Chapter

Plant Phenology and An Assessment of the Effects Regarding Heavy Metals, Nanoparticles, and Nanotubes on Plant Development: Runner Bean, Artichoke, and Chickpea Seedlings

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Abstract

The relationship between environmental pollution and nutrition in particular, which forms the basis of health, is fundamentally important for protecting human health. Therefore, the data obtained from the examination of how plants and animals consumed as food are affected by environmental pollution can be seen as an indicator of their effects on humans. On the other hand, the role of technology and nanotechnology in life has been increasing in this century, and a considerable amount of heavy metals, nanoparticles (NPs), and nanotubes (NTs) are released to the environment. The results of morphological or anatomical examination of runner bean (*Phaseolus coccineus* L) and artichoke (*Cynara scolymus* L.) plants subjected to copper (Cu) and lead (Pb) heavy metals and chickpea (*Cicer arietinum* L) plants subjected to Au nanoparticles and C_{70} single-walled carbon nanotubes (SWNTs) are presented with this study in the point of their phenological development process. The three taxa belonging to Fabaceae and Asteraceae families with high economic status and having flowers with characteristic features were chosen deliberately as representatives. This chapter presents a study that will shed light on future biomonitoring-based studies focusing on the impact of environmental pollution on plants phenology with economic value.

Keywords: heavy metal, nanoparticle, nanotube, runner bean (*Phaseolus coccineus* L), artichoke (*Cynara scolymus* L), chickpea (*Cicer arietinum* L), morphology, anatomy

1. Introduction

It is a known fact that environmental pollution constitutes an important problem in Turkey as well as in the rest of the world. Rapid industrialization and population growth have caused pollution in the atmosphere, pedosphere, and hydrosphere.

Therefore, it is seen that countries pay particular attention to pollution-related studies and health problems caused by pollution and allocate high amounts of resources to deal with the problem.

Heavy metals show toxic effects at certain concentrations for living organisms. However, low concentrations of some heavy metals are essential for normal and healthy plant growth. Furthermore, heavy metals and nanoparticles are causes of concern because they can penetrate into different parts and cells of plants at different rates, and by this way, they enter the food chain and reach the living beings.

There are about 22,000 bryophyte species and 20,000 algae species; however, vascular plants are the dominant plant group in the world with 255,000 species. Land plants, which perform their life cycles completely in the terrestrial environment, are mainly composed of bryophytes and vascular plants. Furthermore, at least a thin film of water is required for fertilization in all taxa except seed plants. Even in the two primitive genera seed plants, cycad and gingko, fertilization is a result of free-swimming spermatozoids released into the liquid medium in the archegonium chamber [1–3].

One of the most important features of vascular plants is the presence of buds at the ends of the trunk and side branches in the gymnosperms and generally in the angiosperms. The bud is an apical meristem coated with protective bud scales. Meristem is the region of cells to which new cells, tissues, and organs are added and has the potential for active cell division and contributes to plant growth. Therefore, despite the limited growth potential in animals, plant growth is limitless due to the presence of apical meristem. However, the development of plant parts, such as leaves, flowers, and fruits, is limited to their shapes and is genetically predetermined [1]. In short, when evaluated from a phenological point of view, plant parts do not show any further growth independent of the time they remain on the plant after completing their development.

Cell development and differentiation take place as the changes occurring in protoplast; for example with the fusion occurring in vacuoles to grow, via structures such as mitochondria, plastids and the golgi body, endoplasmic reticulum, microtubules, and microfilaments in cytoplasm. Cell walls differentiate and increase in thickness due to structural and environmental effects, and they may become permeable. Moreover, the walls may integrate with the lignin, which increases tensile forces. Tissues formed by the differentiation of apical meristem include parenchyma, collenchyma, sclerenchyma, and primary xylem and primary phloem, in which the pith and cortex are formed [1, 2].

Phenological stages are divided into eight possible principal stages: [1] bud development, [2] leaf development, [3] shoot/branch development, [4] inflorescence emergence, [5] flowering, [6] fruit development, [7] fruit maturity, and [8] senescence and the beginning of dormancy [3]. Secondary parts and secondary metabolites occur in the plant during the phenological cycle [1]. Genotype and environmental factors are involved in the emergence of seconder metabolites. In this case, based on the amount of soil, water, and air pollution in the environment in which the plant grows, various deteriorations may occur as a result of morphological and physiological changes whose effects on the plant can be seen with the naked eye or observed only through microscopic examinations. In this chapter, general information about heavy metal and nanoparticles is given, and the effects of heavy metals and nanoparticles on the seedlings of runner bean (*Phaseolus coccinea*), chickpea (*Cicer arietinum*), and artichoke (*Cynara scolymus*) species, which are of economic importance, were examined morphologically and anatomically.

2. Effects of heavy metals, nanoparticles, and nanotubes on plant phenological development

The term heavy metal has been used by scientists with various definitions for about 60 years. An element with a density of more than 7 g/cm^3, in 1987 with a density of more than 4 g/cm^3, in 1992 with a density greater than 5 g/cm^3, and in 1995 with a density of 6 g/cm^3 with a metallic property was classified as heavy metal in 1964. Some scientists have classified heavy metals according to their atomic weights, atomic numbers, other chemical properties, and toxic properties. In biological terms, the term heavy metal is generally used for possible contamination of metals and metalloids on the environment and in terms of their toxicity or ecotoxicity [4].

Heavy metals are released into the atmosphere, pedosphere, and hydrosphere every day due to human activities besides natural causes, such as volcanic activities. Flying ashes from the chimneys of cement plants and thermal power plants; the use of heavy metal paint; the smoke emitted by motor vehicles as well as their plastic-based parts such as brake pads, garbage, and waste sludge incineration plants; and the release of industrial wastes, such as pesticides, fertilizers, paper, batteries, products, etc. are among the main causes of heavy metal pollution [5–7].

The discharge of heavy metal-containing particles released from the factory and plant chimneys onto agricultural lands, their dissolution in the soil by rain or irrigation, or the irrigation of agricultural land mixed with industrial wastewater leads to various diseases in crops grown on such lands and damages the agricultural economy [8–10].

Heavy metals have toxic effects for living organisms at certain concentrations. However, certain critical concentrations of some heavy metals are necessary for normal and healthy plant growth. Therefore, heavy metals are classified as essential elements and nonessential elements according to their participation in life processes. Cobalt (Co), copper (Cu), manganese (Mn), molybdenum (Mo), iron (Fe), nickel (Ni), and zinc (Zn) are heavy metals necessary for the growth and vitality of plants and are considered essential elements. Heavy metals such as barium (Ba), cadmium (Cd), mercury (Hg), antimony (Sb), lead (Pb), and chromium (Cr) are not essential for plants and other living organisms and are called nonessential elements [11].

Essential elements are found as a cofactor in many enzyme systems and as a structural component in biological processes in living organisms. For example, copper is an essential element for normal plant growth at certain concentrations. Copper is an essential cofactor for many metalloproteins in plants and plays a role in photosynthetic electron transport, mitochondrial respiration, cell wall metabolisms, and hormone signal transduction pathways [12, 13].

High concentrations of copper (depending on plant species) show toxicity in plants although it is an essential element. Lead is not an essential element and shows toxic properties for plants. The presence of excess copper and lead in the environment negatively affects phenological development in plants [14].

