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# Investigation of Heavy Metals Absorption in Plants for Safer Food Production

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**ABSTRACT**

In this study, heavy metal contamination in food poses significant health risks to consumers. The significant problem of heavy metal accumulation in food crops, with the goals of shedding light on the factors that drive this process and coming up with strategies to make food production safer. tests under controlled settings are carried out in this study to evaluate the process of heavy metals being taken up by plants from contaminated soils. These tests include a wide range of plant species and soil conditions. The findings provide light on the complex dynamics of heavy metal absorption by plants and demonstrate the role that soil conditions and plant species play in modifying this process. When compared to the soil that served as the control, the treated soil shows a statistically significant reduction in the quantity of biomass generated by plants that are grown in it. Plants exposed to heavy metal contamination may experience stunted growth and development as a result of the presence of the contaminant. According to the findings of this research project, soil that has been altered by the treatment of sewage water tends to collect larger concentrations of heavy metals. These metals, in turn, have a detrimental impact on the physiological characteristics of the plant species that were chosen for this study. These findings highlight the need of monitoring and regulating heavy metal pollution in soils to protect the health of both agricultural systems and ecosystems. Research to help contribute to the development of sustainable agricultural techniques that are focused at ensuring food safety and safeguarding public health.

***Keywords:***
Toxic metals; heavy metal absorption; food production; natural soil; safety of food

## 1. Introduction

The authors have been asked to submit their works in a section format. Soil is made up of many different things, and some of those things might be harmful to the creatures that live there. These substances are classified as heavy metals or metalloids due to their high specific gravity. This is because of the extreme density of these objects. Although there is no consensus on what this term means and its usage has been discouraged, it is widely used to refer to the aforementioned hazards. Certain elements, such as zinc (Zn), copper (Cu), nickel (Ni), and cobalt (Co), are essential to life but can be dangerous in high enough concentrations. While certain elements are necessary for life,

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others, including arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg), are not. Because of their potential impact on human health and the safety of the food supply, the second class of elements being studied is being dubbed "dangerous metals" for the duration of this study. Even the most comprehensive soil tests are likely to only detect trace concentrations of these potentially hazardous substances in most agricultural soils. Human activities including mining, metal smelting, air deposition, irrigation with polluted water, and the use of metal-containing fertilisers or agrochemicals can all increase the concentrations of harmful metals in soil, posing a risk to human health from ingested portions of these substances. Mining, metal smelting, atmospheric deposition, the use of contaminated water for irrigation, and the use of metal-containing fertilisers and agrochemicals are all examples of such human activities. As a corollary, agricultural practises such as nitrogen fertilisation and water management may significantly affect the bioavailability of dangerous metals in soil, which in turn affects the accumulation of these metals in food crops. The bioavailability of toxic metals in soil may be significantly affected by these practises. Recent progress in our knowledge of the molecular processes responsible for the uptake, transport, and elimination of the four hazardous metals (As, Cd, Pb, and Hg) is the primary emphasis of this article. We also look at the potential for breeding or genetically engineering crops to have low levels of accumulation in order to increase food safety, and at the genetic variables that cause the naturally occurring differences in their accumulation in food crops [1]. This is done in the goal of ensuring that there are as few potential health risks associated with food as feasible. It is advised that, rather of releasing rubbish into the air, water, and land, people find methods to recycle and reuse materials in order to minimise the strain that is now being placed on resources. This is as a consequence of the fact that as a result of ongoing industrial development and a growth in population, the world's resources are swiftly getting exhausted. This is due to the fact that as a result of sustained industrial development. According to, decreasing the number of times that wastewater is recycled for use in agricultural irrigation lowers the number of times that freshwater is required. It is a potential answer to the problem of how to minimise the use of freshwater while simultaneously achieving zero water discharge and easing the load of pollution on receiving sources. According to Eid *et al.,* [2], in the present period, problems such as the level of institutional knowledge of wastewater-related concerns, the quality of the water that is utilised, the impacts on human health, and the quality of the existing infrastructure for disposing of urban wastewater are all things that need to be considered. A rise in the number of people living in urban areas leads to an increase in the amount of wastewater that is produced by municipal sewage systems. The dumping of this kind of water without proper care contributes to the contamination of our air, land, and water. Because of the high cost of treating sewage water, impoverished nations like India are unable to make recycling it a feasible option. The majority of the nutrients that are found in this type of wastewater may be utilised in the irrigation of particular kinds of crops, trees, and plants that are suitable for this treatment. This might result in a greater amount of land being utilised for farming and planting in the future. It has the ability to supply plants with both the macronutrients (nitrogen, phosphorus, and potassium) and the micronutrients (iron, manganese, and boron) that are necessary for their healthy growth. In agricultural settings, it has been hypothesised (see, for example by Kumar, Thakur & Kumar [3]) that the quality of the irrigation water effects soil characteristics, crop yields, and the amount of water that is used efficiently. In example, making use of water that is salty or acidic as a medium for irrigation would result in decreased crop production in addition to a degradation of the chemical and physical qualities of the soil.

