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Effects of Phytohormones-Jasmonic Acid and Strigolactone- on the Stress Tolerance of *Triticum aestivum* towards Zinc and Nickel

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Mandana Bahadori Far

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Dedication

I am dedicating this work first, to my beloved husband Roman Ferdinand Reichel, for his patience and persuasion during my study and my son Robin Reichel who gives me always love and power to able finish the study up.

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Abstract

Heavy metals (HMs) are natural trace elements that have a density greater than 5 g cm^{-3}. Among HMs, micronutrients are essential for growth and development and important in the metabolism of plants. For example, Zinc (Zn) is an essential element for plant growth and development and plays a vital role in enzyme activation, and Nickel (Ni) is also an essential element for the normal growth of many species of microorganisms and plants. On the other hand, HMs lead to negative impacts. Excess concentrations of Ni cause chlorosis and necrosis in plants. It is considered one of the most toxic metals in the environment. Elevated concentrations of Zn cause growth inhibition, and prevention of cell division and cell elongation in root system. Once metals enter the food chain they become accumulated in humans and animals. Pollution with HMs from anthropogenic sources increased and become a serious environmental concern all over the world. It is therefore, important to find methods to eliminate the negative effects of these pollutants. One of the possibilities to improve plant growth in toxic environment is to strengthen the plant's resilience to biotic and abiotic stress by phytohormones. Their regulatory influence effects growth and development. In this work, jasmonic acid (JA) and strigolactone (SL) were chosen to observe the possible involvement of these hormones on wheat plants under HMs stress. JA has a vital role in many physiological functions related to growth and development such as elongation in plant height, growth in roots, and flower bud formation. SL effects the shaping of shoot structure and development of roots. In these experiments, we surveyed the possible effects of phytohormones on the growth performance and stress tolerance of wheat plants that had been treated with Ni and Zn. Therefore, first, the effects of different concentrations of Zn and Ni on the plant's parameters, second, the possible involvement of the phytohormones (JA and SL) on the growth performance of wheat, and finally the possible involvement of JA and SL on the ability of wheat plants to withstand Ni and Zn induced stress were measured. This case study was done in two experimental phases, in which the effects of HMs and phytohormones on the growth parameters and stress tolerance of wheat plants were investigated. Findings in plants treated with HMs showed a significant decrease in plant parameters such as root and shoot length, root and shoot weight and the number of leaves at high concentrations of HMs applied to the soil. The most significant effects in root and shoot were expected because the roots are the first organ that gets in contact with the HMs in soil and transport absorbed HMs upwards. The various concentrations of JA and SL didn't show consistent significant effects on the different growth parameters of the plants. Among all concentrations of JA applied in this experiment, only $JA10^{-6}$ M showed a significant effect on the plant's growth parameters. We observed that in plants treated with $Ni10^{-3}$ M and JA, there were significant effects in root and shoot weight and length at concentrations of $JA10^{-6}$ M and $JA10^{-8}$ M. Results of treated plants with SL showed significant effect on shoot length in plants treated with $Ni10^{-3}$ M and $SL10^{-6}$ M, $SL10^{-7}$ M, and $SL10^{-9}$ M. We conclude that the toxicity of Zn and Ni depended on the concentration of these HMs in the soil. Also, external application of the JA and SL showed significant effects on some of the measured parameters (root and shoot length). In plants that were treated with Ni, application of JA reduced the Ni stress in certain concentrations. For Zn treated plants, JA could not reduce the stress effects. We found that SL had the similar effects; it improved the resilience of Ni treated plants whereas it had neither positive nor negative effects on Zn treated plants. Our experiments suggest that the phytohormones- JA and SL- can have significant beneficial effects on growth and physiology of plants treated with HMs, but this cannot be assumed as a general fact. In our case, they strengthened Ni treated plants, but not Zn treated plants. In addition, the right concentrations must be defined before application in the field.

Zusammenfassung

Schwermetalle (HM) Sind natürliche Spurenelemente, die eine Dichte von mehr als 5 g cm^{-3} haben. Unter den Schwermetallen sind Mikronährstoffe wesentlich für Wachstum und Entwicklung und wichtig für den Stoffwechsel der Pflanzen. So ist beispielsweise Zink (Zn) ein wesentliches Element für das Wachstum und die Entwicklung von Pflanzen und spielt eine entscheidende Rolle bei der Aktivierung von Enzymen, und auch Nickel (Ni) ist ein wesentliches Element für das normale Wachstum vieler Arten von Mikroorganismen und Pflanzen. Auf der anderen Seite haben HMs negative Auswirkungen. Überschüssige Konzentrationen von Ni führen bei Pflanzen zu Chlorose und Nekrosen. Es gilt als eines der giftigsten Metalle für Pflanzen in der Umwelt. Erhöhte Zn-Konzentrationen hemmen das Wachstum und verhindern die Zellteilung und Zellstreckung im Wurzelsystem. Sobald Metalle in die Nahrungskette gelangen, reichern sie sich in Menschen und Tieren an. Die Verschmutzung mit Metallen aus anthropogenen Quellen hat zugenommen und ist zu einem ernsthaften Umweltproblem in der ganzen Welt geworden. Daher ist es wichtig, Methoden zur Beseitigung der negativen Auswirkungen dieser Schadstoffe zu finden. Eine der Möglichkeiten zur Verbesserung des Pflanzenwachstums in toxischer Umgebung besteht darin, die Widerstandsfähigkeit der Pflanzen gegenüber biotischem und abiotischem Stress durch Phytohormone zu stärken. Ihr regulierender Einfluss wirkt sich auf Wachstum und Entwicklung aus. In dieser Arbeit wurden Jasmonsäure (JA) und Strigolacton (SL) ausgewählt, um die mögliche Beteiligung dieser Hormone an Weizenpflanzen unter HMs-Stress zu untersuchen. JA spielt eine wichtige Rolle bei vielen physiologischen Funktionen im Zusammenhang mit Wachstum und Entwicklung, z. B. bei der Verlängerung der Pflanzenhöhe, dem Wachstum der Wurzeln und der Bildung von Blütenknospen. SL wirkt sich auf die Formung der Sprossstruktur und die Entwicklung der Wurzeln aus. In diesem Versuch untersuchten wir die möglichen Auswirkungen von Phytohormonen auf die Wachstumsleistung und Stresstoleranz von Weizenpflanzen, die mit Ni und Zn behandelt worden waren. Daher wurden erstens die Auswirkungen verschiedener Konzentrationen von Zn und Ni auf die Pflanzenparameter, zweitens die mögliche Beteiligung der Phytohormone (JA und SL) an der Wachstumsleistung von Weizen und schließlich die mögliche Beteiligung von JA und SL an der Fähigkeit der Weizenpflanzen, Ni und Zn induziertem Stress zu widerstehen, gemessen. Diese Fallstudie wurde in zwei Versuchsphasen durchgeführt, in denen die Auswirkungen von HMs und Phytohormonen auf die Wachstumsparameter und die Stresstoleranz von Weizenpflanzen untersucht wurden. Die Ergebnisse bei Pflanzen, die mit HM behandelt wurden, zeigten eine signifikante Abnahme der Pflanzenparameter wie Wurzel- und Sprosslänge, Wurzel- und Sprossgewicht und die Anzahl der Blätter bei hohen Konzentrationen von HM, die in den Boden eingebracht wurden. Die signifikantesten Auswirkungen auf Wurzeln und Spross wurden erwartet, da die Wurzeln das erste Organ sind, das mit den HMs im Boden in Kontakt kommt und die absorbierten HMs nach oben transportiert. Die verschiedenen Konzentrationen von JA und SL zeigten keine konsistenten signifikanten Auswirkungen auf die verschiedenen Wachstumsparameter der Pflanzen. Von allen JA-Konzentrationen, die in diesem Versuch eingesetzt wurden, zeigte nur $JA10^{-6}$ M eine signifikante Wirkung auf die Wachstumsparameter der Pflanzen. Bei den mit $Ni10^{-3}$ M und JA behandelten Pflanzen wurden signifikante Auswirkungen auf das Gewicht und die Länge von Wurzeln und Trieben bei Konzentrationen von $JA10^{-6}$ M und $JA10^{-8}$ M festgestellt. Die Ergebnisse der mit SL behandelten Pflanzen zeigten signifikante Auswirkungen auf die Trieblänge der mit $Ni10^{-3}$ M und $SL10^{-6}$ M, $SL10^{-7}$ M und $SL10^{-9}$ M behandelten Pflanzen. Auch die externe Anwendung von JA und SL zeigte signifikante Auswirkungen auf einige der gemessenen Parameter (Wurzel- und Sprosslänge). Bei Pflanzen, die mit Ni behandelt wurden, reduzierte die Anwendung von JA den Ni-Stress in bestimmten Konzentrationen. Bei Pflanzen, die mit Zn behandelt wurden, konnte JA die Stressauswirkungen nicht verringern. Wir stellten fest, dass SL ähnliche Auswirkungen hatte; es verbesserte die Widerstandsfähigkeit der mit Ni behandelten Pflanzen, während es weder positive noch negative

Auswirkungen auf mit Zn behandelte Pflanzen hatte. Unsere Experimente deuten darauf hin, dass die Phytohormone JA und SL erhebliche positive Auswirkungen auf das Wachstum und die Physiologie von mit HM behandelten Pflanzen haben können, was jedoch nicht als allgemeine Tatsache angenommen werden kann. In unserem Fall stärkten sie mit Ni behandelte Pflanzen, aber nicht mit Zn behandelte Pflanzen. Außerdem müssen vor der Anwendung auf dem Feld die richtigen Konzentrationen festgelegt werden.

1. Introduction

Our environment and its compartments have been severely polluted by heavy metals in many places. Heavy metals (HMs) are naturally occurring elements, but also anthropogenic activities introduce them into the environment. The presence of high concentrations of HMs in the environment threatens all living organisms. Because of the non-degradable state of HMs, they can accumulate in the food chain and lead to life-threatening (Recatala et al. 2006).

Some HMs are essential for plant metabolism and many physiological functions. For instance, Zinc (Zn) is necessary for Zn-dependent enzymes and Nickel (Ni) is also an essential trace element that has a vital role in normal growth and completion of plant's life cycle (Harasim and Filipek, 2015). On the other side, high concentrations of these elements have negative effects on morphology, physiology, and metabolic process of plants. Excessive concentrations of Zn can damage plant metabolic functions and change mitotic activity. High concentrations of Ni causes chlorosis and necrosis and inhibit cell division (Zaid et al. 2017). Some other HMs such as cadmium (Cd), mercury, lead, chromium and uranium are highly toxic even at low concentrations (Koller and Saleh, 2018).

Plant hormones are signaling molecules that regulate in small quantities plant growth and development activities like cell division, flowering, and seed formation. Other functions include regulation of cell membrane permeability, organ size regulation, enzyme activity, and pathogen defence (Wani et al. 2016). In addition, they help plants to respond to biotic and abiotic environmental conditions, such as sunlight, soil constituents and availability of nutrients. Under abiotic stress, phytohormones act as endogenous substances to modulate physiological responses that are critical for the plant's survival (Ullah et al. 2018).

In this study, we searched for a possibility to improve plant growth and survival under different concentrations of HMs (Zn and Ni) by phytohormones, jasmonic acid (JA) and strigolactone (SL) in two experimental parts; we assumed that HMs would induce different stress levels and phytohormones may increase the performance of the plants. As a study model, we selected wheat, *Triticum aestivum*, since this plant is one of the main agricultural products and used for human food and animal feed around the world.

1.1. *Triticum aestivum*

Wheat (*Triticum aestivum, T. durum*), in the family Poaceae, is the most important food grain and cereal crop cultivated in temperate zones and one of the main agricultural products. It provides approximately 55% of the carbohydrates needed in the whole world. Wheat is also a popular food source for animals (Curtis et al. 2002).

It is a grass which mainly grows as a winter annual, with seeding in the fall and harvest from June through August. Wheat seeding occurs in spring in areas with very cold winters and is harvested during the end of summer and in the autumn. Wheat can tolerate cold and snowy days and grows again in warm days in spring (Shukre et al. 2015).

The optimum growing temperature is 25˚C but wheat can grow in the minimum temperature of 3˚ to 4˚C and maximum temperature of 30˚ to 32˚C. This plant has a broad range of humidity compatibility from xerophytic conditions to littoral conditions. Optimal production occurs in locations with precipitation ranges from 250 to 1750 mm (Curtis et al. 2002).

Physiology

Wheat has two types of root, the seminal and the nodal roots. The deepest roots of wheat are extending as far down as 2 m. At the same time as roots grow, the storage of energy in the form of fructans in the stem occurs and conserves the plant in hard situations such as drought and disease (Zhang et al. 2015). The shoot is a combination of series of phytomers that have a node, a leaf, an internode and a bud; 6 to

16 of these units make a vegetative part of the shoot. Tillers have the same structure as the shoot and they grow from axils of the leaves. Each leaf consists of sheath and lamina (Curtis et al. 2002). The last leaf is known as the flag leaf; it is denser and has a higher photosynthetic rate than the other leaves in order to supply carbohydrates to the grains (Singh et al. 1995). For the investigation of morphological and physiological parameters, it is therefore important that always the same kind of leaves is measured.

Wheat like other plant species is sensitive to HM pollution. When this plant is exposed to HMs, it shows different reactions at different levels of growth and development such as yield losses. The toxic concentration of Zn in wheat plants causes harmful effects on physiological and morphological features. In a study that evaluated application of Cd^{2+} (50 µM) and Zn^{2+} (600 µM) on photosynthetic contents in wheat seedlings, results showed that both of these HMs had an inhibitory effect and decrease relative growth rate and CO_2 assimilation in the leaf of wheat (Rizvi et al. 2020). Samar et al (2020) found a negative impact of Ni on plant growth of wheat. Ni causes changes in biochemical reactions, decrease in vegetative growth, and significant reduction of wheat yield.

1.2. Heavy metals

HMs are elements with a density greater than 5 g cm^{-3}. Because of the highly toxic properties of HMs, they are in the center of attention of chemists and environmentalists. HMs can be released from natural and anthropogenic processes and contaminate environmental areas (soils, water, and air).

Natural emissions of HMs are volcanic activities, stones and rock weathering, and forest fire. Anthropogenic processes include agriculture, wastewater, and metallurgical and other industries and runoffs from these sources (Figure 1.1). For instances, smelting leads to release of Zn, copper and arsenic; burning fossil fuels releases Ni, mercury, selenium. Generally, contribution of human activities to metal pollution is more severe than input from natural sources (Shah et al. 2010).

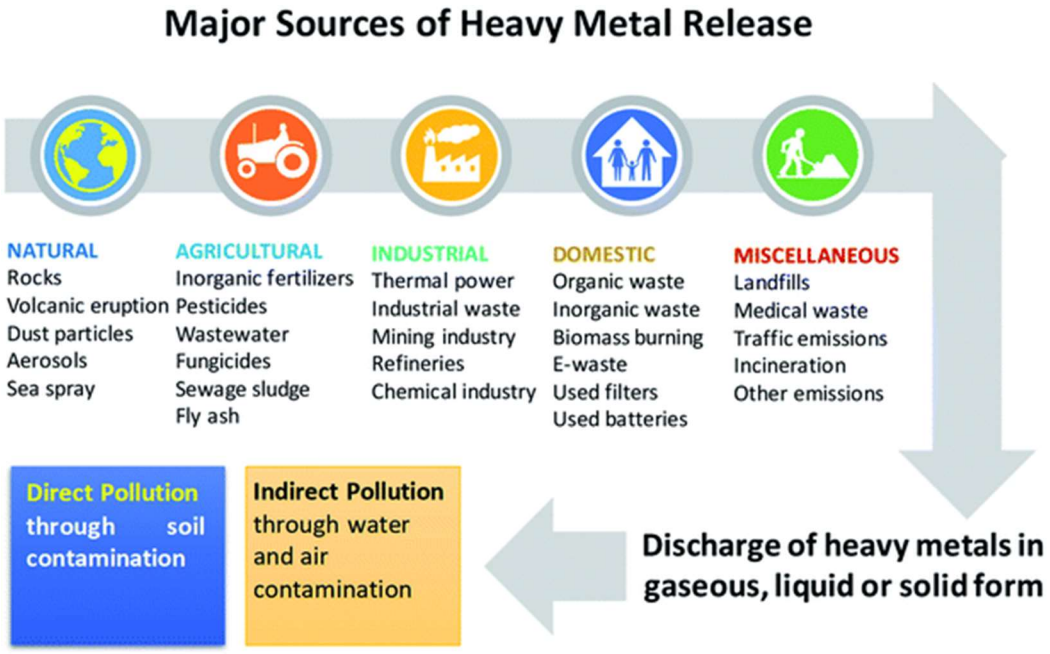


Figure 1.1: Sources of HM pollution in the environment *(Rizvi et al.2020).Natural and anthropogenic sources of heavy metals that effects on plants, animals and human in different forms. We examined the effects of Ni and Zn on some physiological parameters of wheat plant.*

The presence of HMs and their uptake by plants are influenced by soil properties such as soil texture, pH, redox potential, cation exchange, and organic materials. For example, higher organic material and pH in the soil cause hard bounding between HMs and soil and they will be less available in plants.

Some elements such as Zn, Cd, and manganese (Mn) are absorbed easily and moved within plant tissues but some others like iron (Fe), aluminium (Al), and lead (Pb) because of their hard adhesion to soil compounds or roots move less than others (Shah et al. 2010). The most important factor in metal accumulation in plants is soil pH. Metal elements in soils with high pH make compounds with low solubility and their availability in plants reduces (Seregin and Ivanov 2001).

Some HMs such as Ni, Zn, cobalt, copper (Cu), Fe, and Mn are essential for plants and can effect growth and development processes. Essential micronutrients play a vital role in plant metabolism. For example, Zn and Cu are essential for activity of Zn- and Cu-dependent enzymes which are necessary for physiological activities. But their concentrations more than the threshold are toxic and affect plant's life from molecular to physiological levels (Bücker-Neto et al. 2017). In plants, roots have direct contact with toxic elements in the soil and show changes in growth patterns. One of these changes is the inhibition of root elongation that is the first obvious effect of HMs. Similarly, rice seedlings contaminated with Ni have shown a reduction in photosynthesis as a result of growth inhibition (Shah et al. 2010).

One of the significant effects of HMs on plant growth is the overproduction of reactive oxygen species (ROS). Aerobic reactions like respiration lead to ROS production in cells and ROS can damage lipids, DNA and proteins. Thus, when plants are exposed to biotic or abiotic stress, the rate of ROS generation increases (Bücker-Neto et al. 2017) (Figure 1.2).

On the other hand, elements like lead, Cd, arsenic, chromium, and mercury are nonessential for plants and have toxic effects even in very low concentrations (Shah et al. 2010).

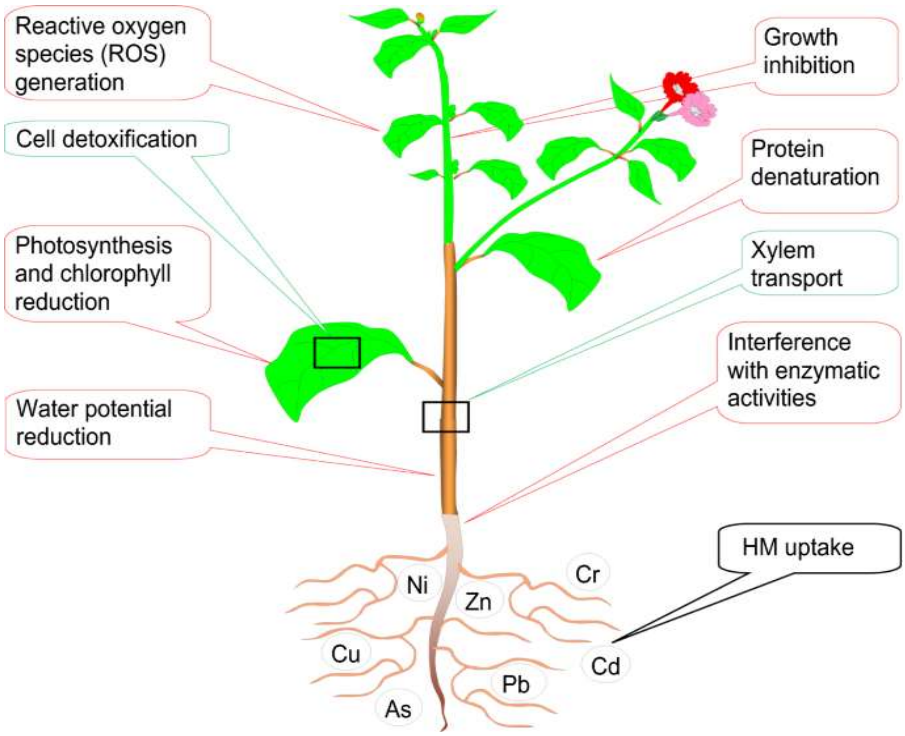


Figure 1.2: Some effects of HMs in plants *(Nedjimi 2021). HMs have positive and negative influences on plants. They uptake from soil by the root system and move to upper parts and effect on length and weight and other physiological and biological parameters of plants.*

With environmental changes, plants use different mechanisms to adapt to changing situations. Perception, transduction, and transmission of stress stimuli are these adaptive mechanisms. Endurance and persistence in stressful situations are mechanisms that plants use to immobilize HMs in their cells. Moreover, plants use different mechanisms to elude HMs toxicity which consist of: (1) production of reactive oxygen species through auto oxidation and Fenton reaction, (2) blocking of functional groups

of enzymes, and (3) movement of metal ions. They are all strategies that help plants to grow on contaminated soil (Shah et al. 2010). The plant's ability to grow in polluted soils is due to; (1) excluding HMs (metal excluders): mechanism that is preventing metals uptake with aerials tissues and/or holding metal concentration above the threshold of HM availability in soils by keeping metals in roots (2) metal indicators: mechanism is an accumulation of HMs through making strong binding between HMs compounds in aerial cells actively or changing in HMs arrangement through accumulation of HMs in non-sensitive sections, and (3) accumulators and hyperaccumulators: mechanism is localizing HMs in the aerial parts more than their concentrations in the soil (Raskin et al. 1994).

1.2.1. Zinc

Zinc is the 23rd most abundant element in the earth's crust and due to human activities, its concentrations are rising unnaturally (Nasernejad et al. 2005). It is a shiny white-bluish element that is crystalline and fragile at normal temperatures. It reacts with oxygen and also with non-metals fairly, so it is a reactive metal.

Galvanization, smelting, pesticide and fertilizer industries as well as combustion of fossil fuel use Zn in the production process and accordingly deposition to air, soil and wastewater from these industries brings Zn in large amounts in to the environment (Raut et al. 2012).

Zn is a necessary element not only in plant growth and development but also plays an important role in the activation and biosynthesis of some plant enzymes involved in carbohydrate metabolism, protein synthesis, activation of nucleic acid metabolism, and hormones (Firdous et al. 2018).

Also, Zn is needed for tryptophan synthesis and it is necessary for producing the growth hormone, auxin. It is needed for integrity of cellular membrane to maintain the structures of macromolecules and transportation of ions. Interaction between Zn with phospholipids and sulfhydryl groups contributes to preserving the membranes (Hafeez et al. 2013). Zn helps plants to produce chlorophyll in leaves (Asati et al. 2016).

Zn has a vital role in HMs homeostasis and the reduction of toxicity of these metals through the limitation of availability in plant cells. Zn can reduce abiotic stresses in different plants, such as reduction of Cu toxicity in plants, improvement of Cd toxicity and reduction of Cu toxicity in rice and wheat (Zaid et al. 2017).

The lack of Zn in soils is an important issue. Some soil characteristics such as pH, amount of organic materials, lime content, and concentration of phosphorus fertilizer can affect Zn concentration (Adiloglu & Adiloglu 2006). A deficiency of this element reduces synthesis of proteins and root development and also plant growth reduction (Zaid et al. 2017).

Zn uptake varies in different plant species but generally it occurs as a divalent cation or complex with organic matters. Roots absorb it through root hairs and cortical cells and then transfer it through xylem to the shoot. Zn translocation in root xylem happens via symplast and apoplast (Hafeez et al. 2013).

High concentrations of Zn in plants lead to decrease in growth and development and cause senescence, reduction of plant biomass and prevention of cell division, inhibition of nutrient uptake and enzyme activity, oxidative damage, and in addition cause chlorosis in the young leaves. Excessive concentrations of this element decrease germination, chlorophyll, carotenoid, amino acids in beans. Toxicity of this element depends on plant type, time of exposure with Zn and growth medium of plants (Zaid et al. 2017) (Asati et al. 2016).

Contrary to negative effects, previous studies about application of Zn on wheat plant showed that Zn increased the number of wheat grains and seed yield. Also, effects of Zn on agronomic traits and grain quality of wheat were investigated and application of Zn at different growth phases increased fertile

spikelet and plant height. An increase in kernel weight, economic yield and harvest index are other effects of Zn application on the wheat plant (Sadeghi et al. 2021).

1.2.2. Nickel

Ni is the 24th most abundant element in the earth's crust; it is silver-white and can be found naturally in different mineral forms. Ni is released from natural and anthropogenic sources and distributed in air, water, and soil. Some natural sources of Ni emission include rocks and soils weathering, volcanos activity, and forests. This element is used in many industries including stainless steel production, metallurgical and chemical processes, and causes major problems for agricultural lands near these industries that receive wastes or sewage sludge from them (Cempel 2006).

Brown et al. (1987) found that Ni is essential for plant growth and development. It is considered as a supplement element in completing the life cycle in plants. Ni takes part in some metabolic reactions including hydrogen metabolism, acetogenesis, and biogenesis. It plays a role in phytoalexin synthesis.

Ni can be absorbed by roots through active transport and passive diffusion. Absorption and bioavailability of Ni to plants depend on pH of soil and soil type. For example, for oat plants grown in soil rich in organic matter, Ni availability is less than for plants grown in clay or sandy soils with pH 4 to 7. (Harasim and Filipek 2015). The mechanism of Ni absorption can be through cation transport system or Mn ion transport system.

Like other HMs that are necessary for plants but toxic at higher levels, there has been more concern about toxicity of Ni in the environment. Excessive Ni causes different physiological changes in different plant species such as necrosis and chlorosis. Reduction of plant growth and inhibition of cell division occur at higher concentrations of Ni in root meristems. Excessive Ni in plants may change plant morphology such as devaluation of mesophyll thickness, the extent of vascular bundles and the expanse of epidermal cells. High concentrations of Ni can reduce the plasticity of cell walls. Another toxicity effect of Ni is inhibition of photosynthesis by reducing chloroplast size and numbers and disorganization of ultrastructure of chloroplast. Elevated concentrations of Ni effect mineral nutrition, enzyme activity, induction of oxidative stress, chlorophyll content and reactive oxygen species (Satish et al. 2015).

On contrary, recent studies have shown that Ni application can also have positive effects such as increasing the grain yield of wheat. It has also been observed that Ni has stimulatory effects on seed germination and seedling growth of wheat plant (Shewti et al. 2018).

