity. According to this review, it is possible to conclude that BLB tests are, on average, enough sensitive to detect compounds that can be toxic to humans and to the whole environment (Vania da Silva Nunes-Halldorson *et al*, 2003).

Monitoring Heavy metals Toxicity and role of Bioluminescence bactria: Sources of Microorganisms

A common gene sequence found in all bioluminescent bacteria is the lux operon, which is defined by the lux CDABE gene organization. Luciferase is encoded by Lux AB, and the fatty- acid reductase complex that produces the aldehydes needed for the bioluminescent process is encoded by LuxCDE. Species differences can be seen despite this shared gene architecture, such as the existence of additional lux genes. The four unique types of the lux operon are the Aliivibrio/ Shewanella type, the Photobacterium type, the Vibrio/Candidatus Photodesmus type, and the Photorhabdus type. These types are based on similarities in gene content and organization. Although this organization's evolutionary history is unknown, it adheres to the genus classification level for Vibrionaceae members (Aliivibrio, Photobacterium, and Vibrio) (E. Newton Harvey *et al*, 1997). The luxG gene, which encodes flavin reductase, is present in all lux operon types except the Photorhabdus operon type. Extra luxI/luxR regulatory genes are found in the majority of

Aliivibrio/Shewanella type operons, and these genes are utilized for autoinduction during quorum sensing. The rib genes that code for riboflavin are present in the Photobacterium operon type, which is known to create the lux-rib operon. In contrast to the Aliivibrio/Shewanella and *Photobacterium* operon types, the Vibrio/ Candidatus, Photodesmus operon type lacks any directly linked regulatory genes (E. Newton Harvey *et al,* 1997). There were seventeen *Vibrio harveyi* isolates in the study group. It had previously been demonstrated that isolate PM47666-1 was extremely pathogenic toward prawn larvae. The residual isolates came from environmental and clinical sources. The Sir George Fisher Center for Tropical Marine Studies kindly provided cultures. Townsville, North Queensland's James Cook University is home to the Australian Collection Marine Microorganisms (ACMM). PM91 isolates were acquired (Morris pizzutto *et al.,* 1991). Fresh seafood, including shrimp, octopus, squid, and cuttlefish, was procured at Maadia Port in Alexandria, Egypt. From Alexandria, Egypt's Eastern Harbor, comb jellyfish were captured (E. Newton Harvey *et al,* 1997). Utilizing certain indicator organisms provides an additional source of microorganisms for tracking the toxicity of heavy metals. These species were chosen due to their sensitivity to exposure to heavy metals and their capacity to react to pollutants in a detectable way, for as by changing their bioluminescence. For instance, because of their quick reaction to environmental stresses like heavy metals, several species of bioluminescent bacteria, notably Vibrio fischeri, have been extensively employed in bioluminescent assays to evaluate the quality of water (Morris Pizzutto, *et al,* 1991).

Monitoring the toxicity of heavy metals can also take into account anthropogenic sources of microorganisms, in addition to natural microbial communities and indicator organisms. Heavy metals can enter water systems through anthropogenic activities such industrial discharge, agricultural runoff, and inappropriate waste disposal . (Elida Nora Ferri, et al., 2007). This could have an impact on the make-up and activity of microbial populations. It is possible to gain important insights into the degree of heavy metal contamination and its possible effects on aquatic ecosystems and human health by keeping an eye on the existence and behavior of microorganism originating from the sources. Overall, bioluminescent assays for monitoring heavy metal toxicity in water samples can be more successful when data from a variety of microorganism sources, such as indicator organisms, natural communities, and human sources, are combined. To protect water quality and ecosystem health, researchers and environmental managers can create effective monitoring systems by utilizing the sensitivity and responsiveness of microorganisms to heavy metal exposure (Morris Pizzutto*, et al,* 1991).

