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Article

Influence of Irrigation with Oxygen Plasma Treated Metal Contaminated Water on Plant Growth

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Abstract: This study aimed to evaluate the effects of plasma treated metal contaminated water, used for irrigation, on plant growth. Zinc (Zn) is one of the more commonly used metals. It can enter the environment from industrial processes as particles released into the atmosphere or as wastewater discharged into waterways or the ground. Exposure to large amounts of zinc, even for a short time, can seriously affect human health. In this experiment, three different DBD O_2 plasma treated zinc contaminated water and a control (tap water) were used, with *Arabidopsis thaliana* as the model plant. The treatments were: i) Control, ii) Zn water, iii) Zn+O_3(30 min), and iv) Zn+O_3(60 min). *Arabidopsis* plant exhibited maximum growth in the Zn+O_3(30 min) treatment. All growth parameters, except leaf area, followed this trend: Zn+O_3(30 min) > Control > Zn water > Zn+O_3(60 min). Gene expression analysis revealed that reduced metal ion stress and controlled oxidation due to active oxygen species contributed to favorable/improved growth of *Arabidopsis* in the Zn+O_3(30 min) treatment. Therefore, 30 minutes of DBD O_2 plasma treated zinc contaminated water [Zn+O_3(30 min)] can mitigate the adverse effects of excess zinc ions and promote the growth of *Arabidopsis* plants.

Keywords: metal dissolved water; DBD; plasma irradiation; ozone; growth performance; gene expression analysis

1. Introduction

The increase in human population has placed a demand for an increased food supply [1]. This has resulted in increased use of pesticides, fertilizers, manures, composts, and wastewater for irrigation. Municipal and industrial wastewater and related effluents have been applied to the land for more than 400 years and now is a common practice in many parts of the world [2]. It is estimated that approximately 20 million hectares of arable land around the world are irrigated with wastewater. In several Asian and African cities, studies suggest that agriculture based on wastewater irrigation accounts for 50 percent of the vegetable supply to urban areas [3]. Although the metal concentrations in wastewater effluents are usually relatively low, long-term irrigation of land with such can eventually result in heavy metal accumulation in the soil. Food crops are grown in metal contaminated soil and by wastewater irrigation can uptake and accumulate metals in quantities high enough to affect food quality and safety. Nowadays, food contamination with heavy metals and their harmful impacts on human and environmental health is a major challenge in many countries. Many studies have shown that heavy metals and metalloids can disturb human metabolomics and contribute to increased morbidity and even mortality [4–6]. In addition, heavy metals are non-biodegradable and, in many cases, can be carcinogenic [7–12]. The degree of toxicity of selected heavy metals to humans varies as follows: Co < Al < Cr < Pb < Ni < Zn < Cu < Cd < Hg, and the toxic effect on humans depends on many variables such as the type of heavy metal and type of compound, and its solubility, dose, method and time of exposure [13]. Therefore, removing heavy metals from water is crucial for the protection of human health and the environment.

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Zinc (Zn) plays a substantial role in many biological processes and is an essential trace element for the proper growth and reproduction of plants and the health of animals and humans; it has also been reported to cause contamination of soil, water, and food chains [14–16]. Zinc enters the environment as a result of both natural processes and human activities. Most zinc is introduced into water by artificial pathways and may enter from numerous sources including mine drainage, industrial and municipal wastes, urban runoff, coal-fired power stations, and burning of waste materials, but the largest input occurs from the erosion of soil particles containing Zn [17,18]. High water Zn levels can be an indication of Zn pollution, from e.g. industrial processes of Zn leaching into the groundwater which may seriously affect human health. According to the Food and Agricultural Organization (FAO) and World Health Organization (WHO) drinking water containing Zn > 3.0 mg/L tends to be opalescent, develops a greasy film when boiled, and has an undesirable astringent taste [19]. Recent studies have shown that polluted waters or industrial wastewaters may contain varying amounts of zinc, for example, 52.8 mg/L (electroplating company) [20], 10 mg/L, being approximately 80% and 20% in the form of Zn^{2+} and $ZnSO_4$ (aq), respectively [21], 33.3 g/L (spent acid solution from the pickling stage of a galvanizing plant) [22], 1392.1 mg/L (zinc plating industry) [23], 22.7 mg/L (galvanic wastewater) and 49.8 mg/L (zinc electroplating industry) [25]. It has been found that the long-term effects of irrigation with wastewater might include pollution of groundwater and soil with heavy metals including Zn ions [26]. The recommended maximum content of Zn in irrigation water is 2.0 mg/L, since above this limit Zn is toxic to many plant species [27] and can pollute water aquifers [26]. Exposure to large amounts of zinc, even for a short time, can cause stomach cramps, nausea, and vomiting. In the long term, exposure to zinc may cause serious health issues that include but are not limited to anemia, pancreas damage, and the decreasing of HDL cholesterol; however, zinc in trace amounts is essential for human health [28]. The toxicological properties of zinc and its compounds, the possibility of migration, and the risk of environmental pollution make it necessary to remove zinc ions from polluted waters and wastewater before they are released into the environment.

The removal of contaminants from wastewater in modern treatment plants is often incomplete. Cold plasma is considered a promising remediation method considering its environmental compatibility, high contaminants removal, and high energy efficiency of the process. Cold atmospheric plasma (CAP) is an advanced oxidation process (AOPs) that has been considered an advantageous and promising remediation technique for both water and soil [29–34]. Non-thermal plasma or cold plasma was produced with numerous methods [35]. Among these methods, dielectric barrier discharges (DBD) and corona discharges (CD) have shown the highest efficiency in water treatment [35]. However, most studies have focused on using low-temperature plasmas to be inactivated microorganisms and to decompose organic compounds for wastewater treatment [35–39]. Dielectric barrier discharge has been commonly generated with the coaxial electrode configuration for water treatment. During the DBD occurs using atmospheric air, energetic free electrons, ultraviolet (UV) light, and a variety of active species are produced in the electrode gap [36]. These active species such as oxygen radicals, OH radicals, and ozone would also oxidize metal ions in liquid effectively. Among these species, ozone is one of the relatively stable active species. Oxidation of the heavy metal ions using oxidation reagents sometimes produces deposits of metal oxide [40–43] and these metal oxide deposits can be removed from the water easily using filtration or deposition [44–47]. Research has been carried out using the DBD (dielectric barrier discharge) oxygen plasma method on metal contaminated water and was confirmed the effective removal of metal (Zn) from contaminated water [48]. This study focuses on treating zinc contaminated water, used for plant cultivation, with ozone. However, no research has been conducted so far on how plasma treated metal contaminated water affects plant growth. In this experiment, metal ions dissolved in water were converted to metal oxides using ozone oxidation treatment. The treated water was then used to irrigate plants to determine the impact of metal ion removal on their growth. Therefore, the objective of this experiment is to observe the effect of DBD oxygen plasma treated zinc contaminated irrigation water on plant growth.

2. Experimental Apparatus and Methods

To determine the effect of oxygen plasma treated Zn contaminated water on plant growth, three different plasma treated zinc contaminated water and a control were used in this experiment. The treatments were: i) Control [tap water], ii) Zn water [zinc contaminated water], iii) Zn+O_3(30 min) [30 minutes of O_2 plasma treated Zn contaminated water], and iv) Zn+O_3(60 min) [60 minutes of O_2 plasma treated Zn contaminated water]. These different treated waters were used to irrigate the plants to observe the effects on growth performance.

2.1. Preparation of Irrigation Water

Zn water (zinc contaminated water) was produced by electrolysis using cylindrical electrodes of 5 mm in diameter and 30 mm in length, which were made of pure zinc. The amount of water used to prepare this metal contaminated water was 0.2 L. When the DC voltage at 8 V was applied to one electrode then metal (zinc) ions dissolved in water. The density of zinc ions in water was obtained using the measurement reagent. The zinc was measured by the colorimetric method using a color-developing reagent for zinc ions [49]. The color variation of the color-developing reagent was quantified using a photonic multichannel spectrometer and converted into the zinc concentration using the standard curve of liquid color of zinc concentration. The concentration of Zn ion in zinc contaminated water was around 39 ppm at pH 10.06.

In this study, the DBD (dielectric barrier discharge) plasma method was used for ozone dissolution into zinc contaminated water. To prepare the oxygen plasma treated of zinc contaminated water, ozone oxidation of the zinc contaminated water was performed separately by the simple ozone bubbling in the water containing the metal ions, as shown in Figure 1. The amount of water was 0.2 L and there was no water flow in the water vessel. The ozone was generated by the torch-shaped dielectric barrier discharge while using pure oxygen gas with a flow rate of 1.0 L/min. The barrier discharge plasma torch used in this experiment has the shape of a cylindrical tube with dimensions of 100 mm in length and 4 mm in inner diameter, which was made from porous alumina. The cylindrical spiral-type discharge electrode was set along the inner wall of the alumina tube, and the copper film as the grounded electrode was wounded on the outer of the alumina tube. When the high voltage with 5 kHz was applied to the discharge electrode, the barrier discharge occurred on the inner surface of the tube, and the oxygen plasma was generated.

