



# Phytoremediation of Arsenic (As), Lead (Pb), and Mercury (Hg) Contaminated Soil Using Sunflower (*Helianthus annuus* L.)

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

Phytoremediation is a cost-effective and environment-friendly approach to remediate or clean our environment using plants by accumulating contaminants. *Helianthus annuus* L., commonly named sunflower is known to be a hyperaccumulator of heavy metals from the soil. This study focuses on using sunflowers for the phytoremediation of heavy metal-contaminated soil, specifically Arsenic (As), Lead (Pb), and Mercury (Hg). The researcher collected soil samples in the months of March –

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April 2024 at the Catarman Sanitary Landfill located at Sitio Banihit, Catarman, Northern Samar, and were analyzed for pH, Nitrogen (N), Phosphorous (P), and Potassium (K) test using a Soil Test Kit (STK) from the Bureau of Soils and Water Management – Department of Agriculture, and detected the presence of As, Pb, and Hg using qualitative analysis. The analysis showed that the soil is slightly acidic, has low nitrogen, high phosphorus, and sufficient potassium, that is an indication of heavy metal contamination, and is good for metal uptake and accumulation in plant. The researcher planted sunflower using the collected soil samples. Leaves were extracted after one (1) month and two (2) weeks, and tested for physical properties in terms of pH, density, and solubility. Results showed that sunflower leaves extract is slightly acidic with an average pH of 6.94., density of 1.00 g/ml, and shows a polar property. The plant sample was also analyzed using Colorimetric analysis for Arsenic, Flame AAS for Lead, and Cold Vapor AAS for Mercury. Findings revealed that the concentration of these three (3) heavy metals are below the detection limit. But in small concentrations, exposure to these heavy metals still poses a great threat to living organisms. Using sunflower plants of about four (4) months or more could further be tested for possible detection of considerable amount of heavy metal uptake.

*Keywords: Arsenic; lead; mercury; phytoremediation; sunflower.*

## 1. INTRODUCTION

The rapid growth of urbanization and industrialization resulted in environmental pollution which became a significant global concern. Contamination of heavy metals, specifically in soil is one of the critical environmental issues that has a great impact on human health and the ecosystem. Once introduced to the soil, these toxic heavy metals perdure for a long period, affecting the environment and, thereafter, communities living near the contaminated areas.

The accumulation of heavy metals in soil is caused by anthropogenic activities such as mining, industrial processes, and improper waste disposal of hazardous substances [1]. The livelihoods of the local communities that depend on land for sustenance was also jeopardized because of these contaminations in soil as heavy metals cause retardation in plant growth, reduce nutritive value, affect photosynthesis [2], and pose a threat to animals and humans through food chain [3,4]. Furthermore, it was found that these heavy metals have neurological and developmental effects on young children, especially on fetuses [5]

The most common environmental heavy metals are copper (Cu), nickel (Ni), chromium (Cr), lead (Pb), cadmium (Cd), mercury (Hg), iron (Fe), and arsenic (As) [6]. However, the most commonly associated with poisoning of humans are As, Pb, and Hg [7]. Most of these heavy metals are non-

biodegradable and toxic to numerous organisms, including humans; therefore, they must be removed from ecosystems [8].

Remediation techniques have been developed to rectify the heavy metal-contaminated sites, including surface capping, encapsulation, landfilling, soil flushing, soil washing, electrokinetic extraction, stabilization, solidification, vitrification, phytoremediation, and bioremediation [9]. Remediation of heavy metals requires special attention to protect soil, air, and water quality, human health, animal health, and the overall environmental condition. However, most of the developed physical and chemical heavy metal remediation technologies are demanding costs that are not feasible, time-consuming, and release additional waste to the environment [7].

In response to this global problem, there is a need for sustainable and eco-friendly methods in mitigating heavy metal contamination in soil. There is a growing interest in one of these methods called Phytoremediation, a plant-based remediation technique.

