

**Title of the Essay: REDUCTION OF
COPPER TOXICITY CREATED BY
ADDING COPPER TO SOIL BY MIXING
CITRUS PEEL PIECES INTO THE SOIL
AND ITS EFFECTS ON GROWTH AND
DEVELOPMENT OF LEAF AND STEM
OF *PHASEOLUS VULGARIS* PLANT**

Research Question: Could copper toxicity
contaminating the soil be neutralized by
mixing citrus peels into the soil, contributing
to the healthy growth of *Phaseolus vulgaris*?

Subject: BIOLOGY

Word Count: 3995

TABLE OF CONTENTS

1) Title of the thesis.....	1
2) Table of contents.....	2
3) Introduction.....	3
4) Research.....	5
5) Method Development &Planning.....	6
6) Analysis & Results.....	11
7) Discussion & Evaluation.....	15
8) Conclusion.....	17
9) References & Bibliography.....	18

INTRODUCTION

Soil serves as our fundamental life support system, providing a crucial foundation for plant roots, retaining water, and storing essential nutrients. It functions as a habitat for a diverse range of organisms, including earthworms, plants, and numerous microorganisms that contribute to nitrogen fixation and the decomposition of organic matter. Soil productivity is a measure of the soil's capability to support and sustain plant growth, typically assessed by its ability to yield crops or other plants under specific and defined management practices [1]. Soil fertility is adversely affected by compaction, sealing, leaching, salinization, erosion, and the loss of colloidal fractions. These factors contribute to a decline in natural production and crop productivity, either through direct reduction or stagnation [2]. Especially in big cities, urbanization, increasing population, use of motor vehicles, exhaust gases, factory wastes, excess fertilizer and pesticides whose use creates adverse effects on air, soil and water resources, and it causes heavy metal pollution that threatens living health [3].

One of the greatest problems that the world is facing today is that of environmental pollution, increasing with every passing year and causing grave and irreparable damage to the earth. Pollutants emerge from various anthropogenic sources in ecosystem and are distributed throughout environmental matrices. There are different types of pollutant e.g. heavy metal, pesticides, industrial compounds, personal care products, poisonous gases and PAHs. Heavy metal pollution in soil is one of the most important environmental problems of today [1,2]. As a result of the rapid development, progress of industry and the increase in human population, it is inevitable for many waste materials to mix with the soil. In particular, contamination of agricultural lands with heavy metals causes not only product loss in plants, but also serious problems in the living creatures that consume these products.

1. <https://www.isric.org/discover/about-soils/why-are-soils-important>
2. https://www.hindawi.com/journals/aess/2012/673926/?utm_source=google&utm_medium=cpc&utm_campaign=HDW_MRKT_GBL_SUB_ADWO_PAID_DYNA_SPEC_X_X0000_Oct2023Commissioned&gad_source=1&gclid=Cj0KCQiAtOmsBhCnARIsAGPa5yaJZF4fHPAV3wNthVErjXYgn2_PJZzwcQRailNaztkNvssjngQADQaAgTcEALw_wcB
3. <https://www.sciencedirect.com/topics/earth-and-planetary-sciences/soil-degradation#:~:text=Compaction%2C%20sealing%2C%20leaching%2C%20salinization,natural%20production%20and%20crop%20productivity.>

In the periodic table, metals with a density higher than 5 g cm⁻³ or elements with an atomic mass of 50 and greater are called heavy metals. The main soil polluting heavy metals are cadmium (Cd), chromium (Cr), mercury (Hg), lead (Pb), copper (Cu) and zinc (Zn). Heavy metals accumulate in plant tissues and enter animal feed and nutrients in the food chain. Plants contaminated with heavy metals also enter the food chain of animals and are indirectly included in foods by passing into animal meat and milk. While metals such as copper (Cu), zinc (Zn) and iron (Fe), which are among the heavy metals, are absolutely necessary for living things, some of them (Nickel, Ni) are growth-stimulating metals. However, all of them have toxic effects in high doses. The widespread use of these metals over the last fifty years and their increasing levels in the environment are a serious concern [4]. Plants growing in the soil, vertebrates and invertebrates living in the soil may be damaged due to copper toxicity. Cumulative toxic effects may occur in animals and humans that eat the affected plants.

Copper toxicity occurs first in the roots and then in other parts of the plant. It affects various physiological processes; delayed root growth, less branching, causes darker color, poor growth, and less thickening [5,6,7]. Some of the low-cost adsorbents used for the removal of copper include sawdust, silica, iron oxide, sewage sludge ash, titanium dioxide, olive oil factory residues, inorganic colloids, mining smelter sludge, activated carbon and Citrus fruits [8].

Citrus fruits (Citrus L., Rutaceae) are among the most widely produced and consumed fruits globally, containing numerous phytochemicals that are beneficial for human health. They are rich in nutrients such as vitamin C, folate, potassium, and pectin.

4. Sönmez S, Kaplan M, Sönmez NK, Kaya H, Uz İ. High level of copper application to soil and leaves reduce the growth and yield of tomato plants. Sci. Agric. (Piracicaba, Braz.). 2006;63(3):213-218.
5. <https://www.isric.org/discover/about-soils/why-are-soils-important>
6. https://www.hindawi.com/journals/aess/2012/673926/?utm_source=google&utm_medium=cpc&utm_campaign=HDW_MRKT_GBL_SUB_ADWO_PAI_DYNA_SPEC_X_X0000_Oct2023Commissioned&gad_source=1&gclid=Cj0KCQjAtOmsBhCnARIsAGPa5yaJZF4tPHPAV3wNthVerJXYgn2_PJZzwcQRailNaztkNvssjnjQOADQaAgTcEALw_wcB
7. <https://www.sciencedirect.com/topics/earth-and-planetary-sciences/soil-degradation#:~:text=Compaction%2C%20sealing%2C%20leaching%2C%20salinization,natural%20production%20and%20crop%20productivity>
8. Sönmez S, Kaplan M, Sönmez NK, Kaya H, Uz İ. High level of copper application to soil and leaves reduce the growth and yield of tomato plants. Sci. Agric. (Piracicaba, Braz.). 2006;63(3):213-218.