Ni concentration in many plant species is very low (0.05-10 mg/kg dry weight). High concentrations of Ni hurt fruit yield and quality of wheat. Parlak as late as 2016 found that Ni concentration at the range of 0 to 50 μgL^{-1} reduce seedling growth, and cause deformation in wheat seedling and reduction of root volume in wheat plant. He also indicated that Ni accumulation in above-ground parts (shoot and leaves) causes growth inhibition of these organs.

Other studies showed that Ni accumulation in roots was more than in shoots and Ni toxicity in wheat decreased plant growth by changing plant metabolism, reduction in enzyme activity and high mobility on this element (Ain et al. 2016).

1.3. Phytohormones

Phytohormones are endogenic molecules that modify physiological and molecular reactions. They have different regulatory functions in low amounts in plants such as enzyme activity, plant growth and development, and cell membrane permeability.

The crucial and necessary phytohormones are jasmonic acid (JA), auxin (IAA), abscisic acid (ABA), gibberellin (GA), cytokinin (CK), ethylene (ET), salicylic acid (SA), brassinosteroid (BR), and

strigolactone (SL) (Fig1.3). These hormones influence seed germination, roots, stems and leaf growth, flowering, and seed development.

In addition, they play an important role in different stages of plant life and have three main categories due to their functions and effects on plants which include control of vegetative growth, reproduction, and stress response (Sytar et al. 2019).

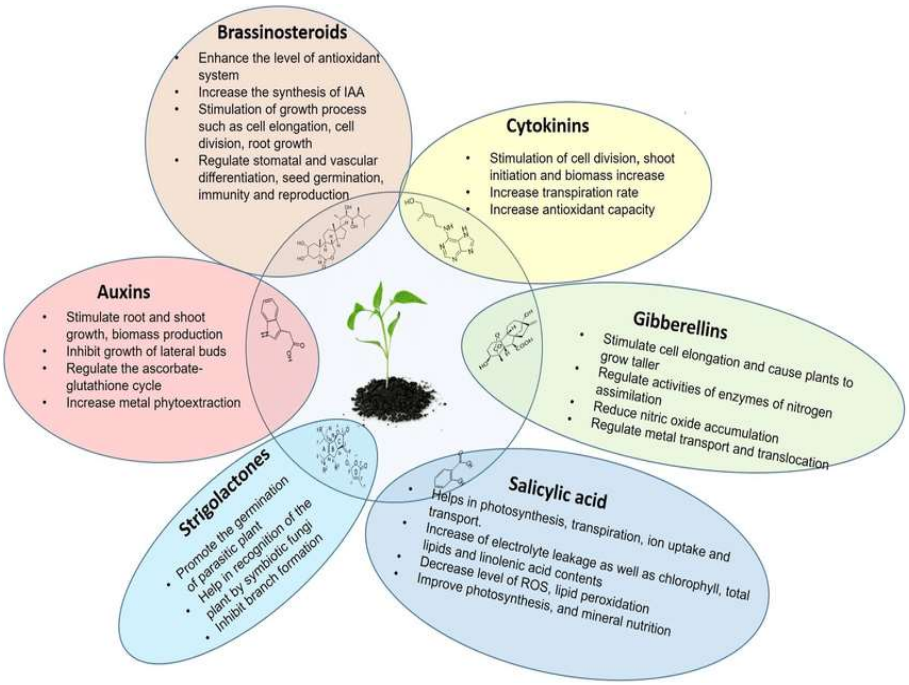


Figure 1.3: Main functions of important phytohormones and their functions in plant growth and development *(Sytar et al. 2019). In this study two phytohormones (JA and SL) were selected to examine the significant and insignificant effects on growth parameters of wheat plant.*

Exogenous application of phytohormones is a safe method for plants under HMs toxicity. These chemical messengers with complicated regulation allow plants to maintain growth and development processes under biotic and abiotic stress. For instance, exogenous application of methyl jasmonate (MeJA) can control stomatal aperture, and inhibit Cd uptake and decrease photosynthetic damage. According to environmental changes, the concentrations of exogenous plant hormones during growth and development can be changed significantly (Sytar et al. 2019).

1.3.1. Jasmonic acid

The source of jasmonate is *Jasmine grandiflorum* which contains esters of MeJA and JA (Sezgin and Kahya 2018). Jasmonates are found in shoot apex, root tips, immature fruits and young leaves. But the biosynthesis of jasmonates occurs in leaves and roots. Production of JA occurs by releasing linolenic acid from plant membranes and lipoxygenases (LOXs) oxidate it to the cyclopentanone form (Cesari et al. 2014) (Fig 1.4). Some important functions of JA include development of reproductive organs, sex determination, seed germination, root growth, leaf movements, fruit ripening, and formation of trichomes (Sabagh et al. 2022).

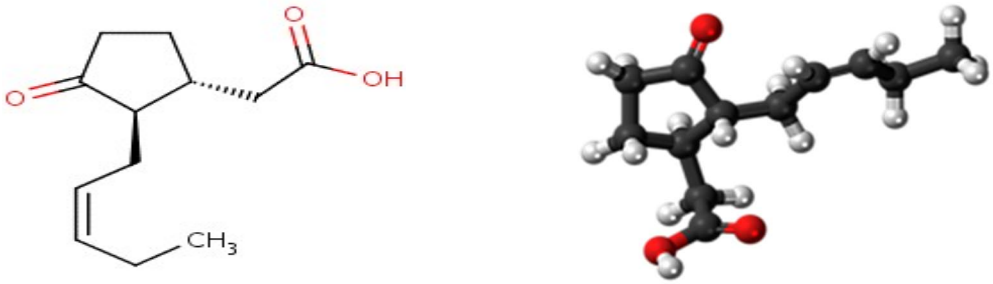


Figure 1.4: Chemical structure of methyl jasmonate- cyclopentaneaceticacid, 3-oxo-2-(2-penten-1-yl)-, methyl ester *(Cesari et al. 2014)**. Methyl jasmonate that used in this study, is a natural cyclopentanone lipid from jasmonates family. It is used to examine its role in growth and development of wheat plant under HMs stress.*

It should be noticed that JA is produced when the plant is exposed to particular stress factors such as elicitation, wounding, osmotic stress, and pathogen attack. JA has an important role in many physiological functions related to growth and development such as effect on seed germination, development of pollen grains, photosynthesis inhibition, plant height elongation, root growth, flower bud formation, and embryogenesis. Also, JA and MeJA act as signals in response to biotic or abiotic stress (Czerpak et al. 2006). Moreover, promoting properties of jasmonate include protein synthesis, adventitious root formation, and synthesis of ethylene (Sezgin and Kahya 2018) (Fig 1.5).

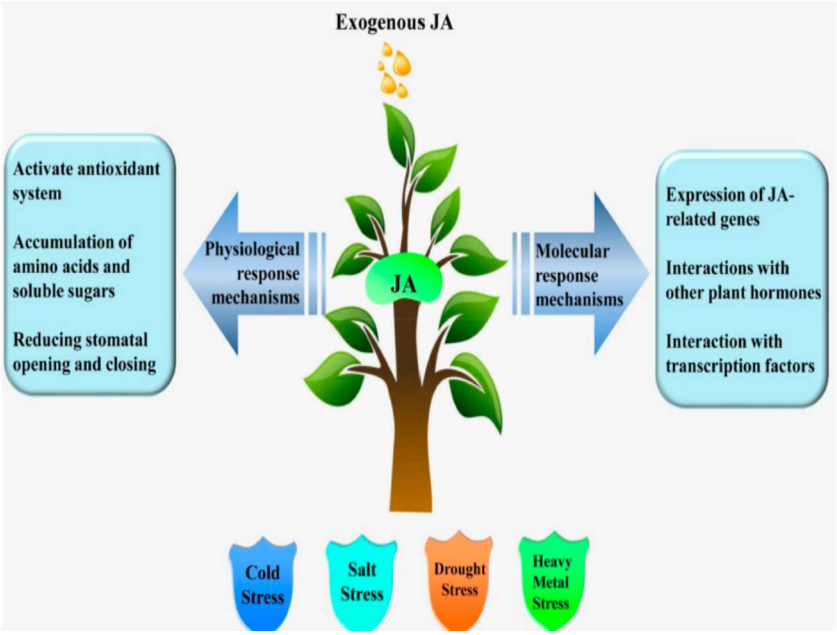


Figure 1.5: The role of JA that showed the molecular and physiological responses of plants under abiotic stress *(Wang et al. 2020). The influence of JA on wheat plant growth and development under HM stress are examined in this study.*

Evidence suggests that JA plays a considerable role in response to HM stress. For example, in Arabidopsis, in response to treatment with Cd, JA levels increased quickly. Similarly, in wheat, overexpression of TaAOS (*Triticum aestivum* allen oxide synthase) activated JA biosynthesis and improved plant tolerance under Zn stress (Kim et al. 2021).

Previous studies about the response of JA to HMs stress demonstrated that exogenous application of JA can reduce harmful effects of oxidative stress on growth parameters, biomass, and protein concentrations in plants treated with Ni through additional production of antioxidant enzyme activity (Wang et al. 2020).

1.3.2. Strigolactone

Strigolactones (SLs), a small class of carotenoid-derived compounds, are plant hormones that first were identified in 2008 as shoot branching factors. They are the most recently known hormones related to branching (Ruyter-Spira et al. 2011).

SLs made up of three connected rings that include ABC scaffold and are connected by enol ether and butenolide that called D-ring. SL structure includes three compounds which are called GR24, GR7, and GR5. The A-ring aromatic produce code GR24, named after inventor Gerald Rosebery. By removing A-ring and cutting B-ring GR7 and GR5 are obtained, respectively (Figure 1.5) (Zwanenburg et al. 2016).

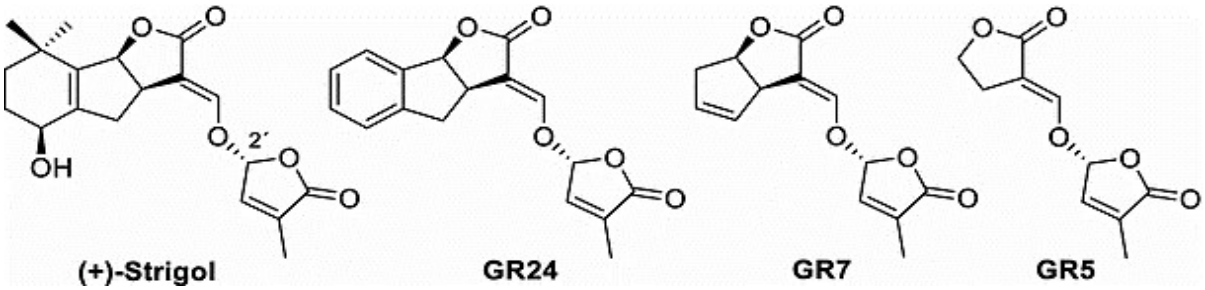


Figure 1.5: Simplified structures of SL. GR24, GR7, and GR5 (Zwanenburg et al. 2016). *SL as a newly identified phytohormones is used to determine the significant or insignificant effects on growth parameters of wheat plant.*

SL is one of the most important hormones in apical dominance formation. It is also impressive in the mechanisms of branching-related genes (Sezgin and Kahya 2018). SL can affect the shaping of shoot structure, development of roots, and senility of leaves in various plant species. Also, by decreasing phosphor and nitrogen in soils production of SL increased and the hormone was secreted to the soil (Marzec 2016).

SL biosynthesis occurs in the root and through PLEIOTROPIC DRUG RESISTANCE 1 carried to the stem but may be synthesized in the upper parts of plants. SL produces in a little amount and is around 25-30 pg per plant. Strigolacton is produced in monocots and dicots. It is synthesized in roots and stems and in two forms root secretion and endogenous form (Kausar and Shahbaz 2017).

As a signalling mediator, it can regulate development of shoot and root, and regulate under and above-ground morphology of plants such as leaf senescence, shoot branching, formation of adventitious root and density of root hair. In addition, SL can increase cell elongation, increase symbiotic reactions, control leaf shape, impact on developmental procedures, and crosstalk with other hormones like ethylene to moderate environmental stress responses.

The adequate number of tillers in wheat is one of the important factors in grain yield improvement and it is related to SL secretion. SL can regulate primary root length in wheat positively (Wu et al. 2022).

Moreover, SL helps plants' tolerance to environmental stresses such as nutrient stress, high salinity, drought, and HM toxicity. SL acts as an effective regulator in response to biotic and abiotic stress in plants (Chien et al. 2014). This function of SL is done by crosstalk between SL and other regulatory plant hormones like CK, ET and ABA. Under abiotic stress, SL persuades tolerance in plant by activating ABA and/or SL with ET and IAA hormones to form a coordinated network to adjust plant growth and respond under abiotic stress (Wu et al. 2022).

1.4. Parameters that indicate possible level of plant stress

Plant performances can be measured by anatomical and physiological parameters which indicate a possible stress level of the plants. For defined growth conditions, pot experiments are made. Concerning

physiology of plants, we measured cellular tolerance by plasmolysis method and the chlorophyll fluorescence of the leaves (FV/FM ratio) by Handy EPA. Regarding anatomical response, morphological parameters such as root and shoot length, root and shoot weight and number of leaves were measured.

1.4.1. Objectives and questions

The main goal of this study was to investigate if the phytohormones JA and SL have positive effects on wheat plants that were stressed with Zn and Ni. Therefore, effects of JA and SL on plants that were treated with different concentrations of Ni and Zn were investigated.

Objective one

- To determine the effects of different concentrations of HM ($ZnSO_4$ and $NiSO_4$) in the soil on wheat.

Objective two

- To investigate the possible impact of phytohormones (JA and SL) on wheat.

Objective three

- To investigate the possible impact of phytohormones on the HM stress tolerance of wheat.

Questions

The following questions rose from the main objectives:

- What is the effect of different concentrations of Zn on the different plant parameters?
- What is the effect of different concentrations of Ni on the different plant parameters?
- Is there a possible impact of JA on the different parameters of plants?
- Is there a possible impact of JA on the different parameters of plants treated with Zn?
- Is there a possible impact of JA on the different parameters of plants treated with Ni?
- Is there a possible impact of SL on the different parameters of plants?
- Is there a possible impact of SL on the different parameters of plants treated with Zn?
- Is there a possible impact of SL on the different parameters of plants treated with Ni?

2. Materials and methods

Biologically controlled wheat seeds from Nestelberger (AT-BIO-301) were seeded into approximately 22 g of soil composed of a homogenized mixture of 25% clay, 14% silt, and 61% sand. This soil was provided by the gardeners of the University of Vienna for this experiment. Five seeds for the first experiment were put into the soil which was treated with different concentrations of $ZnSO_4$ (10^{-3} M, 10^{-2} M, and 5.10^{-3} M) and $NiSO_4$ (10^{-3} M, 5.10^{-3} M, and 10^{-2} M) and for the second experiment was treated with $ZnSO_4$ (10^{-3} M, 10^{-2} M, and 5.10^{-3} M) and $NiSO_4$ (10^{-4} M, 10^{-3} M and 5.10^{-3} M).

The plants of these two experiments were exposed to HM stress for approximately 5 weeks for the first experiment and approximately 4 weeks for the second experiment. The plants were grown in the greenhouse at regularized temperatures variably ranging from 19.8° C to 34.1° C within the growth period. An overview of the experimental outlook of the first part of the experiment during the 5 weeks is provided in figure 2.1. During these experiments, the soil pots were spiked by Zn and Ni weekly to be sure that plants were exposed to HMs in the right concentrations in the progress of this study.

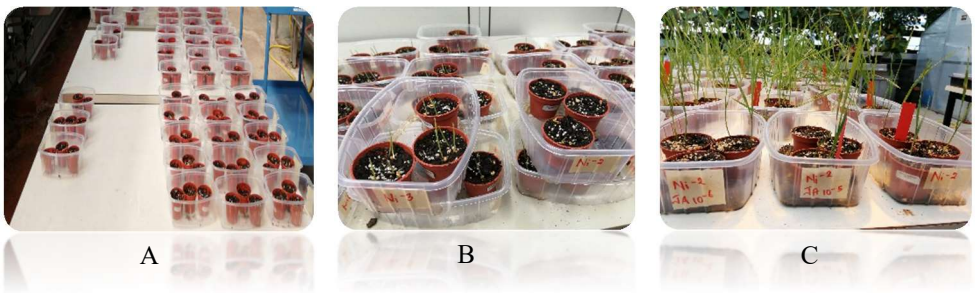


Figure 2.1: *The outlook of the first experimental part of the study. Pictures A, B and C show the process of the experiment from the first day of seedling in the pots and treatment with HMs and phytohormones until third weeks.*

2.1. Treatment with phytohormones

To determine the possible impact of the phytohormones on the HM stress, the plants' leaves were sprayed with JA; for the first experiment, and with SL for the second experiment, at concentrations of 10^{-5} M, 10^{-6} M, 10^{-7} M, 10^{-8} M, and 10^{-9} M, respectively. In the first part of the experiment, the stock solution and method of application of JA were replicated from Awang et al. (2015) and the idea of what concentrations to be used was simulated from Yoon et al. (2009) and Yang et al. (2012). From the second week of experiment, the leaves of wheat plants were sprayed to wetness with JA, once a week. In the second part, the stock solution was prepared as simulated in Soto et al. (2010) and the method of application of SL was simulated from Ha et al. (2014). The idea of what concentrations to use was replicated from Gomez-Roldan et al. (2008), Minakuchi et al. (2010) and Rasmussen et al. (2013). In the second phase, the leaves were sprayed with 5ml of SL, a total of six times within the first three weeks of the experiment. In the progress of this study plants were irrigated twice or three times a week with 20 or 30ml of water depending on the need for water by the plants. The plants were also fertilized once a week with either 20 or 30 ml of WUXAL R super (comprising 8% N, 8% P_2O_5 and 6% K_2O), kindly provided by the gardeners of the University of Vienna.

Treatment groups

- Control group

Group of plants that were neither treated with HMs (Zn or Ni) nor phytohormones (JA and SL).

- HM treatment (Zn and Ni)

Plants that were grown in the soil that was treated only with HMs (Zn or Ni).

- Phytohormone treatment (JA and SL)

Plants that were grown in soil that was treated only with phytohormones (JA or SL) and leaves were sprayed during these experiments.

- HM-Phytohormone treatments (Zn/JA, Zn/SL, Ni/JA, Ni/SL)

Plants that were grown in soil treated with HMs (Zn or Ni); leaves were sprayed with phytohormones (JA or SL) throughout the experiments.

Measured parameters

- Root and shoot length in centimeters (cm)
- Root and shoot weight (dry weight) in grams (g)
- Chlorophyll fluorescence; FV/FM ratio
- Number of leaves
- Cellular tolerance by plasmolysis

2.2. Testing plant physiology

In the third week of each experiment, the cellular tolerance of the plant's leaves was measured by the method of plasmolysis. In the last week of each experiment, before harvesting time, the chlorophyll fluorescence of the leaves was measured using a HANDY PEA meter, by following the instructions from the instrument's manual "the Hansatech instruments HANDY PEA: Field reference guide". The value of the maximal fluorescence and the variable fluorescence were expressed as the FV/FM ratio of the leaves which is a tool to measure the photosynthetic capacity of the plant leaves.

2.3. Testing plant morphology

At the end of the experiments, plants were taken out of the pots and the roots were washed carefully with distilled water. Then, root length, and shoot length were measured in centimeters, and the number of leaves was counted. The plant tissue was oven-dried at 45℃ for two weeks after separating shoots, and roots and weighed in grams. For the statistical analysis and multiple comparisons of treatment groups, mean values of treatments were used to run the ANOVA test (Tukey unequal N HSD, $p < 0.05$) on the software Statistical Package for the Social Sciences (SPSS). To report data, bar charts of excel software were used and they show the calculated standard errors above which are stars indicating significant differences between treatment groups or in comparison with the control group.

2.4. Testing cellular tolerance

Plant development has an important role in plant responses and reactions to environmental changes and stress during their life time. Therefore, plant structures have complex process that allows plants to tolerate and/or adapt to adverse conditions such as salt, drought, HMs. (Wei et al. 2016).

Plasmolysis has been used as a tool to observe and measure many physiological functions in plant cells such as the relation between plant-cell water, description of soluble concentrations of cells, measurement of cell viability and as a tool to destroy intercellular transportation to study the types of growth and separation (Oparka 1994).

Generally speaking, plasmolysis is a reversible reduction of the volume of protoplast cells due to a downward gradient of water flow when they are exposed to a hyperosmotic external water-withdrawing solution. This cell reduction occurs when water comes out from the vacuole and the outer water potential is less than abrogate of the hydrostatic pressure of the turgor and leads to water going out of the protoplast (Cheng et al. 2017).

This experiment used plasmolysis as a measurement tool to observe and measure the sensitivity and viability of the wheat cells (leaf cells) to HMs ($NiSO_4$ and $ZnSO_4$ in solution).

2.5. How to do plasmolysis

Wheat leaves that had been treated with highest and lowest concentration of HMs and phytohormones were cut into slices which were thick enough that the epidermal cells remained alive. These tiny sections of leaves according to treatment with Ni or Zn were put into solutions of $NiSO_4$ and $ZnSO_4$, respectively, in little plastic cups. Cups were kept in darkness for 48 hrs and after that we observed the cytoplasm conditions under the light microscope (Olympus BX41). The withdrawal of the protoplast from the cell wall by hypertonic medium (80% of mannitol) is only possible if the cells are alive and the plasma membrane viable.

Plasmolysis could be observed in the light microscope: the protoplast was detached from the cell wall and visible in the center of the cell wall (Figure 2.2). On the other hand, an unplasmolyzed cell in this experiment is the one that the protoplast did not shrink away from the cell wall because the plasma membrane has lost its semi-permeability due to toxic conditions.

In this study, a section of treated plant was considered to be cellular tolerant when at least 50-90% of the cells were plasmolyzed (+), partially tolerant (±) when not less than 10 to 40% of cells were plasmolyzed and unplasmolyzed (-), when < 5 % of cells were plasmolyzed.

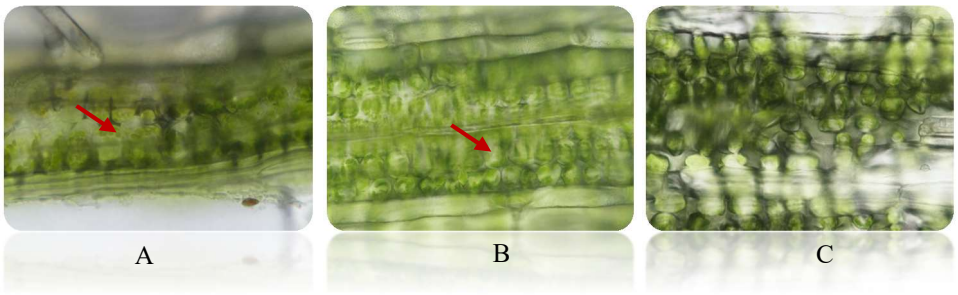


Figure 2.2: Viable and dead cells observed under the light microscope. *Some of the cells that showed the highest and lowest tolerance were observed. Treatment of $Zn10^{-3}$ $JA10^{-5}$M in $Zn10^{-5}$M (A) and treatment of $SL10^{-5}$ in $Ni10^{-1}$M (B) that showed the viable cells and treatment of $Ni10^{-4}$ $SL10^{-9}$ in $Ni10^{-1}$M (C) that showed dead cells. The viable cells showed with red arrow.*

3. Results

Wheat plants are treated with HMs in two different experiments of approximately five and four weeks. Wheat seeds were seeded in 60 ml pots containing approximately 22 g of soil treated with $NiSO_4 10^{-3}$ M, 5.10^{-3} M, and 10^{-2} M and $ZnSO_4 10^{-3}$ M, 10^{-2} M, and 5.10^{-2} M. Every week, defined solution of HMs was applied to the soil. Treated plants with HMs also were sprayed with different concentrations of phytohormones on the leaves, JA in the first phase, and SL in the second phase in concentrations of 10^{-5} M, 10^{-6} M, 10^{-7} M, 10^{-8} M and, 10^{-9} M for each of them. For control C, only water was used.

3.1. Effects of heavy metals

3.1.1. Effects of different concentrations of Zn on wheat

Among concentrations of $Zn10^{-3}$ M, 10^{-2} M, and 5.10^{-2} M that were applied on wheat plants and in comparison with C, plants treated with $Zn5.10^{-2}$ M showed signs of high toxicity. Their roots were short with very little or no root hairs and no lateral root formation. The leaves showed chlorosis and reduction in the number of leaves (Fig 3.1).

On the other side, $Zn10^{-3}$ M and 10^{-2} M treatment did not adversely decrease the growth parameters such as shoot length and weight.

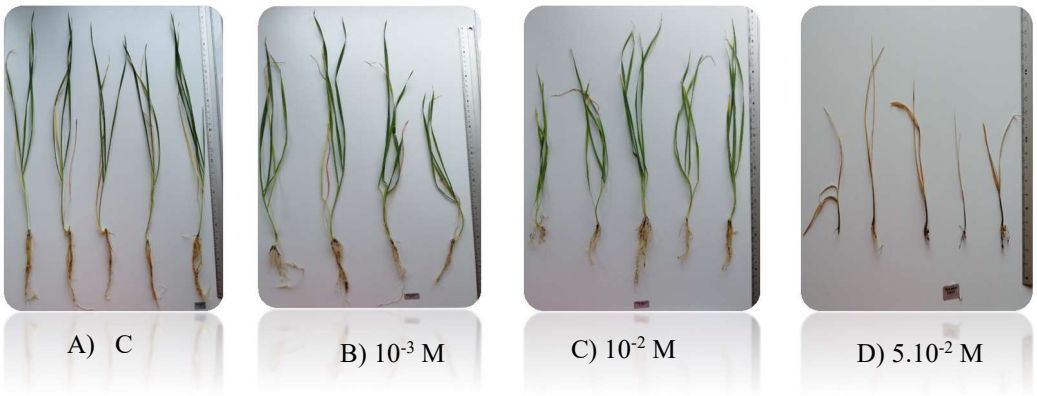


***Figure 3.1*: Comparison of control and Zn treated plants.** *Physical changes in roots and shoots in treatment with different concentrations of HM and compared to C plants. The concentration of $Zn5.10^{-2}$ M (D) showed the significant effects on root and shoot length and weight of wheat plant in comparison with C.*

3.1.1.1. Effects of different concentrations of Zn on root and shoot length

In plants treated with $Zn5.10^{-2}$ M roots length were significantly affected by lowest concentration of Zn in the soil in comparison to C. This result was also same in the shoot length of plants treated with $Zn5.10^{-2}$ M.

The concentration of $Zn10^{-2}$ M showed a significant effect only on root length of wheat plants in comparison with C. Although shoot length was also decreased, it was not significant.

Plants treated with $Zn10^{-3}$ M showed a reduction in root length but it was not significant in comparison with C. But in this concentration of Zn shoot length increased in comparison with C treatment that shows the positive effect of Zn on plant growth (Figure 3.2**)**.