These heavy metals result in lipid peroxidation [15], degradation of cell and thylakoid membrane structure, and a decrease in chlorophyll amount due to the change in the chloroplast structure and thus chlorosis as a result of the oxidative damage they caused [16]. Heavy metals bind to sulfhydryl (-SH) groups of proteins and inhibit enzyme activity [17] and cause oxidative DNA damage [18, 19], chromosomal abnormalities [20], and lack of other essential elements [21–24].

More than 30 base lesions were characterized by DNA exposure to reactive oxygen species [25]. On the DNA, reactive oxygen species can cause

single-nucleobase lesions, single-strand breaks, double-strand breaks, and various oxidative damages such as base connections in the strand [26–28].

Contamination of soils with heavy metals and the accumulation of heavy metals in high concentrations in plants grown here have a genotoxic effect in plants and lead to mutation-like changes in the DNA profile. Therefore, a connection can be established between these changes in the organism and the intensity of pollution in the soil [29].

Sresty and Rao (1999) examined the ultrastructural changes in the nucleolus, nucleus, endoplasmic reticulum, and vacuoles in pea plant stem cells in response to zinc and nickel stress [30].

Zengin and Munzuroğlu (2004) observed the root, stem, and leaf growth in bean (*Phaseolus vulgaris* L.) seedlings exposed to lead and copper stress and examined which tissue was affected more in heavy metal stress [14].

Soudek et al. (2010) exposed flax (*Linum usitatissimum* L.) seeds to different concentrations of lead, nickel, copper, zinc, cadmium, cobalt, arsenic (As), and chromium heavy metals and examined the effects of heavy metal stress on plant germination and root development [31].

Öztürk Çalı and Candan have studied the effects of fungicide on the morphology and viability of pollens of tomato (*Lycopersicon esculentum* Mill.) [32]; the effect of activator application on the anatomy, morphology, and viability of tomato pollen [33]; and influence of activator on meiosis of tomato [34].

Candan and Öztürk Çalı (2015) have observed pollen micromorphology of four taxa of *Anemone coronaria* L. from western Turkey [35]. On the other hand, the authors have compared the pollen morphology and viability of four naturally distributed and commercial varieties of *Anemone coronaria* [36].

Some studies have used various methods based on single-cell gel electrophoresis (comet assay), micronucleus analysis, or cytogenetic analysis in order to investigate the genotoxic effects of pollution on plants.

Steinkellner et al. (1999) treated samples of *Tradescantia* sp. with water from seven regions of Austria where the water was exposed to industrial pollution, and they examined the chromosomal changes in the cells of the root region of the plant by micronucleus analysis. The authors reported negative changes in plant stem cells at the end of the study [37].

Menke et al. exposed the root area of *Arabidopsis thaliana* (L.) to different genotoxic effects. They examined the damage caused by the genotoxic effect in the plant by single-cell gel electrophoresis method and successfully demonstrated the mutagenic effect occurring in stem cell nuclei [38].

The *Comparison of Physiological, Biochemical and Molecular Parameters in Seedlings of Artichoke (Cynara scolymus L.) and Runner Bean (Phaseolus coccineus L.) Seeds Exposed to Lead (Pb) Heavy Metal Stress in the point of Ecological Pollution* was studied with the 2012-057 numbered project supported by Manisa Celal Bayar University [39]. Candan and Batır have presented this scientific important comparison at a conference after the project was completed [40]. On the other hand, Batır has studied on the thesis about determination of the DNA changes in the artichoke seedlings (*Cynara scolymus* L.) subjected to lead and copper stresses [41], and Batır et al. have written an article about that topic [42]. The original PCR photographs of some primers used related runner bean and artichoke samples are given below [39, 41] (**Figures 1–6**).

Today, especially due to increasing demand and changing climatic conditions, many studies have been carried out in plant biotechnology regarding more resistant agricultural plants against factors such as drought, salinity, freezing, and heavy metal contamination. Various biological, chemical, and physical methods are

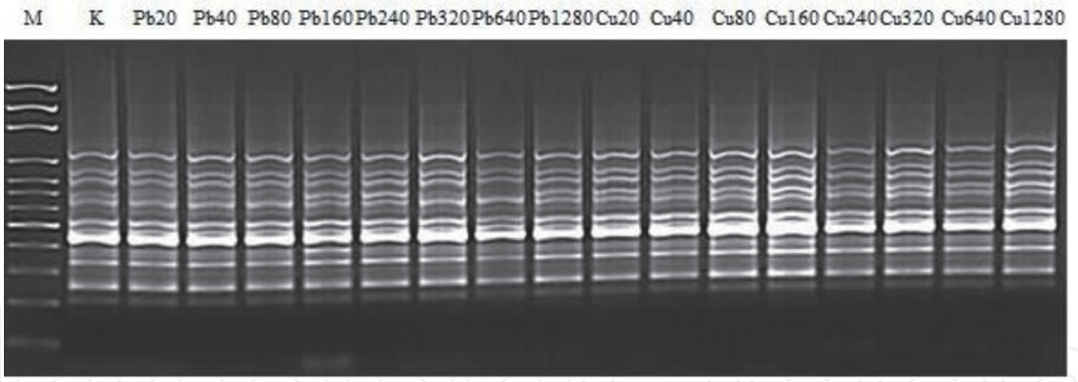

Figure 1.
PCR gel photograph of OP A03 primer used related runner bean samples [39].

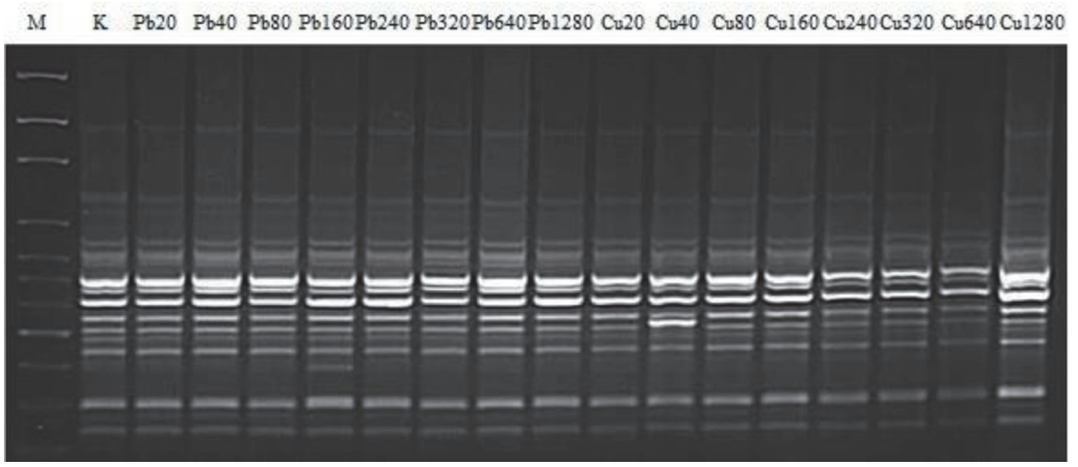

Figure 2.
PCR gel photograph of OP C05 primer used related runner bean samples [39].