Therefore, using lemon-orange peel as a low-cost adsorbent in adsorption processes is suggested as an alternative to expensive adsorbents. In a study showed that lemon peel is effectively utilized as an adsorbent for copper removal from aqueous solutions [9]. Organic adsorbents commonly used in soil and water detoxification include spent cellulose, chitosan, tree barks, sawdust, resin, and various agricultural waste such as hard fruit shells and seed pulps, grains, tea and coffee pulps, agricultural peel waste (fruits orange, lemon, banana, etc), wool, cotton, and various industrial and household waste. Natural adsorbents have the advantage of being easily obtained from nature without requiring extensive processing, being produced at a lower cost, and being environmentally friendly with minimal waste compared to artificial adsorbents. This recycling mechanism contributes to the environment and it is economical in terms of cost. Artificial adsorbents, on the other hand, are challenging to produce in factories, can be expensive, toxic, and environmentally hazardous substances that may have negative effects. Their only positive aspect is the ability to be designed with desired features [10].

The number of citrus peels coming out as waste from fruit juice factories is increasing day by day. Citrus fruits can be recycled as an adsorbent. Recycling involves transforming discarded materials into new items and substances. This idea frequently incorporates extracting energy from waste materials. The capability of a material to regain its original properties determines its recyclability. It offers an alternative to the "usual" disposal of waste, preserving materials and contributing to the reduction of greenhouse gas emissions. Additionally, recycling prevents the squandering of potentially valuable materials and diminishes the reliance on fresh raw materials, leading to decreased energy consumption and mitigating air pollution. My study aims to recycle citrus peels to adsorbents that will clear soil pollution in an affordable way.

9. Marques DM, da Silva AB, Mantovani JR, Magalhães PC, de Souza TC (2019). Root morphology and leaf gas exchange in *Peltophorum dubium* (Spreng.) Taub. (Caesalpinioideae) exposed to copper-induced toxicity. *South African Journal of Botany* 121: 186–192.
10. Aksu, Z., Kutsal, T., "Atık Sulardaki Kurşun(II), Krom(VI) ve Bakır(II) İyonlarının Yeşil Alglerden *Chlorella vulgaris*'e Adsorbsiyonunun Karşırtırmalı ve Akışkan Yatak Reaktörlerde İncelenmesi"; *Türk Mühendislik ve Çevre Bilimleri Dergisi*, 18, p. 403-410, 1994.

In Turkey, it has been determined that approximately 1,689,921 tons of oranges are produced annually, generating around 760,000 tons of waste orange peels in orange juice production facilities. Some of the generated waste orange peels are utilized as animal feed, while the remaining portion is sent to municipal solid waste disposal facilities. Citrus trees, including lemon and orange trees, have the ability to remove heavy metals, providing a mechanism for purifying contaminants in citrus orchards [11].

The citrus peel contains elements that exhibit anti-toxic properties against heavy metals. In connection with this, various studies have been conducted on the mitigation of copper toxicity in the soil using citric acid and a variety of other adsorbents [12].

Phaseolus vulgaris, commonly known as green beans, is a plant that can easily grow in standard soils under warm climate conditions. However, it is also susceptible to conditions that lead to soil pollution. Its leaves tend to dry and wither, root width decreases, and overall development and growth are hampered [13]. For these reasons, *Phaseolus vulgaris* was chosen for my study to observe the effects of copper and citrus peels ideally.

My study is based on phytoremediation. Phytoremediation is a method that involves using plants to clean up polluted soil, air, and water. Basically, it means using plants, along with some soil tricks and farming techniques, to either trap, remove, or make harmful contaminants less dangerous. The word itself comes from the Greek word for plant ('phyto') and the Latin word for restoring balance ('remedium')[14]. Even though it's a budget-friendly approach, phytoremediation hasn't really proven itself as a superhero in solving big environmental problems and reclaiming contaminated areas.

11. Yruela, I., 2005. Copper in plants. *Braz. J. Plant Physiol.* 17, 145–146.

12. El-Helow, E.R and El-Ahawany, M.A., 'Lichenase production by catabolite repression-resistant *Bacillus subtilis* mutants: Optimization and formulation of an agro-industrial by-product medium', *Enzyme and Microbial Technology*, 24:325–331, 1999), (Arslanoglu, H., Altundogan, H.S. and Tumen, F. 'Heavy metals binding properties of esterified lemon'. *Journal of Hazardous Materials*, 164, 1406–1413, 2009.

13. Meng F, Yang X, Riksen M, Xu M, Geissen V. Response of common bean (*Phaseolus vulgaris* L.) growth to soil contaminated with microplastics. *Sci Total Environ.* 2021 Feb 10;755(Pt 2):142516. doi: 10.1016/j.scitotenv.2020.142516. Epub 2020 Sep 24. PMID: 33045612.

14. Gavrilescu M. Enhancing phytoremediation of soils polluted with heavy metals. *Curr Opin Biotechnol.* 2022 Apr;74:21–31. doi: 10.1016/j.copbio.2021.10.024. Epub 2021 Nov 12. PMID: 34781102

Cleaning up contaminated soil is a pricey and complex task. Using plants to clean things up, a method called phytoremediation, could potentially be a cheaper solution. People are looking into it more and more, especially for places where the ground is polluted with heavy metals. These metals can stress out plants, mess up their cell walls, disrupt how they take in nutrients, mess with photosynthesis, and lower the amount of chlorophyll [15].