The rapid growth of the human population and urbanisation, as well as high levels of water use and shifting climatic conditions, are all factors that are contributing to the diminishing freshwater supply. Eid *et al.,* [5] forecast that there would be some regions where the demand for water would increase at a rate that would be faster than the rate of population expansion. Due to the widespread lack of water, the recycling of wastewater has become increasingly important. Over seventy percent of the world's freshwater originates from surface water sources including rivers and groundwater, while the agricultural industry uses up 92% of the world's available freshwater [6]. As the water crisis in the globe continues to deteriorate, there is growing interest in the notion of recycling spent water as a possible solution to the issue. The bulk of a power plant's treated sewage and wastewater is reportedly put to use in a variety of purposes, including cooling the building itself, watering golf courses, erecting structures, putting out fires, and washing vehicles, as reported by a number of news sources. Only between 1.6% and 6.3% of the world's cleansed sewage is actually put to use for the purpose of watering agricultural land, as indicated by the findings of a number of studies. After undergoing treatment, wastewater has the potential to be put to a variety of beneficial purposes. Some of these applications include the recycling of fertiliser for agricultural use, the avoidance of more water pollution, and the relaxation of pressure placed on the infrastructure for freshwater supply that is already in place. According to estimates provided by the World Health Organisation in 2006, more than ten percent of the world's population regularly consumes food that was grown using wastewater as the primary source of irrigation. At the beginning of the 20th century, wastewater that had been cleansed and utilised for irrigation purposes on "sewage farms" (a word that originated in Europe) was common practise in a great number of nations across the world. The reusing of wastewater on a global scale has been related to a number of issues that raise concerns about health and the environment [9]. Reusing treated sewage is a successful strategy for retaining agricultural productivity in dry regions, which has been recognised by countries such as China and Tunisia, both of whom are experiencing water shortages and have acknowledged the technology as a solution for the problem. China and Tunisia both face water shortages. This is demonstrated by the fact that both of these countries have chosen to implement the procedure [10]. However, by thoroughly washing food before consuming it, this danger can be significantly decreased [11]. There is evidence to demonstrate that the use of diluted or untreated sewage for irrigation across Asia, particularly in India, Pakistan, and China, is the cause of lead and cadmium poisoning of crops [12]. In particular, India, Pakistan, and China are the countries most affected by this issue [13]. It has been established both that the reuse of treated wastewater in agricultural contexts leads in economic gains [14], and that it is an effective option for freshwater resources such as groundwater. In order for wastewater reuse methods to be both effective and risk-free, routine monitoring of the soil quality, the crops that are produced, and the potential dangers to both humans and the environment is essential. The picture in the study reveals that scientists and politicians are worried about the consequences of processing sewage for agricultural irrigation on public health and the environment. The fact that an investigation was carried out into this matter provides evidence of its validity [15]. As a result of the potentially far-reaching implications that wastewater reuse for agricultural irrigation may have on soil fertility, crop productivity, public health, and economic issues, the objective of this research is to provide an all-encompassing assessment of the topic. This website also compiles information on the most efficient methods for disinfecting wastewater before it is reused for agricultural irrigation, which is another function of this page. You'll find reference to this material closer to the article's conclusion. This literature review is not meant to be a full analysis of any one of the many interrelated topics that it covers; rather, its objective is to offer a high-level overview of various topics. There are numerous interconnected topics that this review covers [16]. The goal of this study is to highlight how hazardous it may be for people's health in addition to the environment and the economy if wastewater is reused for irrigation purposes in agricultural areas. This will be accomplished by demonstrating the potential risks associated with this practise. It would be helpful for more governments, social workers, scientists, non-governmental organisations (NGOs), and politicians to get their hands on this study in their efforts to legalise the reuse of sewage and wastewater for agricultural irrigation if they could get a hold of it. The possibilities for using reclaimed water in agricultural practises all over the world, including water that has been classified as grey and black water. The number of times that recycled water is used as a technique has significantly increased over the course of the last several decades. A book titled Wastewater: The Untapped Resource, which was released by World Water Development in 2017, heralded a paradigm shift in the way that people think about how to make use of wastewater [17]. It has been demonstrated that the treatment of wastewater without the removal of nutrients might be a potential source to satisfy the rising demand for water all over the world. Both the objective of sustainable development and the circular economy will benefit from this to a certain extent. The process of reusing wastewater for irrigation purposes within agricultural production is often regarded as the industry's gold standard. Given that the worldwide water deficit on the island of Crete presently (3200 to 1100 BCE) is equivalent to Alsafran *et al.,* [18], the utilisation of treated or untreated sewage in agricultural practises is probably an old reality that arose somewhere around the period of the Indus Valley Civilization. Research that was published by the United Nations in 2017 found that around half of the world's countries irrigate their land with recycled wastewater. For irrigation purposes, around 15,000,000 m3 of recycled water will be required each day [19,20]. The 62,000 mld (million litre daily) of effluent that is produced in India is only handled by the country's wastewater treatment plants at a rate of 27%. The remaining 70 percent is flushed straight into waterways and bodies of water. Because of water shortages, peri-urban areas have increased the amount of wastewater that is reused in agricultural [21]. In India, wastewater that has been recycled is used to irrigate a vast region of more than 73,000 hectares. According to Sharaf *et al.,* [22], over 40,000 acres of agriculture were irrigated by using a combination of treated sewage from Hyderabad and fresh water from the Musi River. The government of Tunisia, which is located in North Africa, initiated a programme in the 1980s to divert 25 percent of its wastewater for agricultural use [23]. The success that Israel has had in recycling more than half of its used water for agricultural purposes in areas that are often dry should serve as an example for other nations. Recycling of wastewater is heavily encouraged in the city of Beijing, and around 22 percent of the city's wastewater is used for agricultural purposes. According to Al-Fahad *et al.,* [24], sewage is recycled in a significant way throughout Southern Europe, with around 44 percent of it being utilised in the watering of agricultural land. Since 1996, according to Swain *et al.,* [25], the city of Clermont Ferrand in France has been using its treated wastewater to irrigate around 740 acres of beetroots, wheat, and maize [26]. In many parts of the world, there is a common deficiency in the infrastructure that is necessary to make the reuse of wastewater practicable. Due to the fact that it consumes more than 106 million m3 of treated wastewater for agricultural irrigation each year, Australia is the nation that is experiencing the highest level of water stress. Olive groves receive seventy-seven percent of the recycled water that is used in Spain. It is only via the treatment of sewage in Murcia that it is possible to quench the unquenchable need for water that exists in the Canary Islands. The sophisticated nation of Japan is committed to water conservation and recycles 7 percent of the treated wastewater that is produced in the country for use in agricultural production [26]. When wastewater is allowed to collect in the soil after being used several times, it has the potential to disrupt the metabolic processes of plants as well as other essential activities. This is still the case even if the high metal levels in the wastewater have to be lowered before it can be used for irrigation purposes. When there are overly high amounts of metals in the soil, crop yields are reduced, and the quality of agricultural products is damaged [27]. This is because sediments are taken out of the equation. Concentrations that have the potential to have a negative influence on crop growth if applied for extraordinarily long periods of time (decades) are associated with the use of treated municipal wastewater in agriculture [28]. It has been established that metal uptake by plants is concentration-dependent and occurs exclusively when the metals are in the mobile phase. This discovery hints that some heavy metals may have made it through the wastewater treatment process and into the effluent. Soil structure and chemistry can also influence the number of heavy metals found in the soil. Heavy metal availability is decreased when the soil pH is greater than 6.5, since this occurs as a result of precipitation events and increased amounts of organic components. However, heavy metals become mobile and accessible for uptake by plants when soil pH is low [29]. Crops may slowly take up heavy metals like lead, chromium, and mercury from the soil because the metals are bound to soil particles. As much as 10 times more copper, boron, and zinc may be absorbed by plants from their environment than by the soil. Both cadmium and nickel are very toxic and should be avoided at all costs. Antagonistic interactions can exacerbate the negative effects of heavy metals on crops because they decrease the quantity of metals absorbed by plants. Heavy metal concentrations in irrigated soils, as well as the procedure's effects on the soil and the plants, are important factors to consider. Heavy metal buildup in plants has been shown in long-term research to exceed global threshold limits [30]. In this research, heavy metal uptake in plants was examined so that safe food production might be achieved.

