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„The Effects of Fulvic Acid on Different Plant Species Growing in Nickel and Zinc Contaminated Substrates“

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"And have you seen that seed which you sow?

Is it you who makes it grow,

or are We the causer of growth?"

Quran 56: 63–64

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1. Abstract

Zunehmender Gebrauch von Schwermetallen in Industrie und Haushalt führte in den letzten Jahrzehnten zu einer vermehrten Belastung der Umwelt. Manche Schwermetalle sind wichtige Spurenelemente für Pflanzen und werden daher leicht von ihnen aufgenommen. So gelangen sie schließlich auch in das Nahrungsnetz. Menschen nehmen Schwermetalle vor allem über Nahrung pflanzlichen und tierischen Ursprungs auf. Schwermetalle sind biologisch nicht abbaubar und akkumulieren daher leicht in Geweben und können so gesundheitliche Schäden verursachen. Sie stellen nicht nur in hohen Konzentrationen ein Problem dar, sondern auch wenn es nicht genug von ihnen gibt. So ist Zink ein wichtiges Spurenelement von dessen Mangel etwa 33% der Weltbevölkerung betroffen ist. Zinkmangel ist außerdem der am häufigsten vorkommende Nährstoffmangel in landwirtschaftlich genutzten Böden. Studien über den Einsatz von Biostimulatoren konnten das Potential dieser aufzeigen, diesen Problemen beizukommen. Sie können abhängig von ihrer Zusammensetzung und ihrer Konzentration sowohl die Toleranz von Pflanzen gegenüber erhöhten Schwermetallkonzentrationen steigern als auch die Aufnahme von wichtigen Spurenelementen wie beispielsweise Zink erhöhen. Dies könnte eine Möglichkeit sein dem weltweiten Zinkmangel entgegenzuwirken. In der vorliegenden Arbeit wurde der Effekt von Fulvic Acid (FA) auf Weizen (*Triticum aestivum*), Amaranth (*Amaranthus cruentus* und *A.caudatus*) und *Thlaspi caerulescens* in unterschiedlichen Substraten (In-Vitro-Kulturen, Hydrokulturen und Erdkulturen) unter Zink und Nickel Stress unterschiedlicher Konzentrationen untersucht. Regelmäßiges Besprühen der Blätter mit FA verbesserte unterschiedliche Wachstumsfaktoren (Wurzelhaarlänge, Wurzellänge, Anzahl der Blätter, Länge der Distanz von Wurzelspitze zu erstem Wurzelhaar), jedoch stark abhängig von der gewählten Konzentration der FA, der Pflanzenart und der Konzentration der Schwermetallbelastung durch Zink oder Nickel. Besonders auffallend war die Verbesserung der mechanischen/physikalischen Eigenschaften mit zunehmender FA Konzentration von Weizen- und Amaranthpflanzen in den Erdkulturen. Mit zunehmender FA Konzentration nahm außerdem Blattfläche und Stammdurchmesser zu, sowie die Regenerierungsfähigkeit nach Lausbefall.

The amount of heavy metals released into the environment increased tremendously in the last centuries, due to enhanced use in industry and daily life. Some heavy metals are important trace elements for plants, which therefore easily take them up and facilitate entry of these elements into the food chain. Heavy metals intake by humans mainly happens through consumption of plant- and animal-based food. They are non-degradable and therefore often accumulate in tissues, causing adverse effects to human health. While excess of heavy metals pose a great threat, not enough trace elements are problematic for plants, animals and humans as well. Zinc deficiency for example is the most common deficiency in soils for crop production and 33% of the world's population suffers from the results of zinc deficiency as well. Previous studies suggest that biostimulants can be used to support plants in tolerance of high heavy metal concentrations or increase the content of important trace elements like zinc in crops, to battle deficiency in human nutrition, depending on the concentration and type of the biostimulant used. In this work the effect of foliar application of different concentrations of fulvic acid (FA) on *Triticum aestivum*, *Amaranthus* (A*. cruentus and A. caudatus*) and *Thlaspi caerulescens,* growing in in vitro cultures, hydroponics and soil, contaminated by different concentrations of zinc and nickel, was investigated. FA improved growth performance on several parameters (root hair length, root length, number of leaves, distance between root tip and first root hair) depending on the concentration of the FA applied, the concentration of the heavy metals and the plant species. Especially outstanding was the improvement of the mechanical properties of Amaranth and wheat in soil with increasing concentration of FA. Leaf surface and stem diameter in Amaranth increased visible with increasing FA concentration, as well as the ability to recover faster and better after aphid invasion.

2. Introduction

2.1. Soil Pollution

According to the "Status of the World´s Soil Resources" Report 2015 of the Food and Agriculture Organization of the United Nations (FAO) nutrient imbalance and soil pollution (acidification, contamination and salinization) are among the greatest threats to soil functions. Nutrient imbalance cannot be regulated by mineral fertilizers alone. An increase of the amount of fertilizers used in agriculture would lead to severe damage of soils and cause irreversible environmental harm. Besides utilization of alternative fertilizers, increase of the efficiency of nutrient use and uptake are essential (FAO and ITPS, 2015).

The term "soil pollution" covers the presence of any substance or chemical compound exceeding a normal level of concentration in the soil and causing adverse effects to soil organisms, humans, animals and plants. "Soil contamination" on the other hand, only refers to the presence of any substance or chemical above normal level. The "normal level" is relative to the background levels or baseline levels of a region. Background levels describe the amounts of contaminants in an area depending on the bedrock and other geogenic factors (i.e. landform), while baseline values also include anthropogenic influx. However, this does not necessarily give away toxicity or hazardousness of a contaminant, which is also dependent on climate, weathering and erosion rate in addition to availability and mobility of the elements in question. Hence, recommended values for accepted concentrations of different elements must take all these factors into consideration, as well as country and region-specific challenges. Vulcanic activity or weathering of bedrocks lead to natural occurrence of high levels of contaminants like heavy metals (HMs), but normally do not pose a threat to soil functions, as long as the ecosystem's resilience and response ability are not subjected to destructive (anthropogenic) influence (Rodríguez-Eugenio et al., 2018).

Human health is closely related to soil characteristics. Among the health threatening factors related to soils are toxic contaminants entering the food chain, direct exposure to dust or pathogenic organisms and nutrient-deficient crops, which contribute to malnutrition. Trace elements or heavy metals (HMs) like arsenic (As), lead (Pb), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni) and zinc (Zn) are the most concerning soil contaminants to human health (FAO and ITPS, 2015).

Another crucial consequence of soil contamination, which effects not only human health but a large range of organisms, is eutrophication as well as pollution of ground and drinking water. The greatest impact of increased contaminant levels in the ecosystem is caused by pollutants of anthropogenic origin. Typical anthropogenic sources of HMs are tailings and other by-products of industrial activities, waste rock deposits, smelting and mining operations, usage of pesticides and fertilizers in agriculture and forestry, (livestock) manure, remains of war (i.e. minefields), oil spills, untreated irrigation water, waste water, contaminated sewage sludges and more. Activities connected to urban and transport infrastructures have been underestimated in the last centuries, but now enjoy great attention. Some of the more prevalent examples are emissions from engines, corrosion of metal vehicle parts, tyre and pavement abrasion, foliar deposition and root uptake of city dust. Plastic poses a great threat as well. Not only owing to the fact, that it is so frequently used in industry and households, but because it normally is not biodegradable and therefore only becomes smaller in size. With decreasing size, the surface increases and so does the amount of HMs and other toxic elements binding to the particles, which are easily dispersed by wind and water, enriching the content of these toxic elements in soils, aquatic systems and organisms alike (Rodríguez-Eugenio et al., 2018).

2.2. Heavy Metals

Heavy metals (HMs) are elements that have a high atomic weight and a density of at least five times higher than water. Large use in industrial, domestic, agricultural, medical and technological fields leads to dramatically increased release into the environment as well as human exposure. Bioavailability is influenced by factors like temperature, pH-levels, lipid solubility, species characteristics, trophic interactions, biochemical adaptations, absorption and sequestration. Some HMs are essential for animals, humans and plants, however in a very specific range of concentrations. Anything beyond thresholds causes adverse effects (Tchounwou et al., 2012).

HMs released in the environment may eventually end up in our food chain, and then have a major impact on human health. For the majority of human population, the largest amount of HM absorption comes from animal- and plant-based food (Cimboláková et al., 2019).

Chaney (1980) described the "Soil-Plant Barrier" as the most effective shield against food chain contamination. He argues, if a contaminant is highly toxic for plants at low concentrations, the plant dies before accumulating levels, high enough to become toxic for human (and animal) consumption. Based on the potential food-chain risk he classified four groups (Tab. 1).

Group1	Group2	Group3	Group4
Silver (Ag)	Mercury (Hg)	Boron (B)	Arsenic (As)
Chromium (Cr)	Lead (Pb)	Copper (Cu)	Cadmium (Cd)
Tin (Sn)		Manganese (Mn)	Cobalt (Co)
Titanium (Ti)		Molybdenum (Mo)	Molybdenum (Mo)
Yttrium (Y)		Nickel (Ni)	Selenium (Se)
Zirconium (Zr)		Zinc (Zn)	Thallium (Tl)

Tab. 1: HMs classified in four groups according to food chain contamination risk

Group1 contains elements that pose a low food-chain risk, because these elements have a low solubility in the soil and thus are not taken up by plants. High concentrations of these elements in food suggest direct contamination by dust or soil.
Group2 elements pose a minimal risk for humans. These elements may enter the plant roots but have very low mobility within the plant and therefore do not reach the edible parts of the plant.
Elements in Group3 are easily taken up by plants but are phytotoxic in elevated levels. The Soil-Plant-Barrier protects the food-chain against contamination.
Group4 elements are high-risk elements, which are readily taken up by plants and are not automatically phytotoxic at levels that are hazardous to human health (Rodríguez-Eugenio et al., 2018).

2.3. Zinc

The average Zinc (Zn) content in the soil worldwide lies between 17 and 125 mg/kg (Saha et al., 2017). The critical limit for agricultural soils in Austria (ÖNORM L 1075) given by the "Umweltbundesamt" is 300 mg/kg total Zn content. Zn mobility increases with decreasing pH level of the soil (Saha et al. 2017).

Zn is an essential element for humans, animals and plants. It is estimated to be part of over 3000 enzymes in the human body. Zn plays a vital role in the immune system, reproductive system, physical growth and development. As a neurotransmitter it is especially important in salivary glands, prostate, immune system and intestine. The concentration of Zn in blood does not decline in proportion to its deficiency, causing even more severe effects in humans, especially children (Alloway, 2009).

The daily requirement of Zn is 0.3 mg/kg body weight and the maximum lies at 1 mg/kg. Grains, vegetables and fruits usually contain <5 mg/kg. Food rich in proteins like meat and marine organisms contain higher

concentrations (10–50 mg/kg). Absorption is highly variable, lying between 10–90%. An excess of Zn ingestion (i.e. through overuse or abuse of supplements) results in vomiting, fever, nausea, stomach cramps and diarrhoea. Copper (Cu) deficiency is the main consequence of chronic ingestion of Zn in higher concentrations (WHO, 2003). Zn and Cu are competitive elements, Zn absorption getting favoured over Cu in the gut (Hoffman et al., 1988).

Approximately 33% of the world population suffers from Zn deficiency, in some parts of the world the number raises up to 73%. To bring down this alarming number, methods are sought to increase the Zn content in crops. The idea to fortify the Zn content in staple food is called biofortification and has a decisive advantage over Zn supplements. Crops with increased Zn content would reach the populations most affected by deficiency, that very often have no access to supplements (Alloway, 2009).

Adding to this problem is the fact, that Zn deficiency is the most prominent one in crops worldwide. Zn is an essential element in enzymes regulating the auxin synthesis, cellular membranes, protein synthesis and carbohydrate metabolism, as well as controlling gene expression in response to environmental stress, like high light intensity and high temperatures. Symptoms of Zn deficiency are stunted growth, chlorosis, small leaves, sterility and an increased tendency to get infected by fungal diseases or injured by high light intensities and temperatures. Very often cereal plants suffer from hidden or latent deficiency (Alloway, 2009).

Soil factors responsible for Zn deficiency and low availability are (among others) high soil pH, high calcite and organic matter contents or high concentrations of Na, Ca, Mg, P and HCO_3 in the soil solution. Very often hidden Zn deficiency is due to high yielding crops, needing increased levels of NPK fertilizers, which require elevated pH levels or growing Zn-inefficient varieties on soils with very low Zn availability. Sandy soils or strongly leached tropical soils are primarily affected by low total Zn content. Calcareous soils in arid areas have, in addition to elevated pH levels, very high contents of $CaCO_3$, which contains Zn through chemisorption, amplifying Zn deficiency. High levels of P enhance shoot growth and dilute the absorbed Zn within the plant, as well as reducing root growth and mycorrhizal infection, which results in less Zn uptake. Furthermore, Zn is a key signalling entity in regulating P uptake (Alloway, 2009).

2.4. Nickel

The average Nickel (Ni) content in the soil worldwide lies between 8 and 55 mg/kg (Saha et al., 2017). The critical limit for agricultural soils in Austria (ÖNORM L 1075) given by the “Umweltbundesamt” is 60 mg/kg. Ni mobility increases with increasing pH level of the soil (Saha et al., 2017).

Ni is an essential element and important micronutrient for plants, which require concentrations ranging between 0.05–10 mg/kg. It cannot be replaced by other elements and is required for nodule growth, hydrogenase enzyme activity and increases disease tolerance of plants. Ni deficiency causes reduced growth, chlorosis, meristematic necrosis, disturbance of N assimilation, Fe uptake, lipid metabolism and enzyme activity. Ni deficiency does not occur often. Excess of Ni on the other hand is very common and poses a great threat to crop yield. It leads to reduction of germination, growth, biomass accumulation, cell division, disturbance of photosynthesis and nutrient absorption, causing leaf chlorosis and finally necrosis. Ni binds with pectines, which decreases cell wall plasticity and increases peroxidase activity. It causes removal of Ca from binding sites in oxygen evolving complexes and replaces Mg in chlorophyll, which eventually inhibits the electron transport of the PhotosystemII. Ni stress also leads to reduction of Fe, Cu, Zn, Mg and Mn contents (Hassan et al., 2019).

Translocation of Ni within the plant is linked with Ni-ligand complexes like nicotianamine, histidine, organic acids and proteins. 50% of the Ni taken up by roots stays in the roots, 80% of it is retained in the vascular cylinder and only 20% occurs in the cortical region of the root, which is believed to be one of the reasons why Ni has such a high mobility within the plant (Hassan et al., 2019).

Ni is assumed to be essential for human beings and animals as well, but so far, no evidence has been found nor deficiency symptoms observed. The average total oral intake of Ni in humans lies between 200–300 µg/day. Except cacao products (10 mg/kg) and nuts (3 mg/kg), most food products contain less than 0.5 mg/kg fresh weight Ni (WHO, 2000).

Overall Ni is becoming a major allergen in human population worldwide, causing skin irritation and allergic dermatitis. The risk increases with ear-piercing and contact with Ni in diverse consumer products. Acute inhalation of nickelcarbonyl and high doses of Ni-ingestion cause severe lung damage, gastric irritations and pulmonary oedema. Ni is counted as human carcinogen with assumed linear correlation of dose and carcinogenic effect. Workers of the Ni-refining industry also show elevated levels of sister chromatid exchanges and chromosomal gaps (WHO, 2000).

2.5. Plant and Heavy Metals Interactions

In general, prolonged exposure of plants to HMs leads to accumulation of ROS (Reactive Oxygen Species), AOS (Activated Oxygen Species), marginalization of cell functions and adverse effects on lipids, proteins and thylakoid membranes. Plants respond with immobilization, exclusion, chelation and compartmentalization of the metal ions (Rout and Das, 2003; Singh et al., 2016).

Formation of ROS species, such as H_2O_2, O_2^- and OH^-, affects chloroplasts and mitochondria, leads to phytotoxic effects and induces the antioxidative system.

Stress interferes with the hormone system, resulting in root growth inhibition. It also affects germination and internodal growth, as well as calcium signalling pathways, which influences plant growth, plant development, embryogenesis and facilitates hypersensitive responses caused by pathogens (Kumar and Trivedi, 2016).

Many plants for example regulate metal accumulation with glutathione, which is an antioxidant and precursor of phytochelatins (PCs). PCs chelate free metal ions through formation of thiolates. Thiolates can be transported to vacuoles, which then function as HM storages.

Plants that are classified as accumulators or hyperaccumulators can deal with high levels of HMs and sequester these normally toxic elements in their tissues (Ghori et al., 2016; Singh and Singh, 2016).

2.6. Phytoremediation

Phytoremediation has the aim to use plants and their associated microorganisms to remove or inactivate contaminants from the soil and the environment (Singh and Singh, 2016).

There are several processes that fall under the category of phytoremediation. Some plants cannot only be used for one of these mechanisms, but several of them. In Tab. 2 different phytoremediation processes and their definition are given.

Phytoextraction	Most common practice; plant accumulates HMs in its organs
Phytostabilization	Immobilization and stabilization of pollutants in the soil by providing dense vegetation to avoid movement and further leaching of these elements
Phytofiltration	Adsorption and absorption of pollutants from water bodies (no HM)
Phytovoltalization	Conversion of contaminants into volatile form, enabling it to move from soil into atmosphere (no HM)

Tab. 2: Definition of different phytoremediation technics

The idea of phytoextraction is to use suitable plants that accumulate high levels of HMs in their tissues and grow them on contaminated soils. After harvesting the plant, they are dried and burned, which results in highly concentrated material, containing a higher concentration of the pollutant than the soil.

Excluders can take up HMs from the soil into the below-ground organs, but do not translocate them within the plants. Accumulators and hyperaccumulators on the other hand, are characterized by the ability to translocate HMs in shoots and leaves, in addition to accumulation in the roots.
Plants having the ability to store HMs in their tissues, need mechanisms to manage the following stages:

1. To ensure bioavailability and uptake of HMs from the soil via roots.
2. Translocation of HMs from roots to shoots through xylem.
3. Sequestration of HMs in different plant parts i.e. vacuoles in leaves.

Bioavailability and uptake of HMs is dependent on pH level, water content, type and abundance of organic substances as well as the form of HMs (ions in solution, complexes, in hydroxides, oxides or silicates etc.). Very often root exudates function like chelates or HM reductases, either breaking large organic complexes down or binding ions making them uncharged and thus available and easier accessible for absorbance through roots. Organic acids, microorganisms and fungi can increase (or decrease) bioavailability of HMs in the soil as well. Bioavailability can also be amplified using chelating agents like EDTA ($C_{10}H_{16}N_2O_8$).
HMs entering the roots can either take the apoplastic pathway, travelling through intracellular spaces without entering the cells, or take the symplastic pathway. The symplastic pathway transport is an active transport through the cytoplasm and is mainly used for non-essential metals like Ni, Pb and Cd. Pathways vary between different plant species and different HMs (Ghori et al., 2016).

In order to translocate HMs within the plant, they have to pass the casparian strip, which blocks intracellular movement. From this point on every transport of metals requires energy. The process of elements entering the xylem is called xylem loading. Natural chelates, like PCs and organic acids, are involved in this process by ensuring elements are not charged, which could interfere with and hinder the transport. In *Thlaspi caerulescens* nicotinamide is a chelator required for Ni and Zn translocation.
The final steps are detoxification and sequestration of HMs in shoots and leaves. PCs and organic acids, like histidine and citrate, play a crucial role in these processes. Final storage destination can be intra- or intercellular and is different for every hyperaccumulator and HM (Ghori et al., 2016).
In *Thlaspi caerulescens* for example Cd is stored in the cell wall (Cosio et al., 2005), Zn in the vacuoles (Küpper et al., 1999) and Ni in both (Krämer et al., 2000).

To be able to accumulate HMs, plants need to have a high growth rate, tolerance to high concentrations of HMs, as well as easy adaptation to biotic and abiotic stress. Ideal plants qualified for phytoremediation are hyperaccumulators, that at the same time produce relatively little biomass as they grow. Examples of the 450–500 different plant species, that qualify as hyperaccumulators so far, are *Thlaspi caerulescens* (Pb, Cd, Ni, Zn) and *Arabidopsis halleri* (Zn, Cd).
To assess if a plant is a hyperaccumulator the translocation factor (TF), meaning the ratio of HM "content in shoots/content in roots", has to be greater than 1, likewise the bioconcentration factor (BF), referring to the ratio HM "content in roots/content in soil" (Ghori et al., 2016).
In other works, plants with TF and BF >1 qualify as accumulators, and only when plants contain more than 1000 µg/g (0.1%) Ni or 10 000 µg/g (1%) Zn in dry weight, they classify as hyperaccumulators (Robinson et al., 1998; Küpper et al., 1999).

2.7. Phytohormone Priming and Biostimulants

Phytohormones and biostimulants are substances that are promising candidates for the growing demand of ecological and sustainable intensification in horticulture.

Phytohormones are signalling substances in plants, that are involved in several plant growth and development processes and play a crucial role in management of biotic and abiotic stress. They regulate cell membrane permeability, enzyme activity, growth, reproduction, manage secondary metabolites, regulate germination etc. and work in very low concentrations.

Auxin (IAA), Cytokinin (CK), Ethylene (ET), Abscisic Acid (ABA), Gibberellin (GA), Brassinosteroid (BR), Salicylic Acid (SA), Jasmonic Acid (JA) and Strigolactone (SL) are the phytohormones known so far. Besides natural occurrence within the plants, exogenous application of phytohormones in appropriate concentrations proved to be safe and effective in enhancing the "genetic program" of a plant.
Phytohormone concentrations increase when plants suffer of HM stress. SA for example is an important element of the defence mechanism of plants to HM stress. Besides activation of antioxidant defence systems, SA activates detoxification processes under Cd exposure. When SA was exogenously applicated, a decrease of ROS and lipid peroxidation was observed. It furthermore, led to elevated levels of chlorophyll content, total lipids and linoleic acid, which increases capability of the plant to cope with HM stress. Application of Methyl-JA in *Kandelia obovate* prevented Cd uptake and reduced transpiration rate. Many more examples motivate further development and research in phytohormone priming (Sytar et al., 2019).

Another approach in strengthening plants to cope with stress is the application of biostimulants. Biostimulants are characterized as substances of natural origin or microorganisms, that promote growth and plant-protection, without causing any negative side effects. They are not classified as fertilizers, which serve as direct nutrient suppliers to plants. Instead, they facilitate nutrient uptake by improving mechanisms of the plant to process nutrients or by strengthening mycorrhiza and other microorganisms, that provide their hosts with nutrients. Examples of efficient biostimulants are seaweed extracts, humic and fulvic acids, beneficial bacteria and fungi, amino and carboxylic acids or chitosan (Drobek et al., 2019; Du Jardin, 2015).

The potential in biostimulants lies not only in their efficiency (very small quantity and concentration necessary to achieve effect), but also in the potential to increase the effectiveness of fertilizers, which may reduce the amount of fertilizers dumped on fields. Often highlighted is their multifaceted impact on many different characteristics of a plant, like height, fruit size, amount of yield, improvement of mechanical properties etc. The use on a commercial scale would reduce soil, water and air pollution considerably. It may also be an ecological way to strengthen and protect yield against illness and pests, as well as abiotic stress. The difficulty lies in developing effective methods for agronomical use. Biostimulants seem to work very specifically, in a limited range of concentrations, influenced by soil properties and plant species (Drobek et al., 2019).

2.8. Humic and Fulvic Acids

Humic substances (HS), including humins, humic acids (HA) and fulvic acids (FA), make up more than 80% of the soil organic matter (SOM) and are important for buffering, water retention and bioavailability of nutrients (Talpur et al., 2016).

HA are soluble in aqueous alkaline solutions and precipitate in pH 1–2. HA are huge complexes, consisting of many components with relatively low atomic weight, a great number of hydrogen bonds and stabilizing hydrophilic/hydrophobic interactions. HS are formed through transformation of plant and animal matter and result from microbial metabolism (Canellas et al., 2015).
Most of the time FA occurs in lower concentrations than HA. It has a lower molecular weight, is soluble at all pH levels and has more functional groups, containing oxygen, than HA (Tapur et al., 2016).

Different studies suggest, that the effect of applied humic acids is highly dependent on plant species, application method and rate, sources of the substances and environmental conditions. Nevertheless, in

general HS are growth promoting and have a greater impact on monocotyledonous than on dicotyledonous plants, due to still unknown reasons. Enhanced plant growth seems to be connected to the effect HS have on membrane transporters and changes in other aspects of root architecture. For example, HS stimulates alteration of root surface pH and redox potential, which causes promotion of nutrient uptake, secondary transport and over expression of ion transporters, leading to formation of lateral roots and root hairs. Auxin-like molecules have been observed in HS complexes, that could access receptors in- and outside of plant cells. In some studies HS acting like signalling molecules and inducing release or production of phytohormones has been reported. Maize plants in one experiment changed their profile of exuded organic acids when HA was present. In another study nitrate transport was enhanced by 89% compared with the untreated control.
HMs and HS build complexes by HMs binding on carboxylic and phenolic hydroxyl groups of HS. Depending on the type and concentration of HM or micronutrient, as well as application method and amount of HS used, change in uptake was observed. For example, both decrease and increase of Cd, Cu and Pb uptake in plants was reported, subjugated to the mentioned factors. FA complexes free Pb^{2+} and leads to reduced Pb uptake, if the FA amount added to the soil meets the quantity of Pb available. Translocation factor of Pb from roots to shoots decreased when comparing the control to HS treated plants (Canellas et al., 2015; Du Jardin, 2015).
FA at low concentrations caused increased translocation of Pb in *Vicia faba*, but decreased translocation when treated with higher FA concentration (25 mg/L) (Shahid et al, 2012).

HS building complexes with micronutrients, prevents leaching and may lead to enhanced uptake as well. Addition of HS improves quality to maintain water absorption and cell turgor, even under drought stress. It also increases peroxidase activity from 16% up to 270%, which plays a crucial role in regulating oxidative stress. Under salinity conditions yield parameters positively improves, when treated with humic acids. In horticulture the application of HS, as a foliar spray, proves to be more effective, than adding humic substances to the soil. The major biostimulant effects are promotion of fruit and vegetable growth, followed by decrease of plant disease incidents and reduced length of the production cycle (Canellas et al., 2015). It was also reported, that the average length and diameter of cucumber fruits, as well as the number of leaves and stems increases. In another study HA application caused a tenfold increase in fruit size of apricots. A mixture of HA, FA, polysaccharides and carboxylic acids leads to decrease in apricot acidity (Drobek et al., 2019). Foliar application of FA enhances K levels in plants (Priya et al., 2014).

Radicon®, a biostimulant mainly consisting of FA and HA, leads to increased fruit length, shows a significantly positive effect on antioxidant capacity and a positive effect in fruit set percentage and yield, increasing the number of fruits per tree (around 21 kg/tree compared to the control 12.7 kg/tree in the second year). Radicon treated plants give earlier and longer fruits than the control (Tarantino et al., 2018).

Treatment with 0.5 g/L FA of lettuce plants under 20 µM Cd stress, shows increase of growth in shoots (79.5%) and roots (53.8%), as well as increase in chlorophyll content (60.2%), photosynthesis (39.9%) and reduce in electrolyte leakage (35.0%) (Wang et al., 2019).

Suh et al. (2014) report, that three times foliar application of FA on tomato (*Lycopersicon esculentum* L.) in 0.8 g/L concentration led to significant increase of plant height, dry and fresh weight, P and Ca contents, fruit numbers and fruit size. Concentration of 1.6 g/L on the other hand, led to reduction of plant height, no increase in other parameters, smaller fruit size, however significant reduction of cracking. Three times foliar application of FA on potato plants (*Solanum tuberosum* L.) in 1:1000, 1:750 and 1:500 dilutions showed no effect on chemical composition or number and size of potato tubers (Suh et al., 2014).

2.9. Aim of this work

The purpose of the experiments is to describe the effects of several concentrations of FA on different plant species growing on the control substrates as well as under HM stress (different concentrations of Ni and Zn). Furthermore, effects of Ni and Zn treatment in different concentrations are documented as well.
Both growth parameters (dry weight, maximum root length, maximum root hair length, the distance between root tip and first root hair and number of leaves) and stress parameters (*Fv/Fm*, cell tolerance and Reactive Oxigen Species) are documented, as well as localization of HM within the plants and total HM content in roots and shoots are determined.
Triticum aestivum and two *Amaranthus* species were chosen as agricultural plants (*Amaranthus cruentus* and *A. caudatus*), *Thlaspi caerulescens* and *Thlaspi goesingense* as hyperaccumulators for Zn and Ni and *Saxifraga stellaris*, as a plant growing on Cu contaminated soils. Except for invitro cultures,
Thlaspi goesingense and *Saxifraga stellaris* are not used in any further experiments due to difficulty of germination and therefore will be not discussed in any more detail.

2.9.1. Amaranth (*Amaranthus cruentus* and *Amaranthus caudatus*)

The taxa *Amaranthus* consists of about 60 species. It is a pseudocereal dicotylus non-grass plant, that can be divided in grain Amaranth, vegetable Amaranth and weedy Amaranth, according to its utilization (Maurya and Arya, 2018).

Amaranthus hypocondricus, A. cruentus, A. caudatus and *A. edulis* are the four members of the grain Amaranth group, while *A. tricolor* and *A. lividis* are the vegetable Amaranth species.
Regardless, *A. caudatus* and *A. cruentus* are consumed as vegetables in some part of the worlds as well.
8000 years ago, Amaranth, called “huathli” was an important staple food of the Incas, Aztecs and Mayas in South and Central America. Nowadays it is used worldwide for different purposes. In China it is mainly used as food for livestock. The US is the largest producer of Amaranth, used in the food industry. In Africa and India, it is cultivated as vegetable and in the Himalayan region as a cereal. The food industry makes use of it in bakery, cookies, pasta, crackers, etc. The whole plant is also used as dye in the medicine, food and beverage industry, as well as a medicinal plant in Ayurveda and traditional medicine worldwide.
The charactersitics that make it most attractive for stakeholders and scientists today are its nutritional properties, genetic diversity, phenotypic plasticity and high adaptability to heat, drought and other adverse growing conditions. Efforts are made to separate and characterize the compounds of the plant with medicinal properties.
Amaranth grains consist mainly of starch. Grains of *Amaranthus cruentus* contain 48% glutinous starch and grains of *Amaranthus caudatus* constitute of 62% non-glutinous starch, which is a great characteristic, since world population increasingly suffers from gluten-intolerance and alternatives are intensively sought after. The starch of *A. cruentus* has greater swelling power and absorbance capacity than corn and wheat, as well as lower amylase content.
Fat, coming second to starch, in Amaranth grains is significantly higher than in cereals. It is characterized by more than 75% of unsaturated fatty acids (Rastogi and Shukla, 2013).
Linoleic acid making 33% and oleic acid 34% of the unsaturated fat content, followed by 19% palmitic acid and 3.4% stearic acid (Maurya and Arya, 2018).
The protein content in grain Amaranth is higher than in corn and major cereals like maize, wheat and rice. It is comparable to egg protein and is therefore considered as substitute for protein rich meals. Since Amaranth is a very fast growing and cheap plant, it is one candidate to fight malnutrition in low-income regions and the parts of the world, that are most affected by hidden nutrient deficiencies in crops and cereals. High content of Ca, Mg, Fe, K and Zn, have been reported twice as high as in other cereals. In

grains more vitamin C and B2 are present as well. The fibre content in the grains is slightly lower than in wheat, ranging between 19.5–27.9% in *A. cruentus* and between 35.1–49.3% in *A. caudatus.*

Amaranth leaves are also high in unsaturated fats and contain higher amounts of protein than spinach. They are characterized by large numbers of K, Ca, Mg, Zn, Fe, Mn and Ni. Leaves contain levels of vitamin A and C, that make Amaranth a great source to fight vitamin deficiency. Leaves have an average fibre content of 8.39%.
In the whole plant 0.3–0.6% phytic acids are present, having the property to lower cholesterol levels in humans. Saponins are present in very low, inconceivable concentrations and up to 0.8% oxalates and nitrates, that can easily be removed by boiling seeds and leaves for five minutes. Yet, a breeding effort to reduce or eliminate these contents is made (Rastogi and Shukla, 2013).

Amaranthus cruentus is an important agricultural plant in West Africa and India. When growing on Cd contaminated soil, *A. cruentus* accumulates Cd in its tissues. Concentrations in the plant are higher than in the soil. Pb uptake is in line with soil content and leads to toxic concentrations for consumption (Sola et al., 2003).
In pot experiments of Adekunle et al. (2018) with different Pb and Cd concentrations under greenhouse conditions, *Amaranthus cruentus* showed great accumulation and content far above legal limits, without any signs of toxicity for plants.
In a field study in India analysis of *A. cruentus* plants from Cd and Pb contaminated sites showed highly elevated levels of these HMs as well. Translocation factors were above 1 for both HMs in all tested groups (Kamath et al., 2016).

Amaranthus caudatus is an important agricultural plant in West Africa. High concentrations of Zn are observed, as well as decreased Cd content, when elevated levels of Zn are present in contaminated soil due to its similarity. In the study of Abdu (2010) mean of Zn content was higher in edible parts than it was in roots for all collection sites, except for one. Great uptake of Cu and Cd was reported in former studies as well. Uwah et al. (2011) report TF >1 for PB in *A. caudatus*.

The FFTC (Food and Fertilizer Technology Centre), an ”international information centre for small-scale farmers in Asia” together with the Taiwan Livestock Research Institute, Heng-Chun Branch Institute, carried out a study to investigate the amount of HMs taken out of contaminated soils by eight different species, among them *Amaranthus caudatus*. They concluded that Red Amaranth (*Amaranthus caudatus*) can remove >200 µg Zn per g dry weight and 252 µg Ni per g dry weight, but no details of this study were given.

2.9.2. Bread Wheat (*Triticum aestivum*)

Triticum aestivum, a hexaploid species in the *Triticum* genus (*Poaceae*) is an important cereal grain plant, commonly known as bread wheat. Bread wheat was fostered to adapt to different environments i.e. via time of flowering etc. Beside many other adaptations to dry climate or to certain diseases, distinction of spring wheat and winter wheat is very common. Spring wheat is especially fit for regions with severe winters and flowers in the same year as grains are harvested. Winter wheat on the other hand, is adapted to milder winters, yet requires a cold treatment and needs to be planted in fall, yielding then takes place in spring the following year. Today a lot of efforts is put in breeding varieties, which are characterized by winter hardiness, resistance to a vast range of biotic and abiotic stress, grain quality traits, plant height, harvest ability and many more (CFIA, 2014).

With a yearly production of 600 Million tonnes worldwide, a number increasing with every year, the importance of wheat as one of our staple foods becomes evident (Ponce-García et al, 2016).
Together with rice and maize it provides two third of 90% of the world´s food energy intake, provided by edible plants (Rutledge et al., 2011).

In 2017 in the European Union 309.9 million tonnes of cereal grains have been produced, wheat being on first place with 142.6 million tonnes, followed by only half as much green maize and CCM with 64.7 million tonnes (Da Silva et al., 2018).

This highlights the possible impact micronutrient dense staple crops could have in battling malnutrition by investing in biofortification. Positive attempts have already been made. To name one example, Shabnam Shaikh and Meenu Saraf (2017) estimated that uptake of Zn and Fe in grain can be significantly increased by adding different bacteria. In one study they observed a six-fold enhancement in Gw-366 variety wheat and a three-fold enhancement in LK-1 variety wheat of Zn and Fe content (in addition to other growth promoting effects) by inoculating plants with MS-ZT10 bacteria strain
(Sheikh and Saraf, 2017).

2.9.3. Alpine Pennycress (*Thlaspi caerulescens*)

Thlaspi caerulescens, commonly known as Alpine Pennycress or Alpine Penny-grass, is a key model species for hyperaccumulators. Accumulation for Zn, Cd and Ni has been reported for different populations. Populations can be found throughout Northern Europe on both contaminated and uncontaminated grounds. Molecular analysis did not find any variations between populations growing on different soils regarding HM contamination, justifying a distinction of subspecies according to location (properties). Nonetheless, metallous populations showed no difference in growth performance, independent of soil properties in respect of HMs, yet non-metallous populations performed worse with increasing HM content. This is a phenomenon that has not been found in the hyperaccumulator *Arabidopsis halleri*, where little difference in tolerance between populations is found, but not depending on HM content of original location. Another finding of the comparative studies is, that hyperaccumulation occurs in all populations independent of HM content, yet tolerance varies, indicating that these two traits are independent of each other (Macnair, 2007).

In a study by Richau et al. (2008) four *Thlaspi caerulescens* populations (LC, SF, LE, MP) were compared and different accumulation patterns of Ni observed, most likely due to properties on the locations where plants have been taken from. LC and SF were soils contaminated with Pb, Cd and Zn, LE is a population from a non-metalliferous location and MP population grows on a Ni-enriched serpentinite soil. Ni content increased in the following order: LC <SF <LE <<MP. In LC and SF Ni accumulated mainly in the root tips and less in the mature root parts, whereas in MP it accumulated equally and even more so in the mature root segments. 250 µM Ni caused staining, when treated with dimethylglyoxime, in the root cap and in the rhizodermis of the meristematic zone in MP and LC. In MP Ni was also detected, especially in the rhizodermis and cortex of the elongation zone and most intensely in the rhizodermal and cortical cells of the root hair zone of the root.

When Küpper et al. (1999) looked at the sequestration of Zn in the leaves, Zn concentration in epidermal vacuolar sap was around 6-times higher, than in vacuolar sap of mesophyll. Mesophyll cells showed tolerance to concentrations higher than 60 mM Zn in their sap, but Zn was primarily sequestered in epidermal vacuoles.

Scientists are eager to find the mechanisms, that enable hyperaccumulators to accumulate HMs. Besides increased densities of absorption sites on root surfaces, enhanced internal metal transporters and release of phytohormones, the study of Haines (2002) shows that *Thlaspi caerulescens* has the ability of root foraging. It was not only observed, that this hyperaccumulator is able to accumulate the same amount of Zn when it is unevenly distributed in the soil, but it was suggested that patches of different concentrations can be distinguished. Plants growing in soil with Zn patches grow remarkably larger than plants in pots with same concentrations but homogenous distribution. Both plants however have the same Zn content, so Zn amount is not considered as a reason for this phenomenon. The most important factor aiding in

nutrient uptake is root density. Hence, the increase in root surface through root foraging for Zn patches, may has the side effect of increased nutrient uptake, which results in larger plants. Metal foraging results in increase of root length, root hair length and increase in root branching (Haines, 2002).

Using *Thlaspi caerulescens* as a plant for phytoremediation is an idea that occurred in the 1980s. In pot experiments by Robinson et al. (1997), contaminated soil from an old mining site, containing high amounts of Zn and Pb associated with Cd and thallium, as well as wild plants from the same area to study the accumulation rates, were used. They found that the content of HMs Zn and Cd was 1.16% and 0.16% respectively, which would mean a removal of 60 kg Zn and 8.4 kg of Cd per hectare by one crop. Bioaccumulation is higher for Cd than for Zn, and the accumulation coefficient increases with decreasing HM concentrations in the soil. They propose, remediation is possible for Cd in lower concentrations, as HM contents were significantly higher in the plants than in the soil. For Zn however, soil contents are too high to progress fast enough, and plants take up only a very tiny fraction of these amounts (Robinson et al., 1997).

Newer studies concluded, whether plants are suitable remediation candidates, depends on different factors. First a population must be chosen, which possess both the ability to tolerate high concentrations of HMs and good accumulation quality. The population used in this study, as well as other successful studies before, like Haines (2002), is the “Prayon” population in Belgium, which grows on an old Zn/Cd smelter site, or the “Viviez” population on a Zn/Cd smelter in France. It has been shown that the amount of HM uptake greatly depends on the bioavailability of the HMs in the soil. Growth of *Thlaspi caerulescens* and accumulation ability can be significantly improved with N fertilization, addition of sludge and other mineral or organic fertilizer (Schwartz et al., 2003).

3. Material and Methods

3.1. Invitro Cultures

Goal of the invitro culture experiments was the establishment of HM concentrations, assessment of suitability of plant species and determination of usability of Newport Green™ DCF indicator for HMs localization in plant cells as well as determination of the effect of HM treatment and application of FA on root length, the distance between root tip to first root hair and maximum root hair length.

3.1.1. Preparation of Petridishes and Seeds

1% agar growth medium was prepared in Erlenmeyer flasks containing 2.5 g of agar (Agar-Agar, BioScience, BioScience-Grade, pulv.; Carl ROTH GmbH+Co.KG, density = 0.55), 250 ml double-distilled water and 0.5 MS (Murashige-Skoog, without HMs, to provide nutrients). For the Zn treatment 1 mmol of zincsulfate-heptahydrat (EMSURE® Merck Chemicals) was added, for the Ni treatment 1 mmol of nickel(III)-sulfate hexa/heptahydrate (Aldrich Chemistry, DIN 50970 H, for Ni plating) and nothing was added for the control. The medium was then autoclaved and stored at room temperature.

Seeds of *Triticum aestivum L.*, *Amaranthus cruentus*, *Thlaspi caerulescens, Thlaspi goesingense* and *Saxifraga stellaris* were soaked in distilled water overnight and stored in the fridge (4°C). For sterilization the seeds were put in 70% ethanol (incompletely denatured) for 2–3 minutes, followed by ten minutes in a solution of 50% natriumhypochlorit (DenkMit, 3.6 g NaClO/100 g), 50% sterilized distilled water and a few drops of TritonX-100, to reduce surface tension. Seeds were then transferred into sterilized water and rinsed five times.

To reduce contamination the sterilization of the seeds as well as the following steps were done in the sterile bench.

To liquidate agar medium, Erlenmeyer flasks were put in the microwave and petridishes filled with 30 ml each. 25 petridishes per treatment (Ni, Zn, C) were prepared.

Ten (*Triticum*) to 44 (*Saxifraga*) seeds were put on one petridish and sealed with parafilm. Per treatment and plant species five petridishes were prepared.

Petridishes were put in the fridge (4°C) and stored for two days for stratification. Then petridishes were kept at room temperature under artificial light. After germination petridishes were placed into an angle (approximately 50°) for the roots to grow ± parallel to the surface to ease examination of roots and root hairs (Fig.1). (Except the Zn-containing agar plates. They were not completely solid. In other experiments we tried 2% agar for Zn, which resulted in the same consistency as 1% agar for Ni and the control.)

3.1.2. Germination Rate

Germination rate was determined seven days after sowing, by calculating the ratio:

$$\frac{Seeds\ germinated}{Seeds\ total}.$$

3.1.3. Documentation

Every Week pictures were taken of the whole plate to document growth using a camera (Nikon J1, AF Mikro Nikon 60 mm 1:2.8 D Objective), as well as detailed pictures of the seedlings and roots using a Stereomicroscope (Nikon SMZ1500 with Nikon DS-Ri2 camera) and Nikon ECLIPSE Ni (with Nikon DS-Ri2 camera and Nikon intenselight C-HGFI Fluorescence equipment) for root anatomy (Fig.2).

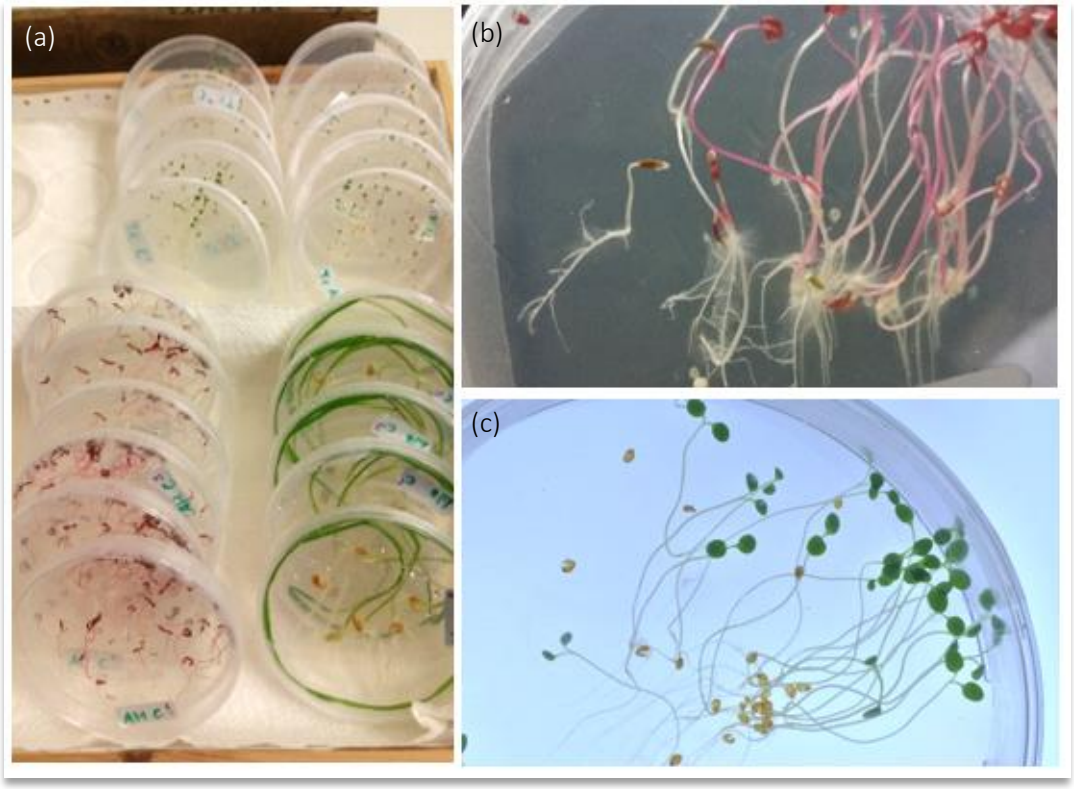


Fig. 1: (a) Petridishes kept under artificial light; close-up of petridishes with (b) Amaranthus cruentus *and (c)* Thlaspi caerulescens

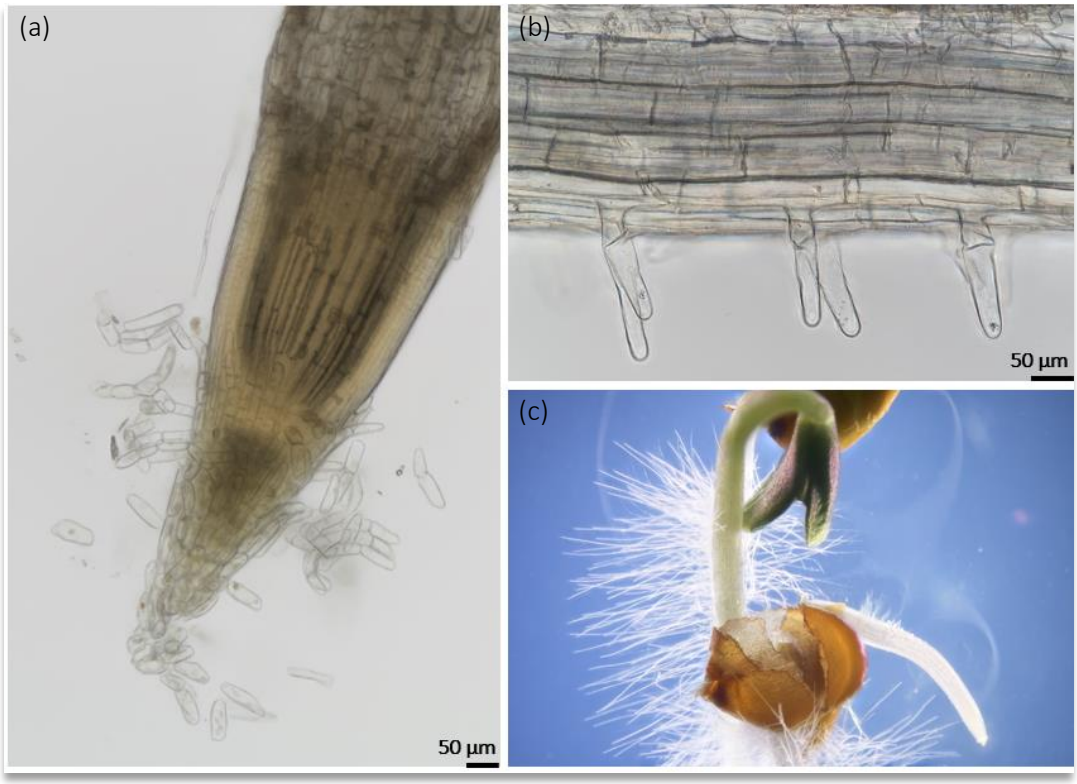


Fig. 2: (a) Root tip of Triticum aestivum *(Nikon eclipse)*
(b) Root hairs of Thlaspi caerulescens *(Nikon eclipse)*
(c) Seedling of Thlaspi caerulescens *(Stereo)*

3.1.4. Treatment with Fulvic Acid

11 days, 17 days and 24 days after sowing, 2 ml of fulvic acid in two concentrations (1:500 = 0.2% and 1:100 = 1%) was added using a syringe (Terumo®, non-pyrogenic) with attached filter (sartorius stedim biotech, Minisart®, single use filter unit, sterile, non-pyrogenic, pore size 0.2 µm). 2 ml of distilled water was added to the control.

