The effects of heavy metal exposure in agriculture soil on chlorophyll content of agriculture crops: A meta-analysis approach

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Abstract. Soil can be contaminated by the accumulation of heavy metals and metalloids through emissions from rapidly developing industrial areas. Due to the possibility of chemical change (speciation) and their bioavailability, the presence of toxic metals in the soil can greatly inhibit the biodegradation of organic contaminants. The researchers aimed to analyse the effect of heavy metal levels in agricultural soil on chlorophyll levels in agricultural crops through a meta-analysis method which is expected to provide results in the form of a summary of data that already exists in journals that have been published so far. Here we present a meta-analysis of 6 studies (56 data collected) published between 1997 and 2020 that reported the effects of heavy metal content on plant chlorophyll content. Based on the meta-analysis of the effect of heavy metal exposure in agricultural soil on the chlorophyll content of agricultural crops, the value "effect size overall" was -0.285 in the range of -0.380 to -0.190, where most of the values "effect size" were on the left. Plants growing on soil contaminated with heavy metals result in decreased growth due to changes in physiological and biochemical activities, especially when heavy metals inhibit plant growth and development. Furthermore, environmental risk assessment due to mercury exposure is very important to control the transport and accumulation of mercury in the biosphere to reduce the impact of mercury on the environment.

1. Introduction
The growing industrialization in the XXI Century has contributed to heavy metal contamination as one of the industrial by-products that can pollute the soil, water and air. Metals can enter the environment through various pathways, including anthropogenic sources playing an important role in increasing metal concentrations. Wastewater from industry is often discharged directly into rivers or other nearby water sources without treatment procedures. These are some of the activities that contribute to heavy metal contamination in the environment. Heavy metals are a pollutant material that is very worried about its presence in the environment due to the bioaccumulation and biomagnification process [1].

Soil can be contaminated by the accumulation of heavy metals and metalloids through emissions from rapidly developing industrial areas, tailings mine, disposal of high metal waste, leaded gasoline and paint, soil application of fertilizers, animal dung, sewage sludge, pesticides, irrigation wastewater, coal combustion residues, petrochemical spills, and atmospheric deposition. Heavy metals are an
obscure inorganic chemical hazard group, and the most commonly found in contaminated sites are lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg), and nickel (Ni). Soil is a major absorber of heavy metals released into the environment by anthropogenic activities and unlike organic contaminants which are oxidized to carbon (IV) oxide by microbial action, most metals do not undergo microbial or chemical degradation, and the total concentration in the soil persists for a long time after their introduction [2][3][4].

Due to the possibility of chemical change (speciation) and their bioavailability, the presence of toxic metals in the soil can greatly inhibit the biodegradation of organic contaminants. Heavy metal contamination in the soil can pose risks and dangers to humans and ecosystems through direct consumption or contact with contaminated soil, food chain (soil-plant-human or soil-plant-animal-human), drinking contaminated groundwater, degradation of food quality (safety and marketability) through phytotoxicity, reduction of land use for agricultural production that causes food insecurity. Excessive accumulation of heavy metals in plant organs can inhibit growth and productivity, and in some cases can even lead to death. Based on several studies, it is stated that there are physiological changes in agricultural crops that are influenced by exposure to heavy metals such as Hg, Cd, Pb, Cu, and Ni [5] [6] [7].

Based on this, the researchers aimed to analyze the effect of heavy metal levels in agricultural soil on chlorophyll levels in agricultural crops through a meta-analysis method which is expected to provide results in the form of a summary of data that already exists in journals that have been published so far. The meta-analysis consists of several independent experiments towards common goals for quantitative research, combined analysis, statistical methods, and summary evaluation. In this study, the researchers conducted a meta-analysis to summarize the adsorption capacity of heavy metals in agricultural crops, with the aim of: (1) analyzing and comparing the levels of chlorophyll content in the leaves of different agricultural crops to provide a reference for future research, and (2) provides reasonable suggestions for agricultural sector by exploiting the varying heavy metal adsorption trends of different types of crops.

2. Materials and methods

2.1 Documentation indexing

Researchers collected data from Google Scholar, Springer, and Elsevier. First, the researcher identified several keywords, such as “heavy metals” “plants”, “agricultural products”, “chlorophyll”, and “meta-analysis”. The researcher only cites complete articles in which all data are available for analysis. Researchers do not include unpublished data or articles that are only a summary of the previous literature. Timeframe for the study on "The effect of heavy metal content on plant chlorophyll content, namely from 1997 - 2020.