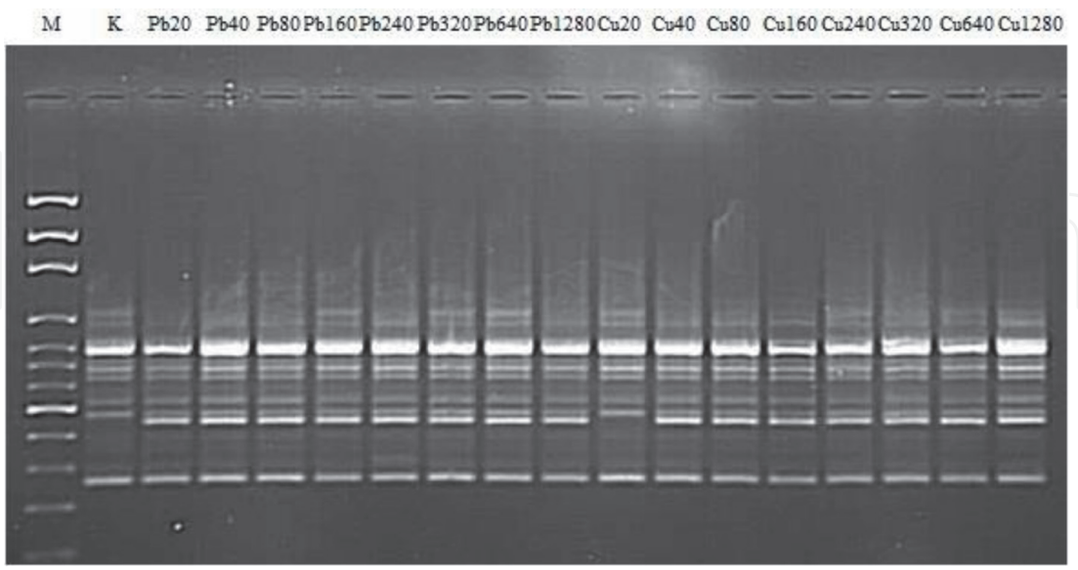

Figure 3.
PCR gel photograph of OP C20 primer used related runner bean samples [39].

available as regards obtaining biomolecules; for example, nanomaterials with their considerable reactions have much attention in biomass. The basis of this study is to determine the genotoxic effect levels of different stress factors on different plant

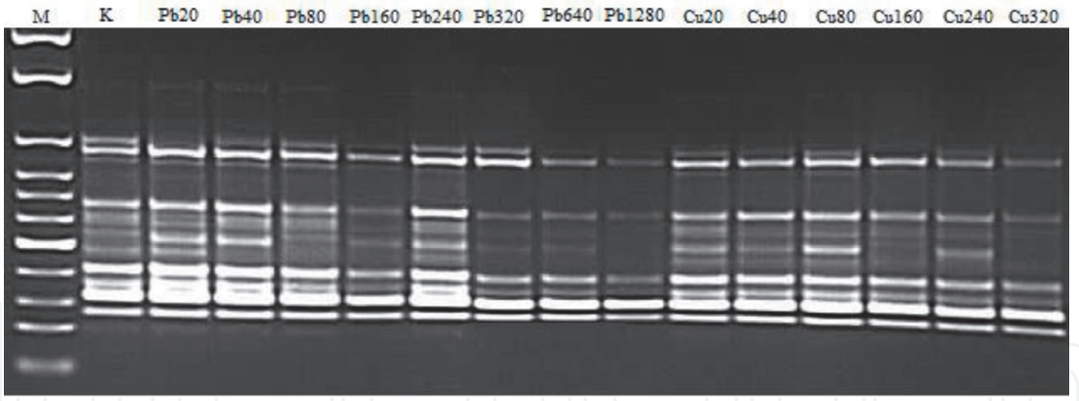

Figure 4.
PCR gel photograph of OP C03 primer used related artichoke samples [39, 41].

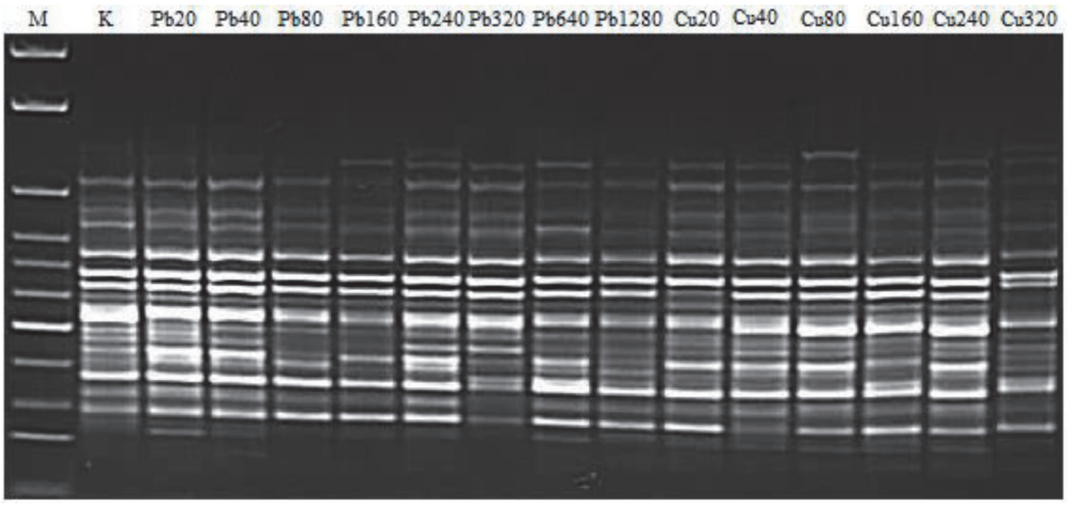

Figure 5.
PCR gel photograph of OP C05 primer used related artichoke samples [39, 41].

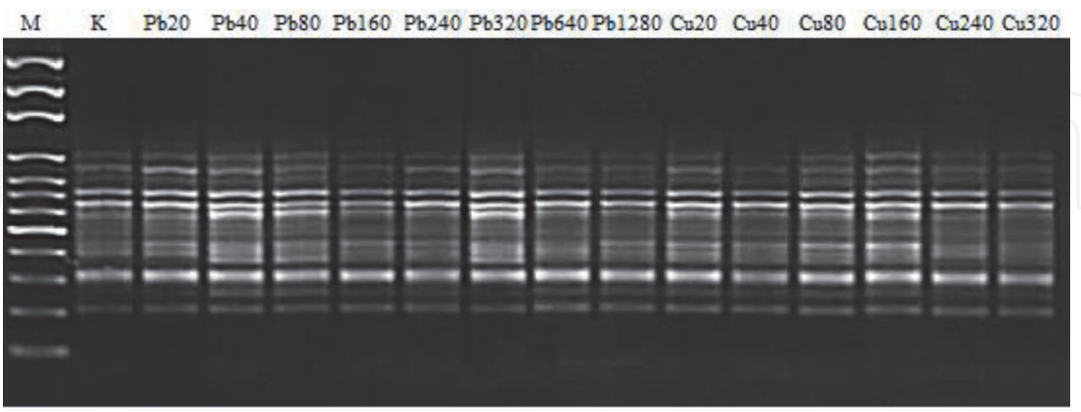

Figure 6.
PCR gel photograph of OP C18 primer used related artichoke samples [39, 41].

species. Until recently, the investigation of the effects of stress factors as heavy metals, nanoparticules, and nanoparticles on plant phenology remained at cellular, morphological, and anatomical levels.