The FA used in the experiments was provided by Ing. Nikolaus Foidl. Nikolaus Foidl invented the following procedure to produce FA:

1) Torrefaction of the biomass (wood materials without bark) by heating it to 220–230°C and keeping it at this temperature for three hours to ensure complete degasification.
2) The remains are grinded to a size of 4–10 µm.
3) Then it is cooked in a 25% mixture of HNO_3, H_2SO_4 and H_3PO_4 for three hours at 160°C under reflux and finally filtered. The liquid is the first fraction of fulvo acid (further referred to as fulvoacid1).
4) The solid remains in the filter are cooked at 160°C with KOH at pH 13–14 for three hours under reflux and filtered again. The remains in the filter are water-insoluble humic substances.
5) The dark brown to black liquid is adjusted to pH 1. The precipitating solids are humic acids and the liquid fulvoacid2.
6) Fulvoacid1 and fulvoacid2 are mixed and make up the fulvo part of the final fulvic acid.
7) The humic acid (precipitating solids of the pH 1 solution in step5) is readjusted to pH 7 to make it water-soluble again. Several salts, including K_2SO_4, nitrate and $CaSO_4$, precipitate at this step in form of a yellow-white solution.
8) Humic acid and fulvo acid are mixed and can now be loaded with different nutrients and salts in soluble state depending on the anticipated use.

3.1.5. Localization of Heavy Metals and Determination of the Usability of Newport Green™ DCF Indicator for Heavy Metals in Plant Cells of *Amaranthus cruentus* and *Thlaspi caerulescens*

To locate depositions of HMs in the plant, seedlings of the untreated (no FA application) plants were taken out of the petridishes, placed on a microscope slide and treated with approximately 125 µl of 5 µM Newport Green™ DCF Indicator. Details of this fluorescent labelling dye can be read in "The molecular Probes® Handbook" (2010). Thresholds (point, where no fluorescence was observed anymore) were determined using fluorescence microscopy (Nikon ECLIPSE Ni with Nikon DS-Ri2 camera and Nikon intenselight C-HGFI Fluorescence equipment, blue excitation, no filters, objective x camera magnification: 10x1, 20x1, 20x2, 40x1, 40x2) after two hours for root tips and root hairs. Slides were put in a moist chamber and stored at 4°C. After 24 hours the same spots were studied using identical settings. Pictures and values were taken for the control plants, as well as Ni (1 mmol) and Zn (1 mmol) treated plants.

3.1.6. Growth Performance

30 days after sowing root length, distance of root tip to first root hair and length of the longest visible root hair were taken, using the measure function of the microscope imaging software NIS-Elements (5.11, 64-bit) on stereomicroscope (Nikon SMZ1500 with Nikon DS-Ri2 camera) and Nikon ECLIPSE Ni (with Nikon DS-Ri2 camera and Nikon intenselight C-HGFI Fluorescence equipment) (Fig.3).

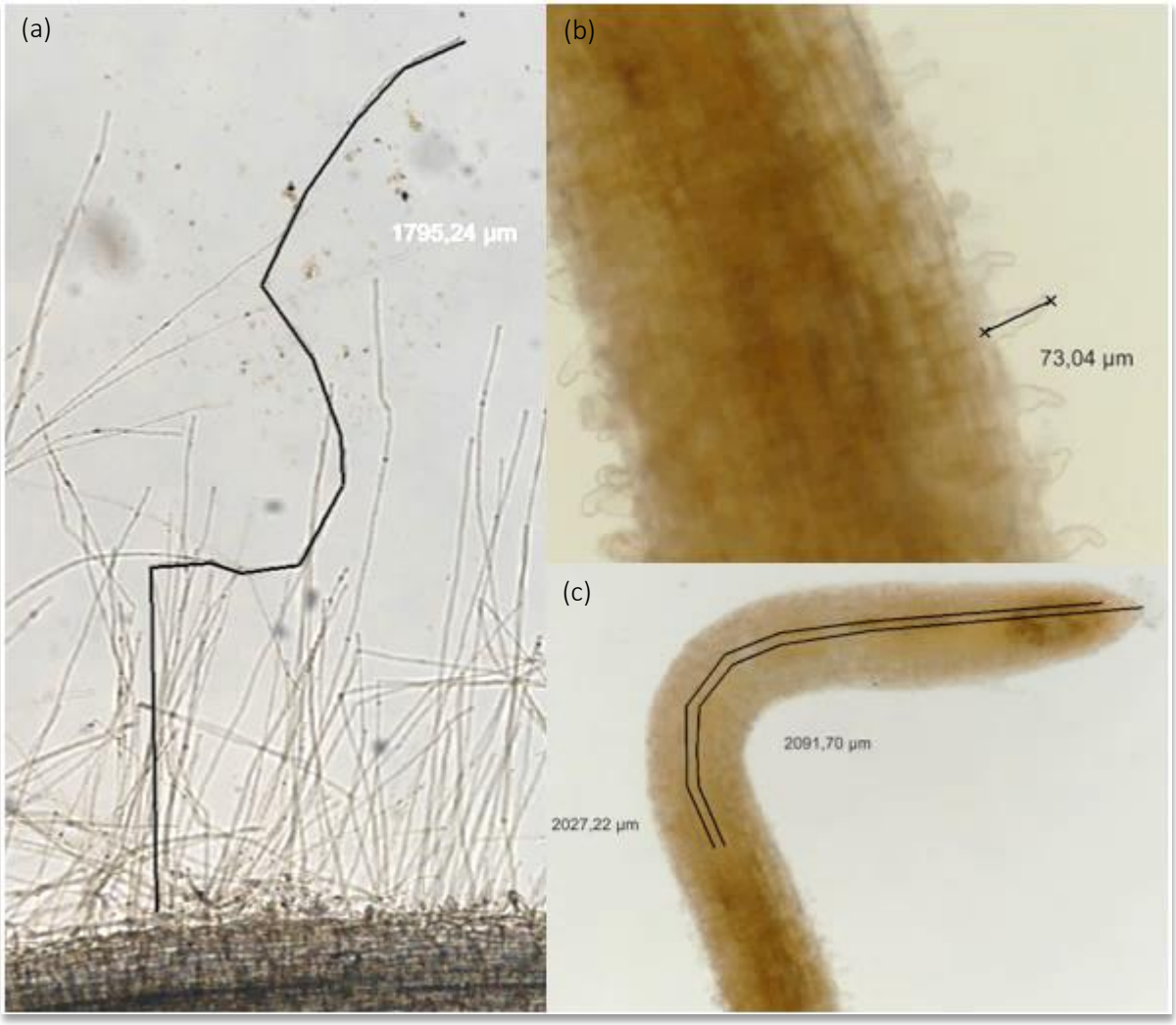


Fig. 3: (a) Maximum root hair length on Amaranthus cruentus *in the control (Nikon eclipse)*
(b) Maximum Root hair length on Amaranthus cruentus *in Ni (Nikon eclipse)*
(c) Root length of Amaranthus cruentus *in Ni (Nikon eclipse)*

3.2. Hydroponics

Goal of the hydroponics experiments was the attainment of higher amount of biomass to examine effect of FA on HM content in plants using ICP, examination of the effects of HM and FA on ROS accumulation and on cell tolerance to HM solutions.

3.2.1. Preparation of Pots and Seeds

135 Pots (Fig.4) were filled with SERAMIS® clay granules and quartz sand.

27 pots were rinsed three times, 30 ml each, with 1 mmol and 0.1 mmol Zn solution (zincsulfateheptahydrat EMSURE® Merck Chemicals), respectively. The same was done with Ni solution (nickel(III)-sulfatehexa/heptahydrate Aldrich Chemistry, DIN 50970 H, for Ni plating).
45 pots were rinsed three times with distilled water (30 ml each, so 90 ml in total per pot).

Ten seeds of *Triticum aestivum L.*, 30 seeds of *Amaranthus caudatus* and 30 seeds of *Thlaspi caerulescens* were soaked in double-distilled water overnight at 4°C and placed on the surface of nine pots for each treatment.
Three pots per plant species were placed together in one box, which was covered with plastic wrap and put under artificial light. See experimental design in Fig.5.

Fig. 4: Measurements of pots used for hydroponics

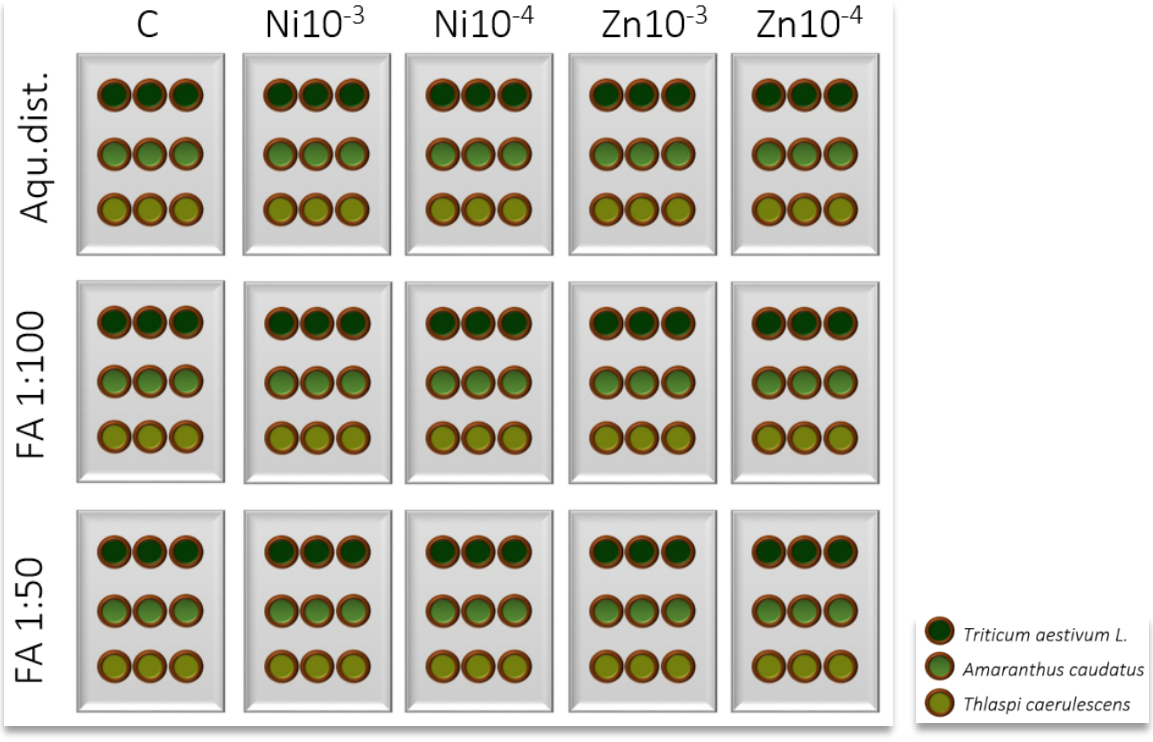


Fig. 5: Experimental design for hydroponics; 10^{-3} = 1 mmol, 10^{-4} = 0.1 mmol; FA = fulvic acid, 1:500 = 0.2%, 1:100 = 1%

Plants were irrigated when needed and approximately 2 ml per pot of FA was sprayed twice a week on the leaves, as soon as the first leaf was seen.

3.2.2. Germination Rate

Germination rate of *Triticum aestivum* was determined 15 days after sowing by calculating the ratio:

$$\frac{Seeds\ germinated}{Seeds\ total}$$

Apart from five plants, seedlings were then removed from the pots and stored for ROS dyes.

3.2.3. Measuring Abiotic-Stress-Induced Increase of ROS in Plant Organs using NBT and DAB

In unfavourable conditions plants accumulate reactive oxygen species (ROS), a by-product of the aerobic metabolism, which can be damaging to lipids (plasma membranes!), nucleic acids (DNA!) and proteins. This causes cell damage and eventually leads to cell death. Increased ROS also activates stress response pathways and therefore can be considered an indicator for (abiotic)stress (Gill and Tuteja; 2010).

3,3'-diaminobenzidine (DAB) and nitrotetrazolium blue chloride (NBT) can be used for the histochemical detection of hydrogen peroxide (H_2O_2) and superoxide (O_2^-), two of the most important ROS in plant cells. Accumulation of H_2O_2 can be visualized using DAB, which is oxidized by hydrogen peroxide and produces a reddish-brown precipitate in presence of haem-containing proteins e.g. peroxidases. Increase of O_2^- can be shown as blue staining, caused by the dark blue formazan-compounds, that result of superoxide reacting with NBT (Kumar et al., 2014).

The protocol of Kumar et.al (2014) was followed to prepare the NBT and DAB solutions. Whole plants (shoots and roots) as well as roots only were put in centrifuge-tubes and covered with DAB or NBT staining solution. Tubes were stored on a rag and put into a dark box overnight at room temperature. On the next day seedlings were removed and washed with distilled water. NBT stained plant materials were directly photographed. DAB stained plant materials were immersed in 70% ethanol and heated in boiling water to remove chlorophyll. Pictures were taken as well (Fig.6).

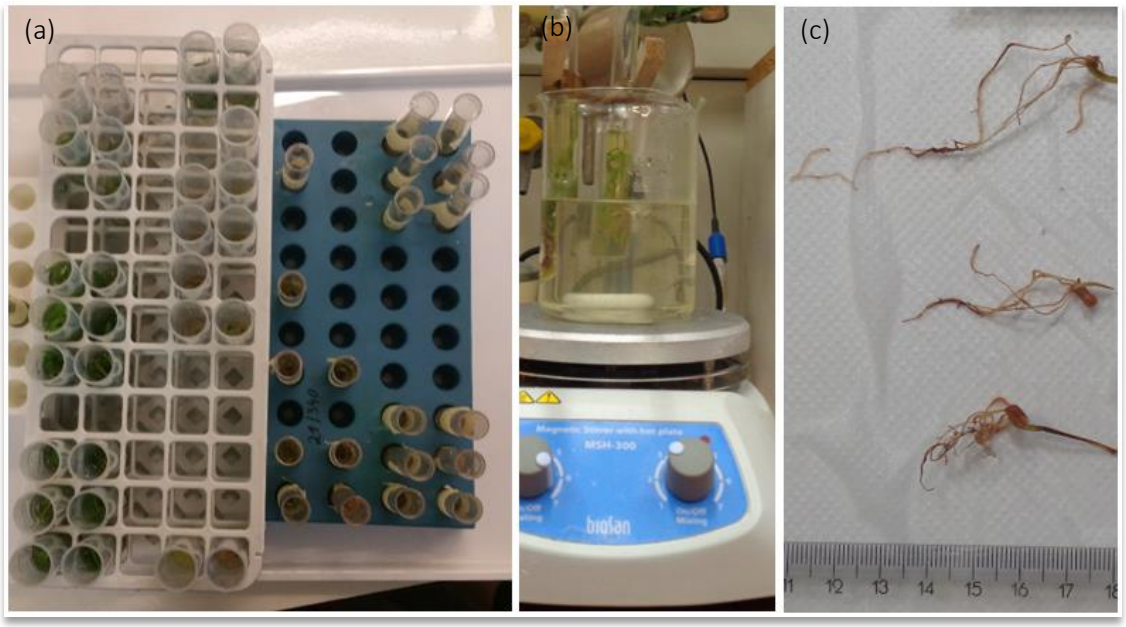


Fig. 6: (a) Triticum aestivum *in DAB and NBT solutions*
(b) Seedlings immersed in 70% ethanol and heated in boiling water to remove chlorophyll
(c) Comparison of the control, Zn and Ni roots dyed after DAB staining and remove of chlorophyll

3.2.4. PEAS Chlorophyll Fluorimeter

33 days after sowing, chlorophyll fluorescence was measured using Handy PEAS Chlorophyll Fluorimeter (Serial Number 2728, Hansatech instruments LTD.). The Handy PEAS is a continuous excitation fluorimeter, which measures the Kautsky Induction, also known as Fast Chlorophyll Fluorescence Induction.

Plants have limited capacity to use the absorbed light energy in their photochemical processes. The amount used is (among other factors) depended on the stress the plant is subjected to. Part of the excessive light energy is re-emitted in form of longer wavelengths in the red to infra-red region, which is referred to as chlorophyll fluorescence. To measure the maximum photochemical efficiency, leaves are darkened with clips, which eventually leads to activation of the reaction centres of the Photosystem II (PSII). When suddenly illuminating the same spot with a very high intensity light a quick rise in chlorophyll fluorescence followed by a slow decline and eventually stabilization of the signal can be measured. This effect is called "Kautsky induction". Handy PEAS measures *Fo* (Fluorescence Origin) and *Fm* (Fluorescence Maximum) and uses these two values to calculate *Fv* (Variable Fluorescence). The ratio *Fv/Fm* is used as indicator for the maximum efficiency of PSII, highly sensitive to stress and therefore used as an indicator for it.

Clips were put on the upper side of the leaf and darkened for 20 minutes. Then measurements were taken. One measurement per pot, which equals three measurements per treatment, were taken.

3.2.5. Growth Performance

On the 34th day after sowing, plants were taken out of the pots, rinsed with distilled water and maximum root length was determined, as well as pictures taken.

Two seedlings were set aside for cell physiology examinations. The rest was split into roots and shoots and put in the drying cabinet at 45°C for four days.

Dry weight in g was then determined using Sartorius CPA2250 (max. 220 g, d = 0.01 mg (100 g)).

3.2.6. Cell Physiology-Determination of Cell Vitality of Wheat Leaves Subjected to HM Stress by Plasmolysis and Deplasmolysis

Several sections of the leaves were put in Ni-solution (10^{-1} =100 mmol, 10^{-2} =10 mmol, 10^{-3} = 1 mmol, 10^{-4} = 0.1 mmol, 10^{-5} = 0.01 mmol, 10^{-6} = 0.001 mmol), Zn-solution (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}) and in water, covered and stored for 48 hours at room temperature without light exposure to prevent photosynthesis.

Sections were then put on a slide, covered with 0.8 mol mannitol and examined after ten minutes. Cells were checked for plasmolysis. If >50% cells showed plasmolysis, section was considered alive, if the number of cells was <50%, section was considered dead. If not sure, deplasmolysis was attempted.

3.2.7. Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES)

Dried plant material was digested using aqua regia (AR) and diluted in double-distilled water, amount of AR was depending on dry weight of the probe (Tab.3).

dry weight	aqua regia	water
50 mg	1 ml	15 ml
300 mg	9 ml	50 ml

Tab. 3: Composition of the probes for ICP

Probes were then filtered twice (Filter Papers Macherey-Nagel, MN616, Ø150 mm) and 15 ml were decantated in final tubes. For ICP-measurement probes with concentrations too high were diluted until the amount of AR was below 7% (Fig.7).

Measurements were taken by Dipl. Ing. Lenitz Herwig, MA (University of Vienna, Department of Environmental Geosciences) using 5110 ICP-OES (Agilent Technology).

Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) is used to determine the concentrations of elements in a liquid. The sample is heated and transforms into hot gases, containing free atoms and ions of elements, which leads to collisional excitation and ionization. When dropping back to ground state, atoms and ions emit light. Each element emits characteristic wavelengths, allowing us to determine the composition of the probe. Furthermore, intensity of the light emitted at the specific wavelength is detected as well. Intensity is correlated to concentration of the element in the probes. The higher the concentration, the higher the intensity (Fitzsimmons, 2015).

In this work a blanc (Standard 0) and seven standards (Standard 1–7) with known concentration (0, 0.1, 0.05, 0.1, 0.5, 1, 5 and 10 mg/L) were measured for each element of interest.

To analyse data, Microsoft® Excel® Office 365 was used. Concentration curves were established, and calibration charts created, following these steps: A scatter plot or point chart was created using the concentrations of the standards, as independent values and intensity of the element in these standards, as depended values. Then trendline was created using a second-degree polynomial function, with the general formula: $y = ax^2 + bx + c$. To determine concentrations in mg/L, the following calculation has been done:

$$Concentration_{[mg/L]} = \frac{-b + \sqrt{b^2 - 4 \cdot a \cdot \left(c - Intensity_{[nm \cdot c/s]}\right)}}{2 \cdot a}$$

To determine the concentration of the elements of interest in the original biomass in mg/kg, the following calculation has been done:

$$\frac{Concentration\ [mg/L]\ \cdot\ End\ Volume\ [ml]}{Sample\ Weight\ [g]}$$

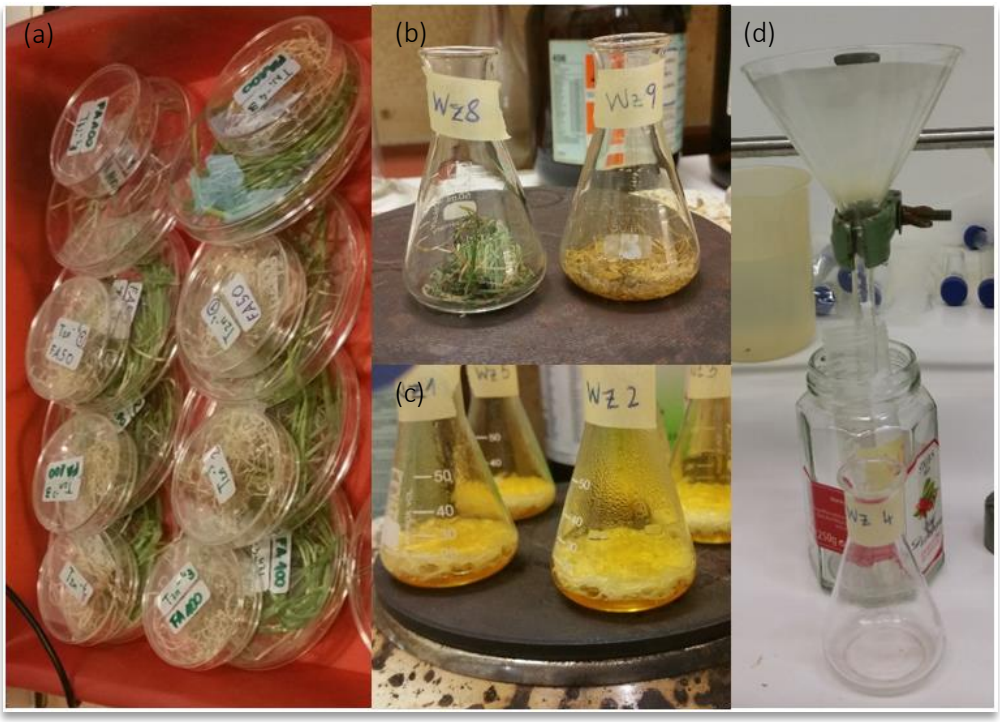


Fig. 7: (a) Dried plant material of Triticum aestivum *in petridishes*
(b) Plant material prepared for digestion, (b) aqua regia added and (c) filter process

3.3. Soil Cultures

Goal of the soil cultures experiment was to create a more realistic setting to see potential effects of FA on plants under HM stress, obtain more repeats to run statistical analysis and determine significant differences between the treatment groups. See experimental design in Fig.8.

3.3.1. Preparation of Pots and Seeds

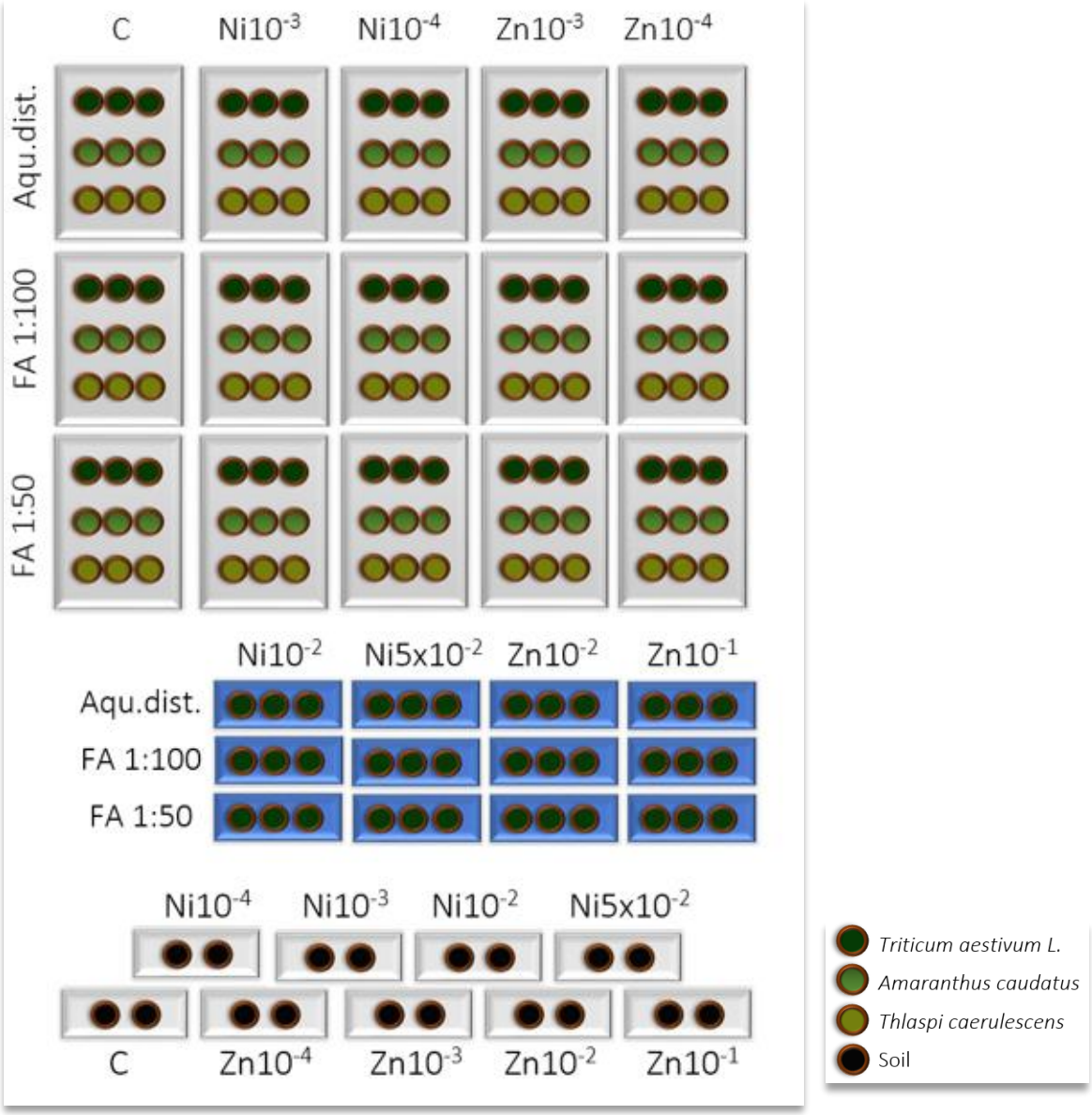


Fig. 8: Experimental design for soil cultures. Each pot contains either five seeds of Triticum aestivum, *ten seeds of* Amaranthus cruentus *or ten seeds of* Thlaspi caerulescens. *HM concentrations are: 10^{-1} = 100 mmol, 5*10^{-2} = 50 mmol, 10^{-2} = 10 mmol, 10^{-3} = 1 mmol and 10^{-4} = 0.1 mmol FA = fulvic acid in different concentrations: 1:100 = 1% and 1:50 = 2%*

135 Pots (same as used for hydroponics) were filled with a mixture of seven parts potting soil (Balkon- und Blumenerde, Kultursubstrat-Substratgruppe II, Franz Kranzinger GmbH, consisting of white peat, bark humus, Original Toresa® wood fibre and clay minerals; pH 5–6.5 and salt content below 3 g/l), two parts perlite (Premium Perlite, Gramoflor) and one part lawn quartz (Min2C, Natural Minerals, fire-dried, grainsize 0.5–2.0 mm). 27 pots were rinsed three times, 30 ml each with 1 mmol or 0.1 mmol Zn solution (zincsulfateheptahydrat (EMSURE® Merck Chemicals)) respectively. The same was done with Ni solution

(nickel(III)sulfatehexa/heptahydrate (Aldrich Chemistry, DIN 50970 H, for Ni plating)) and 45 pots were rinsed three times with distilled water (30 ml each, so 90 ml in total per pot) (Fig.9).

Fig. 9: (a) Pots filled with soil waiting to be watered with HM solution
(b) Pots rinsed three times with 30 ml of HM solution each, before putting seeds in soil

Five seeds of *Triticum aestivum L.*, ten seeds of *Amaranthus caudatus* and ten seeds of *Thlaspi caerulescens respectively,* were soaked in double-distilled water overnight at 4°C and placed in nine pots each treatment, using tweezers for wheat and a pipette for the other plant seeds.

Additionally, three pots per treatment (Adest, FA100 = 1:100 = 1%, FA50 = 1:50 = 2%), i.e. nine pots each HM concentration, were prepared for Ni 10 mmol and 50 mmol and for Zn 10 mmol and 100 mmol, respectively. They were rinsed three times, 30 ml each, with the HM solution, and five wheat seeds were put in each pot.

Pots with the same treatment were put in one box, which then was covered with plastic wrap and placed under artificial light.

For soil analysis two pots, each HM and concentration, were prepared and handled in the same way as the distilled water treated pots.

As soon as the first leaves were visible, pots were moved outside and two weeks later into the greenhouse (minimum temperature 20°C, maximum temperature 28°C, minimum humidity 40%, maximum humidity 65%) and treated with FA. Approximately 2 ml FA per pot were sprayed on leaves.

Plants were watered when needed (approx. every second day 20–30 ml/pot) and FA was applied three times a week. Over the course of the experiment, fertilizer was added thrice (first time 0.1%, second and third 0.2%, 40 ml), using WUXAL® super (NPK-Fertilizer with 8% N, 8% P_2O_4 and 6% K_2O, pH 6–6.5) and plants were watered thrice with HM solutions. (30 ml each; 4–6 days after WUXAL® application).

Throughout the growing period several pictures were taken (Fig.10).

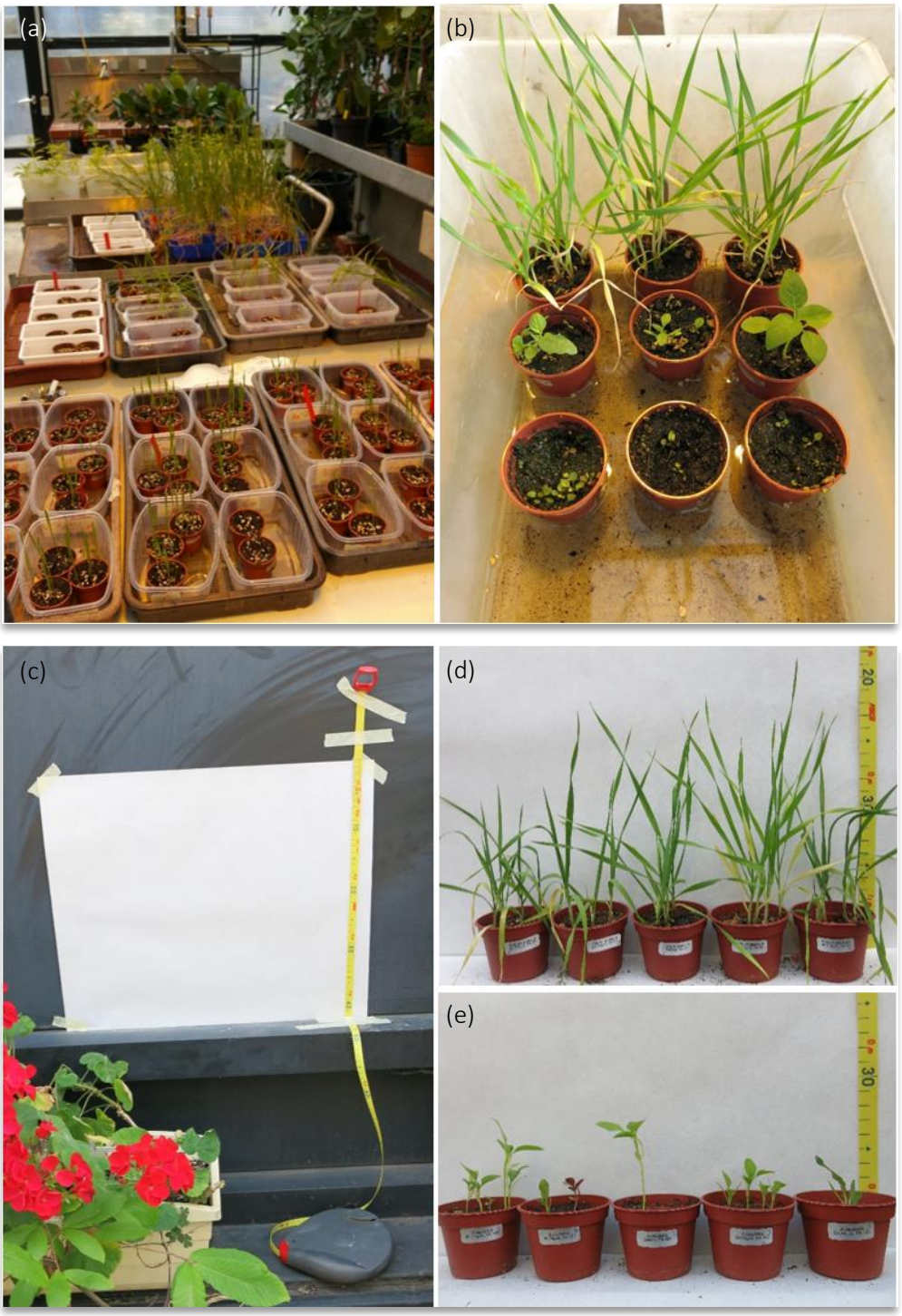


Fig. 10: (a) Boxes with the different pots in greenhouse
(b) picture of one box with Triticum aestivum, Amaranthus caudatus *and* Thlaspi caerulescens
(c) documentation of setting for progress-photographs, pictures to document intermediate status of growth performance of (d) wheat and (e) Amaranth

Nearly two weeks after germination mildew (Fig.12) was observed on the leaves and close-up pictures were taken with the stereomicroscope. Mildew disappeared a few days later and did not cause any problems. Aphids (Fig.13) on *Triticum aestivum* were always removed and on *Amaranthus caudata* aphids and thrips (*Thysanoptera*) (Fig.11) were removed only at the beginning and several pictures were taken with the stereomicroscope (Nikon SMZ1500 with Nikon DS-Ri2 camera). On thrips invaded Amaranth, bitemarks were visible on leaf tips. Butterfly scales (Fig.14) indicate, that plants were probably visited by more animals. As soon as aphids were left alone on Amaranth, no aphids were observed on wheat anymore.

*Fig. 11: Thrips (*Thysanoptera*) on leaves of* Amaranthus caudatus *(Stereomicroscope)*

Fig. 12: Mildew on Triticum aestivum *(Stereomicroscope)*

Fig. 13: Aphids on (b) Triticum aestivum *and (a, c, d)* Amaranthus caudatus

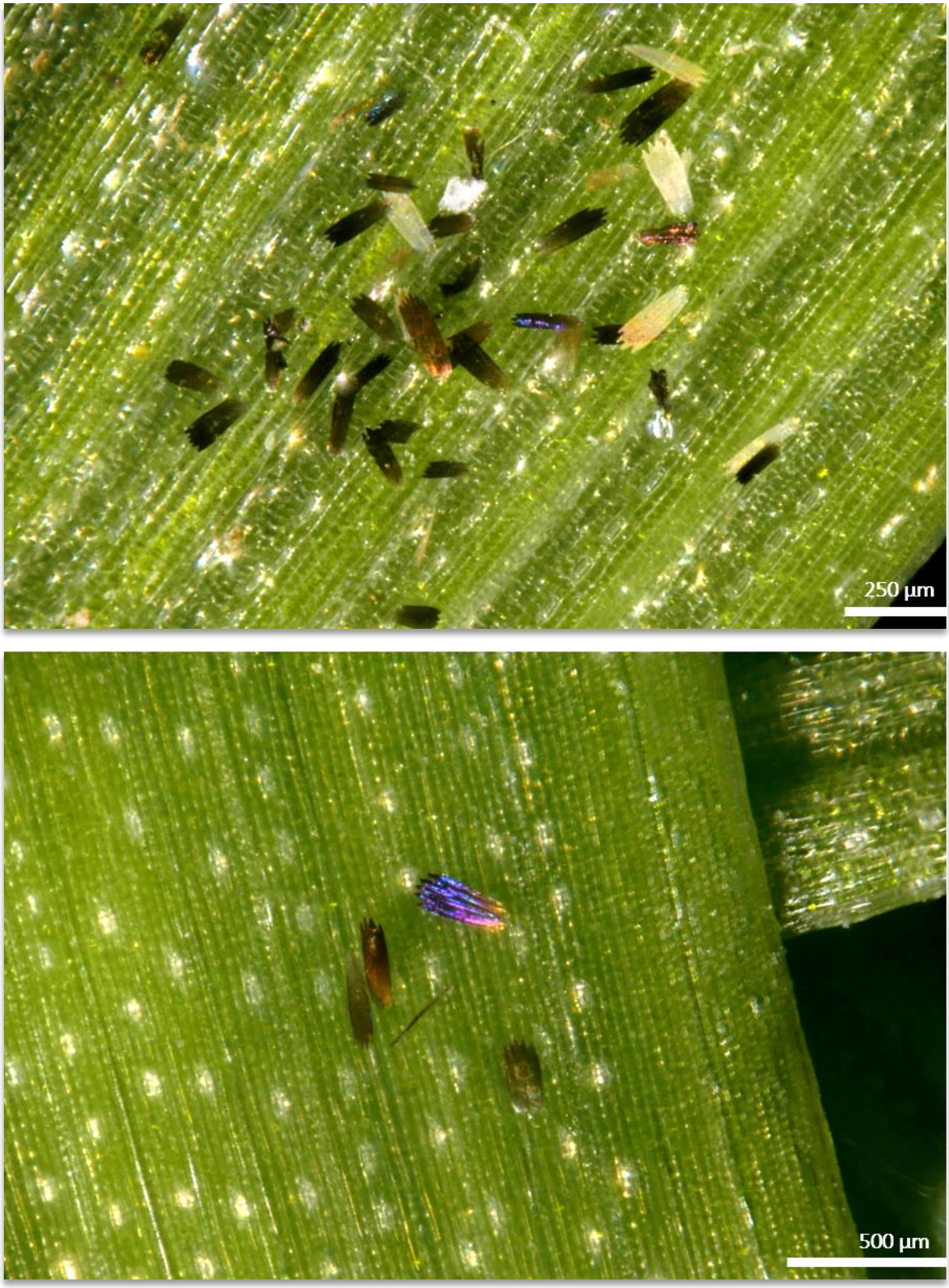


Fig. 14: Butterfly scales on Triticum aestivum *(Stereomicroscope)*

3.3.2. Germination Rate

Germination rate was determined 15 days after sowing, by calculating the ratio:

$$\frac{Seeds\ germinated}{Seeds\ total}.$$

3.3.3. PEAS Chlorophyll Fluorimeter

35 days after sowing chlorophyll fluorescence was measured using Handy PEAS Chlorophyll Fluorimeter (Serial Number 2728, Hansatech instruments LTD.). Clips were put on the upper side of the leaf and darkened for 20 minutes. Then measurements were taken. One measurement per pot, which equals three measurements per treatment, were taken.

3.3.4. Growth Performance

On the 39th day after sowing plants were taken out of the pots, rinsed with distilled water, roots detangled and maximum root length and number of leaves determined, as well as pictures taken (Fig.15).

One plant was set aside for cell physiology examinations. The rest was split into roots and shoots and put in the drying cabinet at 45 °C for four days. Soil was put in the drying cabinet at 45 °C for 14 days.

Dry weight in g was then determined using Sartorius CPA2250 (max. 220 g, d = 0.01 mg (100 g)).

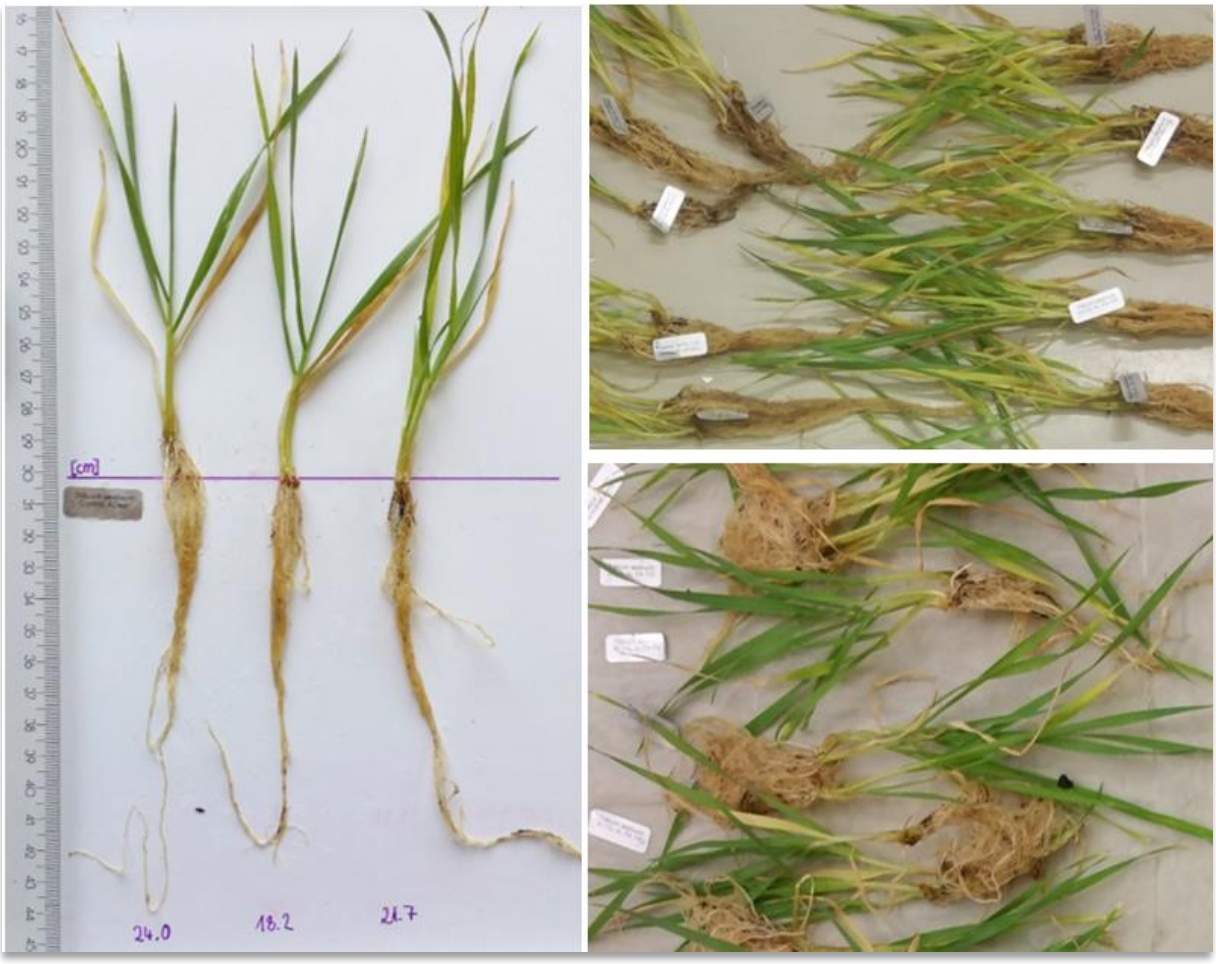


Fig. 15: Triticum aestivum *root length picture, wheat rinsed and detangled*

3.3.5. Cell Physiology-Determination of Cell Vitality of Wheat Leaves Subjected to HM Stress by Plasmolysis and Deplasmolysis

Several sections of the leaves were put in Ni-solution (1, 10^{-1} = 100 mmol, 10^{-2} = 10 mmol, 10^{-3} = 1 mmol, 10^{-4} = 0.1 mmol, 10^{-5} =0.01 mmol, 10^{-6} = 0.001 mmol), Zn-solution (1, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}) and in water, then covered and stored for 48 hours at room temperature, without light exposure to prevent photosynthesis.

Sections were then put on a slide, covered with 0.8 mol mannitol and examined after ten minutes. Cells were checked for plasmolysis. If >50% cells showed plasmolysis, the section was considered alive, if the portion of cells showing plasmolysis was <50%, section was considered dead. If not sure, deplasmolysis was attempted.

3.3.6. Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES)

Dried plant material was digested using aqua regia (AR) and diluted in double-distilled water, amount of AR was depending on dry weight of the probe (Tab.4).

dry weight	aqua regia	water
0.5 g	3.5 ml	50 ml
0.2 g	1 ml	15 ml
in between	2 ml	33 ml

Tab. 4: Composition of probes for ICP

For the digestion of the soil, dried soil was sieved using a 2 mm sieve and then a 1 mm sieve. Following the described procedure in Chen and Ma (1999), 500 mg were digested on a heat plate in 12 ml AR and then diluted with 20 ml 2% HNO_3, filtered (Filter Papers Macherey-Nagel, MN616, Ø150 mm) and water added. To ensure a clear solution, 15 ml were decantated in 15 ml centrifuge tubes for the ICP.

Measurements were taken by Dipl. Ing. Lenitz Herwig, MA (University of Vienna, Department of Environmental Geosciences using 5110 ICP-OES (Agilent Technology).

In this work a blanc (Standard 0) and seven standards (Standard 1–7) with known concentration (0, 0.1, 0.05, 0.1, 0.5, 1, 5 and 10 mg/L) were measured for each element of interest.

To analyse data Microsoft® Excel® Office 365 was used. Concentration curves were established, and calibration charts created, following these steps: A scatter plot or point chart was created using the concentrations of the standards as independent values and intensity of the element in these standards as depended values. Then trendline was created using a second-degree polynomial function with the general formula: $y = ax^2 + bx + c$. To determine concentrations in mg/L, the following calculation has been done:

$$Concentration_{[mg/L]} = \frac{-b + \sqrt{b^2 - 4 \cdot a \cdot \left(c - Intensity_{[nm \cdot c/s]}\right)}}{2 \cdot a}$$

To determine the concentration of the elements of interest in the original biomass in mg/kg, the following formula has been used:

$$\frac{C\ [mg/L]\ \cdot\ End\ Volume[ml]}{Sample\ Weight\ [g]}$$

Content of Ni and Zn in soil, root and shoot were used to calculate the bioconcentration factor (BF), ratio for HM uptake, describing transport from soil to root: $\frac{HM\ Content\ in\ Root}{HM\ Conten\ in\ Soil}$ and translocation factor (TF), describing translocations from HM within the plant: $\frac{HM\ Content\ in\ Shoot}{HM\ Content\ in\ Root}$.

3.4. Statistical Analysis

IBM SPSS Statistics 24 Win. was used to process and evaluate all data.

Two Way ANOVA was used to test influence of HMs treatment and FA treatment on several continues variables such as root length, HM content, distance between root tip and first root hair and maximum length of the root hair.

Levene´s Test of Equality of Error Variances as well as F-Test for Heteroskedasticity was used to determine, if variance was homogenous. When homogeneity-criterion was not met, violation was noted with results presented.

To look further into the nature of the significant main effects, Bonferroni Post-hoc tests or Dunnett's T_3 Post-hoc tests (when variance was inhomogeneous, when tested for effect of one factor between different groups) were carried out. Power of effect size was examined using the value *f*.

$$f = \sqrt{\frac{\eta_p^2}{1 - \eta_p^2}}$$

Cohen (1988) considers $f = 0.10$ a small effect size, $f = 0.25$ a medium effect size and $f = 0.4$ a strong effect size.

For interpretation of significant interactive effects Test of Simple Effects with LSD Test (Fisher´s Least Significant Differences) was performed, as described in IBM Support.[1] Furthermore, when Bar charts showed noticeably differences between FA treatment within a HM group, the same was done as well.

Tables containing descriptive statistics were copied from SPSS Statistics Viewer.

[1] Significant interaction in ANOVA: how to obtain a Simple Effects Test (IBM Support, Document Number 417465, Historical Number 20486, https://www.ibm.com/support/pages/significant-interaction-anova-how-obtain-simple-effects-test; 10.February.2020, 07:54 Central European Standard Time)

4. Results

4.1. In Vitro Cultures

Seeds of *Triticum aestivum L., Amaranthus cruentus*, *Thlaspi caerulescens, Thlaspi goesingense* and *Saxifraga stellaris* were grown in petridishes containing 1% agar with 0.5 MS for the control group and 1 mmol Ni or 1 mmol Zn were added for HM containing groups. Five petridishes each (except for *Triticum aestivum*, only one plate for Ni and two for Zn) were prepared and later FA (FA100 = 1:100 = 1% and FA50 = 1:50 = 2%)) or Adest was added two times in total.