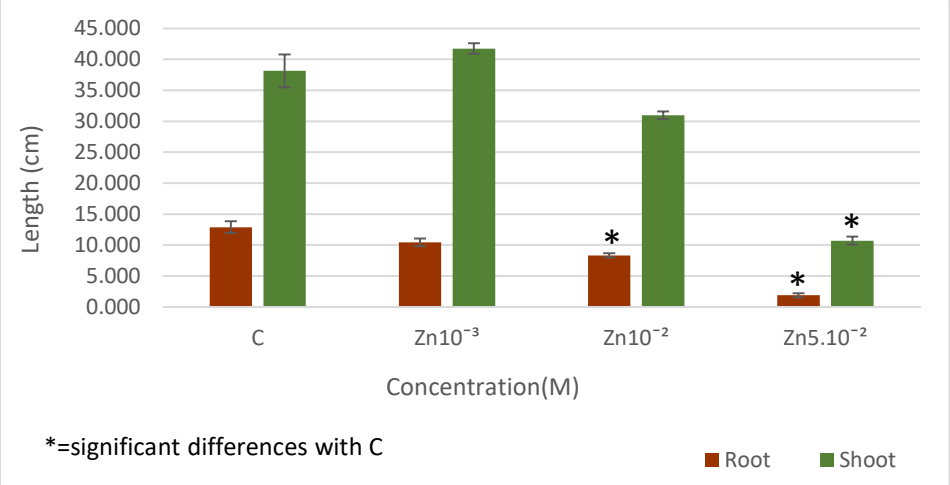


Figure 3.2: Comparison of root and shoot lengths in C and Zn treated plants. *Brown bars show the average root length and green bars show the average shoot length. Stars over the bars indicate significant differences between the mean value of root and shoot length and treatment groups after ANOVA (Tukey unequal N HSD, $p < 0.05$).Significant effects of different concentration of Zn on root and shoot length were observed at $Zn10^{-2}$ M and $Zn5.10^{-2}$ M, respectively.*

3.1.1.2. Effects of different concentrations of Zn on root and shoot dry weight

Plants treated with different concentrations of Zn showed a reduction of dry weight in roots and shoots.

In comparison with C, root weight in treated plants with $Zn10^{-3}$ M, 10^{-2} M and 5.10^{-2} M decreased significantly especially at highest concentration of Zn.

Shoot weight in all concentrations of Zn reduced but not significantly except in the concentration of $Zn5.10^{-2}$ M in comparison with C (Figure 3.3).

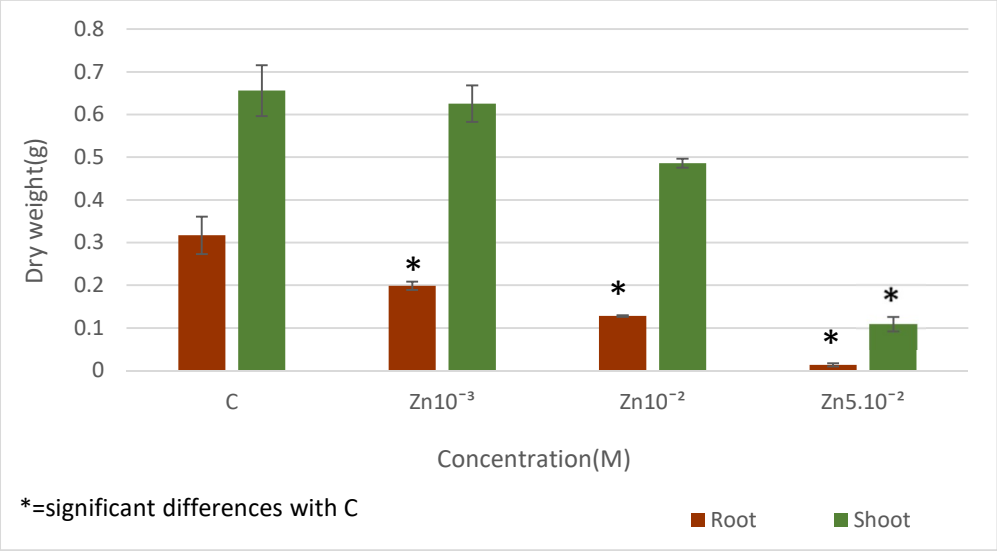


Figure 3.3*: *Comparison of root and shoot dry weights of C and Zn treated plants. *Brown bars show the average root biomass and green bars show the average shoot biomass. Stars over the bars indicate significant differences between the mean value of root and shoot length and treatment groups after ANOVA (Tukey unequal N HSD, $p < 0.05$). Significant effects of different concentration of Zn on root dry weight were observed at $Zn10^{-3}$ M, $Zn10^{-2}$ M and $Zn5.10^{-2}$ M and on shoot dry weight at $Zn5.10^{-2}$ M.*

3.1.1.4. Effects of different concentrations of Zn on the photosynthetic ability

The values of the maximal fluorescence and the variable fluorescence were expressed as the FV/FM ratio of the leaves and in this study it was measured in different concentrations of Zn.

In this experiment, the FV/FM ratio of wheat leaves decreased in Zn5.10^{-2} M, although insignificantly, in comparison with C treatment. Other concentrations of Zn (10^{-3} M and10^{-2} M) applied to the soil showed a very small increase but insignificant in the FV/FM ratios of wheat plants in comparison with C (0.799, 0.800 and 0.789 respectively) (Figure 3.4).

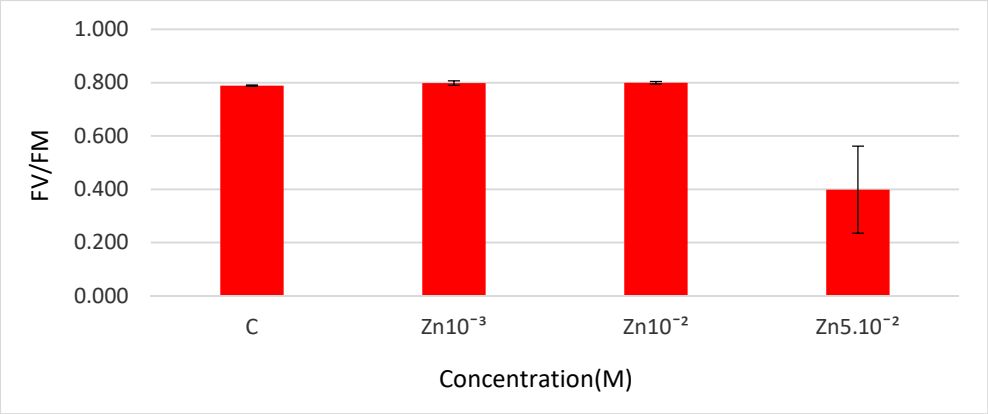


***Figure 3.4**: **Comparison of FV/FM ratio of C and Zn treated plants.** Red bars indicate the mean value of the fluorescence measurement of the leaves (FV/FM ratio). No significant effects of treatment with different concentrations of Zn were observed on the FV/FM ratio.*

3.1.1.5. Effects of different concentrations of Zn on wheat plants to form leaves

Among all concentrations of Zn applied to the soil, only plants treated with Zn5.10^{-2} M showed significant decrease in comparison with C.

The concentrations of Zn10^{-3} M and 10^{-2} M showed also reduction in number of leaves but not significantly (Figure 3.5).

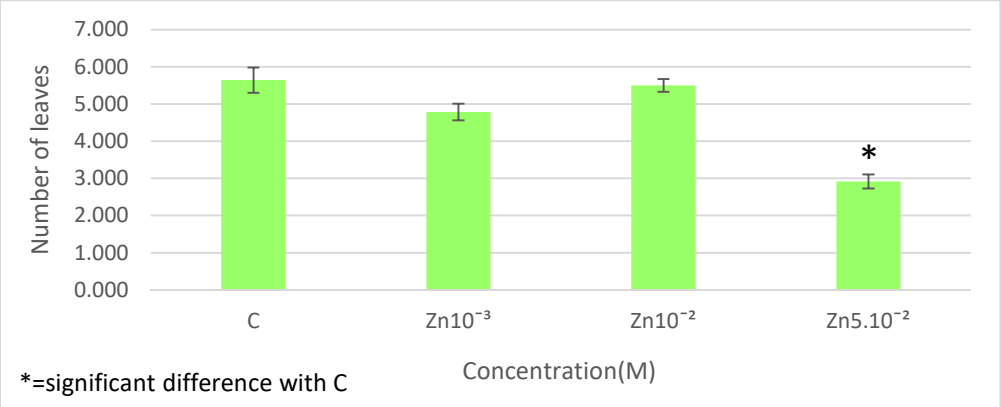


***Figure 3.5**: **Comparison of the number of leaves of C and Zn treated plants.** Green bars indicate the mean value of the number of leaves counted per plant (Numbers). Stars over the bars indicate significant differences between the mean values of the number of leaves between treatment groups after ANOVA (Tukey unequal N HSD, $p < 0.05$). Plants were treated with Zn5.10^{-2} M showed significant decrease in number of leaves.*

3.1.2. Effects of different concentrations of Ni on wheat

In this experiment, the concentrations of Ni that were applied to the plants, showed a significant effect at Ni5.10^{-3} M to 10^{-2} M. Symptoms of Ni toxicity such as chlorosis in leaves and necrotic leaf spots were very common observations in plants treated with Ni5.10^{-3} M in this experiment. In maximal toxicity of Ni10^{-2} M, just one seed completely germinated and had root and shoots but the others were affected by the high concentration of Ni and had no defined root and shoot. The influence of Ni10^{-3} M on wheat plants (all measured parameters) was insignificant in comparison with C.

***Figure 3.6**: **Comparison of C and Ni treated plants.** The physical changes in roots and shoots when treated with different concentrations of Ni are compared to the C plants. Significant effects of Ni on wheat plants were observed at concentrations of Ni5.10^{-3} M and (C) Ni10^{-2}M (D).*

3.1.2.1. Effects of different concentrations of Ni on root and shoot length

The concentration of Ni10^{-3} M didn't show any significant effect on the root and shoot length.

On the other hand, at the concentration of Ni5.10^{-3} M a significant decrease of root length was observed. Plants treated with Ni10^{-2} M showed a significant decrease in root length in comparison with C treatment. Also, treated plants with this concentration showed a significant decrease in shoot length (Figure 3.7).

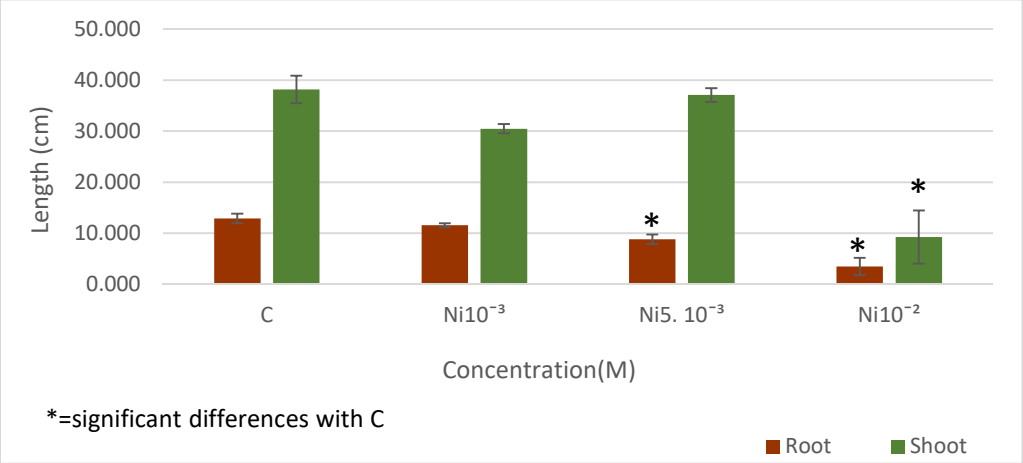


***Figure 3.7: Comparison of root and shoot lengths of C and Ni treated plants.** Brown bars show the root length and green bars show the shoot length. Stars over the bars indicate significant differences between the mean values of root and shoot length between treatment groups after ANOVA (Tukey unequal N HSD, $p < 0.05$). Ni5.10^{-3} M showed significant decrease on root length and Ni10^{-2} M showed significant decrease on root and shoot length.*

3.1.2.2. Effects of different concentrations of Ni on root and shoot dry weight

The root biomass of plants treated with different concentrations of Ni showed a significant decrease in Ni5.10^{-3} M and10^{-2} M in comparison with C. Also, these concentrations of Ni had a significant effects on shoot biomass of wheat plants.

On the other hand, plants treated with Ni10^{-3} M showed an increase in the root and shoot biomass although not significantly (Figure 3.8).

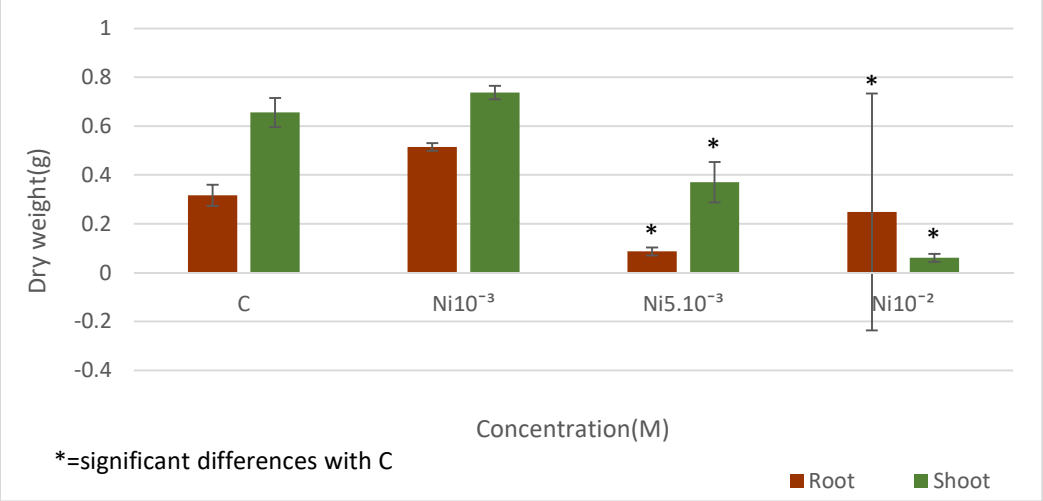


***Figure 3.8**: **Comparison of root and shoot weights of C and Ni treated plants.** Brown bars show the root biomass and green bars show the shoot biomass. Stars over the bars indicate significant differences between the mean values of root and shoot length between treatment groups after ANOVA (Tukey unequal N HSD, $p < 0.05$). Significant decrease of root and shoot dry weight were observed at concentrations of $Ni5.10^{-3}M$ and $Ni10^{-2}$ M.*

3.1.2.3. Effects of different concentrations of Ni on the photosynthetic ability of wheat leaves

In this experiment, no significant effects of different concentrations of Ni on FV/FM ratio were observed. Only at concentration of $Ni10^{-3}$ M FV/FM ratio increased (C: 0.789 and $Ni10^{-3}$M: 0.795) (Figure 3.9).

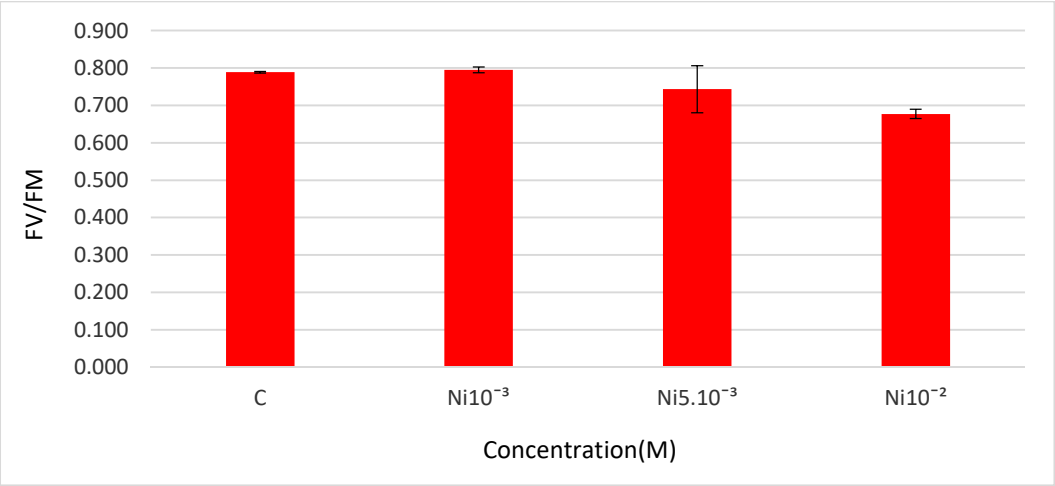


***Figure 3.9**: **Comparison of FV/FM ratio of C and Ni treated plants.** Red bars indicate the mean value of the fluorescence measurement of the leaves (The FV/FM ratio). There were no significant effect of different concentrations of Ni on FV/FM ratio.*

3.1.2.4. Effects of different concentrations of Ni on wheat plants to form leaves

Plants treated with $Ni10^{-3}$ M and 5.10^{-3} M showed an insignificant decrease in the total number of leaves formed by the plants whereas the plants treated with $Ni10^{-2}$ M showed a significant decrease in the number of leaves in comparison with C treatment (Figure 3.10).

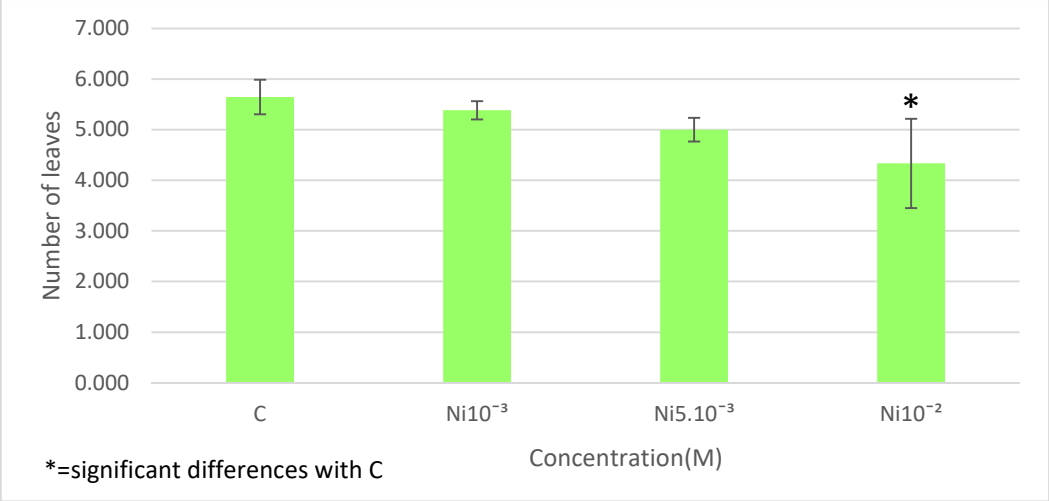


***Figure 3.10**: **Comparison of the number of leaves of C and Ni treated plants.** Green bars indicate the mean value of the number of leaves counted per plant (Numbers). Stars over the bars indicate significant differences between the mean values of the Number of leaves between treatment groups after ANOVA (Tukey unequal N HSD, $p < 0.05$). Only at concentration of $Ni10^{-2}$ M significant decreased of number of leaves were observed.*

3.2. Effects of phytohormones

In this case study, to understand the effects of phytohormones on plant growth, different concentrations of JA and SL were applied to the plants in the optimal conditions and in plants treated with Zn and Ni.

3.2.1. The involvement of phytohormones on wheat plants grown in optimal environmental condition

3.2.1.1. Effects of JA on root and shoot length of wheat plants

All concentrations of JA applied to the wheat plants showed a slightly increased shoot length in comparison with C treatments. A significant increase in shoot length occurred in treated plants with $JA10^{-6}$ M.

Root length of treated plants with different concentrations of JA decreased in all concentrations of JA applied to wheat plant in comparison with C treatments (Figure 3.11).

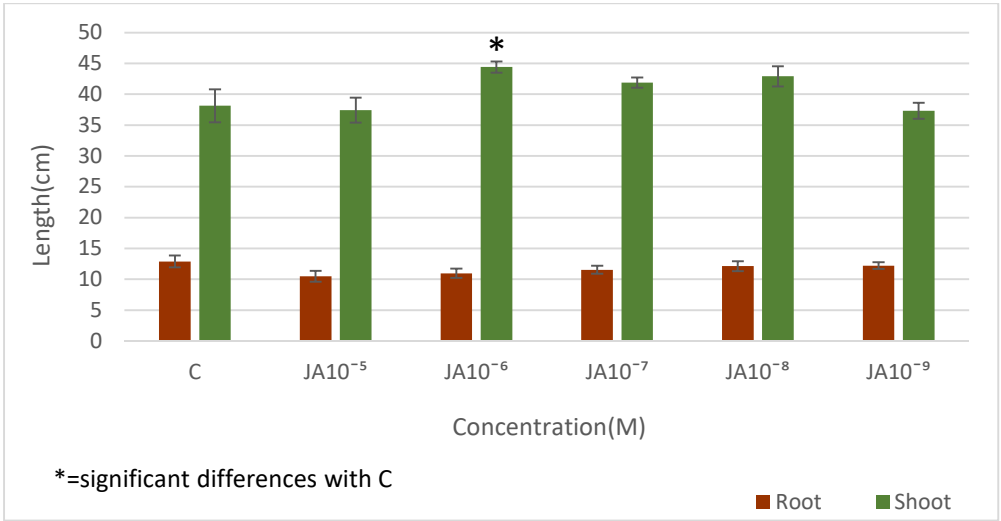


***Figure 3.11: Root and shoot lengths of JA treatments in comparison with C.** Brown bars indicate the mean value of the root length while the green bars indicate the mean value of the shoot length. Stars over the bars indicate significant differences between the mean value of the shoot and root length of all JA treatments in comparison with the C plants, after ANOVA (Tukey*

unequal N HSD, $p < 0.05$). Shoot length of plant treated with JA10^{-6} M showed a significant increase in comparison with C. Other concentrations didn't show any significant effect.

3.2.1.2. Effects of JA on root and shoot root and shoot dry weight of wheat plants

In treated plants with different concentrations of JA, root weight decreased slightly but insignificntly.

On the other side, shoot weight increased in concentration of JA10^{-6} M and JA10^{-7} M. At lower concentration of JA10^{-8} M and JA10^{-9} M shoot biomass decreased in comparison with C treatments slightly but insignificantly, same as in JA10^{-5} M (Figure 3.12).

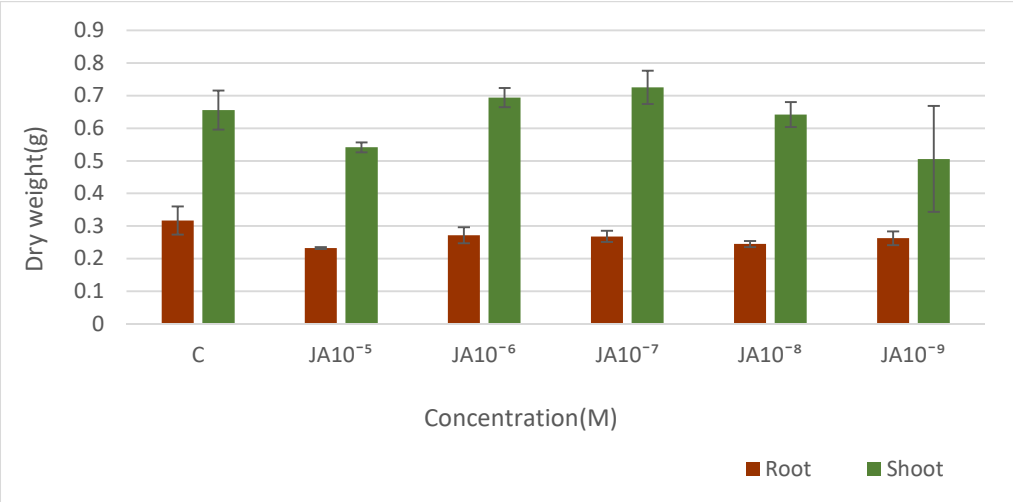


Figure 3.12: Root and shoot weights of JA treatments in comparison with C. *Brown bars indicate the mean value of the root weight while the green bars indicate the mean value of the shoot weight. Stars over the bars indicate significant differences between the mean value of the shoot and root weight of all JA treatments in comparison with the C plants, after ANOVA (Tukey unequal N HSD, $p < 0.05$). Slightly increase in shoot length in concentrations of JA10^{-6} M and JA10^{-7}M and slightly decrease in root length were observed.*

3.2.1.3. Effects of JA on FV/FM ratio of wheat plants

All concentrations of JA applied to the wheat plants showed no significant involvement in the FV/FM ratio in comparison with C group. Only in treated plants with JA10^{-7} M FV/FM ratio increased, whereas JA10^{-8} M caused a slight decrease (Figure 3.13).

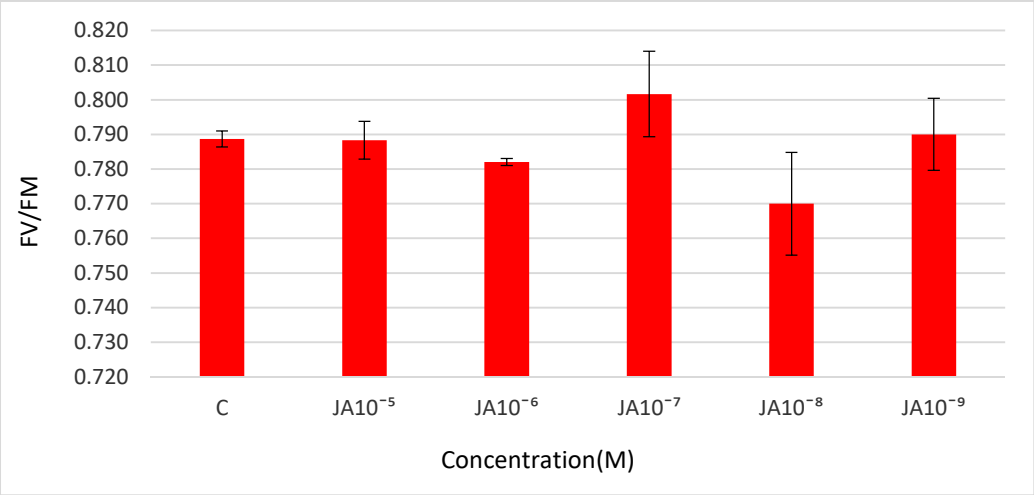


Figure 3.13: FV/FM ratio of treated plants with JA in comparison with C. *Red bars indicate the mean value of the FV/FM ratio. Stars over the bars indicate significant differences between the mean value of the FV/FM ratio of all JA treatments in comparison with the C treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$). A slight decrease in plants treated with JA10^{-8}M and a slight increase in JA10^{-7} M were observed.*

3.2.1.4. Effects of JA on number of leaves of wheat plants

Different concentrations of JA decreased number of leaves formed by wheat plants but not significantly in comparison with C treatments (Figure 3.14)

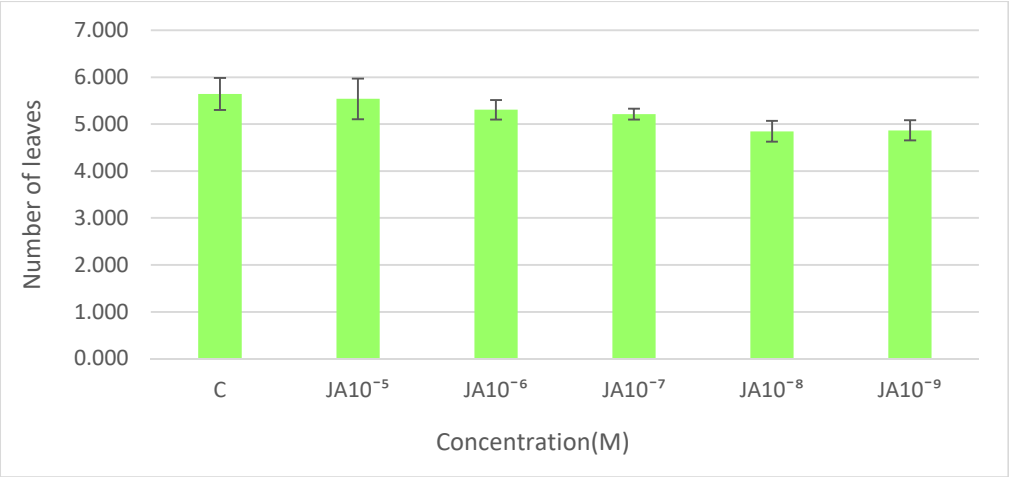


Figure 3.14: The number of leaves of JA treated plants in comparison with C. *Green bars indicate the mean value of the number of leaves. Stars over the bars indicate significant differences between the mean value of the number of leaves of all JA treatments in comparison with the C plants, after ANOVA (Tukey unequal N HSD, $p < 0.05$). There was no significant effect of JA on number of leaves of wheat plant.*

3.2.1.5. Effects of SL on root and shoot length of wheat plants

In plants treated with different concentrations of SL, there was no significant effect in comparison with C treatments.

