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Bioassay of industrial and hazardous waste pollutants: A review

Yung-Tse Hung ^{1,*}, London Voss ¹, Howard H. Paul ² and Christopher R Huhnke ¹

¹ Department of Civil and Environmental Engineering, Cleveland State University, Cleveland, Ohio, USA.

² Department of Information Systems, Cleveland State University, Cleveland, Ohio, USA.

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Abstract

Every industry produces waste pollutants that can cause harm to the environment. Consistent contaminants into the environment affect human health and ecosystems. While these environmental consequences can be monitored through chemical and technological approaches, its effect on living organisms creates another approach. Both plant and animal bioassays have shown promising results to monitoring pollution especially with observing early effects. These biological wastewater treatment processes provide an imperative low cost option to underdeveloped countries because preserving the environment takes every part of this world. This paper will cover the various bioassay methods and its results and feasibility in different industries.

Keywords: Industrial effluent; Biological treatment; Plant bioassay; Contamination; Ecosystem health; Industrial pollution

1. Introduction

Any materials left over after the manufacturing process is completed is considered industrial waste. Almost all industries contribute to waste including textile mills, food production, industrial chemicals, consumer goods, construction, mining, and others. Industrial waste can cause serious damage to the environment, if not properly damaged. While industrial waste can be considered either hazardous or nonhazardous, both can cause harm to the environment if not handled suitably. Precise measurements and knowledge of the waste created in these industries is crucial to ensuring the environment is unharmed from this production. While many say the solution to pollution is dilution, determining accurate measurements of the pollution is the first step. No material is inherently toxic if it is diluted enough, meaning that measurement of how much waste is being dumped into the environment is crucial to keeping it clean.

A way to measure the concentration of a pollutant is bioassay. Bioassay is the “measurement of the concentration or potency of a substance by its effect on living cells or tissues” (1). A bioassay can be quantal if it is binary, or it can be quantitative if it is nonbinary. For example, a quantal response is whether an event occurs, such as death of the subject. While a quantitative response would be classified as a response that is on an ongoing scale such as an increase in blood pressure to the subject. Bioassay is also classified into direct and indirect. A direct bioassay occurs when the living organism in question has a recordable response, and an indirect bioassay is when a fixed stimulus is kept, and the response recorded is waited and reported on. Bioassay can be used to “detect biological hazards or produce a report on the quality of the substance. A bioassay can be used to monitor water quality as well as wastewater discharges and its impact on the surroundings. It is also used to assess the environmental impact and safety of new technologies and facilities” (2).

* Corresponding author: Yung-Tse Hung

1.1. History of Bioassays

The history of bioassays dates back to much earlier than many other environmental controls, all the way into the late 19th century. The interesting thing about bioassays is the ability to determine the danger of a substance without extensive knowledge or expensive lab equipment. The visual effects of bioassay created the ability for bioassays to be able to be used before they were scientifically defined. "One well known example of a bioassay is the "canary in the coal mine" experiment. To provide advance warning of dangerous levels of methane in the air, miners would take methane-sensitive canaries into coal mines. If the canary died due to a build-up of methane, the miners would leave the area as quickly as possible". While the methodologies of using bioassays in today's industrial waste have evolved, the principle behind it remains.

1.2. Dangers of Industrial and Hazardous Waste Pollutants

+As defined above, industrial waste is an all-encompassing term used to describe any material that is left over as a result of a manufacturing process. While it may not seem like all this waste would harm the environment, if not monitored it can have serious harmful effects, even nonhazardous waste. "Many factories and most power plants are located near bodies of water to obtain large amounts of water for manufacturing processes or for equipment cooling. In the US, electric power plants are the largest water users. Other industries using large amounts of water are pulp and paper mills, chemical plants, iron and steel mills, petroleum refineries, food processing plants and aluminum smelters" (3).