3. Materials and methods

Fabaceae family to which runner bean and chickpea plants belong and Asteraceae family to which artichoke belongs were selected as the study material. Both of them are families with large numbers of economically important plants in Turkey as well as in the rest of the world. The flowers of the Fabaceae family are zygomorphic in shape and have legume and lomentum fruit. There are many flowers lined up on the flower tray (receptaculum) and head (capitulum) formed by the bracts surrounding these flowers, and there are achene type fruits in the Asteraceae family [43–46].

The aim of the present study was to investigate the effects of copper and lead heavy metals on runner bean (*Phaseolus coccineus*) and artichoke (*Cynara scolymus*) seedlings. In addition, the effects of Au nanoparticles and C_{70} single-walled carbon nanotubes on chickpea (*Cicer arietinum*) seedlings were investigated morphologically. The tolerability of heavy metal and nanoparticle effects by these plants, the cultivation in heavy metal or nanoparticle-contaminated areas, the morphological and anatomical reflections of the changes in genomes, and to which extent the plant's general structure are preserved compared to controls were evaluated in this way.

3.1 Germination and cultivation of runner bean and artichoke seeds

Runner bean and artichoke seeds were sterilized and then planted for growing. At least 20 seeds were included and observed in the control group and for each heavy metal application. Seeds were planted and germinated in viols with fine-grained perlite [47].

$CuCl_2$ $2H_2O$ and Pb $(CH_3COO)_2$ $3H_2O$ solutions were applied in concentrations of 20, 40, 80, 160, 240, 320, 640, and 1280 ppm to the runner bean and artichoke seeds planted in groups of 3(Cu 20, Cu 40, Cu 80, Cu 160, Cu 320, Cu 640, Cu 1280 and Pb 20, Pb 40, Pb 80, Pb 160, Pb 320, Pb 640, Pb 1280). This procedure was repeated for 21 days. The seeds of the control group were planted and irrigated with distilled water. As a result, seedlings of the control group and those subjected to Cu-Pb heavy metal stress were obtained after 21 days [47] (**Figures 7, 8**).

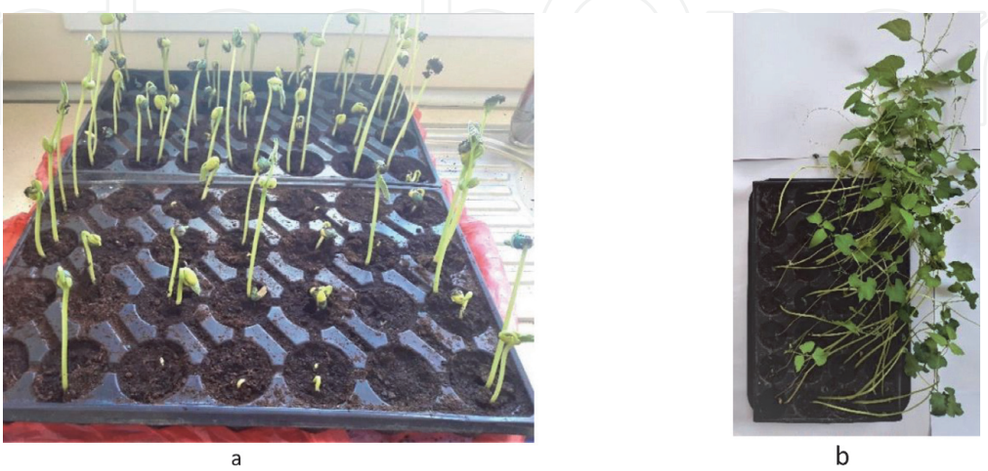

Figure 7.
Runner bean seedlings treated with Pb grown in viol. (a) general view at development phase. (b) general view after development.

Figure 8.
Artichoke seedlings treated with Pb grown in viol. (A) General view. (b) Close-up view of samples treated with 20 ppm Pb.

Figure 9.
General view of the control group grown in the climate cabinet, chickpea seedlings treated with Au NPs and C_{70} SWNTs and pots with late germination.

3.2 Germination and cultivation of chickpea seeds

Twenty chickpea seeds were exposed to 4 ml Au NPs and C_{70} SWNTs and 15 ml deionized water mixture for 2 days, and they were grown in pots with perlite for 21 days in two groups. The control group of 20 seeds was also grown in other pots with perlite. All the plants in this group were watered every day in the morning only with water. Au NPs and C_{70} single-walled carbon nanotubes exposed to chickpea plants and control group were taken from the pots after 3 weeks, and herbarium materials were made. On the other hand, some of them were stored at 70% alcohol for microscopical investigations [48] (**Figure 9**).

3.3 Methods used to obtain anatomical data

Cross sections were taken from taxa to determine and compare the characteristics of root and stem anatomy of runner bean and artichoke seedlings exposed to Cu and Pb heavy metal concentrations. While determining the samples from seedlings to take sections, 3–10 mm from the end of the roots and the middle part of the body above the ground were used. These fragments were used for sectioning with

microtome by the paraffin method. All plant samples were subjected to various treatments to make the sections suitable for microtome removal [47].

These operations were carried out on the samples taken from the abovementioned parts of the plants retained in 70% alcohol. These parts were passed through 80, 90, and 100% alcohol and 2 alcohol/1 xylol, 1 xylol/1 alcohol, 1 alcohol/2 xylol, and 100% xylol solutions, in this order. The paraffin was allowed to penetrate the interior of the samples which were kept in the laboratory drying oven at 60° for 48 hours. Sections of 20, 25, 30, 35, and 40 μm used in the investigations were obtained via samples placed in paraffin blocks. The sections were placed on slides properly by using a hot water bath set at 40 °, and they were fixed onto the slides with adhesive. Sections were cleared off paraffin using 100% xylol, 1 xylol/1 alcohol, absolute alcohol, 95% alcohol, 80% alcohol, 70% alcohol, and purified water, in this order, for 5 minutes each, and then they were stained with safranin and fast green. Samples were kept in pure water, 70% alcohol, 80% alcohol, 95% alcohol, absolute alcohol, 1 xylol: 1 alcohol, and 100% xylol, for 1 minute each, so that water removal from the tissues was completed [49–51]. After removal of all the water, the preparations which were made permanent using Entellan and allowed to dry for 4–5 days at room temperature were examined in general. In this way, the reaction of the samples (with different concentrations of heavy metal in the cells) to dyes and the possible staining status were determined.

During the examination of the sections, the treatment of plant tissues with dye has caused a problem since the samples contain heavy metals, such as copper and lead, and they affect the physiology of the plant. However, it is known that in permanent preparations, an artificial appearance is obtained by losing some of the chemical content of the plant material and pigments due to the fact that the plant materials pass through a considerable amount of chemical stages. Therefore, it has been stated in some studies that permanent preparation methods are not suitable for some plants [52, 53]. It was also tried to make hand cross sections on artichoke and runner bean samples. The anatomical features of the taxa were evaluated and interpreted according to Carlquist, Fahn, and Yentür [2, 54, 55].

Dyes were prepared using different ratios of safranin and fast green, and all of them were tested for staining of heavy metal-treated anatomical specimens in accordance with the literature [50, 51], and examinations were performed on the specimens deemed appropriate.

Furthermore, objectives of 4, 10, 20, and 40 were used for examination and photographing the anatomical structures of the root and stem of the taxa. Runner bean and artichoke root and stem photographs were taken with 4, 10, and 20 objectives, and the unit of measurement was determined as 100 (μm).