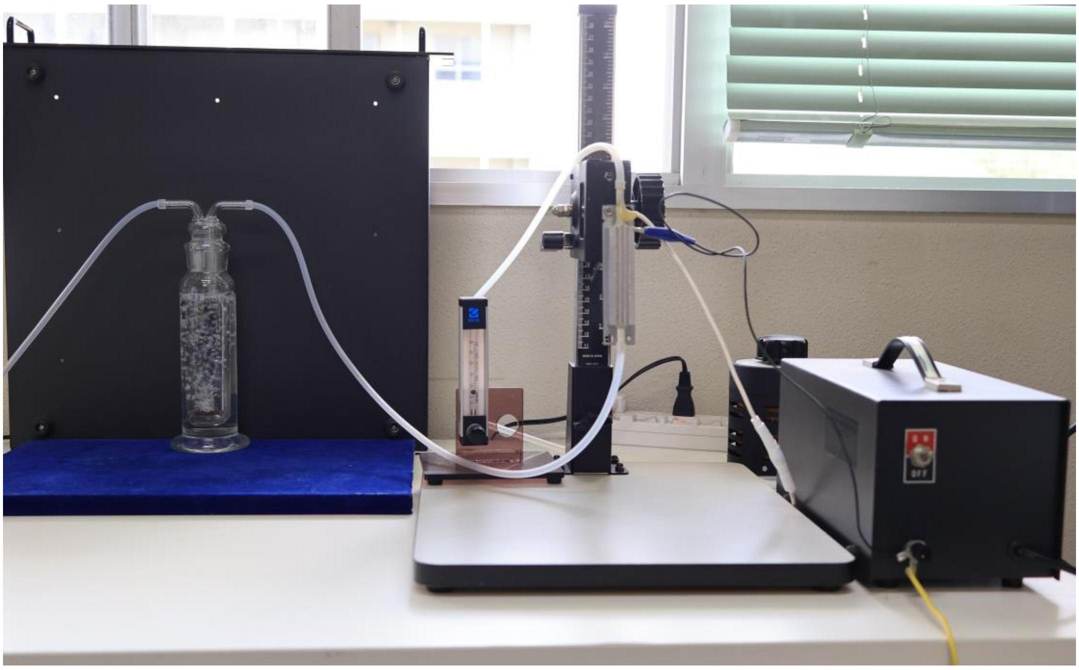

Figure 1. Experimental setup of ozone dissolution into zinc contaminated water.

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The ozone was produced by high-energy electrons in the plasma and was ejected from the opening edge of the tube by the oxygen gas flow. The produced ozone was transported into the water vessel and dissolved in water using a bubbler. Table 1 shows the experimental conditions of this study. The concentration of the gaseous ozone ejected from the dielectric barrier discharge (DBD) device was measured using the gas detection tube (KITAGAWA Gas Detector Tube System, Model AP-20) and was controlled by changing the discharge voltage. This concentration range was almost the same as that used for the practical ozone treatment of tap water.

Table 1. Experimental conditions of ozone dissolution in zinc contaminated water.

Type of water	Zinc contaminated water
Material Gas	Oxygen (O_2)
Gas flow rate	1.0 L/min
Irradiation period	0~120 minutes
Discharge voltage	5.0 kV
Input power	40 W
Frequency	3.5~5.6 MHz

Zn+O_3(30 min) [30 minutes of O_2 plasma treated Zn contaminated water] and Zn+O_3(60 min) [60 minutes of O_2 plasma treated Zn contaminated water] treated water was produced through 30 and 60 minutes of oxygen plasma treatment in Zn contaminated water, respectively. The ozone concentration in the Zn+O_3(30min) treated water was 0.58 ppm at pH 7.4, while in the Zn+O_3(60min) treated water, it was 0.77 ppm at pH 8.7. After treating the zinc contaminated water with oxygen plasma, it was transferred to a conical tube and centrifuged. The deposit accumulated at the bottom was then extracted from the water. Then this deposit-free treated water was used as irrigation water to assess its effect on plant cultivation. All the treated water was prepared three days before beginning irrigation to ensure that any residual ozone, with a lifetime of several hours, would not have an adverse effect on the plants.

2.2. Growth Performance Analysis

Arabidopsis thaliana (wild type, Columbia-01) served as the model plant in this experiment to assess the impact of plasma treated metal contaminated water on plant growth. Each treatment involved 5 pots, with 10 *Arabidopsis* seeds sown in each. After germination, one plant was allowed to grow in each pot. Plants were irrigated with treated water daily along with control measures. The plants were all cultivated in the same large plant incubator to ensure uniform environmental conditions. *Arabidopsis thaliana* cultivated after one month of growth following seed sowing. To assess growth performance, data from 5 plant samples of each treatment were averaged. Plant height and seed weight were measured, and leaf area was calculated using image processing software. Plant height was measured by scale measure and seed weight was measured in precision measure. When the leaf size grows to about 1 cm, select the 5th leaf from the one with the largest leaf area in each strain, that is, measure the area of a total of 5 leaves from 5 plants, and a simple average was obtained. The leaf area was measured from the captured images of leaves using the image processing software (Image J). The total area of 5 leaves was measured in descending order of area and evaluated using the average value. The standard error and error bars were calculated. The growth performance of the plants was assessed using plant height, seed weight, and leaf area as indicators.

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2.3. Gene Expression Analysis

To evaluate the effect of zinc contaminated water on plant growth after treatment with DBD O_2 plasma, the biological responses of plants were examined. One comprehensive method to understand these responses is through gene expression analysis. *Arabidopsis* leaves were selected for gene expression analysis after three weeks of growth, when they reached approximately 1 cm in size. The 5th leaf with the largest area from each treatment plant was collected, and RNA was extracted using RNA extraction reagent. The quality of the extracted RNA was confirmed via electrophoresis. Gene expression in the leaves was analyzed using the microarray method with a microarray scanner (AgilentSurePrintG3GE8x60Kv2). The gene data obtained were organized using functional annotation bioinformatics and pathway analysis methods with the Database for Annotation, Visualization, and Integrated Discovery (DAVID) (http://david.abcc.ncifcrf.gov/home.jsp) [50,51]. The significance level for this analysis was set at $p < 0.05$.

3. Results and Discussion

Arabidopsis thaliana was irrigated with various plasma treated zinc contaminated water alongside a control to observe their effects on growth performance. Various growth parameters were measured to assess the impact on physical appearance, and gene expression analysis was conducted to identify biological changes in the plants' genes.

3.1. Growth Response of Plant

Different growth parameters of *Arabidopsis,* such as plant height, seed weight, and leaf area, were observed. The average data from 5 plant samples of each treatment were used to measure the effects of oxygen plasma treated Zn contaminated water.

3.1.1. Visual Effect on Plant Growth

Plant growth of *Arabidopsis* was affected by different plasma treated zinc contaminated water. The maximum growth of the *Arabidopsis* plants was observed in 30 minutes of O_2 plasma treated zinc contaminated water [Zn+O_3(30min)], followed by control. The poorest growth was found in 60 minutes of O_2 plasma treated zinc contaminated water [Zn+O_3(60min)], followed by zinc contaminated water [Zn water]. The visual effects on the leaves of the *Arabidopsis* plant are shown in Figure 2. It is observed from this figure that most of the leaves turned reddish color and became distorted in the zinc contaminated water. The maximum number of green leaves was found in 30 minutes of O_2 plasma treated zinc contaminated water, followed by the control [tap water]. The trend of plant growth was as follows: Zn+O_3(30min) > Control > Zn+O_3(60min) > Zn water.

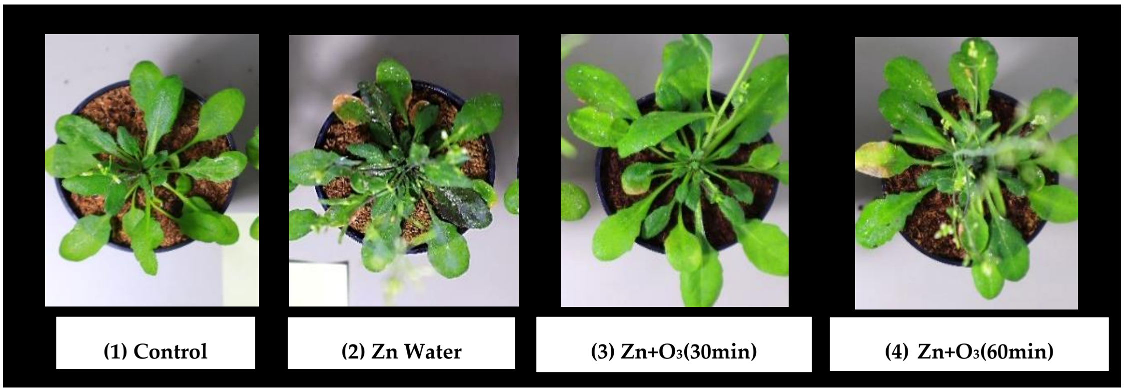

Figure 2. Effect on plant growth by irrigating with plasma treated zinc contaminated water.