2.2 Data search and selection

a. Inclusion Criteria

Researchers used 6 papers selected that provided information on heavy metal content in soil and chlorophyll content in soil, as well as types of studied plants.

b. Exclusion Criteria

Scientific papers that were republished are not included in the criteria of the author. And scientific papers that do not include data in the literature in full or that are not analysed will be dropped out of the sample.

The following information was selected from each article: (1) the name of the first author, the year in which the experiment took place, the location of the experiment; (2) a measure of the concentration of heavy metals in the soil; (3) chlorophyll levels in plants to be compared from the control group with plants exposed to heavy metals. Overall, the researchers reviewed 56 data collected. Overall, researchers collected 9640 references. We reviewed and evaluated each study, eliminating duplicate reports and
studies with poor study quality, low information availability, and incomplete data. Furthermore, the researchers included only 6 papers that could provide information data related to the effect of heavy metals on the chlorophyll content of agricultural crops, which would then be analysed using the OpenMee application.

![Identification Flowchart](image1)

![Screening Flowchart](image2)

![Eligibility Flowchart](image3)

![Includes Flowchart](image4)

**Figure 1.** Flow of sampling technique for meta-analysis study "the effect of heavy metal levels in agricultural soil on chlorophyll levels in agricultural crops".

### 3. Results and discussion

#### 3.1. Statistical analysis

**Table 1.** Description of the characteristics of the included studies.

| Literature | Author, Year | Study | HMs | Chlorophyll Content |
|------------|--------------|-------|-----|---------------------|
|            |              |       |     | Ne | Xe | SDc | Ne | Xe | SDc |
| 1          | Preeti et al., 2011[8] | Cd 1  | 3   | 1.995 | 0.175 | 16 | 1.993 | 0.175 |
| 2          |              | Cd 5  | 3   | 1.995 | 0.175 | 16 | 1.53  | 0.075 |
| 3          |              | Cd 10 | 3   | 1.995 | 0.175 | 16 | 1.443 | 0.015 |
| 4          |              | As 1  | 3   | 2.053 | 0.095 | 16 | 0.93  | 0.05  |
| 5          |              | As 5  | 3   | 2.053 | 0.095 | 16 | 0.913 | 0.035 |
| 6          |              | As 10 | 3   | 2.052 | 0.095 | 16 | 0.563 | 0.205 |
| 7          |              | Pb 1  | 3   | 1.673 | 0.285 | 16 | 1.203 | 0.015 |
| 8          |              | Pb 5  | 3   | 1.673 | 0.285 | 16 | 1.06  | 0.02  |
| 9          |              | Pb 10 | 3   | 1.673 | 0.285 | 16 | 0.513 | 0.095 |
| Literature | Author, Year  | Study | HMs | Chlorophyll Content |
|------------|--------------|-------|-----|---------------------|
|            |              |       |     | Ne     | Xe     | SDc  | Ne     | Xe     | SDc  |
| 2          | Fatoba et al., 2008 [5] | | | | | | | |
| 10         | Cu 5         | 12    | 8.130 | 1.77  | 12    | 7.868 | 2.297 |
| 11         | Cd 5         | 12    | 8.130 | 1.77  | 12    | 4.352 | 1.715 |
| 12         | Fe 5         | 12    | 8.130 | 1.77  | 12    | 5.322 | 1.786 |
| 13         | Pb 5         | 12    | 8.130 | 1.77  | 12    | 6.632 | 1.854 |
| 14         | Va 5         | 12    | 8.130 | 1.77  | 12    | 4.372 | 1.785 |
| 15         | Cu 10        | 12    | 8.130 | 1.77  | 12    | 7.334 | 1.957 |
| 16         | Cd 10        | 12    | 8.130 | 1.77  | 12    | 2.759 | 1.186 |
| 17         | Fe 10        | 12    | 8.130 | 1.77  | 12    | 4.971 | 1.715 |
| 18         | Pb 10        | 12    | 8.130 | 1.77  | 12    | 6.849 | 1.589 |