Availability of Toxicity in Fresh Water: Bioluminescent bacteria are obvious candidates to detect toxicity in wastewater systems. The natural bioluminescent bacteria, however, are too susceptible to some wastewaters, thus their response under certain operational settings cannot accurately represent the condition of the more resilient microbial community that treats wastewaters. Wastewaters, particularly those with an industrial origin, may have concentrations of potent toxicants so high that natural biological logarithmic balances (BLBs) cannot survive and become useful for tracking overall toxicity, the presence of specific compounds, or variations in toxicity levels. Industrial wastewaters frequently overflow treatment facilities with hazardous effluent, sometimes to the point where treatment operations are permanently destroyed (Stefano Girotti*, et al*, 2007). The procedure is done by Microtox ones, the Shk1 assay may be more appropriate for assessing influent wastewater toxicity because it is less sensitive than the Microtox assay. (Lajoie *et al,* 2007) created a procedure for the synthesis, preservation, and application of Shock 1 (Shk1) bioreporter cells. They also investigated the variables that impact Shk1 growth and bioluminescence, such as growth medium, tetracycline concentration, storage conditions, and test media. Standardized time intervals for cell growth, storage, activation, and exposure in the test media are necessary for the effective application of this technique. In nutritive broth, influent wastewater, and from a municipal wastewater treatment facility, the bioluminescence of Shk1 cells was measured. The efficacy of the devised toxicity testing approach using influent wastewater from a municipal wastewater treatment plant to expose exposed individuals to heavy metals (Cd, Zinc) (Stefano Girotti*, et al*, 2007).

Avalability of Toxicity in Sea Water: Actually, very few novel BLB applications for sea sample analysis have been developed in the recent seven years. More than 99 percent of the iron in marine systems is complexed to organic chelates of biotic origin, highlighting the crucial role heterotrophic bacteria play in the marine Fe biogeochemical cycles and the unbreakable relationship between Fe chemistry and the biota. The use of a heterotrophic, halotolerant bacterial reporter that produces light in response to the concentration of bioavailable is explored in the publications by C.E. Tests on the BL reporter were conducted first in a specified seawater medium and subsequently in marine surface waters. How the bioreporter identified the variations in Fe bioavailability is explained in the papers. The outcomes showed how useful this method could be in clarifying the connection between Fe chemistry and bioavailability in intricate marine environments (C.E. Mioni*etal,* 2007*)*.

Avalaibility of Toxicity in Surface and Ground Water: Since freshwaters often have low levels of toxicants, sensitive assays like the marine BLB ones would need to be used. For marine bioluminescent bacteria, however, unsalted waters are not the best habitat because they require a concentration of salts that is equivalent to that of seawater. In order to evaluate freshwater samples, these salts must be added, adding a new variable to the specimen's chemical makeup in each case. In order to circumvent this issue, a number of species have been extracted from a particular setting, altered genetically to incorporate luminescence genes, and developed BL tests (Stefano Girotti*, et al*, 2007). Arsenic concentrations in natural water resources have been measured using atomic absorption spectroscopy (AAS) in conjunction with a bioluminescence-producing, arsenic-inducible bacteria based on *E. coli.* To prevent the influence of iron ions on the availability of arsenic to the bioreporter cells, specific techniques have been designed. When comparing the results of the chemical and biological assays, the bioreporter identified an average of 2.4% false positive and 8.0% false negative identifications at the WHO recommended acceptable arsenic concentration of 10 µg L−1.Compared to the performance of chemical field test kits, these results are far superior (D.Magna*, et* al,2006).