Also the shoot length of plants treated with $SL10^{-5}$ M to $SL10^{-9}$ M was not affected in comparison with C treatments (Figure 3.15).

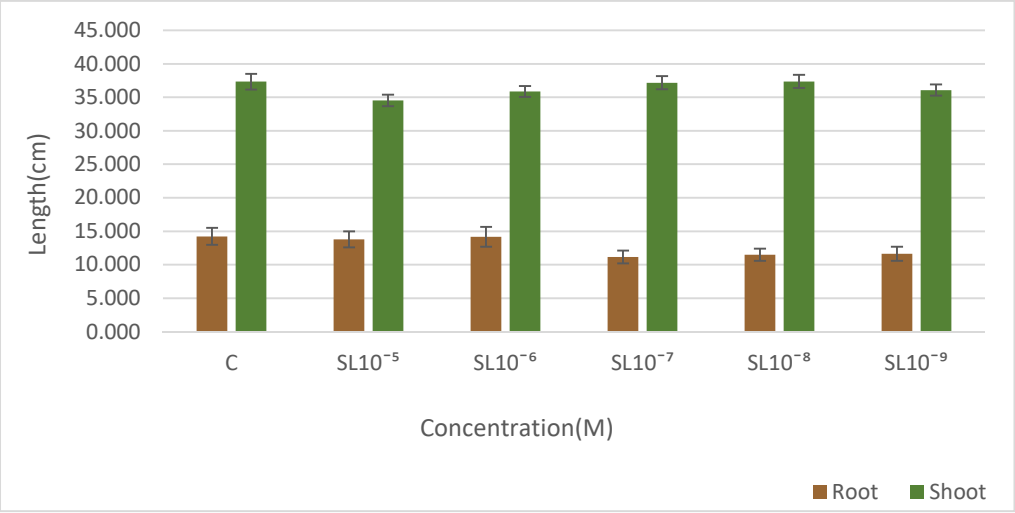


Figure 3.15: Root and shoot length of SL treated plants in comparison with C. *Brown bars indicate the mean value of the root length while the green bars indicate the mean value of the shoot length. Stars over the bars indicate significant differences between the mean value of the shoot and root length of all SL treatments in comparison with the C plants, after ANOVA (Tukey unequal N HSD, $p < 0.05$). There was no significant effect of JA on root and shoot length by applying different concentrations of SL observed.*

3.2.1.6. Effects of SL on root and shoot dry weight of wheat plants

SL increased the shoot biomass of wheat plants slightly though insignificantly at $SL10^{-8}$ M. Root biomass of treated plants with different concentrations of SL decreased in comparison with C plants slightly but insignificantly at $SL10^{-7}$ M (Figure 3.16).

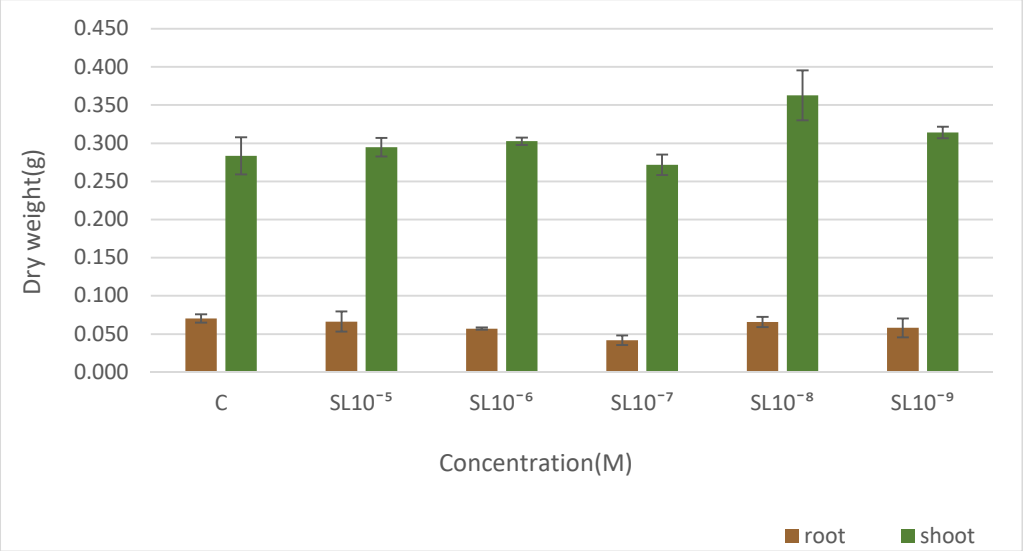


Figure 3.16: Root and shoot weight of C and SL treated plants in comparison. *Brown bars indicate the mean value of the root dry weight while the green bars indicate the mean value of the shoot dry weight. Stars over the bars indicate significant differences between the mean value of the shoot and root dry weight of all SL treatments in comparison with the C plants, after ANOVA (Tukey unequal N HSD, $p < 0.05$). No significant effect of SL on root and shoot dry weight were observed, only slightly increase in $SL10^{-8}$ M on shoot dry weight.*

3.2.1.7. Effects of SL on FV/FM ratio of wheat plants

By increasing the concentrations of SL applied to the wheat plants the FV/FM ratio decreased slightly but insignificantly, while at highest concentration ($SL10^{-9}$ M) this ratio increased in comparison with C treatments slightly but insignificantly (Figure 3.15).

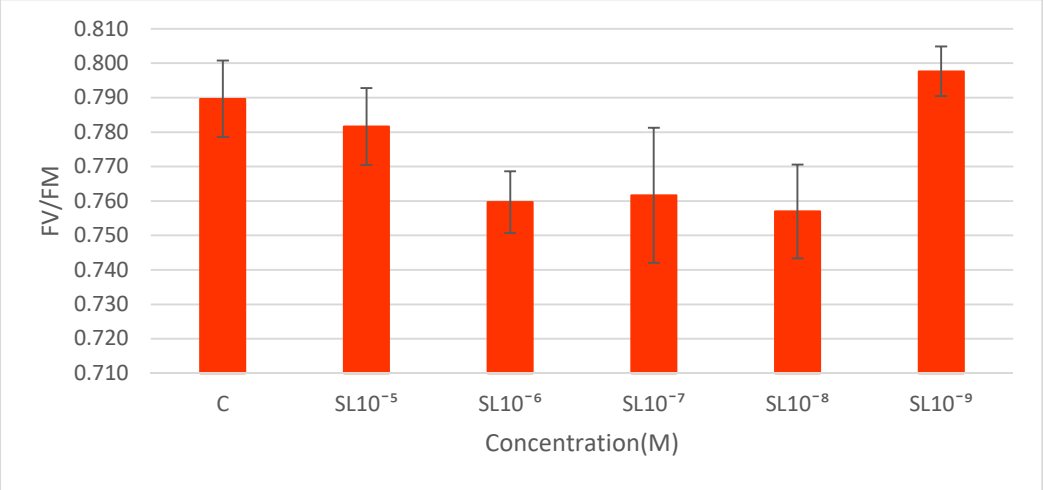


Figure 3.17: FV/FM ratio of C and SL treated plants in comparison. *Red indicates the mean value of the FV/FM ratio while the. Stars over the bars indicate significant differences between the mean value of the FV/FM ratio of all SL treatments in comparison with the C plants, after ANOVA (Tukey unequal N HSD, $p < 0.05$). Results showed that in plants treated with $SL10^{-9}$ M the FV/FM ratio increased slightly but not significantly.*

3.2.1.8. Effects of SL on the number of leaves of wheat plants

Plants treated with different concentrations of SL showed a slight but insignificant increased of leaf number at concentrations of $SL10^{-5}$ M, $SL10^{-6}$ M and $SL10^{-8}$ M.

On the other side, at concentrations of $SL10^{-7}$ M and $SL10^{-9}$ M the number of leaves decreased in comparison with the C group (Figure 3.18).

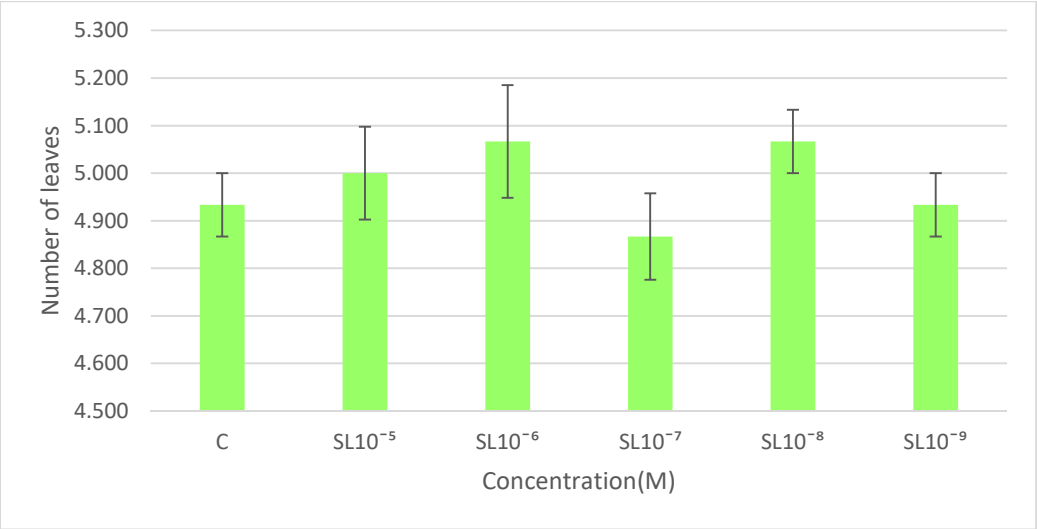


Figure 3.18: The number of leaves of C and SL treated plants in comparison. *Green bars indicate the mean value of the number of leaves, while the Stars above the bars indicate significant differences between the mean value of the number of leaves of all SL treatments in comparison with the C plants, after ANOVA (Tukey unequal N HSD, $p < 0.05$). In all concentrations of SL applied to wheat plant, number of leaves increased but not significantly except in $SL10^{-7}$M.*

3.3. Effects of JA on the HM tolerance of wheat

In this study, we investigated the possible involvement of JA on plants treated with HMs; growth parameters such as the mean values of the root and shoot length, root and shoot dry weight and also FV/FM ratio and the number of leaves were compared to the treated plants with different concentrations of HMs.

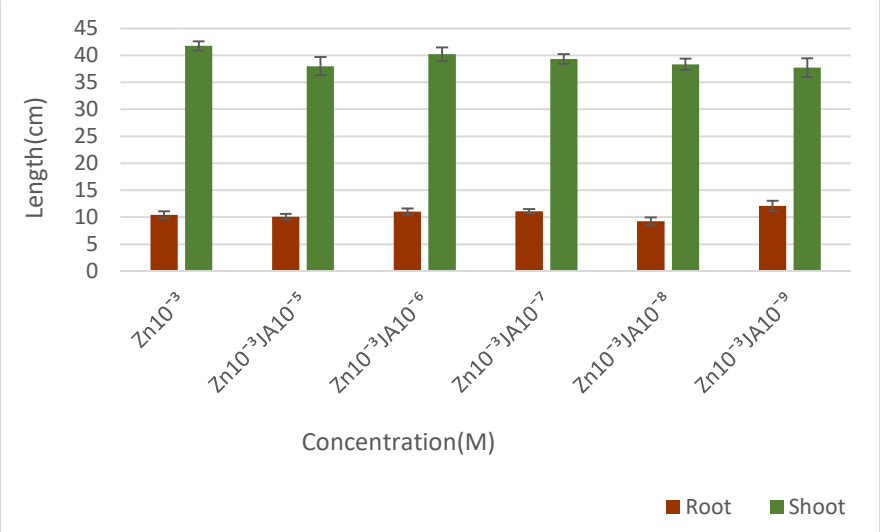
3.3.1. Effects of JA on root and shoot length of plants treated with Zn

In plants treated with $Zn10^{-3}$ M and different concentrations of JA, neither shoot length nor root length were affected significantly.

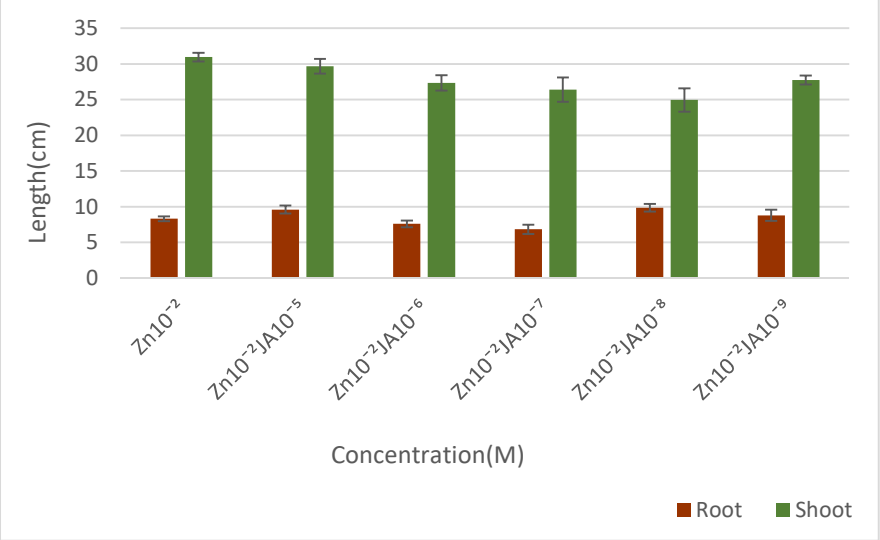
Plants treated with $Zn10^{-2}$ M and different concentrations of JA, shoot length decreased slightly but insignificantly, while root length increased slightly but insignificantly at concentrations of $JA10^{-5}$ M, $JA10^{-8}$ M and $JA10^{-9}$ M in comparison with plants treated only with $Zn10^{-2}$ M.

Plants treated with $Zn5.10^{-2}$ M had severe reactions to the metal. After treatment with different concentrations of JA, root length increased only at concentrations of $JA10^{-6}$ M in comparison with plants treated only with $Zn5.10^{-2}$ M.

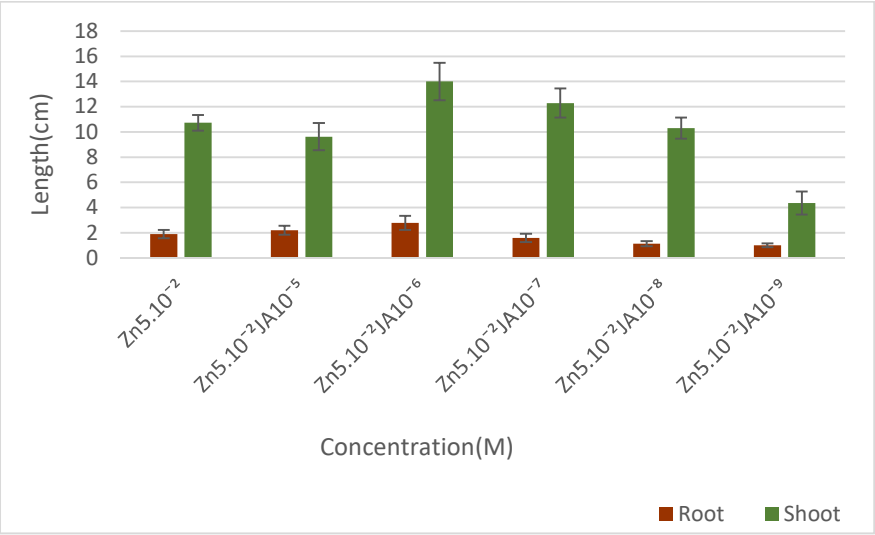
Shoot length also increased at concentrations of $JA10^{-6}$ M and $JA10^{-7}$ M in comparison with plants treated with $Zn5.10^{-2}$ M. The lowest concentration of $JA10^{-9}$ M on contrary had additional negative effects on root and shoot length (Figure 3.19).



a)Zn10^{-3} M treated plants in comparison with Zn 10^{-3} M/JA



b)Zn10^{-2} M treated plants in comparison with Zn 10^{-2} M/JA



c) Zn5.10^{-2} M treated plants in comparison with Zn 5.10^{-2} M/JA

Figure 3.19: Possible involvement of JA on root and shoot length of Zn treated plants. *Brown bars indicate the mean value of the root length while the green bars indicate the mean value of the shoot length. Stars over the bars indicate significant differences between the mean value of the shoot and root length of HM/JA treatments in comparison with the HM treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$). Treatments with $Zn10^{-3}$ M and JA didn't show significant effects on root and shoot length (a). The measured parameters at $Zn10^{-2}$ M and JA decreased but not significantly (b) and in treatments with $Zn5.10^{-2}$ M and JA shoot and root length increased slightly but not significantly in $JA10^{-6}$ M.*

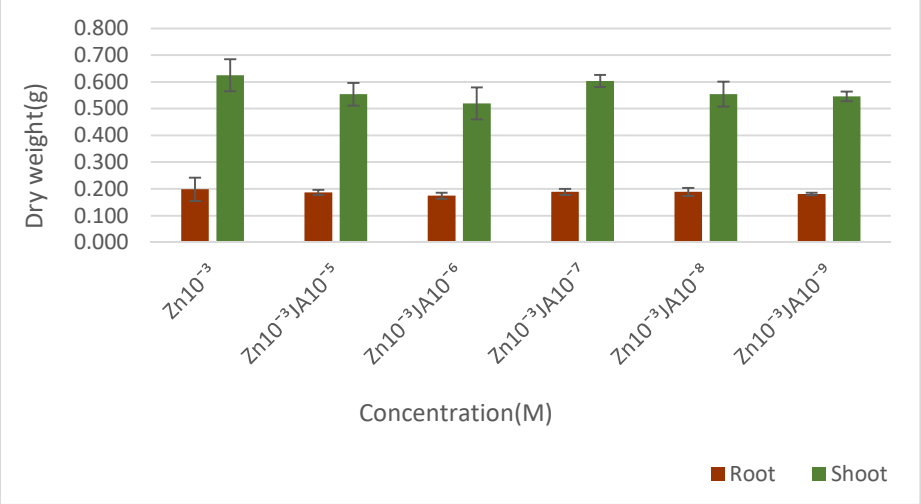
3.3.2. Effects of JA on root and shoot dry weight of plants treated with Zn

In comparison with plants that were treated with different concentrations of Zn, there were no significant effects in plants treated with different concentrations of JA.

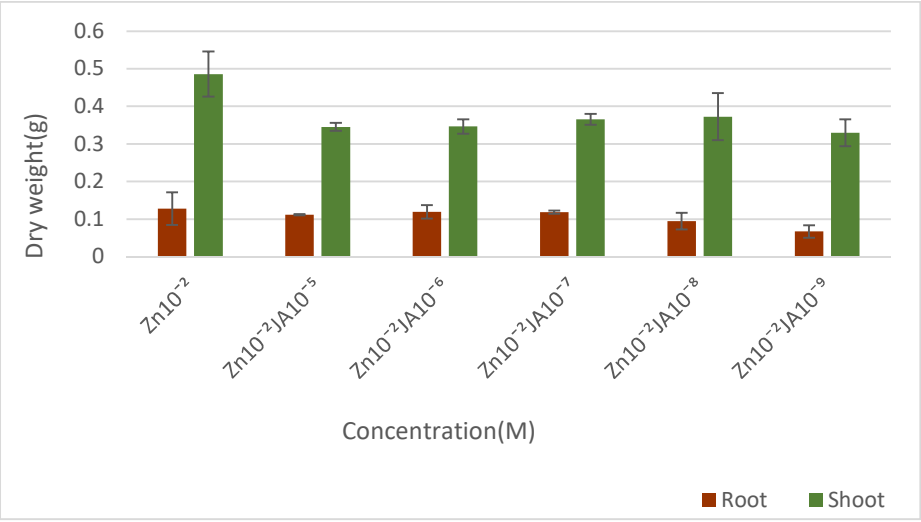
Plants treated with $Zn10^{-3}$ M and different concentrations of JA showed that shoot dry weight slightly but insignificantly decreased. The dry weight of roots was not influenced by JA.

Plants treated with $Zn10^{-2}$ M had insignificantly decreased shoot dry weight. Roots had insignificantly less dry weight when treated with lowest concentrations of $JA10^{-9}$ M.

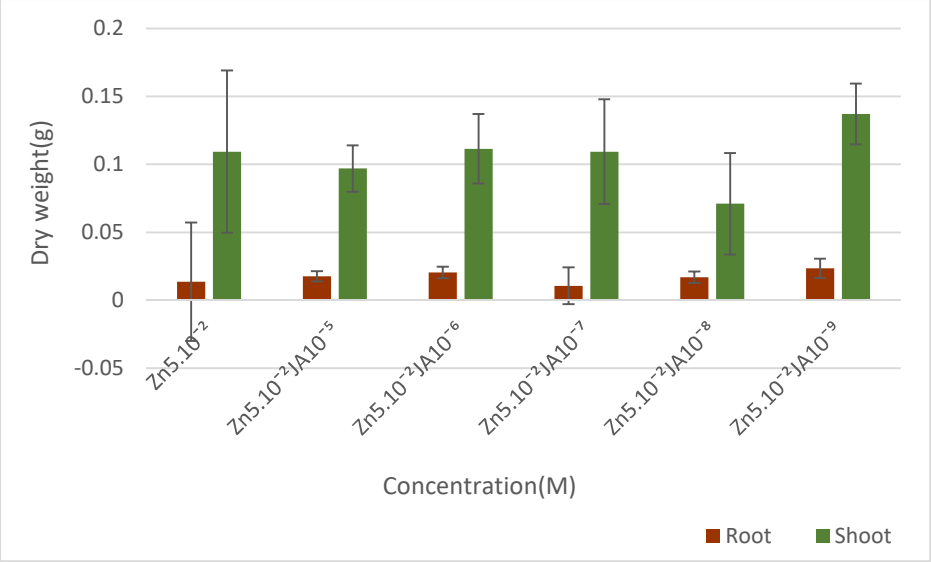
In plants treated with $Zn5.10^{-2}$ M shoot dry weight decreased slightly but insignificantly except at concentration of $JA10^{-9}$ M. Root dry weight increased slightly but insignificantly in all concentrations of JA except at concentration of $JA10^{-7}$ M (Figure 3.20).



a) $Zn10^{-3}$ M treated plants in comparison with $Zn10^{-3}$ M/JA



b) $Zn10^{-2}$ M treated plants in comparison with $Zn10^{-2}$ M/JA



c) $Zn5.10^{-2}$ M treated plants in comparison with Zn 5.10^{-2} M/JA

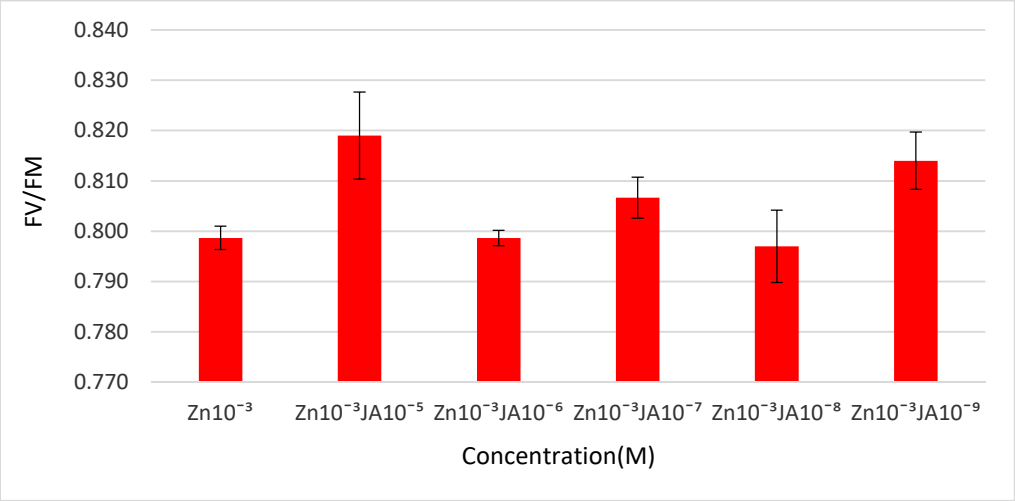
***Figure 3.20**: **Possible involvement of JA on root and shoot weight of Zn treated plants.** Brown bars indicate the mean value of the root weight while the green bars indicate the mean value of the shoot weight. Stars above the bars indicate significant differences between the mean value of the shoot and root weights of HM/JA treatments in comparison with the HM treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$). In comparison with plants that were treated with different concentrations of Zn, there were no significant effects in plants treated with different concentrations of JA.*

3.3.3. Effects of JA on the FV/FM ratio of plants treated with Zn

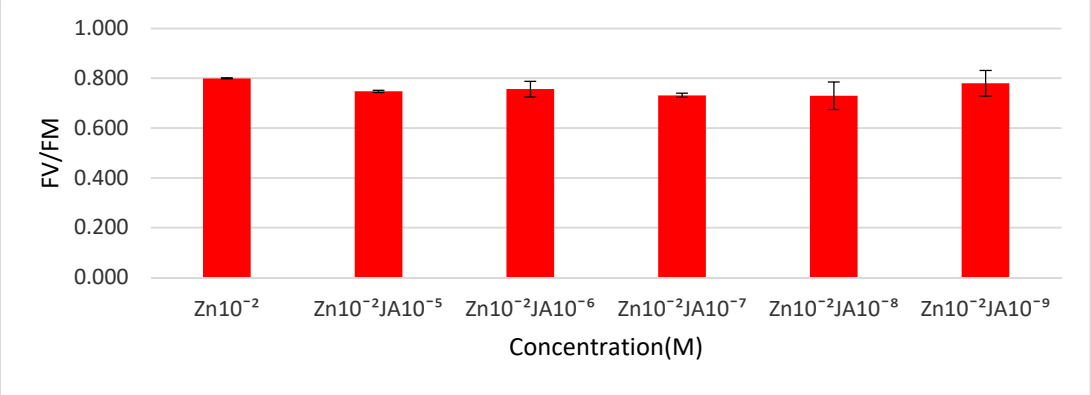
In comparison with plants treated with $Zn10^{-3}$ M, FV/FM ratio increased in concentration of $JA10^{-5}$ M, $JA10^{-7}$ M and $JA10^{-9}$ M, although insignificantly.