*Helianthus annuus* L., commonly known as sunflower, is one such plant species that shows remarkable potential in the remediation of heavy metal-contaminated soils due to its hyper-accumulative property. Sunflower was chosen in this study based on its high biomass, fast growth rate and its ability to remove heavy metals from contaminated soils [10]. Apart from industrial applications of dry sunflower biomass, growing

sunflowers have shown great potential to absorb various metal contaminants such as Ni, Cu, As, Pb, and Cd [11].

This study aims to address the critical issue of heavy metal contamination in soil, by investigating the efficacy of *Helianthus annuus* L. in the phytoremediation of soils contaminated with Arsenic (As), Lead (Pb), and Mercury (Hg). The researcher collected soils from Catarman Sanitary Landfill located at Sitio Banihit, Brgy. Cal-igang, Catarman, Northern Samar, as landfills, are some of the most contaminated soils of heavy metals due to leachates that are generated by the decomposition of waste [12]. Subsequently, the researcher conducted gardening of sunflower using pots with the collected soil sample. Powdered sunflower leaves were then analyzed for quantitative analysis for heavy metals.

## 2. METHODOLOGY

Soil sample was collected at the Catarman Sanitary Landfill located at Sitio Banihit, Brgy.

Cal-igang, Catarman, Northern Samar, Philippines and was used to cultivate sunflower. Preliminary test for detecting the presence of arsenic (As), lead (Pb), and mercury (Hg), extraction of samples, and physicochemical tests for soil and plant samples were conducted at the Technological Innovation Center and Integrated Research Laboratory, both located at the University of Eastern Philippines, University Town, Catarman, Northern Samar.

### 2.1 Preparation of Sample

The researcher cultivated sunflower plants for more than a month. The researcher conducted gardening in the University Town, Catarman, Northern Samar using pots. After full growth, leaves were collected for extraction. Moreover, the plant sample was identified by an expert at the Biological Department, College of Science, University of Eastern Philippines – Main Campus. The sample was then identified as *Helianthus annuus* L. commonly known as sunflower.