Avalability of Toxicity in Tap Water: Online drinking water quality monitoring has seen a sharp rise in popularity in recent years. Of all the techniques available in this field, the most sensitive, dependable, and comprehensive ones must be used for the quality control of water meant for human consumption. The goal is to guarantee to consumers and regulatory bodies that there are no compounds present that could have a negative impact on human health, or that the amounts present are safe. This resulted in the development of new monitors that can offer (almost) real-time data on water quality, for use in the continuous management of river water quality and the deliberate contaminating of drinking water (Stefano Girotti*, et al*, 2007). The cytotoxic and genotoxic potential of water samples from rivers and various sewage treatment plants' primary and secondary effluents has been measured using the Microtox assay. Different amounts of secondary pollutants (20–40%) were given to rainbow trouts. The fish liver's DNA unwinding assay and the muscle's acetyl-cholinesterase activity have both been used to assess the hazardous potential of water samples. It is feasible to conclude from the research's findings that both bacterial techniques can be applied to evaluate the cytotoxicity and genotoxicity of household and industrial wastewater as well as to gauge how effective sewage treatment systems are However, they are unreliable as a stand-alone test for detecting

cytotoxicity and genotoxicity in surface water due to their low sensitivity and high susceptibility. The seawater quality near wastewater pipe discharges from small-rate municipal sewers has been assessed using the Microtox test and a bacterial luminescence bioassay. The ecotoxicological test based on the luminous fraction of epi bacteria seemed to be more successful than these assays (Stefano Girotti*, et al*, 2007).

Heavy metals analysis

Atomic Absorption Spectrometry: Using the method of measuring the radiation that **a** chemical element of interest absorbs, atomic absorption spectroscopy (AAS) can determine the amounts of chemical elements in environmental samples.This is accomplished by analyzing the spectra that are generated when radiation excites the material. Atomic absorption techniques assess the amount of energy in the form of photons of light that are absorbed by the sample. The atoms absorb ultraviolet or visible light and transition to higher energy levels (Vania da Silva Nunes-Halldorson *et al,* 2002). Measurement of metal concentration can be done by a technique called atomic absorption, which is the process of an element of light being absorbed by free atoms at a wavelength specific to that element. Energy is introduced into the atom population by thermal, electromagnetic, chemical, and electrical types of energy in emission, absorption, and fluorescence in atomic spectroscopy. Before being measured, this energy is transformed into light energy by a variety of atomic and electronic processes. Absorption of Atoms Spectrometry is helpful for both the identification and quantitative measurement of several elements found in samples. The method is sensitive enough to detect minute amounts of an element and specific enough to accurately identify particular elements in each sample **(**Norma Letícia Duran, *et al,* 2002). All metals with the exception of Al, Na, Ca, Mg, and K are referred to as "heavy metals," meaning that their density is greater than 5 g/cm3. It contains several elements that are vital to human health, including Fe, Cu, Zn, and Mn; highly hazardous Pb, As, Hg, and Cd are followed by less toxic Au, Ag, and Co. These substances' physiological and toxicological effects stem from a variety of distinct processes. Because heavy metals are not biodegradable, they are a major source of pollution, even at extremely low quantities where they represent a harm to human health and the environment. The most significant non-essential heavy metals affecting surface water systems are antimony, lead, arsenic, chromium, mercury, and cadmium. (Stefano Girotti*, et al*, 2007). In water, heavy metals have the ability to bind to the surface of microorganisms, from where they are transported inside the cell where they can be involved in chemical reactions and change chemically. The majority of known techniques can determine the total amount of heavy metal ions. In addition, laboratory techniques that are routinely used for the analysis of metal ions, such as atomic absorption spectrometry, inductively coupled plasma mass spectrometry, anodic stripping voltammetry, and X-ray fluorescence spectrometry, require sophisticated equipment, pretreatment of samples, or qualified operators(Stefano Girotti*, et al*, 2007).