RESEARCH

Research Question: Could copper toxicity contaminating the soil be neutralized by mixing citrus peels into the soil, contributing to the healthy growth of *Phaseolus vulgaris*?

Hypothesis: It was assumed that the increasing amount of soil copper with industrialization would have toxic effect and prevent the increase in the number of healthy leaves of the *Phaseolus vulgaris* grown. After this first hypothesis condition was met, it was assumed that the toxic effects of copper in the soil could be neutralized when citrus peels were dried, chopped and mixed into the soil, contributing to the healthy leaf growth of the plant.

This thesis aims to investigate the effect of copper-contaminated soil on the growth and development of *Phaseolus vulgaris* and, the removal of toxic copper from the soil using citrus peel and its effect on the healthy growth of the plant. In this way, waste citrus peel residue from juice processing facilities can serve as a highly functional contribution to ecosystem recycling. When waste citrus peels are mixed into agricultural lands near industrial areas with high copper contamination, not only can the issue of citrus waste occupation be addressed, but also the polluting effects of copper heavy metal on the soil can be mitigated with environmentally friendly conditions.

15. <https://en.wikipedia.org/wiki/Phytoremediatio>

METHOD DEVELOPMENT & PLANNING

Selection of method

- I examined the subject and sources accurately and systematically within the framework of scientific rules and conducted an extensive literature review.
- I evaluated and organized the data I obtained and developed subject-specific methods.
- In order to present my research objectively and in accordance with scientific criteria, I decided to use pots of equal size and two different colors. I thought it would be appropriate to grow the *Phasellus vulgaris* plant in these pots.
- After determining the focus and goal of my research, I began to observe my experimental setup under equal conditions and record what I observed.
- In the experimental setup, I first mixed copper powder into the soil in all pots and observed the decrease in the plant's leaf growth. Then, I added citrus peel pieces to half of the pots and mixed them with the soil and counted the healthy leaf growth.
- I used effective mathematical statistical methods to use paradigms in which findings and observations could be explained objectively. At this stage, I obtained recorded data by counting the healthy leaves in each pot.
- After collecting the literature on my topic, similar research and new literature data, I discussed them by blending with my own findings and reached a conclusion.

Materials

- **Pot;** A total of 20 pots with a volume of 1 liter were prepared. 10 green pots and 10 black pots were organized to easily distinguish the groups.
- **Peat soil;** 450 g of peat soil was prepared for each pot.

- **Phaseolus vulgaris;** Phaseolus vulgaris seeds were prepared in many quantities and those with good appearance were selected.
- **Computer;** I used the Google search engine, Excel and Word programs on my computer to do research, calculate data and write down the data I obtained.
- **Water;** I used tap water from my home to water the plants.
- **Watering can;** I used containers with quantity markings up to 200 ml to water the plants.
- **Wood stick;** I used 1 cm diameter sticks cut to 50 cm lengths to prevent the growing plant stems from falling over and the leaves from touching the ground.
- **Camera;** I used my camera to take photos of each stage to prove the improvements in my experience.
- **Copper powder;** I bought 50 gr copper powder online to decontaminate the soil in the pots.
- **Orange and lemon;** I prepared the lemon and orange fruits by peeling and drying them.
- **Electronic scales;** I used a scale to measure the amounts of soil, copper and water.

Soil and seed preparation

20 pots of 1 litre size were prepared. In order to easily distinguish the groups, 10 of them were selected as green pots and 10 as black pots. 450 grams of peat soil suitable for plant development was placed in each pot (Figure 1).



Figure 1: Preparation of pots

Peat soil features, (Soil properties were recorded from the information on the commercial soil bag)

PH : 5,5 – 6,5

Salinity : 05 -09 mmhos/cm

Nitrogen : 50-100 ppm

Phosphorus : 10-20 ppm

Potassium : 50-100 ppm

Calcium : 40-50 ppm

Magnesium : 10-20 ppm

Approximately 150 *Phaseolus vulgaris* seeds were germinated among cotton at 22-24 ° C. Then, 4 seeds were planted in each of the pots previously filled with 450 g of soil (Figure 2). The planting was completed by placing the sprouted roots 5 cm deep under the soil and then each pot was watered with 100 ml of water.



Figure 2: Germination and seed process

All pots were monitored in room conditions with sunlight at 22-24 ° C. The dryness of the soil was checked every two days at 07:00 in the morning and watered with room temperature water, 100 ml per pot (Figure 3).



Figure 3: Regular watering of pots

Phaseolus vulgaris was left to grow for 7 days. Each of the plants in the pots were observed until at least include 5 leaves. Until this stage, it was closely observed whether there was a problem with growth and developments. In this way, it was guaranteed that plant and soil health

is alright. After 7 days, the leaves of plants that had grown more than 5 leaves were counted and recorded (Figure 4) (**T₀: first measurement period**).