4. Results

4.1 Morphological observations

Runner bean seeds were germinated at all concentrations of Cu and Pb. However, there wasn't any germination observed in 640 and 1280 ppm concentrations of Cu in the artichoke seeds, but germination occurred in all concentrations of Pb [39, 47].

It was determined in the morphological observations of runner bean and artichoke plants subjected to heavy metals that significant differences occurred in different doses of phytotoxic effects of Cu and Pb [47].

The control group samples of the runner bean and artichoke plants were photographed in order to compare the heavy metal phytotoxic effects on the

morphological characteristics of the plants. Moreover, the general appearance and close-up photographs of the samples related to runner bean plant irrigated with Cu 20 ppm and Pb 160 and Pb 1280 ppm concentrations and artichoke plant irrigated with Cu 20 and 40 ppm and Pb 20 and 40 ppm were taken (**Figures 10–15**).

Cu and Pb heavy metals caused various phytotoxic effects in cases where the recommended dosage was exceeded or excessive pollution occurred in any other way was determined as the result of the study when the changes in the morphological structures of the plants were examined. Phytotoxicity seen in the morphological structure of the plant emerged as bending, shrinkage, and dark spots on the end of the leaves. On the other hand, while plant root, stem, and leaf lengths increase in low doses, high concentrations (640–280 ppm) cause size reduction and incomplete development [47] (**Figures 10–15**).

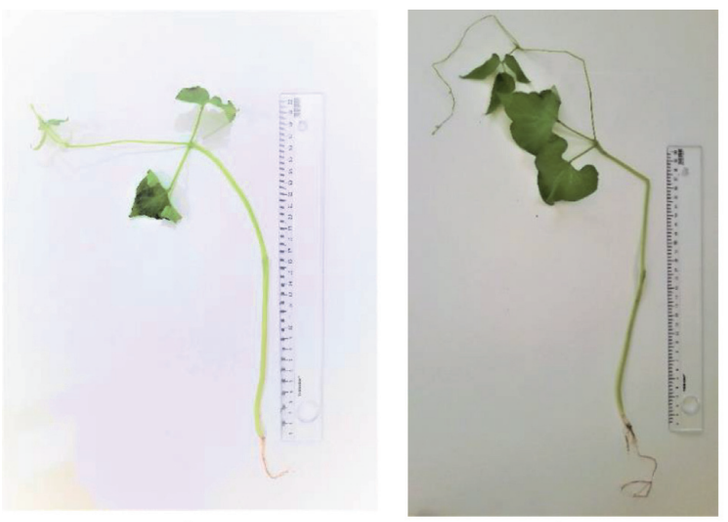

Figure 10.
(a) Control group of runner bean seedling. (b) runner bean seedlings treated with Cu 20 ppm [47].

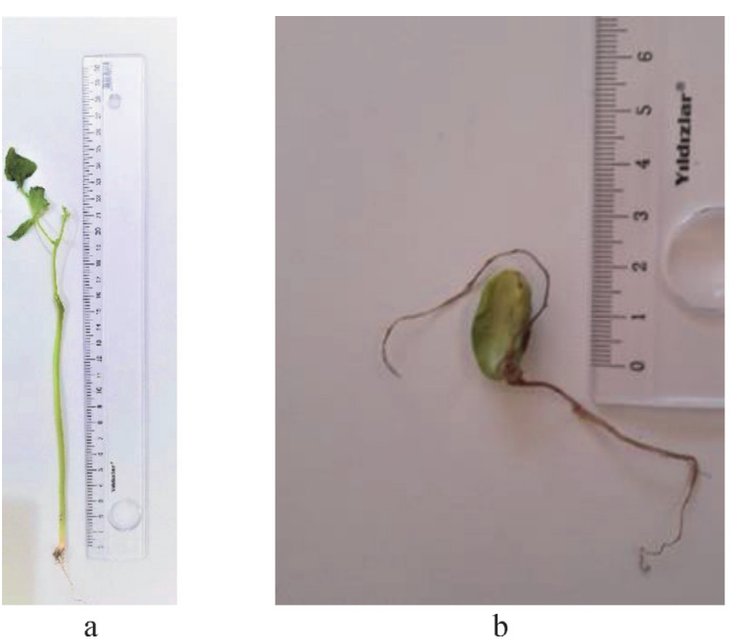

Figure 11.
(a) Runner bean seedling treated with Pb 1280 ppm. (b) Undeveloped runner bean seed treated with Pb 160 ppm [47].

Figure 12.
(a) Runner bean seedlings treated with Pb 1280 ppm. (b) General view of anomalies in terms of shape, chlorosis, and hole [47].

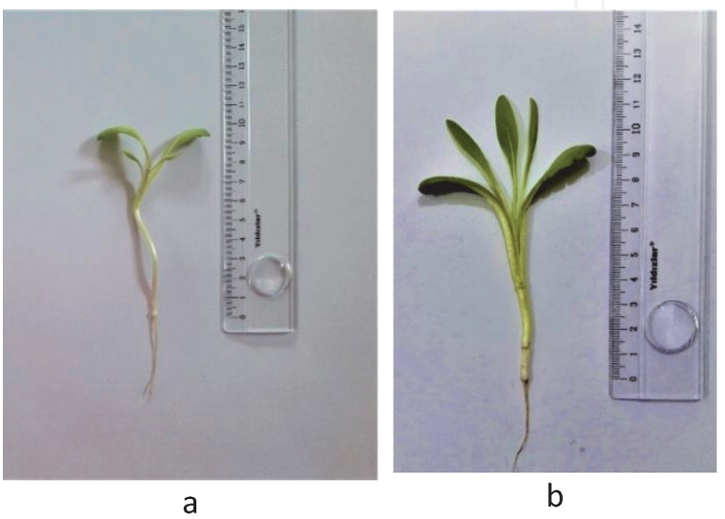

Figure 13.
(a) Artichoke seedling of control group. (b) Artichoke seedling treated with Cu 20 ppm [47].

Figure 14.
Artichoke seedling treated with Cu 40 ppm. (a) General view. (b) Close-up view of the dried leaves [47].

It was observed that there was an increase in plant root, stem, and leaf sizes in both treatment groups when chickpea plant control group and the plants subjected to Au Nps and C_{70} SWNTs were compared. Furthermore, it was determined that there were increases in the number of fibrous roots, nodes, and subbranches in both groups (**Figure 16**).

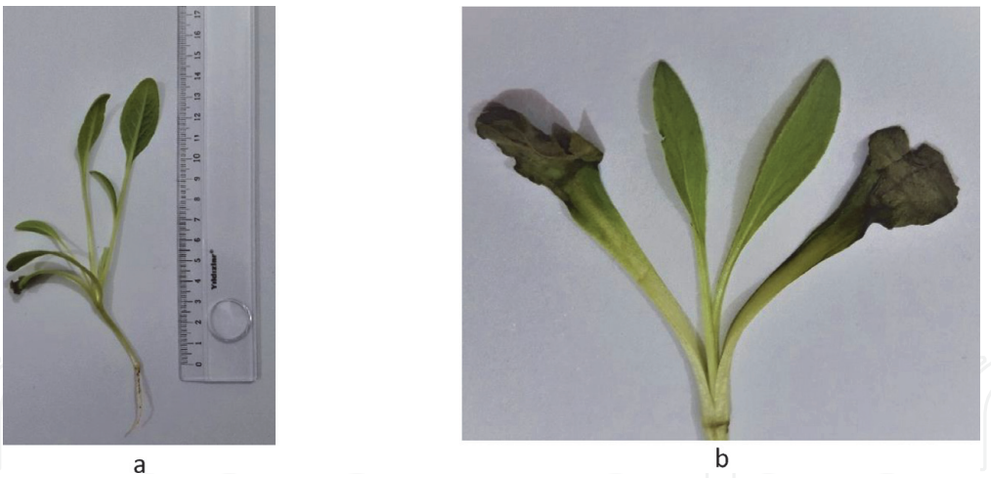

Figure 15.
Artichoke seedling treated with Pb 40 ppm. (a) general view. (b) close-up view of the dried leaves [47].