4.1.1. Germination Rate

Germination rate in % was determined on the seventh day after sowing.

plant species	control					nickel					zinc				
Triticum aestivum	100	83	100	83	50	80					80	60			
Thlaspi caerulescens	86	95	93	89	94	80	100	100	94	88	100	90	100	70	93
Thlaspi goesingense	15	0	11	0	16	0	10	0	8	7	8	8	0	3	4
Amaranthus cruentus	61	93	90	71	88	69	55	72	76	68	75	92	92	84	83
Saxifraga stellaris	<50					<50					<50				
					>70%	70–50%			<50%						

Tab. 5: Gemination rates in %, HM 1 mmol

Thlaspi caerulescens had an average germination rate of 91.4% in the control, 92.4% in 1 mmol Ni and 90.6% in 1 mmol Zn. For *Amaranthus cruentus* 80.6% in the control, 68% in 1 mmol Ni and 85.2% of the seeds germinated in 1 mmol Zn. So, both *Thlaspi caerulescens* and *Amaranthus cruentus* performed well and therefore have been used in the succeeding experiments. *Triticum aestivum* with an average germination rate of 84.2% in the control, will be used in hydroponics and soil cultures, due to its rapid accumulation of biomass and importance as world food crop. HMs had no significant effect on the germination rate (Tab.5).

4.1.2. Growth performance

(A) Overview

Seven days after sowing pictures were taken of the petridishes and growth performance documented by taking pictures with the stereomicroscope. Pictures of the plates were taken again on the 18th day after sowing. In the following paragraphs, an overview and description of these pictures are given, and the trends observed described.

Amaranthus cruentus grew on Zn (1 mmol) substrate not as good as in the control, showing slightly shorter roots in Zn treatment. Amaranth showed no tolerance for Ni (1 mmol). Seedlings in Ni had no colouration, no root hairs, were degenerated, small and did not increase in size after germination (Fig.16).

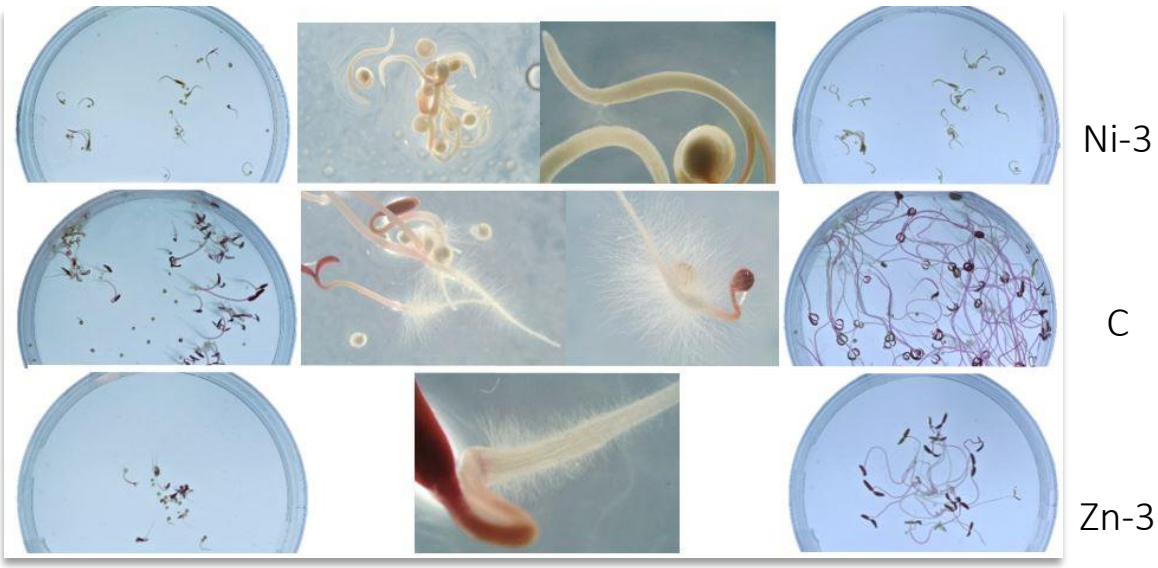


Fig. 16: Amaranthus cruentus, *Ni-3 = 1 mmol Ni, Zn-3 = 1 mmol Zn, C = control, petridishes with Ø 9 cm left petridishes seven days after sowing, right petridishes 18 days after sowing (11 days apart) detailed pictures taken in stereomicroscope*

Saxifraga stellaris (Fig.18) performed poorly on all three substrates.

In each group (Zn 1 mmol, Ni 1 mmol, C) seedlings with and without root hairs of different lengths could be found.

Notably on each seedling, independent of treatment, was the red tip of the root (Fig.17).

Fig. 17: Root tips of Saxifraga stellaris *(Nikon eclipse)*

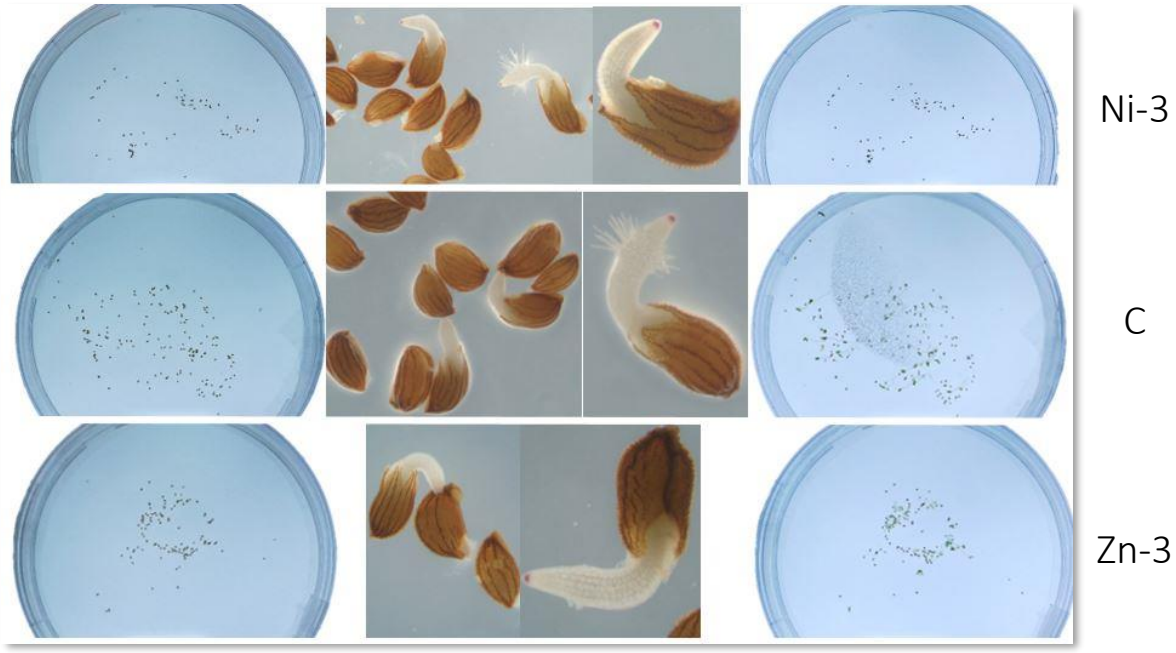


Fig. 18: Saxifraga stellaris, *Ni-3 = 1 mmol Ni, Zn-3 = 1 mmol Zn, C = control, petridishes with Ø 9 cm left petridishes seven days after sowing, right petridishes 18 days after sowing (11 days apart) detailed pictures taken in stereomicroscope*

Thlaspi caerulescens (Fig.19) grew on Ni (1 mmol) as good as in the control, though it seemed that there was an increase in number of root hairs in the Ni plates on some seedlings. In Zn (1 mmol) root hair length reduced remarkably, yet seedling growth was not affected.

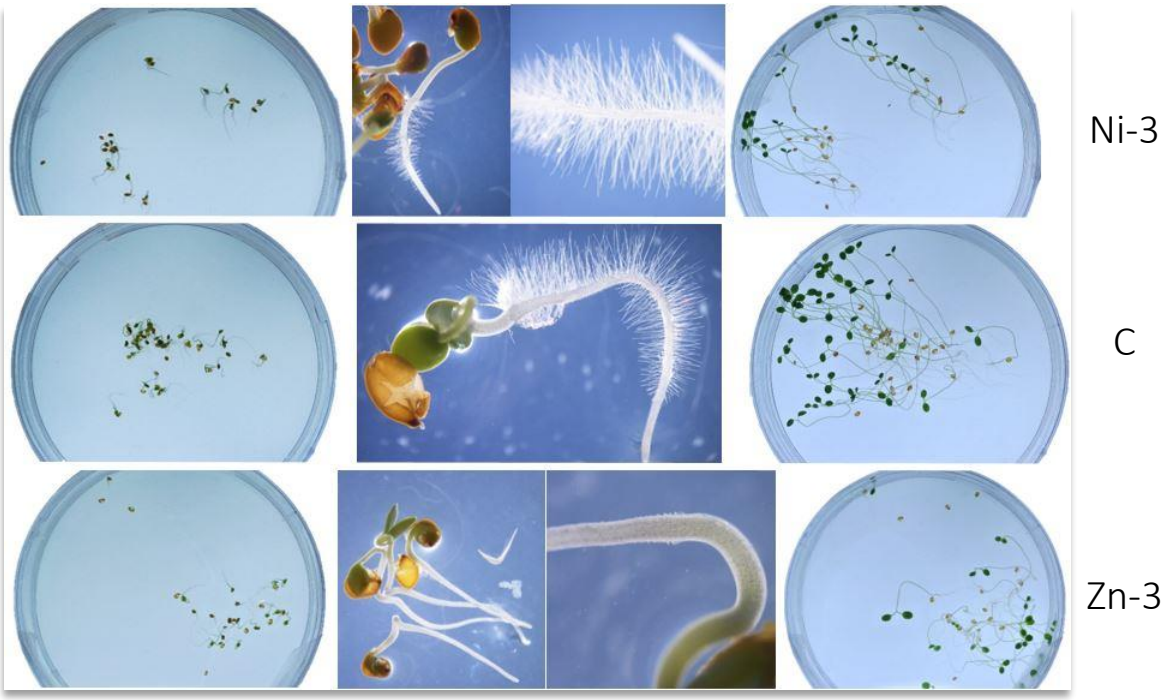


Fig. 19: Thlaspi caerulescens, *Ni-3 = 1 mmol Ni, Zn-3 = 1 mmol Zn, C = control, petridishes with Ø 9 cm left petridishes seven days after sowing, right petridishes 18 days after sowing (11 days apart) detailed pictures taken in stereomicroscope, different magnifications*

Thlaspi goesingense (Fig.20) performed poorly on all three substrates. In each group (Zn 1 mmol, Ni 1 mmol, C) seedlings with view to no root hairs could be found.

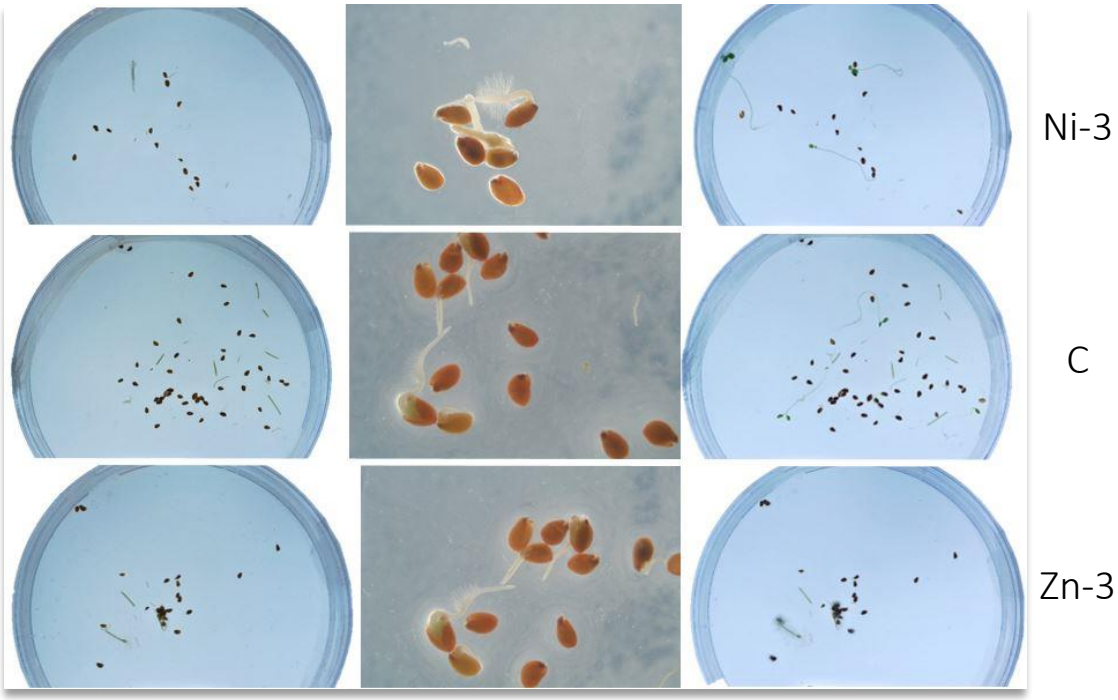


Fig. 20: Thlaspi goesingense*, Ni-3 = 1 mmol Ni, Zn-3 = 1 mmol Zn, C = control, petridishes with Ø 9 cm left petridishes seven days after sowing, right petridishes 18 days after sowing (11 days apart) detailed pictures taken in stereomicroscope*

Germination rate of *Triticum aestivum* (Fig.21) was not affected by HM treatment, but effect of the treatment was observed in root and shoot length. Length of root and shoot of wheat seedlings in Ni substrate (1 mmol) decreased remarkably, relatively to the control, while length of shoots and roots in Zn plates (1 mmol) increased noticeably.

Fig. 21: Triticum aestivum *Ni-3 = 1 mmol Ni, Zn-3 = 1 mmol Zn, C = control, petridishes with Ø 9 cm left petridishes seven days after sowing, right petridishes 18 days after sowing (11 days apart) detailed pictures taken in stereomicroscope, different magnifications*

(B) Growth Parameters

Further analysis and measurement of maximum root length, maximum root hair length and the distance between root tip and first root hair was done for *Thlaspi caerulescens* and *Amaranthus cruenthus* to investigate if HMs and FA influenced these growth parameters and if the measurements show significant differences.

Thlaspi caerulescens – Maximum Root Length

The average maximum root length for *Thlaspi caerulescens* is 34 726 ± 62 354 µm for the control, 13 915 ± 3 054 µm for Ni and 17 727 ± 13 894 µm for Zn.

In the control maximum root length is 78 523 ± 115 123 µm on average. When treated with FA500 it is 18 230 ± 2 386 µm and with FA100 it is 16 184 ± 2 386 µm.

In Ni maximum root length is 12 359 ± 2 181 µm, with FA500 it is 12 465 ± 4 568 µm and treated with FA100 it is 16 341 ± 4 568 µm.

In Zn maximum root length is 11 906 ± 2 393 µm, treated with FA500 it is 13 239 ± 1 799 µm and treated with FA100 28 038 ± 21 601 µm on average.

Statistical analysis of differences between HMs, FA and different treatment combinations ((C, Ni, Zn) x (Adest, FA500, FA100)) showed no significance and is reported in detail in the following paragraph.

Two Way ANOVA showed no significance in the corrected model for maximum root length (Tab.7, Fig.22), depending on HM treatment or treatment with FA ($F(8,33) = 1.481$, $p = 0.202$, $R^2 = 0.264$, $adj.R^2 = 0.086$). No significant main effects could be observed for HM treatment ($F(2,33) = 1.755$, $p = 0.189$, $\eta_p^2 = 0.096$) or treatment with FA ($F(2,33) = 1.07.8$, $p = 0.352$, $\eta_p^2 = 0.061$) and no significant interactive effect was reported ($F(4,33) = 1.824$, $p = 0.148$, $\eta_p^2 = 0.181$) as well.

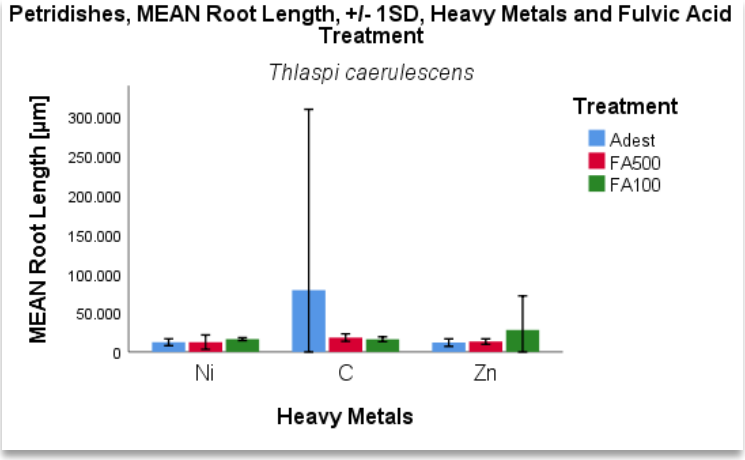


Fig. 22: Bar chart showing mean of root length of Thlaspi caerulescens, *error bars: ± 1SD, no significant differences between treatment groups, FA500 = 0.2% FA, FA100 = 1% FA*

Thlaspi caerulescens – Maximum Root Hair Length

The average maximum root hair length for *Thlaspi caerulescens* is 2 043 ± 1 931 µm for the control, 2 519 ± 1 316 µm for Ni and 1 892 ± 2 486 µm for Zn.

In the control maximum root hair length is 784 ± 907 µm on average.
When treated with FA500 it is 2 502 ± 1 602 µm and with FA100 it is 2 592 ± 2 589 µm.

In Ni maximum root hair length is 3 280 ± 1 008 µm, with FA500 it is 2 918 ± 433 µm and treated with FA100 it is 1 519 ± 1 410 µm.

In Zn maximum root length is 1 159 ± 1 587 µm, treated with FA500 it is 3 496 ± 3 397 µm and treated with FA100 1 023 ± 1 654 µm on average.
Mean in this case is not representative of data. Root hairs were both taken from inside and outside the agar medium. Roots inside the agar had no root hairs, while roots outside had impressively long root hairs. Actual lengths are given in Tab.6.

Adest	FA500	FA100
44.02	0	3801.89
0	0	0
0	5627.16	0
0	0	1310.95
2881.09	4288.39	0
2912.12	7564.77	0

Tab. 6: actual values of root hair length in Zn group of Thlaspi caerulescens

Statistical analysis of differences between HMs, FA and different treatment combinations
((C, Ni, Zn) x (Adest, FA500, FA100)) showed no significance and is discussed in detail in the following paragraph.

Results for the Two Way ANOVA for maximum root hair length (Tab.9, Fig.24) in correlation with HM treatment and FA treatment was not significant for the corrected model on p <0.05 level ($F(8,33) = 1.369$, $p = 0.246$, $R^2 = 0.249$, $adj.R^2 = 0.067$).
No significant main effect for HM treatments was observed ($F(2,33) = 0.505$, $p = 0.608$, $\eta_p^2 = 0.030$),
for treatment with FA as well ($F(2,33) = 1.854$, $p = 0.173$, $\eta_p^2 = 0.101$)
and no significant interaction effect was reported ($F(4,36) = 1.495$, $p = 0.226$, $\eta_p^2 = 0.154$).

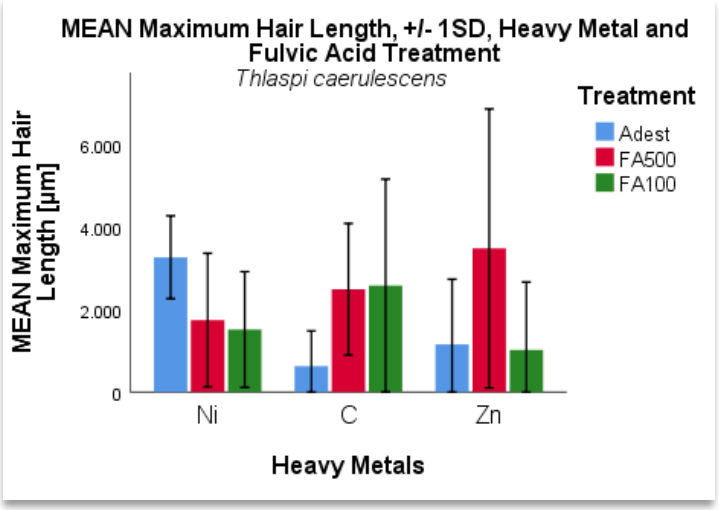


Fig. 23: Bar chart showing mean of maximum root hair length of Thlaspi caerulescens*, error bars: ± 1SD, no significant interactive effects. FA500 = 0.2% FA, FA100 = 1% FA*

Thlaspi caerulescens – Distance Between Root Tip and First Root Hair

The average distance between root tip and first root hair for *Thlaspi caerulescens* is 205 ± 428 µm for the control, 308 ± 315 µm for Ni and 33 ± 24 µm for Zn.

In the control length of the distance between root tip and first root hair is 472 ± 787 µm on average. When treated with FA500 it is 43 ± 34 µm and with FA100 it is 154 ± 147 µm.

In Ni the distance between root tip and first root hair is 452 ± 425 µm, with FA500 it is 292 ± 73 µm and treated with FA100 it is 83 ± 12 µm.

In Zn the distance between root tip and first root hair is 61 ± 33 µm, treated with FA500 it is 23 ± 10 µm and treated with FA100 22 ± 12 µm on average.

Statistical analysis of differences between HMs, FA and different treatment combinations ((C, Ni, Zn) x (Adest, FA500, FA100)) showed no significance and is reported in detail in the following paragraph.

No significant effect could be observed for the distance between root tip to first root hair (Tab.8, Fig.23) ($F(8,23) = 1.084$, $p = 0.409$, $R^2 = 0.274$, $adj.R^2 = 0.021$).

Neither for HM treatment ($F(2,23) = 1.075$, $p = 0.358$, $\eta_p^2 = 0.086$) nor treatment with FA ($F(2,23) = 1.385$, $p = 0.270$, $\eta_p^2 = 0.107$) and no interactive effect ($F(4,23) = 0.434$, $p = 0.782$, $\eta_p^2 = 0.070$) was determined. Fig.24**Fig. 24**

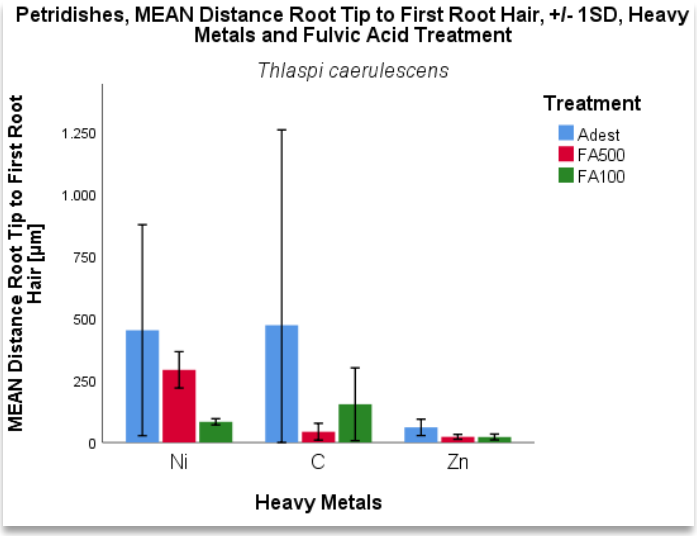


Fig. 24: Bar chart showing mean of distance between root tip and first root hair of Thlaspi caerulescens*, error bars: ± 1SD, no significant differences between treatment groups. FA500 = 0.2% FA, FA100 = 1% FA*

Descriptive Statistics

Maximum Root Length

HM	FA	Mean	Std. Deviation	N
C	Adest	78523,0400	115123,0922	4
	FA100	16183,8000	1553,76558	5
	FA500	18229,5500	2386,20467	5
	Total	34725,6364	62354,36251	14
Ni	Adest	12359,3280	2180,91962	5
	FA100	16340,7780	909,48649	5
	FA500	12464,6567	4568,35339	3
	Total	13914,9615	3053,78274	13
Zn	Adest	11905,7420	2393,41850	5
	FA100	28037,5780	21601,02354	5
	FA500	13238,5600	1708,86781	5
	Total	17727,2933	13894,28809	15

Tab. 7: Descriptive statistics for maximum root length, FA500 = 0.2% FA, FA100 = 1% FA

Distance between Root Tip and First Root Hair

HM	FA	Mean	Std. Deviation	N
C	Adest	472,4175000	786,6419665	4
	FA100	153,6420000	146,8035269	5
	FA500	43,12200000	33,85678027	5
	Total	205,2492857	427,6469825	14
Ni	Adest	451,9720000	425,0127932	5
	FA100	83,22666667	12,48426743	3
	FA500	292,4833333	73,26851598	3
	Total	307,9081818	314,5668146	11
Zn	Adest	60,63000000	33,07845522	2
	FA100	21,96500000	11,80161218	2
	FA500	22,63666667	9,907554357	3
	Total	33,30000000	24,22713286	7

Tab. 8: Descriptive statistics for distance between root tip and first root hair, FA500 = 0.2% FA, FA100 = 1% FA

Maximum Root Hair Length

HM	FA	Mean	Std. Deviation	N
C	Adest	784,0025000	907,1168173	4
	FA100	2592,458000	2589,319708	5
	FA500	2502,004000	1602,081672	5
	Total	2043,451429	1930,659343	14
Ni	Adest	3280,498000	1008,315481	5
	FA100	1518,794000	1410,416983	5
	FA500	2918,226667	432,5362774	3
	Total	2519,318462	1315,854623	13
Zn	Adest	1158,642000	1586,573829	5
	FA100	1022,568000	1654,141084	5
	FA500	3496,064000	3397,375684	5
	Total	1892,424667	2485,902779	15

Tab. 9: Descriptive statistics for maximum root hair length, FA500 = 0.2% FA, FA100 = 1% FA

Amaranthus cruentus – Maximum Root Length

The average maximum root length for *Amaranthus cruentus* is 14 499 ± 3 854 µm for the control, 2 735 ± 297 µm for Ni and 8 222 ± 1 844 µm for Zn.

In the control the maximum root length is 13 503 ± 3 784 µm on average. When treated with FA500 it is 13 340 ± 4 268 µm and with FA100 it is 16 655 ± 3 276 µm.

In Ni the maximum root length is 2 868 ± 215 µm, with FA500 it is 2 528 ± 405 µm and treated with FA100 it is 2 810 ± 128 µm.

In Zn maximum root length is 6 375 ± 548 µm, treated with FA500 it is 8 845 ± 1 298 µm and treated with FA100 9 445 ± 1 816 µm on average.

Statistical analysis of the differences between HMs, FA and different treatment combinations ((C, Ni, Zn) x (Adest, FA500, FA100)) showed significant differences between HM treatments. Average maximum root length increased from Ni (2 735 ± 297) to Zn (8 222 ± 1 844) to C (14 499 ± 3 854).

In the control and Zn significant effect for FA treatment was observed as well. In Zn FA100 treated plants show significant increase in maximum root length when treated with FA100 compared to FA500 and Adest treated plants. In the control FA100 treated plants show significant increase in root length compared to Adest treated plants.

In the following a detailed report of the statistical analysis is given.

Two Way ANOVA showed significance on p<0.05 level in the corrected model for root length (Tab.10, Fig.25) ($F(8,36) = 25.398$, $p < 0.001$ ($1.3179*10^{-12}$), $R^2 = 0.849$, $adj.R^2 = 0.816$), yet homogeneity criterion was not met (F-Test for Heteroskedasticity: $F = 15.645$, $df_1 = 1$, $df_2 = 43$, $p < 0.001$ (0.000281) and Levene´s Test of Equality of Error Variances, based on mean: *Levene statistic* = 4.903, $df_1 = 8$, $df_2 = 36$, $p < 0.001$ (0.000379)).

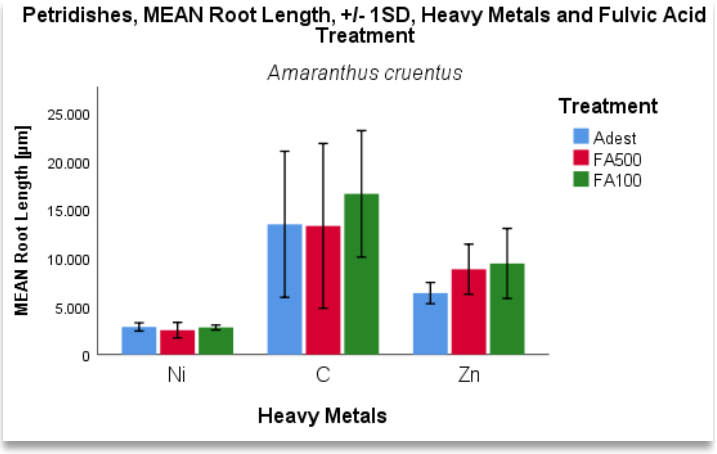


Fig. 25: Bar chart showing mean of max. root length of Amaranthus cruentus*, error bars: ± 1SD, no significant interactive effects. FA500 = 0.2% FA, FA100 = 1% FA*

No significant main effect could be observed for treatment with FA ($F(2,36) = 3.049$, $p = 0.060$, $\eta_p^2 = 0.145$).

HM treatment on root length turned out to be a significant main effect ($F(2,36) = 95.896$, $p < 0.001$ ($3.782*10^{-15}$), $\eta_p^2 = 0.842$) with Cohen´s value ($f = 2.31$) that suggests strong effect size.

No interactive effect of HM treatment and FA treatment was found ($F(4,36) = 1.328$, $p = 0.280$, $\eta_p^2 = 0.128$).

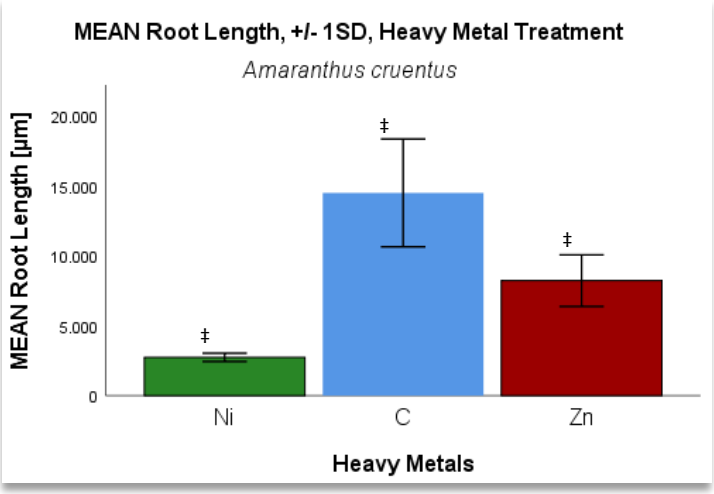


Fig. 26: Bar chart showing mean of max. root length of Amaranthus cruentus*, error bars: ± 1SD, significant differences between all groups marked with ‡*

Descriptive Statistics

Maximum Root Length

HM	FA	Mean	Std. Deviation	N
C	Adest	13503,2260	3784,10366	5
	FA100	16654,7540	3276,04543	5
	FA500	13340,2400	4268,27286	5
	Total	14499,4067	3854,38615	15
Ni	Adest	2867,6620	214,68317	5
	FA100	2810,4820	127,92989	5
	FA500	2528,2160	404,91379	5
	Total	2735,4533	297,11940	15
Zn	Adest	6374,9080	548,24919	5
	FA100	9445,2240	1816,08812	5
	FA500	8845,4900	1297,59036	5
	Total	8221,8740	1844,17623	15

Tab. 10: Descriptive statistics for max. root length (HM and FA treatment) of Amaranthus cruentus*, FA500 = 0.2% FA, FA100 = 1% FA*

Bonferroni Post-hoc Tests showed significant differences between all HM groups:

- Ni to control ($p < 0.001$ ($1.6627*10^{-15}$)),
- Zn to control ($p < 0.001$ ($3.1027*10^{-8}$)) and
- Ni to Zn ($p < 0.001$ ($5.1799*10^{-7}$)),

whereby root length increased from Ni (2 735.45 ± 297.12) to Zn (8 221.87 ± 1 844.18) to C (14 499.41 ± 3 854.39) (Fig.26).

LSD Test for Simple Effects showed significant effects in

- the control between Adest and FA100 ($p = 0.039$) as well as between FA100 and FA500 ($p = 0.031$) (Fig.27) and
- Zn between Adest and FA100 ($p = 0.044$) (Fig.28).

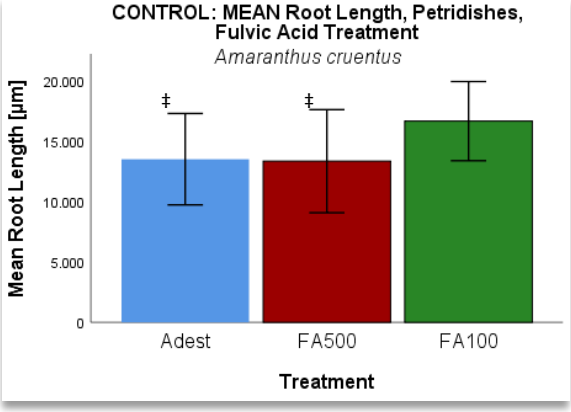


Fig. 27: Bar chart showing mean root length in the control of Amaranthus cruentus *in petridishes, error bars ± 1SD, significant differences between FA100 marked with ‡*

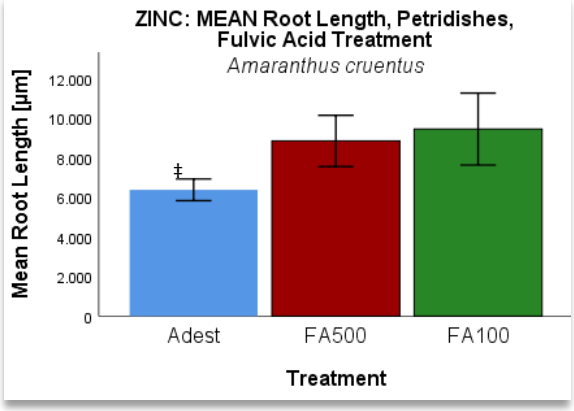


Fig. 28: Bar chart showing mean root length in Zn of Amaranthus cruentus *in petridishes, error bars ± 1SD, significant differences between FA100 marked with ‡*

Amaranthus cruentus – Distance Between Root Tip and First Root Hair

The average distance between root tip and first root hair for *Amaranthus cruentus* is 1 177 ± 443 µm for the control, 0 ± 0 µm for Ni and 607 ± 177 µm for Zn.

In the control the distance between root tip and first root hair is 1 336 ± 720 µm on average. When treated with FA500 it is 1 078 ± 290 µm and with FA100 it is 1 117 ± 189 µm.

In Ni the distance between root tip and first root hair is 0 ± 0 µm, with FA500 it is 0 ± 0 µm and treated with FA100 it is 0 ± 0 µm.

In Zn the distance between root tip and first root hair is 676 ± 42 µm, treated with FA500 it is 577 ± 115 µm and treated with FA100 it is 581 ± 284 µm on average.

Statistical analysis of the differences between HMs, FA and different treatment combinations ((C, Ni, Zn) x (Adest, FA500, FA100)) showed significant differences between HM treatments. Average distance of root tip to first root hair increased from Ni (0 ± 0) to Zn (606.85 ± 176.92) to the control (1176.91 ± 442.78).

In the following a detailed report of the statistical analysis is given.

The same trend was observed for the distance between root tip to first root hair (Tab.11, Fig.29)
($F(8,35) = 15.841$, $p < 0.001$ ($81.4456*10^{-9}$), $R^2 = 0.784$, $adj.R^2 = 0.734$),

yet homogeneity criterion was not met (F-Test for Heteroskedasticity: $F = 6.196$, $df_1 = 1$, $df_2 = 42$, $p = 0.002$ and Levene´s Test of Equality of Error Variances, based on mean: *Levene statistic* = 4.110, $df_1 = 8$, $df_2 = 35$, $p = 0.017$).

Significant main effect on $p < 0.05$ level was determined for HM treatment ($F(2,35) = 62.057$, $p < 0.001$ ($3.0995*10^{-12}$), $\eta_p^2 = 0.780$) with strong effect size ($f = 1.88$) and no significant effect of FA ($F(2,35) = 0.717$, $p = 0.495$, $\eta_p^2 = 0.039$) as well as no interactive effect ($F(4,35) = 0.294$, $p = 0.880$, $\eta_p^2 = 0.032$) was found.

Descriptive Statistics

Distance between root tip and first root hair

HM	FA	Mean	Std. Deviation	N
C	Adest	1336,070000	719,8348380	5
	FA100	1117,062000	188,5686890	5
	FA500	1077,584000	289,8175282	5
	Total	1176,905333	442,7797656	15
Ni	Adest	,0000000000	,0000000000	5
	FA100	,0000000000	,0000000000	5
	FA500	,0000000000	,0000000000	5
	Total	,0000000000	,0000000000	15
Zn	Adest	676,0625000	42,31772865	4
	FA100	580,9040000	283,5149390	5
	FA500	577,4420000	115,2654711	5
	Total	606,8557143	176,9161851	14

Tab. 11: Descriptive statistics for distance between root tip and first root hair (HM and FA treatment) of Amaranthus cruentus*, FA500 = 0.2% FA, FA100 = 1% FA*

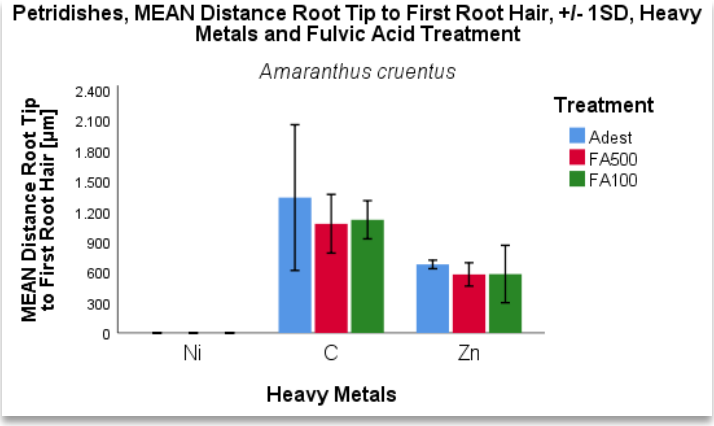


Fig. 29: Bar chart showing mean of distance root tip to first root hair of Amaranthus cruentus*, error bars: ± 1SD, no significant interactive effects. FA500 = 0.2% FA, FA100 = 1% FA*

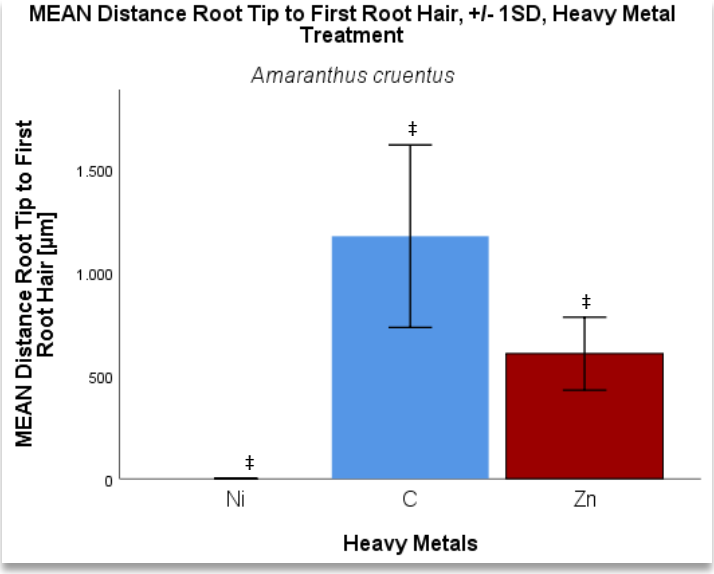


Fig. 30: Bar chart showing mean of root length of Amaranthus cruentus*, error bars: ± 1SD, significant differences between all groups marked with ‡*

Bonferroni Post-hoc Tests showed significant differences between all HM groups:

- Ni to control ($p < 0.001$ ($1.4084*10^{-12}$)),
- Zn to control ($p < 0.001$ (0.000019)) and
- Ni to Zn ($p < 0.001$ (0.000007)),

whereby distance of root tip to first root hair increased from Ni (0 ± 0) to Zn (606.85 ± 176.92) to C (1176.91 ± 442.78) (Fig.30).

Amaranthus cruentus – Maximum Root Hair Length

The average maximum root hair length for *Amaranthus cruentus* is 724 ± 520 µm in the control, 0 ± 0 µm in Ni and 616 ± 244 µm in Zn.

In the control maximum root hair length is 877 ± 590 µm on average. When treated with FA500 it is 1 010 ± 470 µm and with FA100 it is 283 ± 56 µm.

In Ni max. root hair length is 0 ± 0 µm, with FA500 it is 0 ± 0 µm and treated with FA100 it is 0 ± 0 µm.

In Zn maximum root hair length is 455 ± 138 µm, treated with FA500 it is 773 ± 299 µm and treated with FA100 568 ± 54 µm on average.

Statistical analysis of differences between HMs, FA and different treatment combinations ((C, Ni, Zn) x (Adest, FA500, FA100)) showed significant differences.

Maximum root hair length increased on average from Ni (0 ± 0) to Zn (616 ± 244) and from Ni to C (724 ± 520).

Seedlings cultivated on Zn containing agar show an increase of root hair length when treated with FA100 (568 ± 54) compared to seedlings cultivated on the control agar treated with FA100 (283 ± 56), while the opposite effect can be observed when treated with the lower FA concentration FA500 (Zn: 773 ± 299; C: 1 010 ± 470).

In the control (724 ± 520) maximum root hair length decreased from Adest (877 ± 135) to FA100 (283 ± 135) and increased, when treated with lower concentration FA500 (1 010 ± 135).

In Zn FA500 showed highest maximum root hair length (773 ± 135), decreasing compared to Adest (364 ± 135) or FA100 (341 ± 135).

Following a detailed report of the statistical analysis.

Results for the Two Way ANOVA for maximum root hair length (Tab.12, Fig.31) in correlation with HM treatment and FA treatment was significant for the corrected model on p <0.05 level ($F(8,33) = 9.467$, p <0.001 (0.000001), $R^2 = 0.697$, $adj.R^2 = 0.623$),
yet homogeneity criterion was not met
(F-Test for Heteroskedasticity:
$F = 14.515$, $df_1 = 1$, $df_2 = 40$, p <0.001 (0.000469)
and Levene´s Test of Equality of Error Variances, based on mean:
Levene statistic = 9.204, $df_1 = 8$, $df_2 = 33$, p <0.001 (0.000001)).

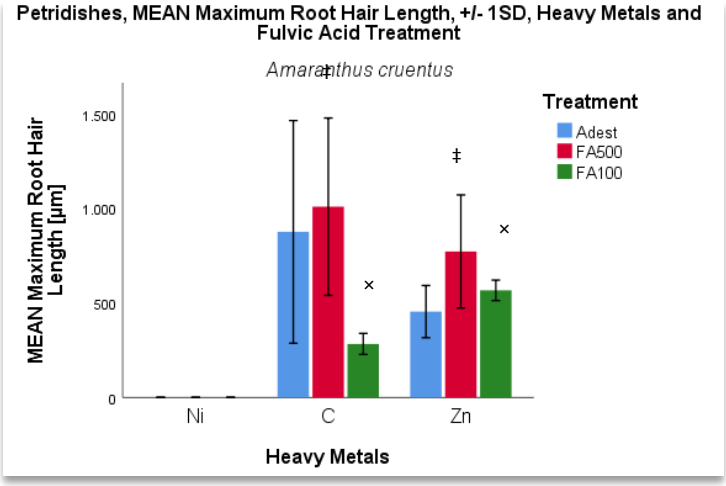


Fig. 31: Bar chart showing mean of maximum root hair length of Amaranthus cruentus*; error bars: ± 1 SD; Significant increase of length from C to Zn when treated with FA100 = 1% FA marked × and significant decrease of length from C to Zn when treated with FA500 marked ‡. FA500 = 0.2% FA, FA100 = 1% FA*

Significant interactive effect was found
($F(4,33) = 2.965$, $p = 0.034$, $\eta_p^2 = 0.697$).
Main effects for HM treatments
($F(2,33) = 26.778$, p <0.001 ($1.2303*10^{-7}$), $\eta_p^2 = 0.619$)
as well as treatment with FA
($F(2,33) = 3.984$, $p = 0.028$, $\eta_p^2 = 0.195$)
were significant.

Cohen´s values suggest a strong effect size for HM treatment ($f = 1.27$), FA treatment ($f = 0.49$) and interaction effects ($f = 1.52$).

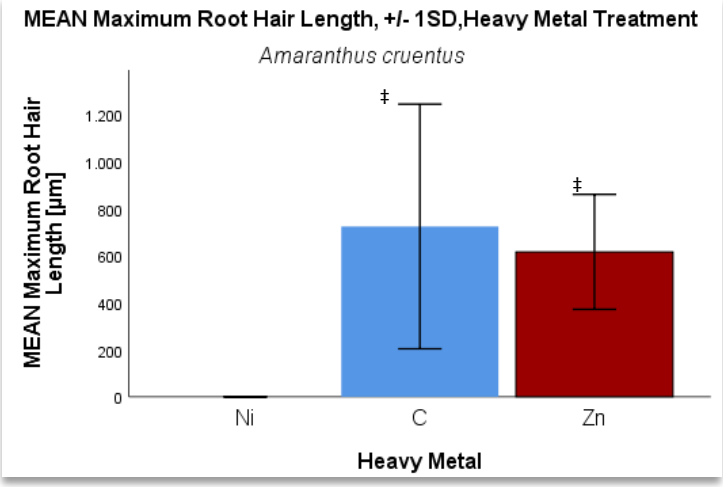


Fig. 32: Bar chart showing mean of maximum root hair length of Amaranthus cruentus*, error bars: ± 1SD, Significant increase of length from Ni groups marked with ‡*

Bonferroni Post-hoc Tests showed significant differences of
Ni to the control (p <0.001 ($1.9854*10^{-7}$)) and Ni to Zn (p <0.001 (0.000011)), whereas maximum root hair length increased on the average from Ni (0 ± 0) to Zn (615.65 ± 244.33) and from Ni to C (723.68 ± 519.81).
It also showed a significant difference of FA500 to FA100 ($p = 0.008$).
Seedlings cultivated on Zn containing agar show an increase of root hair length when treated with FA100 (567.51 ± 54.264) compared to seedlings cultivated on the control agar treated with FA100 (283.49 ± 55.62), while the opposite effect can be observed when treated with the lower FA concentration FA500 (Zn: 773.13 ± 299.37; C: 1 010.49 ± 469.59).

LSD Test for Simple Effects showed significant effects in

- the control between Adest and FA100 (p = 0.004) as well as between FA100 and FA500 (p = 0.001) (Fig.33)
- Zn between Adest and FA500 (p = 0.038) as well as FA500 and FA100 (p = 0.029) (Fig.34)

Descriptive Statistics

Maximum Root Hair Length

HM	FA	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
C	Adest	877,070	134,552	604,185	1149,955
	FA100	283,492	134,552	10,607	556,377
	FA500	1010,486	134,552	737,601	1283,371
Ni	Adest	,000	134,552	-272,885	272,885
	FA100	,000	134,552	-272,885	272,885
	FA500	3,411E-13	134,552	-272,885	272,885
Zn	Adest	363,742	134,552	90,857	636,627
	FA100	340,506	134,552	67,621	613,391
	FA500	773,312	134,552	500,427	1046,197

Tab. 12: Descriptive statistics for maximum root hair length (HM and FA treatment) of Amaranthus cruentus*, FA500 = 0.2% FA, FA100 = 1% FA*

In the control (723.68 ± 519.81) maximum root hair length decreased from Adest (877.07 ± 134.55) to FA100 (283.49 ± 134.55) and increased, when treated with lower concentration FA500 (1010.49 ± 134.55) (Fig.33).
In Zn FA500 showed highest maximum root hair length (773.31 ± 134.55), decreasing when compared to Adest (363.74 ± 134.55) or FA100 (340.51 ± 134.55) (Fig.34).