From Figure 2, it is also observed that plant growth and leaf color are good in 30 minutes of O_2 plasma treated zinc contaminated water [Zn+O_3(30min)]. This improvement might be due to Zn+O_3(30min) mitigating the toxic effects of zinc on *Arabidopsis* growth. The second healthiest growth

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was found in the control, which used tap water. In contrast, 60 minutes of O_2 plasma treated zinc contaminated water [Zn+O_3(60min)] showed leaf damage. Poor growth with distorted leaves was observed in *Arabidopsis* plants irrigated with zinc contaminated water [Zn water]. The leaf distortion and poor growth can be attributed to the toxic effect of zinc.

3.1.2. Effect on Plant Height

Different plasma treated zinc contaminated water affected the growth of the *Arabidopsis* plant. The maximum plant height (36.75 cm) was observed in 30 minutes of O_2 plasma treated zinc contaminated water [Zn+O_3(30min)] (Figure 3). The second highest plant height was found in control, followed by zinc contaminated water [Zn water]. The lowest plant height (31.9 cm) was observed in 60 minutes of O_2 plasma treated zinc contaminated water [Zn+O_3(60min)].

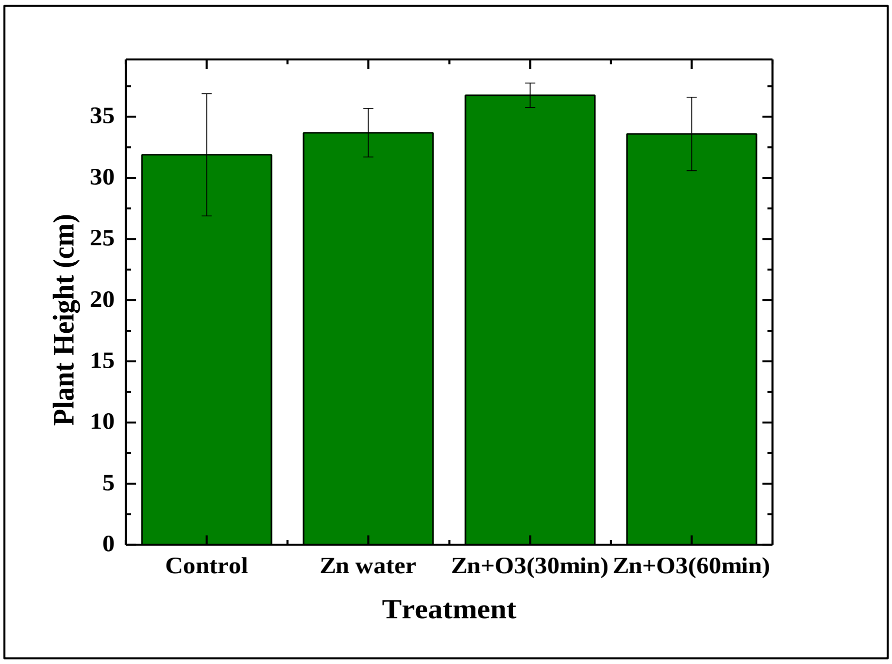

Figure 3. Effect on plant height by irrigating with plasma treated zinc contaminated water.

From Figure 3, it is identified that Zn+O_3(30min) treatment mitigates the negative effects of zinc on the *Arabidopsis* plant and showed the highest plant height. On the other hand, the lowest plant height was observed in Zn+O_3(60min) treatment, which may be attributed to both the effects of zinc and prolonged plasma exposure on *Arabidopsis*. In zinc contaminated water, the plant height was lower than in the control. This decrease might be due to the excessive zinc ion effect on plant growth, resulting in reduced plant height.

3.1.3. Effect on Seed Weight

Seed weight was measured in this experiment, and the maximum seed weight was found in 30 minutes of O_2 plasma treated zinc contaminated water [Zn+O_3(30min)]. The minimum seed weight was found in 60 minutes of O_2 plasma treated zinc contaminated water [Zn+O_3(60min)], as shown in Figure 4. The trend of seed weight was as follows: Zn+O_3(30min) > Control > Zn water > Zn+O_3(60min).

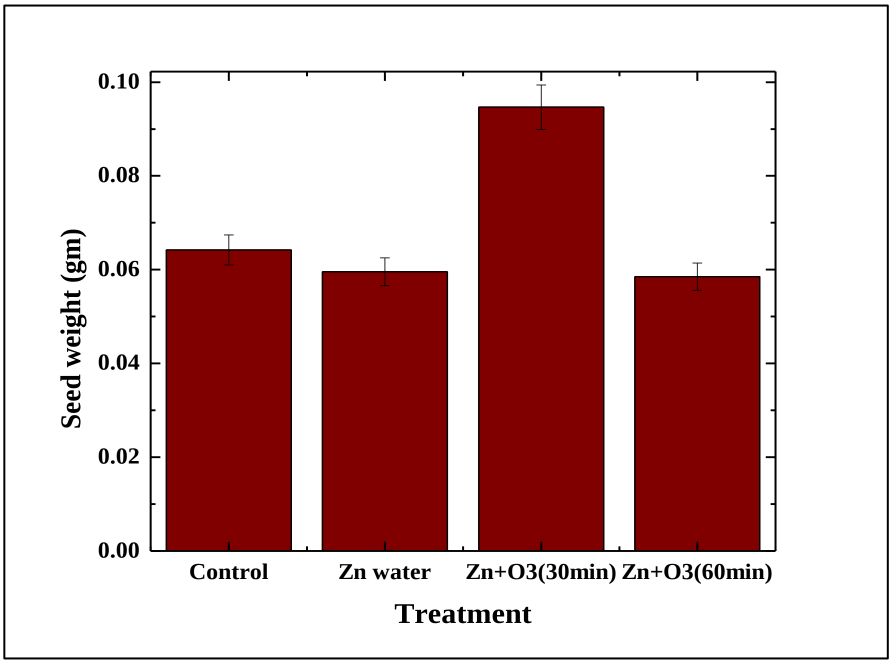

Figure 4. Effect on seed weight by irrigating with plasma treated zinc contaminated water.

From Figure 4 it is evident that seed weight decreased in both Zn water and Zn+O_3(60min) treatments compared to the control. Excessive zinc ions in the Zn contaminated water might cause zinc toxicity within the plant, damaging seed production. The lowest seed weight was found in the Zn+O_3(60min) treatment, which might be due to the combined effects of zinc and prolonged ozone exposure. Long-term oxygen plasma application in zinc contaminated water might damage seed production in *Arabidopsis*, resulting in reduced seed weight. The maximum seed weight was found in the Zn+O_3(30min) treatment. This might be due to the 30 minutes of oxygen plasma application can mitigate the damage caused by zinc toxicity, leading to the highest seed weight in *Arabidopsis*.

3.1.4. Effect on Leaf Area

The largest leaf area was observed in 30 minutes of O_2 plasma treated zinc contaminated water [Zn+O_3(30min)], as shown in (Figure 5). The second largest leaf area was found in 60 minutes of O_2 plasma treated zinc contaminated water [Zn+O_3(60min)]. The smallest leaf area was found in control. The trend of leaf area is as follows: Zn+O_3(30min) > Zn+O_3(60min) > Zn water > Control.

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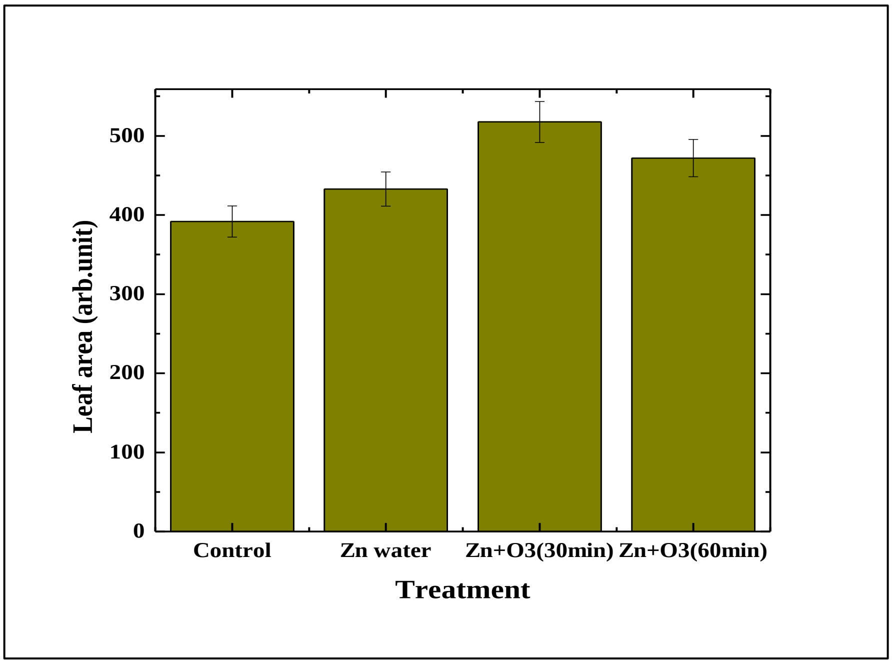

Figure 5. Effect on leaf area by irrigating with plasma treated zinc contaminated water.