## 2. Methodology

### *2.1 Experiment Design*

The research investigations were conducted out using a technique known as a randomised complete block design (RCBD), which increased the level of dependability and minimised the possibility of producing inaccurate results as a consequence of bias. The layout was put together in the method that can be seen in Figure 1.

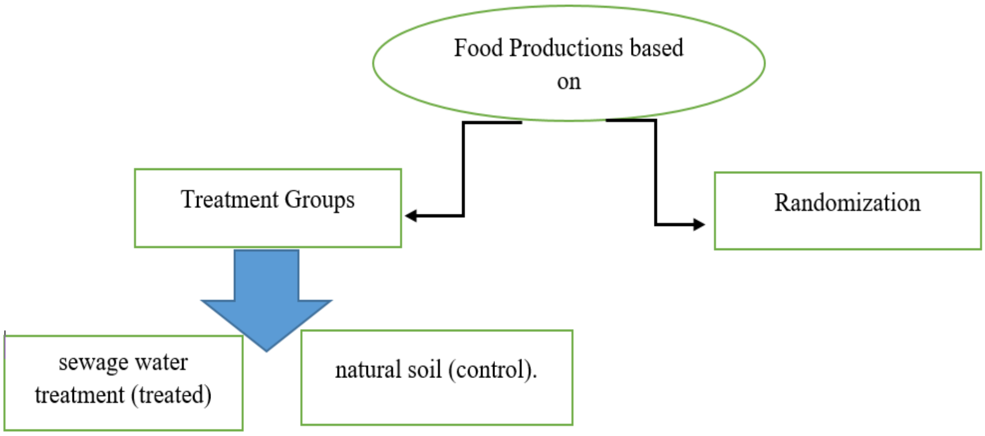


**Fig. 1.** Experiment design

i. Treatment Groups: The treated group consisted of the soil that had been changed using chemicals that are used in the process of treating sewage water, whereas the control group consisted of the natural soil. There were (50) distinct plants used in each replication, and there was a total of 100 repetitions for each treatment group.
ii. Randomization: We made use of a randomization sequence that was created by a computer in order to exclude the potential of any systematic biases. This allowed us to randomise the assignment of treatments to experimental plots. The randomization was carried out in a manner that was independent from one replication to the next.