Hazardous Metals

The Lead: The primary human sources of lead pollution in the environment are the burning of fossil fuels, landfills and their fires, industrial sludge waste, phosphate-based fertilizers, pesticides, and vehicle exhaust fumes. It can be found as carbonates, sulphides, and sulphates. It is regarded as the primary environmental contaminant and is posing a growing threat to all life, particularly in the vicinity of sizable industrial facilities, busy roadways, and urban areas. (Amra Odobasic, Indira Sestan et al, 2018). The amount of organic matter present, the pH of the soil, the ratio of cations to anions, and other environmental conditions all affect how much lead is adopted and to what extent. In addition to breathing in lead-containing particulate matter, humans are exposed to the harmful effects of lead by consuming tainted food and water. The amount of organic matter present, the pH of the soil, the ratio of cations to anions, and other environmental conditions all affect how much lead is adopted and to what extent. In addition to breathing in lead-containing particulate matter, humans are exposed to the harmful effects of lead by consuming tainted food and water. Tetraethyl and tetramethyl lead are the only compounds that can be absorbed via the skin. About 90% of the lead that enters the bloodstream through blood is deposited in the bones as Pb3(PO4)2, where it attaches to red blood cells as Pb2+. Lead is quickly absorbed into the blood stream. Lead can move from the bones in the form of Pb2+ in acidosis (high acidity), which can be harmful to the kidneys, central nervous system, and circulatory and immune systems (Amra Odobasic, Indira Sestan et al, 2018).

Chromium: Chromium can be found in a variety of oxidation states in its compounds, ranging from bivalent to hexavalent. Chromium can exist in trivalent and hexavalent forms in solutions. In most compounds, hexavalent chromium exists as the chromate (CrO4)2− or dichromate (Cr2O7)2− ions. Because of its high degree of oxidation and ease of entry into cellular membranes, Cr (VI) is hazardous. Consequently, it is thought that this type of chromium is carcinogenic. Before wastewater is released into natural recipients, chromium (VI) must be eliminated due to its toxicity, carcinogenicity, and mutagenicity towards living species. It also damages the liver and can cause lung congestion, skin irritation, and ulcer formation. However, compared to chromium (VI), trivalent chromium, or Cr(III), is 300 times less hazardous. Although chromium is an essential component for many plant and animal species, it can also be carcinogenic and induce allergic skin reactions. (Amra Odobasic, Indira Sestan et al, 2018).

Mercury: As a heavy metal, mercury is a part of the periodic table's transition element series. It is distinct in that it can be found in nature in three different forms: organic, inorganic, and elemental, each with a different toxicity profile (Clarkson TW, Magos L, Myers GJ *et al,* 2003). Elemental mercury is a liquid with a high vapor pressure that is present at room temperature and is emitted into the atmosphere as mercury vapor. Additionally, mercury can be found as a cation in the oxidation states of +2 (mercuric) or +1 (mercurous). As a result of microorganisms present in soil and water methylating inorganic (mercuric) forms of mercury, methyl mercury is the most commonly encountered chemical of the organic form found in the environment (D. E, Hartmann LM *et al,* 2004).

Luminous Bacteria: As a versatile and incredibly helpful reporter technology, bioluminescence offers a sensitive, non-destructive, real-time assay that enables temporal and spatial measurement. Numerous scientists have examined the genetic, physiological, and biochemical mechanisms governing bacterial bioluminescence over the years. By employing luminous genes as biosensors for environmental research, these findings have completely changed the field of environmental microbiology. The capacity to transfer the lux phenotype - coupled with particular promoters that permit its expression only in the presence of the appropriate analyte - into various bacterial species offers a practical way to increase the number of options for quick, easy, and sensitive environmental screening (Chatragadda Ramesh *et al,* 2021). There were seventeen *Vibrio harveyi* isolates in the study group. It had previously been demonstrated that isolate PM47666-1 was extremely pathogenic toward prawn larvae. The residual isolates came from environmental and clinical sources. The Sir George Fisher Center for Tropical Marine Studies kindly provided cultures. Townsville, North Queensland's James Cook University is home to the Australian Collection Marine Microorganisms (ACMM). PM91 isolates were acquired. (Raju Mohanraju *et al,* 2021). Fresh seafood, including shrimp, octopus, squid, and cuttlefish, was procured at Maadia Port in Alexandria, Egypt. From Alexandria, Egypt's Eastern Harbor, comb jellyfish were captured.