Figure 16.
(a) Chickpea seedling of the control group and the sample treated with Au NPs. (b) Chickpea seedlings treated with C_{70} SWNTs.

4.2 Anatomical observations

Micrometer was selected as the unit for measurements taken from root and stem cross sections of the runner bean and artichoke seedlings. Cross sections taken from the roots and stem parts of the plants were considered suitable for evaluation. The roots and stems, epidermis, vascular bundle elements, secretary canals, sclerenchyma, starch sheath, cortex and pith cells, and cambium cells were measured, and the presence and variety of crystals were examined and compared [47].

Photographs of root and stem cross sections of the runner bean and artichoke plants were taken in order to compare the anatomical effects of heavy metal phytotoxic effects on the morphological characteristics of the plants. Runner bean root cross-sectional photographs were taken from root samples subjected to Cu 80 ppm and 640 ppm, and Pb 640 ppm concentrations and stem cross-section photographs

were taken from the samples subjected to Cu 20, 80, and 640 ppm concentrations. Artichoke seedlings of root cross sections treated with Cu 160 ppm and Pb 320 and 640 ppm concentrations and stem cross sections treated with Cu 20 and 160 ppm and Pb 1280 ppm concentrations were examined [47] (**Figures 17–25**).

However, diseases caused by heavy metal stress in the plant, such as chlorosis and necrosis, and epidermal thickening, density of crystallization, increase in hairiness, and thinning in vascular bundles had negative effects on staining in anatomical studies and caused the tissues not to absorb the dye. Furthermore, the presence of heavy metals in the plant content and crystallization prevented the retention of the dye and made staining process difficult. Thus, a large number of experiments with different dyes and dye concentrations have been carried out for the tissue to absorb the dye into the cell [47].

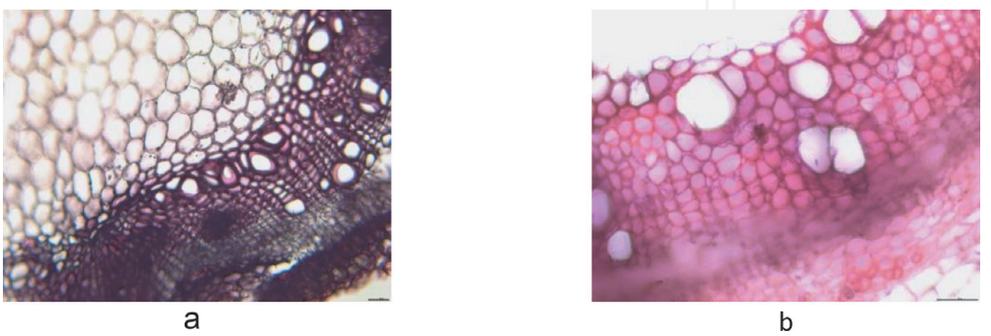

Figure 17.
(a) Control group of runner bean seedling root cross section; vascular bundles, cambium, and glandular primordium. (b) Cross section of runner bean seedling treated with Cu 80 ppm; vascular bundles, cambium, sclerenchyma, endodermis, pericycle, and casparian strip [47].

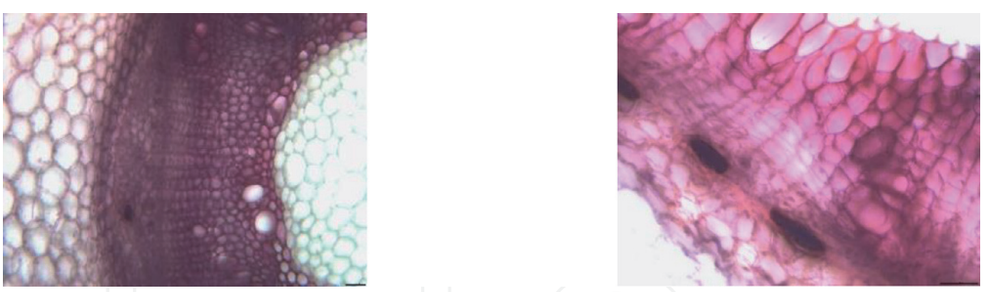

Figure 18.
(a) Runner bean seedling treated with Cu 640 ppm: (a) general view of root cross section, vascular bundles, endodermis, pericycle, and cambium. (b) Close-up view of root cross section, secretion canals, cambium, endodermis, casparian strip [47].

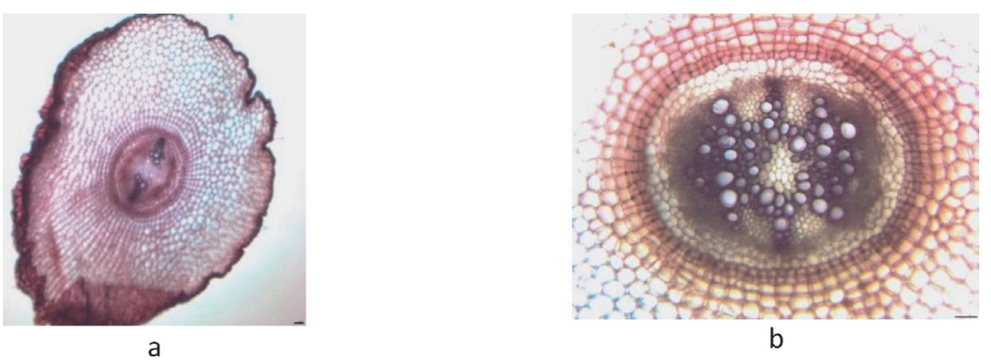

Figure 19.
(a) Control group of artichoke seedling root cross section. (b) Cross section of artichoke seedling treated with Cu 160 ppm root cross-section central cylinder and conduction bundles [47].

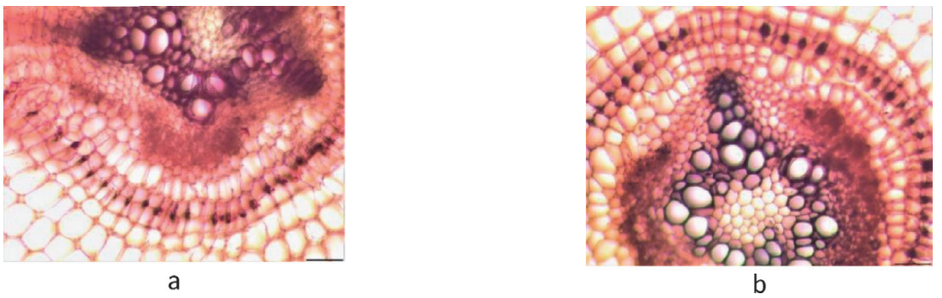

Figure 20.
(a) Close-up view of artichoke seedling root treated with Pb 320 ppm: Central cylinder, endodermis, pericycle, and crystals. (b) Close-up view of root artichoke seedling treated with Pb 640 ppm: Vascular bundles and crystals [47].