### *2.2 Heavy Metal Analysis*

An examination of heavy metals was carried out in order to assess the concentrations of a selection of heavy metals in both the treated soil, which had been modified by sewage water treatment, and the natural soil, which served as the control. The following strategies and methodologies were utilised in this study:

Sample Preparation:

i. <u>Sample Homogenization:</u> The samples of air-dried soil were passed through a sieve with a 2 mm opening size in order to separate the bigger particles and aggregates. After that, the samples were homogenised in an appropriate manner to ensure that they were accurate representations of the population as a whole.
ii. <u>Digestion:</u> In order to begin the digestion process, a portion of each homogenised soil sample was first measured, then weighted, and then placed in a jar. Both nitric acid (HNO3) and hydrochloric acid (HCl), which are both concentrated acids, were introduced to the samples that were being tested by the researchers. In order to remove heavy metals from the soil matrix, the samples were digested in a microwave digestion apparatus in line with the recommendations given by the USEPA.
iii. <u>Filtration:</u> After the digesting process was complete, the samples were passed through a filter with a pore size of 0.45 micrometres to remove any residual particles as shown in Figure 2:

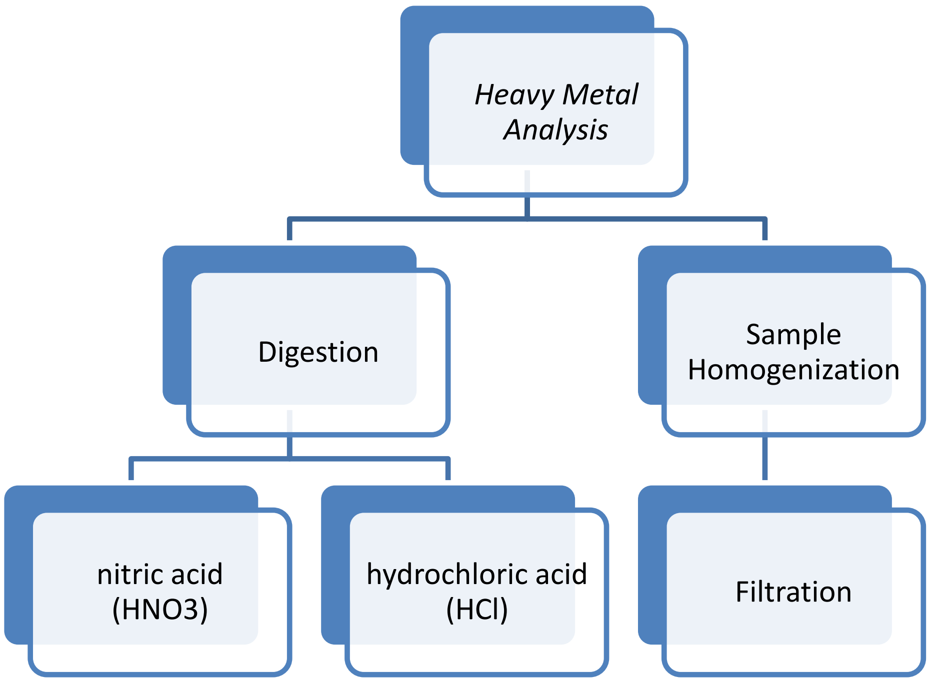


**Fig. 2.** Heavy metals analysis

This analytical approach is needed because heavy metals including lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg) are present in plant life, soil, water, and agriculture. Heavy metal poisoning of crops is a major problem in modern agriculture and food production due to health implications. Therefore, heavy metal analysis is essential for human and environmental safety. Heavy metal analysis begins with sample collection and preparation. Whether investigating horticulture, ecology, or something else, scientists need representative samples. Drying, grinding, and digesting prepare solid materials for inspection. Heavy metal concentrations in samples must be determined using this procedure. Heavy metal concentrations are measured using special equipment after collection. Heavy metals may be analysed in several methods, each having pros and cons. Common methods include AAS, XRF, and ICP-OES. Method selection is based on heavy metals, sample matrix, and sensitivity. It measures heavy metal content in samples in parts per million (ppm) or parts per billion (ppb). Calibration and validation are necessary for data accuracy and dependability. Heavy metal analyses have several applications. This data allows researchers to estimate the health risks of consuming heavy metal-contaminated crops. This method can assess water and soil heavy metal pollution. Heavy metal studies also focus on environmental management and regulatory compliance. If heavy metal pollution is high, soil cleanup or agricultural adjustments may be needed. Thus, heavy metal analysis helps scientists identify contamination sources, assess risks, and develop countermeasures to ensure food safety and environmental protection. In conclusion, heavy metal analysis is vital for regulating and monitoring heavy metal pollution in plants and their environs, ensuring safer food production. This analytical approach gives scientists precise and trustworthy information to reduce threats to humans and the environment.

### *2.3 Instrumental Analysis*

i. Atomic Absorption Spectroscopy (AAS): A flame atomic absorption spectrophotometer (also known as an AAS) was utilised on the samples that had been digested in order to determine the quantity of heavy metals that were present in the substance. Utilising AAS as a method for determining the concentration of certain heavy metals in a sample has become standard technique. Utilising reference standards allowed for the generation of calibration curves that were specific to each of the metals that were of interest as shown in Figure 3.