All concentrations of JA applied to wheat plants that treated with $Zn10^{-2}$ M showed an insignificantly decreased FV/FM ratio.

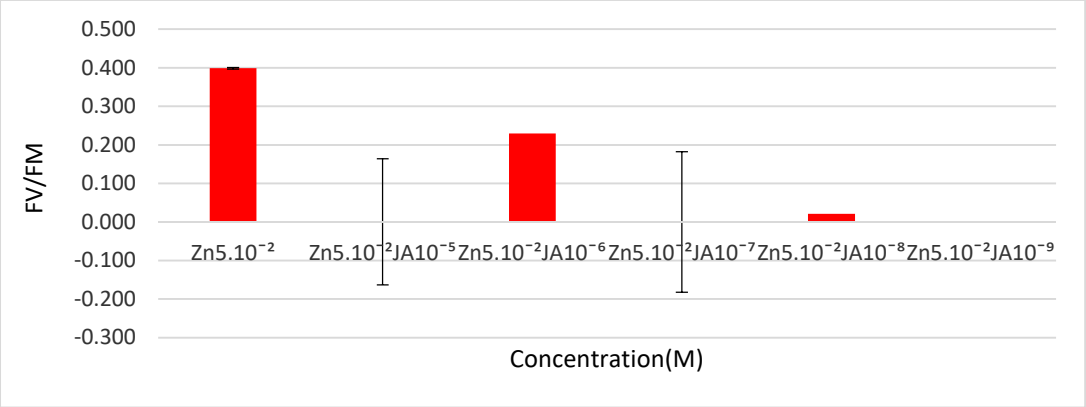
In comparison with plants treated with $Zn5.10^{-2}$ M, FV/FM ratio in all concentrations of JA applied to wheat plants decreased in FV/FM ratio, although insignificantly (Figure 3.21).



a)$Zn10^{-3}$ M treated plants in comparison with $Zn10^{-3}$ M/JA



b)$Zn10^{-2}$ M treated plants in comparison with $Zn10^{-2}$ M/JA



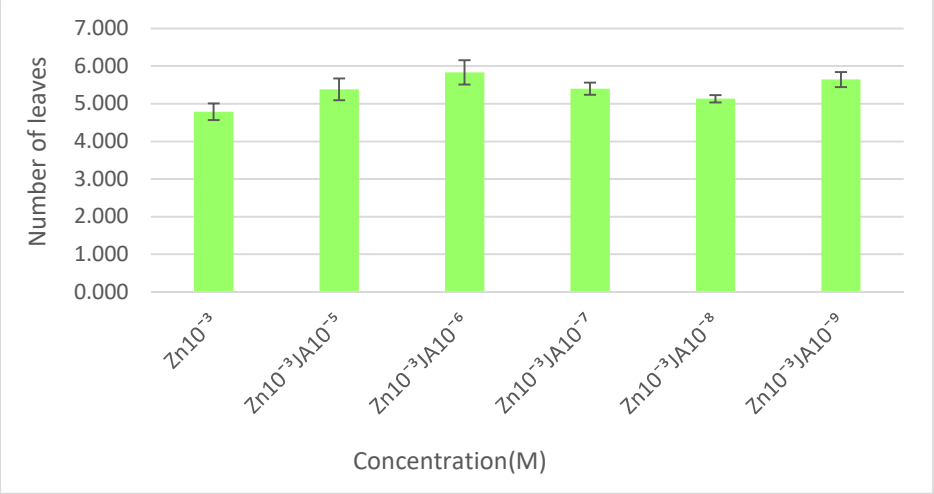
c) $Zn5.10^{-2}$ M treated plants in comparison with $Zn5.10^{-2}$ M/JA

***Figure 3.21**: **Possible involvement of JA on the FV/FM ratio of Zn treated plants.** Red bars indicate the mean value of the FV/FM ratio. Stars above the bars indicate significant differences between the mean value of the FV/FM ratio of HM/JA treatments in comparison with the HM treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$). In treatment with $Zn10^{-3}$ M and $JA10^{-5}$ M, $JA10^{-7}$ M and $JA10^{-9}$ M FV/FM ratio increased but not significantly(a). At concentration of $Zn10^{-2}$ M and different concentrations of JA FV/FM ratio decreased but insignificantly (b and c).*

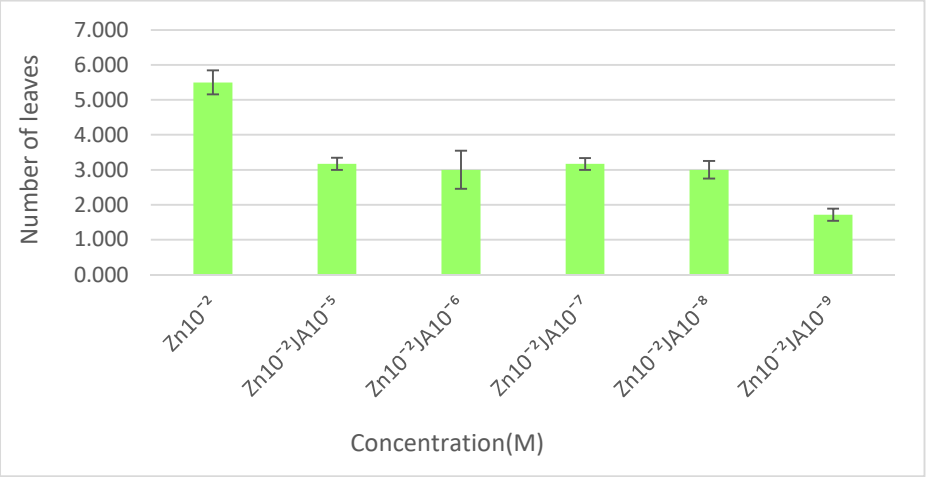
3.3.4. Effects of JA on the number of leaves formed by plants treated with Zn

The number of leaves in plants that were treated with $Zn10^{-3}$ M and different concentrations of JA increased slightly but insignificantly. The same results were observed in plants treated with $Zn5.10^{-2}$ M.

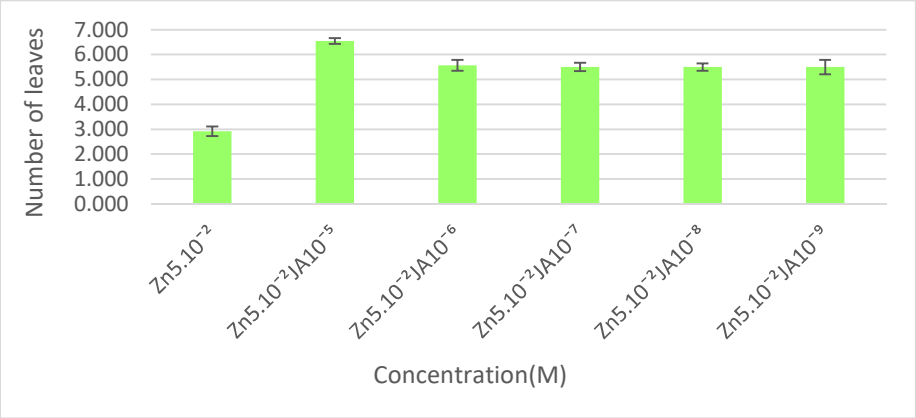
On the other side, the number of leaves in plants treated with different concentrations of JA decreased slightly but insignificantly in comparison with plants treated with $Zn10^{-2}$ M (Figure 3.22).



a) $Zn10^{-3}$ M treated plants in comparison with $Zn10^{-3}$ M/JA



b) $Zn10^{-2}$ M treated plants in comparison with $Zn10^{-2}$ M/JA



c) $Zn5.10^{-2}$ M treated plants in comparison with $Zn5.10^{-2}$ M/JA

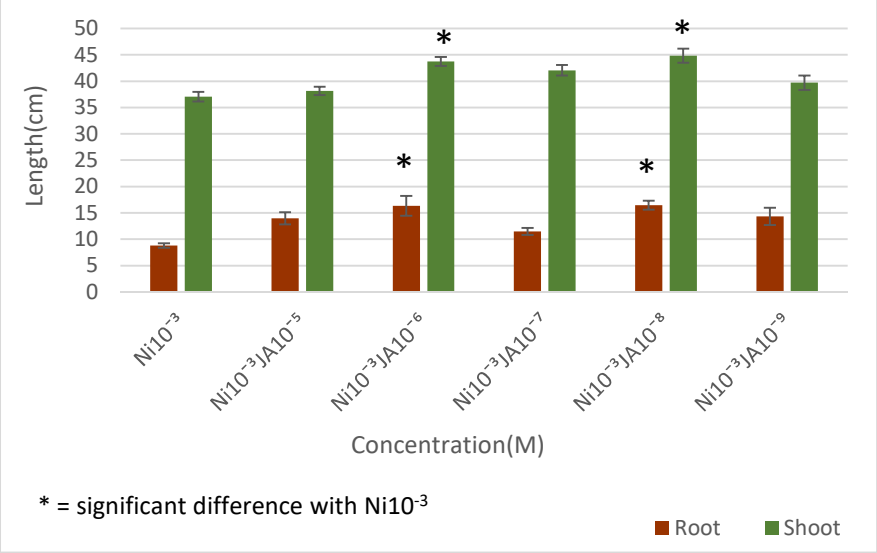
***Figure 3.22**: **Possible involvement of JA on the* number of leaves formed by Zn treated plants.** *Green bars indicate the mean value of the number of leaves. Stars above the bars indicate significant differences between the mean value of the number of HM/JA treatments in comparison with the HM treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$). The nu,ber of leaves in treatments with $Zn10^{-3}$ M and $Zn5.10^{-2}$ M increased but not significantly (a and c) and in treatment with $Zn10^{-2}$ M decreased but insignificantly.*

3.3.5. Effects of JA on root and shoot length of plants treated with Ni

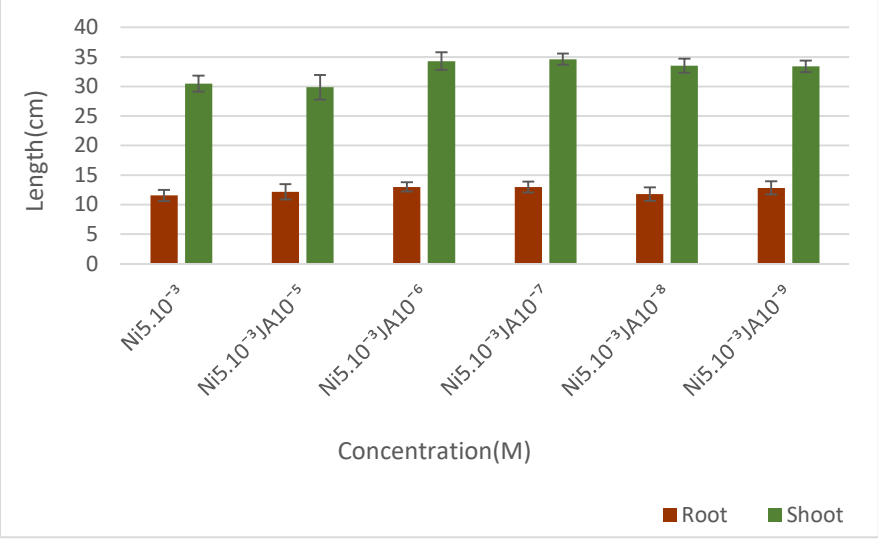
At concentration of $Ni10^{-3}$ M, root and shoot length of plants treated with $JA10^{-6}$ M and 10^{-8} M increased significantly. In other concentrations of JA applied to wheat plants root and shoot length increased but not significantly.

Shoot length of plants treated with $Ni5.10^{-3}$ M increased slightly but insignificantly with application of low concentrations of JA between 10^{-6} M and 10^{-9} M, with while root length was not affected.

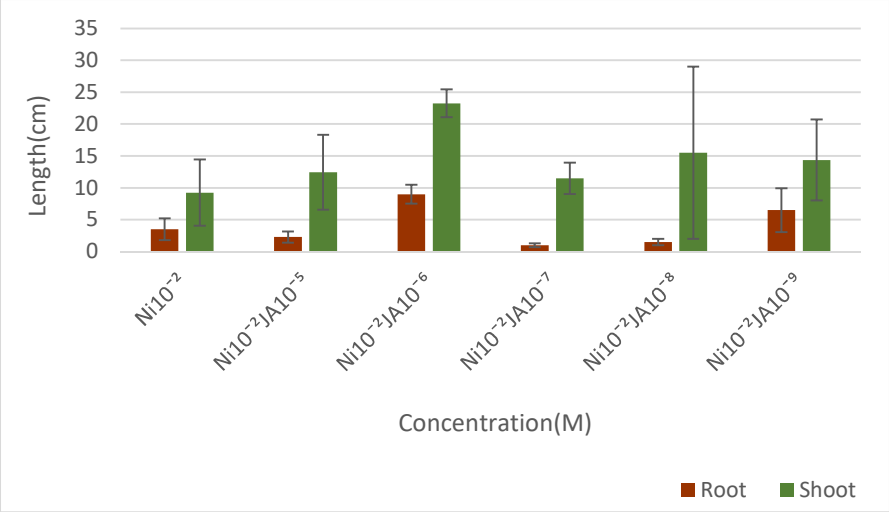
At concentration of $Ni10^{-2}$ M severe effects on the plants were observed. JA of all concentrations had slightly but insignificantly positive effects on the shoot length. Roots grew slightly better in $JA10^{-6}$ M and $JA10^{-9}$ M (Figure 3.23).



a)$Ni10^{-3}$ M treated plants in comparison with $Ni10^{-3}$ M/JA



b)$Ni5.10^{-3}$ M treated plants in comparison with $Ni5.10^{-3}$ M/JA



c) $Ni10^{-2}$ M treated plants in comparison with $Ni10^{-2}$ M/JA

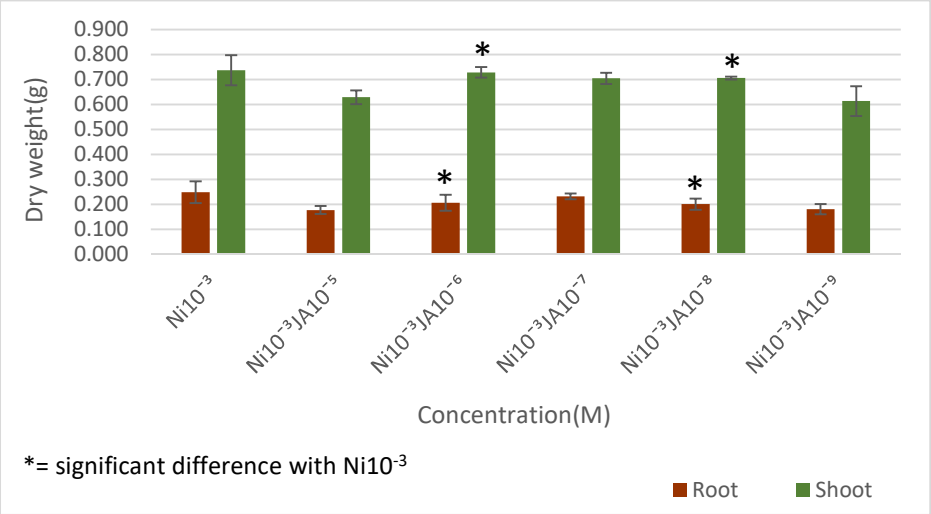
***Figure 3.23**: **Possible involvement of JA on root and shoot length of Ni treated plants.** Brown bars indicate the mean value of the root length while the green bars indicate the mean value of the shoot length. Stars over the bars indicate significant differences between the mean value of the shoot and root length of HM/JA treatments in comparison with the HM treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$).Only in plants treated with $Ni10^{-3}$ with $JA10^{-6}$ M and $JA10^{-9}$ M significant increase in root and shoot length were observed(a). In other concentrations increase slightly but insignificantly (b and c).*

3.3.6. Effects of JA on root and shoot dry weight of plants treated with Ni

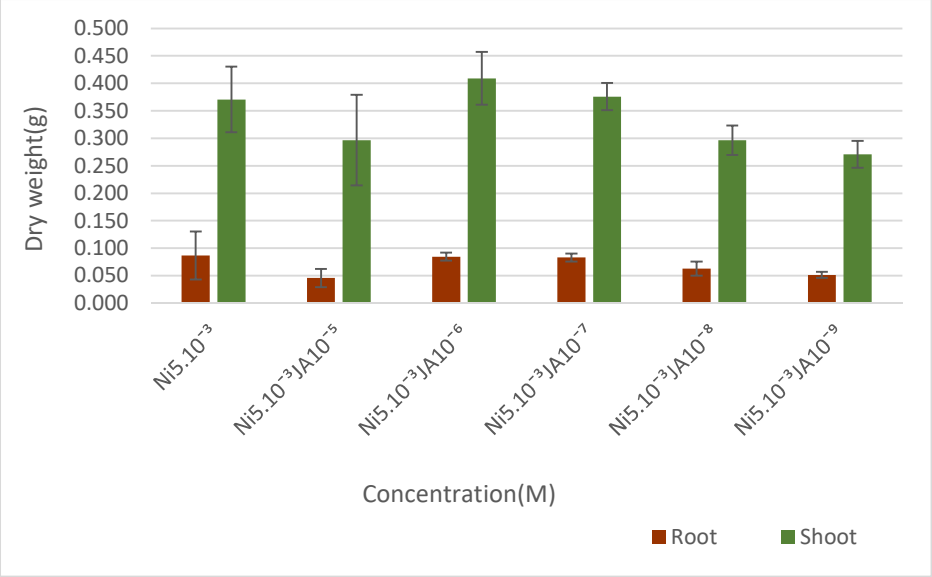
In treated plants with $Ni10^{-3}$ M and different concentrations of JA that were applied to wheat plants, root and shoot weight showed significant differences at concentrations of $JA10^{-6}$ M and 10^{-8} M.

At concentration of $Ni5.10^{-3}$ M, shoot dry weight of plants treated with concentration of $JA10^{-6}$ M and $JA10^{-7}$ M increased but not significantly. Root dry weight of wheat plants decreased in all concentrations of JA but insignificantly.

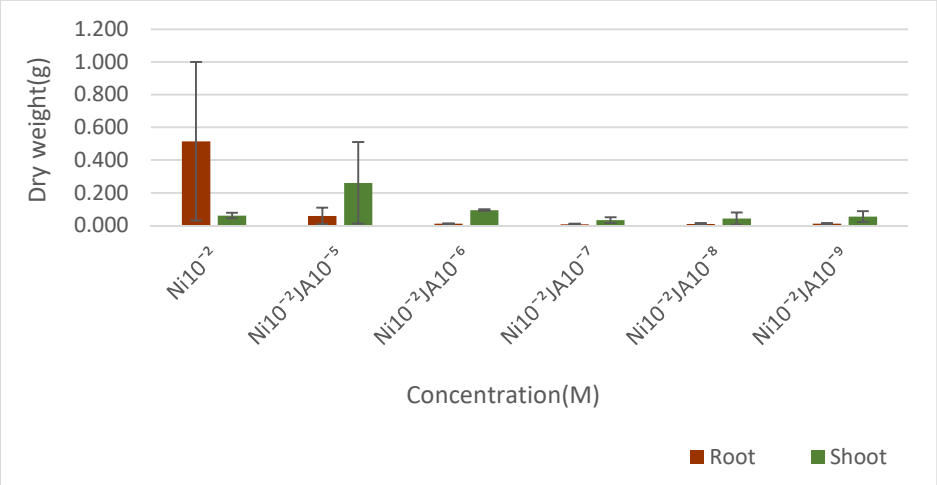
Treated plants with $Ni10^{-2}$ M and different concentrations of JA showed a decrease in root dry weight but not significantly. Shoot dry weight increased slightly but insignificantly by in $JA10^{-5}$ M and 10^{-6} M (Figure 3.24).



a)$Ni10^{-3}$ M treated plants in comparison with $Ni10^{-3}$ M/JA



b)Ni5.10^{-3} M treated plants in comparison with Ni5.10^{-3} M/JA



c) Ni10^{-2} M treated plants in comparison with Ni10^{-2} M/JA

***Figure 3.24**: **Possible involvement of JA on root and shoot weight of Ni treated plants.** Brown bars indicate the mean value of the root weight while the green bars indicate the mean value of the shoot weight. Stars above the bars indicate significant differences between the mean value of the shoot and root weights of HM/JA treatments in comparison with the HM treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$). The significant decrease in root dry weight were observed in treatments with Ni10^{-3} with JA10^{-6} M and JA10^{-9} M and the same results were observed for shoot fry weight (a). In other treatments with Ni and JA no significant effects were observed.*

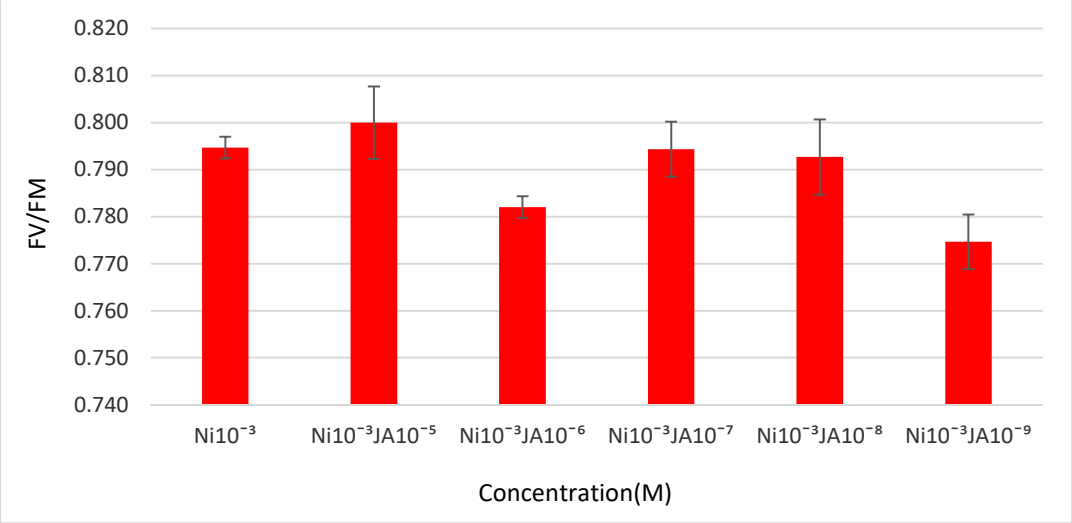
3.3.7. Effects of JA on the FV/FM ratio of plants treated with Ni

Supplement of JA to Ni treated plants had slight effects on the FV/FM ratio at all applied concentrations. However, they were not significant.

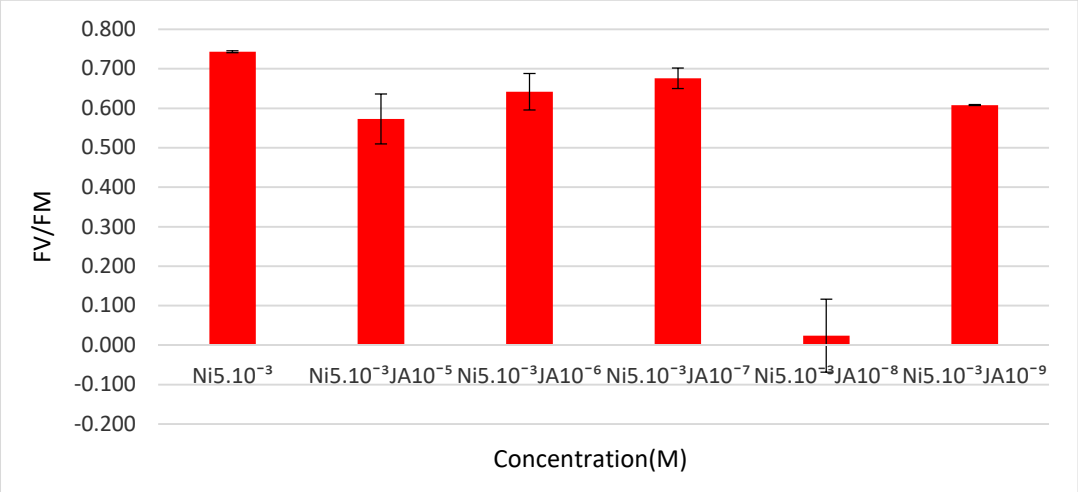
FV/FM ratio of treated plants with Ni10^{-3} M increased by applying JA10^{-5} M but not significantly. In concentrations of JA10^{-6} M and10^{-9} M FV/FM ratio decreased insignificantly.

Plants treated with Ni5.10^{-3} M showed a decrease in all concentrations of JA applied to the wheat plants.

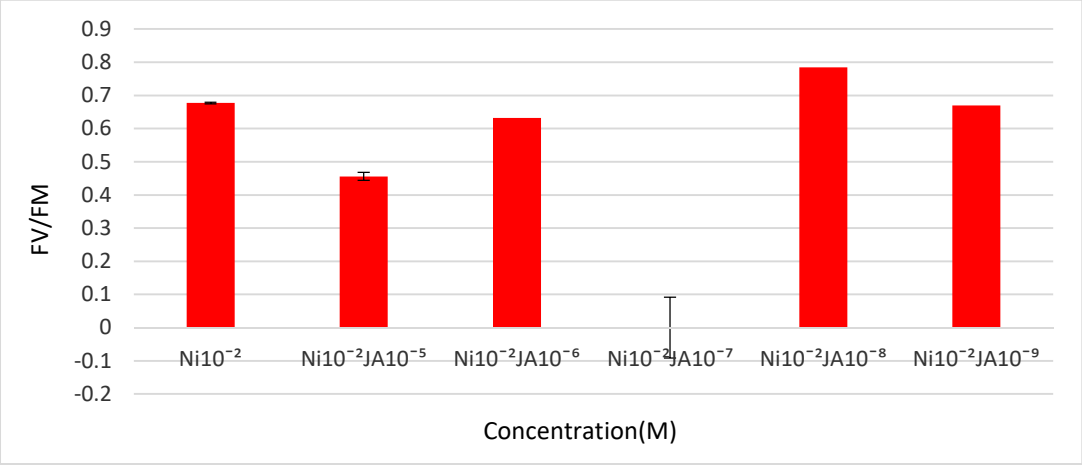
In plants treated with Ni10^{-2} M and JA10^{-5} M and 10^{-6} M the FV/FM ratio decreased. At concentration of JA10^{-8} M increased. In treatment with JA10^{-7} M, it didn't reported any ratio and in JA10^{-9} M showed the same ratio as treatment with Ni10^{-2} (Figure 3.25).



a)Ni10^{-3} M treated plants in comparison with Ni10^{-3} M/JA



b) Ni5.10^{-3} M treated plants in comparison with Ni5.10^{-3} M/JA



c) Ni10^{-2} M treated plants in comparison with Ni10^{-2} M/JA

***Figure 3.25**: **Possible involvement of JA on the FV/FM ratio of Ni treated plants.** Red bars indicate the mean value of the FV/FM ratio. Stars above the bars indicate significant differences between the mean value of the FV/FM ratio of HM/JA treatments in comparison with the HM treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$). Supplement of JA to Ni*

treated plants had slight effects on the FV/FM ratio at all applied concentrations but not significant. There were no significant effects of treatment with different concentrations of JA observed.