### Photo Documentation

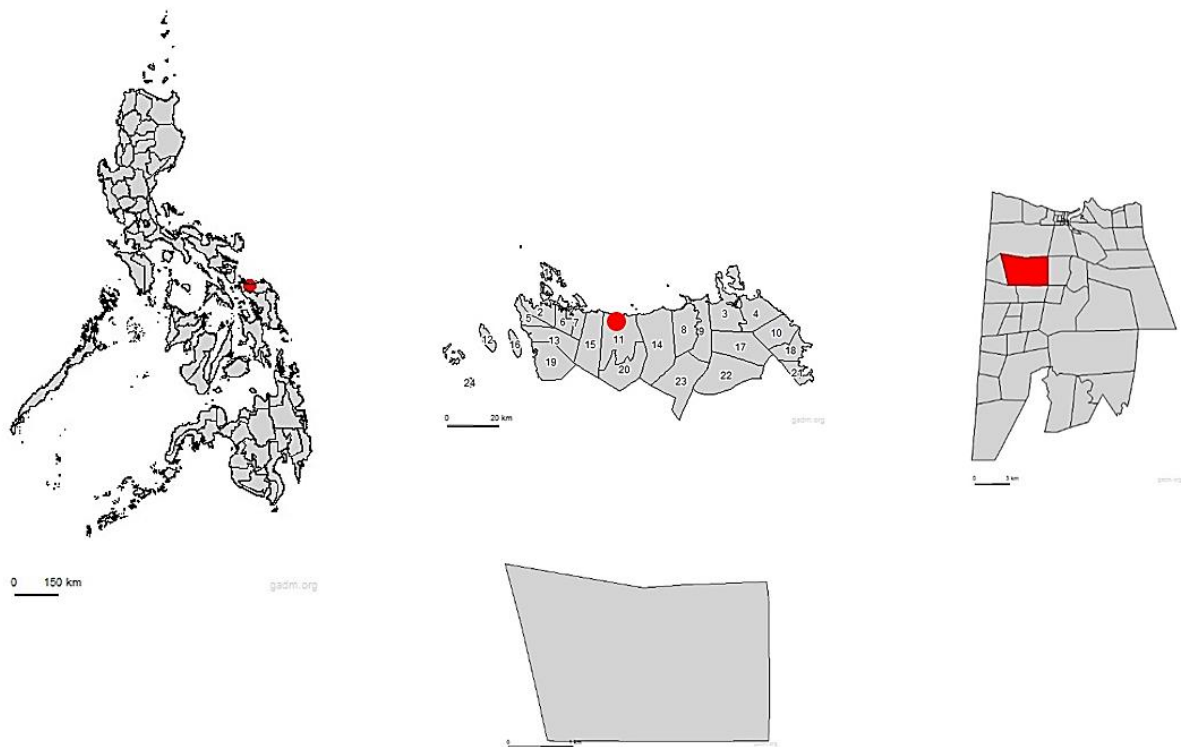


Fig. 1. Map of Brgy. Cal-igang, Catarman, Northern Samar [13]



**Fig. 2. Cultivation of sunflower sample**

## **2.2 Physicochemical Properties of Soil Sample**

To assess the NPK content in the soil, samples collected at the site were airdried and cleaned of debris. Samples were then homogenized using mortar and pestle. Soil Test Kit (STK) from the Philippine Bureau of Soils and Water Management (BSWM) – Department of Agriculture were used to qualitatively determine

the nitrogen (N), phosphorous (P), and potassium (K) present in the soil.

## **2.3 Soil pH**

The soil sample were tested for pH using a Soil pH-moisture tester with an accuracy of  $\pm 0.2$  pH. The sample were cleaned of obstruction such as pebbles grasses, and leaves. The tester was thoroughly cleaned before the test. The meter

was then inserted into the soil embedding its metallic surface completely. After 10 minutes, the pointer indicated the correct value of pH. This was repeated three (3) times.

## 2.4 Qualitative Detection of Heavy Metals in Soil Sample

Test for qualitative detection of heavy metals followed Gilreath's [14] method with modifications. The soil sample was first digested to dissolve minerals and extract the heavy metals from the soil matrix.

## 2.5 Digestion of Soil Sample

Airdried, homogenized, and sieve soil sample was prepared for digestion. One (1) gram of soil was transferred to a beaker, and added with (10) mL of concentrated nitric acid ( $\text{HNO}_3$ ). The mixture was heated for 10 minutes with a lid. The heated sample was then allowed to cool down for 10 minutes. Afterwards, a 5 mL concentrated  $\text{HNO}_3$  was mixed into the beaker and heated again for 30 minutes. If a brown gas is still observed, another 5 mL of  $\text{HNO}_3$  was added until the reaction is complete. Then the mixture is allowed to cool down. After a while, 2 mL of distilled water and 3 mL of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) were added into the mixture, then heated. One (1) mL of  $\text{H}_2\text{O}_2$  was again added until no changes happen. The mixture was continuously heated until its final volume reduced to 5 mL. It was allowed to cool down. Afterwards, 10 mL of conc. hydrochloric acid (HCl) was added then heated for 15 minutes. The resulting mixture was filtered using a filter paper. Finally, the filtrate is diluted to 100 mL and ready for metal element analysis.

## 2.6 Qualitative Detection of Arsenic (As)

To the prepared digested soil solution, a volume of 3F HCl was added. It was centrifuged and the centrate was discarded. The residue was then added with ten (10) drops of concentrated HCl, then stirred. Afterwards, the mixture was heated in a hot water bath for a minute, the centrifuged. The centrifugate was again removed. The residue was washed with a prepared mixture of eight (8) drops of water and four (4) drops of concentrated HCl. It was centrifuged and washing was added to the centrifugate of the concentrated HCl treatment. After, the residue and centrifugate were separated. The residue was washed with hot water three (3) times, and then four (4) drops of concentrated  $\text{HNO}_3$  were

added to the solution. After that, it was heated in a water bath. After heating, 5 drops of 0.5F  $\text{AgNO}_3$  was added and then stirred. Fifteen drops of 2.5F NaAc solution was also added. The formation of silver arsenate ( $\text{Ag}_3\text{AsO}_4$ ) a reddish-brown precipitate indicates the presence of arsenic.