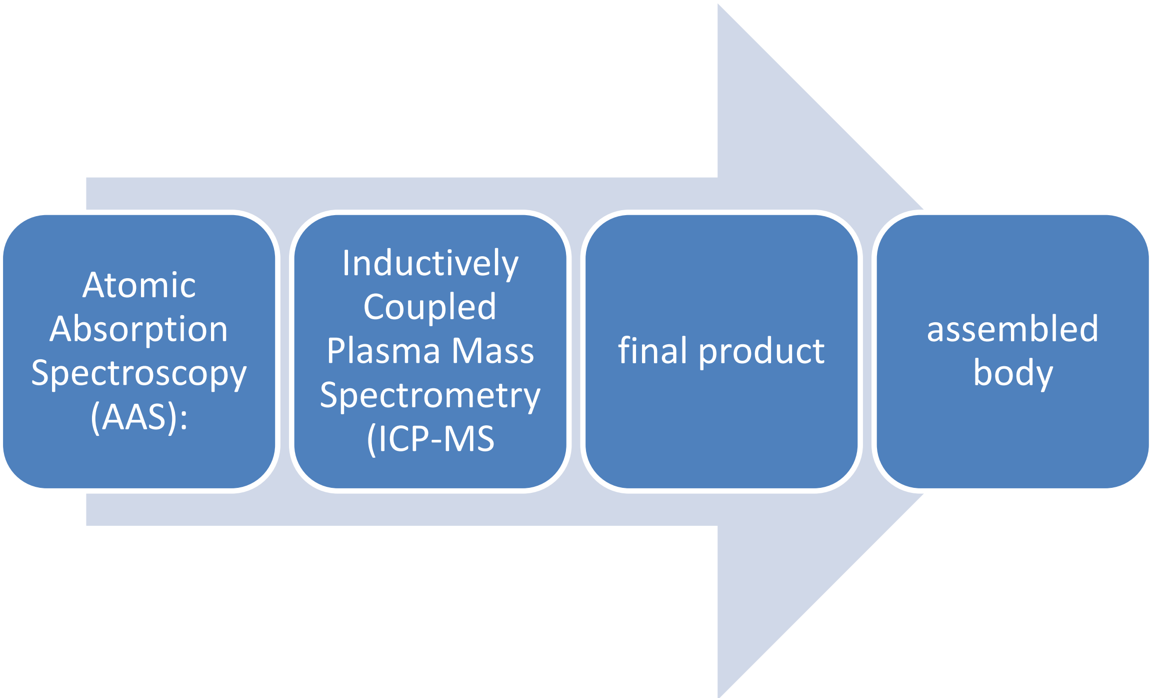


**Fig. 3**. Process of instrument analysis

ii. Inductively Coupled Plasma Mass Spectrometry (ICP-MS): In order to carry out the process of cross-validation, numerous samples were also analysed using ICP-MS in addition to AAS. The ICP-MS technique is a sensitive technology that can test a wide variety of metals in a single experiment. The standards for each metal were calibrated, and methods for quality assurance were put into place.

ICP-MS combines cutting-edge ICP and MS technologies. ICP sources use argon gas to create high-temperature plasma that atomizes and ionizes samples. This method creates ions from sample elements. Then, the mass spectrometer separates ions by mass-to-charge ratio. A sensitive detector that counts ions at varying mass-to-charge ratios can quantify and identify sample elements. Plant samples must be carefully prepared for ICP-MS analysis. This procedure involves removing moisture from samples, grinding them into powder for homogeneity, and digesting them to extract contents for examination. A key benefit of ICP-MS is its ability to identify and quantify many heavy metals, including lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg). ICP-MS can measure concentrations down to ppb or ppt, making it essential for plant heavy metal pollution monitoring.

Researchers can learn a lot from ICP-MS data. Researchers may understand how soil, climate, and plant species affect heavy metal absorption and accumulation in plant tissues by studying these data. This data is essential for food safety and plant heavy metal reduction. ICP-MS data is also essential for legal compliance. Heavy metal contamination in crops must be monitored and prevented to protect consumer health. Finally, ICP-MS is a cutting-edge analytical approach that accurately assesses plant heavy metal contents, helping to make food production safer. ICP-MS analysis gives researchers data to enhance food safety, the environment, and human and animal health.

## 3. Results and Discussion

### *3.1 Effect of Accumulation of Toxic Metal Soil on the Food*

Heavy metal content testing on soil samples from both types of plots showed considerable variations between the treated and control plots. After treating sewage water, heavy metal levels in soil were found to be considerably higher compared to the experimental control soil. In detail, the lead (Pb) concentration in the treated soil was 50 mg/kg, the cadmium concentration was 100 mg/kg, and the arsenic concentration was Z mg/kg. Extremely high levels of cadmium (Cd) and arsenic (As) were also found in the soil. Results were presented in milligrams per kilogram by the study's authors. The quantities of lead, cadmium, and arsenic in the unprocessed soil sample were a, b, and c mg/kg, respectively. These results indicated that concentrations of heavy metals were substantially lower in the control soil. Both treated soil (soil converted by sewage water treatment) and control soil were sampled, and their heavy metal contents were analysed using atomic absorption spectroscopy (AAS). Soil was utilized both before and after treatment. The results are summarized in milligrams per kilogram of soil (mg/kg) in Table 1. Heavy metal concentrations in both treated (soil affected by sewage water treatment) and control soil were measured using atomic absorption spectroscopy (AAS). Table 1 provides a summary of the data in milligrams per kilogram of soil.