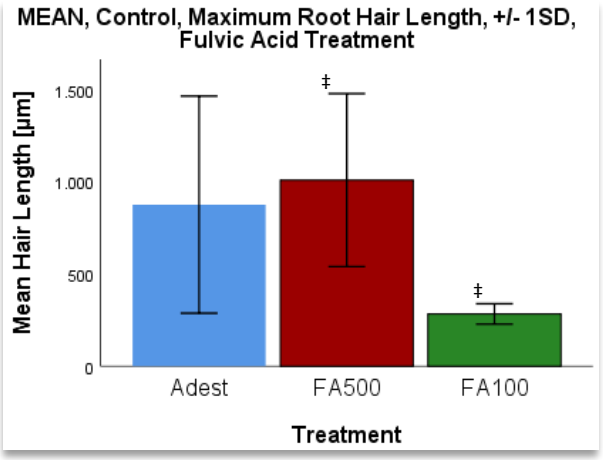


Fig. 33: Bar chart showing mean of maximum root hair length of Amaranthus cruentus *in the control, error bars: ± 1SD, Significant differences to Adest marked with ‡. FA500 = 0.2% FA, FA100 = 1% FA*

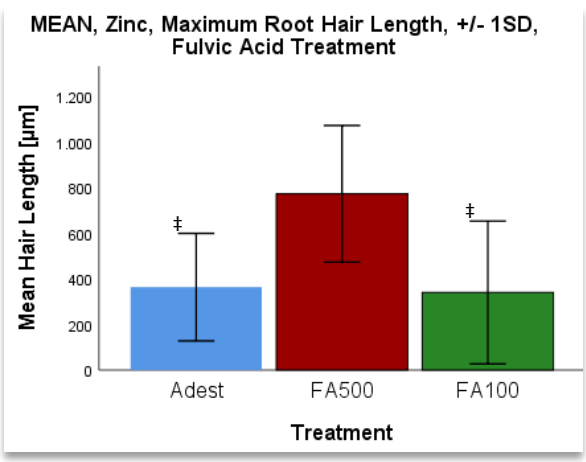


Fig. 34: Bar chart showing mean of maximum root hair length of Amaranthus cruentus *in Zn, error bars: ± 1SD, Significant differences to FA500 marked with ‡. FA500 = 0.2% FA, FA100 = 1% FA*

4.1.3. Localization of Heavy Metals using NewportGreen

Localization of Zn and Ni within *Amaranthus cruentus* and *Thlaspi caerulescens* seedlings has been visualized by complexing with NPG staining solution. Seedlings have been drenched with the staining solution and were checked after two hours using fluorescence microscopy. Threshold was determined for the control as level were barely no autofluorescence was observed anymore.

Amaranthus cruentus

When comparing root tips of *Amaranthus cruentus* in the control to Ni and Zn, two hours after drenching seedlings with NPG at threshold level, Ni and Zn plants show stronger fluorescence in cell walls and cells of calyptra (root cap) and Ni as well in vacuoles in cortex. Differences become more difficult to see, when checking the same spot 20 hours later (Fig.35–40). Only, when comparing fluorescence of root hairs in the control and Zn treated group, differences could be observed better after 20 hours. Matrix of root hairs shows fluorescence in Zn treatment. In Ni treatment on the other hand, only tiny compartments within the root hairs as well as epidermis of root hair emitted light (Fig.41–43).

Fig. 35: Amaranthus cruentus*, root tip, fluorescence microscopy, two hours after staining with NPG, (a) C, (b) Zn, (c) Ni*

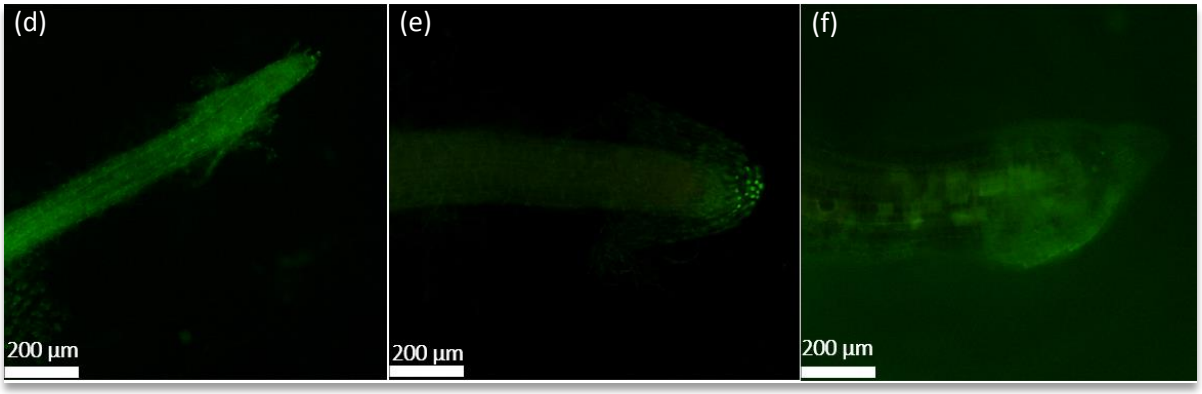


Fig. 36: Amaranthus cruentus*, same frame and settings as in first row, 20 hours after staining with NPG, (d) C, (e) Zn, (f) Ni*

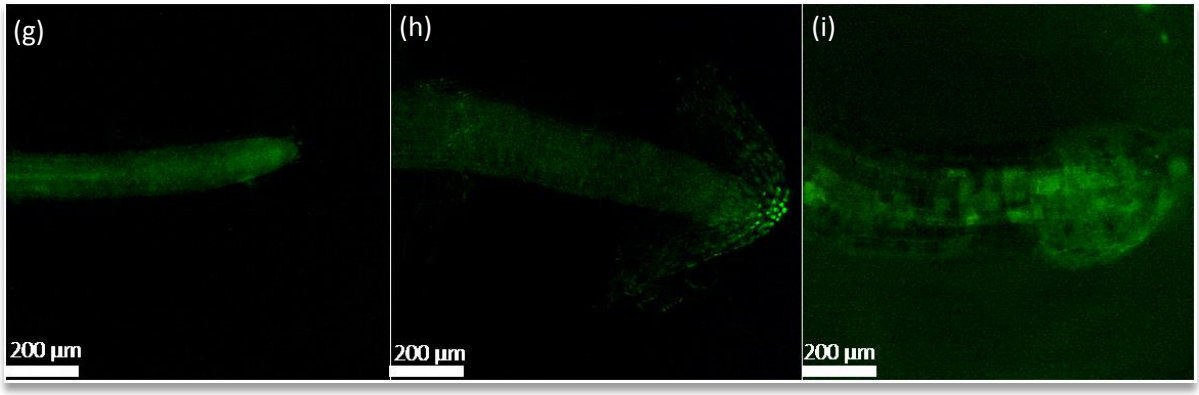


Fig. 37: Amaranthus cruentus*, same frame, but new threshold, 20 hours after staining with NPG, (g) C, (h) Zn, (i) Ni*

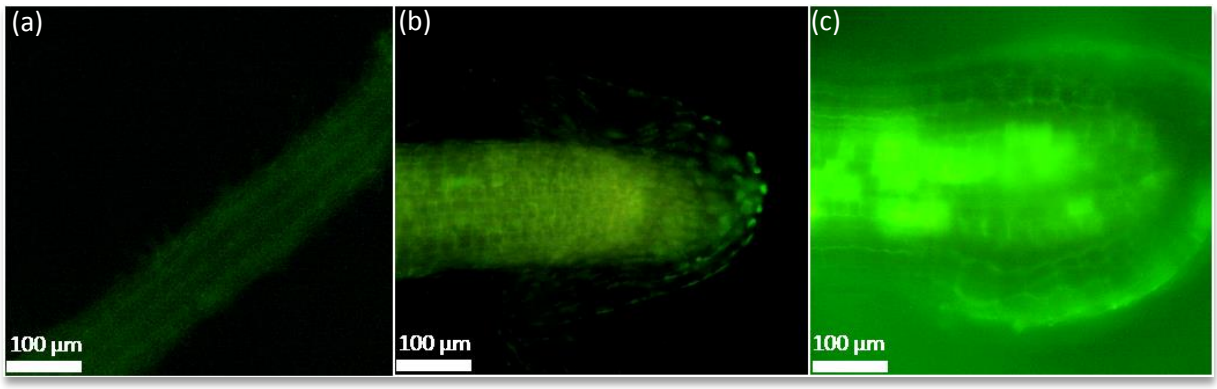


Fig. 38: Amaranthus cruentus*, root tip, fluorescence microscopy, two hours after staining with NPG, (a) C, (b) Zn, (c) Ni*

Fig. 39: Amaranthus cruentus*, same frame and settings as in first row, 20 hours after staining with NPG, (d) C, (e) Zn, (f) Ni*

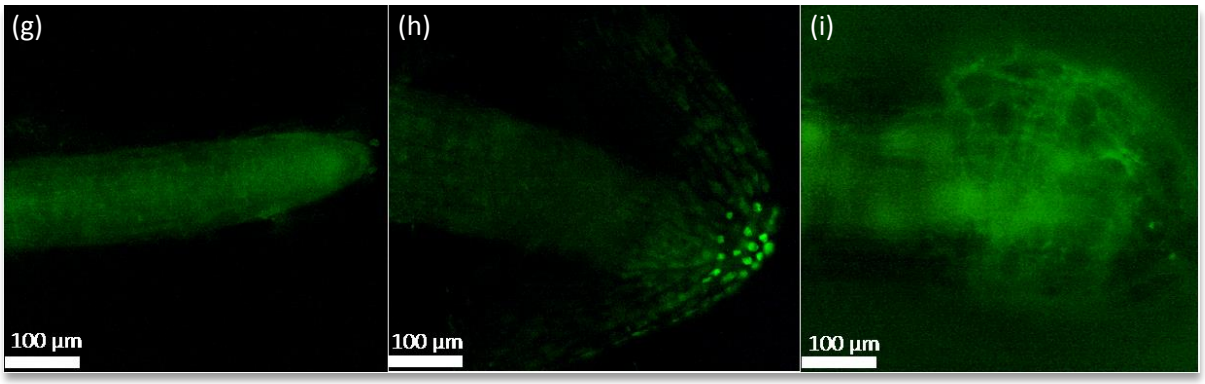


Fig. 40: Amaranthus cruentus*, same frame, but new threshold, 20 hours after staining with NPG, (g)C, (h) Zn, (i) Ni*

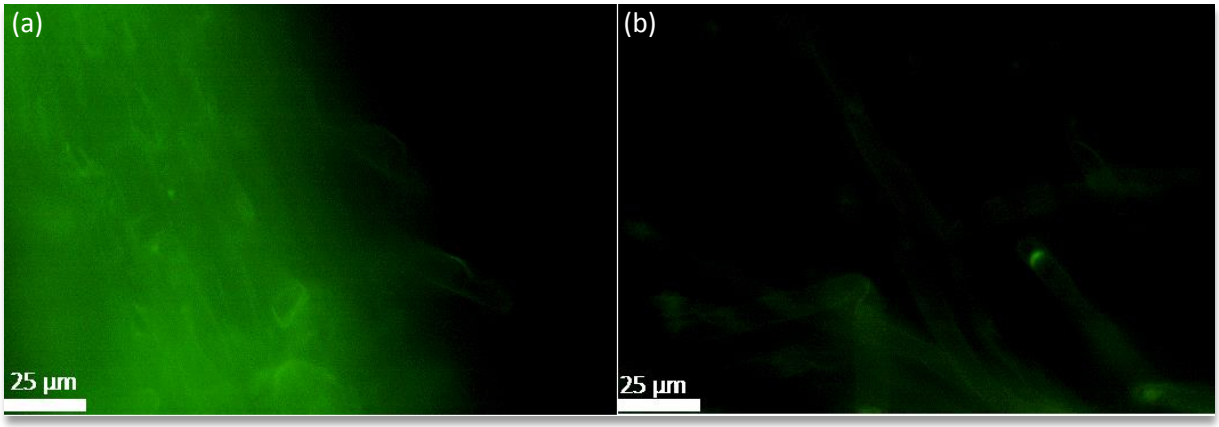


Fig. 41: Amaranthus cruentus*, root fairs, fluorescence microscopy, two hours after staining with NPG, (a)C, (b) Zn*

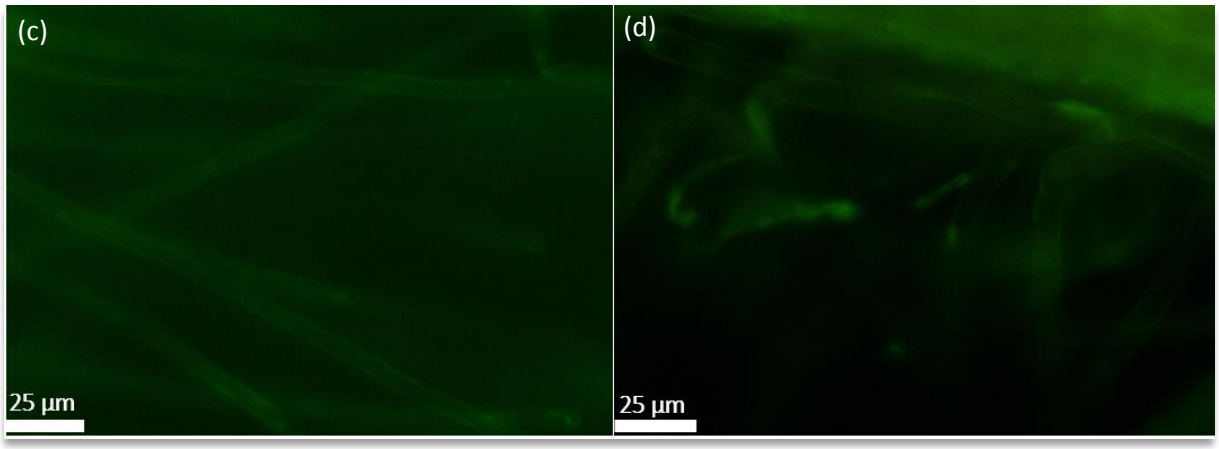


Fig. 42: Amaranthus cruentus*, same frame and settings as in first row, 20 hours after staining with NPG, (c)C, (d) Zn*

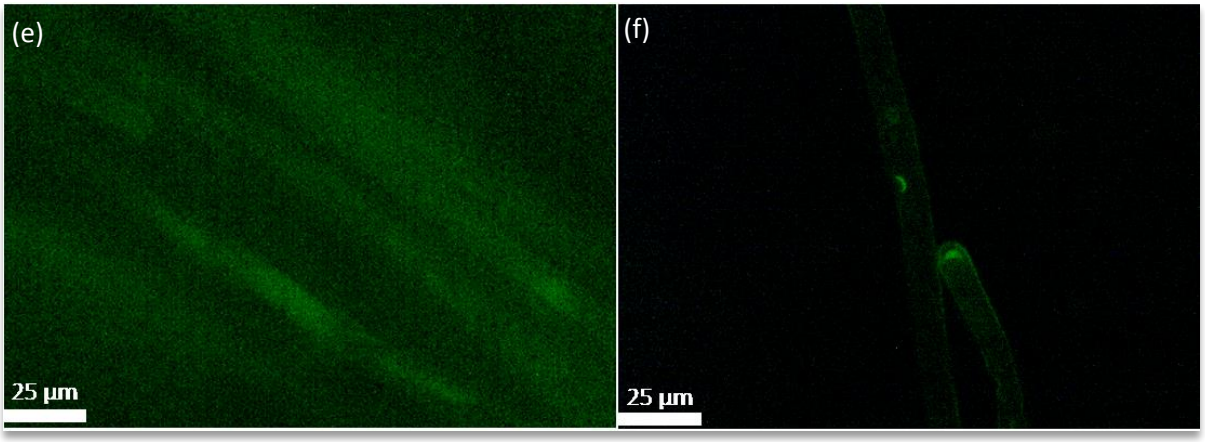


Fig. 43: Amaranthus cruentus*, same frame, but new threshold, 20 hours after staining with NPG, (e) C, (f) Zn*

Thlaspi caerulescens

NewportGreen (NPG) fluorescence dye worked better, when absorbed for two hours in higher magnifications and for lower magnifications the longer period of 20 hours were more effective. Roots of *Thlaspi caerulescens* in the control were compared to roots of plants, which grew in Ni and Zn, two hours after drenching seedlings with NPG at threshold level.

The control and Ni showed little to no difference. At threshold level of the control, very weak green light could be seen, emitted by cell walls of Ni. Roots on Zn agar emitted light from the cell walls, calyptra and central cylinder. Cell walls were most intense in root elongation zone (Fig.44–46).

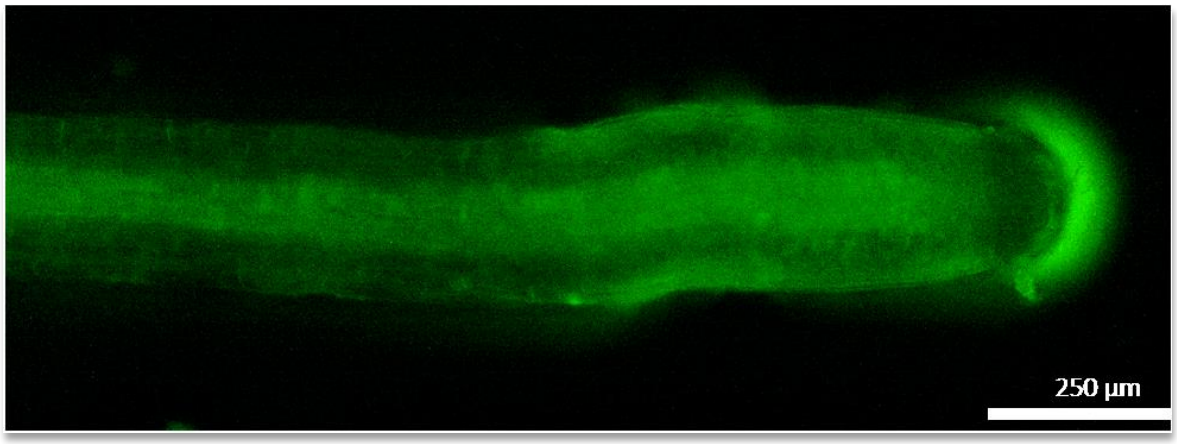


Fig. 44: Thlaspi caerulescens*, Zn, fluorescence microscopy, 20 hours after staining with NPG*

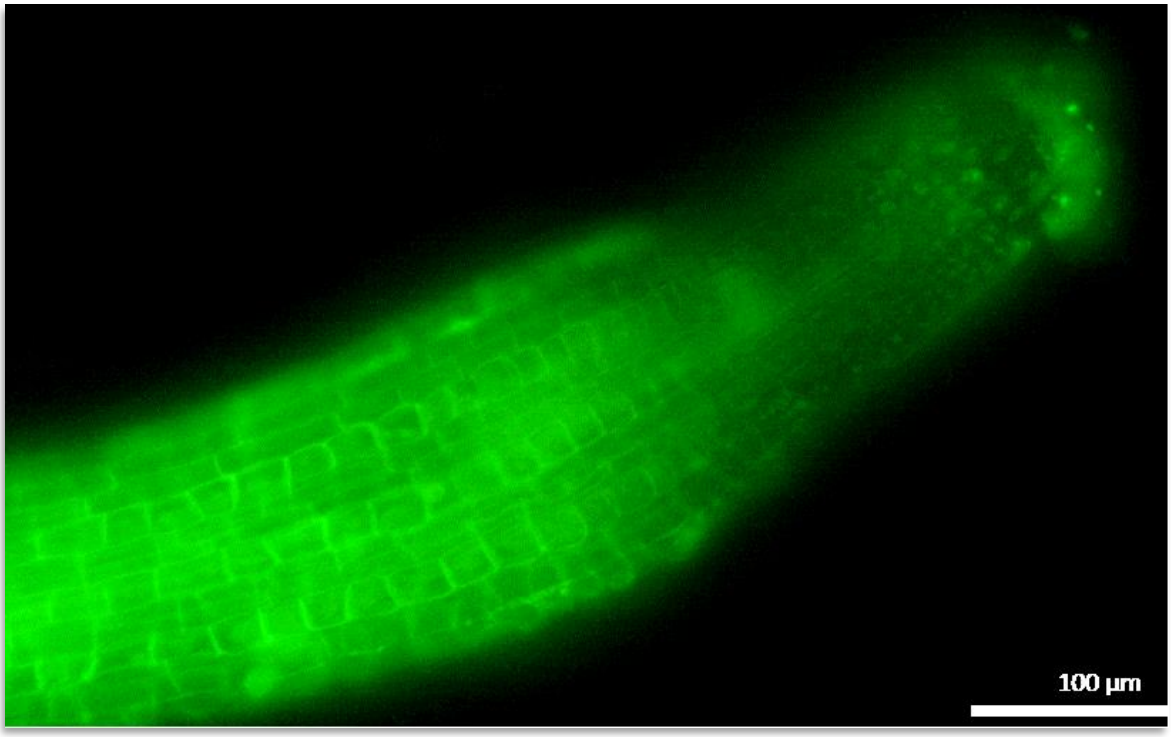


Fig. 45: Thlaspi caerulescens*, Zn, fluorescence microscopy, 2 hours after staining with NPG*

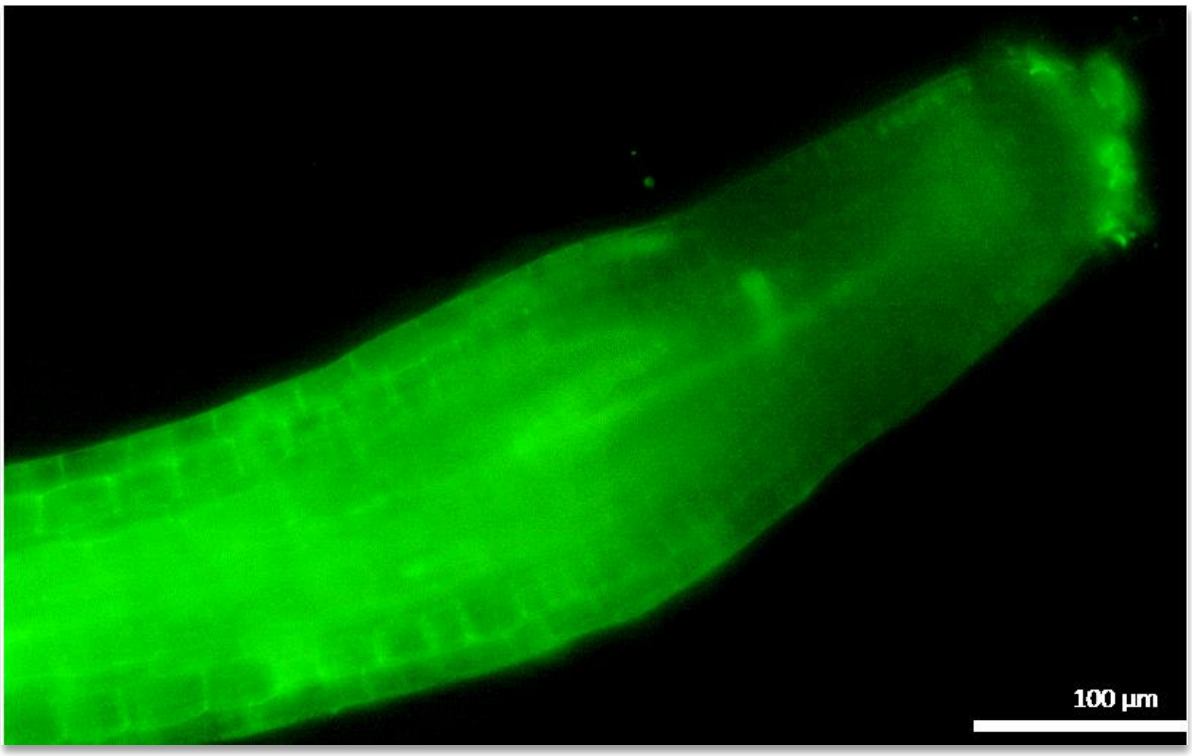


Fig. 46: Thlaspi caerulescens*, Zn, fluorescence microscopy, 2 hours after staining with NPG*

4.2. Hydroponics

4.2.1. Germination Rate

Germination rate in % was determined on the 15th day after sowing (Tab.13).

Ni-3 (1 mmol)			**Ni-4** (0.1 mmol)			C			**Zn-4** (0.1 mmol)			**Zn-3** (1 mmol)		
Triticum aestivum														
70	70	90	100	90	90	100	80		90	100	90	80	100	90
70	80	70	100	90	80	90	90	70	90	100	100	80	100	100
100	90	90	100	90	90	100	90	100	80	90	100	90	100	60
Amaranthus caudatus														
50	30	20	63	53	93	50	57	63	57	83	60	60	30	43
33	27	13	73	67	70	83	63	63	50	100	70	100	80	50
37	80	30	67	60	67	50	90	63	60	93	93	60	80	67
Thlaspi caerulescens														
23	27	70	13	43	57	50	67	53	67	70	27	40	53	47
20	50	43	33	43	17	40	27	47	17	50	27	80	37	33
20	43	37	33	13	57	50	37	33	80	30	30	77	33	50

>70%	70–50%	<50%

Tab. 13: Germination rates in %

Triticum aestivum performed well in all groups, slightly worse in Ni-3. *Amaranthus caudatus* performed worse than *Triticum aestivum*, but still okay in all HM groups except Ni-3. In Ni-3 germination rate was noticeably affected by Ni. *Thlaspi caerulescens* did not germinate well independent of HM treatment. One Way ANOVA showed no significant differences between HM groups on p <0.05 level for *Triticum aestivum* ($F(4,39) = 1.959$; $p = 0.120$) (Fig.48, Tab.14) or
Thlaspi caerulescens ($F(4,40) = 1.161$; $p = 0.343$) (Fig.49, Tab.16), but significant differences for *Amaranthus caudatus* ($F(4,40) = 6.690$, p <0.001 (0.000321)) (Fig.47, Tab.15).

Bonferroni Post-hoc Tests showed significant differences of

- Ni-3 to Ni-4 ($p = 0.003$),
- Ni-3 to C ($p = 0.009$),
- Ni-3 to Zn-4 (p <0.001 (0.000283) and
- Ni-3 to Zn-3 ($p = 0.15$),

whereas germination rate is significantly lower in Ni-3 (35.56 ± 19.62) compared to all other HM groups (Ni-4: 68.11 ± 11.02, C: 64.67 ± 13.59, Zn-4: 74.0 ± 18.55, Zn-3: 63.33 ± 21.34).

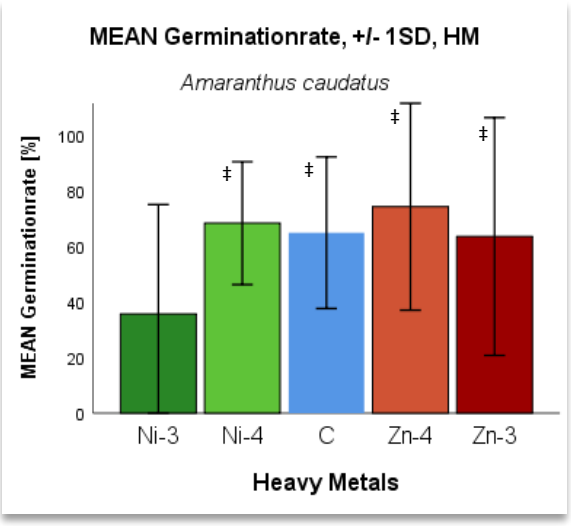


Fig. 47: Bar chart showing mean of germination rate of Amaranthus caudatus*, error bars: ± 1SD, Significant increase of germination rate from Ni-3 to any other group marked with ‡*

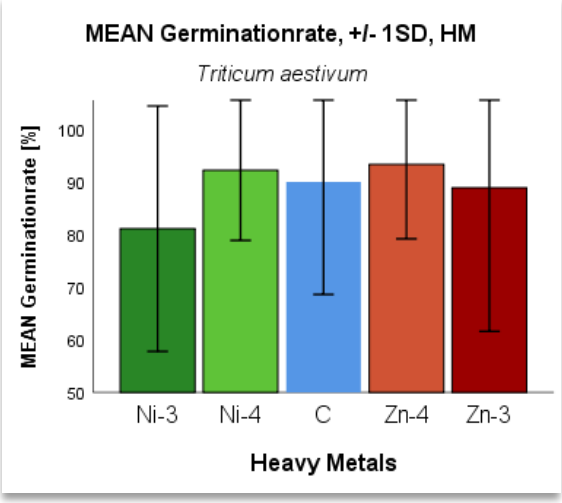


Fig. 48: Bar chart showing mean of germination rate of Triticum aestivum*, error bars: ± 1SD, no significant difference of germination rate between HM groups.*

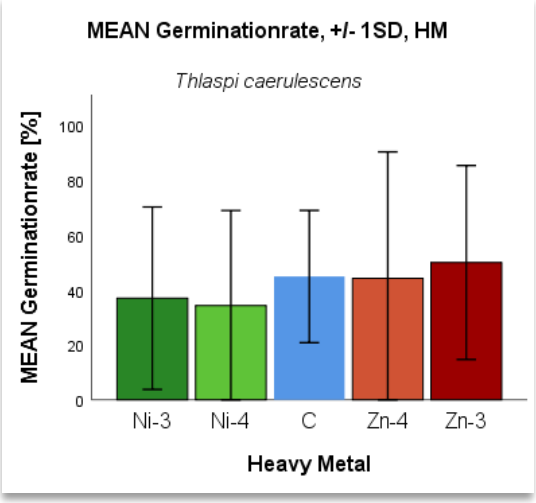


Fig. 49: Bar chart showing mean of germination rate of Thlaspi caerulescens*, error bars: ± 1SD, no significant difference of germination rate between HM groups.*

Descriptive statistics

Germination Rate in Hydroponics

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Ni-3	9	81,1111	11,66667	3,88889	72,1433	90,0789	70,00	100,00
Ni-4	9	92,2222	6,66667	2,22222	87,0978	97,3467	80,00	100,00
C	8	90,0000	10,69045	3,77964	81,0626	98,9374	70,00	100,00
Zn-4	9	93,3333	7,07107	2,35702	87,8980	98,7686	80,00	100,00
Zn-3	9	88,8889	13,64225	4,54742	78,4025	99,3753	60,00	100,00

Tab. 14: Descriptive statistics for germination rate (HM treatment) of Triticum aestivum

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Ni-3	9	35,5556	19,61575	6,53858	20,4776	50,6336	13,00	80,00
Ni-4	9	68,1111	11,01640	3,67213	59,6432	76,5791	53,00	93,00
C	9	64,6667	13,59228	4,53076	54,2187	75,1146	50,00	90,00
Zn-4	9	74,0000	18,54724	6,18241	59,7433	88,2567	50,00	100,00
Zn-3	9	63,3333	21,32487	7,10829	46,9416	79,7251	30,00	100,00

Tab. 15: Descriptive statistics for germination rate (HM treatment) of Amaranthus caudatus

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Ni-3	9	37,0000	16,59819	5,53273	24,2415	49,7585	20,00	70,00
Ni-4	9	34,3333	17,29162	5,76387	21,0418	47,6248	13,00	57,00
C	9	44,8889	12,03583	4,01194	35,6373	54,1404	27,00	67,00
Zn-4	9	44,2222	23,00966	7,66989	26,5354	61,9090	17,00	80,00
Zn-3	9	50,0000	17,65644	5,88548	36,4281	63,5719	33,00	80,00

Tab. 16: Descriptive statistics for germination rate (HM treatment) of Thlaspi caerulescens

4.2.2. Growth Performance

(A) Overview

Shoots of *Triticum aestivum* had similar length in lower concentration of Ni, the control and both Zn groups, and decreased noticeably in Ni-3 compared to all other groups. Enough biomass was accumulated and execution of further experiments (ROS-dyes, cell plasmolysis tests and ICP) was possible (Fig.50(a)).

Amaranthus caudatus grew at the beginning nicely, yet seedlings eventually died, and no further experiments could be carried out. Amaranth did not grow in Ni-3 and grew seemingly better in Zn than in the control (Fig.50(b)).

Same goes for *Thlaspi caerulescens*. When plants were taken out of the pots, seedlings were found slumped under the first layer of the substrate, some still carrying green leaves, but with very weak stem (Fig.50(c)).

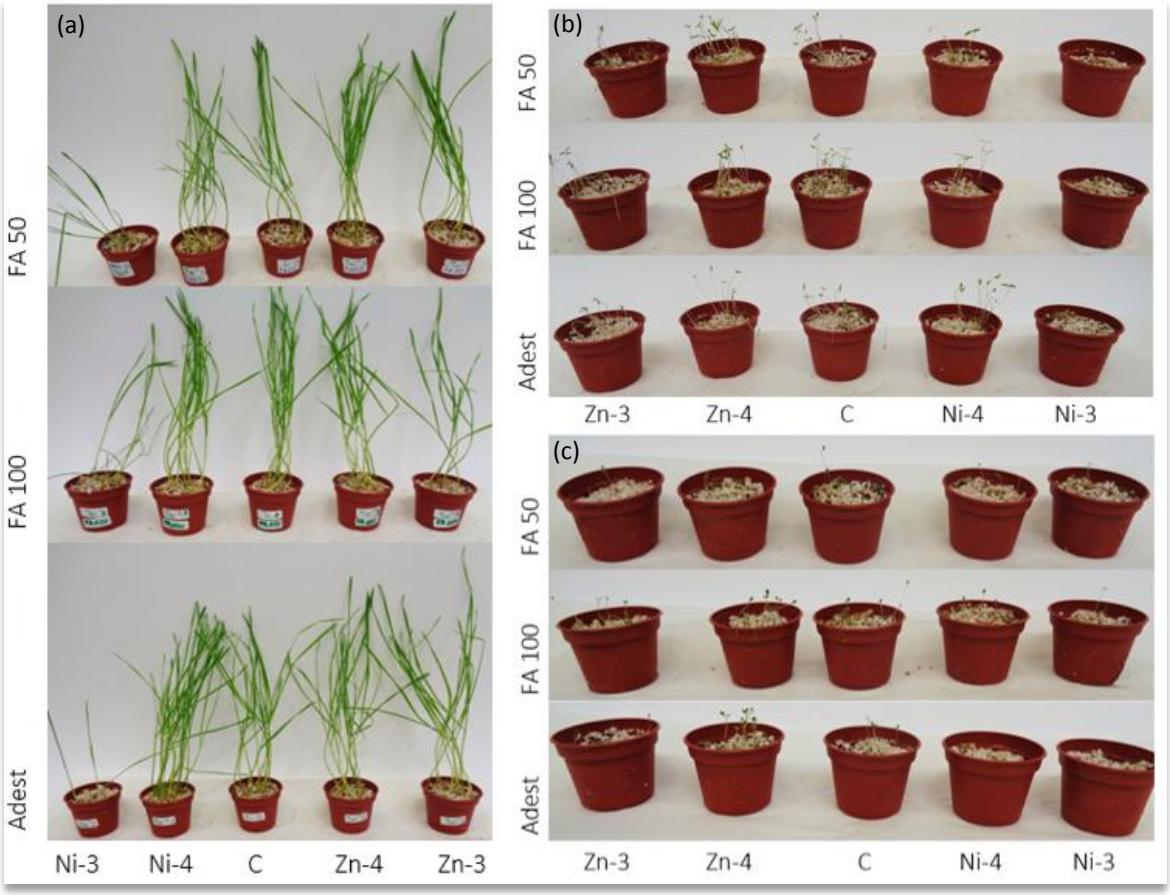


Fig. 50: Overview growth performance of Triticum aestivum *(a),* Amaranthus caudatus *(b) and* Thlaspi caerulescens *(c)*
FA50 = 2% FA, FA100 = 1% FA

Further analysis of plant performance was done with *Triticum aestivum*, measuring and analysing different growth and stress parameters.

(B) Growth Parameters

Triticum aestivum – Maximum Root Length

The average maximum root length for *Triticum aestivum* is 7.1 ± 2.3 cm for the control, 6.6 ± 2.9 cm for Ni-4, 1.8 ± 0.8 cm for Ni-3, 6.6 ± 2.9 cm for Zn-4 and 6.4 ± 2.2 cm for Zn-3.

In the control maximum root length is 7.3 ± 2.3 cm on average. When treated with FA100 it is 6.5 ± 2.1 cm and with FA50 it is 7.8 ± 2.1 cm.

In Ni-4 maximum root length is 6.7 ± 3.2 cm, with FA100 it is 7.2 ± 2.3 cm and treated with FA50 it is 5.9 ± 3.1 cm. In Ni-3 maximum root length is 1.7 ± 0.7 cm, with FA100 it is 2 ± 0.9 cm and treated with FA50 it is 1.7 ± 0.6 cm.

In Zn-4 maximum root length is 7.1 ± 2.4 cm, treated with FA100 it is 6.4 ± 1.5 cm and treated with FA50 6.3 ± 1.8 cm on average. In Zn-3 maximum root length is 7.1 ± 1.9 cm, treated with FA100 it is 5.7 ± 2.4 cm and treated with FA50 6.3 ± 2.4 cm on average.

Statistical analysis of differences between HMs, FA and different treatment combinations ((C, Ni, Zn) x (Adest, FA500, FA100)) showed significant differences between HM treatments. Mean of maximum root length increased from Ni-3 (1.8 ± 0.8) to all other HM groups (C: 7.1 ± 2.2, Ni-4: 6.6 ± 2.9, Zn-4: 6.6 ± 1.9, Zn-3: 6.4 ± 2.3).

In the following a detailed report of the statistical analysis is given.

Two Way ANOVA showed significance on p <0.05 level in the corrected model for root length (Tab.17, Fig.51) ($F(14,151) = 9.251$, p <0.001 ($1.7653*10^{-14}$), $R^2 = 0.462$, $adj.R^2 = 0.412$), yet homogeneity criterion was not met (F-Test for Heteroskedasticity: $F = 8.630$, $df_1 = 1$, $df_2 = 164$, $p = 0.004$ and Levene´s Test of Equality of Error Variances, based on mean: *Levene statistic* = 2.250, $df_1 = 14$, $df_2 = 151$, $p = 0.008$.

No significant main effect could be observed for treatment with FA ($F(2,151) = 0.583$, $p = 0.559$, $\eta_p^2 = 0.008$) and no interactive effect of HM treatment and FA treatment was found ($F(8,151) = 0.733$, $p = 0.662$, $\eta_p^2 = 0.037$). HM treatment on root length turned out to be a significant main effect ($F(4,151) = 29.101$, p <0.001 ($6.1852*10^{-18}$), $\eta_p^2 = 0.435$) with Cohen´s value ($f = 0.88$) that suggests strong effect size.

Descriptive Statistics

Maximum Root Length

Heavymetals	Treatment	Mean	Std. Deviation	N
C	Adest	7,333	2,3179	9
	FA100	6,536	2,0541	14
	FA50	7,750	2,1288	10
	Total	7,121	2,1489	33
Ni-4	Adest	6,746	3,2459	13
	FA100	7,230	2,2652	10
	FA50	5,867	3,1288	12
	Total	6,583	2,9255	35
Ni-3	Adest	1,663	,7482	8
	FA100	1,979	,8523	14
	FA50	1,729	,6211	7
	Total	1,831	,7626	29
Zn-4	Adest	7,133	2,3685	12
	FA100	6,489	1,4512	9
	FA50	6,307	1,7842	15
	Total	6,628	1,9117	36
Zn-3	Adest	7,083	1,9343	12
	FA100	5,740	2,4213	10
	FA50	6,336	2,4043	11
	Total	6,427	2,2480	33

Tab. 17: Max. root length of Triticum aestivum *(hydroponics), FA50 = 2% FA, FA100 = 1% FA*

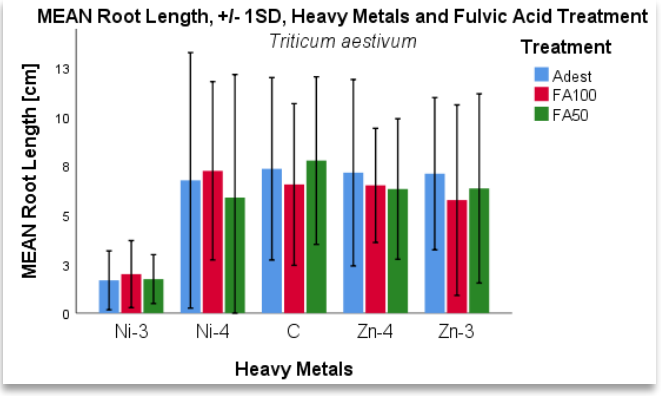


Fig. 51: Bar chart showing mean of root length of Triticum aestivum*, error bars: ± 1SD, no significant interactive effects, FA50 = 2% FA, FA100 = 1% FA*

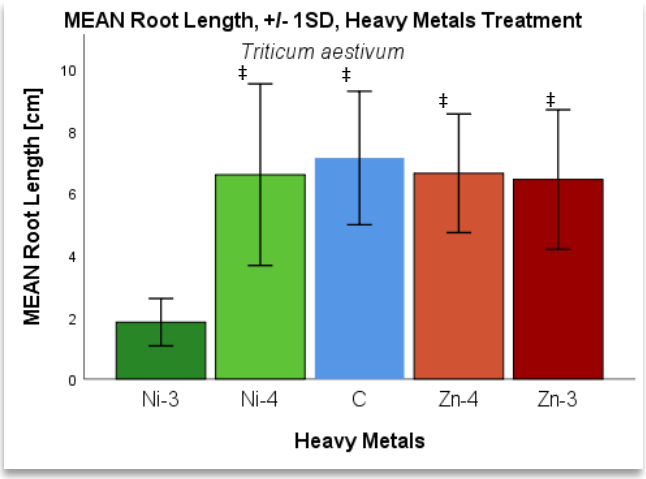


Fig. 52: Bar chart showing mean of root length of Triticum aestivum*, hydroponics, error bars: ± 1SD, Significant increase of mean root length from Ni-3 to groups marked with ‡.*

Bonferroni Post-hoc Tests showed significant differences of (Fig.52):

- Ni-3 to control (p <0.001 ($2.6628*10^{-16}$)),
- Ni-3 to Ni-4 (p <0.001 ($4.4118*10^{-14}$)),
- Ni-3 to Zn-4 (p <0.001 ($1.9513*10^{-14}$)) and
- Ni-3 to Zn-3 (p <0.001 ($4.533*10^{-13}$)),

Whereby mean of maximum root length increased, when comparing Ni-3 (1.83 ± 0.76) to all other groups

(C: 7.12 ± 2.15;
Ni-4: 6.58 ± 2.93;
Zn-4: 6.63 ± 1.91;
Zn-3: 6.43 ± 2.25).

4.2.3. Determination of Stress Levels

Different methods were used to evaluate if HM influenced parameters, indicating stress in *Triticum aestivum* plants.

(A) PEAS Chlorophyll Fluorimeter

Handy PEAS Chlorophyll Fluorimeter was used to measure *Fv/Fm*, an indicator for the maximum efficiency of the PSII. This ratio is very sensitive to stress and low values are indicators for the stress levels of plants. The upper side of the leaves was covered with clips, darkened for 20 minutes and then one measurement per pot – therefore three measurements per treatment – were taken.

The mean of *Fv/Fm* for *Triticum aestivum* in the control is 0.67 ± 0.06, in Ni-4 0.62 ± 0.08, in Ni-3 0.32 ± 0.26, in Zn-4 0.57 ± 0.2 and in Zn-3 it is 0.61 ± 0.11.

In the control *Fv/Fm* is 0.68 ± 0.08 on average, treated with FA100 it is 0.72 ± 0.01 and with FA50 it is 0.62 ± 0.03.

In Ni-4 the ratio amounts to 0.54 ± 0.05, with FA100 treatment to 0.65 ± 0.02 and with FA50 to 0.66 ± 0.11. In Ni-4 it is way lower with 0.26 ± 0.21, treated with FA100 it is 0.30 ± 0.40 and with FA50 it is 0.39 ± 0.31.

In Zn-4 *Fv/Fm* is 0.46 ± 0.13, treated with FA100 it is 0.51 ± 0.27 and with FA50 it is 0.74 ± 0.04. The *Fv/Fm* ratio in Zn-3 is 0.67 ± 0.03, treated with FA100 it is 0.52 ± 0.16 and treated with FA50 it is 0.64 ± 0.07.

Statistical analysis of differences between HMs, FA and different treatment combinations ((C, Ni, Zn) x (Adest, FA100, FA50)) showed significant differences between HM treatments. *Fv/Fm* ratio was significantly lower in Ni-3 (1.8 ± 0.8) compared to all other HM groups (C: 0.67 ± 0.06, Ni-4: 0.62 ± 0.08, Zn-4: 0.57 ± 0.2, Zn-3: 0.61 ± 0.11).

In the following a detailed report of the statistical analysis is given.

Two Way ANOVA showed significance on p <0.05 level in the corrected model for *Fv/Fm* (Tab.18, Fig.53) ($F(14, 29) = 2.472$, $p = 0.019$, $R^2 = 0.544$, $adj.R^2 = 0.324$), yet homogeneity criterion was not met (F-Test for Heteroskedasticity: $F = 27.476$, $df_1 = 1$, $df_2 = 42$, $p = 0.001$ (0.000005) and Levene´s Test of Equality of Error Variances, based on mean: *Levene statistic* = 5.240, $df_1 = 14$, $df_2 = 29$, p <0.001 (0.000084)).

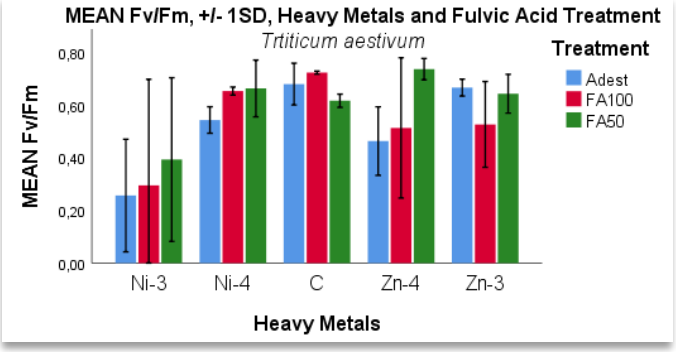


Fig. 53: Bar chart showing mean of Fv/Fm *of* Triticum aestivum *(hydroponics), error bars: ± 1SD, no significant interactive effects. FA50 = 2% FA, FA100 = 1% FA*

No significant main effect could be observed for treatment with FA ($F(2,29) = 1.270$, $p = 0.296$, $\eta_p^2 = 0.081$). HM treatment on *Fv/Fm* turned out to be a significant main effect ($F(4, 29)=6.266$, $p=0.001$, $\eta_p^2 =0.464$) with Cohen´s value (f=0.93) that suggests strong effect size.
No interactive effect of HM treatment and FA treatment was found ($F(8,29) = 0.837$, $p = 0.578$, $\eta_p^2 = 0.188$).

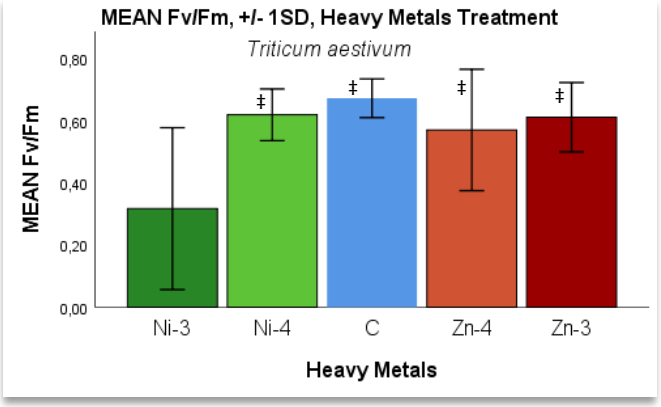


Fig. 54: Bar chart showing mean of Fv/Fm *of* Triticum aestivum*, hydroponics, error bars: ± 1SD, significant increase of mean* Fv/Fm *from Ni-3 to groups marked with ‡.*

Bonferroni Post-hoc Tests showed significant differences of
Ni-3 to control (p = 0.001),
Ni-3 to Ni-4 (p = 0.005),
Ni-3 to Zn-4 (p = 0.027) and
Ni-3 to Zn-3 (p = 0.007),

whereby plants are more stressed in Ni-3 (0.32 ± 0.13) compared to all other groups
(C: 0.67 ± 0.06;
Ni-4: 0.62 ± 0.08;
Zn-4: 0.57 ± 0.20;
Zn-3: 0.61 ± 0.11.
Given the Means of each group all plants show a *Fv/Fm* suggesting stress (Tab.18).

Descriptive Statistics

PEAS Values

Heavymetals	Treatment	Mean	Std. Deviation	N
C	Adest	,6800	,07937	3
	FA100	,7233	,00577	3
	FA50	,6167	,02517	3
	Total	,6733	,06245	9
Ni-4	Adest	,5433	,05033	3
	FA100	,6533	,01528	3
	FA50	,6633	,10786	3
	Total	,6200	,08322	9
Ni-3	Adest	,2567	,21385	3
	FA100	,2950	,40305	2
	FA50	,3933	,31086	3
	Total	,3175	,26092	8
Zn-4	Adest	,4633	,13013	3
	FA100	,5133	,26652	3
	FA50	,7367	,04041	3
	Total	,5711	,19567	9
Zn-3	Adest	,6667	,03215	3
	FA100	,5267	,16289	3
	FA50	,6433	,07371	3
	Total	,6122	,11167	9

Tab. 18: Descriptive statistics for Fv/Fm (HM and FA Treatment) of Triticum aestivum *(hydroponics), FA50 = 2% FA, FA100 = 1% FA*

LSD Test for Simple Effects showed significant effects in Zn between
Adest and FA50 (p = 0.044).