| 19         | Va 10        | 12    | 8.130 | 1.77  | 12    | 3.091 | 1.605 |
| 3          | Gurpreet et al., 2012 [9] | | | | | | | |
| 20         | Pb 10        | 6     | 0.485 | 0.004 | 6     | 0.418 | 0.003 |
| 21         | Pb 50        | 6     | 0.485 | 0.004 | 6     | 0.38  | 0.003 |
| 22         | Pb 100       | 6     | 0.485 | 0.004 | 6     | 0.351 | 0.001 |
| 23         | Ni 10        | 6     | 0.485 | 0.004 | 6     | 0.342 | 0.002 |
| 24         | Ni 50        | 6     | 0.485 | 0.004 | 6     | 0.149 | 0.004 |
| 25         | Ni 100       | 6     | 0.485 | 0.004 | 6     | 0.125 | 0.002 |
| 4          | Romika et al., 2015 [10] | | | | | | | |
| 26         | Mix1-5       | 4     | 22.61 | 1.31  | 6     | 18.71 | 2.14  |
| 27         | Mix1-5       | 4     | 22.61 | 1.31  | 6     | 19.1  | 2.27  |
| 28         | Mix1-5       | 4     | 22.61 | 1.31  | 6     | 18.96 | 1.74  |
| 29         | Mix2-5       | 4     | 24.48 | 1.69  | 6     | 25.05 | 1.51  |
| 30         | Mix2-5       | 4     | 24.48 | 1.69  | 6     | 20.46 | 1.61  |
| 31         | Mix2-5       | 4     | 24.48 | 1.69  | 6     | 23.51 | 1.31  |
| 32         | Mix3-5       | 4     | 25.87 | 1.53  | 6     | 22.56 | 1.58  |
| 33         | Mix3-5       | 4     | 25.87 | 1.53  | 6     | 21.68 | 1.15  |
| 34         | Mix3-5       | 4     | 25.87 | 1.53  | 6     | 22.34 | 1.66  |
| 35         | Mix4-5       | 4     | 22.55 | 1.75  | 6     | 21.29 | 1.92  |
| 36         | Mix4-5       | 4     | 22.55 | 1.75  | 6     | 19.8  | 1.76  |
| 37         | Mix4-5       | 4     | 22.55 | 1.75  | 6     | 19.06 | 1.33  |
| 5          | Manios et al., 2002 [11] | | | | | | | |
| 38         | Cu A         | 5     | 9.17  | 3.44  | 5     | 10.83 | 4.48  |
| 39         | Cu B         | 5     | 9.17  | 3.44  | 5     | 10.83 | 5.34  |
| 40         | Cu C         | 5     | 9.17  | 3.44  | 5     | 14.17 | 4.48  |
| 41         | Cu D         | 5     | 9.17  | 3.44  | 5     | 15    | 7.64  |
| 42         | Ni A         | 5     | 17.5  | 6.92  | 5     | 21.67 | 8.98  |
| Literature | Author, Year | Study | HMs | Chlorophyll Content |
|------------|--------------|-------|-----|---------------------|
|            |              |       |     | Nc  | Xc  | SDc  | Ne  | Xe  | SDe  |
| 44         | Ni C         | 5     | 17.5| 6.92 | 5   | 27.67 | 4.53 |
| 45         | Ni D         | 5     | 17.5| 6.92 | 5   | 27.5  | 3.82 |
| 46         | Zn A         | 5     | 3.418|15.38 | 5   | 48.33 | 12.14|
| 47         | Zn B         | 5     | 3.418|15.38 | 5   | 58.33 | 10.68|
| 48         | Zn C         | 5     | 3.418|15.38 | 5   | 55.83 | 8.38 |
| 49         | Zn D         | 5     | 3.418|15.38 | 5   | 60.83 | 13.04|
| 44         | Ni C         | 5     | 17.5| 6.92 | 5   | 27.67 | 4.53 |
| 45         | Ni D         | 5     | 17.5| 6.92 | 5   | 27.5  | 3.82 |
| 46         | Zn A         | 5     | 3.418|15.38 | 5   | 48.33 | 12.14|
| 47         | Zn B         | 5     | 3.418|15.38 | 5   | 58.33 | 10.68|
| 48         | Zn C         | 5     | 3.418|15.38 | 5   | 55.83 | 8.38 |
| 49         | Zn D         | 5     | 3.418|15.38 | 5   | 60.83 | 13.04|
| 6          | Ewais, 1997  | 50    | Cd 5| 3.57 | 0.09 | 10   | 3.3  | 0.04 |
|            |              | 51    | Cd 10|3.57 | 0.09 | 10   | 2.61 | 0.09 |
|            |              | 52    | Cd 20|3.57 | 0.09 | 10   | 2.07 | 0.08 |
|            |              | 53    | Ni 50|3.57 | 0.09 | 10   | 3.42 | 0.06 |
|            |              | 54    | Ni 100|3.57 | 0.09 | 10   | 3.07 | 0.09 |
|            |              | 55    | Pb 50|3.57 | 0.09 | 10   | 3.41 | 0.07 |
|            |              | 56    | Pb 100|3.57 | 0.09 | 10   | 2.95 | 0.11 |