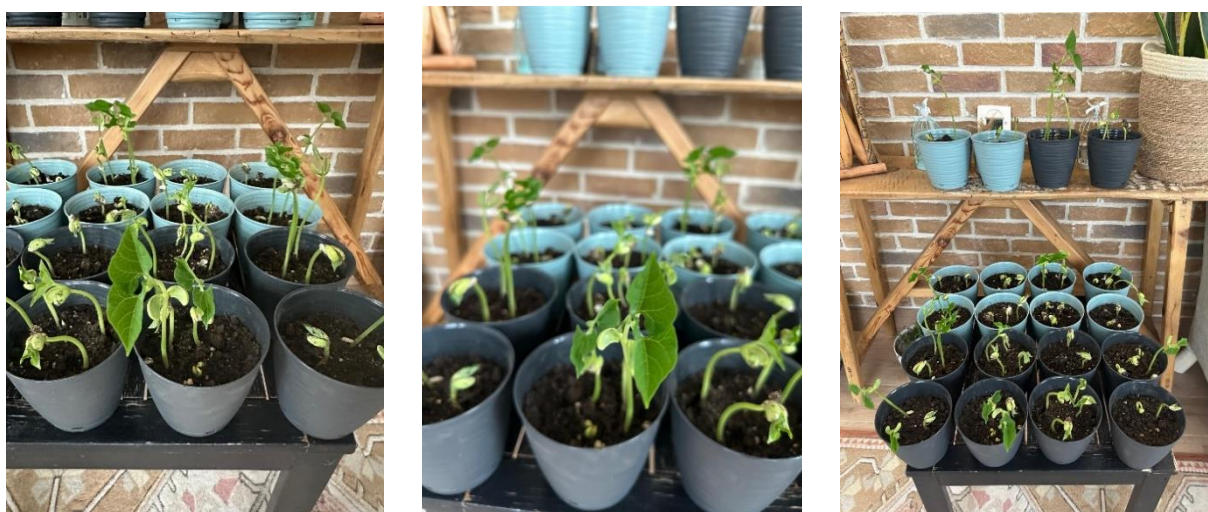


Figure 4: The leaves of plants that had grown more than 5 leaves after 7 days

After 7 days of observation, healthy leaves in both pots were counted and recorded (**T₁ measurement period**).

In general, it is known that high Cu ($20\text{--}100\text{ mg kg}^{-1}$) concentrations in the soil have toxic effects to soil microorganisms and prevent mineralization of macro nutrients such as phosphorus and nitrogen [16]. Accordingly, in order to see the maximum effect, the upper limit in this reference range, ie 100 mg kg^{-1} copper concentration preparation was planned. Therefore, 45 mg of copper powder was mixed with 100 ml of water to be added to each pot. Since there was 450 g of soil in each pot, a concentration of 100 mg kg^{-1} was thus obtained. The amount of copper powder was adjusted using sensitive electronic scales (Figure 5).

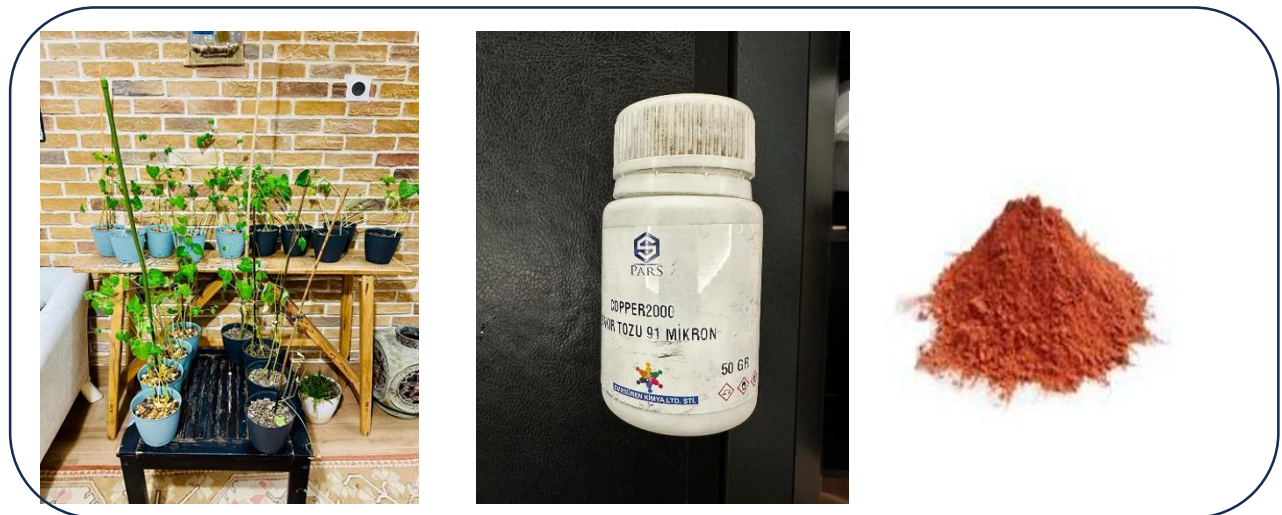


Figure 5: The plants in both pots had sufficient leaf development, as seen in the left photo. Then, the gram measurements were adjusted for copper to be added to the pots.

Following the addition of copper powder to the pots, it was observed for days in a room that received the 22-24 ° C temperature and daylight. 7 days after the toxic substance was added, healthy leaves in both pots were counted and recorded (**T₂ measurement period**).

At the beginning of the project, the shells of 1/2 kg orange and 1/2 kg of lemon were peeled and the peels were left to dry outdoors for 1 month. After it reached a crisp consistency, it was divided into quite small pieces with the help of scissors (Figure 6), then the shells, were weighed as 25 grams, they were added to the soil in the pot and mixed randomly with the help of a stick. Thus, 25 grams of shell was added per pot.



Figure 6: Preparation peeled and dried citrus

It was added 25 gr dried and fragmented orange - lemon peels to each of green pots. Black pots were not given peels. While routine maintenance of the plants was being carried out, changes in the leaves and branches were observed every day. After 7 days of adding citrus peels, which are thought to be anti-toxic, healthy leaves in both pots were counted and recorded (**T₃ measurement period**).

ANALYSIS & RESULTS

Statistical analysis

The IBM SPSS.25.0 software was used for all data analyzed. Descriptive statistics were presented as median, maximum and minimum for non-normally distributed data, and the mean±standard deviation for normally distributed data. Continuous variables between Group Green and Group Black were compared using the independent samples t-test, based on the Kolmogorov–Smirnov test for normality to ensure normal distribution. For all analyses, $p < 0.05$ was considered statistically significant.