The Bioluminescence Inhibition Assay (bia) to determine toxicity: Using the bioluminescence inhibition assay (BIA), the impact of

many pollutants at varying doses on the cellular bioluminescence of the chosen strain *Vibrio sp.* 6HFE was investigated. First, standard solutions of heavy metals (NiSO4.6H2O, CuCl2.2H2O, HgCl2, CoCl2.6H2O, AgNO3, FeSO4.7H2O, ZnCl2, and K2Cr2O7), hydrocarbons (benzene, phenol, penta-chlorophenol sodium salt, and catechol), and organic solvents (acetic acid, ethyl acetate, acetone, methanol, chloroform, isoamyl, acetonitrile, formamide, and DMSO) were first prepared in HPLC grade sterile water. In order to perform bioluminescence experiments, 100 µl of each toxicant was mixed uniformly with luminescent *Vibrio sp.* 6HFE (OD = 0.4 ~ 4 × 106 CFU) that was in a microtiter plate well (Howaida Hassan, et al., 2022). Additionally, a control experiment with sterile water and bacterial culture was conducted in tandem with every toxicity well. Every toxicant concentration was tested using three replicates. The microplate was kept at 25°C for the entire night in the luminometer shacked. A luminometer (LUMISTAR, Galaxy, BMG, Germany) was used to evaluate the bioluminescence of the culture and treated wells at regular intervals. Finally, using the following equations (4 and 5), where (t) is the period in minutes, the percent of bioluminescence inhibition (BI%) was calculated (Howaida Hassan, *et al,* 2022).

The Microtox Assay: Frequent toxicity tests that use Daphnia or fish as test organisms are costly and time-consuming. As a result, microbial bioassays are becoming more and more common for determining how harmful environmental contaminants are to living things. Microbial bioassays have better reproducibility, a cheap cost, and a quick response. Many agencies have evaluated the effects of chemicals in the environment over the past eighteen years using the Microtox system. This toxicity bioassay is available for purchase and employs both naturally occurring bioluminescent bacteria and, more recently, *Vibrio harveyi*. In *V.harveyi*, light emission is dependent upon a functional metabolism. Toxic substances that interfere with metabolism or bacterial survival thus result in a corresponding decrease in light output that is directly related to the sample's level of toxicity. The effective concentrations (EC50) at which light emission is reduced by 50% are used to express the results (Vânia da Silva Nunes- Halldorson, *et al,* 2003). They postulated that conformational changes in bacterial chromosomes promoted the transcription of luminescence genes (lux operon), boosting the expression of genes involved in aldehyde production and leading to an increase in intracellular K+ concentration. As a result, potassium, calcium, and magnesium ions have been added to the testing media for the Microtox assay. Another external component that influences light intensity in the bioassay is sodium chloride, which needs to be carefully controlled. Since *V. harveyi* are marine organisms, it is advised to add NaCl to the test solution in order to achieve a saline concentration of roughly 20 g/L (Vânia da Silva Nunes- Halldorson, *et al,* 2003).

Due to low external osmotic pressure, *V.harveyi* cell membranes can burst in concentrations of salt less than 5 g/L. The recommended limits for pH and temperature are 6.5-7.0 and 10-25ºC, respectively as they can also have an impact on the bioassay results and need to be carefully controlled. It has been suggested that bioluminescence may be influenced by cell density-related parameters; we now know that these are the results of auto induction. As a result, it is advised to use recently made bacterial suspensions and to regulate cell density (Vania da Silva Nunes-Halldorson, *et al,* 2003). When experimental conditions are appropriate, the Microtox bioassay can have equal or great erprecision than the traditional fish and Daphnia bioassays. Microtox is a simple bioassay that has several advantages over other toxicity tests (Vania da Silva Nunes- Halldorson, *et al,* 2003). One of the advantages is its good statistical significance since the observed response is produced by a large number of cells (~10^6). Also, this bioassay is amenable to automated toxicity data collection that can be used in mathematical models. The Microtox system is currently being employed for toxicity testing in complicated environmental conditions because to its great sensitivity and rapid response. For instance, it has been applied to evaluate the toxicity of several pesticides and their main metabolites in soil that has been cleaned up from a contaminated site. The Microtox method has also been used for preliminary screening of cyanobacteria blooms and to assess the efficacy of toxicity reduction during wastewater treatment. The offshore oil and gas industry may find further applications for this technology, such as source monitoring of discharges from offshore operations. The potential uses of this apparatus for environmental research will grow as additional organizations and scholars investigate the applicability of the Microtox bioassay in their respective fields of study (Stefano Girotti *etal*, 2007).