It is identified from Figure 5 that leaf area increased in all treatments compared to the control. Zinc deficiency might be the reason for the smallest leaf area in the control, which was treated with tap water. However, the quality of the leaves deteriorated in both the Zn water and Zn+O_3(60min) treatments, as shown in Figure 2. Reddish, distorted leaves with the smallest leaf area were found in the zinc contaminated water, likely due to zinc toxicity. It is possible to think that the maximum leaf area with good quality observed in the Zn+O_3(30min) treatment was due to 30 minutes of O_2 plasma treated zinc contaminated water mitigated the negative effects of zinc, resulting in the highest leaf area of *Arabidopsis*.

3.2. Evaluation of Zinc Effects on Biological Reactions of Plant

One of the comprehensive methods to understand biological reactions in living organisms is to analyze gene expressions. Gene expression analysis of *Arabidopsis* leaves was conducted to identify the changes in genes. RNA was extracted from the leaves of *Arabidopsis thaliana* after three weeks of growth, and microplates for the gene expression analysis were prepared. The lists of expressed genes were analyzed using the DAVID software, and an annotation table of the functions of the expressed genes was obtained. From the annotation table and signaling pathways in response to zinc ions, the effects of plasma treated zinc contaminated water on *Arabidopsis* were clarified. All gene expression data showed significant differences at $p < 0.05$.

3.2.1. Gene Expression Analysis of Plant Fed with Zn Water

When zinc ion concentration in the water increased, the zinc ion penetrated the cells. Table 2 shows the functional annotation of expressed genes in *Arabidopsis thaliana,* which was irrigated with zinc contaminated water (Zn water). The most significant annotation of expressed gene functions was related to cell wall strengthening. Feeding the *Arabidopsis* plants with Zn water caused excessive zinc ion uptake, which damaged the cell walls. As a response, the plants attempted to enhance cell wall strengthening activity to minimize this damage. Therefore, *Arabidopsis* plants strengthened their cell walls to overcome the detrimental effects of excessive zinc ions.

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Table 2. Annotation table of Zn contaminated water effect on *Arabidopsis* plant.

Book	Sheet	Cell	Value
data20221227.xlsx	c1	Q3	GO:0005737(cytoplasm)丨 GO:0005886 (plasma membrane)丨 GO:0008270 (zinc ion binding)丨 GO:0009617 (response to bacterium)丨 GO:0009626 (plant-type hyper sensitive response)丨 GO:0009809 (lignin biosynthetic process)丨 GO:0016616 (oxidoreductase activity, acting on the CH-OH group)
data20221227.xlsx	c1	Q65	GO:0005515 (protein binding)丨 GO:0005739 (mitochondrion)丨 GO:0005773 (vacuole)丨 GO:0005774 (vacuolar membrane)丨 GO:0006793 (phosphorus metabolic process)丨 GO:0006814 (sodium ion transport)丨 GO:0006874 (cellular calcium ion homeostasis)丨 (GO:0006882 (cellular zinc ion homeost)
data20221227.xlsx	c1	C261	Arabidopsis thaliana C2H2-type zinc finger family protein (AT3G53600), mRNA [NM_115220]
data20221227.xlsx	c1	Q309	GO:0005737(cytoplasm)丨 GO:0008270 (zinc ion binding)丨 GO:0008898 (S-adenosyl methionine-homocysteine S-methyl transferase activity)丨 GO:0009086 (methionine biosynthetic process)丨 GO:0033528 (S-methyl methionine cycle)丨 GO:0047150 (betaine-homogsteine S-methyl transferase)
data20221227.xlsx	c1	Q344	GO:0001666 (response to hypoxia)丨 GO:0004022 (alcohol dehydrogenase (NAD+) activity)丨 GO:0004024 (alcohol dehydrogenase activity, zinc-dependent)丨 GO:0005737 (cytoplasm)丨 GO:0005794 (Golgi apparatus)丨 GO:0005829 (cytosol)丨 GO:0005886 (plasma membrane)丨
data20221227.xlsx	c1	Q364	GO:0005737 (cytoplasm)丨 GO:0005829 (cytosol)丨 GO:0008270 (zinc ion binding)丨 GO:0009809 (lignin biosynthetic process)丨 GO:0016616 (oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor)丨 GO:0045551 (cinnamyl-alcohol dehydrogenase activity)
data20221227.xlsx	c1	C365	Arabidopsis thaliana Dof-type zinc finger DNA-binding family protein (AT2G28510), mRNA [NM_128411]
data20221227.xlsx	c1	Q365	GO:0000976 (transcription cis-regulatory region binding)丨 GO:0003700 (DNA-binding transcription factor activity)丨 GO:0005634 (nucleus)丨 GO:0005730 (nucleolus)丨 GO:0006355 (regulation of transcription, DNA-templated)丨 GO:0008270 (zinc ion binding)
data20221227.xlsx	c1	Q424	G0:0000325 (plant-type vacuole)丨 GO:0004364 (glutathione transferase activity)丨 GO:0005507 (copper ion binding)丨 GO:0005634 (nucleus)丨 GO:0005739

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			(mitochondrion)l GO:0005777 (peroxisome)l GO:0005829 (cytosol)l GO:0005886 (plasma membrane)l GO:0009507 (chloroplast)l
data20221227.xlsx	c1	C441	Rep: Pspzf zinc finger protein- like- Arabidopsis thaliana (Mouse-ear cress), partial-18% [TC400366]
data20221227.xlsx	c1	Q523	GO:0005737(cytoplasm)l GO:0008270 (zinc ion binding)l GO:0008898 (S-adenosyl methionine-homocysteine S-methyl transferase activity)l GO:0009086 (methionine biosynthetic process)l GO:0033528 (S-methyl methionine cycle)l GO:0047150 (betaine-homogsteine S-methyl transferase)
data20221227.xlsx	c1	Q588	GO:0000976 (transcription cis-regulatory region binding)l GO:0003677 (DNA-binding)l GO:0003700 (DNA-binding transcription factor activity)l GO:0005515 (protein binding)l GO:0005634 (nucleus)l GO:0008270 (zinc ion binding) l GO:0009733 (response to auxin).
data20221227.xlsx	c1	Q605	GO:0001666 (response to hypoxia)l GO:0004022 (alcohol dehydrogenase (NAD+) activity)l GO:0004024 (alcohol dehydrogenase activity, zinc-dependent)l GO:0005737 (cytoplasm)l GO:0005794 (Golgi apparatus)l GO:0005829 (cytosol)l GO:0005886 (plasma membrane)
data20221227.xlsx	c1	C632	Arabidopsis thaliana Gro ES-like zinc-binding dehydrogenase family protein (AT5G24760), mRNA [NM_001085150]
data20221227.xlsx	c1	Q632	GO:0004024 (alcohol dehydrogenase activity, zinc-dependent)l GO:0005737 (cytoplasm)l GO:0005829 (cytosol)l GO:0008270 (zinc ion binding)l GO:0009507 (chloroplast)l GO:0046294 (formaldehyde catabolic process)l GO:0051903 (S-(hydroxymethyl) glutathione dehydrogenase activity)
data20221227.xlsx	c1	C653	Arabidopsis thaliana Dof-type zinc finger DNA-binding family protein (AT1G64620), mRNA [NM_105137]
data20221227.xlsx	c1	C658	Arabidopsis thaliana GATA zinc finger protein (AT1G28400), mRNA [NM_102608]
data20221227.xlsx	c1	C659	Arabidopsis thaliana Zinc finger (C3HC4-type RING finger) family protein (VIM1), mRNA [NM_202316]
data20221227.xlsx	c1	Q690	G0:0003729 (mRNA binding)l GO:0003871 (5-methyl tetra hydro pteroyl triglutamate-homoqsteine S-methyl transferase activity)l GO:0005737 (cytoplasm)l GO:0005829 (cytosol)l GO:0005886 (plasma membrane)l GO:0008270 (zinc ion binding)l GO:0008705 (methionine synthase activity).