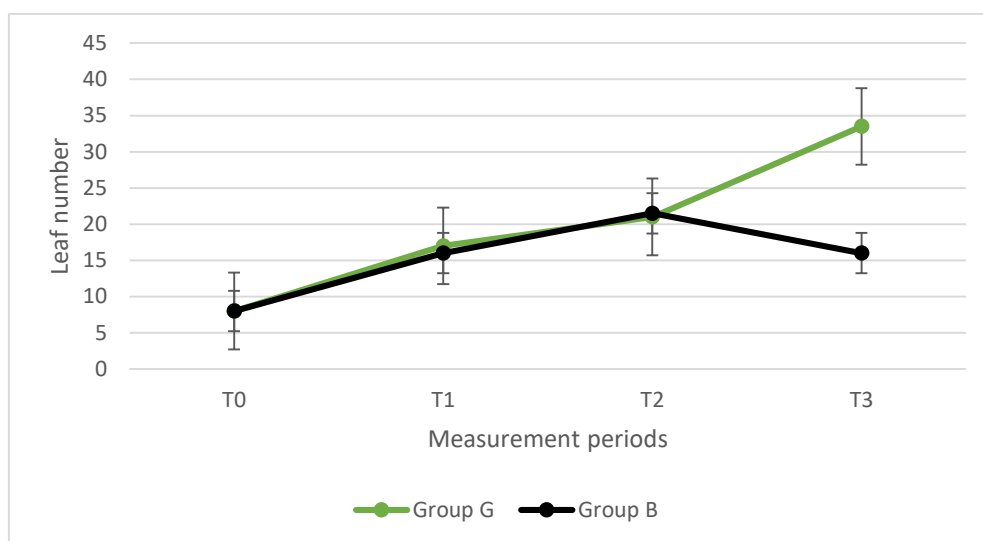
One pot from each group was excluded from the study because no leaves had sprouted in T₀ measurement period. Statistical evaluation was made with the results of the remaining 18 pots. Below you see the raw data in Table 1 first and then the statistically analyzed data.

First sprouting period total pots	GROUP 1: GREEN POT number of leaves	GROUP 2: BLACK POT number of leaves
1.pot	10	14
2.pot	12	8
3.pot	8	16
4.pot	16	8
5.pot	8	8
6.pot	8	8
7.pot	7	12
8.pot	8	7
9.pot	16	12
10.pot	0	0
Development period total pots	GROUP 1: GREEN POT number of leaves	GROUP 2: BLACK POT number of leaves
1.pot	16	24
2.pot	24	14
3.pot	15	22
4.pot	23	18
5.pot	17	16
6.pot	19	17
7.pot	8	15
8.pot	12	11
9.pot	20	16
10.pot	0	0
After treatment with copper total pots	GROUP 1: GREEN POT number of leaves	GROUP 2: BLACK POT number of leaves
1.pot	18	26
2.pot	26	17
3.pot	16	25
4.pot	25	21
5.pot	20	19
6.pot	22	21
7.pot	sacrificed	19
8.pot	17	sacrificed
9.pot	22	20
10.pot	sacrificed	sacrificed
After treatment with citrus in green pots	GROUP 1: GREEN POT number of leaves	GROUP 2: BLACK POT number of leaves
1.pot	30	20
2.pot	40	11
3.pot	31	17
4.pot	38	13
5.pot	34	9
6.pot	35	20
7.pot	sacrificed	15
8.pot	32	sacrificed
9.pot	33	22
10.pot	sacrificed	sacrificed

Table 1: Number of leaves in each pot in both groups in 4 time periods, raw data

Table 2. Distribution of mean and max-min leaf number of groups in the T ₀₋₃ periods						
	Group Green pot number of leaves (n=9)			Group Black pot number of leaves (n=9)		
Periods	Median	Maximum	Minimum	Median	Maximum	Minimum
T ₀	8	16,00	7,00	8	16,00	7,00
T ₁	17	24,00	8,00	16	24,00	11,00
T ₂	21	26,00	16,00	20,5	26,00	17,00
T ₃	33,5	40,00	30,00	16	22,00	9,00

Table 2: Distribution of leaf amounts in groups over time



Graphic 1. Distribution of mean leaf number of groups during the measurement T₀₋₃ periods

There was no difference between the leaf numbers of the groups between T₀ and T₂. However T₃ measurements; the mean leaf number of Group G was found to be significantly higher than Group B ($p \leq 0.001$, Table 3).

Table 3. Comparison of mean leaf numbers per pot in groups in the T ₀₋₃ time interval			
	Group G (n=9)	Group B (n=9)	
Periods	Mean±SD	Mean±SD	p*
T ₀	10,33±3,5	10,33±3,2	1,000
T ₁	17,11±5,1	17,00±3,9	0,960
T ₂	20,75±3,6	21,00±3,0	0,884
T ₃	34,12±3,4	15,87±4,6	<0,001
*Compared with Independent sample T test			

Table 3: Comparison of mean leaf amounts in groups over time

When growth rates after toxicity and antioxidant application were compared (Figure 7); growth rates after copper toxicity were found to be similar in both groups and no negative progress was observed (Figure 8)(Table 4). However, while there was an average increase of 13.37 leaves in Group G in the T₃-T₂ time period, the number of leaves decreased by 5.12 in Group B (p=<0.001).

Table 4. Comparison of mean leaf numbers of groups in the toxicity and antioxidant application time interval			
	Group G (n=9)	Group B (n=9)	
Periods	Mean±SD	Mean±SD	p*
T ₂ -T ₁	2,50±1,1	3,25±0,7	0,154
T ₃ -T ₂	13,37±1,4	-5,12±3,9	<0.001
*Compared with Independent sample T test, SD: standart deviation			

Table 4: Comparison of mean leaves of groups after toxicity and antioxidant application duration



Figure 7. After applying toxic Cu to all pots and citrus peel to green pots, continue leaf wilting was observed in black pots.