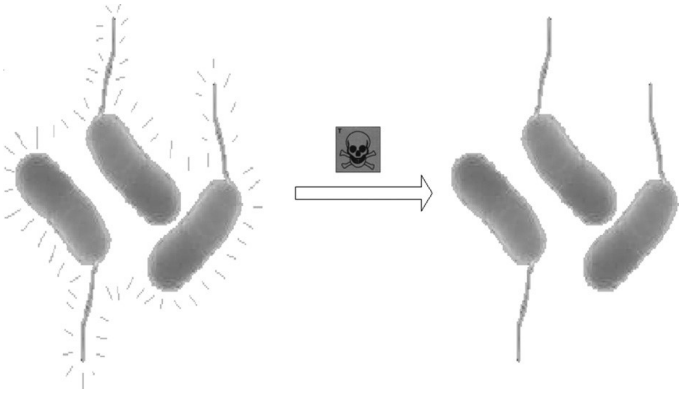

Fig. 4. Naturally bioluminescent bacteria reduce or stoplight emission in presence of toxic compounds that impair their metabolism (Stefano Girotti *etal*, 2007).

Identification of the Chosen Bright Isolation by Molecular Means: Using the EZ-10 Genomic DNA kit (Bio Basic Inc., USA), chromosomal DNA was extracted from a freshly formed culture in order to identify the chosen luminous bacterial isolate. Universal forward and reverse primers (27F; 5′AGAGTTTGATCCTGGCTCAG3 ′ and 1429 R, 5′TACGGYTACCTTGTTACGACTT3) were used to amplify the 16S rDNA gene. Amplify Taq DNA polymerase and an Applied Biosystem (Thermo Fisher Scientific, USA) were used in the ABI PRISM dye terminator cycle sequencing kit to sequence the purified 1500-bp amplicon. The produced sequence was sent to GenBank in order to receive the relevant entry number. The sequence was analyzed using the National Centre for Biotechnology Information's N-BLAST tool, which compares sequence similarity with other sequences in the database. MEGA-X was the software package utilized to obtain phylogenetic tree and multiple alignment (Howaida Hassan *et al,* 2022).

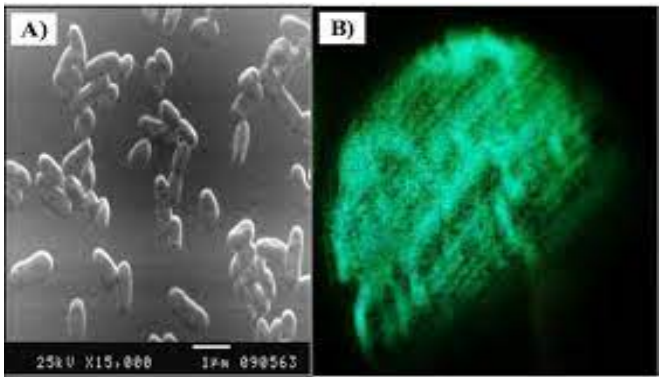

Fig. 2. A SEM micrograph of Vibrio sp. 6EFE (×15,000); B bioluminescence of Vibrio sp. 6HFE grown on LA medium agar plates for 12 h at 30°C (Howaida Hassan et al., 2022)