2. Effects on living organisms

While discussed above, the fundamental idea behind a bioassay is a living organism's tendency to react to the presence of certain substances. These reactions can range from the living organism in question dying to an increase of certain quantities within the subject. These reactions can also be seen in plants, or even the offspring of the subject that was exposed to the contaminant. "Some biological contaminants trigger allergic reactions, including hypersensitivity pneumonitis, allergic rhinitis, and some types of asthma. Infectious illnesses, such as influenza, measles, and chickenpox are transmitted through the air. Molds and mildews release disease-causing toxins. Symptoms of health problems caused by biological pollutants include sneezing, watery eyes, coughing, shortness of breath, dizziness, lethargy, fever, and digestive problems" (3).

While this sounds extreme, "allergic reactions occur only after repeated exposure to a specific biological allergen. However, that reaction may occur immediately upon re-exposure or after multiple exposures over time. As a result, people who have noticed only mild allergic reactions, or no reactions at all, may suddenly find themselves very sensitive to allergens. While these reactions can range from a variety of broad senses of visual and extremity, the concept of the bioassay remains the same. This paper will further look into the specific types of reaction observed in living organisms through bioassays.

2.1. Mutagenicity, Teratogenicity, Bioaccumulation Potential, and Phototoxicity

Mutagenicity is defined as the changing of evolutionary genetics from generation to generation. In studying bioassays, this can be seen by newborns showing responses and reactions due to the exposure of contaminants of their mothers. "Mutagenicity is an important toxicological endpoint that requires thorough evaluation during the industrial chemical registration process. Regulatory requirements for mutagenicity assessment in registration of industrial chemicals vary in geographic regions (and in some cases by intended application). The mutagenicity testing requirements for registration of industrial chemicals from representative geographic regions. Mutagenicity data requirements are tailored to the tonnage band". This method of testing can be useful in identifying these carcinogens and identifying the chemicals and other substances that pose a serious risk to the environment.

While mutagenicity focuses on the genetic changes from generation to generation, teratogenicity is the study of the changes that happen to a living organism throughout the whole progression of life of that organism. It is defined as "the study of abnormalities of physiological development in all organisms including plants during the entire lifespan. It is a sub-discipline in medical genetics which focuses on the classification of congenital abnormalities as dysmorphology" (4). These are very serious and harmful examples that show concerning effects that can be the cause of hazardous pollutants from industries. These devastating effects completely change people's lives and pose a harm to the people, animals, and all other living organisms that inhabit the earth. While the effects of hazardous waste are clearly horrific, understanding how it occurs is key in improving the current state of industrial waste. It is important to realize that careful study of these principles can help in early identification for potential pollutants.

The effects on living organisms from hazardous pollutants can be seen in many forms. Bioaccumulation is “the gradual accumulation of substances, such as pesticides or other chemicals, in an organism. Bioaccumulation occurs when an organism absorbs a substance at a rate faster than that at which the substance is lost or eliminated by catabolism and excretion. Thus, the longer the biological half-life of a toxic substance, the greater the risk of chronic poisoning, even if environmental levels of the toxin are not very high. Bioaccumulation, for example in fish, can be predicted by models. Hypothesis for molecular size cutoff criteria for use as bioaccumulation potential indicators are not supported by data. Naturally, the process of bioaccumulation is necessary for an organism to grow and develop; however, accumulation of harmful substances can also occur” (4). In the context of bioassay, it is also possible to observe and determine the bioaccumulation potential in hazardous waste pollutants in certain organisms.

As the figure indicates, bioaccumulation is often used to model toxins in fish that can easily spread to humans eating these fish. It is just another example of how effects of living organisms can be used to determine potency of hazards or pollutants in the environment. The crucial part of this modeling is knowing when it has reached toxic and harmful levels and the identification of these pollutants. While bioaccumulation focuses on the overall ecosystem health, phytotoxicity focuses on specifically the health of the plants. Phytotoxicity “describes any adverse effects on plant growth, physiology, or metabolism caused by a chemical substance, such as high levels of fertilizers, herbicides, heavy metals, or nanoparticles.