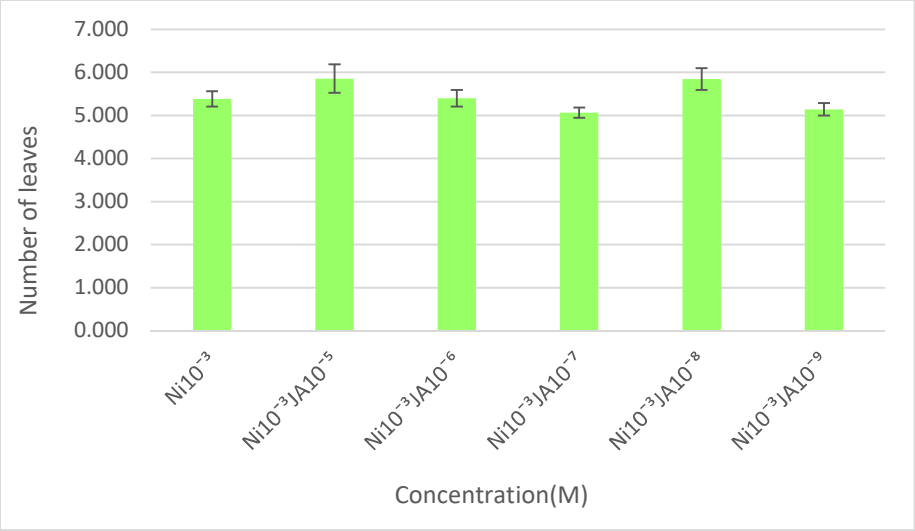
3.3.8. Effects of JA on the number of leaves by plants treated with Ni

All the concentrations of JA applied to the Ni treated plants showed only slight effects on the number of leaves that were not significant.

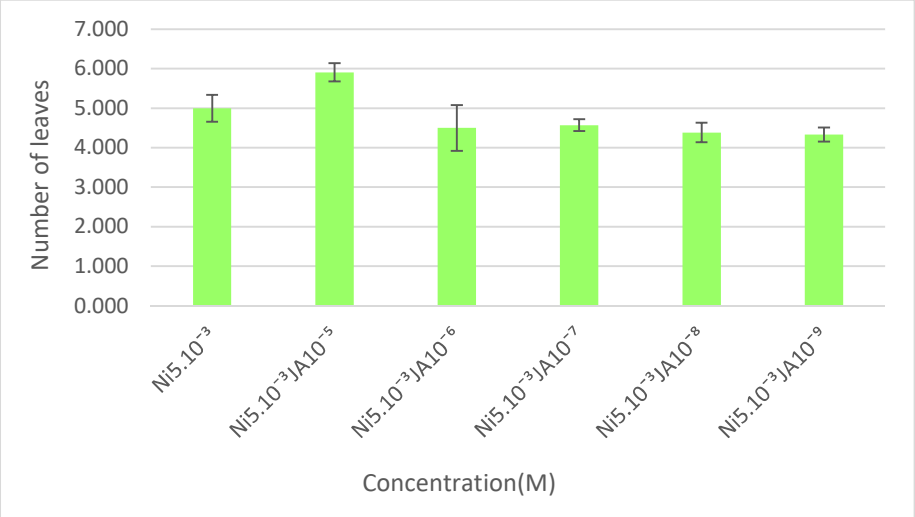
In plants treated with $Ni10^{-3}$ M, number of the leaves increased slightly at concentration of $JA10^{-5}$ and $JA10^{-8}$ M.

At concentration of $Ni5.10^{-3}$ M number of the leaves decreased except in plant treated plants $JA10^{-5}$ M.

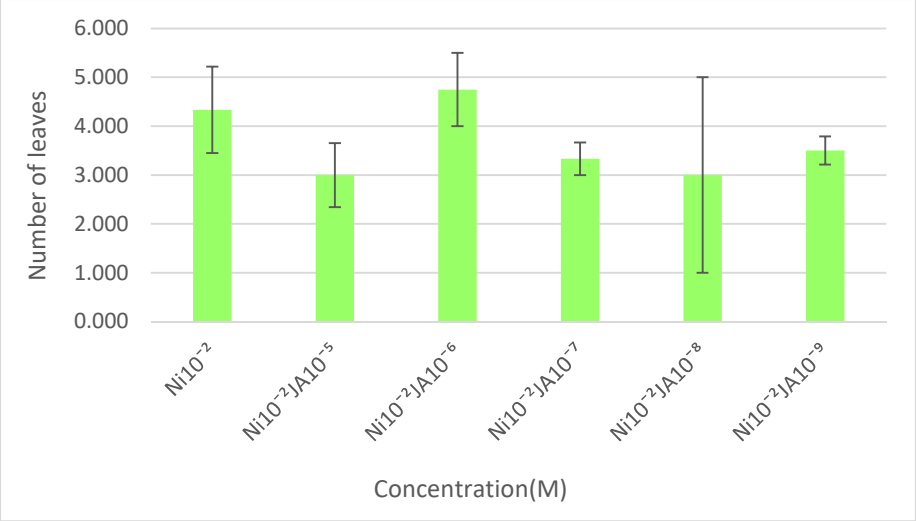
Number of the leaves in plants treated with $Ni10^{-2}$ M and different concentrations of JA decreased except at concentrations of $JA10^{-6}$ M (Figure 3.26).



a)$Ni10^{-3}$ M treated plants in comparison with $Ni10^{-3}$ M/JA



b)$Ni5.10^{-3}$ M treated plants in comparison with $Ni5.10^{-3}$ M/JA



c) $Ni10^{-2}$ M treated plants in comparison with $Ni10^{-2}$ M/JA

***Figure 3.26**: **Possible involvement of JA on the number of leaves formed by Ni treated plants.** Green bars indicate the mean value of the number of leaves. Stars above the bars indicate significant differences between the mean value of the number of leaves of HM/JA treatments in comparison with the HM treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$). All the concentrations of JA applied to the Ni treated plants showed only slight effects on the number of leaves that were not significant.*

3.4. Effects of SL in HMs induced stress on wheat

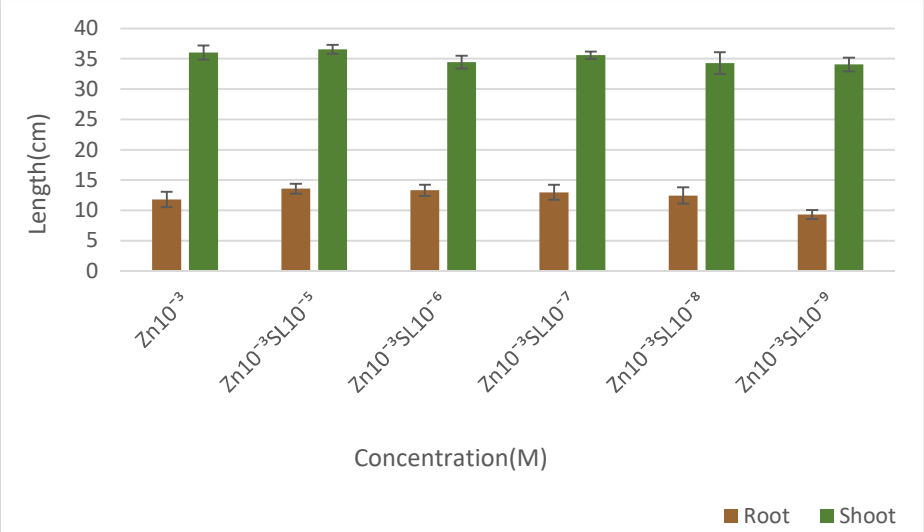
In this study, the possible involvement of SL on HMs-induced stress of wheat was investigated. With suggestions from previous studies that SL is involved in causing structural changes to the root, shoot, and leaves in plants exposed to stress conditions, this experiment was designed to investigate the possible involvement of SL on parameters such as root and shoot length, root and shoot weight, FV/FM ratio and number of leaves in plants which were stress induced by HMs.

3.4.1. Effects of SL on root and shoot length of plants treated with Zn

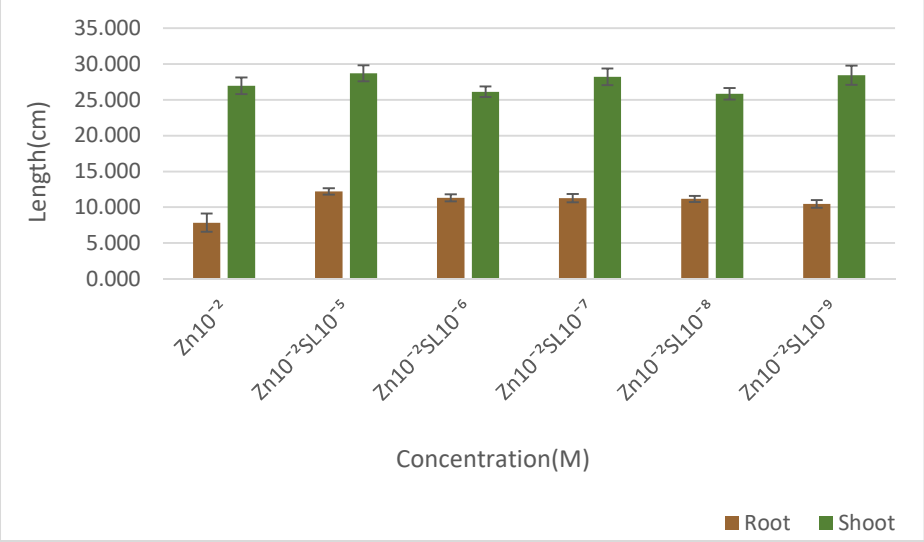
Shoot length of plants supplemented with different concentrations of SL were not effected significantly in comparison with plants treated with $Zn10^{-3}$ M alone. On the other side, root length increased slightly but insignificantly in all concentrations of SL except at $SL10^{-9}$ M.

In comparison between plants treated with $Zn10^{-2}$ M and plants supplemented with SL, shoot length increased slightly but insignificantly at concentrations of $SL10^{-5}$ M, $SL10^{-7}$ M and $SL10^{-9}$ M. By increasing the concentrations of SL root length increased slightly but insignificantly.

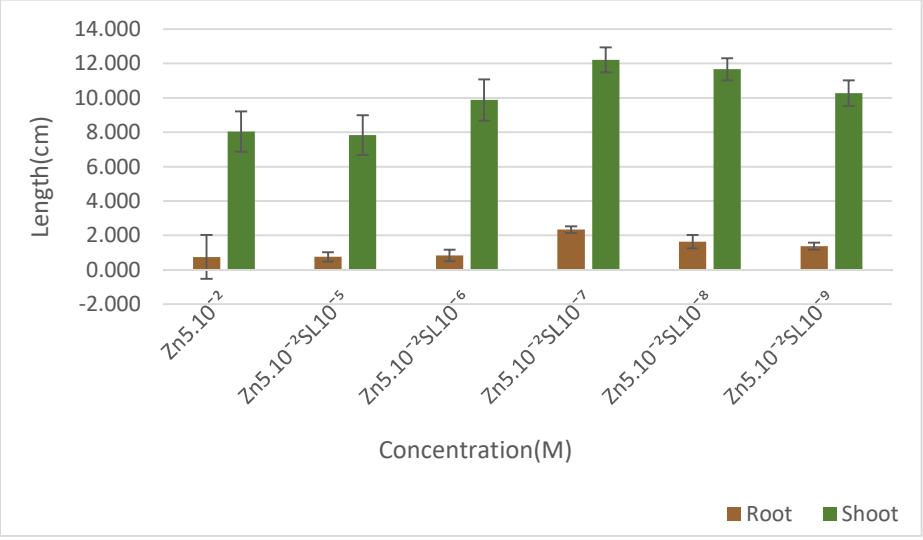
Treatment of plants with $Zn10^{-2}$ M had severe effects in growth of plants. Additional treatment with SL increased root and shoot length in the lower concentrations of $SL10^{-6}$ M to $SL10^{-9}$ M (Figure 3.27).



a)$Zn10^{-3}$ M treated plants in comparison with $Zn10^{-3}$ M/SL



b)$Zn10^{-2}$ M treated plants in comparison with $Zn10^{-2}$ M/SL



c) $Zn5.10^{-2}$ M treated plants in comparison with $Zn15.10^{-2}$ M/SL

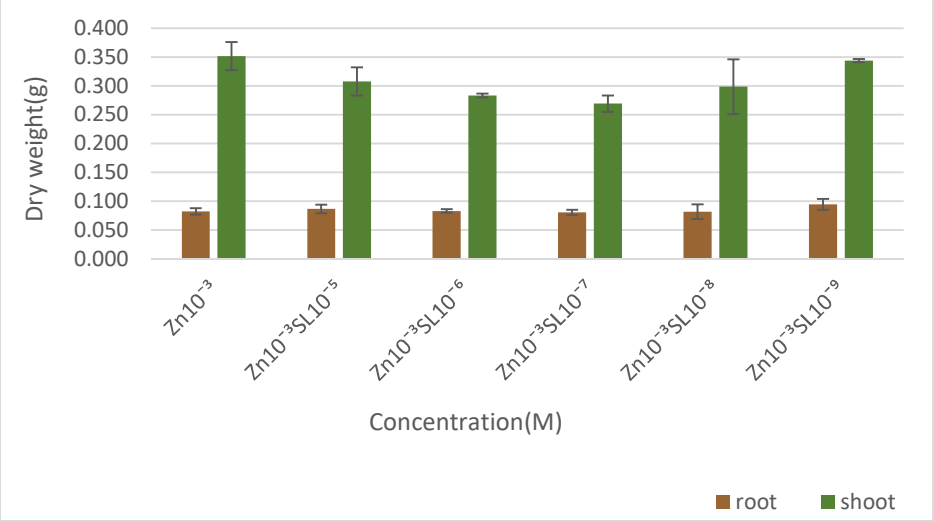
***Figure 3.27**: **Possible involvement of SL on root and shoot length of Zn treated plants**. Brown bars show the mean value of the root length while green bars show the mean value of the shoot length. Stars above the bars indicate significant differences between the mean value of the root and shoot length of HM/SL treatments in comparison with the HM treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$).In all treatments with Zn and different concentrations of SL, root and shoot length increased but not significantly (a, b and c)*

3.4.2. Effects of SL on root and shoot dry weight of plants treated with Zn

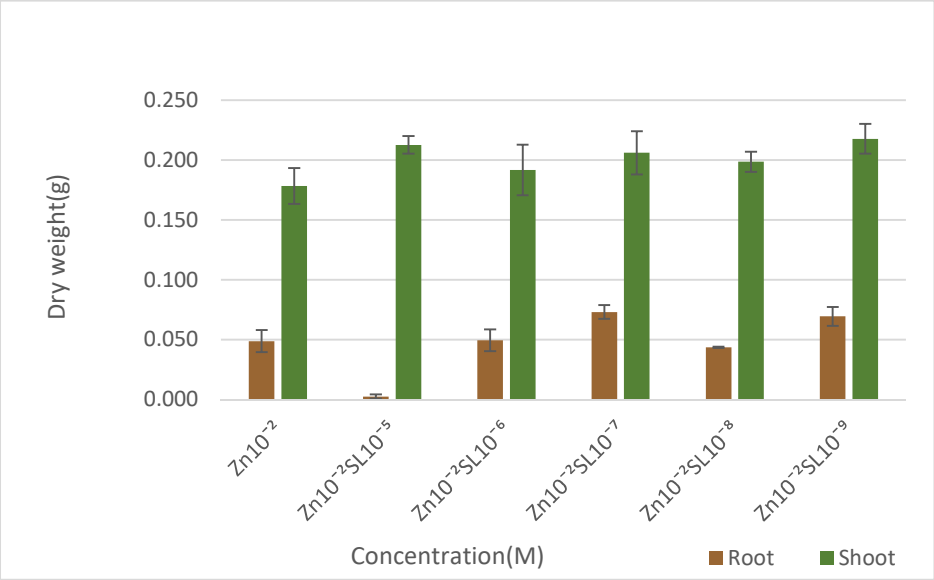
Application of SL to Zn treated did cause effects for the biomass in some cases, but these effects were not significant.

Shoot dry weight of wheat plants treated with different concentrations of SL and exposed to concentrations of $Zn10^{-3}$ and $Zn10^{-2}$ M decreased slightly but insignificantly. Root dry weight at concentrations of $Zn10^{-3}$ M was not effected by SL. Root dry weight in $Zn10^{-2}$ M treated plants increased slightly but insignificantly by increasing the concentrations of SL except at $SL10^{-5}$ M.

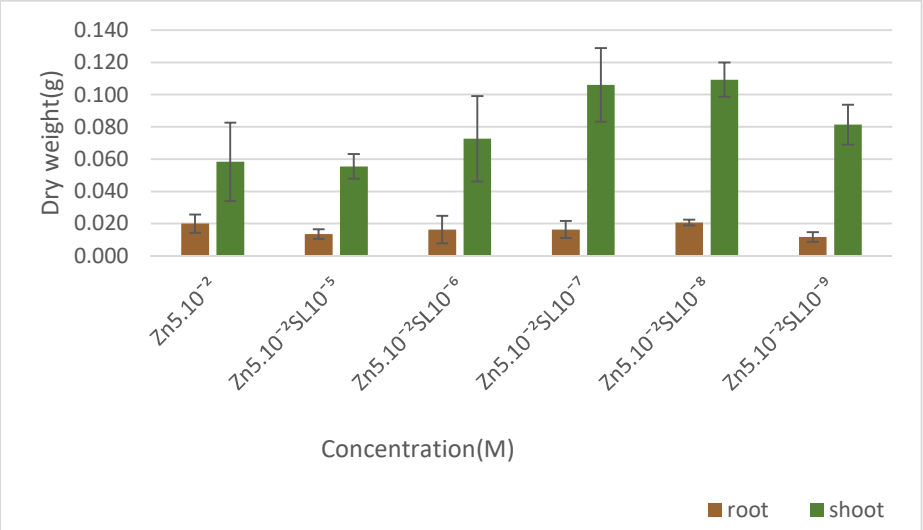
By increasing the concentrations of SL root biomass of plants treated with $Zn5.10^{-2}$ M was not affected. Shoot weight of plants at this concentration increased slightly but insignificantly except at concentration of $SL10^{-5}$ M (Figure 3.28).



a)$Zn10^{-3}$ M treated plants in comparison with $Zn10^{-3}$ M/SL



b)$Zn10^{-2}$ M treated plants in comparison with $Zn10^{-2}$ M/SL



c) $Zn5.10^{-2}$ M treated plants in comparison with $Zn5.10^{-2}$ M/SL

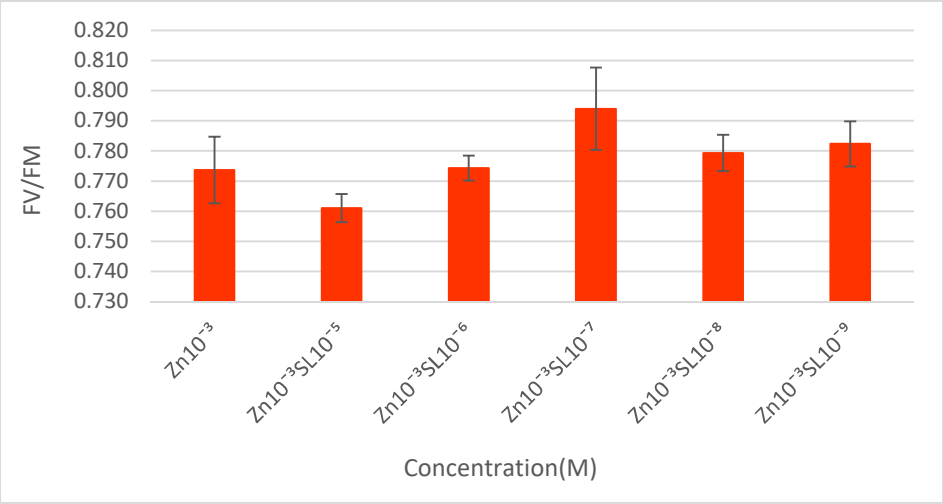
Figure 3.28: Possible involvement of SL on root and shoot weight of Zn treated plants. *Brown bars show the mean value of the root weight while green bars show the mean value of the shoot weight. Stars above the bars indicate significant differences between the mean value of the root and shoot weight of HM/SL treatments in comparison with the HM treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$). Application of SL to Zn treated did cause effects for the biomass in some cases, but these effects were not significant (a, b and c).*

3.4.3. Effects of SL on the FV/FM ratio of plants treated with Zn

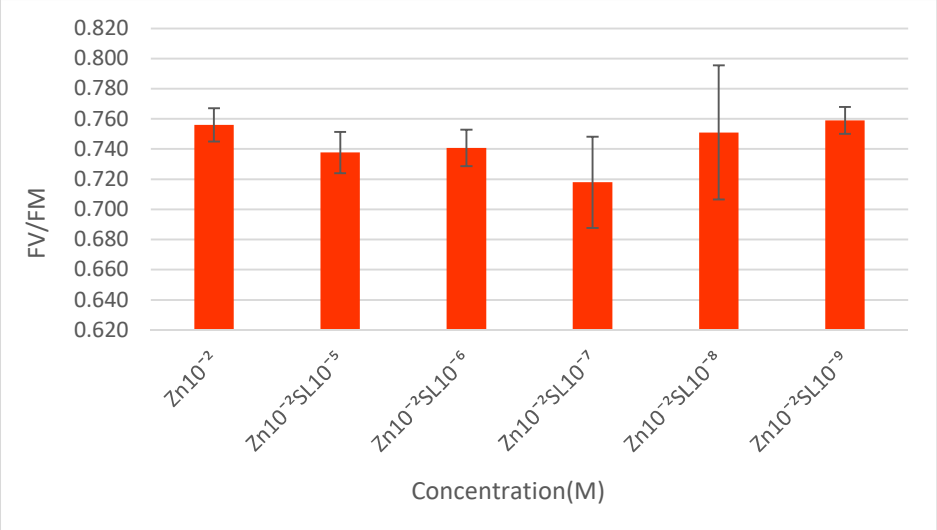
When SL was applied to the plants that were treated with $Zn10^{-3}$ M, $Zn10^{-2}$ M, and $Zn5.10^{-2}$ M, it had slight effect on the FV/FM ratio in some concentrations, they were, however, not significant.

In treated plants with $Zn10^{-3}$ M and different concentrations of SL, FV/FM ratio increased, although insignificantly, except at the highest concentration of $SL10^{-5}$ M. This ratio decreased slightly but insignificantly at concentration of $Zn10^{-2}$ M with different concentrations of SL except for the lowest concentration of $SL10^{-9}$ M.

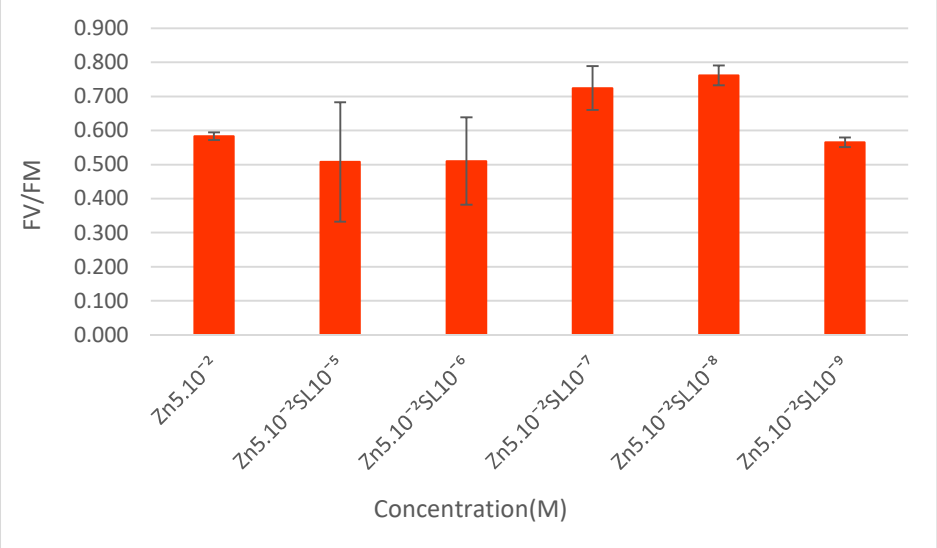
In plants treated with $Zn5.10^{-2}$ M the FV/FM ratio decreased at concentrations of $SL10^{-5}$ M and $SL10^{-6}$ M and then increased at concentrations of $SL10^{-7}$ M and $SL10^{-8}$ M and decreased again at concentration of $SL10^{-9}$ M slightly but insignificantly (Figure 3.29).



a)$Zn10^{-3}$ M treated plants in comparison with $Zn10^{-3}$ M/SL



b)$Zn10^{-2}$ M treated plants in comparison with $Zn10^{-2}$ M/SL



c) $Zn5.10^{-2}$ M treated plants in comparison with $Zn15.10^{-2}$ M/SL

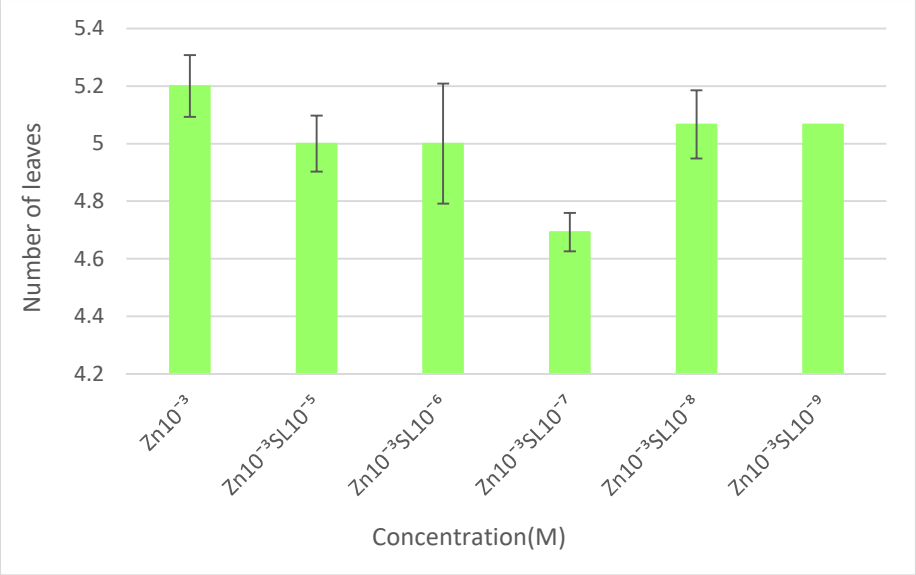
***Figure 3.29**: **Possible involvement of SL on the FV/FM ratio of Zn treated plants**. Red bars indicate the mean value of the FV/FM ratio. Stars above the bars indicate significant differences between the mean value of the FV/FM ratio of HM/SL treatments in comparison with the HM treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$). No significant effects of supplement of SL in treatments with Zn were observed.*

3.4.4. Effects of SL on the number of leaves by plants treated with Zn

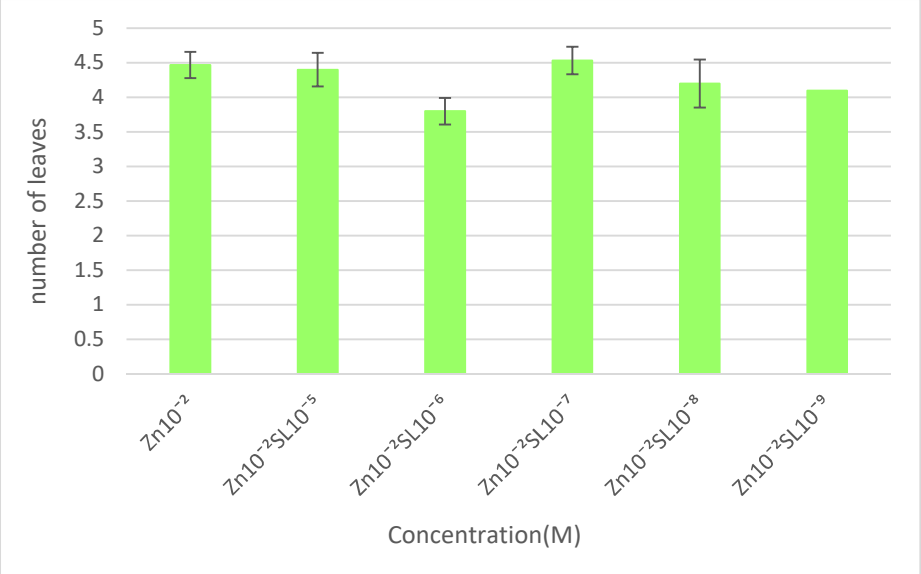
Application of SL to plants treated with Zn 10^{-3} M, $Zn10^{-2}$ M and $Zn5.10^{-2}$ M had slight effects on the number of leaves, they were, however, not significant effect.