Measure Bioluminescence Intensity with Luminous Bacteria: The affirmative candidates' bioluminescence (BL) was assessed using a luminometer (LUMISTAR, Galaxy, BMG, Germany) at regular

intervals. The count per second (CPS) was used to measure the intensity. 200 µl of bacterial suspension were added to the transparent, v-bottom Greiner plate to perform the measurement. Three copies of each reading were taken. The isolate chosen for further identification, characterisation, optimization, and application stages was the one with the maximum light emission (Howaida Hassan *et al,* 2022). Assay for luminosity fresh V. harveyi broth culture was inoculated in luminous broth and cultured at 35°C to measure luminescence activity. The initial OD of the broth culture was observed. As a blank, broth medium devoid of culture inoculation was employed. (Mohd Izuan Effendi Halmi *et al*, 2014). Prior to taking readings, 200 µ L of samples were pipetted into 96-well DTX microplates. Relative Luminescence Unit (RLU) was the luminescence unit utilized. The luminometer (Hidex) was used to record luminescence readings every hour.

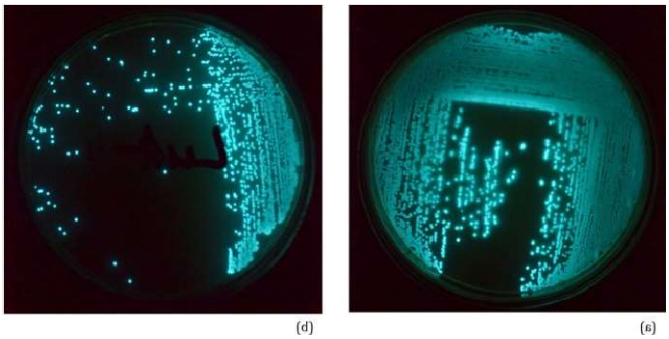

Fig. 3. Images showing luminescence expressed by (a) PBR1 and (b) PBL1 in dark room (Paritosh Parmar *et al*, 2020)

Bioluminescence Intensity Optimization: The light intensity in fast time reaching with maintaining bioluminescence stability is the main criteria in such step. The experiment setup was conducted in a 96-well sterile tissue culture plate with 10 µl of the bacterial culture (0.5McFarland) of selected strain mixed with 190 µl of sterile broth. The optimization step in this study was carried out through two stages: one — variable at a time design (OVAT) and statistical designs including Plackett-Burman design (PBD) and central composite design (CCD) (Howaida Hassan*, et al,* 2022).

CONCLUSION

It is feasible to draw the conclusion that BLB tests are, on average, sufficiently sensitive to detect substances that may be hazardous to humans and the environment as a whole based on the data given in the large collection of papers that make up this study. This claim is supported by the ongoing development and use of bioluminescent alarm tests. When compared to the features of other bioassays, the BL test systems' sensitivity, ease of use, rapidity, versatility, and low cost make luminescent assays appear to be a preferable option—at least in relation to some of the stated attributes. The usefulness and reliability of GM BLB assays were further enhanced by genetic manipulation techniques, which transformed native bacteria from the environmental matrix into luminescence emitters for analysis. For the first time, the current study showed how to separate, identify, and characterize luminous bacteria from marine species. Quantitative analysis revealed that the strain V. sp. 6HFE that was chosen had the maximum bioluminescence intensity. OVAT, PBD, and CCD were used to optimize the cultural conditions in order to maximize light intensity. The luminous V. sp. 6HFE was used to determine the toxicity of a variety of toxicants, such as hydrocarbons, heavy metals, and solvents, by using optimum circumstances. The effectiveness of the strain V. sp. 6HFE as a biosensor for observing pollution in actual ecosystems was validated. Using bioluminescent tests to monitor heavy metal toxicity helps to both protect human health and the environment. Heavy metal concentrations in soil or water can be found and measured using these assays, which make use of bioluminescent organisms. They contribute to the assessment of environmental contamination levels and the creation of efficient pollution control strategies by accomplishing this. As exposure to high concentrations of heavy metals can have detrimental effects on health, these assays also help to protect human health. In environmental monitoring and risk assessment, the bioluminescent technique is a useful tool since it provides a sensitive and quick method of detection.

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