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data20221227.xlsx	c1	C711	Arabidopsis thaliana Zinc-binding dehydrogenase family protein (AT5G16960), mRNA [NM_001343474]
data20221227.xlsx	c1	C737	Arabidopsis thaliana Gro ES-like zinc-binding dehydrogenase family protein (AT5G24760), mRNA [NM_001085150]
data20221227.xlsx	c1	Q737	GO:0004024 (alcoholdehydrogenase activity, zinc-dependent)| GO:0005737 (cytoplasm)| GO:0005829 (cytosol)| GO:0008270 (zinc ion binding)| GO:0009507 (chloroplast)| GO:0046294 (formaldehyde catabolic process)| GO:0051903 (S-(hydroxymethyl) glutathione dehydrogenase activity)
data20221227.xlsx	c1	C789	Arabidopsis thaliana catalytic/ metal ion binding/ metalloendopeptidase/ zinc ion binding protein (AT3G57460), mRNA [NM_001339869]
data20221227.xlsx	c1	Q862	GO:0001666 (response to hypoxia)| GO:0004022 (alcoholdehydrogenase (NAD) activity)| GO:0004024 (alcohol dehydrogenase activity, zinc-dependent)| GO:0005737 (cytoplasm)| GO:0005794 (Golgi apparatus)| GO:0005829 (cytosol)| GO:0005386 (plasma membrane)
data20221227.xlsx	c1	Q868	GO:0005886 (plasma membrane)| GO:0006826 (iron ion transport)| GO:0010039 (response to iron ion)| GO:0010043 (response to zinc ion)| GO:0016020 (membrane)| GO:0035673 (oligopeptide transmembrane transporter activity)| GO:0048316 (seed development)
data20221227.xlsx	c1	C900	Arabidopsis thaliana Gro ES-like zinc-binding dehydrogenase family protein (ATS5G24760), mRNA [NM_001085150]
data20221227.xlsx	c1	Q900	GO:0004024 (alcohol dehydrogenase activity, zinc-dependent)| GO:0005737 (cytoplasm)| GO:0005829 (cytosol)| GO:0008270 (zinc ion binding)| GO:0009507 (chloroplast)| GO:0046294 (formaldehyde catabolic process)| GO:0051903 (S-(hydroxymethyl) glutathione dehydrogenase activity)
data20221227.xlsx	c1	C909	Arabidopsis thaliana Yippee family putative zinc-binding protein (AT3G55890), mRNA [NM_001339759]
data20221227.xlsx	c1	Q912	GO:0005634 (nucleus)| GO:0005737 (cytoplasm)| GO:0009506 (plasmodesma)| GO:0010043 (response to zinc ion)
data20221227.xlsx	c1	Q970	GO:0005737 (cytoplasm)| GO:0005829 (cytosol)| GO:0005886 (plasma membrane)| GO:0008270 (zinc ion binding)| GO:0009809 (lignin biosynthetic process)| GO:0016616 (oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor)

data20221227.xlsx	c1	Q972	GO:0005737 (cytoplasm)ǀ GO:0005829 (cytosol)ǀ GO:0005886 (plasma membrane)ǀ GO:0008270 (zinc ion binding)ǀ GO:0009809 (lignin biosynthetic process)ǀ GO:0016616 (oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor)
Number of increases in expressed genes - 31.			
Book	Sheet	Cell	Value
data20221227.xIsx	c1	C1015	Arabidopsis thaliana RING/FYVE/PHD zinc finger super family protein (PIT1), mRNA [NM_116439]
data20221227.xIsx	c1	Q1015	GO:0004842 (ubiquitin-protein transferase activity)ǀ GO:0005737 (cytoplasm)ǀ GO:0005794 (Golgi apparatus)ǀ GO:0005886 (plasma membrane)ǀ GO:0008270 (zinc ion binding)ǀ GO:0016567 (protein ubiquitination)
data20221227.xlsx	c1	C1033	Arabidopsis thaliana B-box type zinc finger family protein (BBX30), mRNA [NM_001036568]
data20221227.xIsx	c1	Q1033	GO:0005515 (protein binding))ǀ GO:0005634 (nucleus)ǀ GO:0007623 (circadian rhythm)ǀ GO:0008270 (zinc ion binding)ǀ GO:0009909 (regulation of flower development)ǀ GO:0030674 (protein-macromolecule adaptor activity)
data20221227.xlsx	c1	C1037	Arabidopsis thaliana zinc-finger protein 2 (ZF2), partial mRNA [NM_001125191]
data20221227.xlsx	c1	C1042	Arabidopsis thaliana DOF zinc finger protein1 (DOF1), mRNA [NM_104048]
data20221227.xIsx	c1	C1058	Arabidopsis thaliana zinc finger (CCCH-type) family protein (CZF1), mRNA [NM_001036440]
data20221227.xIsx	c1	$CS1066	Arabidopsis thaliana Zinc finger C-x8-C-x5-C-x3-H type family protein (ATCTH), mRNA [NM_001202675]
data20221227.xIsx	c1	Q1073	GO:0004352 (glutamate dehydrogenase (NAD) activity)ǀ GO:0004353 (glutamate dehydrogenase [NAD(P] activity)ǀ GO:0005507 (copper ion binding)ǀ GO:0005524/ATP binding)ǀ GO:0005737 (cytoplasm)ǀ GO:0005739 (mitochondrion)ǀ GO:0006538 (glutamate catabolic process)
data20221227.xIsx	c1	Q1088	GO: 0000325 (plant type vacuole)ǀ GO:0002239 (response to oomycetes)ǀ GO:0004364 (glutathione transferase activity)ǀ GO:0005737 (cytoplasm)ǀ GO:0005829 (cytosol) ǀ GO:0005886 (Plasma membrane) ǀ GO:0006749 (glutathione metabolic process) ǀ GO:0009407 (toxin catabolic process)
data20221227.xIsx	c1	C1116	Arabidopsis thaliana DOF zinc finger protein1 (DOF1), mRNA [NM_104048]

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data20221227.xlsx	c1	C1171	Arabidopsis thaliana mini zinc finger1 (MIF1), mRNA [NM_106124]
data20221227.xlsx	c1	C1187	Arabidopsis thaliana Zinc finger C-x8-C-x5-C-x3-H type family protein (ATCTH), mRNA [NM_001202675]
data20221227.xlsx	c1	SCS1190	Arabidopsis thaliana DHHC-type zinc finger family protein (AT3G22130), mRNA [NM_113115]
data20221227.xlsx	c1	C1200	Arabidopsis thaliana DOF zinc finger protein1 (DOF1), mRNA [NM_104048]
data20221227.xlsx	c1	C1214	Rep: EDA18 (embryo sac development arrest13); protein binding/ zinc ion binding-Arabidopsis thaliana, complete [TC378430]
data20221227.xlsx	c1	C1216	Arabidopsis thaliana zinc finger (C3HC4-type RING finger) family protein (RDUF2), mRNA [NM_001345358]
data20221227.xlsx	c1	C1264	Arabidopsis thaliana zinc finger (CCCH-type) family protein (CZF1), mRNA [NM_001036440)]
data20221227.xlsx	c1	Q1269	GO:0004222 (metalloendopeptidase activity)ǀ GO:0005576 (extracellular region)ǀ GO:0008270 (zinc ion binding)ǀ GO:0030198 (extracellular matrix organization)ǀ GO:0030574 (collagen catabolic process)ǀ GO:0031012 (extracellular matrixǀ GO:0031225 (anchored component of membrane)
data20221227.xlsx	c1	C1270	Arabidopsis thaliana zinc ion binding protein (AT1G74770) mRNA [NM_1334666]
data20221227.xlsx	c1	Q1270	GO:0005515 (protein binding)ǀ GO:0005634 (nucleus)ǀ GO:0006511 (ubiquitin-dependent protein catabolic process)ǀ GO:0006879 (cellular iron ion homeostasis)ǀ GO:0008270 [zinc ion binding)ǀ GO:0016567 (protein ubiquitination)ǀ GO:0033212 (iron import into cell)
data20221227.xlsx	c1	C1305	Arabidopsis thaliana Zinc finger (C3HC4-type RING finger) family protein (RDUF1), mRNA [NM_114529]
data20221227.xlsx	c1	C1308	Arabidopsis thaliana CHY-type/ CTCHY-type/ RING-type Zinc finger protein (AT5G22920), mRNA [NM_122198]
data20221227.xlsx	c1	Q1308	GO:0005634 (nucleus)ǀ GO:0006511 (ubiquitin-dependent protein catabolic process)ǀ GO:0008270 (zinc ion binding)ǀ GO:0016567 (protein ubiquitination)ǀ GO:0061630 (ubiquitin protein ligase activity)ǀ GO:1902456 (regulation of stomatal opening)
data20221227.xlsx	c1	C1332	Arabidopsis thaliana RING/FYVE/PHD zinc finger super family protein (AT1G02610), mRNA [NM_001331347]
data20221227.xlsx	c1	Q1332	GO:0004842 (ubiquitin-protein transferase activity)ǀ GO:0005634 (nucleus)ǀ GO:0005737 (cytoplasm)ǀ

			GO:0008270 [zinc ion binding)∣ GO:0016567 (protein ubiquitination)
data20221227.xIsx	c1	C1355	Arabidopsis thaliana CHY-type/ CTCHY-type/ RING-type Zinc finger protein (AT5G22920), mRNA [NM_001343781]
data20221227.xIsx	c1	Q1355	GO:0005634 (nucleus)∣ GO:0006511 (ubiquitin-dependent protein catabolic process)∣ GO:0008270 (zinc ion binding)∣ GO:0016567 (protein ubiquitination)∣ GO:0061630 (ubiquitin protein ligase activity)∣ GO:1902456 (regulation of stomatal opening)
data20221227.xlsx	c1	C1363	Arabidopsis thaliana Zinc finger C-x8-C-x5-C-x3-H type family protein (ATCTH), mRNA [NM_001202675]
data20221227.xIsx	c1	C1391	Arabidopsis thaliana salt tolerance zinc finger (STZ), mRNA [NM_102538]
data20221227.xlsx	c1	C1430	Arabidopsis thaliana salt-inducible zinc finger 1(SZF1), mRNA [NM_115456]

Number of decreases in expressed genes - 31.