Treatments with $Zn10^{-3}$ M the number of leaves decreased by SL slightly but insignificantly. The same result was observed in plants treated at concentration of $Zn10^{-2}$ M except at concentration of $SL10^{-7}$ M.

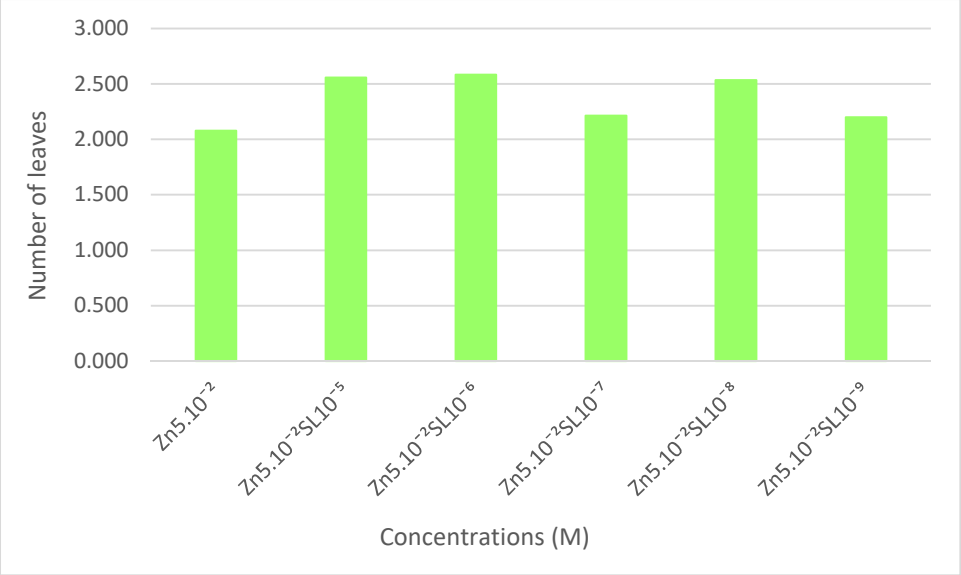
On the other side, plants treated with $Zn5.10^{-2}$ M showed an increase in the number of leaves slightly but insignificantly (Figure 3.30).



a)$Zn10^{-3}$ M treated plants in comparison with $Zn10^{-3}$ M/SL



b)$Zn10^{-2}$ M treated plants in comparison with $Zn10^{-2}$ M/SL



c) $Zn5.10^{-2}$ M treated plants in comparison with $Zn5.10^{-2}$ M/SL

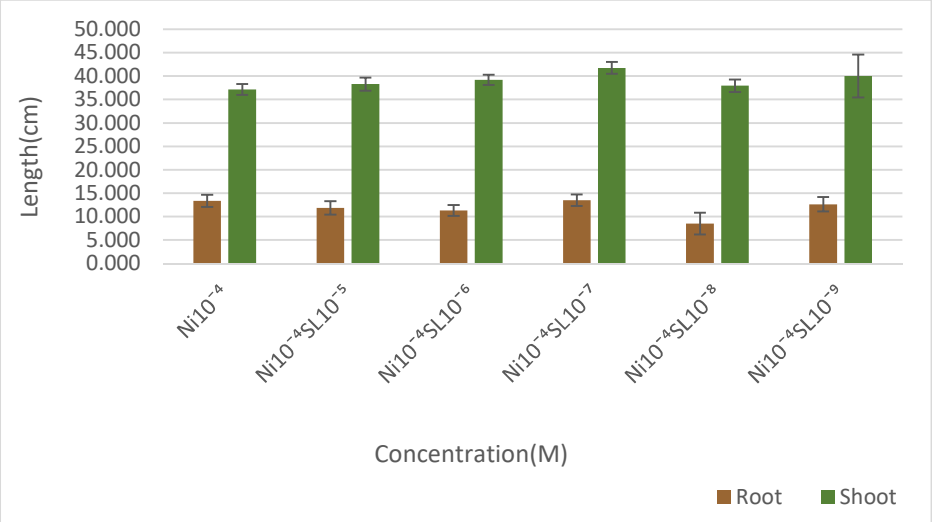
***Figure 3.30**: **Possible involvement of SL on the number of leaves formed by Zn treated plants**. Green bars indicate the mean value of the number of leaves. Stars above the bars indicate significant differences between the mean value of the number of leaves of HM/SL treatments in comparison with the HM treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$). Application of SL in treatments with different concentrations of Zn didn't show any significant effects on number of leaves.*

3.4.5. Effects of SL on root and shoot length of plants treated with Ni

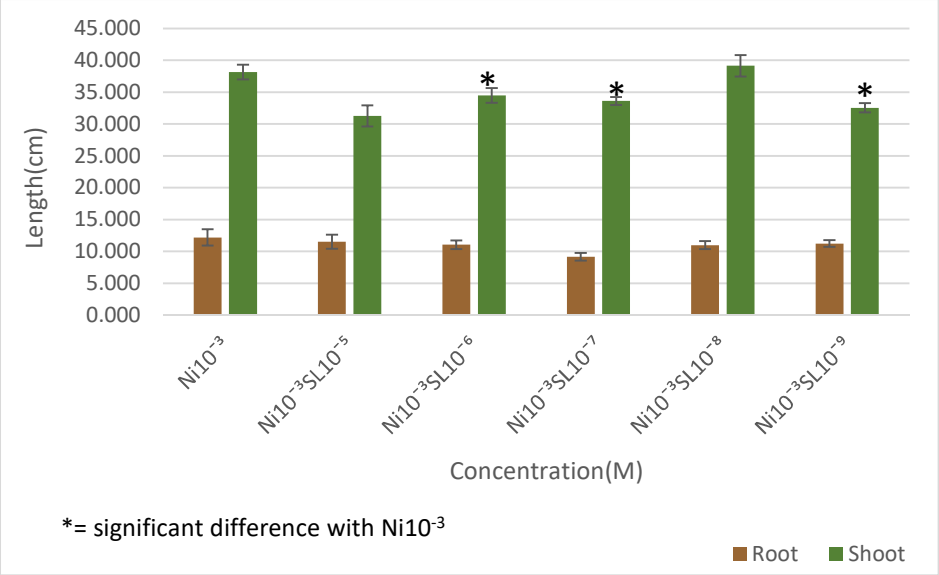
In plants treated with $Ni10^{-4}$ M, shoot length increased by using different concentrations of SL but not significantly. Root length decreased except in plants treated with $SL10^{-7}$ M.

Plants treated with $Ni10^{-3}$ M showed significant decrease in shoot length when $SL10^{-6}$ M, $SL10^{-7}$ M and $SL10^{-9}$ M applied to the plants. At this concentration root length decreased but not significantly.

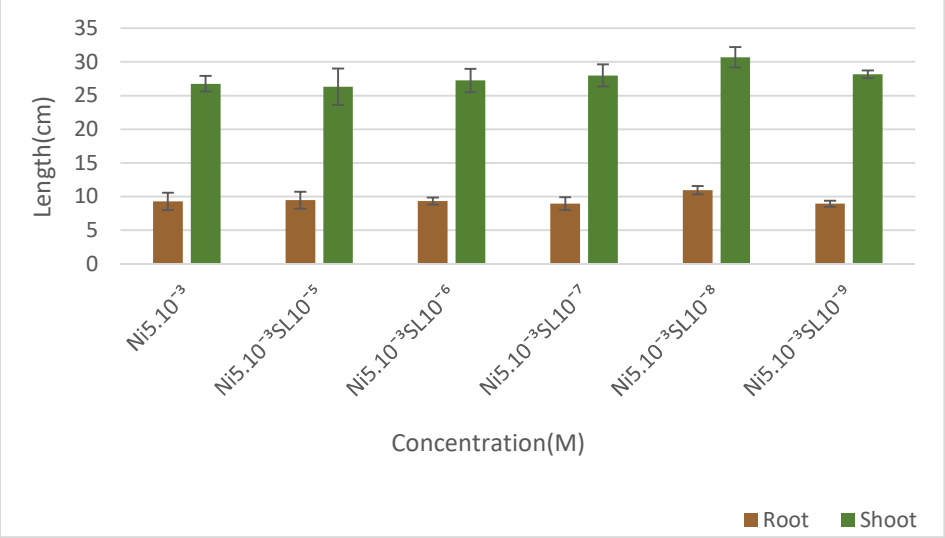
Shoot and root length of plants that were treated with $Ni5.10^{-3}$ M were not affected except for treatment with $SL10^{-8}$ M, which yielded a small though insignificant increase in both root and shoot length (Figure 3.31).



a)$Ni10^{-4}$ M treated plants in comparison with $Ni10^{-4}$ M/SL



b)Ni10^{-3} M treated plants in comparison with Ni10^{-3} M/SL



c) Ni5.10^{-3} M treated plants in comparison with Ni5.10^{-3} M/SL

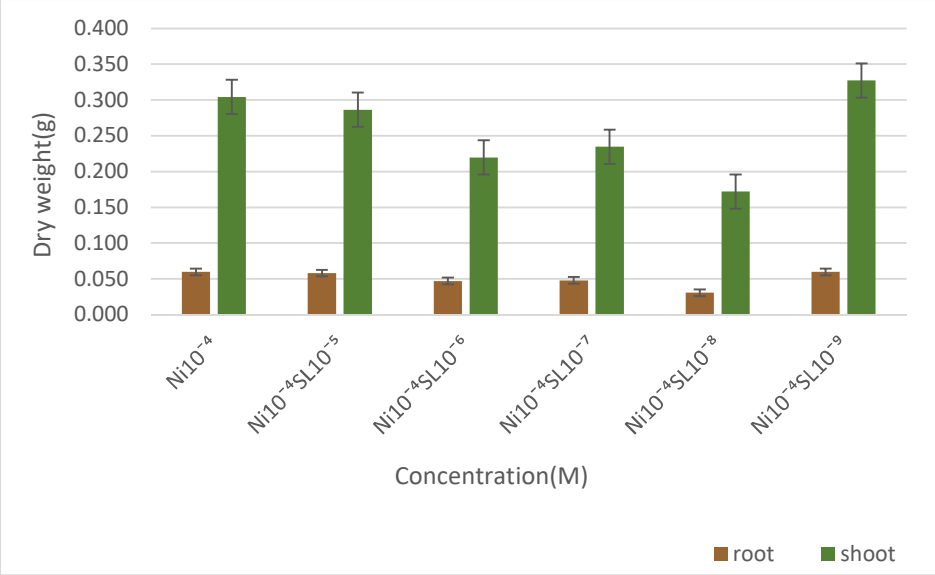
***Figure 3.31**: **Possible involvement of SL on root and shoot length of Ni treated plants**. Brown bars show the mean value of the root length while green bars show the mean value of the shoot length. Stars above the bars indicate significant differences between the mean value of the root and shoot length of HM/SL treatments in comparison with the HM treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$). Root and shoot length of plants treated with Ni10^{-3} and SL10^{-6} M, SL10^{7} M and SL10^{-9} M showed a significant decrease in comparison with treatment only with Ni10^{-3} (b). Other treatments didn't show any significant effects (a and c).*

3.4.6. Effects of SL on root and shoot dry weight of plants treated with Ni

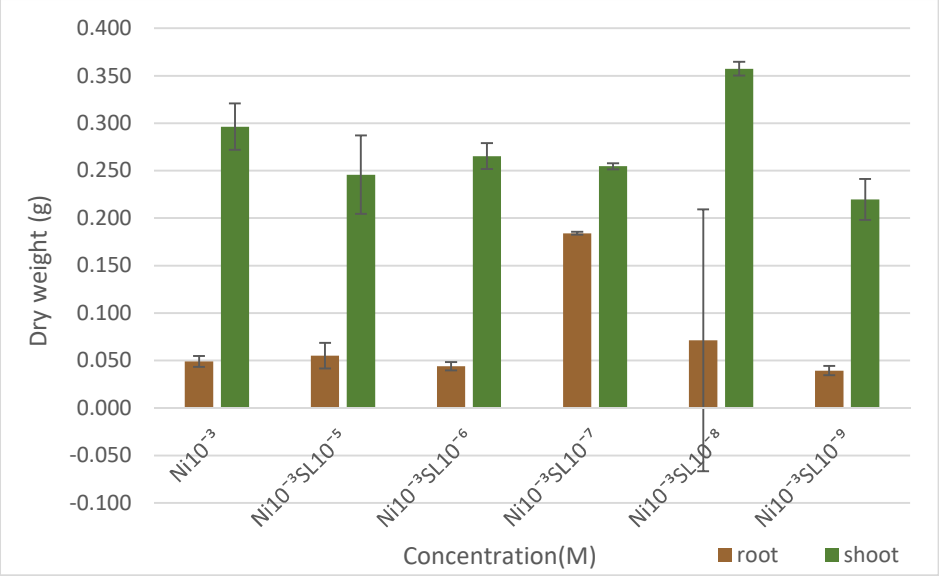
In plants treated with Ni10^{-4} M root and shoot biomass decreased except in plants treated with SL10^{-9} M where root and shoot biomass were increased slightly but insignificantly.

In comparison of plants treated with 10^{-3} M and different concentrations of SL, root biomass increased, though insignificantly, except at concentration of SL10^{-6} M and SL10^{-9} M. On the other hand, shoot biomass decreased insignificantly except at concentration of SL10^{-8} M.

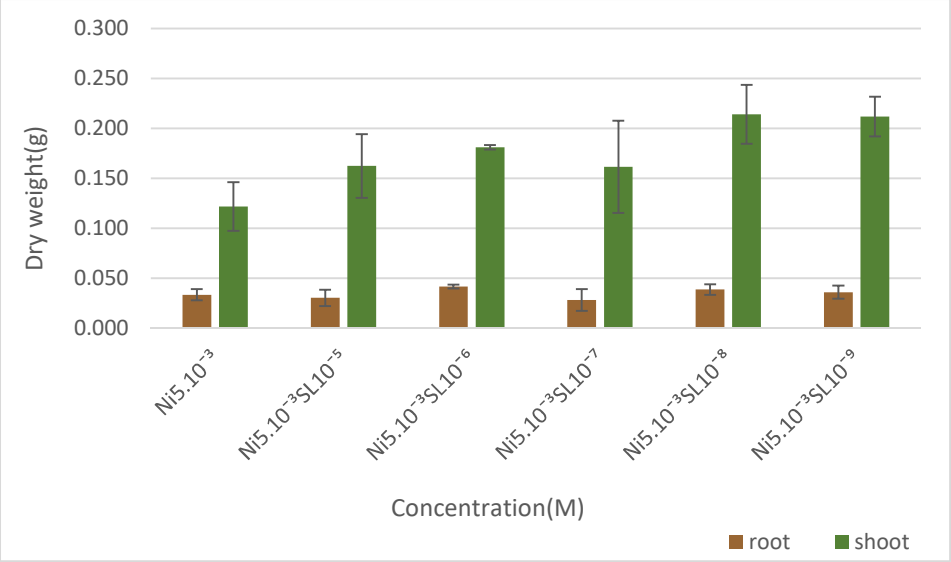
In plants treated with Ni5.10^{-3} M, by increasing SL concentrations shoot biomass increased slightly but insignificantly, whereas root biomass was not affected (Figure 3.32).



a)Ni10^{-4} M treated plants in comparison with Ni 10^{-4} M/SL



b)Ni10^{-3} M treated plants in comparison with Ni 10^{-3} M/SL



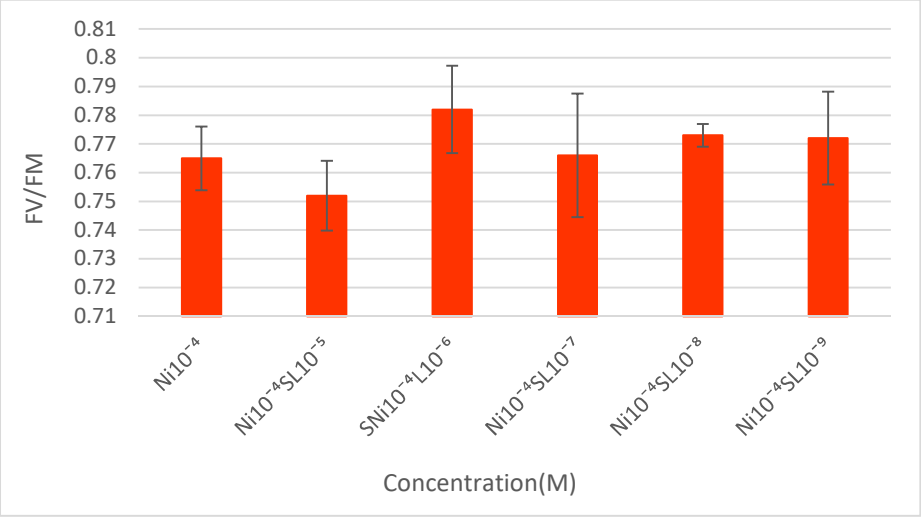
c) Ni5.10^{-3} M treated plants in comparison with Ni5.10^{-3} M/SL

***Figure 3.32**: **Possible involvement of SL on root and shoot weight of Ni treated plants.** Brown bars show the mean value of the root weight while green bars show the mean value of the shoot weight. Stars above the bars indicate significant differences between the mean value of the root and shoot weight of HM/SL treatments in comparison with the HM treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$). In each treatment with Ni, results showed increase in shoot dry weight in concentrations of SL10^{-9} M, 10^{-8} M, SL10^{-8} M and 10^{-9} M (a, b and c) respectively.*

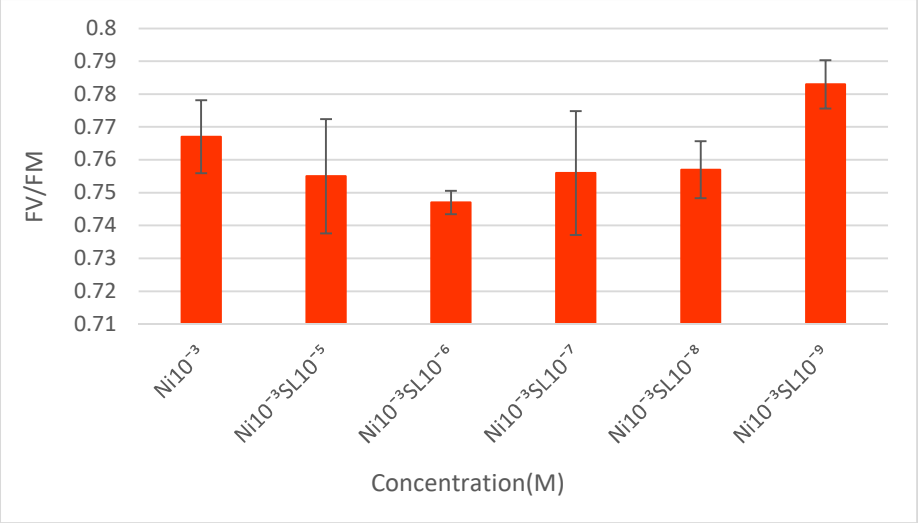
3.4.7. Effects of SL on the FV/FM ratio of plants treated with Ni

The FV/FM ratio of plants treated with Ni10^{-4} M decreased at concentration of SL10^{-5} M and then increased, both not significantly.

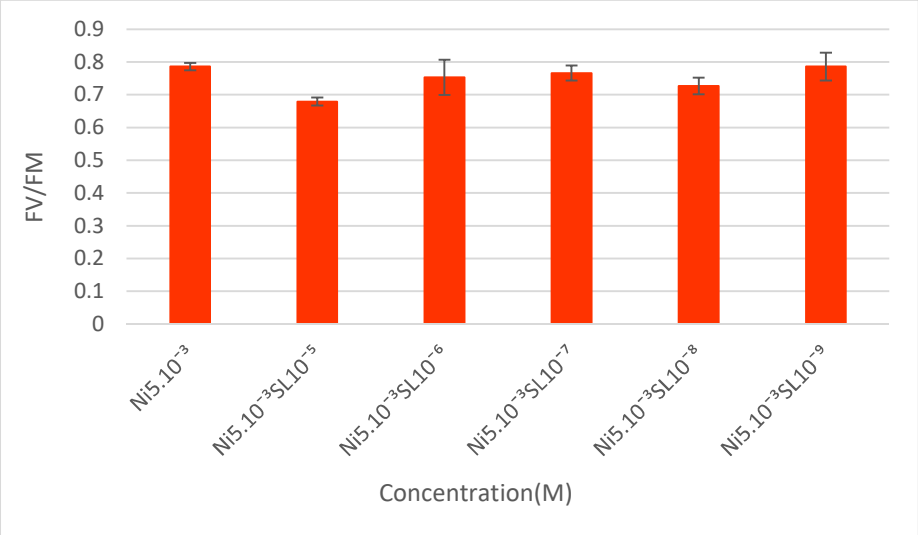
At concentration of Ni10^{-3} M, FV/FM ratio decreased slightly but at the highest concentration of SL (10^{-9} M) increased slightly. It was the same result for plants treated with Ni5.10^{-3} M and different concentrations of SL (Figure 3.33).



a)Ni10^{-4} M treated plants in comparison with Ni10^{-4} M/SL



b)Ni10^{-3} M treated plants in comparison with Ni10^{-3} M/SL



c) Ni5.10^{-3} M treated plants in comparison with Ni5.10^{-3} M/SL

***FIGURE 3.33**: **Possible involvement of SL on the FV/FM ratio of Ni treated plants.** Red bars indicate the mean value of the FV/FM ratio. Stars above the bars indicate significant differences between the mean value of the FV/FM ratio of HM/SL treatments in comparison with the HM treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$). There were no significant effects of treatments with different concentrations of Ni and SL on FV/FM ratio observed.*

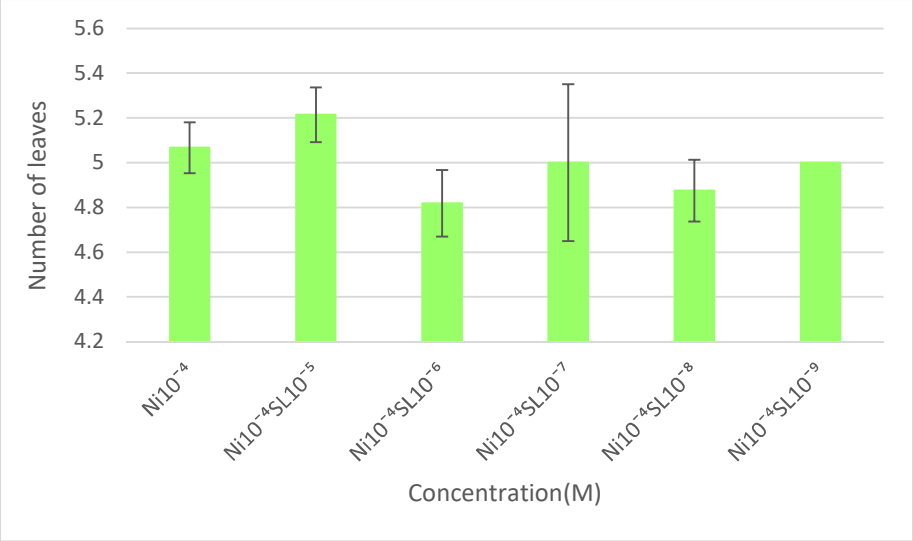
3.4.8. Effects of SL on the number of leaves by plants treated with Ni

There was no significant effect on the number of the leaves in plants treated with different concentrations of SL and exposed to different concentrations of Ni.