Plants had significant higher Fv/Fm in FA50 (0.74 ± 0.04) treated plants than in Adest group (0.46 ± 0.13) (Fig.55).

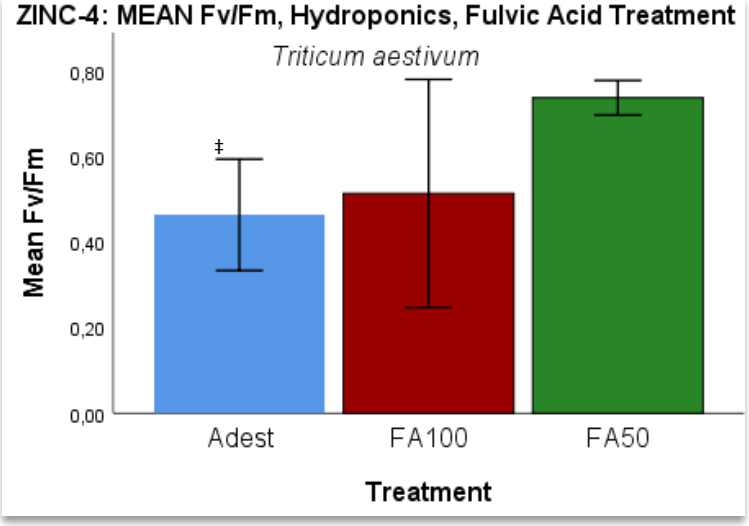


Fig. 55: Bar chart showing mean of Fv/Fm for Triticum aestivum in Zn-4, hydroponics, Significant differences to FA50 marked with ‡.

(B) ROS-Dyes

3,3´-Diaminobenzidine (DAB) and nitrotetrazolium blue chloride (NBT) were used to detect stress related H_2O_2 and O_2^- in roots of *Triticum aestivum* plants.

DAB

Plants of the control showed view brown staining in lower part of the roots to root tip, amount of staining visibly reduced with increasing FA concentration. Plants treated with FA50 only showed brown discolouration in the very last part of the root tips (Fig.56).

Slightly increased effect of DAB was seen in Ni-4, FA reduced the surface covered with DAB staining (Fig.57). In Zn-4 without FA roots were completely brown, gradually shading to black towards root tips (Fig.59). Again, FA treated groups showed discoloration only at root tips, in higher FA concentration visibly less.

While FA50 showed least staining in Ni-4, Zn-4 and the control, in higher HM concentrations (1 mmol) FA100 performed best. In Ni-3 and Zn-3 stains were darker than in other groups, suggesting higher stress levels. FA50 showed slightly better results, and in FA100 only root tips were brown (Fig.58, Fig.60).

NBT

Different results could be observed in NBT stained plant roots. In the control plants non-treated roots showed less blue staining than FA100 and FA50. FA50 showed more blue discolouration than FA100 (Fig.61).

In Zn-4 FA100 showed NBT effect only on one root tip (out of five), while roots of FA50 treated plants and the control were completely light blue (Fig.64).

In Ni-4 non-treated plants showed least blue discolouration. No difference between FA100 and FA50 was observed (Fig.63).

In Zn-3 all three treatment groups showed equal blue staining of the roots (Fig.62).

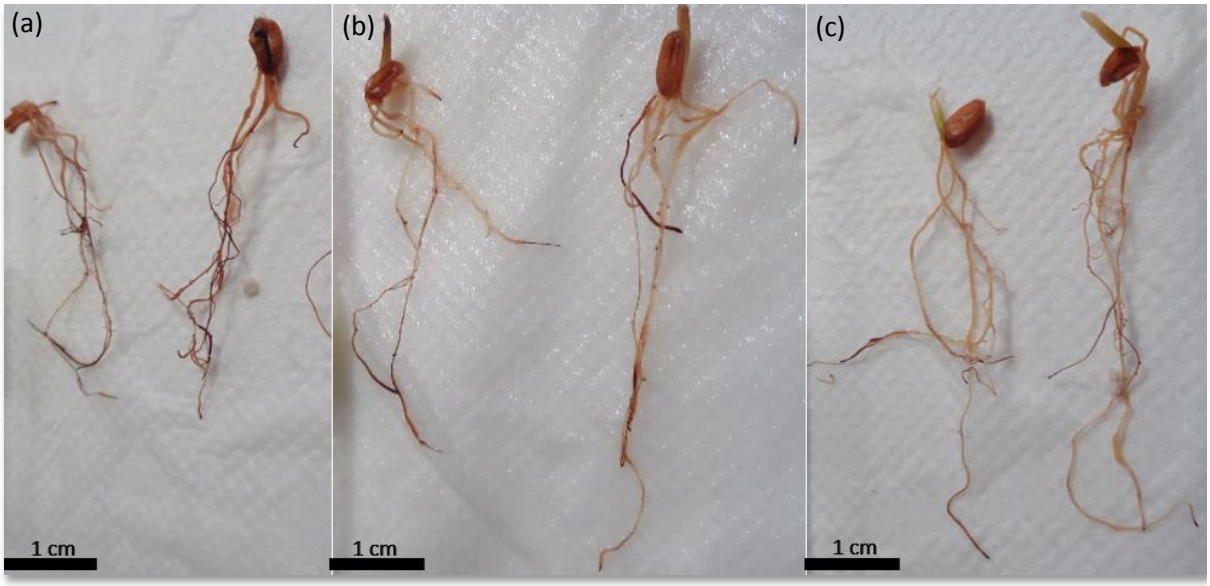


Fig. 56: Triticum aestivum*, control, DAB, chlorophyll removed (a) Adest (b) FA100 = 1% FA (c) FA50 = 2% FA*

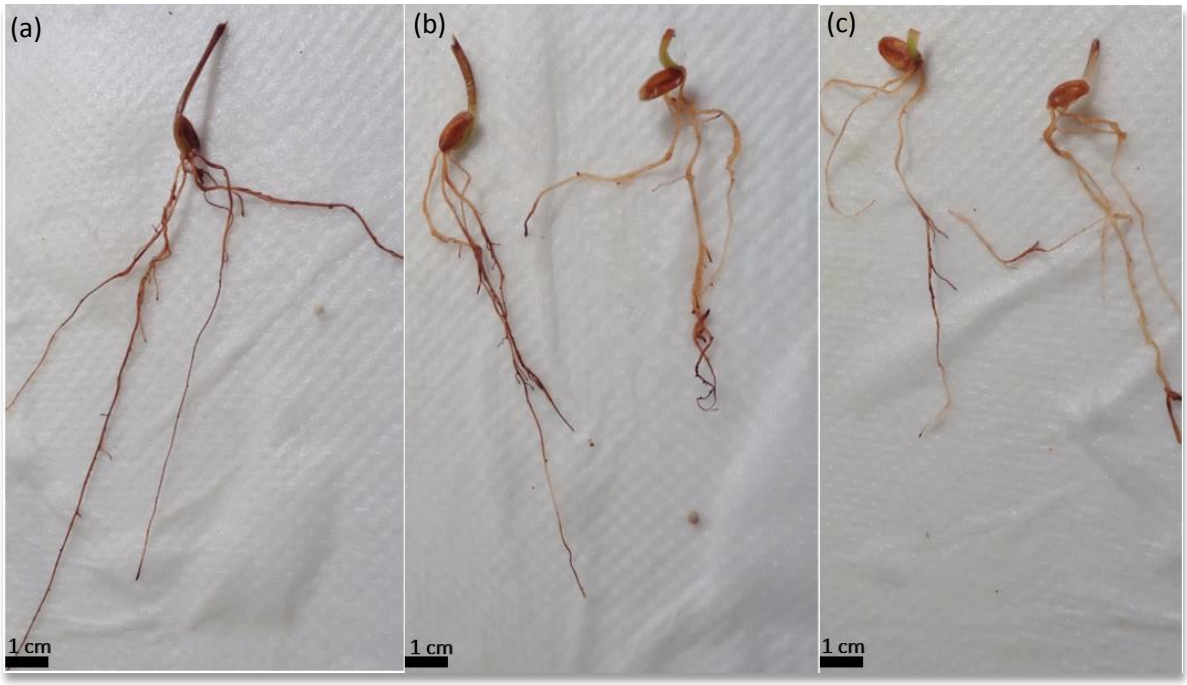


Fig. 57: Triticum aestivum*, Ni-4, DAB, chlorophyll removed (a) Adest (b) FA100 = 1% FA (c) FA50 = 2% FA*

Fig. 58: Triticum aestivum*, Ni-3, DAB, chlorophyll removed (a) Adest (b) FA100 = 1% FA (c) FA50 = 2% FA*

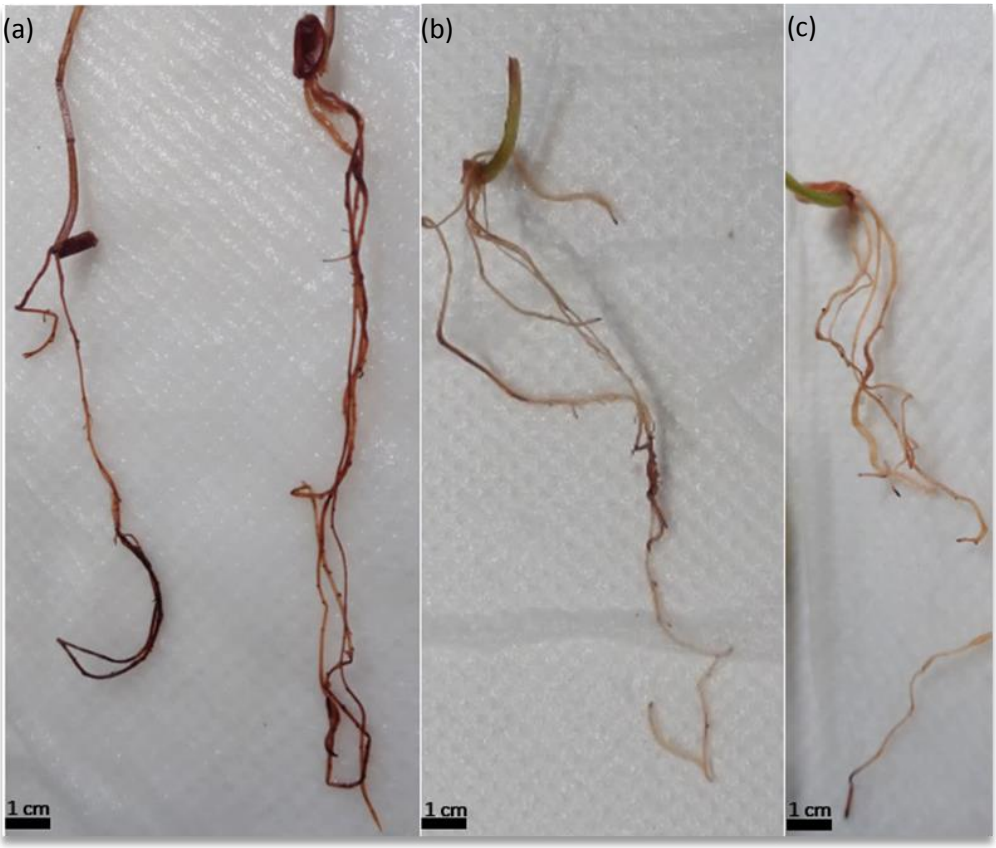


Fig. 59: Triticum aestivum*, Zn-4, DAB, chlorophyll removed (a) Adest (b) FA100 = 1% FA (c) FA50 = 2% FA*

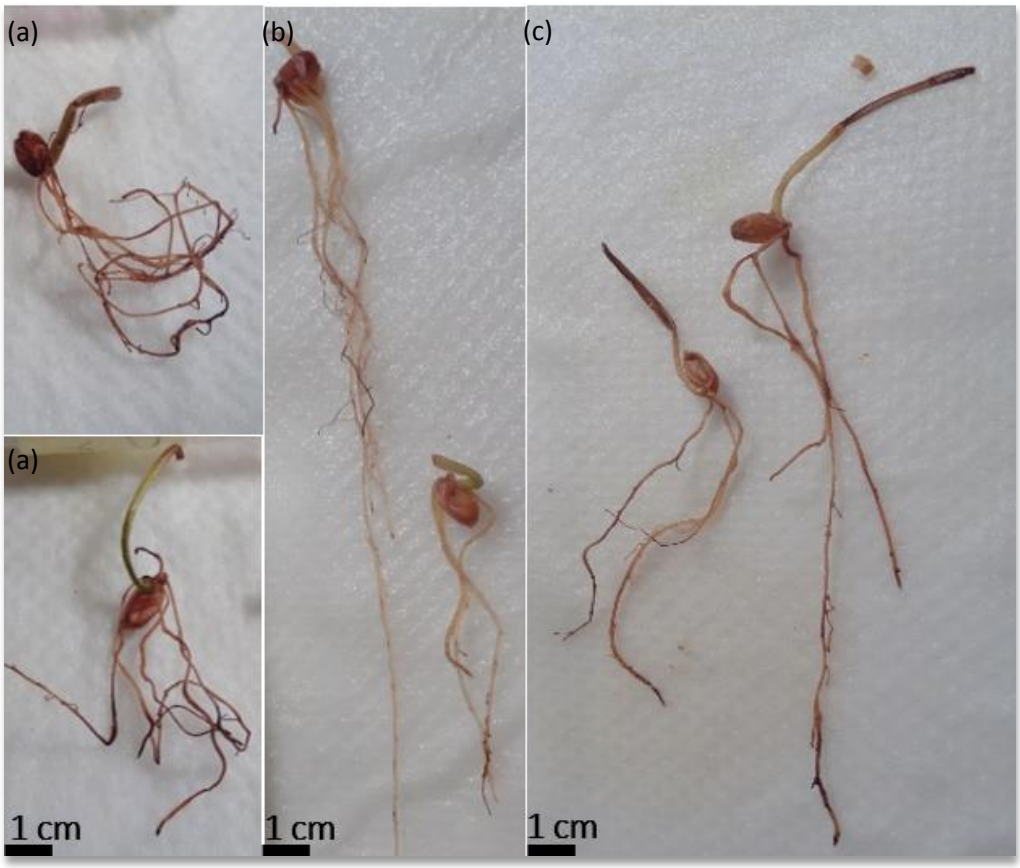


Fig. 60: Triticum aestivum*, Zn-3, DAB, chlorophyll removed (a) Adest (b) FA100 = 1% FA (c) FA50 = 2% FA*

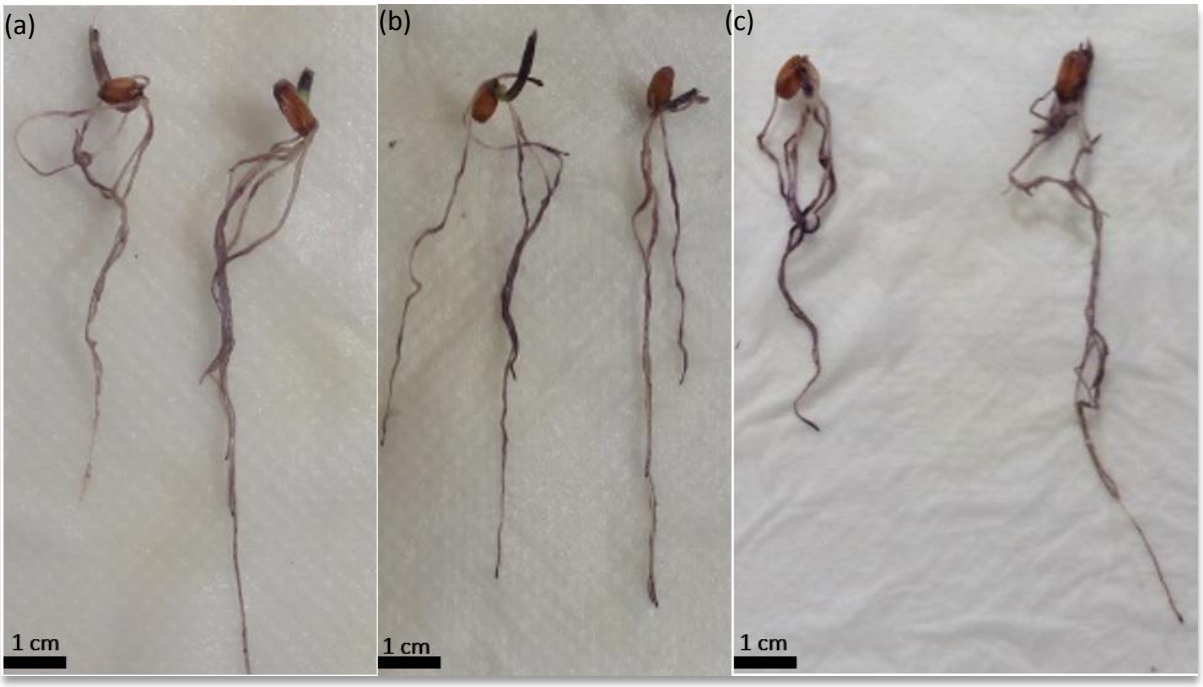


Fig. 61: Triticum aestivum*, C, NBT, (a) Adest (b) FA100 = 1% FA (c) FA50 = 2% FA*

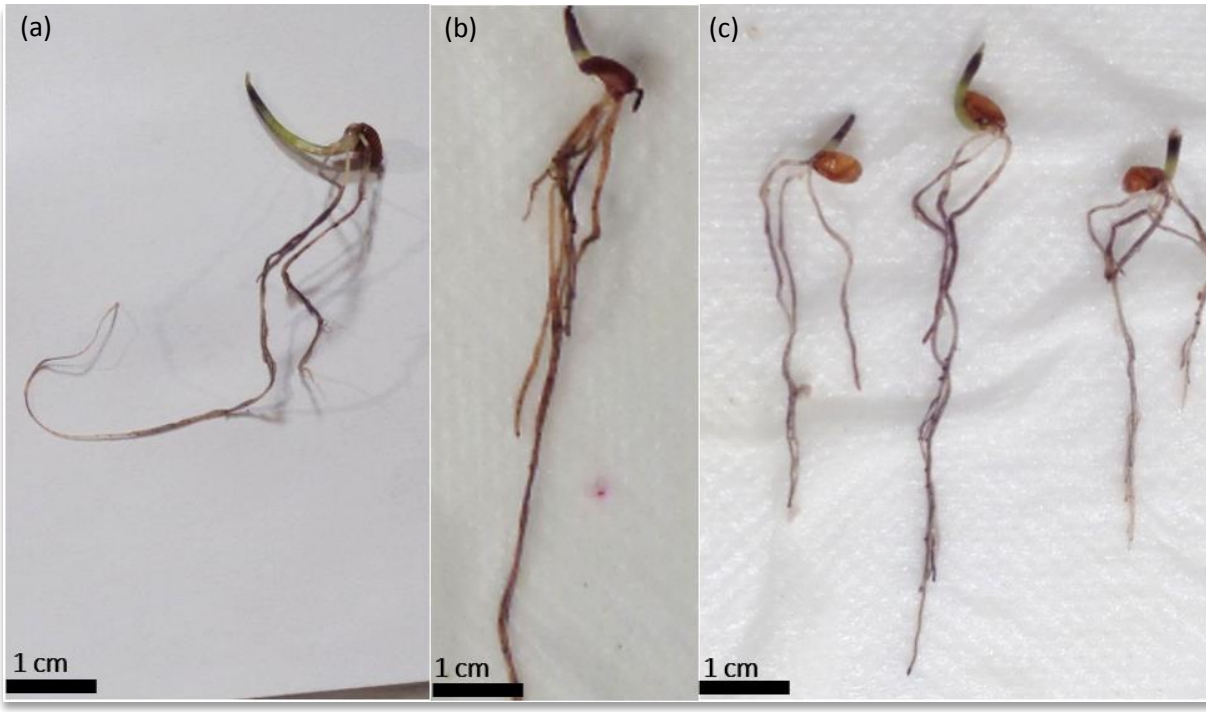


Fig. 62: Triticum aestivum*, Zn-3, NBT, (a) Adest (b) FA100 = 1% FA (c) FA50 = 2% FA*

Fig. 63: Triticum aestivum*, Ni-4, NBT, (a) Adest (b) FA100 = 1% FA (c) FA50 = 2% FA*

Fig. 64: Triticum aestivum*, Zn-4, NBT, (a) Adest (b) FA100 = 1% FA (c) FA50 = 2% FA*

(C) Heavy Metal Tolerance Tests to Determine Cell Vitality of Wheat Leaves Subjected to HM Stress

Living plant cells react to different concentration gradients in and outside their cells with plasmolysis. Sections of *Triticum aestivum* leaves have been put in different concentrations of Zn and Ni to determine cell tolerance to these HM solutions.

Plants	Solutions							
	C	Zn-6	Zn-5	Zn-4	Zn-3	Zn-2	Zn-1	Zn 1
Control	+	+	+	±	-	-	-	-
Zn(-3)	+	+	+	+	-	-	-	-
Ni(-4)	+	+	+	+	+	+	-	-
	C	Ni-6	Ni-5	Ni-4	Ni-3	Ni-2	Ni-1	Ni 1
Control	+	+	+	+	+	-	-	-
Zn(-3)	+	+	+	+	±	-	-	-
Ni(-4)	+	±	±	±	+	+	+	-

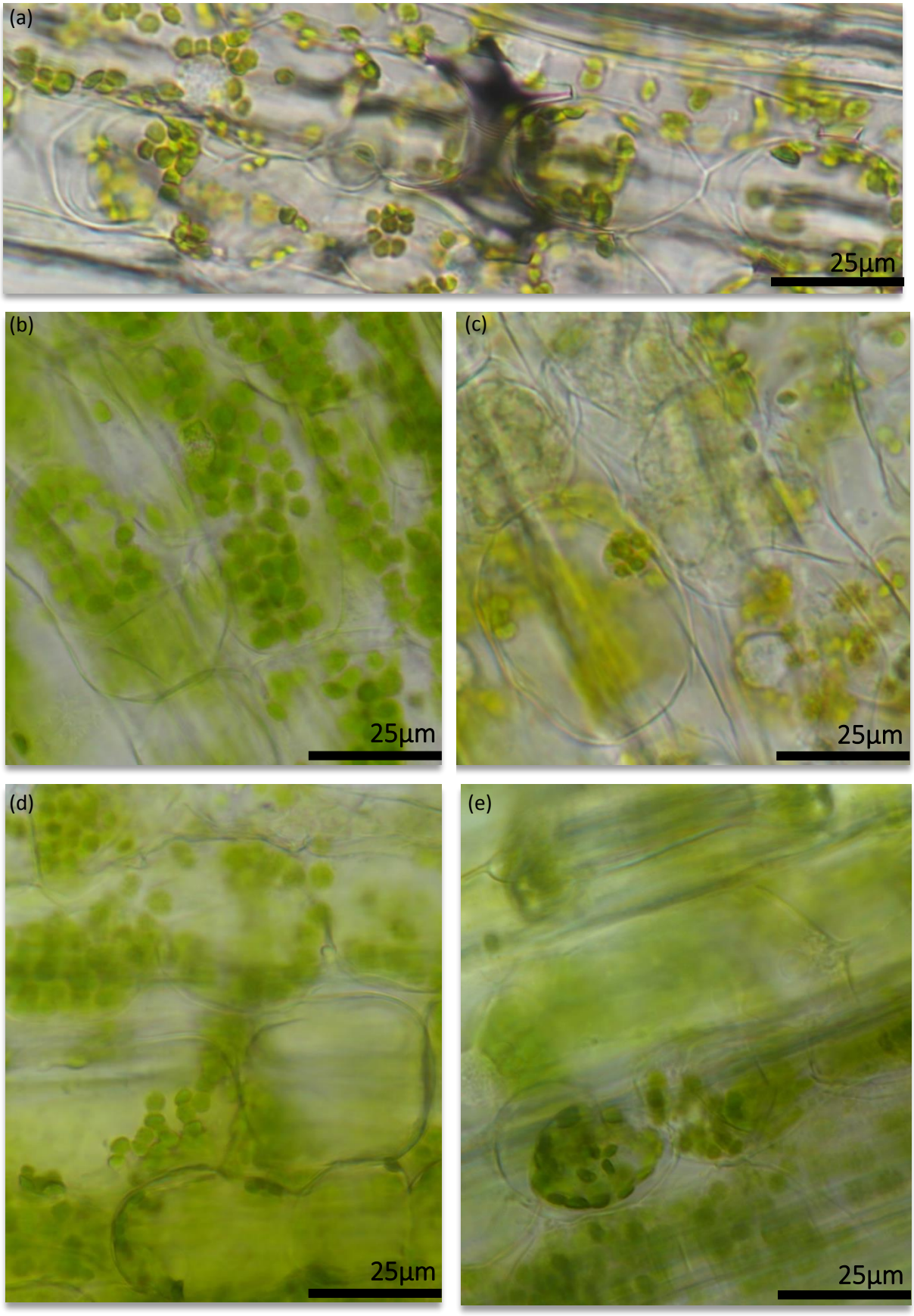
Tab. 19: Vitality of plant cells after immersing them in Zn and Ni solutions of different concentrations for 48 hours. Cells alive: >50% | 50% | <50%

Sections have been put into HM solution for 48 hours and were checked afterwards for plasmolysis when being drenched with mannitol.

Tolerance for different concentrations of Ni and Zn of plants growing in hydroponics, containing 1 mmol Ni, 0.1 mmol Zn as well as in the control, was compared.

Plants, independent of treatment, survived 10-times higher concentrations of Ni than Zn.
Most of cells in sections of the control and 1 mmol Zn are alive. In Zn concentrations up to 0.1 mmol and Ni concentrations up to 1 mmol.
Leaf cells of 0.1 mmol Ni treated plants are tolerant to concentrations up to 100 mmol Zn and 0.1 M Ni.

Plants previously treated with 1 mmol Ni were more tolerant to HM solutions than the control and 0.1 mmol Zn treated plants, dealing with 100-times higher concentrations in both Zn and Ni solutions.


(a)
25µm
(b)
25µm
(c)
25µm
(d)
25µm
(e)
25µm

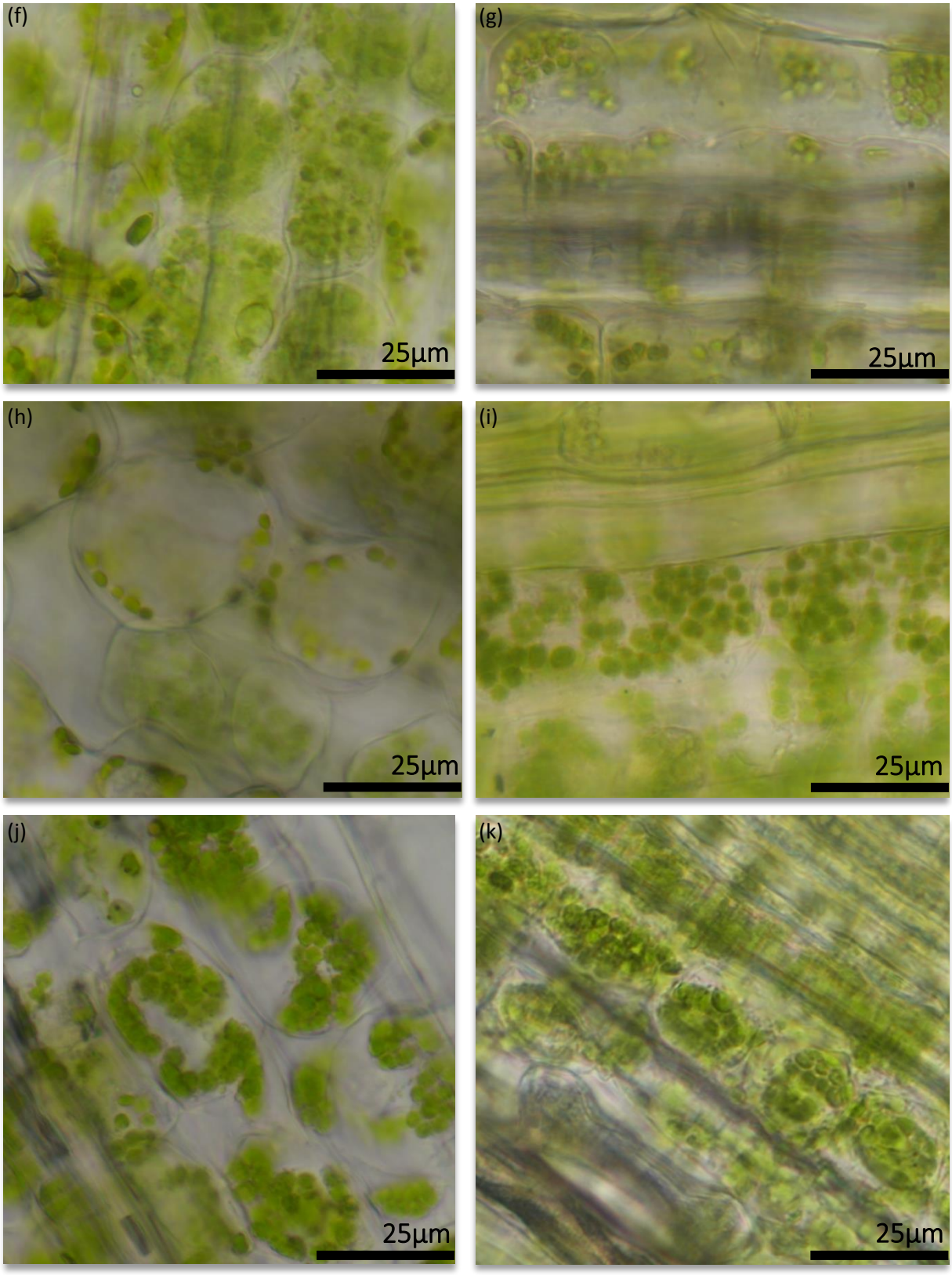


Fig. 65: Triticum aestivum, *Ni-4, sections in (a)C, (b) Ni-6, (c) Zn-6, (d) Ni-5, (e) Zn-5, (f) Ni-4, (g) Zn-4, (h) Ni-3, (i) Zn-3, (j) Ni-1, (k) Zn-1 solution, after drenching with mannitol*

4.2.4. ICP Values and Transfer Factor

To evaluate effect of the different HM and FA treatments on the total Zn and Ni content within the plants, plant material was split into shoots and roots, dried, digested in aqua regia (AR) and HM content measured by ICP-OES.

One AR digest per treatment-combination of HM (Ni-3, Ni-4, C, Zn-4, Zn-3) and FA (Adest, FA50) was prepared, intensity was measured using ICP-OES and finally concentration in mg/kg for each HM of interest was calculated based on solutions (standards) with known concentration. Visualization of the correlation between these concentrations and the intensity measured for Zn and Ni can be seen in the calibration charts (Fig.66).

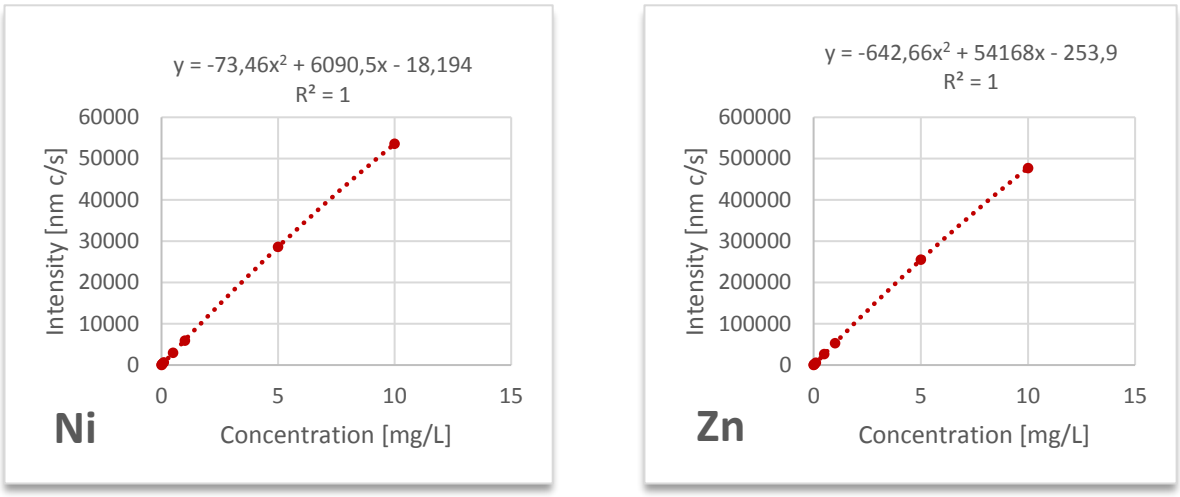


Fig. 66: Calibration charts visualizing correlation of intensity, measured by ICP-OES and (known) conc. of Ni and Zn solutions.

	Nickel					
	[mg/kg]	Ni-3	Ni-4	C	Zn-4	Zn-3
Adest	Root	4706.41	454.80	54.71	79.02	62.45
	Shoot	1328.91	279.52	12.85	16.17	20.43
FA50	Root	5699.70	458.44	115.01	27.26	41.96
	Shoot	2275.48	247.12	25.07	9.93	18.73
	Zinc					
	[mg/kg]	Ni-3	Ni-4	C	Zn-4	Zn-3
Adest	Root	389.96	84.28	150.08	460.49	4771.42
	Shoot	58.24	185.98	186.34	288.50	6006.10
FA50	Root	450.00	138.04	224.30	521.52	3187.60
	Shoot	101.25	109.36	176.19	189.85	3963.67

Tab. 20: MEAN content of Ni and Zn in the different treated groups, plant organs and added HM solutions. FA50 = 2% FA, FA100 = 1% FA, Ni-3 = 1 mmol, Ni-4 = 0.1 mmol, Zn respectively

Ni content is higher in Ni treated groups, decreasing with decreasing Ni concentration. Zn content is higher in Zn treated groups, increasing with increasing Zn concentrations (Tab.20). Ni content decreases from root to shoot, which is reflected in the translocation factor, always below 1. Translocation factors for Zn is most of the time above 1, distinctly decreasing when treated with FA50. FA treatment has no effect on Ni content in wheat plants (Tab.21).

TF (Ni, Adest)	
	Rt→St
Ni-3	0.28
Ni-4	0.61
C	0.23
Zn-4	0.20
Zn-3	0.33

TF (Ni, FA50)	
	Rt→St
Ni-3	0.40
Ni-4	0.54
C	0.22
Zn-4	0.36
Zn-3	0.45

TF (Zn, Adest)	
	Rt→St
Ni-3	0.15
Ni-4	2.21
C	1.24
Zn-4	0.63
Zn-3	1.26

TF (Zn, FA50)	
	Rt→St
Ni-3	0.23
Ni-4	0.79
C	0.79
Zn-4	0.36
Zn-3	1.24

TF	>1	<1

Tab. 21: Translocation factor for Triticum aestivum *(hydroponics) for groups of different treatments (HM, FA)*

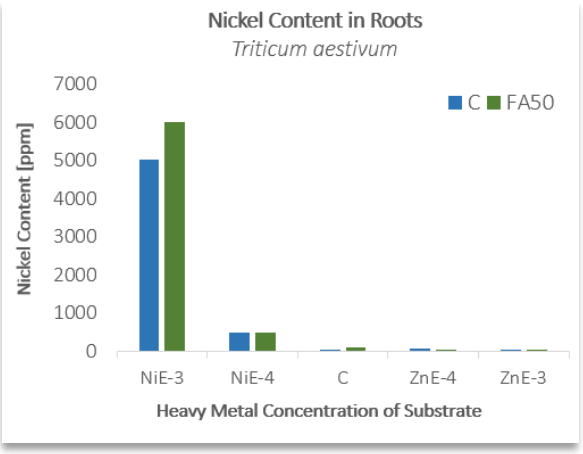


Fig. 67: Bar chart showing Ni content in root of wheat, FA50 = 2% FA, Zn/Ni-3 = 1 mmol, Zn/Ni-4 = 0.1 mmol

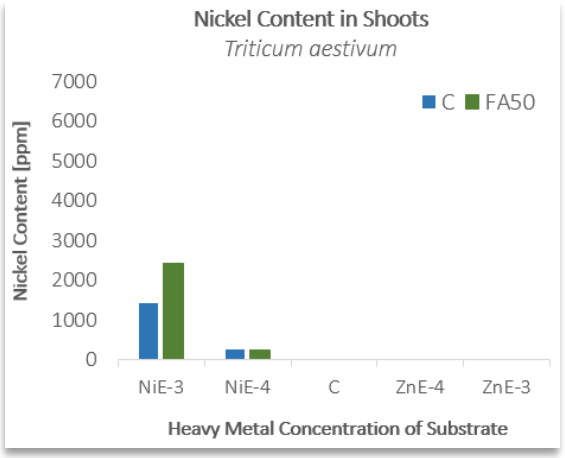


Fig. 68: Bar chart showing Ni content in shoot of wheat, FA50 = 2% FA, Zn/Ni-3 = 1 mmol, Zn/Ni-4 = 0.01 mmol

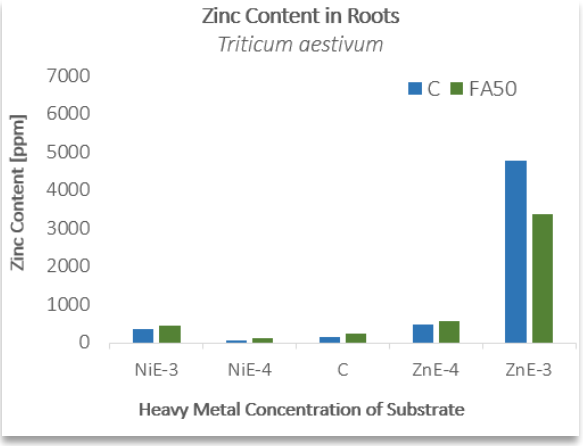


Fig. 69: Bar chart showing Zn content in root of wheat, FA50 = 2% FA, Zn/Ni-3 = 1 mmol, Zn/Ni-4 = 0.1 mmol

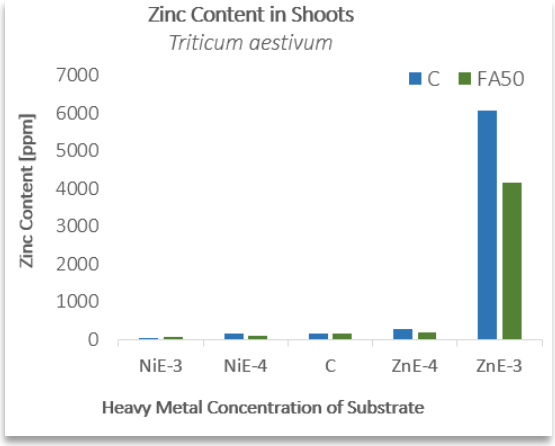


Fig. 70: Bar chart showing Zn content in shoot of wheat, FA50 = 2% FA, Zn/Ni-3 = 1 mmol, Zn/Ni-4 = 0.01 mmol

When comparing total HM content in highest concentration (1 mmol), FA50 causes increase in Ni content of shoots and roots (Fig.67–68) and decrease of Zn content (Fig.69–70).

Difference between 0.1 mmol and 1 mmol HM solution, approximately corresponds to the difference between the HM content of these two concentrations within the plant (Fig.67–70).

4.3. Soil Cultures

Aim of the soil experiments was to create a setting closer to reality and to compare it with the effects of HM and FA observed in the hydroponics. More repeats and concentrations were prepared for *Triticum aestivum* for statistical analysis.

4.3.1. Germination rate

Germination rate in % was determined on the 15th day after sowing.

Ni5(-2) (50 mmol)			Ni-2 (10 mmol)			Ni-3 (1 mmol)			Ni-4 (0.1 mmol)			C			Zn-4 (0.1 mmol)			Zn-3 (1 mmol)			Zn-2 (10 mmol)			Zn-1 (100 mmol)		
Triticum aestivum																										
0	0	0	40	20	20	100	100	0	80	60	80	60	60	60	80	60	80	80	80	80	60	60	80	20	40	0
0	0	0	40	20	40	100	40	20	80	100	80	60	60	80	100	60	40	100	20	20	100	80	80	0	0	0
0	0	0	40	20	0	20	100	40	40	100	80	80	80	80	20	80	80	100	80	80	100	60	80	0	0	0
Amaranthus caudatus																										
No plants of *Amaranthus caudatus* in this concentration planted						20	20	30	20	10	40	30	40	40	20	10	40	10	70	50	No plants of *Amaranthus caudatus* in this concentration planted					
						40	20	30	30	50	30	50	50	30	30	30	60	50	30	30						
						60	30	50	60	50	20	10	0	50	100	70	40	80	70	50						
Thlaspi caerulescens																										
No plants of *Thlaspi caerulescens* in this concentration planted						10	40	10	50	100	50	30	50	20	70	70	0	40	50	40	No plants of *Thlapsi caerulescens* in this concentration planted					
						60	50	0	80	10	50	40	60	70	60	60	40	50	40	70						
						70	60	20	60	50	50	10	90	40	30	10	30	0	70	70						

colours defined: >70% | 70–50% | <50%

Tab. 22: Germination rates in %

Two Way ANOVA showed no significant differences of germination rate between HM groups on p <0.05 level for *Thlaspi caerulescens* ($F(4,40) = 0.821$, $p = 0.519$)(Fig.72, Tab.24), and *Amaranthus caudatus* ($F(4,40) = 1.142$, $p = 0.351$)(Fig.73, Tab.25), but was significant for *Triticum aestivum* ($F(8,72) = 15.075$, p <0.001 ($9.7475*10^{-13}$), $R^2 = 0.626$, $adj.R^2 = 0.585$).

Variance was not homogenous (Levene´s Test of Equality of Error Variances, based on mean: *Levene statistic* = 7.810, $df_1 = 8$, $df_2 = 72$, p <0.001 ($1.7201*10^{-7}$)

Dunnett´s T_3 Post-hoc Tests showed significant differences of

Ni5(-2) to Ni-2 ($p = 0.011$), Ni5(-2) to Ni-4 (p <0.001 (0.000039)), Ni5(-2) to C (p <0.001 (0.000001)), Ni5(-2) to Zn-4 ($p = 0.001$), Ni5(-2) to Zn-3 ($p = 0.003$), Ni5(-2) to Zn-2 (p <0.001 (0.000011))

Ni-2 to Ni-4 (p <0.001 (0.000299)), Ni-2 to C (p <0.001 (0.000115)), Ni-2 to Zn-4 ($p = 0.028$), Ni-2 to Zn-3 ($p = 0.05$), Ni-2 to Zn-2 (p <0.001 (0.000067)),

Zn-1 to Ni-4 (p <0.001 (0.000273)), Zn-1 to C ($p = 0.001$), Zn-1 to Zn-4 ($p = 0.008$), Zn-1 to Zn-3 ($p = 0.014$), Zn-1 to Zn-2 (p <0.001 (0.000187)),

Whereby mean germination rate is significantly lower in Ni5(-2) (0 ± 0), Ni-2 (26.67 ± 14.14) and Zn-1 (15.56 ± 21.86) compared to Ni-4 (77.78 ± 18.56), C (68.89 ± 10.54), Zn-4 (66.67 ± 24.5), Zn-3 (71.11 ± 30.19) and Zn-2 (77.78 ± 15.64) and significant lower in Ni5(-2) than in Ni-2 (Fig.71, Tab.23).

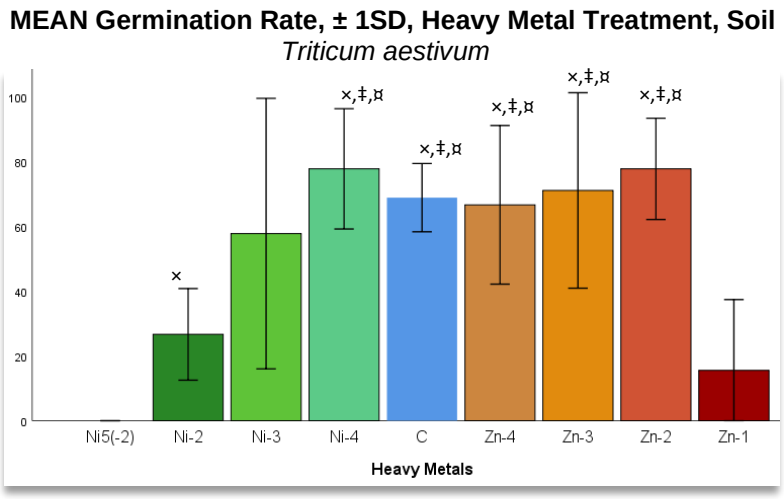


Fig. 71: Bar chart showing mean of germination rate of Triticum aestivum, *soil cultures, error bars: ± 1SD.*
Significant increase of mean germination rate from Ni5(-2) to all bars marked with ×
Significant increase of mean germination rate from Ni-2 to all bars marked with ‡
Significant increase of mean germination rate from Zn-1 to all bars marked with ¤

Descriptive Statistics

Germination Rate
Triticum aestivum

hm	Mean	Std. Deviation	N
Ni5(-2)	,00	,000	9
Ni-2	26,67	14,142	9
Ni-3	57,78	41,767	9
Ni-4	77,78	18,559	9
C	68,89	10,541	9
Zn-4	66,67	24,495	9
Zn-3	71,11	30,185	9
Zn-2	77,78	15,635	9
Zn-1	15,56	21,858	9

Tab. 23: Descriptive statistics for germination rate (HM treatment) of Triticum aestivum *(soil culture)*

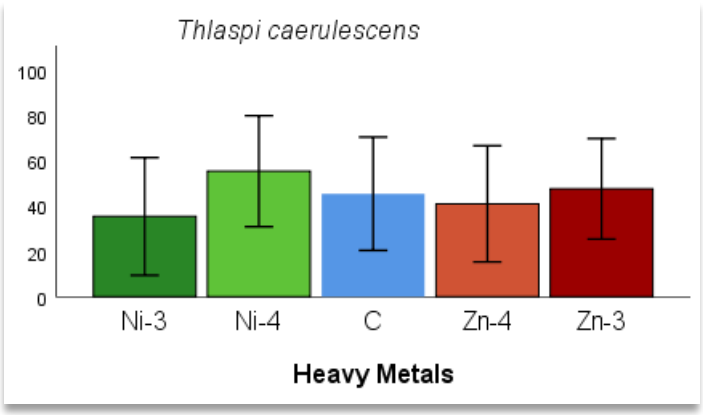


Fig. 72: Bar chart showing mean of germination rate of Thlaspi caerulescens, *soil cultures, error bars: ± 1SD, no significant differences*

Germination Rate
Thlaspi caerulescens

hm	Mean	Std. Deviation	N
Ni-3	35,56	26,034	9
Ni-4	55,56	24,552	9
C	45,56	25,055	9
Zn-4	41,11	25,712	9
Zn-3	47,78	22,236	9

Tab. 24: Descriptive statistics for germination rate (HM treatment) of Thlaspi caerulescens *(soil culture)*

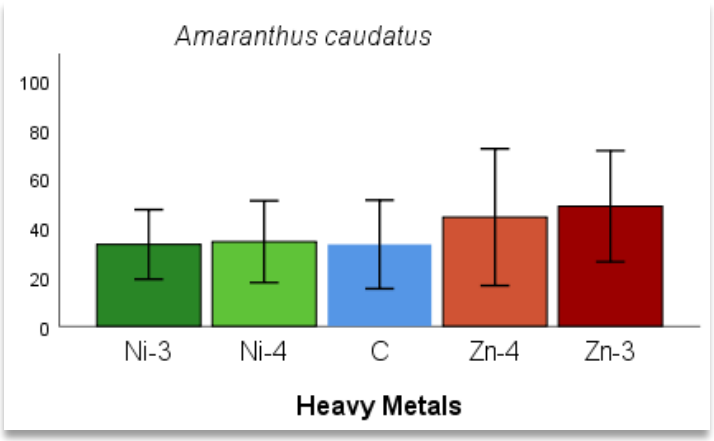


Fig. 73: Bar chart showing mean of germination rate of Amaranthus caudatus, *soil cultures, error bars: ± 1SD, no significant differences*

Germination Rate
Amaranthus caudatus

hm	Mean	Std. Deviation	N
Ni-3	33,33	14,142	9
Ni-4	34,44	16,667	9
C	33,33	18,028	9
Zn-4	44,44	27,889	9
Zn-3	48,89	22,608	9

Tab. 25: Descriptive statistics for Germination rate (HM treatment) of Amaranthus caudatus *(soil culture)*

4.3.2. Growth Performance

(A) Overview

Thlaspi caerulescens grew very slowly and even after three weeks, new seeds germinated, so it was not possible to compare growth parameters as anticipated. Furthermore, no observations concerning FA were possible (Fig.74).