The principles of bioassay focus on the biological health of living organisms. Living organisms are created to be able to sustain their life and know when there is a presence of a substance that should not be there. “Biomonitoring of industrial chemicals in human tissues and fluids has shown that all people, not just those working in or living near major pollution sources, carry a “body burden” of synthetic chemicals in their blood, fat, mother’s milk, semen, urine, and breath” (5). Different organisms have different responses to a variety of contaminants, but their responses give knowledge of the identification of these pollutants and can be used for a range of applicable environmental resources for industrial waste.

3. Application of bioassay

While the discussion of how a bioassay works and the different modes and facets it can take, the application is the most important aspect to consider through an environmental lens. “Bioassay is used to test the effects of compounds being considered for use in drugs or skin care products. Before a chemical compound receives FDA approval as an ingredient in products for human use, it must be thoroughly tested on laboratory animals. For environmental testing, bioassays provide an integrated picture of overall toxicity of an effluent or a sample of water, sediment, or soil from a contaminated site. Fathead minnows, various aquatic invertebrates, earthworms, protozoans, and seeds all are used for bioassays of aquatic samples.

The range of organisms used in bioassay tests is large and within each of these organisms exists a large range of responses that can be observed. This creates an abundant amount of information that can be collected from the effects of living organisms. This means the application of these studies offers a broad range of outcomes and this paper will discuss specifically how this can improve and manage hazardous and industrial waste.

3.1. Lettuce Seed Bioassays

A common living organism chosen for the current day bioassay test is the lettuce seed. Lettuce seed “bioassays have proven to be an easy and inexpensive means of testing the toxicity of some types of contaminants of concern in water and sediments, including heavy metals and some pesticides and other organic toxicants” (6). These factors create an inclination to use this bioassay in favor of other options which is important when the options are endless. The lettuce seed bioassay data are so versatile they are even able to determine a dose response relationship that is evident to record. It is such a simple explanation on bioassays, that it is even a common high school chemistry high school experiment to determine a dose response relationship using lettuce seeds.

3.2. When Chemical Assay Is Not Available

A chemical assay has the same goal of a bioassay of determining the substance and also the potency and exposure in an environment. “Chemical assays analyze the quality of raw materials. Organic chemicals, for example, are assayed using gas chromatography and infrared spectroscopy. Raw materials in manufacturing may be assayed using titration or gravimetric analysis. Generally, these seek to determine the quantity of a constituent in a sample” (7). While chemical bioassays provide precise and intricate results, there are times where it is not practical or optional to conduct.

3.3. Insulin Bioassays

Insulin in the body regulates glucose levels within the bloodstream and allows glucose to be stored in the liver, muscles, and adipose tissue, resulting in overall weight gain. Pacific BioLabs has extensive experience in performing in vivo cGMP bioassays. “The in vivo bioidentity and the biopotency tests for insulin utilize the rabbit blood sugar method in which four rabbit test groups are injected with insulin and their blood glucose levels are measured over time. The bioidentity test is a qualitative test which requires only two rabbits per test group. The blood glucose levels of the rabbits should indicate an insulin potency above a specific threshold (15 USP Units/mg) to qualitatively identify the product as insulin” (8). This bioassay process is important in developing appropriate insulin doses for millions of people that need it to survive.

4. Utilization of bioassays with industrial waste

Every waste created from industrial processes poses a risk to biological health. The environmental agencies of the world are charged with assessing these wastes to create a policy that prevents the harm of ecosystems as a result of these pollutants. Toxicity bioassays are an important tool in determining these policies. “Biological toxicity tests are widely used for evaluating the toxicants contained in the waste. Most toxicity bioassays have been developed for liquid waste. These cases need to be considered to respect the environmental policies that they provide and the biological treatment it creates.