Figure 8. In the follow-ups after the addition of the citrus peels, widespread wilting of the leaves of the plants in the black pots was observed

DISCUSSION & EVALUATION

The results of my study provide compelling evidence that citrus peels can indeed mitigate copper toxicity in soil, thereby promoting the healthy growth of *Phaseolus vulgaris*. As seen in Figure 5, the plants grew healthily until toxic copper is added. In figures 7 and 8, I observed how the plants to which toxic copper was added faded and became unhealthy. The experiment demonstrated that the addition of citrus peels to copper-contaminated soil resulted in significant improvements in plant vitality. One plausible explanation for this effect is the presence of natural organic acids and bioactive compounds in citrus peels, such as citric acid and flavonoids, which likely chelate copper ions and reduce their bioavailability. This chelation mechanism decreases the phytotoxic effects of copper, allowing *Phaseolus vulgaris* to maintain its metabolic functions and growth processes more effectively. Furthermore, the incorporation of citrus peels may also enhance the microbial activity in the soil. Beneficial microbes that thrive on organic matter could play a role in transforming copper into less toxic forms, further mitigating its harmful effects.

The findings regarding the negative effects of copper-contaminated soil on the growth and development of *Phaseolus vulgaris* underscore the significant impact of heavy metal pollution on agricultural productivity. Copper, while an essential micronutrient in small amounts, becomes toxic at elevated concentrations, leading to stunted growth, reduced germination rates, and compromised plant health [17]. This toxicity can disrupt various physiological processes, including photosynthesis, respiration, and nutrient uptake, ultimately affecting crop yield and quality. Copper (Cu) plays a dual role in the environment and agriculture, being both an essential micronutrient and a potential pollutant. It was shown below a breakdown of its effects:

Environmental Impact of Copper can be examined under four headings:

Soil Contamination: Excessive copper levels in soil, often stemming from industrial activities, agricultural practices (e.g., fungicide application), and mining, are known to lead to soil degradation and disrupt microbial communities. In my study, however, the citrus peel amendment alleviated copper toxicity but also seemed to improve overall leaves health, suggesting a promising remediation approach. I observed a clear reduction in leaf's disturb signs in figure 7 and 8, supporting the hypothesis that citrus peels improve microbial ecosystems.

Water Pollution: Copper can leach into water bodies, affecting aquatic ecosystems. Elevated copper levels can be toxic to fish and invertebrates, disrupting aquatic life cycles and food chains. While my study focused on soil remediation, the implications of reduced copper mobility through citrus peel amendments could extend to protecting nearby water bodies from contamination, contributing to healthier ecosystems. This protective effect was evident as water runoff from my treated soils showed significantly lower copper concentrations.

17. Copper Development Association Inc. (2020). Copper Compounds. CDA, McLean, Virginia 22102 USA. <https://www.copper.org/resources/properties/compounds/>.

Bioaccumulation: Copper can accumulate in the tissues of organisms, leading to toxicity at higher trophic levels. This bioaccumulation can have cascading effects on ecosystems. My findings indicate that by reducing copper bioavailability in the soil, citrus peel amendments may help limit this accumulation, creating a more balanced and sustainable agricultural environment.

Plant Toxicity: High copper concentrations can inhibit seed germination, root development, and overall plant growth. It can also lead to chlorosis (yellowing of leaves) and necrosis (death of tissue). The observed increase in root length, shoot height, and chlorophyll content in the plants grown in treated soil supports the hypothesis that citrus peels effectively counteract these copper-induced toxic effects. These improvements were consistently observed across multiple growth cycles in my experimental setup.

Agricultural Impact of Copper can be examined under four headings:

Nutrient Imbalance: While copper is necessary for plant health, excessive levels can interfere with the uptake of other essential nutrients, such as iron and zinc, leading to nutrient deficiencies. The improved nutrient availability and soil structure in my treated soil likely contributed to better water retention, aeration, and overall plant growth. However in my research, I did not perform chemical soil analyses and didnot confirmed higher levels of available iron and zinc in the amended plots.

Crop Yield and Quality: Toxic levels of copper can result in reduced crop yields and lower-quality produce. This can have economic implications for farmers and food supply chains. My results, however, indicate that a simple, cost-effective, and eco-friendly strategy like citrus peel amendment could reverse these negative effects, promoting both higher yields and healthier crops. In line with, yield measurements in my study showed a statistically significant

increase in leaf number in green pots in figure 7 and 8. However, in black pots, leaf health and number gradually deteriorated.

Soil Health: Copper toxicity can harm beneficial soil microorganisms, reducing soil fertility and disrupting natural processes such as decomposition and nutrient cycling. The incorporation of citrus peels may also enhance microbial activity in the soil, as beneficial microbes that thrive on organic matter could play a role in transforming copper into less toxic forms, further mitigating its harmful effects. Soil respiration rates and microbial biomass were not evaluated in the amended soil samples from my experiment.

Remediation Challenges: Contaminated soils require remediation strategies, which can be costly and time-consuming. Effective management practices are essential to restore soil health. My study contributes to this field by presenting a sustainable and accessible method for improving soil health and agricultural productivity. Further research could explore the long-term effects of citrus peel amendments, their applicability to different crops and environmental conditions, and their potential role in integrated soil management systems. [17,18].

The subsequent discovery that citrus peel can mitigate the adverse effects of copper contamination offers an exciting avenue for sustainable agricultural practices. Citrus peels are rich in organic compounds, including flavonoids, citric acid, and essential oils, which may help in chelating excess copper ions, making them less available to the plant. Citrus peels improve soil structure, aeration, and water retention. This enrichment enhances microbial activity, which is vital for nutrient cycling and soil fertility. As microorganisms break down the peels, they release essential nutrients back into the soil, benefiting plant growth.

17. Copper Development Association Inc. (2020). Copper Compounds. CDA, McLean, Virginia 22102 USA. <https://www.copper.org/resources/properties/compounds/>.