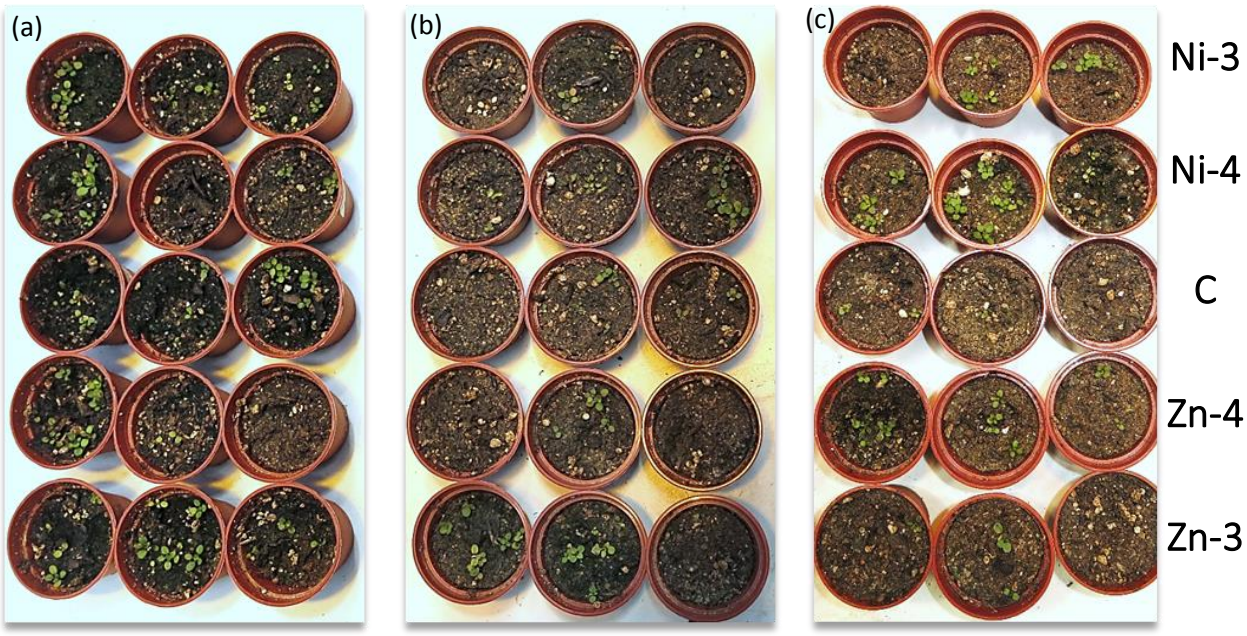


Fig. 74: Thlaspi caerulescens*, exactly one month after sowing (a) FA50 = 2% FA, (b) FA100 = 1% FA, (c) control*

Amaranthus caudatus grew very heterogeneously. Seeds germinated weeks after sowing and plants were not comparable. Roots developed well and detangling would have been impossible without tearing or damaging them. When comparing Amaranth plants two months after germination, leaves of FA50 treated plants had more surface area than the control plants. Shoots of higher FA concentration were more stable than plants without FA treatment and had higher shoot diameter, as can be seen in Fig.39. Plants treated with FA recovered faster after aphid invasion, if aphids were removed regularly (once every two weeks) – when left alone, all plants started to show signs of stunted, distorted and crumpled growth (Fig.75).

Germination of *Triticum aestivum* was completed within the first two weeks. Plant growth went smoothly, and enough biomass was accumulated for further examination. While no difference in shoot and root length was visible for the different FA concentrations (Fig.76), groups could be distinguished by their mechanical properties and touch. Roots of higher FA concentrations were more robust, and plants could be detangled just by pulling them apart without tearing. While roots of plants that have just been treated with water were easily damaged. The two concentrations of FA (FA100 = 1% FA and FA50 = 2% FA) could be easily told apart by the thickness and grip of their roots. The same was true for the shoots. Leaves of FA50 treated plants felt harder and thicker than leaves of the other two groups and surface was not as smooth as of the control plants. Unfortunately, no parameters to quantify these observations were recorded (Fig.76).

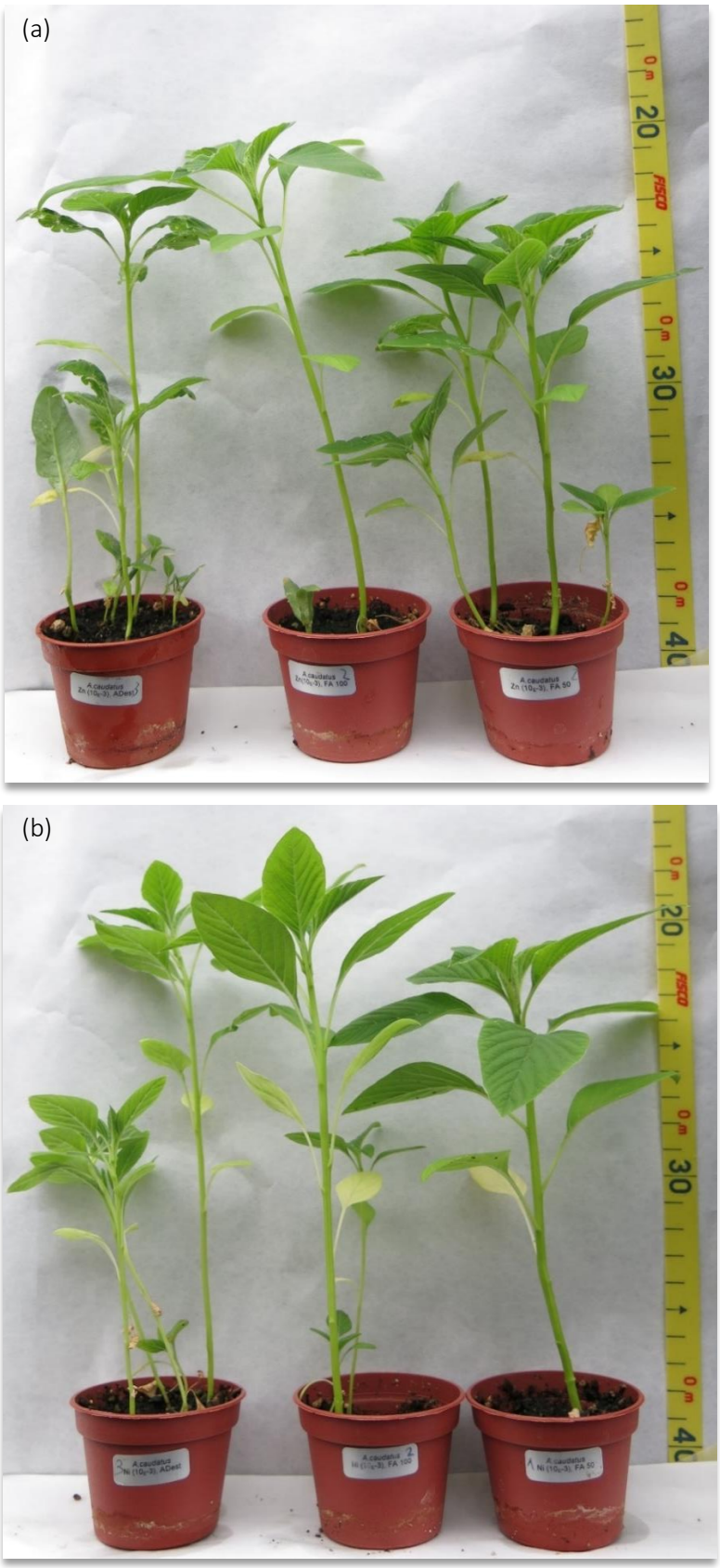


Fig. 75: Amaranthus caudatus*, exactly two months after sowing,*
(a) Zn-3 (b) Ni-3 (from left to right: Adest, FA100, FA50)

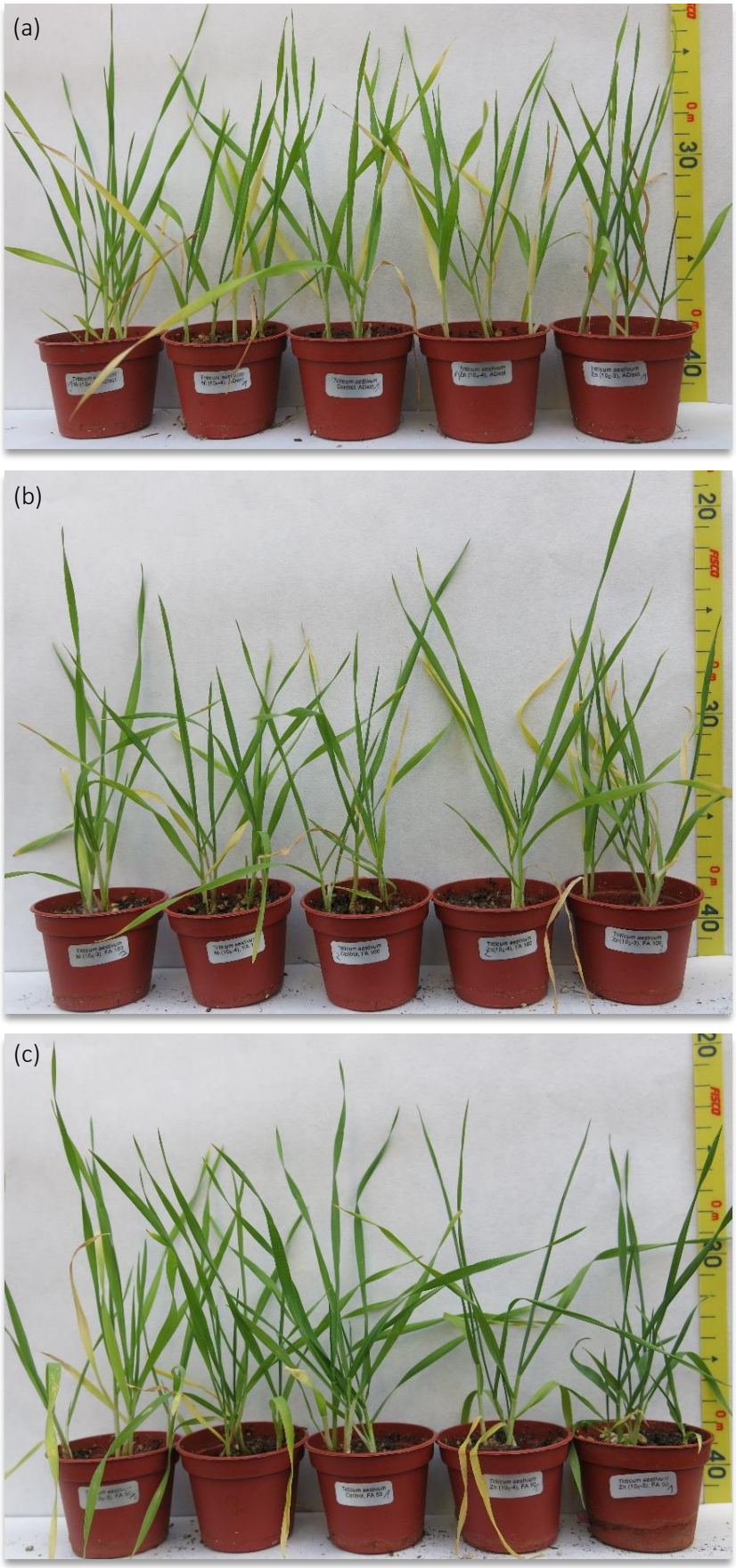


Fig. 76: Triticum aestivum*, exactly one month after sowing, (a) Adest, (b) FA100, (c) FA50, (from left to right: 1 mmol Ni, 0.1 mmol Ni, C, 0.1 mmol Zn, 1 mmol Zn)*

Further analysis of plant performance was done for *Triticum aestivum*, measuring and analysing different growth and stress parameters.

(B) Growth Parameters

Dry Weight

Plant dry weight was determined to see if different mechanical properties of the FA treatments are found in this parameter as well.

Seeds did not germinate in Ni5(-2), in Zn-1 only shoots and in Ni-2 only roots grew. Therefore, dry weight was analysed for whole plants and additionally for shoots and roots separately. Plants or roots and shoots weight per pot were measured and divided per number of plants in this pot. So statistical tests were run over average weight of one plant/shoot/root per pot.

The average dry weight for the whole plant in the control is 0.149 ± 0.019 mg. In Ni-2 it is 0.012 ± 0.019, in Ni-3 0.155 ± 0.089 and in Ni-4 it is 0.158 ± 0.065. In Zn-1 the average dry weight is 0.016 ± 0.005, in Zn-2 it is 0.188 ± 0.018, in Zn-3 it is 0.129 ± 0.005 and in Zn-4 it is 0.155 ± 0.050. Approximately the same values are measured for the two FA treatments, FA100 and FA50. Means are given in Tab.26.

Statistical analysis of differences between HMs, FA and different treatment combinations showed significant differences between HM treatments. Plants growing in Ni-2 contaminated soil have significant lower dry weight than plants of the control, Ni-3, Ni-4, Zn-2, Zn-3 and Zn-4. The same was found for Zn-1. Also, Zn-2 had significant higher dry weight than Ni-3. Furthermore, in the control FA50 led to a significant increase of plant dry weight, compared to FA100.

Comparison of dry weight of shoots and roots separately was not possible for plants which grew on Ni-2 and Zn-1 contaminated substrate, due to poor development. Significant difference between Zn-2 and Ni-3 was found in shoot dry weight as well, in root dry weight no significant difference was observed.

In the control dry weight of shoots is 0.094 ± 0.023 and dry weight of roots is 0.064 ± 0.015. For Ni-3 dry weight for shoots is 0.101 ± 0.035 and for roots it is 0.062 ± 0.019. In Ni-4 average dry weight for shoots amounts to 0.081 ± 0.021 and for roots 0.061 ± 0.018. In Zn-2 for shoots it is 0.118 ± 0.043 and for roots 0.069 ± 0.018. In Zn-3 dry weight of shoots is 0.073 ± 0.007 on average, and for roots it is 0.054 ± 0.007. In Zn-4 mean dry weight of shoots is 0.104 ± 0.027 and of roots it is 0.068 ± 0.013.

Statistical analysis found significant differences between HM treatment in shoot dry weight. Dry weight of shoots in Zn-2 was significantly higher than in Zn-3.
In Zn-2 significant increase of dry weight of roots was found, when comparing roots of plants treated with FA100 to Adest or FA50 treated plants.

Further means and standard deviations of the dry weight of shoots are given in Tab. 27 and in Tab.28 for roots. Following, a detailed report of the statistical analysis.

Plant Dry Weight

Two Way ANOVA showed significance on p <0.05 level in the corrected model for plant dry weight (Tab.26, Fig.77) ($F(23.41) = 6.275$, p <0.001 ($1.9388*10^{-7}$), $R^2 = 0.779$, $adj.R^2 = 0.655$),
yet homogeneity criterion was not met
(Levene´s Test of Equality of Error Variances, based on mean:
Levene statistic = 2.801, $df_1 = 21$, $df_2 = 41$, $p = 0.002$)

No significant main effect could be observed for treatment with FA
($F(2,41) = 0.486$, $p = 0.619$, $\eta_p^2 = 0.023$)
or interactive effects
($F(14,41) = 0.580$, $p = 0.865$, $\eta_p^2 = 0.165$).
But main effect for HM treatment on plant dry weight was found ($F(7,41) = 19.053$, p <0.001 ($4.8165*10^{-11}$), $\eta_p^2 = 0.765$) with Cohen´s value ($f = 1.8$) that suggests strong effect size.

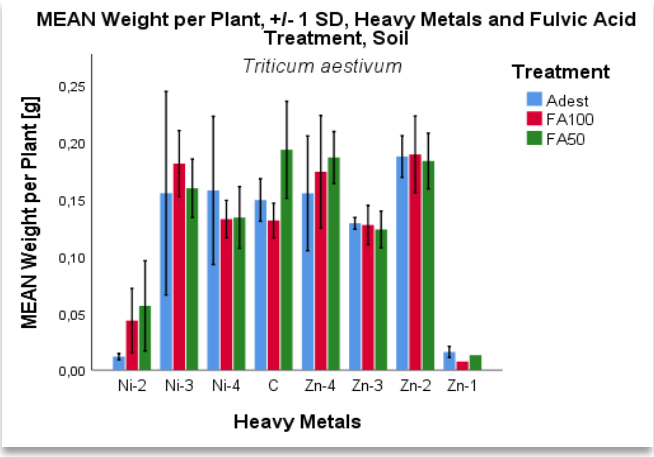


Fig. 77: Bar chart showing mean of dry weight per plant of Triticum aestivum*, soil cultures, error bars: ± 1SD, no significant differences, FA100 = 1% FA, FA50 = 2% FA*

Descriptive Statistics

Dry Weight Per Plant

Treatment	HM	Mean	Std. Deviation	N
Adest	C	,14944444	,018709277	3
	Ni-2	,01180000	,002828427	2
	Ni-3	,15540000	,089491229	3
	Ni-4	,15786111	,065167537	3
	Zn-1	,01598733	,004950690	2
	Zn-2	,18766278	,018300020	3
	Zn-3	,12900000	,005105144	3
	Zn-4	,15531667	,050482183	3
	Total	,12998317	,069796258	22
FA100	C	,13141667	,015232230	3
	Ni-2	,04346667	,028309951	3
	Ni-3	,18150000	,028991378	2
	Ni-4	,13268333	,016460584	3
	Zn-1	,00741000	.	1
	Zn-2	,18954278	,033763752	3
	Zn-3	,12745000	,017321446	3
	Zn-4	,17421667	,049593758	3
	Total	,13174944	,059384158	21
FA50	C	,19366667	,042618462	3
	Ni-2	,05643333	,039633961	3
	Ni-3	,15980000	,025637278	3
	Ni-4	,13410000	,027216355	3
	Zn-1	,01306000	.	1
	Zn-2	,18370367	,024492202	3
	Zn-3	,12355000	,016249077	3
	Zn-4	,18677778	,022903986	3
	Total	,14214338	,058691046	22

Tab. 26: Descriptive statistics for mean dry weight per plant of Triticum aestivum *(soil culture), FA100 = 1% FA, FA50 = 2% FA*

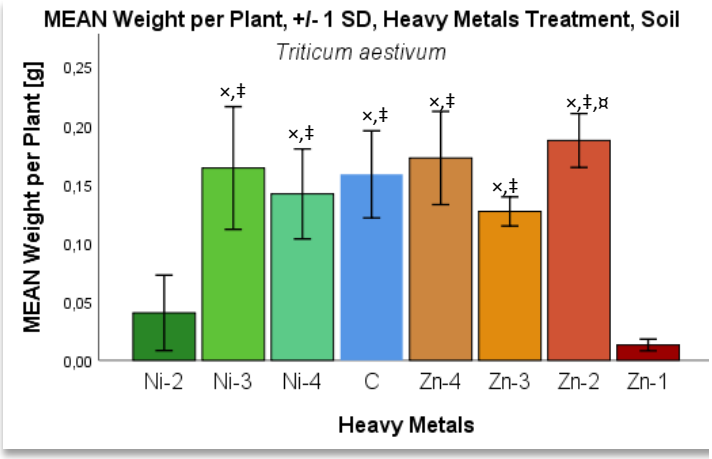


Fig. 78: Bar chart showing mean of dry weight per plant of Triticum aestivum*, soil cultures, error bars: ± 1SD.*

Significant increase of mean dry weight per plant from Ni-2 to all bars marked with ×

Significant increase of mean dry weight per plant Zn-1 to all bars marked with ‡

Significant increase of mean dry weight per plant from Zn-3 to group marked with ¤

Bonferroni Post-hoc Tests showed significant differences of:

- Ni-2 to C ((p <0.001 (0.000002)),
- Ni-2 to Ni-3 (p <0.001 (0.000001)),
- Ni-2 to Ni-4 (p <0.001 (0.000032)),
- Ni-2 to Zn-2 (p <0.001 ($8.16*10^{-9}$)),
- Ni-2 to Zn-3 (p <0.001 (0.00049)),
- Ni-2 to Zn-4 (p <0.001 ($1.175*10^{-7}$),
- Zn-2 to Zn-3 (p = 0.031)
- Zn-1 to C (p <0.001 (0.000002)),
- Zn-1 to Ni-3 (p <0.001 (0.000001)),
- Zn-1 to Ni-4 (p <0.001 (0.00002)),
- Zn-1 to Zn-2 (p <0.001 ($2.365*10^{-8}$)),
- Zn-1 to Zn-3 (p <0.001 (0.000178)),
- Zn-1 to Zn-4 (p <0.001 ($2.0763*10^{-7}$)

whereby plant dry weight increases from Zn-1 (0.013 ± 0.005) and Ni-2 (0.040 ± 0.032) compared to the control (0.158 ± 0.037), Ni-3 (0.164 ± 0.052), Ni-4 (0.142 ± 0.038), Zn-2 (0.187 ± 0.023), Zn-3 (0.127 ± 0.012) and Zn-4 (0.172 ± 0.040) and increases from Zn-3 to Zn-2 (Fig.78).

LSD Test for Simple Effects showed significant differences in the control between FA100 and FA50 (p = 0.012).

FA50 (0.19 ± 0.04) treated plants had significant higher dry weight per plant than FA100 (0.13 ± 0.02) treated plants (Fig. 79)

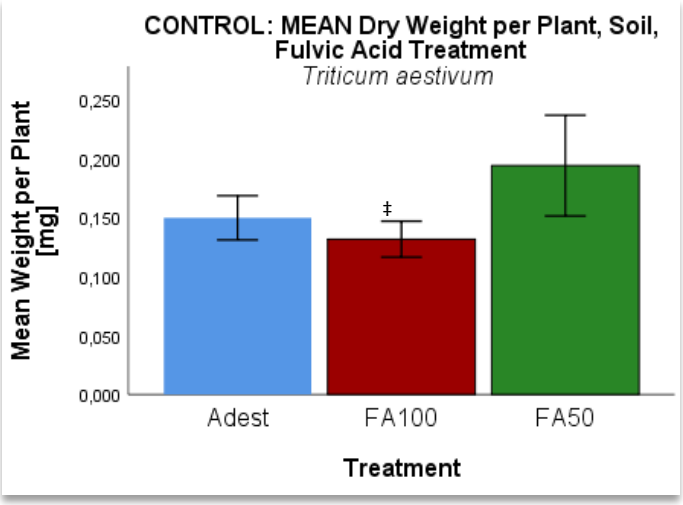


Fig. 79: Bar chart showing mean of dry weight per plant of Triticum aestivum *in the control, ± 1SD, significant differences to FA50 marked with ‡.*

Shoot Dry Weight

Two Way ANOVA showed no significance on p <0.05 level in the corrected model for shoot dry weight (Tab.27, Fig.80)
($F(17,35) = 1.458$, $p = 0.169$, $R^2 = 0.415$, $adj.R^2 = 0.130$), yet homogeneity criterion was not met
(Levene´s Test of Equality of Error Variances, based on mean:
Levene statistic = 4.331, $df_1 = 17$, $df_2 = 35$,
p <0.001 (0.000121))

No significant main effect could be observed for treatment with FA
($F(2,35) = 0.745$, $p = 0.482$, $\eta_p^2 = 0.944$)
or interactive effects
($F(10,35) = 0.850$, $p = 0.586$, $\eta_p^2 = 0.195$).

But main effect on p <0.05 level for HM treatment on dry weight per shoot was found
($F(5,35) = 2{,}945$, $p = 0.025$, $\eta_p^2 = 0.296$)
with Cohen´s value ($f = 0.65$) that suggests strong effect size.
Bonferroni Post-hoc Tests showed significant differences of Zn-2 to Zn-3 ($p = 0.033$).
Zn-2 (0.013 ± 0.005) has significant higher dry weight per shoot than Zn-3 (0.040 ± 0.032) (Fig.81).

Descriptive Statistics

Weight Per Shoot

Treatment	HM	Mean	Std. Deviation	N
Adest	C	,08700000	,014240006	3
	Ni-3	,09340000	,058370198	3
	Ni-4	,08808333	,033294456	3
	Zn-2	,13284139	,015177532	3
	Zn-3	,07183333	,003165570	3
	Zn-4	,09061667	,034380094	3
	Total	,09396245	,033049834	18
FA100	C	,07700000	,008884678	3
	Ni-3	,11750000	,021213203	2
	Ni-4	,07693333	,010519664	3
	Zn-2	,08809444	,069297981	3
	Zn-3	,07338333	,010504325	3
	Zn-4	,10856667	,032938782	3
	Total	,08864314	,032785327	17
FA50	C	,11744444	,021670085	3
	Ni-3	,09840000	,014793326	3
	Ni-4	,07670000	,018519449	3
	Zn-2	,13213950	,018858964	3
	Zn-3	,07426667	,008964560	3
	Zn-4	,11177778	,015210864	3
	Total	,10178806	,025885414	18
Total	C	,09381481	,022817847	9
	Ni-3	,10130000	,034721720	8
	Ni-4	,08057222	,020549530	9
	Zn-2	,11769178	,042894063	9
	Zn-3	,07316111	,007163745	9
	Zn-4	,10365370	,026872280	9
	Total	,09491401	,030600804	53

Tab. 27: Descriptive statistics for mean dry weight per shoot of Triticum aestivum *(soil culture)*

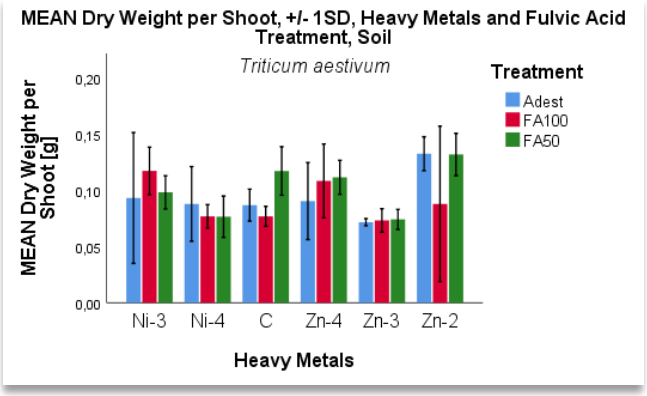


Fig. 80: Bar chart showing mean of dry weight per shoot of Triticum aestivum*, soil cultures, error bars: ± 1SD, no significant differences*

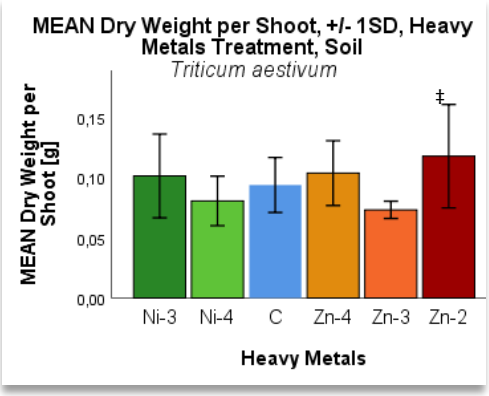


Fig. 81: Bar chart showing mean of dry weight per shoot of Triticum aestivum*, soil cultures, error bars: ± 1SD.*
Significant increase of mean dry weight per shoot from Zn-3 to all bars marked with ‡

Root Dry Weight

Two Way ANOVA showed no significance on p <0.05 level in the corrected model for root dry weight (Fig.82, Tab.28)
($F(17,35) = 1.559$, $p = 0.131$, $R^2 = 0.431$, $adj.R^2 = 0.155$),

and homogeneity criterion was not met (Levene´s Test of Equality of Error Variances, based on mean:
Levene statistic = 3.044, $df_1 = 17$, $df_2 = 35$, $p = 0.003$

No significant main effect could be observed for treatment with FA
($F(2,35) = 0.331$, p=0.720, $\eta_p^2 = 0.019$),
nor HM treatment
($F(5,35) = 1.045$, $p = 0.407$, $\eta_p^2 = 0.130$)
or interactive effects
($F(10,35) = 2.057$, $p = 0.056$, $\eta_p^2 = 0.370$).

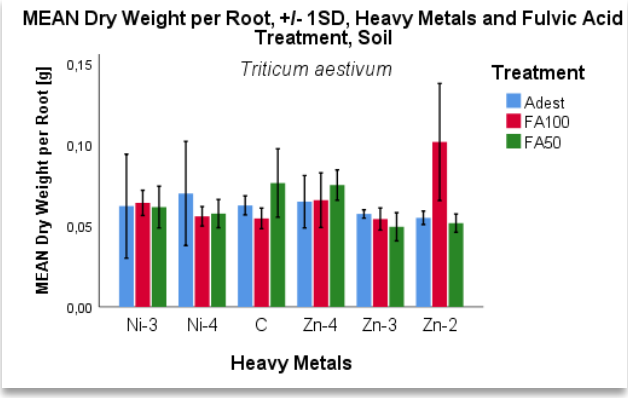


Fig. 82: Bar chart showing mean of dry weight per shoot of Triticum aestivum*, soil cultures, error bars: ± 1SD, no significant differences*

Descriptive Statistics

Dry Weight Per Root

Treatment	HM	Mean	Std. Deviation	N
Adest	C	,06244444	,005872093	3
	Ni-3	,06200000	,032000000	3
	Ni-4	,06977778	,032069947	3
	Zn-2	,05482139	,004161038	3
	Zn-3	,05716667	,002565801	3
	Zn-4	,06470000	,016120484	3
	Total	,06181838	,017438113	18
FA100	C	,05441667	,006350853	3
	Ni-3	,06400000	,007778175	2
	Ni-4	,05575000	,006005206	3
	Zn-2	,10144833	,035997002	3
	Zn-3	,05406667	,006831057	3
	Zn-4	,06565000	,016813462	3
	Total	,06599971	,022894614	17
FA50	C	,07622222	,021019391	3
	Ni-3	,06140000	,012856613	3
	Ni-4	,05740000	,008710913	3
	Zn-2	,05156417	,005634066	3
	Zn-3	,04928333	,008700048	3
	Zn-4	,07500000	,009351173	3
	Total	,06181162	,014842957	18
Total	C	,06436111	,014845091	9
	Ni-3	,06227500	,018698969	8
	Ni-4	,06097593	,018143726	9
	Zn-2	,06927796	,030337258	9
	Zn-3	,05350556	,006638074	9
	Zn-4	,06845000	,013483457	9
	Total	,06315726	,018346895	53

Tab. 28: Descriptive statistics for mean dry weight per root of Triticum aestivum *(soil culture)*

Nevertheless, LSD Test for Simple Effects showed significant effects in Zn-2 between Adest and FA100 (p = 0.002) as well as between FA100 and FA50 (p = 0.001) (Fig.83).

Dry weight of roots is significantly higher, when plants were treated with FA100 (0.10 ± 0.04), than in Adest (0.05 ± 0.00) and when treated with FA50 (0.05 ± 0.01).

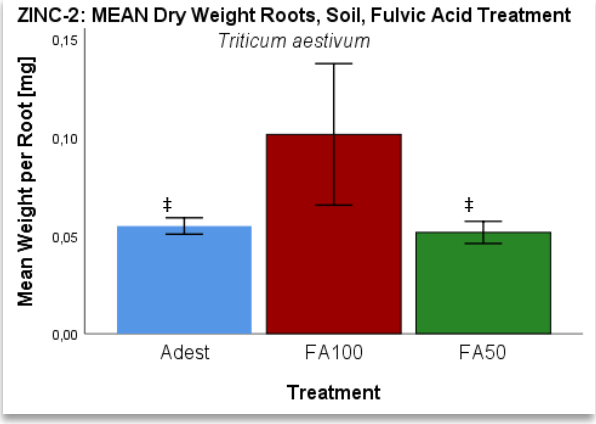


Fig. 83: Bar chart showing mean of dry weight per root of Triticum aestivum in Zn-2, soil, ± 1SD, significant difference to FA100 marked with ‡.

Maximum Root Length

The average maximum root length for *Triticum aestivum* is 15.1 ± 3.8 cm for the control, 14.7 ± 4.0 cm for Ni-4, 15.7 ± 3.2 cm for Ni-3, 3.7 ± 3.5 cm for Ni-2, 15.4 ± 3.8 cm for Zn-4, 13.7 ± 2.7 cm for Zn-3, 11.0 ± 3.1 cm for Zn-2 and 0.0 ± 0.0 cm for Zn-1.

Statistical analysis showed significant differences for HM as well as for FA in the control. Ni-3, Ni-4 and Zn-3.

Average maximum root length increases significantly from Zn-1 (0 ± 0) and Ni-2 (3.7 ± 3.5) compared to the control (15.1 ± 3.8), Ni-3 (15.7 ± 3.2), Ni-4 (14.7 ± 4.0), Zn-2 (11.0 ± 3.1), Zn-3 (13.7 ± 2.7) and Zn-4 (15.4 ± 3.8) and increases from Zn-2 to Ni-3, Ni-4, Zn-3 and Zn-4.

In the control (18.8 ± 1.1) mean root length decreased significantly, when treated with FA100 (13.7 ± 0.9) or FA50 (13.6 ± 1.0) and in Zn-3 (15.5 ± 1.1), FA100 (12.5 ± 0.9) led to significant decrease as well.

In Ni-3 (12.8 ± 1.2) the opposite effect of FA100 (18.8 ± 1.6) and FA50 (16.3 ± 0.9) on root length was observed.

In Ni-4 root length increases significantly from FA100 (13.2 ± 0.9) to FA50 (16.4 ± 0.9) as well.

Even though statistical analysis showed no significant differences for FA treatment in Ni-2 and Zn-1 on root length, when looking at Fig.87 and Fig.91, influence can be seen in the above ground organs. While FA increases effect of Zn stress, it decreases effect of Ni stress in plants.

Following pictures of the seedlings and a detailed report of the statistical analysis.

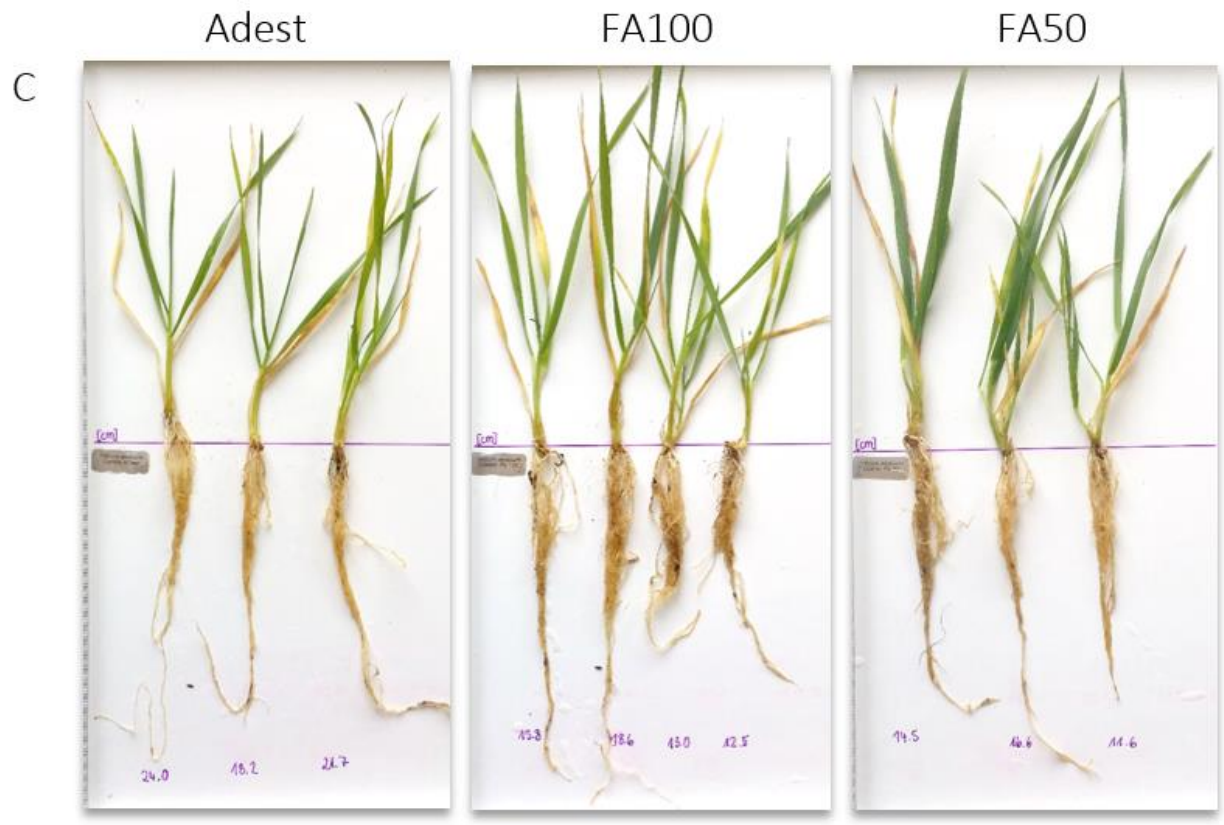


Fig. 84: Comparison of root length of Triticum aestivum *in control treated with a) Adest b) FA100 = 1% FA c) FA50 = 2% FA*

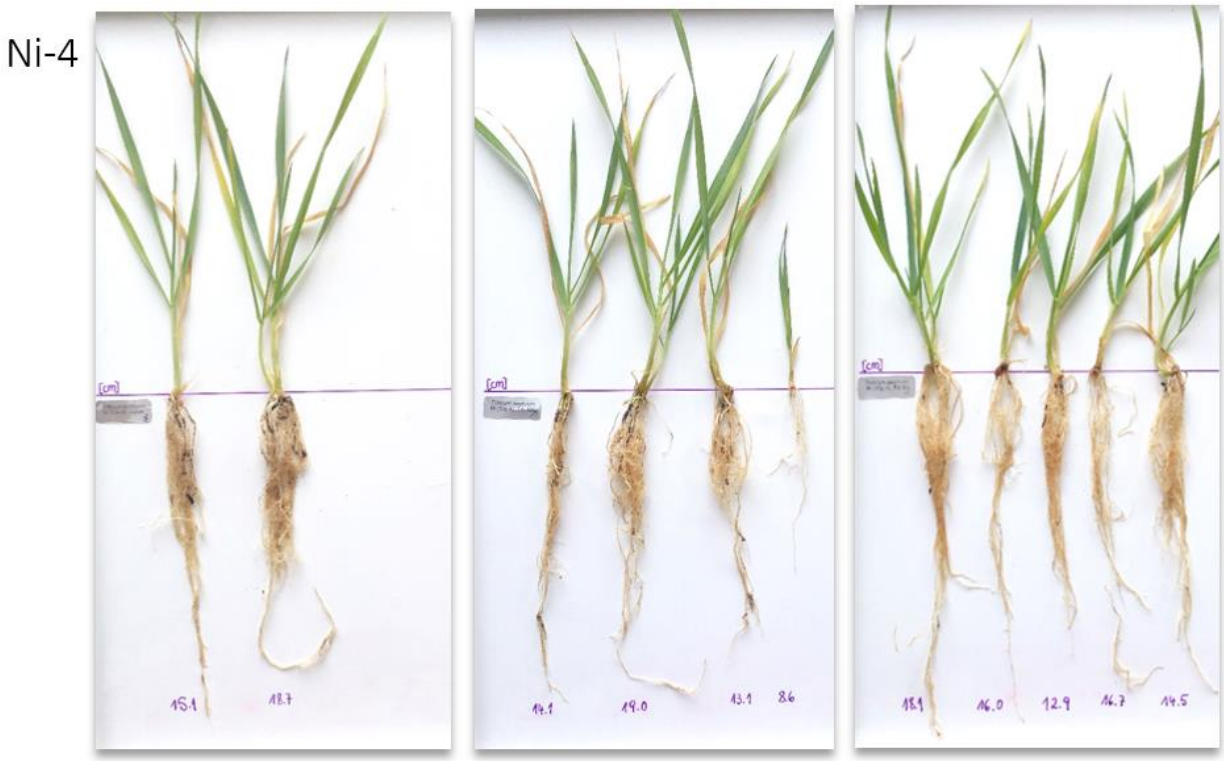


Fig. 85: Comparison of the root length of Triticum aestivum *in Ni-4 treated with a) Adest b) FA100 = 1% FA c) FA50 = 2% FA*

Fig. 86: Comparison of the root length of Triticum aestivum *in Ni-3 treated with a) Adest b) FA100 = 1% FA c) FA50 = 2% FA*

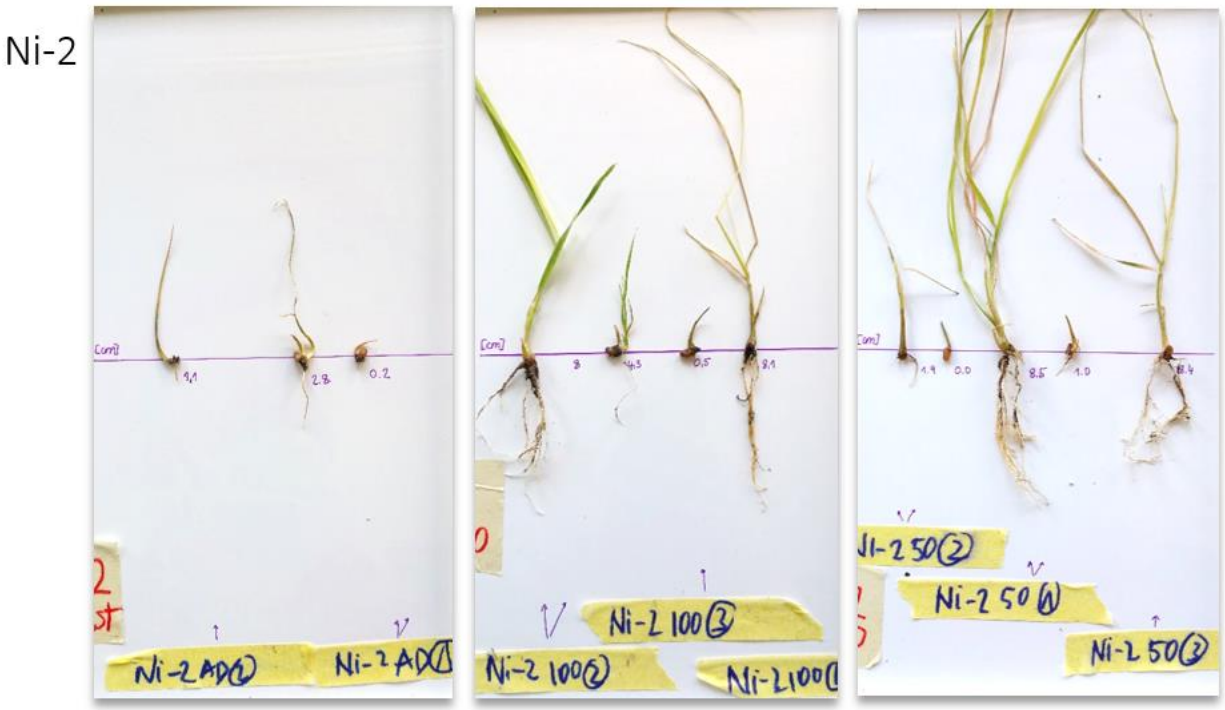


Fig. 87: Comparison of the root length of Triticum aestivum *in Ni-2 treated with a) Adest b) FA100 = 1% FA c) FA50 = 2% FA*

Fig. 88: Comparison of the root length of Triticum aestivum *in Zn-4 treated with a) Adest b) FA100 = 1% FA c) FA50 = 2% FA*

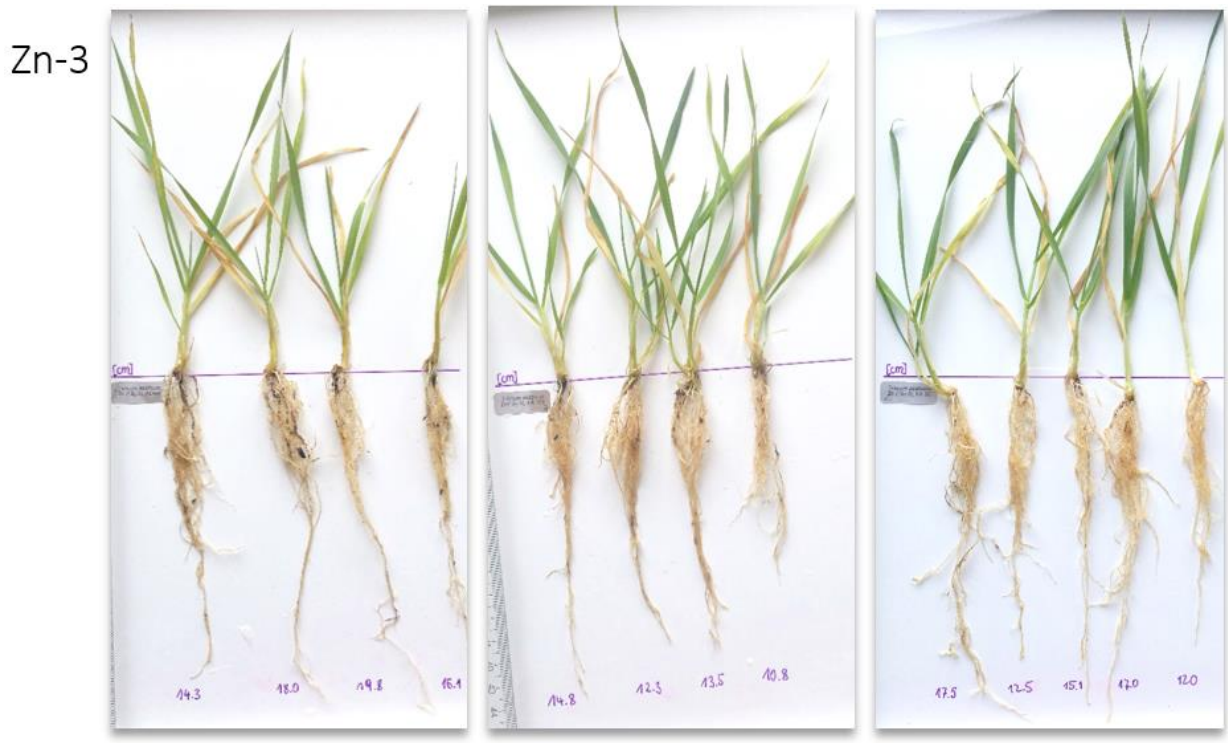


Fig. 89: Comparison of the root length of Triticum aestivum *in Zn-3 treated with a) Adest b) FA100 = 1% FA c) FA50 = 2% FA*

Fig. 90: Comparison of the root length of Triticum aestivum *in Zn-2 treated with a) Adest b) FA100 = 1% FA c) FA50 = 2% FA*

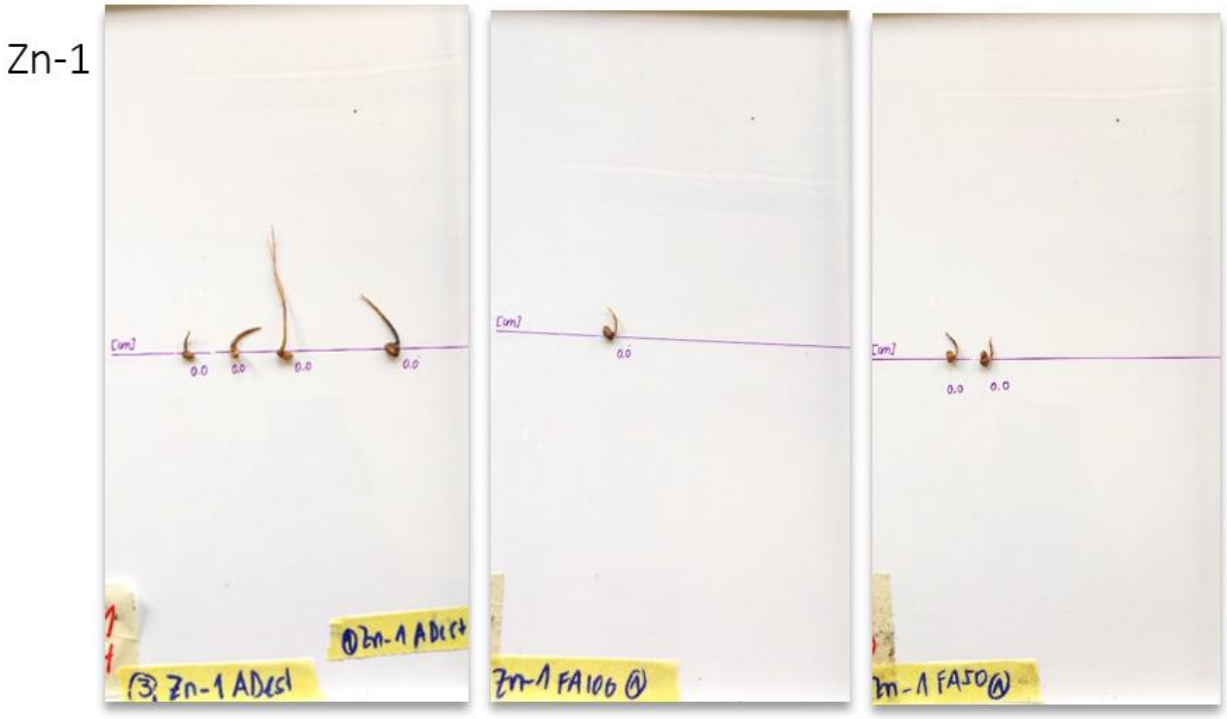


Fig. 91: Comparison of the root length of Triticum aestivum *in Zn-1 treated with a) Adest b) FA100 = 1% FA c) FA50 = 2% FA*

Two Way ANOVA showed significance on p <0.05 level in the corrected model for maximum root length (Tab.29, Fig.92)
($F(23,182) = 14.546$, p <0.001 ($3.8964*10^{-30}$),
$R^2 = 0.648$, $adj.R^2 = 0.603$),
yet homogeneity criterion was not met
(Levene´s Test of Equality of Error Variances, based on mean:
Levene statistic = 2.237, $df_1 = 22$, $df_2 = 182$, $p = 0.002$)

No significant main effect could be observed for treatment with FA
($F(2,182) = 0.259$, $p = 0.772$, $\eta_p^2 = 0.003$).