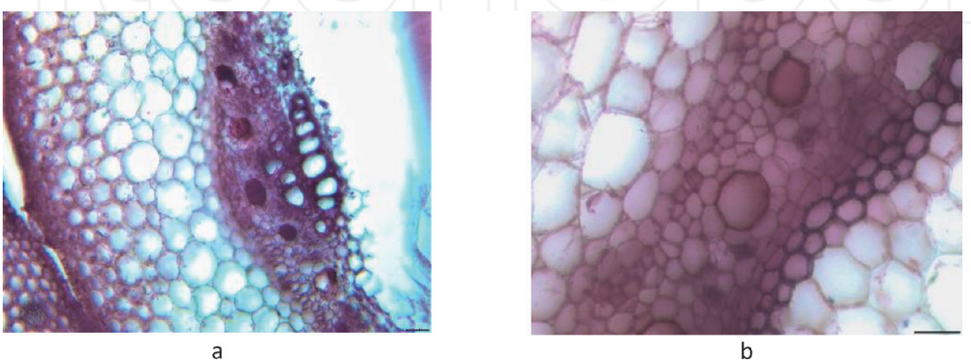

Figure 21.
(a) General view of control group runner bean seedling stem cross section; close-up view of epidermis, cortex bundles, and secretion canals. (b) Close-up view of runner bean seedling stem treated with Cu 20 ppm: Xylem, phloem, secretion canals, and starch scabbard [47].

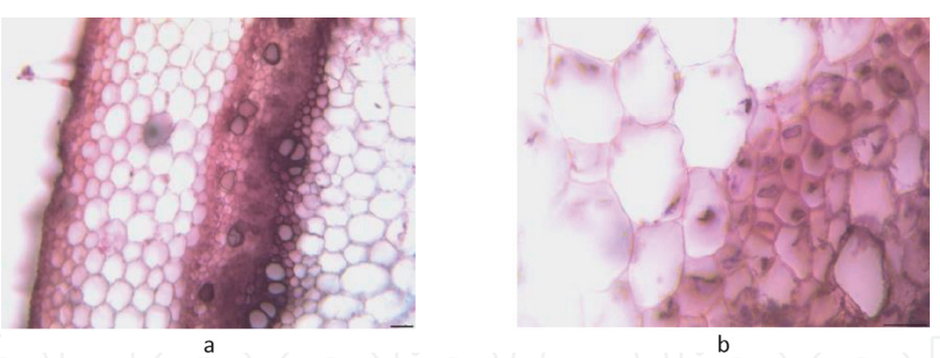

Figure 22.
(a) General view of runner bean seedling stem treated with Cu 80 ppm: Vascular bundles, secretion canals, and crystals. (b) Close-up view of runner bean seedling stem treated with Cu 640 ppm: Secretion canals and crystals [47].

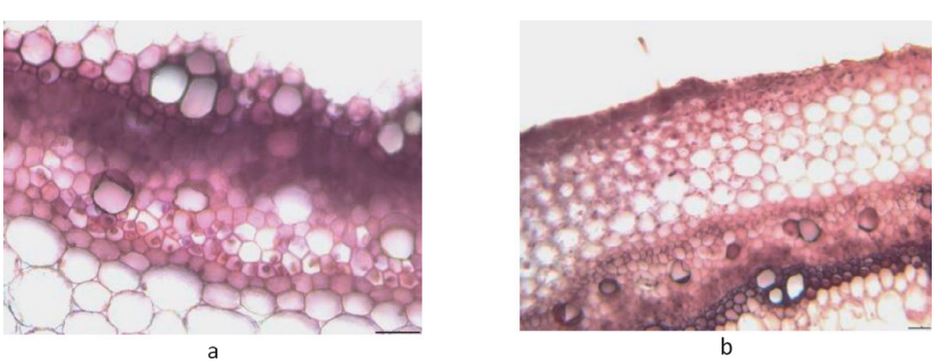

Figure 23.
(a) Close-up view of runner bean seedling stem treated with Pb 40 ppm: Xylem, phloem, secretion canals, and crystals. (b) General view of runner bean seedling stem treated with Pb 640 ppm: Vascular bundle, secretion canals [47].

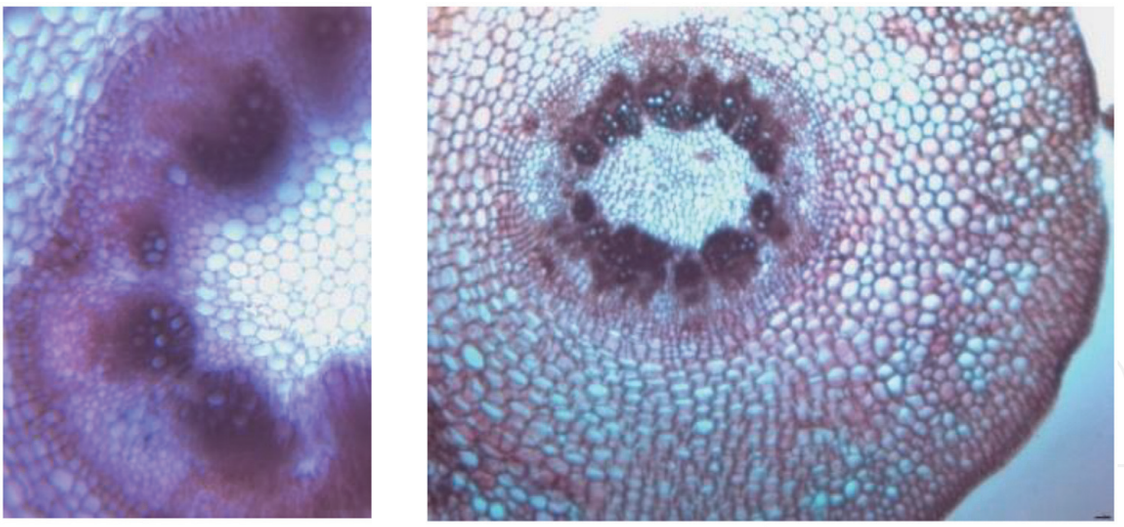

Figure 24.
(a)general view of artichoke seedling stem treated with Cu 20 ppm stem: Vascular bundles and (b) general view of artichoke seedling stem treated with Cu 160 ppm.

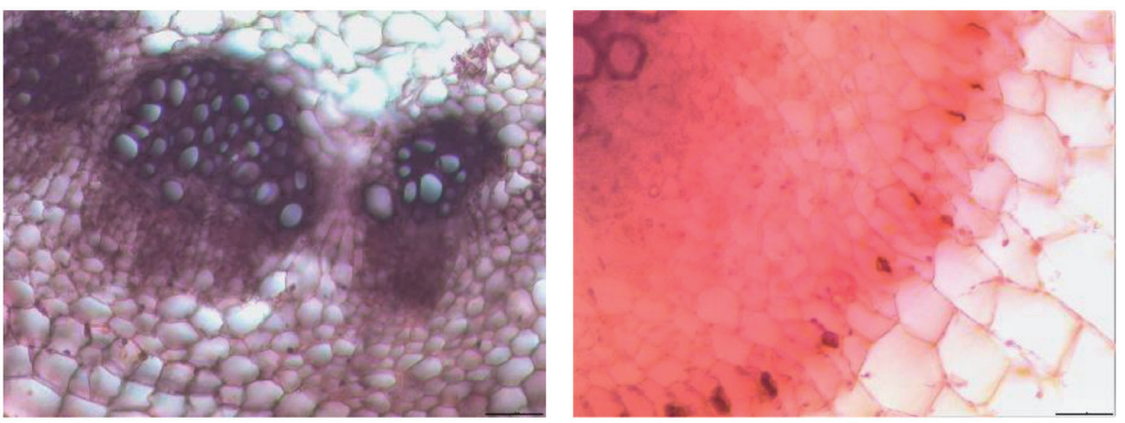

Figure 25.
(a) Close up view of artichoke seedling stem treated with Cu 160 ppm:Xylem, phloem, and crystals and (b) close up view of artichoke seedling stem treated with Pb 1280 ppm cortex and crystals.