## 2.7 Qualitative Detection of Lead (Pb)

To the digested sample, four (4) drops of 3F HCl were added, mixed thoroughly and centrifuged. Test for complete precipitation were done by adding another drop of the HCl to the supernatant liquid. Then solution was centrifuged again, and the centrifugate and precipitate were separated. The precipitate remaining was washed with ten (10) drops of cold water with one (1) drop of 3F HCl. The wash water was discarded. After that, ten (10) drops of water was added and heated with stirring for three (3) minutes, in water bath. It was again centrifuge quickly and centrifugate was removed while the mixture is still hot in the water bath. To the centrifugate, four (4) drops of 1F  $\text{K}_2\text{CrO}_4$  was added. The confirmation of lead presence is indicated by a formation of lead chromate ( $\text{PbCrO}_4$ ), a yellow precipitate [14,15,5].

## 2.8 Qualitative Detection of Mercury (Hg)

About 3 mL of the prepared digested soil samples was used in the detection of mercury ions in the samples. To the prepared sample, 6M ammonia ( $\text{NH}_3$ ) was added. The appearance of gray to black precipitate is positive to mercury ion.

## 2.9 Extraction of *Helianthus annuus* L. (Sunflower) Leaves

Sunflower leaves were collected and washed using distilled water. Samples were then airdried and grinded by an electrically powered blender. Afterwards, it was pressed by a mechanical presser. The extract was filtered using a Whatman filter paper, and the residue was discarded. The extract was appropriately labeled and used within the same day for physical properties.

## 2.10 Determination of Physical Properties

Physical properties determination was done to further describe the extract obtained from sunflower leaves. The physical properties like pH, solubility, and density were determined.

### 2.11 pH

The pH measurement was conducted using a digital pH meter, with 3 mL of Sunflower extract placed in a beaker. The digital pH meter was immersed in the sample, and the reading was recorded after a two-minute interval. This process was repeated three times.

### 2.12 Density

To determine the density, approximately 3 mL of sunflower extract was weighed, and the weight in grams (g) was recorded. This value was then divided by the volume of the extract used in milliliters (mL). The entire procedure was repeated three times.

### 2.13 Solubility

For testing the solubility of sunflower extract, three solvents—ethanol, hexane, and water—were used. Nine test tubes were prepared each contain 3 mL of the sample. Subsequently, three test tubes were filled with 3 mL of each solvent. The nine test tubes were shaken, and their solubility observed. The results, indicating miscibility or immiscibility based on the formation of multiple phases, was recorded.

### 2.14 Quantitative Detection of Heavy Metals in Plant Sample

Dried, powdered, and homogenized plant samples were sent to F.A.S.T. Laboratories in Mandaue City, Cebu, Philippines for the analysis of heavy metal concentration. For the quantitative detection of Arsenic (As), modified silver diethyldithiocarbamate-colorimetric method was used to identify and quantify its concentration, while Cold Vapor Atomic Absorption Spectroscopy (AAS) was used to

determine the concentration of Mercury (Hg) in the sample. For Lead (Pb) quantitative detection, Flame AAS was utilized.

## 3. RESULTS AND DISCUSSION

As shown in Table 1, soil sample collected from the sample site has low nitrogen, high phosphorus, and sufficient amount of potassium. Soil with low amount of nitrogen has low nitrogen-fixing bacteria that may be inhibited by heavy metal contamination (Zhang, et al. (2023). High phosphorus [16] and sufficient amount of potassium in soil [17] also enhances the bioavailability and heavy metal uptake of plants. This implies that the soil sample has an indication of heavy metal contamination, and is good for metal uptake and accumulation in plant.