But main effect for HM treatment on maximum root length was found
($F(7,182) = 37.409$, p <0.001 ($3.9709*10^{-32}$),
$\eta_p^2 = 0.590$)
with Cohen´s value ($f = 1.20$)
as well as a significant interactive effect
($F(14,182) = 2.842$, $p = 0.001$, $\eta_p^2 = 0.179$)
with Cohen´s value ($f = 0.47$),
suggesting that both hold strong effect size.

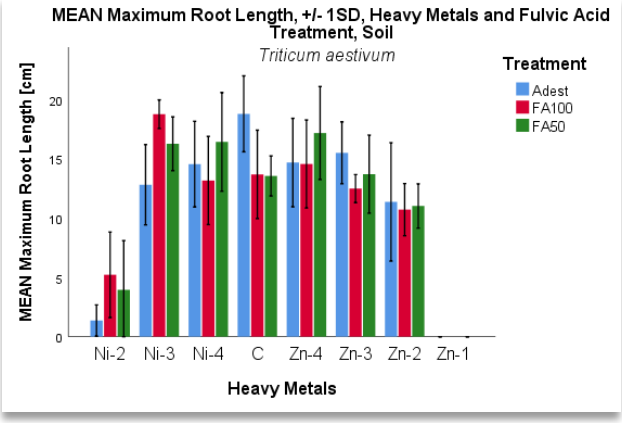


Fig. 92: Bar chart showing mean of maximum root length of Triticum aestivum*, soil cultures, error bars: ± 1SD, no significant interactive effect*

Descriptive Statistics

Maximum Root Length

Treatment	HM	Mean	Std. Deviation	N
Adest	C	18,8111	3,20876	9
	Ni-2	1,3667	1,32035	3
	Ni-3	12,8286	3,38955	7
	Ni-4	14,5714	3,61604	7
	Zn-1	,0000	,00000	4
	Zn-2	11,3800	4,99840	10
	Zn-3	15,5222	2,61810	9
	Zn-4	14,7000	3,73631	10
	Total	12,9780	6,09460	59
FA100	C	13,7000	3,73558	12
	Ni-2	5,2250	3,61236	4
	Ni-3	18,7825	1,20848	4
	Ni-4	13,1846	3,72466	13
	Zn-1	,0000	.	1
	Zn-2	10,7273	2,20095	11
	Zn-3	12,5000	1,18322	13
	Zn-4	14,5818	3,71371	11
	Total	12,6483	4,19487	69
FA50	C	13,5700	1,69250	10
	Ni-2	3,9600	4,15367	5
	Ni-3	16,2846	2,27297	13
	Ni-4	16,4417	4,16772	12
	Zn-1	,0000	,00000	2
	Zn-2	11,0357	1,86861	14
	Zn-3	13,7231	3,29625	13
	Zn-4	17,1889	3,92347	9
	Total	13,4885	4,94011	78
Total	C	15,1419	3,79682	31
	Ni-2	3,7333	3,53690	12
	Ni-3	15,6929	3,19971	24
	Ni-4	14,7094	4,02559	32
	Zn-1	,0000	,00000	7
	Zn-2	11,0371	3,07228	35
	Zn-3	13,7314	2,71486	35
	Zn-4	15,4033	3,84066	30
	Total	13,0608	5,06375	206

Tab. 29: Descriptive statistics for mean maximum root length of Triticum aestivum *(soil culture)*

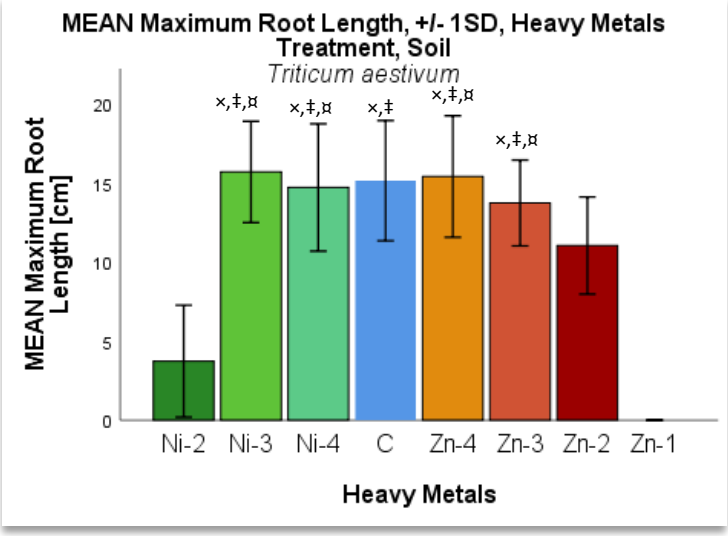


Fig. 93:
Bar chart showing mean of maximum root length of Triticum aestivum, *soil cultures, error bars: ± 1SD.*

Significant increase of mean maximum root length from Ni-2 to all bars marked with ×

Significant increase of mean maximum root length from Zn-1 to all bars marked with ‡

Significant increase of mean maximum root length from Zn-2 to group marked with ¤

Bonferroni Post-hoc Tests showed significant differences of:

- Ni-2 to C (p <0.001 ($4.5102*10^{-19}$)),
- Ni-2 to Ni-3 (p <0.001 ($2.5741*10^{-19}$)),
- Ni-2 to Ni-4 (p <0.001 ($4.6332*10^{-18}$)),
- Ni-2 to Zn-2 (p <0.001 ($3.1781*10^{-9}$)),
- Ni-2 to Zn-3 (p <0.001 ($7.9736*10^{-16}$)),
- Ni-2 to Zn-4 (p <0.001 ($1.2728*10^{-19}$),
- Zn-2 to Ni-3 (p <0.001 (0.000003)),
- Zn-2 to Ni-4 (p <0.001 (0.000139)),
- Zn-2 to Zn-3 (p =0.015) and
- Zn-2 vs. Zn-4 (p <0.001 (0.000004).
- Zn-1 to C (p <0.001 ($1.8809*10^{-21}$)),
- Zn-1 to Ni-3 (p <0.001 ($9.0535*10^{-22}$)),
- Zn-1 to Ni-4 (p <0.001 ($1.3249*10^{-20}$)),
- Zn-1 to Zn-2 (p <0.001 ($4.5335*10^{-13}$)),
- Zn-1 to Zn-3 (p <0.001 ($1.0125*10^{-18}$)),
- Zn-1 to Zn-4 (p <0.001 ($6.412*10^{-22}$)

Maximum root length increases from Zn-1 (0 ± 0) and Ni-2 (3.7 ± 3.5) compared to the control (15.1 ± 3.8), Ni-3 (15.7 ± 3.2), Ni-4 (14.7 ± 4.03), Zn-2 (11.04 ± 3.07), Zn-3 (13.7 ± 2.71) and Zn-4 (15.4 ± 3.8) and increases from Zn-2 to Ni-3, Ni-4, Zn-3 and Zn-4 (Fig.93, Tab.29).

LSD Test for Simple Effects showed significant effects in

- the control between Adest and FA100 (p <0.001 (0.000364)) and FA50 (p <0.001 (0.000447)) as well
- Ni-4 between FA100 and FA50 (p = 0.03)
- Ni-3 between Adest and FA100 (p = 0.003) and FA50 (p = 0.022)
- Zn-3 between Adest and FA100 (p = 0.03)

Descriptive Statistics

Maximum Root Length

Treatment	HM	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Adest	C	18,811	1,063	16,713	20,909
	Ni-2	1,367	1,842	-2,267	5,001
	Ni-3	12,829	1,206	10,450	15,208
	Ni-4	14,571	1,206	12,192	16,950
	Zn-1	1,776E-15	1,595	-3,147	3,147
	Zn-2	11,380	1,009	9,390	13,370
	Zn-3	15,522	1,063	13,424	17,620
	Zn-4	14,700	1,009	12,710	16,690
FA100	C	13,700	,921	11,883	15,517
	Ni-2	5,225	1,595	2,078	8,372
	Ni-3	18,783	1,595	15,635	21,930
	Ni-4	13,185	,885	11,439	14,930
	Zn-1	1,332E-15	3,190	-6,294	6,294
	Zn-2	10,727	,962	8,830	12,625
	Zn-3	12,500	,885	10,754	14,246
	Zn-4	14,582	,962	12,684	16,480
FA50	C	13,570	1,009	11,580	15,560
	Ni-2	3,960	1,427	1,145	6,775
	Ni-3	16,285	,885	14,539	18,030
	Ni-4	16,442	,921	14,625	18,259
	Zn-1	2,132E-14	2,256	-4,451	4,451
	Zn-2	11,036	,853	9,354	12,718
	Zn-3	13,723	,885	11,977	15,469
	Zn-4	17,189	1,063	15,091	19,287

Tab. 30: Descriptive statistics for maximum root length (HM and FA treatment) of Triticum aestivum, *FA50 = 2% FA, FA100 = 1% FA*

In the control (18.8 ± 1.1) average maximum root length decreased significantly, when treated with FA100 (13.7 ± 0.9) or FA50 (13.6 ± 1.0) (Fig.94, Tab.30) and in Zn-3 (15.5 ± 1.1), FA100 (12.5 ± 0.9) led to significant decrease as well (Fig.96).
In Ni-3 (12.8 ± 1.2) the opposite effect of FA100 (18.8 ± 1.6) and FA50 (16.3 ± 0.9) on maximum root length was observed (Fig.95, Tab.30). In Ni-4 root length increases significantly from FA100 (13.2 ± 0.9) to FA50 (16.4 ± 0.9) as well (Fig.97, Tab.30).

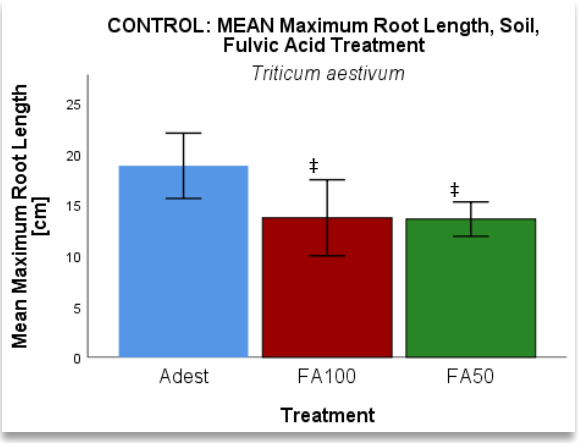


Fig. 94: Bar chart showing mean of maximum root length of Triticum aestivum *in the control, error bars: ± 1SD, Significant differences to Adest marked with ‡. FA50 = 2% FA, FA100 = 1% FA*

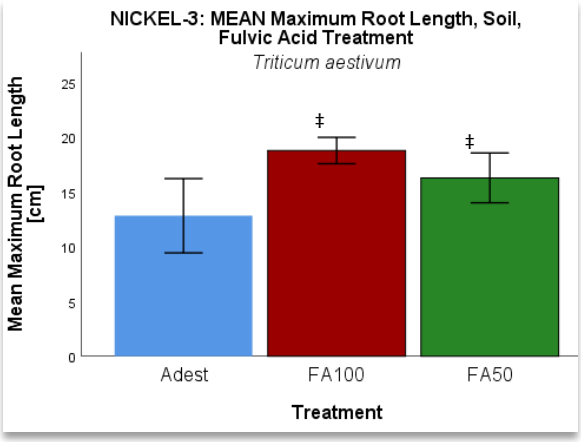


Fig. 95: Bar chart showing mean of maximum root length of Triticum aestivum *in Ni-3, error bars: ± 1SD, Significant differences to Adest marked with ‡. FA50 = 2% FA, FA100 = 1% FA*

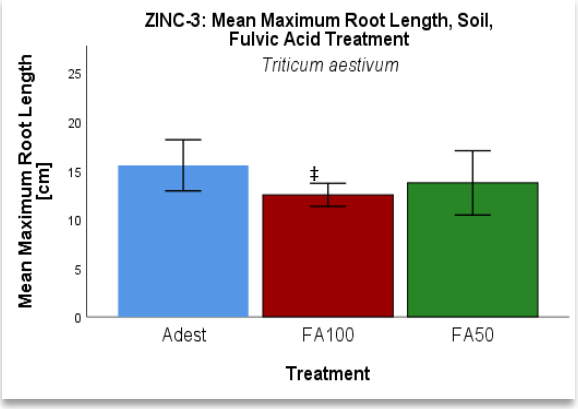


Fig. 96: Bar chart showing mean of maximum root length of Triticum aestivum *in Zn-3, error bars: ± 1SD, Significant differences to Adest marked with ‡. FA50 = 2% FA, FA100 = 1% FA*

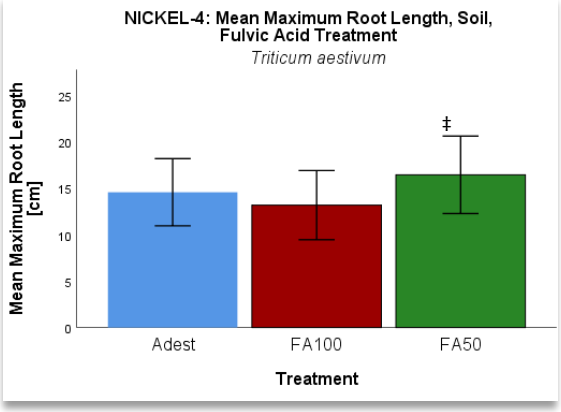


Fig. 97: Bar chart showing mean of maximum root length of Triticum aestivum *in Ni-4, error bars: ± 1SD, Significant differences to FA100 marked with ‡. FA50 = 2% FA, FA100 = 1% FA*

Number of Leaves

The average number of leaves per shoot for *Triticum aestivum* is 7.0 ± 1.7 for the control, 7.6 ± 2.0 for Ni-4, 7.6 ± 2.3 for Ni-3, 3.6 ± 2.6 for Ni-2, 7.8 ± 2.4 for Zn-4, 6.9 ± 1.8 for Zn-3, 6.1 ± 1.7 for Zn-2 and 1.1 ± 0.4 for Zn-1.

In the control the average number of leaves per shoot is 6.3 ± 0.9, treated with FA100 it is 6.3 ± 1.5 and with FA50 it is 8.6 ± 1.6 average leaves per shoot.

In Ni-2 the average number of leaves per shoot is 2 ± 1.7, treated with FA100 it is 4.3 ± 2.2 and with FA50 4 ± 3.3. In Ni-3 it is 6.3 ± 2.2, treated with FA100 10.3 ± 3.3 and with FA50 treatment it is 7.5 ± 1.4.
The average number of leaves per shoot in Ni-4 is 6.6 ± 2.1, treated with FA100 it is 7 ± 1.7 and with FA50 it is 8.2 ± 2.1.

In Zn-1 the average number of leaves per shoot is 1.3 ± 0.5, treated with FA100 it is 1.0 and with FA50 it is 1.0 ± 0.0. In Zn-2 it is 5.8 ± 2.2, treated with FA100 it is 6.1 ± 1.8 and with FA50 it is 6.3 ± 1.4.
The average number of leaves per shoot in Zn-3 is 5.8 ± 1.1, treated with FA100 it is 7.2 ± 1.5 and with FA50 it is 7.5 ± 2.1. In Zn-4 it is 6.5 ± 2.3, treated with FA100 it is 7.9 ± 16. And with FA50 9.2 ± 2.6.

Statistical analysis generally showed a significant increase of average number of leaves from Adest (5.6 ± 2.3) to FA100 (6.8 ± 2.2) and FA50 (7.3 ± 2.5). Number of leaves increased also when comparing Ni-2 and Zn-1 to the control, Ni-3, Ni-4, Zn-2, Zn-3 and Zn-4, as well as from Zn-2 to Zn-3.

In the control number of leaves was significantly higher in FA50 than in FA100 and Adest. In Ni-3 it was significantly higher in FA100 than in Adest and FA50. In Zn-4 mean of number of leaves per plant was significant higher of FA50 treated plants than Adest and the same effect was found for Zn-3.

Following a detailed report of the statistical analysis.

Two Way ANOVA shows significance on p <0.05 level in the corrected model for number of leaves (Tab.31, Fig.98)
($F(23,182) = 7.179$, p <0.001 ($8.4153*10^{-16}$), $R^2 = 0.476$, $adj.R^2 = 0.409$) and Variances homogenous (Levene´s Test of Equality of Error Variances, based on mean:
Levene statistic = 1.467, $df_1 = 22$, $df_2 = 182$, $p = 0.090$)

Significant main effects can be observed for treatment with FA (Fig.99)
($F(2,182) = 8.506$, p <0.001 (0.000294), $\eta_p^2 = 0.085$)
and treatment with HM (Fig.100)
($F(7,182) = 15.833$, p <0.001 ($3.681*10^{-16}$), $\eta_p^2 = 0.378$).

Main effect for FA treatment has Cohen´s value $f = 0.3$ and therefor medium effect size, while main effect for HM treatment ($f = 0.78$) has strong effect size.
No significant interactive effect was found
($F(14,182) = 1.582$, $p = 0.088$, $\eta_p^2 = 0.108$).

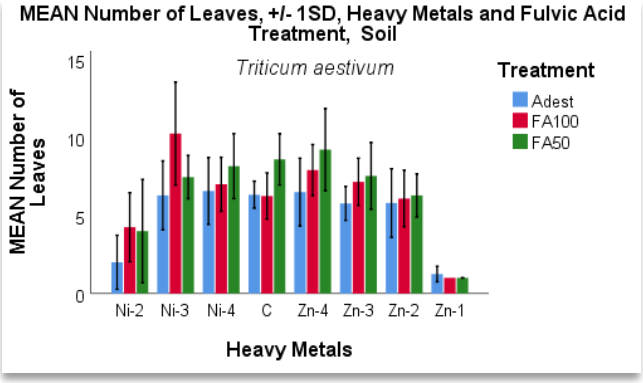


Fig. 98: Bar chart showing mean of maximum root length of Triticum aestivum*, soil, no significant interactive effect FA50 = 2% FA, FA100 = 1% FA*

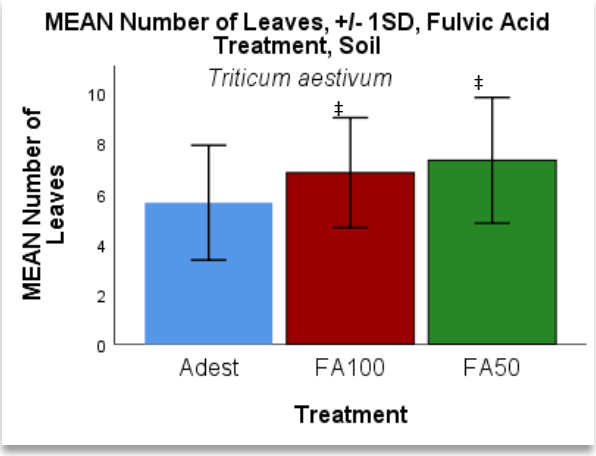


Fig. 99: Bar chart showing mean of number of leaves of Triticum aestivum*, soil, error bars: ± 1SD. Significant increase of mean maximum root length from Adest to all bars marked with ‡, FA50 = 2% FA, FA100 = 1% FA*

Descriptive Statistics

Number of Leaves Per Plant

Treatment	HM	Mean	Std. Deviation	N
Adest	C	6,33	,866	9
	Ni-2	2,00	1,732	3
	Ni-3	6,29	2,215	7
	Ni-4	6,57	2,149	7
	Zn-1	1,25	,500	4
	Zn-2	5,80	2,201	10
	Zn-3	5,78	1,093	9
	Zn-4	6,50	2,173	10
	Total	5,64	2,288	59
FA100	C	6,25	1,485	12
	Ni-2	4,25	2,217	4
	Ni-3	10,25	3,304	4
	Ni-4	7,00	1,732	13
	Zn-1	1,00	.	1
	Zn-2	6,09	1,814	11
	Zn-3	7,15	1,519	13
	Zn-4	7,91	1,640	11
	Total	6,84	2,194	69
FA50	C	8,60	1,647	10
	Ni-2	4,00	3,317	5
	Ni-3	7,46	1,391	13
	Ni-4	8,17	2,082	12
	Zn-1	1,00	,000	2
	Zn-2	6,29	1,383	14
	Zn-3	7,54	2,145	13
	Zn-4	9,22	2,635	9
	Total	7,33	2,505	78
Total	C	7,03	1,741	31
	Ni-2	3,58	2,610	12
	Ni-3	7,58	2,339	24
	Ni-4	7,34	2,010	32
	Zn-1	1,14	,378	7
	Zn-2	6,09	1,738	35
	Zn-3	6,94	1,798	35
	Zn-4	7,83	2,350	30
	Total	6,68	2,432	206

Tab. 31: Descriptive statistics for mean number of leaves of Triticum aestivum *(soil), FA50 = 2% FA, FA100 = 1% FA*

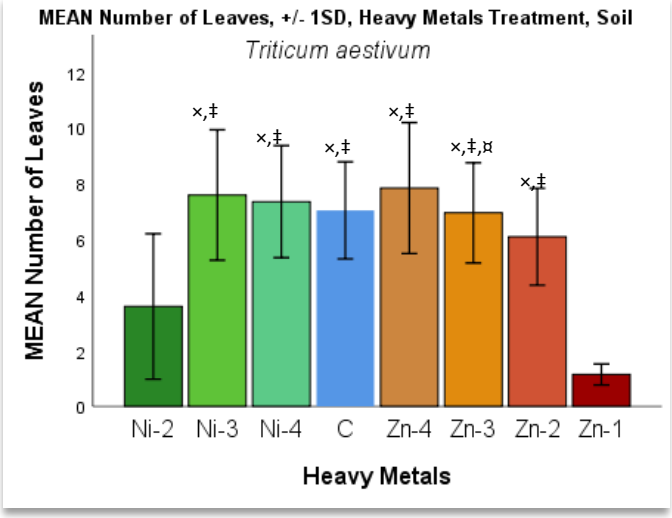


Fig. 100:
Bar chart showing mean of number of leaves of Triticum aestivum*, soil cultures, error bars: ± 1SD.*

Significant increase of mean number of leaves from Ni-2 to all bars marked with ×

Significant increase of mean number of leaves from Zn-1 to all bars marked with ‡

Significant decrease of mean number of leaves from Zn-2 to group marked with ¤

Bonferroni Post-hoc Tests showed significant differences of:

- Ni-2 to C (p <0.001 (0.000005)),
- Ni-2 to Ni-3 (p <0.001 (2.2172*10^{-7})),
- Ni-2 to Ni-4 (p <0.001 (3.8928*10^{-7}),
- Ni-2 to Zn-2 (p = 0.003),
- Ni-2 to Zn-3 (p <0.001 (0.000007)),
- Ni-2 to Zn-4 (p <0.001 (8,9069*10^{-9}),

- Zn-2 to Zn-3 (p <0.001 (3.8316*10^{-8})

- Zn-1 to C (p <0.001 (6.3797*10^{-11})),
- Zn-1 to Ni-3 (p <0.001 (3.4535*10^{-12})),
- Zn-1 to Ni-4 (p <0.001 (5.2801*10^{-12})),
- Zn-1 to Zn-2 (p <0.001 (3.8316*10^{-8})),
- Zn-1 to Zn-3 (p <0.001 (7.8239*10^{-11})),
- Zn-1 to Zn-4 (p< 0.001 (1.5778*10^{-13})

- Adest to FA100 (p = 0.001)
- Adest to FA50 (p <0.001 (0.000001)

whereby the average number of leaves increases from plants treated only with Adest (5.6 ± 2.3) to plants treated with FA100 (6.8 ± 2.2) and FA50 (7.3 ± 2.5) (Fig.99). The average number also increases from Ni-2 (3.6 ± 2.6) and Zn-1 (1.1 ± 0.4) compared to the control (7.0 ± 1.7), Ni-3 (7.6 ± 2.3), Ni-4 (7.3 ± 2.0), Zn-2 (6.1 ± 1.7), Zn-3 (6.9 ± 1.8) and Zn-4 (7.8 ± 2.4) and decreases from Zn-3 to Zn-2 (Fig.100).

LSD Test for Simple Effects showed significant effects in

- the control between Adest and FA50 (p = 0.009) as well as between FA100 and FA50 (p = 0.004)
- Ni-3 between Adest and FA100 (p = 0.001) as well as between FA100 and FA50 (p = 0.010)
- Zn-4 between FA50 and Adest (p = 0.002)
- Zn-3 between FA50 and Adest (p = 0.031)

In the control number of leaves decrease when treated with FA50 (8.6 ± 1.6), compared to FA100 (6.3 ± 1.5) and Adest (6.3 ± 0.9) (Fig.101). In Ni-3 FA100 (10.3 ± 3.3) has significant higher number of leaves per plant than plants treated with FA50 (7.5 ± 1.4) and Adest (6.3 ± 2.2) (Fig.102). Number of leaves per plant in Zn-4 increase when treated with FA50 (9.2 ± 2.6) compared to Adest treated plants (6.5 ± 2.2) (Fig.103). The same effect for FA50 (7.5 ± 2.1) and Adest (5.8 ± 1.1) was found in Zn-3 (Fig.104).

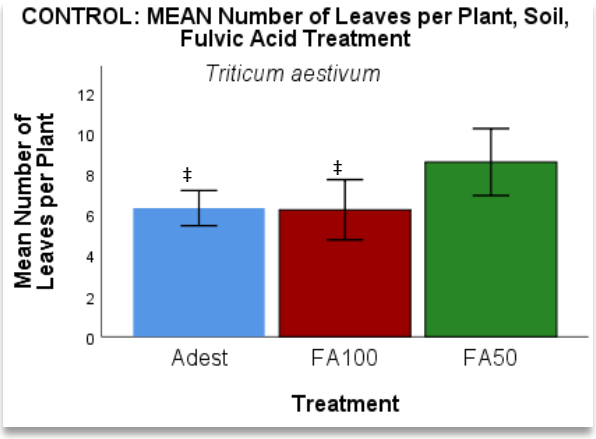


Fig. 101: Bar chart showing mean number of leaves per Triticum aestivum plant in the control, ± 1SD, soil. Significant differences to FA50 marked with ‡.

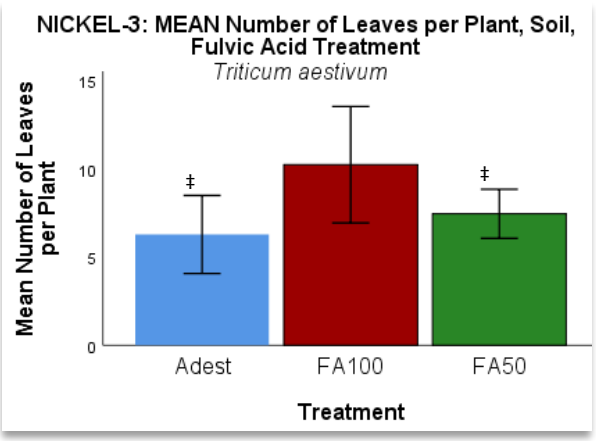


Fig. 102: Bar chart showing mean number of leaves per Triticum aestivum plant in Ni-3, ± 1SD, soil. Significant differences to FA100 marked with ‡.

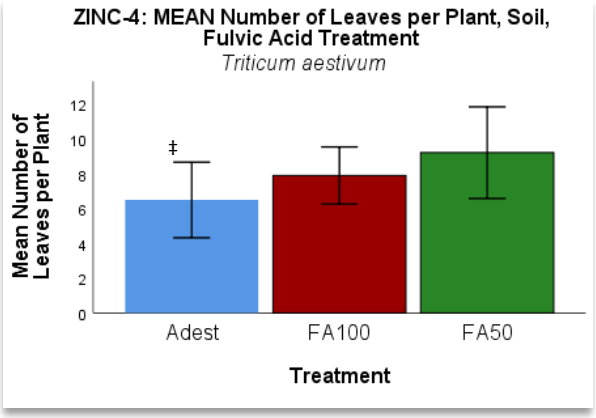


Fig. 103: Bar chart showing mean number of leaves per Triticum aestivum plant in Zn-4, ± 1SD, soil. Significant difference to FA50 marked with ‡.

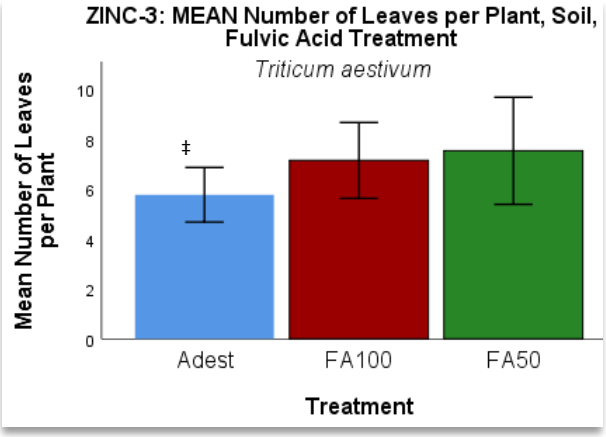


Fig. 104: Bar chart showing mean number of leaves per Triticum aestivum plant in Zn-3, ± 1SD, soil. Significant difference to FA50 marked with ‡.

4.3.3. Determination of Stress Levels

Different methods were used to evaluate if HM influenced parameters, indicating stress in *Triticum aestivum* plants.

(A) PEAS Chlorophyll Fluorimeter

Handy PEAS Chlorophyll Fluorimeter was used to measure *Fv/Fm*, an indicator for the maximum efficiency of the PSII. This ratio is very sensitive to stress and low values are indicators for the stress levels of plants. The upper side of the leaves was covered with clips, darkened for 20 minutes and then one measurement per pot and therefore three measurements per treatment were taken.

The mean of *Fv/Fm* for *Triticum aestivum* in the control is 0.69 ± 0.17, in Ni-4 0.67 ± 0.10, in Ni-3 0.60 ± 0.13, in Ni-2 0.69 ± 0.17, in Zn-4 0.71 ± 0.06, in Zn-3 it is 0.68 ± 0.09 and in Zn-2 0.81 ± 0.01.

Fv/Fm in Zn-2 was significant higher than in Zn-3.

Besides that, no other differences were significant, FA treatment had no significant effect in any group. Means and Standard Deviations are given in Tab.32.

In the following a detailed report of the statistical analysis is given.

Two Way ANOVA showed significance on p <0.05 level in the corrected model for *Fv/Fm* (Tab.32, Fig.105) ($F(19,39) = 1.984$, $p = 0.035$, $R^2 = 0.492$, $adj.R^2 = 0.244$), yet homogeneity criterion was not met (Levene´s Test of Equality of Error Variances, based on mean:
Levene statistic = 4.331, $df_1 = 17$, $df_2 = 35$, p <0.001 (0.000121))

No significant main effect could be observed for treatment with FA
($F(2,39) = 0.252$, $p = 0.779$, $\eta_p^2 = 0.013$)
or interactive effects
($F(11,39) = 1.591$, $p = 0.140$, $\eta_p^2 = 0.310$).

But main effect on p <0.05 level for HM treatment for *Fv/Fm* was found
($F(6,39) = 3.268$, $p = 0.011$, $\eta_p^2 = 0.335$)
with Cohen´s value ($f = 0.71$) that suggests strong effect size.

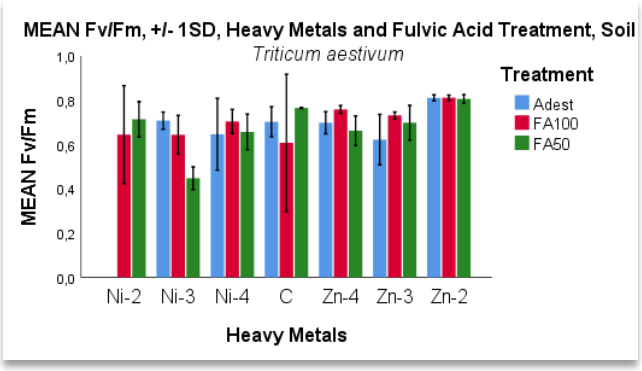


Fig. 105: Bar chart showing mean of Fv/Fm Triticum aestivum*, soil cultures, error bars: ± 1SD, no significant differences, FA50 = 2% FA, FA100 = 1% FA*

Descriptive Statistics

Fv/Fm

Treatment	HM	Mean	Std. Deviation	N
Adest	C	,70267	,068061	3
	Ni-3	,70767	,038889	3
	Ni-4	,64633	,162531	3
	Zn-2	,81067	,013868	3
	Zn-3	,62200	,113961	3
	Zn-4	,69833	,050013	3
	Total	,69794	,097089	18
FA100	C	,60767	,309865	3
	Ni-2	,64450	,221324	2
	Ni-3	,64400	,087607	3
	Ni-4	,70400	,054617	3
	Zn-2	,81100	,012288	3
	Zn-3	,73167	,015308	3
	Zn-4	,75833	,018148	3
	Total	,70295	,136683	20
FA50	C	,76467	,003055	3
	Ni-2	,71367	,080376	3
	Ni-3	,44767	,051423	3
	Ni-4	,65700	,080225	3
	Zn-2	,80600	,019157	3
	Zn-3	,69800	,078823	3
	Zn-4	,66233	,067099	3
	Total	,67848	,120661	21
Total	C	,69167	,172784	9
	Ni-2	,68600	,130044	5
	Ni-3	,59978	,129356	9
	Ni-4	,66911	,098310	9
	Zn-2	,80922	,013544	9
	Zn-3	,68389	,085004	9
	Zn-4	,70633	,059977	9
	Total	,69271	,118411	59

Tab. 32: Descriptive statistics for mean Fv/Fm *of* Triticum aestivum *(soil culture), FA50 = 2% FA, FA100 = 1% FA*

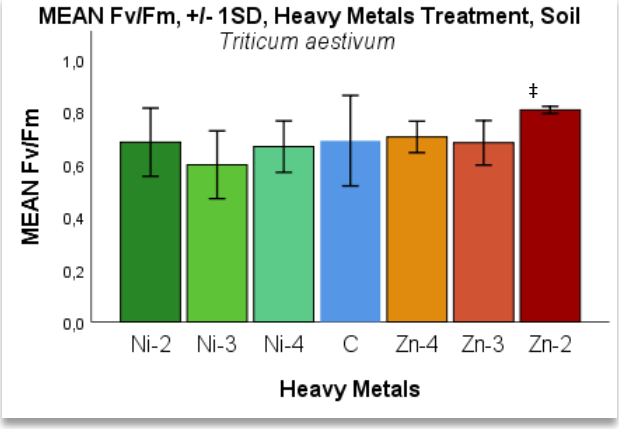


Fig. 106: Bar chart showing mean of Fv/Fm *of* Triticum aestivum*, soil cultures, error bars: ± 1SD. Significant increase from Zn-3 to all bars marked with ‡*

Bonferroni Post-hoc Tests showed significant differences of Zn-2 to Ni-3 (p = 0.002).

Zn-2 (0.81 ± 0.01) has significant higher *Fv/Fm* than Zn-3 (0.68 ± 0.09) (Fig.106).

(A) Heavy Metal Tolerance Tests to Determine Cell Vitality of Wheat Leaves Subjected to HM Stress

Zn-2	C	Zn-6	Zn-5	Zn-4	Zn-3	Zn-2	Zn-1	Zn 1
Adest	+	+	-	+	±	-	-	-
FA100	+	+	-	-	±	-	-	-
FA50	+	+	-	±	-	-	-	-
	C	Ni-6	Ni-5	Ni-4	Ni-3	Ni-2	Ni-1	Ni 1
Adest	+	+	+	+	±	-	-	-
FA100	+	+	+	-	-	-	-	-
FA50	+	+	+	±	-	-	-	-

Tab. 33: Vitality of plant cells after immersing them in Zn and Ni solutions of different concentrations for 48 hours. FA50 = 2% FA, FA100 = 1% FA, Cells alive: >50% | 50% | <50%

Sections of *Triticum aestivum* leaves, treated with 10 mmol Zn, have been put into HM solution for 48 hours and were checked for ability to perform plasmolysis (Fig.107) after soaking in mannitol for ten minutes.

Tolerance for different concentrations of Ni and Zn of the control plants, as well as plants treated with two concentrations of FA, were compared.

Independent of FA treatment, cells in leaf sections of 100 mmol Zn treated plants survived solutions with concentrations as high as 10^{-6} M Zn and 10^{-5} M Ni.
The control plants survived concentrations up to 1 mmol Ni and Zn respectively. FA treated plants performed worse in Zn, in Ni slightly better, than untreated plants.
Most cells in the sections were alive in 10^{-5} M Ni, while less than 50% of cells did so in Zn 10^{-5} M (Tab.33).

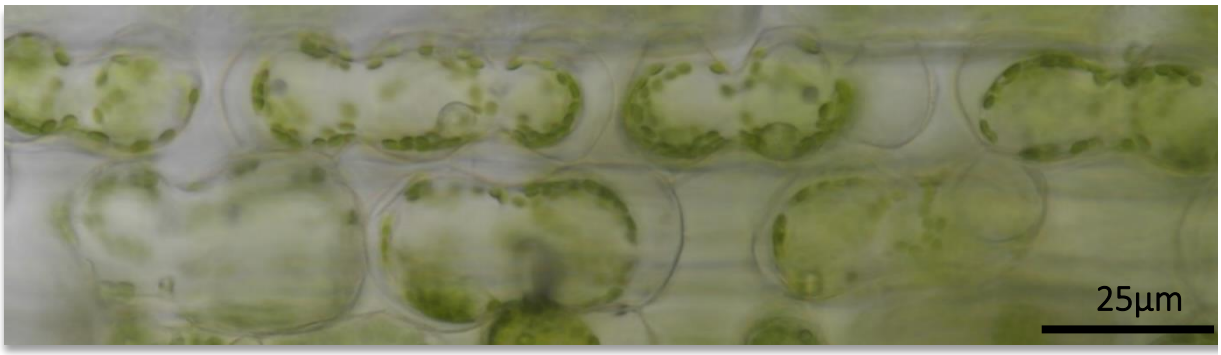


Fig. 107: Plasmolysis of Triticum aestivum *cells (Zn-2, Adest) in Zn-4 solution*

4.3.4. ICP-OES: Determination of Heavy Metal Content

To evaluate effect of the different HM and FA treatments on the total K, Zn and Ni content within the plants, plant material was split into shoots and roots, dried, digested in aqua regia (AR) and HM content measured by ICP-OES.

One AR digest per pot was prepared and intensity was measured using ICP-OES. Concentration in mg/kg for each HM of interest was calculated based on solutions (standards) with known concentration. Visualization of the correlation between these concentrations and the intensity measured can be seen in the calibration charts (Fig.108).

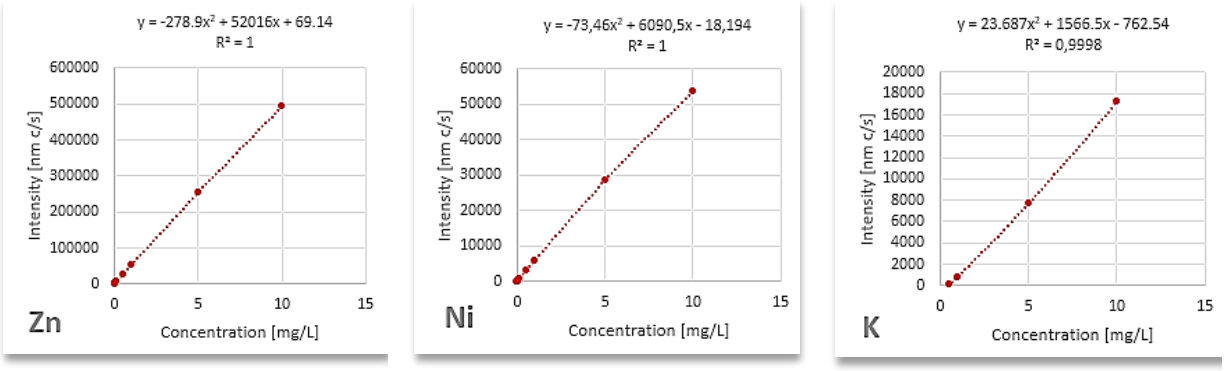


Fig. 108: Calibration charts, visualizing correlation of intensity measured and (known) concentration of Ni, Zn and K solutions.

(A) Zn, Ni and K Content in Plants

Seedlings in Ni-2 had nearly no shoots and seedlings in Zn-1 had no roots. So only one whole plant measurement was possible in these cases. Not enough seeds germinated (Tab.34) to run statistics. An overview of the K, Ni and Zn content is given in Fig.109 and Fig.110.
As expected, the Ni amount is remarkably higher in the Ni-2 treated plants than in the Zn-1 treated plants, surprisingly K amount as well. K shows little to no difference between the FA concentrations as well as the control, only treated with Adest.

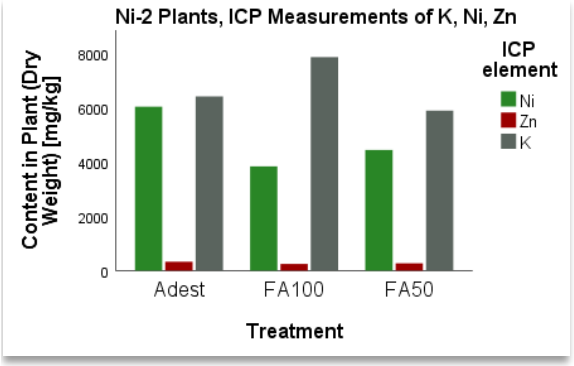


Fig. 109: Content of Zn, Ni and K (mg/kg dry weight) of plants growing in Ni-2 (10 mmol), FA50 = 2% FA, FA100 = 1% FA,

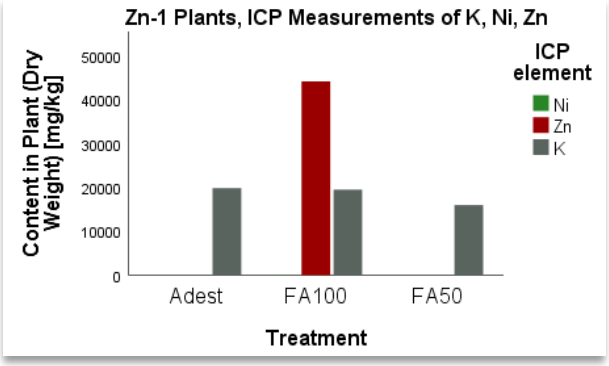


Fig. 110: Content of Zn, Ni and K (mg/kg dry weight) of plants growing in Zn-1 (100 mmol), FA50 = 2% FA, FA100 = 1% FA

The Ni content in Ni-2 is 6 060 ± 3 068 mg/kg dry weight and in Zn-1 it is 2.5 ± 0.31 mg/kg dry weight. The Zn content in Ni-2 is 331 ± 83 mg/kg dry weight. In Zn-1 taking measurements was not possible. The K content in Ni-2 was 15 960 ± 1 977 mg/kg dry weight and in Zn-1 it was 5 912 ± 573 mg/kg dry weight. FA treatment seemingly had no effect on the content of the three elements in the dried plant material, when considering the high standard deviations. Means and standard deviations are indicated in Tab.34.

Descriptive Statistics

Nickel

HM	Treatment	Mean	Std. Deviation	N
Ni-2	Adest	6059,564779	3068,469701	2
	FA 100	3851,028262	1810,118521	3
	FA 50	4460,462140	1367,167305	3
	Total	4631,700095	1916,125751	8
Zn-1	Adest	2,485355069	,3131143573	2
	FA 100	12,74054903	.	1
	FA 50	1,079424505	.	1
	Total	4,697670918	5,405747522	4

Potassium

HM	Treatment	Mean	Std. Deviation	N
Ni-2	Adest	15960,80673	1976,809204	2
	FA 100	19495,79015	2496,955717	3
	FA 50	19847,77950	2440,062632	3
	Total	18744,04030	2649,205919	8
Zn-1	Adest	5911,921338	572,5698770	2
	FA 100	7890,483829	.	1
	FA 50	6440,505690	.	1
	Total	6538,708049	991,7158341	4

Zinc

HM	Treatment	Mean	Std. Deviation	N
Ni-2	Adest	330,9727926	82,58048029	2
	FA 100	243,9662073	106,7290476	3
	FA 50	270,1227780	195,7265502	3
	Total	275,5265676	128,4210816	8

Tab. 34: Descriptive statistics for Ni, Zn and K content in dry weight of plants of Triticum aestivum *(soil) growing in Zn-1 (100 mmol) and Ni-2 (10 mmol), FA50 = 2% FA, FA100 = 1% FA*

In all the other concentrations roots and shoots had developed enough to prepare separated digests and to run statistical tests.

(B) Zn, Ni and K Content in Roots

Nickel

The average total Ni content in the roots of the control is 4.5 ± 3.4, of Ni-4 it is 20.1 ± 22.2, in Ni-3 it is 75.6 ± 41.9, in Zn-4 it is 26.3 ± 42.8, in Zn-3 it is 6.1 ± 3.8 and in Zn-2 the total Ni content in the roots is 7.1 ± 6.1 mg/kg dry weight.

In the control the average total Ni content in the roots is 6.8 ± 5.7 mg/kg dry weight, when treated with FA100 it is 3.1 ± 0.8 mg/kg dry weight and with FA50 it is 3.5 ± 0.5 mg/kg dry weight.

In Ni-4 the average total Ni content in the roots is 10.1 ± 4.4 mg/kg dry weight, with FA100 treatment it is 13.1 ± 3.9 and with FA50 treatment it is 37.0 ± 35.8 mg/kg dry weight. In Ni-3 it is 54.3 ± 2.2 mg/kg dry weight, treated with FA100 total Ni content in the roots is 124.0 ± 16.9 mg/kg dry weight and with FA50 it is 64.8 ± 53.0 mg/kg dry weight.

The average total Ni content in the roots of Zn-4 is 34.2 ± 53.9 mg/kg dry weight, treated with FA100 the Ni content is 42.3 ± 55.6 mg/kg and with FA50 it is 2.6 ± 0.7 mg/kg dry weight. In Zn-3 it is 6.0 ± 3.6 mg/kg dry weight, with FA100 it is 7.8 ± 0.6 and with FA50 it is 4.6 ± 2.3 mg/kg dry weight. In Zn-2 the average total Ni content in the roots is 5.5 ± 1.4 mg/kg dry weight, treated with FA100 it is 3.9 ± 0.6 mg/kg dry weight and with FA50 11.9 ± 9.7 mg/kg dry weight.

Statistical analysis showed significant decrease of average total Ni content in the roots of all groups compared to Ni-3.

Following a detailed report of the statistical analysis.

Results for the Two Way ANOVA for mean Ni content (Fig.111) in correlation with HM treatment and FA treatment (Tab.35) was significant for the corrected model on p <0.05 level ($F(17,35) = 4{,}014$, p <0.001 (0.000248), $R^2 = 0.661$, $adj.R^2 = 0.496$),

Homogeneity criterion was not met
(Levene´s Test of Equality of Error Variances, based on mean:
Levene statistic = 10.368, $df_1 = 17$, $df_2 = 35$,
p <0.001 ($4.4751*10^{-9}$).

No significant differences between different FA treatment groups
($F(2,35) = 1.445$, $p = 0.249$, $\eta_p^2 = 0.076$)
and no interactive effect between the two factors (HM, FA) was observed
($F(10,35) = 1.564$, $p = 0.159$, $\eta_p^2 = 0.309$).
But differences between HM treatments are a significant main effect
($F(5,35) = 11.410$, p <0.001 (0.000001), $\eta_p^2 = 0.620$),
Cohen´s value ($f = 1.78$) suggest a strong effect size.