4.1. Allium Cepa

Allium Cepa is “commonly known as onion, is a worldwide culinary and therapeutic spice belonging to the family Liliaceae. Onion is an essential ingredient in many African sauces and is mostly produced locally, with Egypt being the first producer in the continent. Onion is a source of various biologically active compounds, such as phenolic acids, thiosulfates, and flavonoids. The plant has a variety of pharmacological activities including anticancer, antidiabetic, antimicrobial, cardiovascular, antioxidant effects, etc., justifying its possible use in the treatment of various human ailments” (9). The use of *Allium Cepa* in bioassays is extensive in their ability to change growth rate and other factors that are easily observable from a scientific standpoint.

4.2. Phaseolus Aureus

A mung bean is a plant species in the legume family that can be used as an ingredient in both sweet and savory dishes (10). Its previous genus name was *Phaseolus Aureus* but was one of many species that moved from the genus *Phaseolus* to *Vigna* in the 1970s. The experiments that are explored further took place in the 1970s and used this plant species as a bioassay testing method. This is referring to the mung bean, now known as *Vigna Radiata*.

In this experiment, “a bioassay employing green or etiolated cuttings of *Phaseolus aureus* Roxb. was developed for determining metformin-induced growth stimulation in light. Growth enhancement of green cuttings was more rapid and relatively greater than that of etiolated cuttings. (11). The principle of the reactions of living organisms fueled this study and the applicability it can have in this testing process. The set up and sampling decisions follow the principles of bioassay reinforced throughout this paper.

5. Bioassays in wastewater treatment

The key concept of the environmental engineering field is the applicability of monitoring and assessing water and wastewater treatment. The application of bioassay in wastewater treatment is evident. “Bioassays rely on measuring the response of organisms exposed to contaminants relative to a control. They have been used to establish the toxicity levels of target contaminants and complex aqueous matrices. While all chemicals are no hazardous under a certain dilution, the accumulation poses serious threat to the environment and needs to be monitored through the biological methods.

While this study monitors both the biodegradability and toxicity of the wastewater effluent, it is important to understand how they affect one another. “The dualism toxicity/biodegradability is particularly relevant when AOPs are investigated in industrial wastewater pretreatment because the main objective is to improve the biodegradability of a mixture of pollutants refractory to biological treatment. In this regard some authors use toxicity tests to infer the behavior of treated wastewater in terms of biodegradability in relation to a subsequent biological process as well as to set up optimum operating conditions of the investigated AOPs. But this approach is incorrect because, depending on the organisms used in toxicity tests, the results may either underestimate or overestimate the effect of AOPs on the biodegradability of wastewater” (12).

5.1. Biomonitoring Methods

The environmental and social impact of wastewaters left untreated due to improper management or unsuitable methods is a large issue. It is important to select a proper method in each project to ensure accurate and relevant results. "The rapid increase in industrialization is alarming the survival of living creatures and damaging the ecological balance. Continued release of untreated industrial wastes/leachates to the rivers contaminates the entire ecosystem. The continuous exposure of organisms to dangerous chemical substances may show chromosomal abnormalities which can cause diseases and can affect the human offspring. This includes careful and precise preparation and understanding each case individually. It is also important to understand the factors that influence the decision in which method to choose.

Because of their ability to monitor and expand on the end results of an environment, bioassays offer an insight into the impact of industrial waste that other monitoring methods cannot. "Bioassays are very important in environmental monitoring and assessment because these assays allow us to screen and evaluate the different endpoints related to toxicity. New versatile, fast and kit-based assays are needed to explore the onsite monitoring of the pollutants for assessment of the environmental degradation. The integrated use of chemical and biological bioassays to monitor the environmental contamination will enable the expansion of more accurate environmental monitoring systems. Therefore, this [study] intends to provide updated research findings regarding the applicability and usefulness of plant based bioassays and recommend its use in monitoring of industrial effluents and wastes" (13). With as many options that exist in this realm of study, it is important to understand why some methods are recommended over others.