Table 2 identifies 62 expressed genes, with 31 showing increased expression and 31 showing decreased expression. The gene expression annotation revealed an increased response to the auxin hormone in the *Arabidopsis* plants. Auxin is a plant growth hormone that influences various growth responses. In the Zn water (zinc contaminated water) treatment, the plants faced growth problems due to the high concentration of zinc. Based on the gene expression, it is possible to think that the plants increased auxin hormone production to mitigate the growth problems caused by zinc toxicity.

3.2.2. Gene Expression Analysis of Plant Fed with Zn+O_3(30min)

Table 3 shows the functional annotations of expressed genes in *Arabidopsis* plants irrigated with 30 minutes of plasma-treated zinc-contaminated water. The gene expressions coding for plant hormones such as auxin, jasmonic acid, and ethylene were enhanced by the treatment. Zinc is a major component of auxin and jasmonic acid. This result suggests that the defense response in plants was improved. Jasmonic acid modulates defense response activity in plants. The 30 minutes of O_2 plasma treatment might cause some damage inside the plant, prompting an increase in defense hormones to recover. It also helps the plant recover from O_3 stimulation.

Table 3. Annotation table of Zn+O_3(30min) effect on *Arabidopsis* plant.

Book	Sheet	Cell	Value
data20221227.xlsx	c2	C161	Arabidopsis thaliana RING/ FYVE/ PHD zinc finger super family protein (AT2G37950), mRNA [NM_129351]
data20221227.xlsx	c2	Q161	GO:0005634 (nucleus)∣ GO:0008270 (zinc ion binding}∣ GO:0009555 (pollen development).
data20221227.xlsx	c2	C165	Arabidopsis thaliana CCCH-type zinc finger family protein (OZF1), mRNA [NM_127539]
data20221227.xlsx	c2	Q177	GO:0005886 (plasma membrane)∣ GO:0006826 (iron ion transport)∣ GO:0010039 (response to iron ion)∣ GO:0010043 (response to zinc ion)∣ GO:0016020 (membrane)∣ GO:0035673

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			(oligopeptide transmembrane transporter activity)Ι GO:0048316 (seed development)Ι
data 20221227.xlsx	c2	Q260	GO:0005737 (cytoplasm)Ι GO:0008270 (zinc ion binding)Ι GO:0009809 (lignin biosynthetic process)Ι GO:0016616 (oxido reductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor)Ι GO:0045551 (cinnamyl-alcohol dehydrogenase activity)Ι
data 20221227.xIsx	c2	C281	Arabidopsis thaliana DHHC-type zinc finger family protein (AT3G56920), mRNA [NM_115551]
data20221227.xIsx	c2	C293	Arabidopsis thaliana Zinc-binding dehydrogenase family protein (AT5G37980), mRNA [NM_123157]
data20221227.xIsx	c2	Q311	GO:0003729 (mRNA binding)Ι GO:0005634 (nucleus) Ι GO:0005829 (cytosol)) Ι GO:0008270 (zinc ion binding) Ι GO:0009409 (response to cold)Ι GO:0009793 (embryo development ending in seed dormancyΙ GO:0010154 (fruit development)
data20221227.xlsx	c2	C333	Arabidopsis thaliana DHHC-type zinc finger family protein (AT5G41060), mRNA [NM_001344380]
data20221227.xlsx	c2	C341	Arabidopsis thaliana zinc finger protein 4 (ZFP4), mRNA[NM_105285]
data20221227.xlsx	c2	C373	Arabidopsis thaliana Dof-type zinc finger DNA-binding family protein (DOF6), mRNA [NM_114430]
data20221227.xlsx	c2	C393	Arabidopsis thaliana GATA zinc finger protein (AT1G28400), mRNA [NM_102608]
data20221227.xlsx	c2	Q396	GO:0000976 (transcription cis-regulatory region binding)Ι GO:0003700 (DNA-binding transcription factor activity)Ι GO:0005515 (protein binding)Ι GO:0005634 (nucleus)Ι GO:0006355 (regulation of transcription, DNA-templated)Ι GO:0007623 (circadian rhythm)Ι GO:0008270 (zinc ion binding)
data 20221227.xIsx	c2	C410	Arabidopsis thaliana C2H2 and C2HC zinc fingers super family protein (AT4G17810), mRNA [NM_001341243]

Number of increases in expressed genes – 14.

Book	Sheet	Cell	Value
data20221227.xlsx	c2	C476	Arabidopsis thaliana zinc finger (CCCH-type) family protein (CZF1), mRNA [NM_001036440]
data20221227.xlsx	c2	Q487	GO:0005634 (nucleus)Ι GO:0005737 (cytoplasm)Ι GO:0009506 (plasmodesma)Ι GO:0010043 (response to zinc ion)
data20221227.xlsx	c2	C552	Rep: protein binding/ zinc ion binding- Arabidopsis thaliana, partial-89% [TC398910]
data20221227.xlsx	c2	Q586	GO:0005634 (nucleus)Ι GO:0005730 (nucleolus)Ι GO:0005886 (plasma membrane)Ι GO:0008270 (zinc ion

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			binding)丨 GO:0009910 (negative regulation of flower development)
data20221227.xlsx	c2	C598	Arabidopsis thaliana zinc ion binding protein (AT2G44580), mRNA [NM_130022]
data20221227.xlsx	c2	C634	Arabidopsis thaliana catalytic/ metal ion binding/ metalloendopeptidase/ zinc ion binding protein (AT3G57460), mRNA [NM_001339869]
data20221227.xlsx	c2	Q670	GO:0000325 (plant-type vacuole)丨 GO:0002239 (response to oomycetes)丨 GO:0004364 (glutathione transferase activity)丨 GO:0005737 (cytoplasm)丨 GO:0005829 (cytosol)丨 GO:0005886 (plasma membrane)丨 GO:0006749 (glutathione metabolic process)丨 GO:0009407 (toxin catabolic process)丨
data 20221227. xlsx.	c2	Q679	GO:0005737 (cytoplasm)丨 GO:0006511 (ubiquitin-dependent protein catabolic process)丨 GO:0008270 (zinc ion binding)丨 GO:0016567 (protein ubiquitination)
data20221227.xlsx	c2	C683	Arabidopsis thaliana zinc-finger protein 3 (ZF3), mRNA [NM_123683]
data20221227.xlsx	c2	Q711	GO:0000325 (plant-type vacuole)丨 GO:0002239 (response to oomycetes)丨 GO:0004364 (glutathione transferase activity)丨 GO:0005737 (cytoplasm)丨 GO:0005829 (cytosol)丨 GO:0005886 (plasma membrane)丨 GO:0006749 (glutathione metabolic process)丨 GO:0009407 (toxin catabolic process)丨
data 20221227.xlsx	c2	C712	Arabidopsis thaliana salt-inducible zinc finger 1 (SZF1), mRNA [NM_115456]
data20221227.xlsx	c2	C725	Arabidopsis thaliana zinc finger (CCCH-type) family protein (CZF1), mRNA [NM_001036440]
data20221227.xlsx	c2	C734	Arabidopsis thaliana zinc finger (C3HC4-type RING finger) family protein (RDUF2), mRNA [NM_001345358]
data20221227.xlsx	c2	C771	Arabidopsis thaliana zinc-finger protein 3 (ZF3), mRNA [NM_123683]
data20221227.xlsx	c2	Q779	GO:0004126 (cytidine deaminase activity)丨 GO:0005634 (nucleus)丨 GO:0005739 (mitochondrion)丨 GO:0005829 (cytosol)丨 GO:0006216 (cytidine catabolic process)丨 GO:0008270 (zinc ion binding)丨 GO:0009972 (cytidine deamination)丨 GO:0042803 (protein homodimerization activity)丨
data 20221227.xlsx	c2	Q914	GO: 0000325 (plant-type vacuole)丨 GO:0002239 (response to oomycetes)丨 GO:0004364 (glutathione transferase activity)丨 GO:0005737 (cytoplasm)丨 GO:0005829 (cytosol)丨 GO:0005886 (plasma membrane)丨 GO:0006749