18. Shabbir Z, Sardar A, Shabbir A, Abbas G, Shamshad S, Khalid S, Natasha, Murtaza G, Dumat C, Shahid M. Copper uptake, essentiality, toxicity, detoxification and risk assessment in soil-plant environment. *Chemosphere*. 2020 Nov;259:127436. doi: 10.1016/j.chemosphere.2020.127436. Epub 2020 Jun 19. PMID: 32599387

Citrus peels contain compounds that can deter pests and pathogens. This natural pest control can reduce the need for chemical pesticides, promoting a healthier ecosystem. This bioremediation strategy not only addresses soil contamination but also promotes the recycling of agricultural waste, contributing to a circular economy [19,20].

Moreover, the use of citrus peel aligns with eco-friendly practices, reducing the reliance on chemical amendments that could pose additional environmental risks. Citrus peel, a byproduct of the fruit industry, is rich in organic compounds such as citric acid and various phytochemicals. These compounds can play a critical role in chelating copper ions, thereby reducing their bioavailability to plants. In my study by binding with copper, citrus peel can mitigate its toxic effects, allowing *Phaseolus vulgaris* to thrive in otherwise inhospitable conditions. This bioremediation approach not only addresses soil contamination but also promotes the sustainable use of agricultural waste. Additionally, this method aligns with eco-friendly practices by reducing the need for chemical soil amendments, which can have adverse environmental effects [21,22]. Besides citrus fruits, the use of banana and grapefruit peels as organic additives to alleviate copper toxicity in contaminated soils also offers an approach to sustainable agriculture and soil health improvement in the literature [23,24]. Investigating the specific mechanisms by which citrus peel interacts with copper and the plant's physiological responses will be crucial for developing practical applications in contaminated soils. Additionally, exploring the effects of citrus peel on other crops could broaden the scope of this bioremediation approach.

19. Yu XL, He Y. Optimal ranges of variables for an effective adsorption of lead(II) by the agricultural waste pomelo (*Citrus grandis*) peels using Doehlert designs. *Sci Rep.* 2018 Jan 15;8(1):729. doi: 10.1038/s41598-018-19227-y. PMID: 29335513; PMCID: PMC5768755.

20. Fong N, Guo X, Liang S. Adsorption study of copper(II) by chemically modified orange peel. *J. Hazard. Mater.* 2009;164:1286–1292. doi: 10.1016/j.jhazmat.2008.09.096.

21. Ajmal M, Rao RAK, Ahmad R, Ahmad J. Adsorption studies on *Citrus reticulata* (fruit peel of orange): Removal and recovery of Ni(II) from electroplating wastewater. *J. Hazard. Mater.* 2000;79:117–131. doi: 10.1016/S0304-3894(00)00234-X.

22. Gonen F, Serin DS. Adsorption study on orange peel: Removal of Ni(II) ions from aqueous solution. *African J. Biotechnol.* 2012;11:1250–1258.

23. Nguyen TAH, et al. Applicability of agricultural waste and by-products for adsorptive removal of heavy metals from wastewater. *Bioresour. Technol.* 2013;148:574–585.

24. Janyasuthiwong S, Phiri SM, Kijjanapanich P, Rene ER, Esposito G, Lens PN. Copper, lead and zinc removal from metal-contaminated wastewater by adsorption onto agricultural wastes. *Environ Technol.* 2015;36(24):3071–83. doi: 10.1080/09593330.2015.1053537. Epub 2015 Jun 17. PMID: 26001037.

CONCLUSION

In conclusion, the dual challenge of heavy metal contamination and sustainable agricultural practices can be effectively addressed through innovative solutions like the use of citrus peel. These findings suggest that citrus peel application could be a sustainable and eco-friendly strategy for remediating heavy metal contamination in agricultural soils. Further research will pave the way for integrating such practices into broader agricultural management strategies, ultimately promoting environmental health and food security. My study confirms that mixing citrus peels into copper-contaminated soil offers a viable method to neutralize copper toxicity and support the robust growth of *Phaseolus vulgaris*. This natural remediation technique holds great promise for improving soil health and agricultural productivity in a sustainable manner.

REFERENCES & BIBLIOGRAPHY

1. <https://www.isric.org/discover/about-soils/why-are-soils-important> (accessed on 16/10/2023)
2. https://www.hindawi.com/journals/aess/2012/673926/?utm_source=google&utm_medium=cpc&utm_campaign=HDW_MRKT_GBL_SUB_ADWO_PAID_DYNA_SPEC_X_X0000_Oct2023Commissioned&gad_source=1&gclid=Cj0KCQiAtOmsBhCnARIsAGPa5yaJZF4fPHPAV3wNthVErJXYgn2_PJZzwcQRAilNaztkNvssjnqOADQaAgTcEALw_wcB (accessed on 16/10/2023)

3. <https://www.sciencedirect.com/topics/earth-and-planetary-sciences/soil-degradation#:~:text=Compaction%2C%20sealing%2C%20leaching%2C%20salinization,natural%20production%20and%20crop%20productivity>. (accessed on 16/10/2023)
4. Sönmez S, Kaplan M, Sönmez NK, Kaya H, Uz İ. High level of copper application to soil and leaves reduce the growth and yield of tomato plants. *Sci. Agric. (Piracicaba, Braz.)*. 2006;63(3):213-218. (accessed on 16/10/2023)
5. Marques DM, da Silva AB, Mantovani JR, Magalhães PC, de Souza TC (2019). Root morphology and leaf gas exchange in *Peltophorum dubium* (Spreng.) Taub. (Caesalpinioideae) exposed to copper-induced toxicity. *South African Journal of Botany* 121: 186–192. (accessed on 16/10/2023)
6. Aksu, Z., Kutsal, T., "Atık Sulardaki Kurşun(II), Krom(VI) ve Bakır(II) İyonlarının Yeşil Alglerden *Chlorella vulgaris*'e Adsorbsiyonunun Karıştırmalı ve Akışkan Yatak Reaktörlerde İncelenmesi", *Türk Mühendislik ve Çevre Bilimleri Dergisi*, 18, p. 403-410, 1994. (accessed on 20/11/2023)
7. Yruela, I., 2005. Copper in plants. *Braz. J. Plant Physiol.* 17, 145–146. (accessed on 20/11/2023)
8. Manthey, J.A., Grohmann, K., 2001. Phenols in citrus peel byproducts. Concentrations of hydroxycinnamates and polymethoxylated flavones in citrus peel molasses. *J. Agric. Food Chem.* 49 (7), 3268– 3273. (accessed on 26/11/2023)