5. Discussion

Heavy metal pollution in soil and water is one of the most important environmental problems in industrialized countries. Various heavy metals such as cadmium, lead, copper, mercury, and chromium from various industrial establishments such as leather, paint, fertilizer, textile, cement, and chemical industry are released onto soil and aquatic environments and cause environmental pollution [56–58]. Since most of the heavy metals do not undergo biodegradation in the environment, they can easily accumulate and increase their toxic effects on living things by forming very complex structures [59].

High-structured plants are equipped with advanced features that allow them to adapt to changes in nature, one of which is the retention of metals in the roots [60]. Retention and deposition of metals in roots have more negative effect on the root area and seed germination than stem and leaf growth. Zengin and Munzuroğlu (2004) reported that the most negative effect was in the root area of the bean (*Phaseolus vulgaris*) seedlings exposed to increasing concentration of lead and copper solutions; stem and leaf growth was negatively affected; however, they stated that the most negative effect was in the root area of the seedlings [14].

Soudek et al. (2010) treated linen (*Linum usitatissimum* L.) seeds with different concentrations of lead, nickel, copper, zinc, cadmium, cobalt, arsenic, and chromium heavy metals and reported that heavy metal stress had negative effects on the number of germinating seeds and seedling root development. The negative effect of heavy metal stress on root length in plants may result from the division of cells in the root region or the prolongation of the cell cycle [31]. The root, stem, and leaf structures of the runner bean and artichoke seedlings grown in high Cu and Pb concentrations (160, 320, 640, 1280 ppm) examined in this study were degraded as a result of oxidative damage. Therefore, the epidermal cells forming the surface of these parts were damaged, which negatively affected root, stem, and leaf growth. However, it also caused adverse conditions such as dryness, shrinkage, and necrosis in the leaves although Cu and Pb heavy metals applied at low concentrations generally stimulated growth and increase the number of leaves in the plant [39, 47]. Furthermore, browning caused by heavy metal stress was observed in the roots of the runner bean and artichoke seedlings in which high concentrations of heavy metals were applied. This color change occurs with the increase in the amount of suberin in stem cells. Therefore, suberin stem cells will limit the uptake of water, and plant growth inhibition occurs [61, 62].

The defense mechanisms developed by plants against heavy metal stress may vary in the level of family, genus, species, subspecies, and variety [54–57]. The defense mechanisms that allow plants to be tolerant to heavy metals have not yet been fully understood. However, the mechanisms of tolerance include vacuolar phenomena [58], enzymatic and nonenzymatic antioxidant systems [59, 60], metal-binding ligands such as metallothionein [61], and alternative oxidase pathways [62].

Although copper is an essential element, it is more toxic when it is present in high doses compared to cadmium, a nonessential element. This is explained by the direct influence of copper on the formation of reactive oxygen species [superoxide radical ($O_{2}\cdot-$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH-$)] because it is a trace element. Since copper and iron transition metals are involved in oxidoreduction reactions, they act as catalysts that accelerate the formation of reactive oxygen species [63–66]. As in previous studies, it was found that artichoke seedlings are negatively affected mostly by copper in molecular terms [39, 41, 67, 68]. The highest negative effect was observed in groups subjected to copper solutions in terms of root length, root dry weight, total soluble protein amount, and genomic mold stability [39–42] (**Figures 17–22**).

Reactions to heavy metal stress in this study emerged as different anatomical results in both plants. However, it can be determined that the response of copper-treated samples was not always greater than that of the lead-treated samples even though they responded differently to different concentrations in terms of anatomical results. Previously the effect rate observed at the molecular level in Cu-treated samples has been found to be higher than the effect rate in Pb-treated samples in both plants [39–42]. However, it was not the case for the present anatomical study because the roots and stems of the plants were examined in terms of many parameters, so heavy metals did not show the same effect [47] (**Figures 17–25**).

It was observed that seedlings belonging to runner bean species showed high tolerance against lead and copper stress. The genome of the plants was preserved at 94–95%, and no significant reduction in total soluble protein was observed especially at 1280 ppm concentration of lead and copper solutions. This situation has led to the conclusion that runner bean species has a strong defense mechanism against heavy metal contamination [40–42, 49]. In this anatomically based study, it would be wrong to say that one heavy metal is always superior to another in terms of its effects because plant parts were examined anatomically in terms of many parameters and similar results were not observed in all. The reaction of the plants as

crystallization is particularly important and has shown similar responses in both heavy metal treatments. The differences varied in terms of crystal density, location, and shapes (**Figures 17–25**) [47].

As a result, various anatomical differences determined in this study regarding the characteristic features, such as root and stem vascular bundles thicknesses, cell size and fragmentation in the pith region, formation or thickening of cambium and sclerenchyma, shape and size of secretary canals, differences in cortex cell sizes, the sizes of the vascular bundles, the formation of crystals and deposits in the phloem layer according to the heavy metal concentration applied, the number of epidermis cells per unit area, and the epidermal wall shapes, can be used to reveal the phytotoxic effects of Cu and Pb heavy metals.

The morphology of roots and shoots is extremely important for the growth and development of all plants, and each factor that changes their morphology has positive/negative effects [69].

On the other hand, Candan and Lu (2017) have shown that there are more differences on the pea green (*Pisum sativum*) anatomy under the effects of C_{70} nanomaterial [70]. Candan and Markushin have studied about spectroscopic study of the gold nanoparticles (Au NPs) distribution in leaf, stem, and root of the pea green plant [71]. In this chapter, the effects of Au nanoparticles and C_{70} nanotubes on the morphology of roots, leaves, and stems are investigated, and positive results on the development of chickpea have been observed (**Figure 16**).

6. Conclusion

It is the fact that the application of some materials which are not used consciously or at the recommended dosage and which contain heavy metals in order for the crop to be attractive for the consumers actually yields negative results. Therefore, this study is important about examining the extent of heavy metal phytotoxic effects related determining them on plants phenological development in the point of morphologically and anatomically changes. The information given in this study is valuable as it presents the negative molecular effect of heavy metal pollution on the plant in terms of morphological and anatomical aspects. Furthermore, this study will guide the researchers on the effects of environmental pollution in relation with the phenological development of economic plants and hence on human health.

Heavy metal and nanoparticule-nanotube-induced plants must be evaluated in the point of biochemistry and examined via scanning electron microscope (SEM) and transmission electron microscope (TEM) regarding their root, stem, and leaf structure and apical tip, leaf-bud primordiums, and provascular tissue in detailed ways for interdisciplinary studies according to plants' phenological development progress.

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