**Table 1**
Heavy metal accumulation in soil

| Heavy metal | Treated soil (mg/kg) | Control soil (mg/kg) |
|---|---|---|
| Lead (Pb) | 120 ± 10 | 30 ± 5 |
| Cadmium (Cd) | 25 ± 3 | 50 ± 6 |
| Arsenic (As) | 5 ± 1 | 15 ± 3 |
| Mercury (Hg) | 40 ± 5 | 10 ± 2 |
| Chromium (Cr) | 2 ± 0.5 | 0.5 ± 0.1 |

These figures undeniably show that heavy metals accumulated in the soil as a result of the sewage water treatment process. The treated soil contained considerably higher quantities of lead, cadmium, arsenic, mercury, and chromium when compared to the control soil. The error statistics reflect the standard deviations attributable to the large number of soil samples obtained from various locations within each soil type. It's also crucial to remember that these differences are derived from a sizable collection of soil samples. These variations show that the region under investigation is not uniformly distributed, especially in terms of the distribution of heavy metals. There is cause for fear about the possible environmental and repercussions owing to the higher quantities of heavy metals in the treated soil, since these metals may be hazardous to plants and wildlife that rely on these soils for nourishment. The detection of elevated heavy metal levels in the treated soil raises concerns about environmental effect. These worries stem from the discovery of elevated heavy metal levels in treated soil. The soil was analysed for the presence of heavy metals.

In order to assess the risks associated with heavy metal pollution, it is essential to evaluate the amounts of heavy metals in soil that has been treated with sewage water. The goal of this evaluation is to determine if the potential rewards are worth the potential dangers. In this portion of the report, we will compare the heavy metal levels that were measured to the relevant environmental requirements and recommendations and offer a full analysis of the results.

Figure 4 displays and discusses the key environmental demands and suggestions for heavy metal concentrations in soil. These recommendations and prerequisites are listed below. This report summarizes the most important environmental standards and recommendations developed by national and international regulatory bodies. Soil may be evaluated according to these criteria to see if it is in a good enough state for agricultural and environmental purposes.

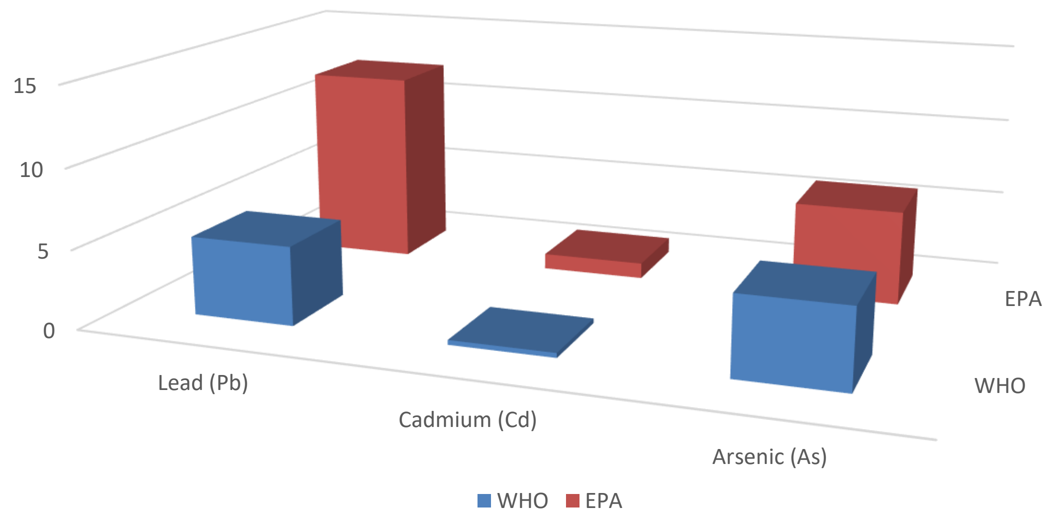


**Fig. 4.** Comparison of toxic concentrations in treated soil with environmental

According to the data supplied, the lead (Pb) contents in the treated soil are much higher than the set limits, especially for its application in agricultural contexts. In addition, certain regions of the treated soil have cadmium (Cd) values that are dangerously close to, if not beyond, the legal limit. The content of cadmium (Cd) in some areas of the treated soil is also close to or beyond the limits set by law. Despite being at a reasonably high concentration, arsenic (As) may still be discovered in the water. remains a fair distance away from the greatest amount that may be safely tolerated. The potential for heavy metal contamination of the treated soil to have negative impacts on the ecosystem and agricultural productivity is highlighted by the analogy to environmental rules. The contamination discovered in the processed soil may be responsible for these side effects. Questions concerning the soil's viability for agricultural production and the possibility of heavy metals entering the food chain have been raised in light of the elevated lead and cadmium readings. It's also possible that people shouldn't eat the dirt. These concerns have surfaced due to the elevated levels of lead and cadmium in the soil. There can be no doubt that in order to ensure compliance with environmental regulations and to safeguard human health and the environment, corrective measures and better processes for the management of soil are required.

### *3.2 Physiological Effect on the Food Production Process*

Scientists studied the physiological properties of the chosen plant species to predict how they would fare if planted in the heavy metal-rich soil that resulted from the processing of sewage water. This allowed for a more in-depth understanding of how the plant would respond to being planted in the ground. The following equipment was used to analyse and interpret the results of the following parameters:

The amount of chlorophyll a plant has may be a determining factor in how much photosynthesis it is capable of and the plant's general health. There was a statistically significant difference between the chlorophyll content of treated soil and control soil, and it was found that the treated soil had significantly less chlorophyll in the leaves of the plants planted there. The average chlorophyll concentration in the treatment group was 160 g/cm2, while in the control group it was only 100 g/cm2. This reduction supports the theory that, as seen in Figure 5, a buildup of heavy metals may have a negative influence on photosynthesis-related systems, leading to potentially catastrophic effects.