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			(glutathione metabolic process)ǀ GO:0009407 (toxin catabolic process)ǀ
data 20221227.xlsx	c2	Q919	GO: 0000325 (plant-type vacuole)ǀ GO:0002239 (response to oomycetes)ǀ GO:0004364 (glutathione transferase activity)ǀ GO:0005737 (cytoplasm)ǀ GO:0005829 (cytosol)ǀ GO:0005886 (plasma membrane)ǀ GO:0006749 (glutathione metabolic process)ǀ GO:0009407 (toxin catabolic process)ǀ
data 20221227.xlsx	c2	C1129	Arabidopsis thaliana salt tolerance zinc finger (STZ), mRNA [NM_102538]
data20221227.xlsx	c2	C1157	Arabidopsis thaliana C2H2 and C2HC zinc fingers super family protein (ZAT11), mRNA [NM_129298]
data20221227.xlsx	c2	Q1194	GO:0004222 (metalloendopeptidase activity)ǀ GO:0005576 (extracellular region)ǀ GO:0008270 (zinc ion binding)ǀ GO:0030198 (extracellular matrix organization)ǀ GO:0030574 (collagen catabolic process)ǀ GO:0031012 (extracellular matrix)ǀ GO:0031225 (anchored component of membrane.
data 20221227.xlsx	c2	C1263	Arabidopsis thaliana zinc finger (C3HC4- type RING finger) family protein (RDUF1), mRNA [NM_114529]
data20221227.xlsx	c2	C1299	Rep: Zinc finger AN1 domain-containing stress-associated protein 12-Arabidopsis thaliana (Mouse-ear cress), partial-76% [TC390759]
data20221227.xlsx	c2	C1386	Arabidopsis thaliana C2H2-type zinc finger family protein (RHL41), mRNA [NM_125374]
data20221227.xlsx	c2	C1433	Arabidopsis thaliana zinc finger (AN1-like) family protein (PMZ), mRNA [NM_113740]
data20221227.xlsx	c2	Q1433	GO:0005634 (nucleus)ǀ GO:0005737 (cytoplasm)ǀ GO:0008270 (zinc ion binding)ǀ GO:0009737(response to abscisic acid)

Number of decreases in expressed genes – 25.

The number of expressed genes for the Zn+O_3(30min) treatment was reduced to 39, compared to the Zn water treatment, which had 62 expressed genes. From the gene expression annotation in Table 3, it was identified that the response to the abscisic acid hormone was decreased in the Arabidopsis plant. Abscisic acid is a major phytohormone that plays a crucial role in regulating plant growth and development, stress responses, and multiple physiological processes [52]. Based on the gene expression data, it is possible to consider that the plant might have been attempting to overcome various growth-related problems.

Gene expression annotation of this treatment also revealed a decrease in glutathione metabolic processes and glutathione transferase activity in the Arabidopsis plant. Glutathione is an essential metabolite for plant life, well-known for its role in controlling reactive oxygen species (ROS) [53]. Based on gene expression analysis, it can be inferred that the plant may regulate oxidation caused by active oxygen species produced during the 30 minutes plasma application.

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Gene expression analysis further indicated that reduced metal ion stress and controlled oxidation due to active oxygen species are likely factors contributing to the healthy growth of Arabidopsis plants. This condition appears achievable with the $Zn+O_3$(30min) treatment.

3.2.3. Gene Expression Analysis of Plant Fed with $Zn+O_3$(60min)

The redox annotation is presented in the functional annotation table (Table 4), demonstrating the effects when zinc ion water was treated with oxygen plasma for 60 minutes. In this case, ozone and active oxygen species accumulate in the water. Genes encoding superoxide dismutase, glutathione, and Thioredoxin are highlighted in the annotation table. Additionally, typical gene expressions related to plant hormones show similarities with the 30 minutes plasma treatment.

Arabidopsis plant grown with $Zn+O_3$(60min) water showed actual damage from active oxygen species. Excessive oxidation in this treatment leads to significant plant damage, prompting an increase in antioxidative substances. These hormones are produced to neutralize active oxygen species, thereby enhancing the plant's defense response. In comparison, plant damage is less severe with Zn+O3 (30 min), allowing for better plant growth recovery.

Table 4. Annotation table of $Zn+O_3$(60min) effect on *Arabidopsis* plant.

Book	Sheet	Cell	Value
data20221227.xlsx	c3	Q144	GO:0005634 (nucleus)ǀ GO:0005737 (cytoplasm)ǀ GO:0006511 (ubiquitin-dependent protein catabolic process)ǀ GO:0008270 (zinc ion binding)ǀ GO:0016567 (protein ubiquitination)
data20221227.xIsx	c3	Q162	G0:0005737 (cytoplasm)ǀ GO:0008270 (zinc ion binding)ǀ GO:0008898 (S-adenosyl methionine-homocysteine S-methyl transferase activity)ǀ GO:0009086 (methionine bio synthetic process)ǀ GO:0033528 (S-methyl methionine cycle)ǀ GO:0047150 (betaine-homocysteine S-methyl transferase)
data 20221227.xlsx	c3	C171	Arabidopsis thaliana Yippee family putative zinc-binding protein (AT3G55890), mRNA [NM_001339759]
data20221227.xlsx	c3	Q243	GO:0000976 (transcription cis-regulatory region binding)ǀ GO:0003700 (DNA-binding transcription factor activity)ǀ GO:0005634 (nucleus)ǀ GO:0006355 (regulation of transcription, DNA-templated)ǀ GO:0008270 (zinc ion binding)ǀ GO:0009908 (flower development)
data20221227.xlsx	c3	C273	Arabidopsis thaliana Transcription factor jumonji (jmj) family protein/ zinc finger (C5HC2 type) family protein (AT5G46910), mRNA [NM_001344705]
data20221227.xIsx	c3	Q337	GO:0005739 (mitochondrion)ǀ GO:0008270 (zinc ion binding)ǀ GO:0009451 (RNA modification)
data20221227.xIsx	c3	Q350	GO:0004497 (monooxygenase activity)ǀ GO:0005506 (iron ion binding)ǀ GO:0007275 (multicellular organism development)ǀ GO:0008270 (zinc ion binding)ǀ GO:0010268 (brassino steroid homeostasis)ǀ GO:0016125 (sterol metabolic process)ǀ GO:0016132 (brassino steroid biosynthetic process)
data 20221227.xIsx	c3	Q352	GO:0004222 (metalloendopeptidase activity)ǀ GO:0005576 (extracellular region)ǀ GO:0008270 (zinc ion binding)ǀ

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			GO:0030198 (extracellular matrix organization)ǀ GO:0030574 (collagen catabolic process)ǀ GO:0031012 (extracellular matrix)ǀ GO:0031225 (anchored component of membrane)
data 20221227.xIsx	c3	C459	Arabidopsis thaliana zinc transporter 11 precursor (ZIP11), mRNA [NM_104468]
data20221227.xIsx	c3	Q459	GO:0005385 (zinc ion transmembrane transporter activity)ǀ GO:0005634 (nucleus)ǀ GO:0009507 (chloroplast)ǀ GO:0016020 (membrane)ǀ GO:0046873 (metal ion transmembrane transporter activity)ǀ GO:0071577 (zinc ion transmembrane transport)

Number of increases in expressed genes – 10.

Book	sheet	Cell	Value
data20221227.xlsx	c3	C475	Arabidopsis thaliana CHY-type/ CTCHY-type/ RING-type Zinc finger protein (AT5G22920), mRNA [NM_001343781]
data20221227.xIsx	c3	Q475	GO:0005634 (nucleus)ǀ GO:0006511 (ubiquitin-dependent protein catabolic process)ǀ GO:0008270 (zinc ion binding)ǀ GO:0016567 (protein ubiquitination)ǀ GO:0061630 (ubiquitin protein ligase activity)ǀ GO:1902456 (regulation of stomatal opening)
data20221227.xlsx	c3	C514	Arabidopsis thaliana zinc finger (CCCH-type) family protein (CZF1), mRNA [NM_001036440]
data20221227.xlsx	c3	C517	Arabidopsis thaliana salt-inducible zinc finger1 (SZF1), mRNA [NM_115456]
data20221227.xIsx	c3	Q522	GO:0003729 (mRNA binding)ǀ GO:0004089 (carbonate dehydratase activity)ǀ GO:0005737 (cytoplasm)ǀ GO:0005829 (cytosol)ǀ GO:0005886 (plasma membrane)ǀ GO:0008270 (zinc ion binding)ǀ GO:0009409 (response to cold)ǀ GO:0009507 (chloroplast)ǀ GO:0009535 (chloroplast thylakoid membrane.
data 20221227.xlsx	c3	C530	Arabidopsis thaliana DOF zinc finger protein 1 (DOF1), mRNA [NM_104048]
data20221227.xlsx	c3	Q540	GO:0004222 (metalloendopeptidase activity)ǀ GO:0005576 (extracellular region)ǀ GO:0008270 (zinc ion binding)ǀ GO:0030198 (extracellular matrix organization)ǀ GO:0030574 (collagencatabolic process)ǀ GO:0031012 (extracellular matrix)ǀ GO:0031225 (anchored component of membrane.
data 20221227.xlsx	c3	C547	Arabidopsis thaliana zinc finger (C3HC4-type RING finger) family protein (RDUF2), mRNA [NM_001345358]
data20221227.xlsx	c3	C590	Arabidopsis thaliana B-box type zinc finger protein with CCT domain-containing protein (BBX16), mRNA [NM_106047]