In terms of pH, the soil was 6.74 that is considered as slightly acidic. This is within the normal range of pH in growing plant [18]. In relation to heavy metal solubility, Briffa et al. [1] study shows that metals are more bound to the soil if the pH is higher. This implies that a slightly acidic soil makes heavy metals soluble to water, thus easier uptake and making them available to plants.

Table 2 shows that the soil was positive for arsenic and lead in the qualitative detection of heavy metals. This indicates that the soil sample was more exposed to sources of arsenic and lead, than mercury. This also implies that soil from Catarman Sanitary Landfill is dangerous for plants, animals, and humans. The presence of harmful chemicals in the soil can lead to inhibited plant growth, reduced crop yields, and the potential accumulation of toxins in edible plants, which can further affect food safety and human health.

**Table 1. Summary of physicochemical properties of soil sample**

Parameter	Results
Nitrogen	0-2 % (Low)
Phosphorus	16-20 ppm (High)
Potassium	Sufficient
Soil pH	6.7 (Slightly Acidic)

**Table 2. Qualitative detection of Arsenic (As), Lead (Pb), and Mercury (Hg) in soil sample**

Heavy Metals	TRIAL 1	TRIAL 2	TRIAL 3	REMARKS
As	Positive	Positive	Positive	<b>Positive</b>
Pb	Positive	Positive	Negative	<b>Positive</b>
Hg	Negative	Negative	Negative	<b>Negative</b>

**Table 3. Physical properties of sunflower extract**

Parameters	Results		
pH	6.94 (Almost Neutral)		
Density	1.00 g/mL		
Solubility	Distilled Water	Miscible	Polar
	Ethanol	Miscible	
	Hexane	Immiscible	

**Table 4. Concentration of Arsenic (As), Lead (Pb), and Mercury (Hg) in Sunflower Leaves**

Heavy Metals	Concentration (mg/kg)
Arsenic	< 0.2
Lead	< 2.5
Mercury	< 0.05

Table 3 shows the physical properties of sunflower leaves extract. Results shows that the extract is almost neutral, has a density of 1.00 g/mL which is comparable to water, and is miscible to ethanol and water, thus has a polar property.

Table 4 shows that arsenic, lead, and mercury were detected in the powdered sunflower leaves, however the levels are below detection limit. This indicates that these heavy metals are between the limit of detection and 0 concentration. Moreover, low concentration was detected may be because Catarman is not that urbanized yet to be exposed to higher sources of these heavy metals. Moreover, the plant sample is still young, thus heavy metal concentration is still low as older sunflowers have higher metal uptake based on the study of Kötschau et al. (2013) about a time-series phytoremediation experiment. Even in low concentration, consumption of contaminated plant biomass is concerning as contaminants may still enter the food chain through insects, animals and humans [3].

#### 4. CONCLUSION

The findings of this study revealed that the soil sample collected from Catarman Sanitary Landfill has a low presence of Nitrogen (N), high presence of Phosphorus (P), sufficient amount of Potassium (K), and a slightly acidic pH. This implies that the soil sample has an indication of heavy metal contamination. Furthermore, the qualitative analysis resulted to a positive contamination of Arsenic (As) and Lead (Pb). Extract from sunflower grown in the soil sample is almost neutral, polar and with a density of 1.00 g/mL comparable to water. Colorimetric test, Flame Atomic Absorption (AAS), and Cold Vapor

AAS for arsenic, lead, and mercury revealed concentrations that are below detection limit. Sunflower is good in remediating Arsenic (As), Lead (Pb), Mercury (Hg) in the collected sample.

Based on the findings, actions are necessary from the local government and environmental conservation organizations to monitor and strictly implementation of proper waste management especially for hazardous substances. There is also a need to continue testing and monitoring the use of products with the presence these heavy metals. The researcher also wants to promote the use of sunflower in remediating areas susceptible to heavy metal contamination. Lastly, more research must be conducted on post-phytoremediation methods like the proper disposal of plant biomass used for remediating contaminated soil.

#### DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declared that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during writing or editing of manuscripts.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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