6. Range of application of bioassays

While this paper focuses on a bioassays ability to monitor and remediate industrial effluents, it has applicability across many fields, especially pharmaceutical and clinical medical trials. While this strays away from the goal of identifying and preventing industrial waste pollutants, the processes involved introduce interesting conversations when it comes to the fundamentals of bioassay sampling methods. The processes that are used in these fields can also be replicated in an industrial setting given the right circumstances. The following sections will review some alternative uses of bioassays and discuss the possibility of applying it to the environmental field in preventing or identifying industrial waste, or in wastewater treatment.

6.1. Dose Response Relationship

A fundamental concept in the field of toxicology is the relationship between how much of a toxin, dose, and how that affects living organisms' response. It is essentially a quantal form of bioassay that receives its own name and is widely used. "The dose–response relationship, or exposure–response relationship, describes the magnitude of the response of an organism, as a function of exposure or doses to a stimulus or stressor (usually a chemical) after a certain exposure time. Dose–response relationships can be described by dose–response curves. Studying dose response, and developing dose–response models, is central to determining "safe", "hazardous" and (where relevant) beneficial levels and dosages for drugs, pollutants, foods, and other substances to which humans or other organisms are exposed. These conclusions are often the basis for public policy" (14).

7. Case studies

To best understand the progress and results of bioassays and their application in industrial and hazardous waste, as well as wastewater treatment processes, it is best to discover and discuss a variety of case studies. This paper will explore the broad range of industries and effluents while also the range of applications of bioassay testing to provide a proper overview of the tests conducted using the biological responses of ecosystems, individual organisms, and test subjects to determine and identify the harm and potency of a variety of substances, focusing on the pollutants of multiple industries.

7.1. Textile Industry Waste Bioassays

India is one of the world's largest producers of textiles and apparel. This massive production of clothing also creates a massive production of waste. "In almost all developing nations and in many of the highly developed nations, water pollution due to discharge of inadequately treated wastewater into the environment is a major issue of concern. The wastewater generation is mainly attributed to the rapid increase in the industrial sector, which is growing substantially for the economic development of a country. Among the various industries, textile industry is considered as one of the major contributors of water pollution, since the waste generated is very complex in nature containing color content and

toxic components.” (15). The effects on human and environmental health provide an insight to the study of bioassay and using plant and microorganisms to study the effects and determine thresholds of certain byproducts.

The applicability of bioassay tests has a variety of uses that can be beneficial for biological environmental improvements. It is important to understand how the range of these tests can be used to improve the environmental health of the areas surrounding industries that produce harmful wastes. The goal of these environmental studies is to keep the biological ecosystems healthy.

8. Conclusion

The principle of bioassay is the beauty of living organisms and their ability to respond to the substances they are exposed to. These reactions can come in a variety of reactions and can be an evaluation of the biological health of an area. The method for choosing a bioassay depends on the contaminant that is being tested to choose a bioassay that could be sensitive to its presence. Many common day tests for industrial wastewaters are done with plant and microorganism bioassays. Bioassays are also commonly used in clinical trials with rodent subjects to determine carcinogens and other dose response relationships with a variety of chemicals.

The application for bioassays is seen in the many studies discussed in this paper. They create the ability to create certain toxic thresholds of chemicals which helps determine environmental standards. Site cleanup plans can be determined and guided through testing done through bioassays which are able to identify where certain contaminants have traveled. Its application in industrial and hazardous pollutants is evident and has been tested and used for decades, while the principles of these ideas continue to advance. Because the idea of a bioassay directly can create a visual for the health and, country, the harm that occurs on the environment in direct result of the pollutants deposited from the manufacturing of products throughout the globe.

While the biomonitoring of organisms creates an intuitive understanding of the harm a contaminant has on the environment, it does have its limits and should be used in conjunction with chemical analyses. The benefits of bioassay include the emotional impact some of the effects on living organisms can have on people and truly understanding the harm industries can cause to the environment. While the testing done today is methodical and statistical, the principles remain the same that have been used for decades and will continue to be the same even as the technologies advance the processes to even more improvement and exactness.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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