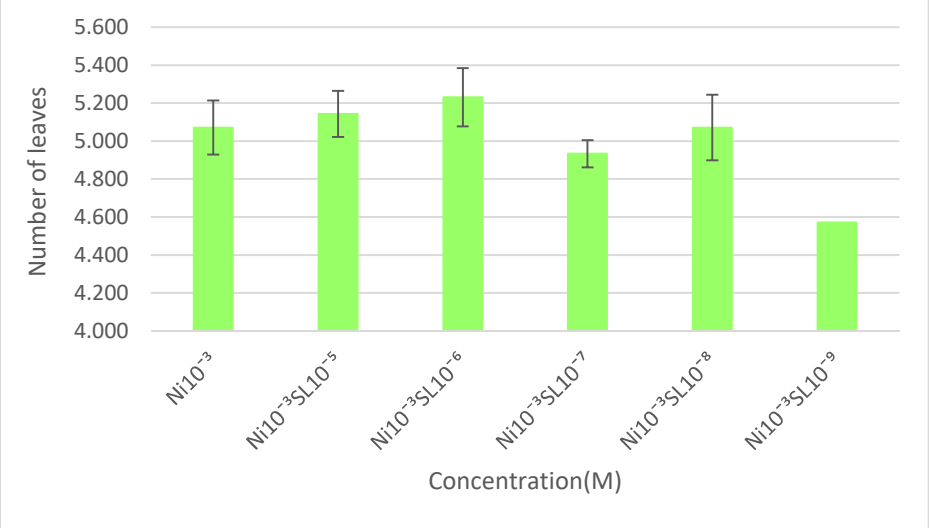
Number of the leaves in plants treated with Ni10^{-4} M increased at concentration of SL10^{-5} M and then decreased by lower concentrations of SL.

In treatments with Ni10^{-3} M, the number of leaves increased slightly but at concentration of SL10^{-7} M and SL10^{-9} M decreased.

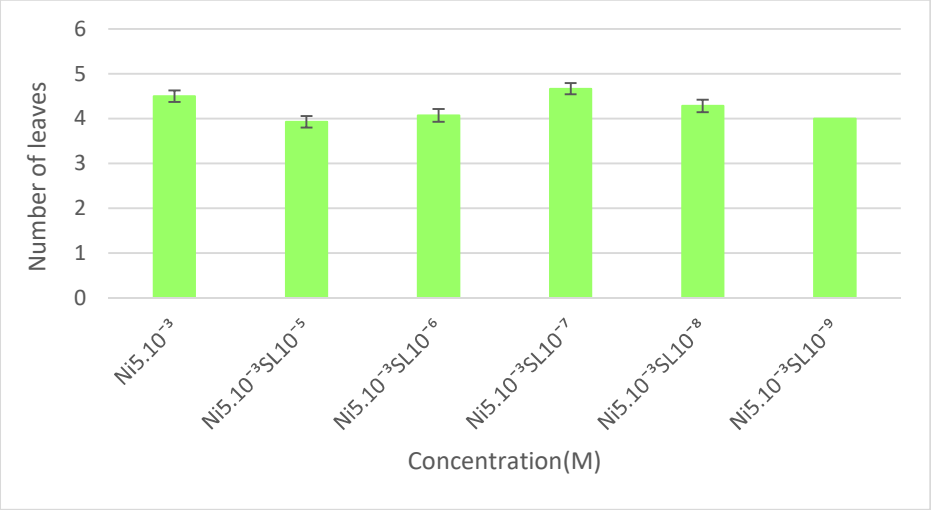
At concentration of Ni5.10^{-3} M, the number of leaves decreased but at concentrations of SL10^{-7} M increased (Figure 3.34).



a)Ni10^{-4} M treated plants in comparison with Ni10^{-4} M/SL



b)Ni10^{-3} M treated plants in comparison with Ni10^{-3} M/SL



c) Ni5.10^{-3} M treated plants in comparison with Ni5.10^{-3} M/SL

***Figure 3.34**: **Possible involvement of SL on the number of leaves formed by Ni treated plants**. Green bars indicate the mean value of the number of leaves. Stars above the bars indicate significant differences between the mean value of the number of leaves of HM/SL treatments in comparison with the HM treatment, after ANOVA (Tukey unequal N HSD, $p < 0.05$).*

3.5. Results of cellular tolerance

In this experiment plasmolysis was the method that was used to measure the sensitivity, resilience, and viability of wheat plants (cells of leaves) in different concentrations of the various treatments. The following treatments were used for the cellular tolerance test:

1. The lowest and highest concentration of HMs ($NiSO_4$ and $ZnSO_4$).
2. The lowest and highest concentration of phytohotmones (JA and SL).
3. Lowest and highest treatment of HMs and phytohormones treatments.
4. Control (C).

The results of the cellular tolerance test of the selected treatments in different concentrations of HMs solutions ($ZnSO_4$ and $NiSO_4$) and tap water acting as a C solution was shown in the Tables 3 and 4. They are color-coded due to the viability as indicated by plasmolysis of the cells that explain in following:

- At least 50-90% of the cells were plasmolyzed (+); green color in the tables.
- Partially tolerant (±) when between 10 to 40% of cells were plasmolyzed; yellow in the tables.
- Unplasmolyzed (-) <5% of cells were plasmolyzed; red color in the tables.

3.5.1. Cellular tolerance test for Zn and JA treatments

The cells treated with $Zn10^{-3}$ $JA10^{-5}$ M showed the lowest cellular tolerance in different concentrations of $ZnSO_4$ solutions. The alive and plasmolyzed cells were observed only at concentrations of $Zn10^{-5}$ M and $Zn10^{-6}$ M.

The highest cellular tolerance occurred in the cells of plants treated with $Zn5.10^{-2}$ M, $Zn10^{-3}$ $JA10^{-9}$ M and $Zn5.10^{-2}$ M. The plant cells in these concentrations were diplasmolyzed at $Zn10^{-1}$ M, $Zn10^{-2}$ M and Zn10 M (Table 1).

	Concentrations of $ZnSO_4$ in M						
Growth conditions of plants	Zn10	$Zn10^{-1}$	$Zn10^{-2}$	$Zn10^{-3}$	$Zn10^{-4}$	$Zn10^{-5}$	$Zn10^{-6}$
$Zn10^{-3}$	+	-	-	+	+	+	+
$Zn10^{-3}JA10^{-5}$	-	-	±	-	-	+	+
$Zn10^{-3}JA10^{-9}$	-	+	+	±	+	+	+
$Zn5.10^{-2}$	+	-	±	+	+	+	+
$Zn5.10^{-2}JA10^{-5}$	-	-	±	+	+	+	+
$Zn5.10^{-2}JA10^{-9}$	-	±	-	-	+	+	+
$JA10^{-5}$	-	-	+	+	+	+	+
$JA10^{-9}$	-	-	±	+	+	+	+

***Table 1: Cellular tolerance test for Zn and JA treatments.** This table shows the results of the cellular tolerance of the selected treatments ($Zn\ 10^{-3}$, $Zn\ 10^{-3}$/ $JA\ 10^{-5}$, $Zn\ 10^{-3}$/ $JA\ 10^{-9}$, $Zn\ 5.10^{-2}$, $Zn\ 5.10^{-2}$/ $JA\ 10^{-5}$, $Zn\ 5.10^{-2}$/ $JA\ 10^{-9}$, $JA\ 10^{-5}$, $JA\ 10^{-9}$) M. The highest tolerance were observed in treatments with $Zn10^{-3}$ M, $Zn10^{-3}$ $JA10^{-5}$ M and $Zn10^{-3}$ $JA10^{-9}$ M, $Zn5.10^{-2}$ M and $JA10^{-5}$ M.*

3.5.2. Cellular tolerance test for Ni and JA treatments

The cells that were treated with $Ni10^{-2}$ M and $Ni10^{-2}$ $JA10^{-9}$ M showed the lowest cellular tolerance in comparison with other concentrations of HMs and phytohormones. On the other hand, in the cells that were treated with $JA10^{-9}$ M the highest cellular tolerance was observed (Table 2).

	Concentrations of $NiSO_4$ in M						
Growth conditions of plants	Ni10	$Ni10^{-1}$	$Ni10^{-2}$	$Ni10^{-3}$	$Ni10^{-4}$	$Ni10^{-5}$	Ni 10^{-6}
$Ni10^{-3}$	-	-	-	+	+	+	+
$Ni10^{-3}$ $JA10^{-5}$	-	-	±	+	+	+	+
$Ni10^{-3}$ $JA10^{-9}$	-	-	-	+	+	+	+
$Ni10^{-2}$	-	-	-	-	+	+	+
$Ni10^{-2}$ $JA10^{-5}$	-	-	-	±	+	+	+
$Ni10^{-2}$ $JA10^{-9}$	-	-	-	-	+	+	+
$JA10^{-5}$	-	-	±	+	+	+	+
$JA10^{-9}$	-	-	+	+	+	+	+

Table 2: Cellular tolerance test for Ni and JA treatments. *This table shows the results of the cellular tolerance of the selected treatments (Ni 10^{-3}, $Ni10^{-3}$/ JA 10^{-5}, $Ni10^{-3}$/ JA 10^{-9}, Ni 10^{-2}, Ni 10^{-2}/ JA 10^{-5}, Ni 10^{-2}/ JA 10^{-9}, JA 10^{-5}, JA 10^{-9}) M. Treatments with $Ni10^{-2}$M and $Ni10^{-2}$ $JA10^{-9}$ M showed the lowest cellular tolerance.*

3.5.3. Cellular tolerance test for Zn and SL treatments

Only leaf cells that were treated with $Zn10^{-3}$ $SL10^{-9}$ M showed the lowest cellular tolerance. Other cells had higher cellular tolerance in different concentrations of Zn and C except in treatments with $Zn5.10^{-2}$ M and $Zn10^{-3}$ M (Table 3).

	Concentrations of $ZnSO_4$ in M						
Growth conditions of plants	$Zn10^{-1}$	$Zn10^{-2}$	$Zn10^{-3}$	$Zn10^{-4}$	$Zn10^{-5}$	$Zn10^{-6}$	Control
$Zn10^{-3}$	-	-	-	±	+	+	+
$Zn10^{-3}$ $SL10^{-5}$	-	-	-	+	+	+	+
$Zn10^{-3}$ $SL10^{-9}$	-	-	-	-	+	+	+
$Zn5.10^{-2}$	-	-	-	±	+	+	+
$Zn5.10^{-2}$ $SL10^{-5}$	-	-	-	+	+	+	+
$Zn5.10^{-2}$ $SL10^{-9}$	-	-	-	+	+	+	+
$SL10^{-5}$	-	-	-	+	+	+	+
$SL10^{-9}$	-	-	-	+	+	+	+
Control	-	-	-	+	+	+	+

Table 3: Cellular tolerance test for Zn and SL treatments. *This table shows the results of the cellular tolerance of the selected treatments (Zn 10^{-3}, Zn 10^{-3}/ SL 10^{-5}, Zn 10^{-3}/ SL 10^{-9}, Zn 5.10^{-2}, Zn 5.10^{-2}/ SL 10^{-5}, Zn 5.10^{-2}/ SL 10^{-9}, SL 10^{-5}, SL 10^{-9}) M, and*

C in different solutions of $ZnSO_4$ M, and water (H_2O) as C solution respectively. The highest tolerance were observed in treatments with $Zn10^{-3}SL10^{-5}$ M and $Zn5.10^{-2}SL10^{-5}$ M.

3.5.4. Cellular tolerance test for Ni and SL treatments

Leaf cells that were treated with $SL10^{-5}$ M and $SL10^{-9}$ M showed the highest cellular tolerance in all treatments with different concentrations of Ni and C. The cells that were treated with $Ni10^{-4}$ M and C showed lower cellular tolerance than other treatments.

The treatments with $Ni10^{-4}$ M, $Ni10^{-4}$ $SL10^{-5}$ M and $Ni10^{-4}$ $SL10^{-9}$ M showed the lowest tolerance, respectively (Table 4).

	Concentrations of $NiSO_4$ in M						
Growth conditions of plants	$Ni10^{-1}$	Ni 10^{-2}	Ni 10^{-3}	Ni 10^{-4}	Ni 10^{-5}	Ni 10^{-6}	Control
$Ni10^{-3}$	-	-	+	+	+	+	+
$Ni10^{-3}SL10^{-5}$	-	-	-	+	+	+	+
$Ni10^{-3}SL10^{-9}$	-	-	±	+	+	+	+
$Ni10^{-4}$	±	±	-	±	+	+	+
Ni 10^{-4} $SL10^{-5}$	-	±	±	+	+	+	+
$Ni10^{-4}$ $SL10^{-9}$	-	-	-	+	+	+	+
$SL10^{-5}$	+	+	+	+	+	+	+
$SL10^{-9}$	+	+	+	+	+	+	+
Control	-	-	+	+	+	+	+

Table 4: Cellular tolerance test for Ni and SL treatments. *This table shows the results of the cellular tolerance of the selected treatments (Ni 10^{-3}, Ni 10^{-3}/ SL 10^{-5}, Ni 10^{-3}/ SL 10^{-9}, Ni.10^{-4}, $Ni10^{-4}$/ SL 10^{-5}, $Ni10^{-4}$/ SL 10^{-9}, SL 10^{-5}, SL 10^{-9}) M, and C in different solutions of $NiSO_4$ M, and water (H_2O) as C solution respectively. Treatments with highest and lowest concentrations of SL showed the highest tolerance.*

4. Discussion

Investigation of HMs (Ni and Zn) in different concentrations on growth parameters and stress tolerance of wheat plant and also the role of phytohormones (JA and SL) in reducing the toxicity effects of HMs were the primary aims of this study. Wheat seeds were grown in soil that contained Zn or Ni. Then, JA or SL were applied to the leaves of wheat plants. Root and shoot length, root and shoot weight, FV/FM ratio and the number of leaves were measured. First, the results of measured parameters of plants treated with HMs and phytohormones were compared to the C group, separately. Then, results of HMs and phytohormones treatments were compared to treatment with HMs.

4.1. Plants treated with Zn

Zn is an essential micronutrient and plays an important role in maintaining HMs homeostasis in plant cells. It improves plant growth and development processes. Activation of different enzymes that are responsible for protein synthesis, lipid, and nucleic acid is one of the functions of Zn (Zaid et al. 2017). In contrast, this HM can be assimilated easily by plants and thus reaches toxic concentrations; thereby, it inhibits the plant's development and growth. Moreover, Zn can damage cell membranes within the plants, decreasing transpiration, and destroying protein synthesis. Based on physiochemical properties, Zn is a non-redox metal and plays indirect roles as an oxidative stressor by glutathione depletion, binding to sulfhydryl groups of proteins, inhibiting anti-oxidative enzymes, or inducing ROS-producing enzymes (Bücker et al. 2017). In this study, the effects of different concentrations of Zn (10^{-3} M, 10^{-2} M, and 5.10^{-2} M) on the wheat plants were investigated.

Root weight of wheat plants that were treated with Zn10^{-3} M decreased significantly in comparison with C plants. The other parameters such as shoot weight, root and shoot length, FV/FM ratio, and the number of leaves decreased but not significantly.

The concentration of Zn10^{-2} M had a significant effect on root length and root weight but other parameters didn't show any significant effect.

Plants treated with Zn5.10^{-2} M showed a significant decrease only in root and shoot length, root weight, and the number of leaves.

HMs such as Zn by the production of free radicals and ROS mainly have an effect on plant growth that occurs through the degeneration of cellular components. The first obvious effect of HMs toxicity is the inhibition of root elongation. It occurs in the elongation zone by inhibition of root cell division or the reduction of cell expansion. Moreover, root length can be also an important tolerance index in reduction of plant height and it is the result of the reduction in root growth and uptake of nutrient and water transport less than normal (Shah, et al. 2010). Zn adversely affect the plant height and shoot growth as well (Rout et al. 1999).

4.2. Plants treated with Ni

In plant growth, Ni acts as an essential micronutrient and it is also a component of the enzyme urease which is necessary for nitrogen metabolism. Inhibition of growth, seed germination and photosynthesis, chlorosis, necrosis, and wilting are some of the common signs of Ni toxicity in plants. (Satish et al. 2015).

Toxicity of Ni mainly appears in plant growth inhibition as a result of environmental pollution. The accumulation of Ni mostly occurs in the roots and it is more severe than in other plant tissues (Seregin and Kozhevnikova 2006).

In this experiment, in comparison with C, plants treated with Ni10^{-2} M and Ni5.10^{-3} M showed a significant decrease in root and shoot length. At these concentrations biomass of wheat plants (root and shoot dry weight) decreased significantly. The number of leaves showed a significant decrease at concentration of Ni10^{-2} M.

The inhibition mechanism of Ni^{2+} is supposed to be a decrease in the plasticity of cell walls (maybe by making junction with pectin and raising activity of peroxidase in the cell walls and intercellular space) (Satish et al. 2015).

4.3. Effects of phytohormones

Phytohormones are the internal molecules in plants that regulate plant growth under normal conditions, and improve or change physiological responses to biotic and abiotic stress; they play a key role in plant sustainability under stress situations (Wani et al. 2016). Plant hormones control the permeability of cell membranes, activation of enzymes, plant growth, and development in a small extent. One of the abiotic stress for plants is HMs toxicity which reduces growth and development and is due to accumulation in the plant's organ (Sytar et al. 2018) (Figure 4.1).

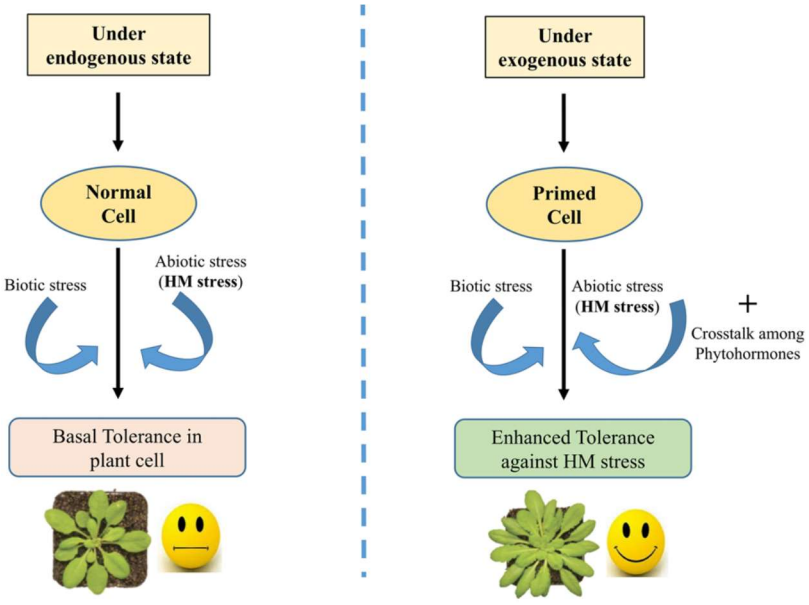


Figure 4.1. Probable mechanism of phytohormone priming regulation under HM toxicity*. (Sytar et al. 2018). The enhanced capacity of cells to mobilize induced cellular defense called priming. Endogenous and exogenous states are the situation that phytohormones produced in plants and exogenous application of phytohormones to the plants.*

In this experiment phytohormones, JA and SL were applied to wheat plants to find the following possible outcomes;
(1) The effects of JA and SL on wheat plants grown in optimal conditions and
(2) The effects of the JA and SL on the stressful conditions (HMs induced).
To observe the outcomes of phytohormones on wheat plants, first, JA and SL were applied on plants that grow under optimal conditions and results were compared with the C group. Then, the possible effects of JA and SL on the stress tolerance of wheat plants under HMs treatments were distinguished and compared to the HM-phytohormone treatments.

4.3.1. Effects of JA on control plants and on plants treated with HM

Jasmonates obtained from jasmine plants and are esters of JA and MeJA. Some functions of external application of JA are inhibition of photosynthesis, pollen germination, bud formation, embryogenesis, seed germination, and height elongation (Sezgin and Kahya 2018).

In this study, different concentration of JA were applied to the wheat plants and results compared to the control. There weren't any significant effects in concentrations of JA10^{-5} M, 10^{-6} M, 10^{-7} M, 10^{-8} M, and 10^{-9} M on all measured parameters except a significant increase in shoot length at concentration of JA10^{-6} M in comparison with C.

By administering JA to plants that were treated with Zn, root and shoot length at $Zn5.10^{-2}$ M increased then decrease only slightly but not significantly at only one JA concentrations ($JA10^{-9}$ M), whereas the other JA concentrations had slightly negative effects. Also in plants treated with $Zn10^{-2}$ M no positive effects could be observed after JA treatments, rather slightly negative effect concerning the number of leaves.

Plants treated with $Ni10^{-3}$ M showed significant increase in root and shoot length by application of JA at concentrations of 10^{-6} M and 10^{-8} M. The dry biomass of wheat plants treated with $Ni10^{-3}$ M showed that applying $JA10^{-6}$ M and $JA10^{-8}$ M resulted to significant increase in root and shoot weight. Other parameters didn't show any significant effects by using JA.

The main reason of significant effect on the shoot length and weight by applying JA is due to the high stress level brought on the plants by exposure to $Ni10^{-3}$ M, which is the highest concentration, therefore, JA acted as a repressor of shoot growth and enabling the plant to focus more energy on defending itself against the HM stress (Gasperini et al.2015).

A possible reason for significant effect of JA on the roots is the cross-talks between ABA and JA signaling pathways which participate in plant's responses to stress. Specifically here the MYC TFs (JAZs-MYC2) participates in the crosstalk between JA and ABA signaling pathways, affecting plant root growth and defense (Chen et al. 2011).

4.3.2. Effects of SL on control plants and on plants treated with HM

SL was discovered more than 45years ago and first defined as a provocative factor in seed germination. This hormone is produced in roots and can move to upper organ through xylem, thus preventing lateral bud growth. It seems that SL plays a relatively important role in responding to environmental changes and is a key regulator in signalling molecules in plant's developmental adaptation (Wani et al. 2016).

In this study, plants treated with different concentrations of SL showed no significant effects on root and shoot length, root and shoot weight, FV/FM ratio and the number of leaves in comparison with plants in optimum situation.

Results of plants that were exposed to different concentrations of Zn and treated with SL showed that there were no significant influence on wheat plant.

By applying different concentrations of SL to plants treated with $Ni10^{-3}$ M, a significant increase in shoot length at concentrations of $SL10^{-6}$ M, 10^{-7} M and 10^{-9} M was observed in comparison with plants that were treated with $Ni10^{-3}$ M.

An explanation for significant influence of SL on shoot length is the ability of SL to make a crosstalk with other phytohormones such as cytokinin that effect on shoot architecture (Ruyter-Spira et al. 2011).

At lower concentrations of $Ni10^{-4}$ M and $Ni5.10^{-3}$ M, there were no significant effects on measured parameters of plants that were treated with different concentrations of SL.

4.4. Cellular tolerance of plants treated with HMs-phytohormones

Previous studies in plant physiology showed that the development of the plant is an important factor in plant response to environmental stress and also it structured for other stimuli that will happen in the future (Dinneny 2014). In this experiment, plasmolysis used as a measurement tool to observe the sensitivity and resilience of the wheat cells to HMs stress.

The cell wall is one of the most important structures that affect water uptake. Mechanical resistance to water uptake acts as a pressure inside the cell (turgor pressure) and limits water absorption. In balanced conditions, there is an equality between external and internal water potential. The differences in the

elasticity of cell wall and cell membrane lead to attenuation of association of these cells and when plasmolysis occurred, it may separate them at a little external water potential (Wei et al. 2016).

To investigate the resilience of cells to HMs, leaves were collected from the highest and lowest concentrations of HMs and phytohormones treatments. The tiny sections from the upper epidermis were cut and put into graded concentrations of ZnSO4 and NiSO4. They were kept in darkness for 48hrs and then they were observed under the light microscope. This should indicate HMs stress directly to the cells. Findings of this viability test were necessary to determine the influence of HMs and putative effect of phytohormones on the cell's ability to resist under HMs stress.

In plants treated with Zn and JA the lowest cellular tolerance at treatments group with $Zn10^{-2}$ $JA10^{-5}$ M and the highest tolerance was at $Zn5.10^{-2}$ M were observed. Treatment with SL showed that in all concentrations except $Zn10^{-3}$ M and $Zn5.10^{-2}$ M, plant cells had highest tolerance but at concentration of $Zn10^{-3}$ $SL10^{-9}$ M, they had lowest tolerance.

Leaf cells that were treated with different concentrations of Ni and JA, showed highest tolerance at concentrations of $JA10^{-5}$ M, $JA10^{-9}$ M, and $Ni10^{-3}$ $JA10^{-5}$ M. On the other hand, lowest tolerance was at concentrations of $Ni10^{-2}$ M and $Ni10^{-2}$ $JA10^{-9}$ M. The wheat cells that were treated with SL showed the highest tolerance at concentrations of $SL10^{-5}$ M and $SL10^{-9}$ M and the lowest tolerance was at concentrations of $Ni10^{-4}$ $SL10^{-9}$ M.

The possible reason for the highest tolerance at $JA10^{-5}$ M and 10^{-9} M might be due to the sense of the presence of exogenous application of $JA10^{-5}$ and 10^{-9} M in the environment and lead to the JA receptor CORONATINE INSENSITIVE1 (COI1) mediating the ubiquitination and degradation of JAZ proteins via the 26S proteasome (Yan et al. 2007).

The possible reason of the highest tolerance in the treated cells with $SL10^{-9}$ M could be due to the presence of SL on the ultrastructure of the cell wall of the plants which could participate in the formation of cellulose microfibrils, pectin and glycoproteins, therefore can fasten the cell wall or increase ROS scavenging and cellular redox homeostasis significantly (Anjum et al. 2015).

5. Conclusion

Environmental changes – natural or anthropogenic- threaten the survival of plant life and influence growth and development in different ways. But plants have specific signals and modifying systems to respond to biotic and abiotic stress and to reduce the negative and harmful effects. In the last decades, HMs contamination in the environment causes many concerns around the world, and it needs much attention to diminishing this issue (Bücker-Neto et al, 2017).

Improvement of defense against toxicity effects of HMs in plants carries out by phytohormones. They are chemical messengers and endogenous molecules that produced in very little amount and regulate many physiological processes and they are critical hormones for plant survival under HM stress (Sytar et al. 2019).

In this study, the effects of HMs, (Zn and Ni) and phytohormones (JA and SL) on the growth improvement and stress tolerance of wheat which is an important agricultural plant and food source were investigated. The experiment was done in two phases approximately five and four weeks respectively. To find that wheat plants are able to afford the HMs stress in the soil, JA and SL were sprayed on the plant's leaves in the first and second parts, respectively.

Treated plants with HMs showed a significant decrease in plant parameters such as root and shoot length and root and shoot weight at highest concentration. On the other hand, an insignificant decrease in all other parameters was observed. The most significant finding effects were in root and shoot that was predictable because roots are the first plant's organ that contact with the HMs in soil and thus must addicted and accumulate them and/or prevent uptake into the shoots (Sytar et al. 2019). A positive effect of Zn in small concentration was not observed in the here applied concentrations. Also Ni in the here applied concentrations had no positive effect.

The various concentrations of JA and SL that applied to the wheat plants didn't show a consistently significant effect on the different growth parameters of the plants when applied in optimal growth conditions.

In plants treated with different concentrations of JA and exposed to HMs, just JA10^{-6} M and JA10^{-8} M showed significant effects on the various plant parameters (root and shoot length and weight) when administered to Ni10^{-3} M . Concerning SL, when sprayed on treated plants with Ni10^{-3} M, we observed significant effects on shoot length only at concentrations of SL10^{-6} M, 10^{-7} M and 10^{-9} M.

We conclude, that different phytohormones are effective in improving plant performance under heavy metal stress in different concentrations and depend on the kind and toxicity of the heavy metal. A general rule for a putative beneficial role of the phytohomones JA and SL cannot be given.

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