Note:  
Author: Researcher  
SDc: Standard deviation in control  
Year: Year of publication  
Ne: Number of samples/replicates in experiment  
HMs: Heavy metals's treatment  
Xe: Average chlorophyll content in experiment  
Nc: Number of samples/replicates in control  
SDe: Standard deviation in experiment  
Xc: The average chlorophyll content in the control

### 3.2. Discussion

Based on the analysis of the OpenMee application, the results of the different plant chlorophyll levels were obtained. The researcher extracted and counted 56 (fifty-six) data points that met the screening and elimination criteria for various types.

Figure 2 shows that most of the data distribution is in the left range/minus area. This indicates that the control value is greater than the experiment, so the hypothesis is accepted that the "Chlorophyll Content" in the control is greater than the experiment. This is due to a decrease in chlorophyll in experimental plants exposed to heavy metals. With an “overall” value is -0285 (entry range), which means a real difference because the “overall” value is not cut at the zero points. This shows that there is a physiological change in chlorophyll in plants.

Heavy metals that affect the decrease in chlorophyll levels are Hg, Pb, Cd, and Cu. Excessive accumulation of heavy metals in plant organs can inhibit growth and productivity, and in some cases can even lead to death. The absorption of heavy metals by plants can be through plant roots through the infiltration process and can also be through leaves that absorb heavy metals from the atmosphere[12]. The effects of HM contamination in chlorophyll are influenced by soil physicochemical properties. Thus, physicochemical soil properties such as pH, organic matter, and texture can strongly change the effects of HM contamination in chlorophyll [13,14].

This meta-analysis shows that increased levels of heavy metals in the soil can be absorbed by plants and accumulate in chlorophyll, thereby disrupting the levels of chlorophyll produced by plants. The ability of certain plant species to absorb metal is highly correlated with its ability to absorb other metals. However, absorption its ability varies according to plant organs, with the tendency for the highest
accumulation to be in chlorophyll. pH and the living habits of aquatic plants also affect the ability to absorb heavy metals by plants [4,15]. We declare that this meta-analysis will help identify the effects of heavy metals on plant chlorophyll living in heavy metal polluted areas.

This study can be a preliminary study for the development of comparative research on the impact of heavy metals on the biosphere. Therefore, it was concluded that plants can absorb heavy metals from both the soil and the atmosphere. The season can also affect the absorption of heavy metals by plants. Due to seasonal changes, plant root systems can change their accumulation ability for heavy metals. Seasons can also affect the speciation of heavy metals in the soil. Thus, further studies are needed for environmental risk assessment to see the extent of the effects of heavy metals on the environment. So that it can determine the appropriate steps for mitigating environmental impacts due to heavy metals for environmental sustainability.

**Forest Plot**

![Forest Plot](image)

**Figure 2.** The effect of heavy metal levels in agricultural soil on chlorophyll levels in agricultural crops.
4. Conclusions
Based on the meta-analysis of the effect of heavy metal exposure in agricultural soil on the chlorophyll content of agricultural crops, the value "effect size overall" was -0.285 in the range of -0.380 to -0.190, where most of the values "effect size" were on the left. This indicates that the chlorophyll content in the control is greater than in the experiment. This proves that exposure to heavy metals in agricultural soil has a significant effect on the chlorophyll content of plants.

Plants growing on soil contaminated with heavy metals result in decreased growth due to changes in physiological and biochemical activities, especially when heavy metals inhibit plant growth and development. Therefore, it is important to intensify research for a better understanding of the toxicity of heavy metals in agricultural crops, because the results of this meta-analysis show that growth, photosynthesis, and chlorophyll content in plants are affected by the presence of heavy metals.

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