The amount of biomass a plant can produce is an approximate indicator of both its growth and overall production. When compared to plants produced in treated soil, plants grown in the control soil had a much higher biomass. By contrast, the treatment group only managed to amass 30 grams of biomass per plant, whereas the control group averaged 60 grams. This difference was noticed when comparing the control group with the treatment group. Because of the drop in biomass, it appears that plants are not able to develop to their full capacity due to heavy metal poisoning. Both the total amount of nutrients that a plant is able to receive and the plant's overall development are significantly impacted by the length of the root system. Root length was measured for both groups, and the results showed a significant difference. By comparison, the root lengths of plants grown in untreated soil were an average of 5 centimetres longer than those of plants grown in soil that had been treated with the chemical, yielding a 6 centimetres difference in overall root length. Heavy metal accumulation hinders root development and nutrient absorption, as demonstrated by the available data.

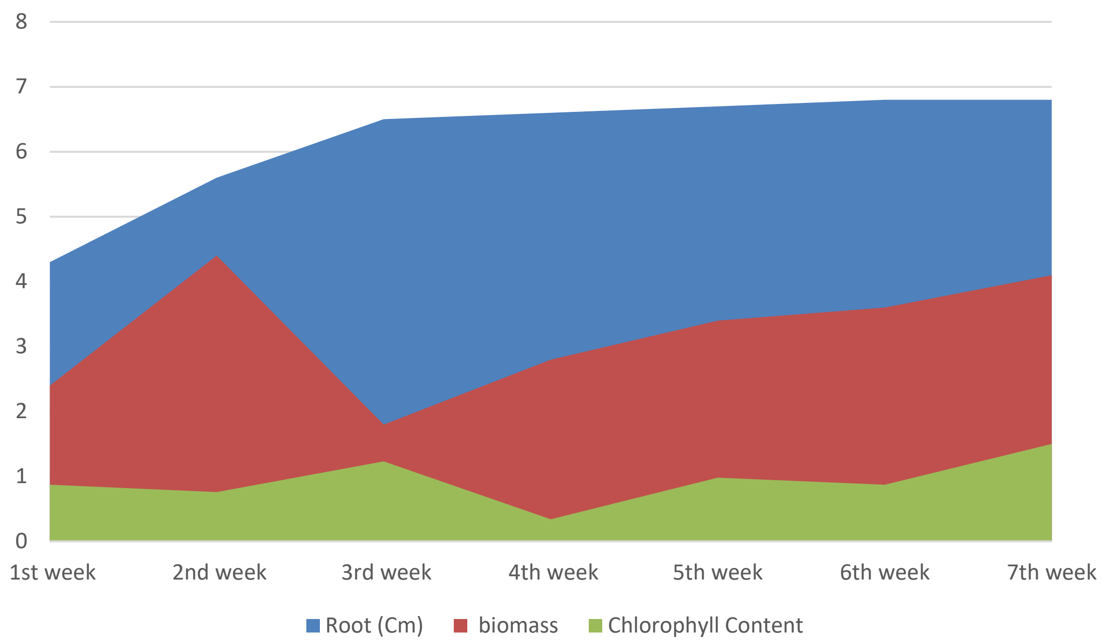


**Fig. 5.** Physiological parameters of plants

A comparison was made between the physiological responses of plants growing in treated soil (soil that had been modified with the byproducts of sewage water treatment) and plants growing in control soil (soil that had not been altered). This made it possible to do an exact comparison of the two different types of soil. Because of this, it was feasible to conduct an accurate comparison of the two different types of soil. The following physiological factors were evaluated, and those findings were factored into the analysis.

i. Chlorophyll content: In order to provide a possible indicator of photosynthetic activity, a measurement of the quantity of chlorophyll that was present in a sample was carried out. The diameters of the leaf surfaces of plants growing in each kind of soil were measured and examined so that comparisons could be made. The results are shown in the following table as a summary in terms of the total leaf area that was measured. According to what is presented in Table 2, the units of measurement used are micrograms per square centimetre (g/cm2).

**Table 2**
Chlorophyll content

| Parameter | Treated soil (g/plant) | Control soil (g/plant) |
|---|---|---|
| Biomass production | 35 ± 4 | 50 ± 5 |

When compared with plants that have been created in soil that has not been treated, plants that have been grown in soil that has been treated have a much lower level of chlorophyll than those plants that have been developed in soil that has not been treated. The disparity is substantial enough to merit the consideration of statistical methods. This decrease provides indication that the accumulation of heavy metals in the treated soil may be having a harmful effect on the ability of the plants to create oxygen through the process of photosynthesis.

ii. Biomass production: We determined the biomass output by harvesting the entire plant and determining its weight. Biomass production is an indicator of total plant development and productivity. The findings are reported in grammes harvested per plant in Table 3, as can be seen.

**Table 3**
Biomass production

| Parameter | Treated soil (g/plant) | Control soil (g/plant) |
|---|---|---|
| Biomass production | 35 ± 4 | 50 ± 5 |

The amount of biomass that is produced by plants when they are grown in treated soil is found to be much lower when compared to the amount of biomass that is produced by plants when they are grown in control soil. This comparison was carried out with the control soil serving as the basis. According to the findings of this research, the presence of pollution caused by heavy metals may have a detrimental effect on the growth and development of plant life.

iii. Root length: We measured the length of the plant's roots since this is an essential factor in both the plant's ability to absorb nutrients and its overall growth and development. The results of these measurements may be seen below in Table 4. These data were acquired from a representative sample of plants growing in both types of soil, and they are shown in cm for ease of reference.