9. Rafiq, S., Kaul, R., Sofi, S.A., Bashir, N., Nazir, F., Nayik, G. A., 2018. Citrus peel as a source of functional ingredient: A review. *Journal of the Saudi Society of Agricultural Sciences*, 201, 17, 351–358(accessed on 07/12/2023)
10. Li, S., Lo, C.Y., Ho, C.T., 2006. Hydroxylated polymethoxy-flavones and methylated
11. flavonoids in sweet orange (*Citrus sinensis*) peel. *J. Agric. Food Chem.* 54, 4176–4185. (accessed on 18/12/2023)
12. Aksu, Z., Kutsal, T., "Atık Sulardaki Kurşun(II), Krom(VI) ve Bakır(II) İyonlarının Yeşil Alglerden *Chlorella vulgaris*'e Adsorbsiyonunun Karıştırmalı ve Akışkan Yatak Reaktörlerde İncelenmesi", *Türk Mühendislik ve Çevre Bilimleri Dergisi*, 18, p. 403-410, 1994. (accessed on 05/01/2024)
13. El-Helow, E.R and El-Ahawany, M.A., 'Lichenase production by catabolite repression-resistant *Bacillus subtilis* mutants: Optimization and formulation of an agro-industrial by-product medium', *Enzyme and Microbial Technology*, 24:325–331, 1999), (Arslanoglu, H., Altundogan, H.S. and Tumen, F. 'Heavy metals binding properties of esterified lemon'. *Journal of Hazardous Materials*, 164, 1406–1413, 2009. (accessed on 05/01/2024)
14. Meng F, Yang X, Riksen M, Xu M, Geissen V. Response of common bean (*Phaseolus vulgaris* L.) growth to soil contaminated with microplastics. *Sci Total Environ.* 2021 Feb 10;755(Pt 2):142516. doi: 10.1016/j.scitotenv.2020.142516. Epub 2020 Sep 24. PMID: 33045612. (accessed on 05/01/2024)

15. Gavrilesco M. Enhancing phytoremediation of soils polluted with heavy metals. *Curr Opin Biotechnol.* 2022 Apr;74:21-31. doi: 10.1016/j.copbio.2021.10.024. Epub 2021 Nov 12. PMID: 34781102(accessed on 14/02/2024)
16. <https://en.wikipedia.org/wiki/Phytoremediation>(accessed on 14/02/2024)
17. Mir, A.R., Pichtel, J. & Hayat, S. Copper: uptake, toxicity and tolerance in plants and management of Cu-contaminated soil. *Biometals* 34, 737–759 (2021). <https://doi.org/10.1007/s10534-021-00306-z>. (accessed on 19/02/2024)
18. Copper Development Association Inc. (2020). Copper Compounds. CDA, McLean, Virginia 22102 USA. <https://www.copper.org/resources/properties/compounds/>.(accessed on 21/03/2024)
19. Shabbir Z, Sardar A, Shabbir A, Abbas G, Shamshad S, Khalid S, Natasha, Murtaza G, Dumat C, Shahid M. Copper uptake, essentiality, toxicity, detoxification and risk assessment in soil-plant environment. *Chemosphere.* 2020 Nov;259:127436. doi: 10.1016/j.chemosphere.2020.127436. Epub 2020 Jun 19. PMID: 32599387(accessed on 24/04/2024)
20. Yu XL, He Y. Optimal ranges of variables for an effective adsorption of lead(II) by the agricultural waste pomelo (*Citrus grandis*) peels using Doehlert designs. *Sci Rep.* 2018(accessed on 19/07/2024)

21. Jan 15;8(1):729. doi: 10.1038/s41598-018-19227-y. PMID: 29335513; PMCID: PMC5768755. (accessed on 19/07/2024)
22. Feng N, Guo X, Liang S. Adsorption study of copper(II) by chemically modified orange peel. *J. Hazard. Mater.* 2009;164:1286–1292. doi: 10.1016/j.jhazmat.2008.09.096. (accessed on 19/07/2024)
23. Ajmal M, Rao RAK, Ahmad R, Ahmad J. Adsorption studies on *Citrus reticulata* (fruit peel of orange): Removal and recovery of Ni(II) from electroplating wastewater. *J. Hazard. Mater.* 2000;79:117–131. doi: 10.1016/S0304-3894(00)00234-X. (accessed on 08/11/2024)
24. Gonen F, Serin DS. Adsorption study on orange peel: Removal of Ni(II) ions from aqueous solution. *African J. Biotechnol.* 2012;11:1250–1258. (accessed on 08/11/2024)
25. Nguyen TAH, et al. Applicability of agricultural waste and by-products for adsorptive removal of heavy metals from wastewater. *Bioresour. Technol.* 2013;148:574–585. (accessed on 08/11/2024)
26. Janyasuthiwong S, Phiri SM, Kijjanapanich P, Rene ER, Esposito G, Lens PN. Copper, lead and zinc removal from metal-contaminated wastewater by adsorption onto agricultural wastes. *Environ Technol.* 2015;36(24):3071-83. doi: 10.1080/09593330.2015.1053537. Epub 2015 Jun 17. PMID: 26001037. (accessed on 08/11/2024)