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data20221227.xIsx	c3	Q590	GO:0000976 (transcription cis-regulatory region binding)丨GO:0003700 (DNA-binding transcription factor activity)丨 GO:0005634 (nucleus)丨 GO:0005730 (nucleolus)丨 GO:0006355 (regulation of transcription, DNA-templated)丨 GO:0008270 (zinc ion binding)丨 GO:0009641 (shade avoida).
data 20221227.xlsx	c3	Q597	GO:0003729 (mMRNA binding)丨GO:0004089 (carbonate dehydratase activity)丨 GO:0005737 (cytoplasm)丨 GO:0005829 (cytosol)丨GO:0005886 (plasma membrane)丨 GO:0008270 (zinc ion binding)丨GO:0009409 (response to cold)丨 GO:0009507 (chloroplast)丨 GO:0009535 (chloroplast thylakoid membrane.
data 20221227.xlsx	c3	C626	Arabidopsis thaliana DOF zinc finger protein 1(DOF1), mRNA [NM_104048]
data20221227.xlsx	c3	Q629	GO:0004407 (histonedeacetylase activity)丨 GO:0005515 (protein binding)GO:0005634 (nucleus)丨 GO:0008270 (zinc ion binding)丨GO:0009651 (response to salt stress)丨 GO:0009737 (response to abscisic acid)丨 GO:0009793 (embryo development ending in seed dormancy)丨
data 20221227.xlsx	c3	C631	Arabidopsis thaliana B-box type zinc finger family protein (BBX30), mRNA [NM_001036568]
data20221227.xlsx	c3	Q631	GO:0005515 (protein binding)丨 GO:0005634 (nucleus)丨 GO:0007623 (circadian rhythm)丨 GO:0008270 (zinc ion binding)丨 GO:0009909 (regulation of flower development)丨 GO:0030674 (protein-macro molecule adaptor activity)
data20221227.xlsx	c3	C632	Arabidopsis thaliana C2H2-type zinc finger family protein (AT3G46080), mRNA [NM_114477]
data20221227.xlsx	c3	C647	Arabidopsis thaliana DOF zinc finger protein 1 (DOF1), mRNA [NM_104048]
data20221227.xlsx	c3	Q648	GO:0003729 (mRN Abinding)丨 GO:0004089 (carbonate dehydratase activity)丨 GO:0005737 (cytoplasm)丨 GO:0005829 (cytosol丨 GO:000586 (plasma membrane)丨 GO:0008270 (zinc ion binding)丨GO:0009409 (response to cold)丨 GO:0009507 (chloroplast)丨 GO:0009535 (chloroplast thylakoid membrane).
data 20221227.xlsx	c3	Q655	GO:0003729 (mRN Abinding)丨 GO:0004089 (carbonate dehydratase activity)丨 GO:0005737 (cytoplasm)丨 GO:0005829 (cytosol丨 GO:000586 (plasma membrane)丨 GO:0008270 (zinc ion binding)丨GO:0009409 (response to cold)丨 GO:0009507 (chloroplast)丨 GO:0009535 (chloroplast thylakoid membrane).

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data 20221227.xIsx	c3	C689	Arabidopsis thaliana Zinc finger C-x8-C-x5-C-x3-H type family protein (ATCTH), mRNA [NM_001202675]
data20221227.xIsx	c3	C692	Arabidopsis thaliana zinc ion binding protein (AT1G74770), mRNA [NM_001334666]
data20221227.xlsx	c3	Q692	GO:0005515 (protein binding)丨 GO:0005634 (nucleus)丨 GO:0006511 (ubiquitin-dependent protein catabolic process)丨 GO:0006879 (cellular iron ion homeostasis)丨 GO:0008270 (zinc ion binding)丨 GO:0016567 (protein ubiquitination)丨 GO:0033212 (iron import into cell)丨
data 20221227.xlsx	c3	C724	Arabidopsis thaliana GATA type zinc finger transcription factor family protein (WLIM2a), mRNA [NM_129548]
data20221227.xlsx	c3	C781	Arabidopsis thaliana A20/AN1-like zinc finger family protein (AT3G52800), mRNA [NM_001339589]
data20221227.xlsx	c3	Q781	GO:0003677 (DNA binding)丨 GO:0005634 (nucleus)丨 GO:0008270 (zinc ion binding)丨 GO:0071456 (cellular response to hypoxia)
data20221227.xlsx	c3	C796	Arabidopsis thaliana zinc ion binding protein (AT2G44580), mRNA [NM_130022]
data20221227.xlsx	c3	C812	Arabidopsis thaliana zinc-finger protein 3 (ZF3), mRNA [NM_123683]
data20221227.xlsx	c3	C853	Arabidopsis thaliana GATA type zinc finger transcription factor family protein (WLIM2a), mRNA [NM_129548]
data20221227.xlsx	c3	C856	Arabidopsis thaliana zinc-finger protein 2 (ZF2), mRNA [NM_112848]
data20221227.xIsx	c3	C868	Arabidopsis thaliana zinc finger (CCCH-type) family protein (CZF1), mRNA [NM_001036440]
data20221227.xIsx	c3	C901	Rep: EDA 18 (embryo sac development arrest 18); protein binding/ zinc ion binding- Arabidopsis thaliana, complete [TC378430]
data20221227.xlsx	c3	C941	Arabidopsis thaliana zinc finger (C3HC4-type RING finger) family protein (RDUF2), mRNA [NM_001345358]
data20221227.xlsx	c3	C958	Rep: Pspzf zinc finger protein-like- Arabidopsis thaliana (Mouse-ear cress), partial-18% [TC400366]
data20221227.xlsx	c3	C1017	Arabidopsis thaliana zinc-finger protein 3 (ZF3), mRNA [NM_123683]
data20221227.xlsx	c3	C1053	Arabidopsis thaliana HCP-like super family protein with MYND-type zinc finger (AT5G50450), mRNA [NM_124427]
data20221227.xlsx	c3	C1063	Arabidopsis thaliana C2H2 and C2HC zinc fingers super family protein (ZAT7), mRNA [NM_114478]
data20221227.xlsx	c3	C1113	Arabidopsis thaliana zinc-finger protein 2 (ZF2), partial mRNA [NM_001125191]

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data20221227.xlsx	c3	C1187	Arabidopsis thaliana zinc finger (AN1-like) family protein (PMZ), mRNA [NM_113740]
data20221227.xIsx	c3	Q1187	GO:0005634 (nucleus)丨 GO:0005737 (cytoplasm)丨 GO:0008270 (zinc ion binding)丨 GO:0009737 (response to abscisic acid)
data20221227.xIsx	c3	C1200	Rep: Zinc finger AN1 domain-containing stress-associated protein 12- Arabidopsis thaliana (Mouse-ear cress), partial-76% [TC390759]
data20221227.xIsx	c3	C1257	Arabidopsis thaliana zinc finger (C3HC4-type RING finger) family protein (RDUF1), mRNA [NM_114529]
data20221227.xlsx	c3	C1333	Arabidopsis thaliana C2H2 and C2HC zinc fingers super family protein (ZAT11), mRNA [NM_129298]
data20221227.xlsx	c3	C1350	Arabidopsis thaliana salt tolerance zinc finger (STZ), mRNA [NM_102538]
data20221227.xIsx	c3	C1363	Arabidopsis thaliana C2H2-type zinc finger family protein (RHL41), mRNA [NM_125374]

Number of decreases in expressed genes – 44.

In Zn+O_3(60min), the number of expressed genes decreased to 54, compared to 62 in the Zn water treatment. According to gene expression annotation in Table 4, it was observed that the expression of genes responsive to abscisic acid decreased twofold in *Arabidopsis* plants. Abscisic acid is a major phytohormone crucial for regulating plant growth, development, stress responses, and various physiological processes. Based on gene expression data, it can be inferred that *Arabidopsis* experienced impaired growth under this treatment. Physical growth parameter analysis also confirmed poor growth outcomes in this treatment.

4. Conclusions

Arabidopsis thaliana plants were irrigated with different DBD oxygen plasma treated zinc contaminated water to evaluate their effect on growth performance. This study found that the maximum growth performance occurred with 30 minutes of O_2 plasma treated zinc contaminated water [Zn+O_3(30min)]. Visual observations of *Arabidopsis* showed maximum plant height and green leaves in the Zn+O_3(30min) treatment, while a distorted reddish color was observed in the zinc contaminated water [Zn water]. Growth parameters, except for leaf area, followed this trend: Zn+O_3(30min)> Control> Zn water> Zn+O_3(60min). Based on the gene expression data from the Zn water treatment, it is evident that plants increased auxin hormone production to mitigate the growth problems caused by zinc toxicity, which explains the poor growth performance of *Arabidopsis* under this treatment. Also, in the Zn+O_3 (60 min) treatment, the expression of genes responsive to abscisic acid decreased twofold in *Arabidopsis* plants, indicating poor growth. Gene expression data in the Zn+O_3(30min) treatment indicated that reduced metal ion stress and controlled oxidation by active oxygen species contributed to the improved growth of *Arabidopsis*. Therefore, it can be concluded that 30 minutes of DBD O_2 plasma treated zinc contaminated water [Zn+O_3(30min)] can help alleviate the negative effects of excess zinc ions and promote the growth of *Arabidopsis* plants.

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