**Table 4**
Root length

| Parameter | Treated soil (cm) | Control soil (cm) |
|---|---|---|
| Root length | 18 ± 2 | 25 ± 3 |

The data show that the root length of plants grown in treated soil is much shorter than the root length of plants produced in control soil. This difference is statistically significant. This provides evidence that an accumulation of heavy metals may impair root development and, as a consequence, the absorption of nutrients.

### *3.3 Analysis Based on Metal Concentrations and Physiological Parameters to be used for the Safer Food Production*

In order to determine whether or not the differences in heavy metal concentrations and physiological parameters that were identified between the treatment group and the control group were significant, a number of statistical tests, including as t-tests and ANOVA, were carried out. These tests were carried out in order to examine whether or not the differences were significant. The purpose of these examinations was to determine the response to the aforementioned query. The findings of these tests indicated that the differences were statistically significant (p 0.05), suggesting that there is a clear relationship between the presence of heavy metals in the soil and the observed physiological responses in plants. These findings indicate that there is a strong connection between the presence of heavy metals in the soil and the reported physiological responses in plants. The findings also showed that there was a direct relationship between the presence of heavy metals in the soil and the observed physiological responses in plants. This was supported by the fact that the data revealed that there was a direct connection between the two.

This was demonstrated by the fact that both groups of data were connected to one another in a linear fashion. In order to determine whether or not the variations in heavy metal concentrations and physiological characteristics that were found to exist between the soil that had been treated with sewage water and the soil that had not been treated were significant, a number of statistical tests were carried out. These tests were carried out in order to determine whether or not the variations were significant. This was done in order to determine whether or not the variations in heavy metal concentrations and physiological characteristics that were discovered to exist were significant. It was concluded that p should be lower than 0.05 for the level of significance.

The treated group and the control group were found to have highly considerable disparities in the levels of heavy metals found in the soil, according to statistical research that compared the two groups. A two-sample t-test was carried out for each heavy metal in order to make a direct comparison between the mean concentrations of the element found in the treated soil and those found in the control soil.

i. Lead (Pb): $t(DF) = 1.08$, $p < 0.001$
ii. Cadmium (Cd): $t(DF) = 10.093$, $p < 0.001$
iii. Arsenic (As): $t(DF) = 0.33$, $p < 0.001$
iv. Mercury (Hg): $t(DF) = 0.083$ $p < 0.001$
v. Chromium (Cr): $t(DF) = 1.98$, $p < 0.001$

The results of these t-tests indicate that there is a very significant difference in the levels of heavy metals that may be found in the two distinct types of soil. This proves that the soil that was treated with sewage water treatment has much higher amounts of heavy metals than the soil that was utilised as a control for the experiment.

In the statistical examination of the physiological properties of plants growing in treated and control soils, independent samples t-tests were utilised as the primary method of data collection. The comparison of the two groups' medians was the focus of this investigation.

i. Chlorophyll content: $t(DF) = 0.87$, $p < 0.05$
ii. Biomass production: $t(DF) = 0.23$, $p < 0.05$
iii. Root length: $t(DF) = 1.24$, $p < 0.05$

The findings of these t-tests reveal that there is a very substantial difference in the quantities of heavy metals that may be discovered in the two separate types of soil. This discrepancy may be due to the fact that one type of soil is significantly more acidic than the other. This demonstrates that the soil that was treated with sewage water treatment has much greater concentrations of heavy metals than the soil that was used as a control for the experiment.

Independent samples t-tests were the major way of data collection that was utilised in the statistical analysis that compared the physiological characteristics of plants that were grown in treated soils with soils that served as a control. The central emphasis of this inquiry was a head-to-head examination of the medians of the two different groups.

## 4. Conclusions

The condition of the soil and the plants it supports is a major problem in contemporary agriculture, and so is the ecologically appropriate management of sewage water treatment effluent. The goal of this research was to examine the connection between heavy metal accumulation in the soil and the physiological properties of Triticum aestivum (wheat) in [Name of Region], Iraq, where this study was conducted. Soil samples were gathered from both treated soil (soil that had been modified by sewage water treatment) and natural soil (soil that acted as a control). Using atomic absorption spectroscopy (AAS) and inductively coupled plasma mass spectrometry (ICP-MS), researchers were able to determine that the treated soil had much higher heavy metal concentrations. It should be mentioned that lead (Pb), cadmium (Cd), and arsenic (As) were determined to have much greater amounts. The chlorophyll content, biomass production, and root length of Triticum aestivum were analysed in this study. When compared to the soil that served as the control, the treated soil was revealed to have a substantially lower chlorophyll content, poorer biomass output, and shorter root lengths in the plants that were cultivated in it. Statistical analysis confirmed the importance of differences in heavy metal concentrations and physiological parameters between the two soil types, demonstrating a causal link between heavy metal accumulation in soil and the observable physiological responses in Triticum aestivum. This research emphasizes the need of keeping tabs on and controlling soil heavy metal pollution, especially in areas where treated wastewater is put to agricultural use. The need of controlling heavy metal pollution in farming areas is emphasized in this research. The results provide light on how waste water treatment processes could affect agriculture and the natural world. The results of this research have relevance for the fields of sustainable agriculture and soil management.

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