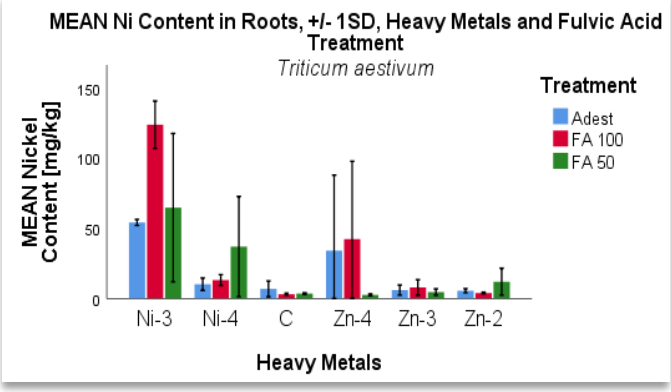


Fig. 111: Content of Ni (mg/kg dry weight) in roots growing in different HMs, FA50 = 2% FA, FA100 = 1% FA

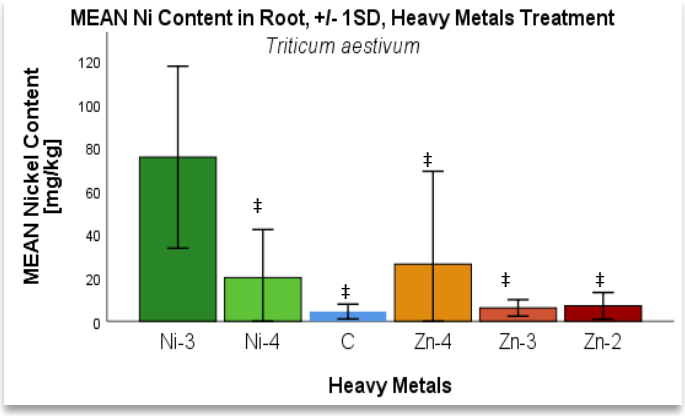


Fig. 112: Significant decrease of Ni content in roots (dry weight) compared to Ni-3 marked with ‡

Descriptive Statistics

Nickel Content in Roots

Treatment	HM	Mean	Std. Deviation	N
Adest	Ni-3	54,25837961	2,167469568	3
	Ni-4	10,14011128	4,422845351	3
	C	6,849660874	5,684486340	3
	Zn-4	34,15711259	53,90207040	3
	Zn-3	5,976248457	3,644281637	3
	Zn-2	5,524678214	1,449386109	3
	Total	19,48436517	26,68026222	18
FA 100	Ni-3	124,0035168	16,92162249	2
	Ni-4	13,14375801	3,878666602	3
	C	3,054869152	,7763883684	3
	Zn-4	42,31896805	55,63658444	3
	Zn-3	7,840850284	5,569583343	3
	Zn-2	3,885387729	,5933388322	3
	Total	26,98461961	44,08428057	17
FA 50	Ni-3	64,76543754	52,96683211	3
	Ni-4	37,02559276	35,78446431	3
	C	3,454886307	,5403715263	3
	Zn-4	2,568656533	,6793983669	3
	Zn-3	4,564782897	2,308808197	3
	Zn-2	11,93776471	9,655663702	3
	Total	20,71952012	32,43572788	18
Total	Ni-3	75,63481064	41,93712380	8
	Ni-4	20,10315402	22,17109616	9
	C	4,453138777	3,400391205	9
	Zn-4	26,34824572	42,78899145	9
	Zn-3	6,127293880	3,799093274	9
	Zn-2	7,115943550	6,123979016	9
	Total	22,30959375	34,42998278	53

Tab. 35: Descriptive statistics for Ni content in dry weight of roots of Triticum aestivum (soil) growing in different HM concentrations, FA50 = 2% FA, FA100 = 1% FA

Bonferroni Post-hoc Tests showed significant differences of

Ni-3 to C (p <0.001 (0.000012)),
Ni-3 to Ni-4 ($p = 0.001$),
Ni-3 to Zn-4 ($p = 0.003$),
Ni-3 to Zn-3 (p <0.001 (0.000018)),
Ni-3 to Zn-2 (p <0.001 (0.000023)),

Ni content decreased from
Ni-3 (75.63 ± 41.94)
to all other groups
(C: 4.45 ± 3.40;
Ni-4: 20.10 ± 22.17;
Zn-4: 26.35 ± 42.79;
Zn-3: 6.13 ± 3.80 and
Zn-2: 7.12 ± 6.12) (Fig.112).

LSD Test for Simple Effects showed significant effects in Ni-3 between FA 100 and Adest (p = 0.004) as well as FA50 (p = 0.012).

FA100 (124.0 ± 16.9) had significant higher Ni content in roots than Adest (54.3 ± 2.2) and FA50 (64.8 ± 53.0) (Fig.113).

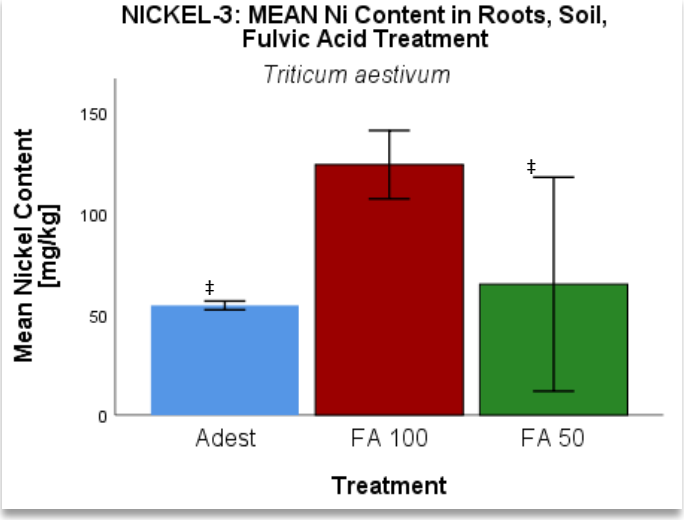


Fig. 113: Bar charts showing mean Ni content in roots of Triticum aestivum in Ni-3 contaminated soil. Error bars are ± 1SD. Significant differences to FA100 marked with ‡.

Zinc

The average total Zn content in the roots of the control is 115.5 ± 42.7, of Ni-4 it is 141.4 ± 109.1, in Ni-3 it is 123.8 ± 96.4, in Zn-4 it is 110.7 ± 34.4, in Zn-3 it is 275.8 ± 74.8 and in Zn-2 the total Zn content in the roots is 5 979.4 ± 1 041.2 mg/kg dry weight.

In the control the average total Zn content in the roots is 127.3 ± 77.7 mg/kg dry weight, when treated with FA100 it is 109.4 ± 5.5 mg/kg dry weight and with FA50 it is 109.7 ± 30.1 mg/kg dry weight.

In Ni-4 the average total Zn content in the roots is 215.3 ± 173.7 mg/kg dry weight, with FA100 treatment it is 84.8 ± 4.4 and with FA50 treatment it is 124.2 ± 63.0 mg/kg dry weight. In Ni-3 it is 181.1 ± 155.4 mg/kg dry weight, treated with FA100 total Zn content in the roots is 96.2 ± 7.5 mg/kg dry weight and with FA50 it is 84.8 ± 19.7 mg/kg dry weight.

The average total Zn content in the roots of Zn-4 is 118.9 ± 44.3 mg/kg dry weight, treated with FA100 the Zn content is 115.1 ± 46.2 mg/kg and with FA50 it is 98.1 ± 16.1 mg/kg dry weight. In Zn-3 it is 275.4 ± 121.5 mg/kg dry weight, with FA100 it is 289.8 ± 53.8 and with FA50 it is 262.3 ± 64.5 mg/kg dry weight. In Zn-2 the average total Zn content in the roots is 6 193.2 ± 250.6 mg/kg dry weight, treated with FA100 it is 6 723.0 ± 413.5 mg/kg dry weight and with FA50 5 022.0 ± 1 352.7 mg/kg dry weight.

Statistical analysis showed significant decrease of average total Zn content in the roots (mg/kg dry weight) of all groups compared with Zn-2. Zn content decreased significantly from FA100 (1 303.5 ± 2 591.3) to FA50 (950.2 ± 1 931.6) and Adest (1 185.2 ± 250.6).

Following a detailed report of the statistical analysis.

Results for the Two Way ANOVA for mean Zn content in the roots (Fig.114) in correlation with HM treatment and FA treatment (Tab.36) was significant for the corrected model on p <0.05 level
($F(17,35) = 123.358$, p <0.001 ($3.8364*10^{-26}$), $R^2 = 0.984$, $adj.R^2 = 0.976$),
Homogeneity criterion was not met
(Levene´s Test of Equality of Error Variances, based on mean:
Levene statistic = 5.584, $df_1 = 17$, $df_2 = 35$, p <0.001 (0.000009).

Significant main effect for HM
($F(5,35) = 411.555$, p <0.001 ($4.8298*10^{-26}$), $\eta_p^2 = 0.983$)
with Cohen´s value $f = 7.6$, and significant main effect for FA treatment
($F(2,35) = 3.331$, $p = 0.047$, $\eta_p^2 = 0.160$)
with Cohen´s value $f = 0.44$ as well as significant interactive effect between HM and FA
($F(10,35) = 3.029$, $p = 0.007$, $\eta_p^2 = 0.464$), with
Cohen´s value ($f = 0.93$). All three Cohen´s values are above 0.4, suggesting strong effect sizes.

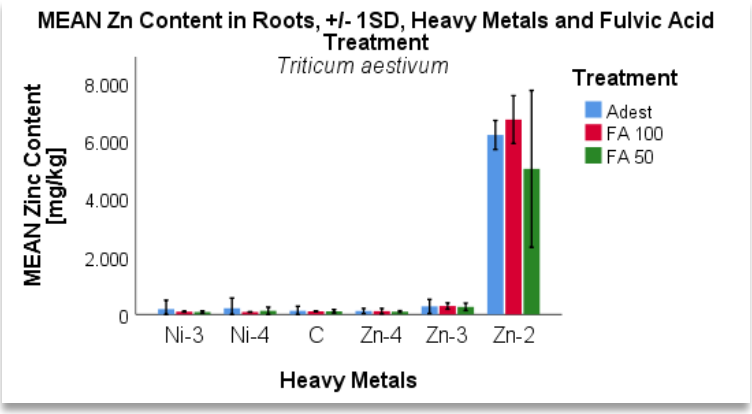


Fig. 114: Content of Zn (mg/kg dry weight) in roots growing in different HMs, FA50 = 2% FA, FA100 = 1% FA

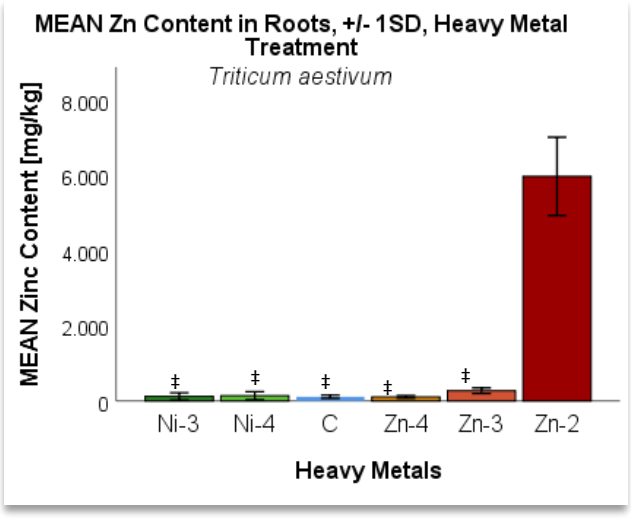


Fig. 115: Significant decrease of Zn content in roots (dry weight) compared to Zn-2 marked with ‡

Descriptive Statistics

Zn Content in Roots

Treatment	HM	Mean	Std. Deviation	N
Adest	Ni-3	181,1161792	155,3580948	3
	Ni-4	215,3416645	173,7012069	3
	C	127,3262158	77,69354225	3
	Zn-4	118,8822570	44,28922357	3
	Zn-3	275,3752292	121,4699336	3
	Zn-2	6193,168585	250,5850085	3
	Total	1185,201688	2308,769968	18
FA 100	Ni-3	96,18396228	7,537149577	2
	Ni-4	84,80576239	4,372807148	3
	C	109,4058222	5,507114395	3
	Zn-4	115,1464560	46,25674586	3
	Zn-3	289,7529293	53,80511528	3
	Zn-2	6723,007632	413,5420705	3
	Total	1303,454337	2591,253359	17
FA 50	Ni-3	84,81335221	19,71317938	3
	Ni-4	124,1671991	62,96439582	3
	C	109,7403712	30,05315204	3
	Zn-4	98,07567190	16,10341963	3
	Zn-3	262,2872294	64,48999777	3
	Zn-2	5021,960341	1352,734058	3
	Total	950,1740275	1931,589323	18
Total	Ni-3	123,7695648	96,39716631	8
	Ni-4	141,4382087	109,0946947	9
	C	115,4908031	42,67629302	9
	Zn-4	110,7014616	34,38634510	9
	Zn-3	275,8051293	74,79151747	9
	Zn-2	5979,378853	1041,238367	9
	Total	1143,311068	2247,294347	53

Tab. 36: Descriptive statistics for Zn content in dry weight of roots of Triticum aestivum *(soil) growing in different HM concentrations, FA50 = 2% FA, FA100 = 1% FA*

Bonferroni Post-hoc Tests showed significant differences of

Zn-2
to Ni-3 (p <0.001 ($2.2717*10^{-27}$)),
to Ni-4 (p <0.001 ($8.9716*10^{-28}$)),
to C (p <0.001 ($7.7134*10^{-28}$)),
to Zn-4 (p <0.001 ($7.5018*10^{-28}$)),
to Zn-3 (p <0.001 ($1.9823*10^{-27}$)).

And significant difference between FA100 (1303.45 ± 2591.25) to FA50 (950.17 ± 1931.59) ($p = 0.016$), effect depending on HM and its concentration.
Zn content decreased from Zn-2 (5 979 ± 1 041) to all other groups (C: 115 ± 43; Ni-4: 141 ± 109; Ni-3: 124 ± 96; Zn-4: 111 ± 34 and Zn-3: 276 ± 75) (Fig.115).

LSD Test for Simple Effects showed significant effects in Zn-2 between Adest and FA50 (p <0.001 (0.000242)) and between FA50 and FA100 (p<0.001 ($9.37*10^{-7}$)) as well (Fig.116).

Zn content in roots is significantly lower in FA50 treated plants (5 021.96 ± 202.63) compared to Adest (6 193.17 ± 202.62) or FA100 (6 723.01 ± 202.62) (Tab.37).

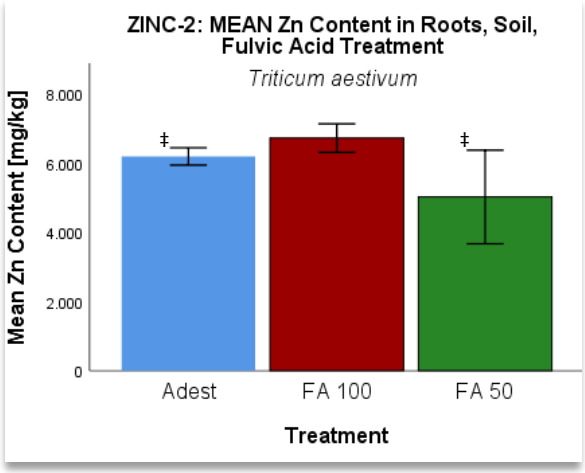


Fig. 116: Bar chart showing mean of Zn content in roots of Triticum aestivum *in Zn-2, error bars: ± 1SD, Significant differences to FA100 marked with ‡. FA50 = 2% FA, FA100 = 1% FA*

Descriptive Statistics

Zinc Content in Roots

HM	Treatment	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Ni-3	Adest	181,116	202,628	-230,240	592,473
	FA 100	96,184	248,167	-407,623	599,991
	FA 50	84,813	202,628	-326,543	496,170
Ni-4	Adest	215,342	202,628	-196,015	626,698
	FA 100	84,806	202,628	-326,551	496,162
	FA 50	124,167	202,628	-287,189	535,524
C	Adest	127,326	202,628	-284,030	538,683
	FA 100	109,406	202,628	-301,951	520,762
	FA 50	109,740	202,628	-301,616	521,097
Zn-4	Adest	118,882	202,628	-292,474	530,239
	FA 100	115,146	202,628	-296,210	526,503
	FA 50	98,076	202,628	-313,281	509,432
Zn-3	Adest	275,375	202,628	-135,981	686,732
	FA 100	289,753	202,628	-121,603	701,109
	FA 50	262,287	202,628	-149,069	673,644
Zn-2	Adest	6193,169	202,628	5781,812	6604,525
	FA 100	6723,008	202,628	6311,651	7134,364
	FA 50	5021,960	202,628	4610,604	5433,317

Tab. 37: Descriptive statistics for Zn content in roots (HM and FA treatment) of Triticum aestivum*, FA50 = 2% FA, FA100 = 1% FA*

Potassium

The average total K content in the roots of the control is 9 121.9 ± 1 696.7, of Ni-4 it is 7 606.9 ± 1696.5, in Ni-3 it is 9 013.2 ± 1696.7, in Zn-4 it is 5 917.4 ± 1937.5, in Zn-3 it is 7 081.7 ± 2 176.4 and in Zn-2 the total K content in the roots is 10 051.4 ± 1 133.7 mg/kg dry weight.

In the control the average total K content in the roots is 7 237.7 ± 1235.3 mg/kg dry weight, when treated with FA100 it is 10 071.1 ± 529.1 mg/kg dry weight and with FA50 it is 10 056.9 ± 678.4 mg/kg dry weight.

In Ni-4 the average total K content in the roots is 6 303.3 ± 1 541.9 mg/kg dry weight, with FA100 treatment it is 9 061.3 ± 720.4and with FA50 treatment it is 7 456.0 ± 1 691.1 mg/kg dry weight. In Ni-3 it is 8 595.3 ± 1 147.7 mg/kg dry weight, treated with FA100 total K content in the roots is 10 822.3 ± 120.7 mg/kg dry weight and with FA50 it is 8 224.9 ± 2 070.0 mg/kg dry weight.

The average total K content in the roots of Zn-4 is 6 003.9 ± 702.2 mg/kg dry weight, treated with FA100 the K content is 8 575.8 ± 1 543.6 mg/kg and with FA50 it is 9 172.4 ± 1 906.6 mg/kg dry weight. In Zn-3 it is 4 819.6 ± 1 100.4 mg/kg dry weight, with FA100 it is 8 378.9 ± 606.2 and with FA50 it is 8 046.5 ± 2 402.5 mg/kg dry weight. In Zn-2 the average total K content in the roots is 10 495.6 ± 2 115.7 mg/kg dry weight treated with FA100 it is 11 038.9 ± 167.0 mg/kg dry weight and with FA50 8 619.8 ± 450.9 mg/kg dry weight.

Statistical analysis showed that K content significantly decreased from Zn-2 to Ni-4, Zn-4 and Zn-3 and increased from Zn-3 to Ni-3 and C. In the control plants and in Zn-4 K content significantly increased, when treated with FA100 or FA50. Foliar application of FA100 or FA50 significantly increased K content in Zn-3 and significantly decreased K content in Zn-2 when comparing FA100 with FA50. In Ni-3 there also was a significant decrease in K content from FA50 to FA100. In Ni-4 treatment with FA100 led to an increase in K content.

Following a detailed report of the statistical analysis.

Results for the Two Way ANOVA for mean K content in roots (Tab.38) in correlation with HM treatment and FA treatment (Fig.117) was significant for the corrected model on p <0.05 level
($F(17,35) = 123.358$, p <0.001 ($3.8364*10^{-26}$), $R^2 = 0.984$, $adj.R^2 = 0.976$),
Homogeneity criterion was not met
(Levene´s Test of Equality of Error Variances, based on mean:
Levene statistic = 2.480, $df_1 = 17$, $df_2 = 35$, $p = 0.011$.

Significant main effect for HMs
($F(5,35) = 7.226$, p <0.001 (0.000097), $\eta_p^2 = 0.508$)
with Cohen´s value $f = 1.02$, and significant main effect for FA treatment
($F(2,35) = 16.007$, p <0.001 (0.000012), $\eta_p^2 = 0.478$)
with Cohen´s value $f = 0.96$ as well as significant interactive effect between HM and FA ($F(10,35) = 2.278$, $p = 0.035$, $\eta_p^2 = 0.394$), with Cohen´s value ($f = 0.81$).
All three Cohen´s values are above 0.4, suggesting strong effect sizes.

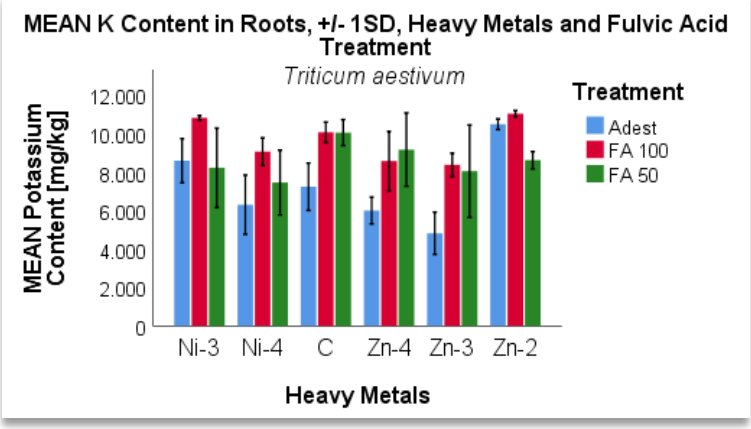


Fig. 117: Content of K (mg/kg dry weight) in roots growing in different HM

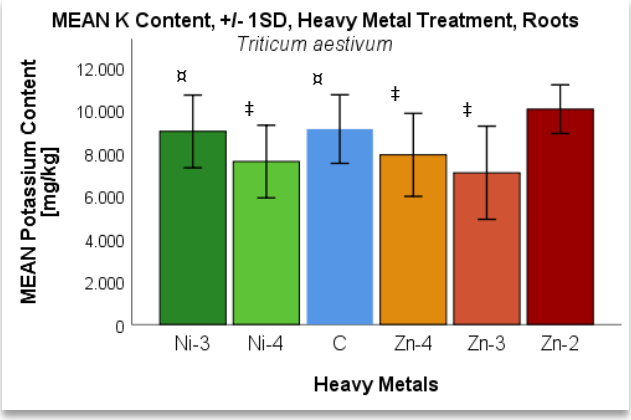


Fig. 118: Significant decrease of K content in roots (dry weight) compared to Zn-2 marked with ‡ and increase from Zn-3 to groups marked with ¤

Descriptive Statistics

Potassium Content in Roots

Treatment	HM	Mean	Std. Deviation	N
Adest	Ni-3	8595,305581	1147,668096	3
	Ni-4	6303,334110	1541,853148	3
	C	7237,688439	1235,284534	3
	Zn-4	6003,904883	702,2058869	3
	Zn-3	4819,593503	1100,428869	3
	Zn-2	10495,47549	277,4249004	3
	Total	7242,550334	2115,746435	18
FA 100	Ni-3	10822,26708	120,6997161	2
	Ni-4	9061,314008	720,4280146	3
	C	10071,06297	529,1267151	3
	Zn-4	8575,777088	1543,632162	3
	Zn-3	8378,945252	606,1828886	3
	Zn-2	11038,91043	167,0283962	3
	Total	9589,562554	1262,996101	17
FA 50	Ni-3	8224,923745	2070,016044	3
	Ni-4	7456,018326	1691,128936	3
	C	10056,87446	678,3535789	3
	Zn-4	9172,402986	1906,555968	3
	Zn-3	8046,515042	2402,487989	3
	Zn-2	8619,817603	450,8617602	3
	Total	8596,092026	1663,605390	18
Total	Ni-3	9013,152767	1696,731243	8
	Ni-4	7606,888815	1696,488937	9
	C	9121,875290	1601,099444	9
	Zn-4	7917,361652	1937,510238	9
	Zn-3	7081,684599	2176,385111	9
	Zn-2	10051,40117	1133,672293	9
	Total	8455,058979	1948,233194	53

Tab. 38: Descriptive statistics for K content in dry weight of roots of Triticum aestivum *(soil) growing in different HM concentrations, FA50 = 2% FA, FA100 = 1% FA*

Bonferroni Post-hoc Tests showed significant differences of
Adest to FA100
(p <0.001 (0.000010) and Adest to FA50 (p = 0.008) as well as
Zn-2 to Ni-4 (p = 0.003), to
Zn-4 (p = 0.015), to Zn-3
(p <0.001 (0.000242)) and Zn-3 to Ni-3 (p = 0.050) and to C (p = 0.023).

K content decreased from
Zn-2 (10 051.40 ± 1 133.67)
to Ni-4 (7 606.89 ± 1 695.49),
Zn-4 (7 917.36 ± 1 937.51) and
Zn-3 (7 081.68 ± 2 176.39) and increased from Zn-3 to Ni-3
(9 013.15 ± 1 696.73) and the control (9 121.88 ± 1 601.10) (Fig.118).

LSD Test for Simple Effects showed significant effects in

- the control between Adest and FA100 (p = 0.009) and FA50 (p = 0.01)
- Zn-4 between Adest and FA100 (p = 0.017) and FA50 (p = 0.004)
- Zn-3 between Adest and FA100 (p = 0.001) and FA50 (p = 0.003)
- Zn-2 between FA100 and FA50 (p = 0.025)
- Ni-3 between FA100 and FA50 (p = 0.03)
- Ni-4 between Adest and FA100 (p = 0.011)

Descriptive Statistics

Potassium Content in Roots

HM	Treatment	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Ni-3	Adest	8595,306	727,698	7118,001	10072,610
	FA 100	10822,267	891,244	9012,946	12631,588
	FA 50	8224,924	727,698	6747,619	9702,228
Ni-4	Adest	6303,334	727,698	4826,030	7780,639
	FA 100	9061,314	727,698	7584,010	10538,618
	FA 50	7456,018	727,698	5978,714	8933,323
C	Adest	7237,688	727,698	5760,384	8714,993
	FA 100	10071,063	727,698	8593,758	11548,367
	FA 50	10056,874	727,698	8579,570	11534,179
Zn-4	Adest	6003,905	727,698	4526,600	7481,209
	FA 100	8575,777	727,698	7098,473	10053,082
	FA 50	9172,403	727,698	7695,099	10649,707
Zn-3	Adest	4819,594	727,698	3342,289	6296,898
	FA 100	8378,945	727,698	6901,641	9856,250
	FA 50	8046,515	727,698	6569,211	9523,820
Zn-2	Adest	10495,475	727,698	9018,171	11972,780
	FA 100	11038,910	727,698	9561,606	12516,215
	FA 50	8619,818	727,698	7142,513	10097,122

Tab. 39: Descriptive statistics for K content in roots (HM and FA treatment) of Triticum aestivum*, FA50 = 2% FA, FA100 = 1% FA*

In the control plants K content (7 237.69 ± 727.698) increased, when treated with FA100 (10 071.06 ± 727.698) or FA100 (10 056.87 ± 727.698) (Fig.119).
The same effect could be observed in Zn-4 (6 003.91 ± 727.698) for FA100 (8 575.78 ± 727.698) and FA50 (9 172.4 ± 727.698) (Fig.120).
Foliar application of FA100 (8 378.95 ± 727.698) or FA50 (8 046.52 ± 727.698) increase K content in Zn-3 as well (4 819.59 ± 727.698) (Fig. 122).
Significant decrease of K content from FA100 (11 028.9 ± 727.698) to FA50 (8 619.818 ± 727.698) could be observed in Zn-2 (Fig. 121).
In Ni-3 there was also a decrease in K content from FA100 (10 822.27 ± 891.24) to FA50 (8 224.9 ± 727.698) (Fig.123).
In Ni-4 (6 303.33 ± 727.698) treatment with FA100 led to an increase in K content (9 061.31 ± 727.698) (Fig.124, Tab.39).

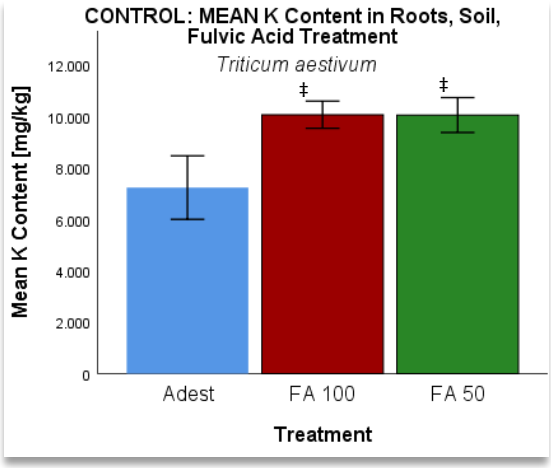


Fig. 119: Bar chart showing mean of K content in roots of Triticum aestivum *in C, error bars: ± 1SD, significant differences to Adest marked with ‡. FA50 = 2% FA, FA100 = 1% FA*

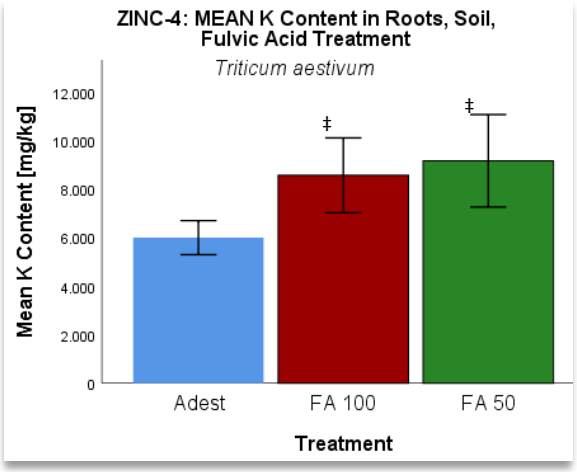


Fig. 120: Bar chart showing mean of K content in roots of Triticum aestivum *in Zn-4, error bars: ± 1SD, significant differences to Adest marked with ‡. FA50 = 2% FA, FA100 = 1% FA*

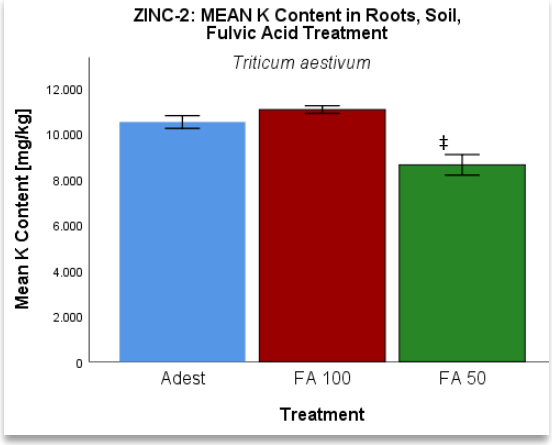


Fig. 121: Bar chart showing mean of K content in roots of Triticum aestivum *in Zn-2, error bars: ± 1SD, significant differences to FA100 marked with ‡. FA50 = 2% FA, FA100 = 1% FA*

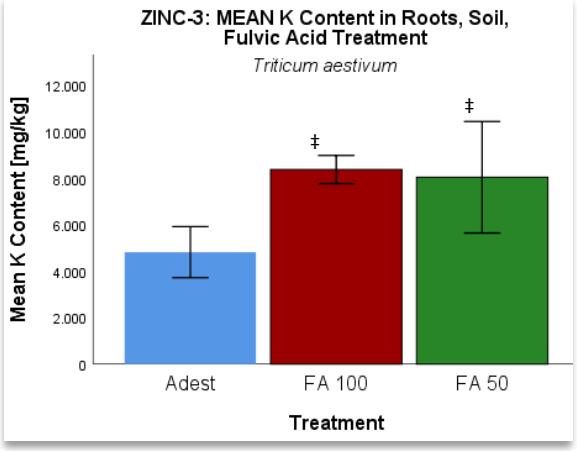


Fig. 122: Bar chart showing mean of K content in roots of Triticum aestivum *in Zn-3, error bars: ± 1SD, significant differences to Adest marked with ‡. FA50 = 2% FA, FA100 = 1% FA*

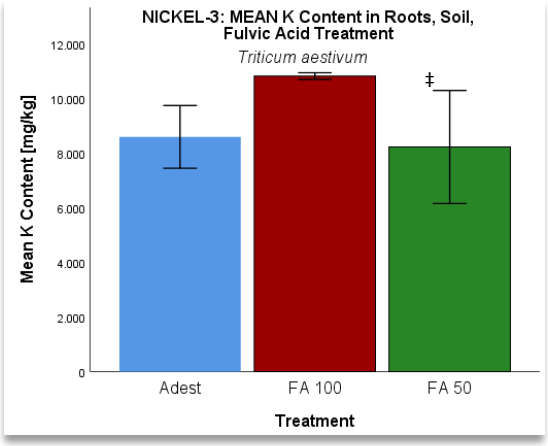


Fig. 123: Bar chart showing mean of K content in roots of Triticum aestivum *in Ni-3, error bars: ± 1SD, significant differences to FA100 marked with ‡. FA50 = 2% FA, FA100 = 1% FA*

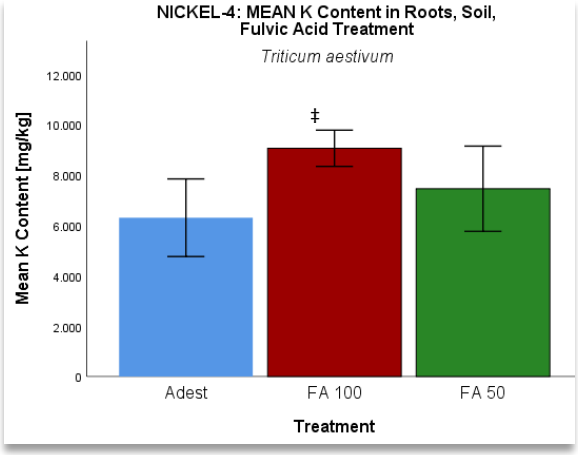


Fig. 124: Bar chart showing mean of K content in roots of Triticum aestivum *in Ni-4, error bars: ± 1SD, significant differences to Adest marked with ‡. FA50 = 2% FA, FA100 = 1% FA*

(C) Zn, Ni and K Content in Shoots

Nickel

The average total Ni content of the shoots in the control is 1.4 ± 1.6 mg/dry weight, in Ni-4 it is 3.1 ± 0.8 mg/kg, in Ni-3 it is 14.1 ± 0.8 mg/kg, in Zn-4 it is 5.5 ± 7.6 mg/kg dry weight, in Zn-3 it is 1.0 ± 0.9 mg/kg and in Zn-2 the total Ni content in the shoots is 20.0 ± 50.8 mg/kg dry weight.

In the control the average total Ni content in the shoots is 0.6 ± 0.3 mg/kg dry weight, when treated with FA100 it is 2.7 ± 2.6 mg/kg dry weight and with FA50 it is 1.4 ± 1.6 mg/kg dry weight.

In Ni-4 the average total Ni content in the shoots is 2.6 ± 1.2 mg/kg dry weight, with FA100 treatment it is 3.7 ± 0.6 and with FA50 treatment it is 3.0 ± 0.2 mg/kg dry weight. In Ni-3 it is 8.8 ± 5.9 mg/kg dry weight, treated with FA100 total Ni content in the shoots is 25.5 ± 12.7 mg/kg dry weight and with FA50 it is 11.9 ± 9.7 mg/kg dry weight.

The average total Ni content in the shoots of Zn-4 is 8.5 ± 11.6 mg/kg dry weight, treated with FA100 the Ni content is 5.4 ± 7.3 mg/kg and with FA50 it is 2.6 ± 3.7 mg/kg dry weight. In Zn-3 it is 1.4 ± 1.3 mg/kg dry weight, with FA100 it is 1.1 ± 0.6 and with FA50 it is 0.4 ± 0.2 mg/kg dry weight. In Zn-2 the average total Ni content in the shoots is 2.1 ± 1.5 mg/kg dry weight, treated with FA100 it is 1.9 ± 1.2 mg/kg dry weight and with FA50 56.1 ± 86.0 mg/kg dry weight.

Statistical analysis of HMs, FA and different treatment combinations ((C, Ni, Zn) x (Adest, FA100, FA50)) generally showed significant higher Ni content in shoots of *Triticum aestivum* growing in Zn-2 contaminated soil. FA50 treated plants had significantly higher amount of Ni in shoots than Fa100 or Adest treated plants.

Following a detailed report of the statistical analysis.

Results for the Two Way ANOVA for mean Ni content in shoots in correlation with HM treatment and FA treatment (Tab.40) was not significant for the corrected model on p <0.05 level ($F(17,35) = 1.179$, $p = 0.329$, $R^2 = 0.364$, $adj.R^2 = 0.055$).

Homogeneity criterion was not met (Levene´s Test of Equality of Error Variances, based on mean:
Levene statistic = 13.833, $df_1 = 17$, $df_2 = 35$, p <0.001 ($8.1378*10^{-11}$).

No significant differences between different FA treatment groups ($F(2,35) = 0.752$, $p = 0.752$, $\eta_p^2 = 0.041$),
nor between different HM treated groups
($F(5,35) = 1.262$, $p = 0.302$, $\eta_p^2 = 0.153$
and no interactive effect between the two factors (HM, FA) was observed ($F(10,35) = 1.245$, $p = 0.298$, $\eta_p^2 = 0.262$) (Fig.125).

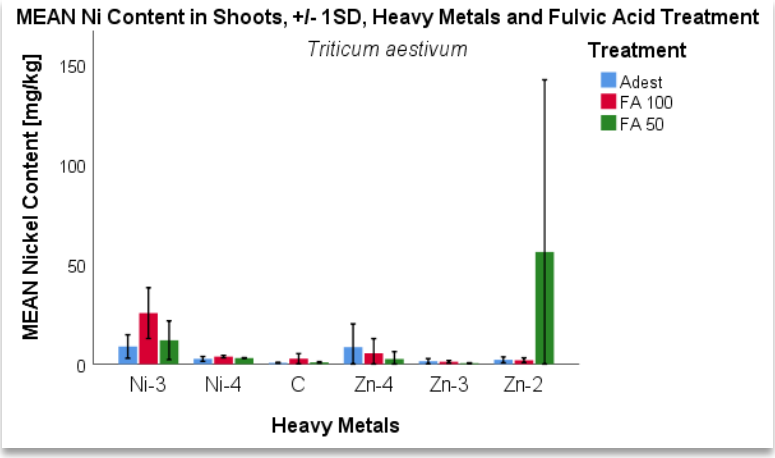


Fig. 125: Content of Ni (mg/kg dry weight) in shoots growing in different HM, no significant differences between any groups, FA50 = 2% FA, FA100 = 1% FA

Descriptive Statistics

Nickel Content in Shoots

Treatment	HM	Mean	Std. Deviation	N
Adest	Ni-3	8,777955330	5,882946362	3
	Ni-4	2,593951161	1,197825918	3
	C	,6499269178	,2507870583	3
	Zn-4	8,455846196	11,64984360	3
	Zn-3	1,439247495	1,316301499	3
	Zn-2	2,110279961	1,473524655	3
	Total	4,004534510	5,685349132	18
FA 100	Ni-3	25,48253983	12,71079411	2
	Ni-4	3,705817244	,6072288193	3
	C	2,680526578	2,574394922	3
	Zn-4	5,442828879	7,269437171	3
	Zn-3	1,148307285	,6486586754	3
	Zn-2	1,928286808	1,183352423	3
	Total	5,628375297	8,702766992	17
FA 50	Ni-3	11,91334395	9,706109805	3
	Ni-4	3,007645758	,2421428352	3
	C	,8861613085	,3774829774	3
	Zn-4	2,561690126	3,663370634	3
	Zn-3	,4201089608	,2370321933	3
	Zn-2	56,07525180	85,98923742	3
	Total	12,47736698	36,06411010	18
Total	Ni-3	14,12987219	10,53994916	8
	Ni-4	3,102471388	,8380876054	9
	C	1,405538268	1,622678415	9
	Zn-4	5,486788400	7,550563090	9
	Zn-3	1,002554580	,8712595985	9
	Zn-2	20,03793952	50,79319126	9
	Total	7,402954848	21,74889971	53

Tab. 40: Descriptive statistics for Ni content in dry weight of shoots of Triticum aestivum *(soil) growing in different HM concentrations, FA50 = 2% FA, FA100 = 1% FA*

Nevertheless, LSD Test for Simple Effects showed significant effects in Zn-2 between
FA50 and Adest (p = 0.004) as well as
between FA100 (p = 0.003).

Ni content in shoots of FA50 (56.1 ± 86.0) treated plant was significantly higher than in
Adest (2.1 ± 1.5) or FA100 (1.9 ± 1.2) treated plants (Fig.126).

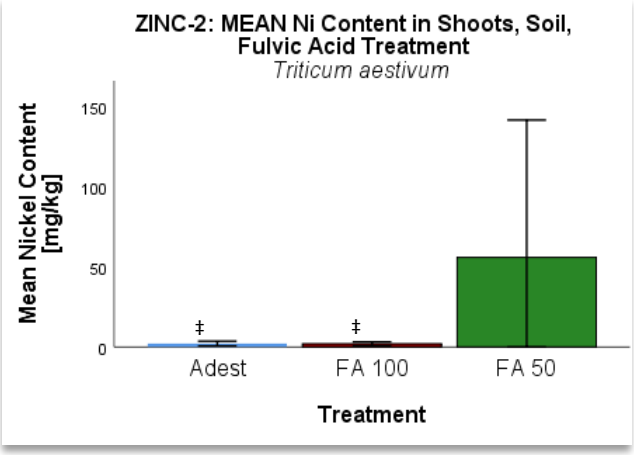


Fig. 126: Bar chart showing mean Ni content in shoots of Triticum aestivum in Zn-2 treated soil. Error bars are ± 1SD. Significant differences to FA50 are marked with ‡.

Zinc

The average total Zn content of the shoots in the control is 50.8 ± 17.8 mg/dry weight, in Ni-4 it is 59.6 ± 45.8 mg/kg, in Ni-3 it is 99.4 ± 115.8 mg/kg, in Zn-4 it is 50.6 ± 21.8 mg/kg dry weight, in Zn-3 it is 90.1 ± 33.8 mg/kg and in Zn-2 the total Zn content in the shoots is 1 138.6 ± 249.7 mg/kg dry weight.

In the control the average total Zn content in the shoots is 54.6 ± 19.3 mg/kg dry weight, when treated with FA100 it is 56.5 ± 26.1 mg/kg dry weight and with FA50 it is 41.3 ± 3.7 mg/kg dry weight.

In Ni-4 the average total Zn content in the shoots is 99.5 ± 66.4 mg/kg dry weight, with FA100 treatment it is 47.8 ± 13.9 and with FA50 treatment it is 31.5 ± 2.7 mg/kg dry weight. In Ni-3 it is 178.6 ± 176.3 mg/kg dry weight, treated with FA100 total Zn content in the shoots is 69.7 ± 20.8 mg/kg dry weight and with FA50 it is 40.0 ± 2.4 mg/kg dry weight.

The average total Zn content in the shoots of Zn-4 is 61.6 ± 35.4 mg/kg dry weight, treated with FA100 the Ni content is 42.9 ± 6.9 mg/kg and with FA50 it is 47.2 ± 17.5 mg/kg dry weight. In Zn-3 it is 99.8 ± 62.8 mg/kg dry weight, with FA100 it is 80.0 ± 8.6 and with FA50 it is 90.5 ± 15.7 mg/kg dry weight. In Zn-2 the average total Zn content in the shoots is 1 317.5 ± 187.4 mg/kg dry weight, treated with FA100 it is 1 214.1 ± 181.9 mg/kg dry weight and with FA50 884.2 ± 166.0 mg/kg dry weight.

Statistical analysis of HMs, FA and different treatment combinations ((C, Ni, Zn) x (Adest, FA100, FA50)) showed no significant differences.

Following a detailed report of the statistical analysis.

Results for the Two Way ANOVA for mean Zn content (Fig.127) in correlation with HM treatment and FA treatment (Tab.41) was significant for the corrected model on p <0.05 level ($F(17,35) = 66.143$, p <0.001 ($1.4802*10^{-21}$), $R^2 = 0.970$, $adj.R^2 = 0.955$),
Homogeneity criterion was not met
(Levene´s Test of Equality of Error Variances, based on mean:
Levene statistic = 4.246, $df_1 = 17$, $df_2 = 35$, p <0.001 (0.000146).

Significant main effect for HMs
($F(5,35) = 216.143$, p <0.001 ($2.8521*10^{-25}$), $\eta_p^2 = 0.969$)
with Cohen´s value $f = 5.59$,
and significant main effect for FA treatment
($F(2,35) = 7.260$, $p = 0.002$, $\eta_p^2 = 0.293$)
with Cohen´s value $f = 0.64$ as well as significant interactive effect between HM and FA
($F(10,35) = 2.931$, $p = 0.009$, $\eta_p^2 = 0.456$),
with
Cohen´s value ($f = 0.92$). All three Cohen´s values are above 0.4, suggesting strong effect sizes.

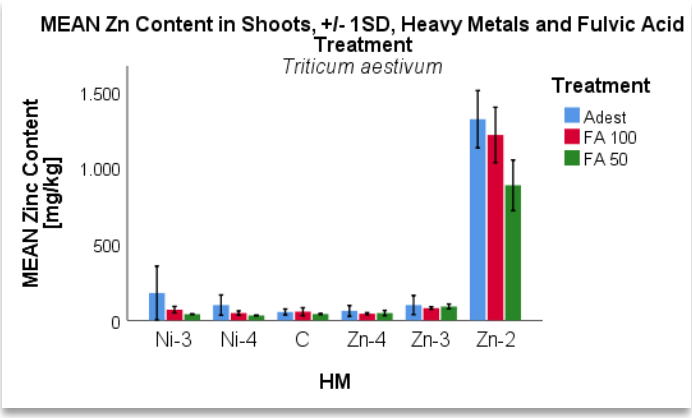


Fig. 127: Content of Zn (mg/kg dry weight) in shoots growing in different HMs decrease of Zn content from Adest to FA100 in all groups, FA50 = 2% FA, FA100 = 1% FA

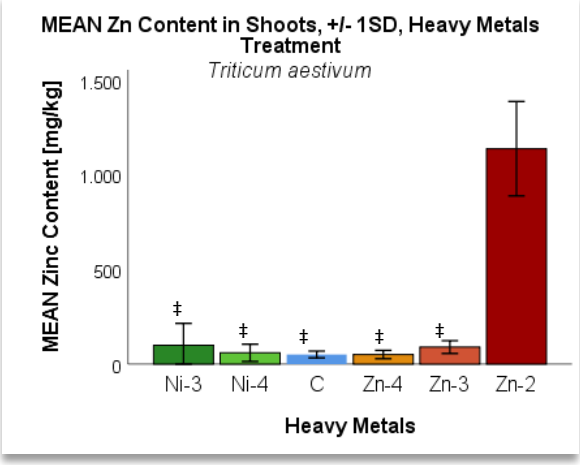


Fig. 128: Significant decrease of Zn content in shoots (dry weight) compared to Zn-2 marked with ‡

Discriptive Statistics

Zinc Content in Shoots

Treatment	HM	Mean	Std. Deviation	N
Adest	Ni-3	178,6329339	176,3225158	3
	Ni-4	99,50602607	66,36159461	3
	C	54,62015498	19,32848271	3
	Zn-4	61,64653495	35,39485664	3
	Zn-3	99,76130518	62,84759548	3
	Zn-2	1317,511209	187,3931019	3
	Total	301,9463606	478,6274285	18
FA 100	Ni-3	69,65726363	20,86952905	2
	Ni-4	47,83505370	13,85580184	3
	C	56,46816880	26,10962710	3
	Zn-4	42,92994141	6,874605907	3
	Zn-3	79,97502844	8,601161752	3
	Zn-2	1214,093007	181,9393491	3
	Total	262,5422428	458,9228708	17
FA 50	Ni-3	40,03578324	2,404482401	3
	Ni-4	31,48035253	2,663386305	3
	C	41,35997990	3,692062185	3
	Zn-4	47,16687770	17,48309052	3
	Zn-3	90,47347158	15,65550506	3
	Zn-2	884,2248769	165,9671988	3
	Total	189,1235570	325,5882231	18

Tab. 41: Descriptive statistics for Zn content in dry weight of shoots of Triticum aestivum *(soil) growing in different HM concentrations*

Bonferroni Post-hoc Tests showed significant differences of
Zn-2
to Ni-3 (p <0.001 ($3.6558*10^{-22}$)),
to Ni-4 (p <0.001 ($3.8432*10^{-23}$)),
to C (p <0.001 ($2.9332*10^{-23}$)),
to Zn-4 (p <0.001 ($2.9122*10^{-23}$)),
to Zn-3 (p <0.001 ($9.9571*10^{-23}$)).

And significant difference between Adest and FA50 ($p = 0.002$).

Zn decreases from Adest to FA50 in all groups.
Zn content decreased from
Zn-2 (1 138.61 ± 249.69)
to all other groups
(C: 50.82 ± 17.84; Ni-4: 59.61 ± 45.79; Ni-3: 99.42 ± 115.76; Zn-4: 50.58 ± 21.76 and Zn-3: 90.07 ± 33.77) (Fig.128).

LSD Test for Simple Effects showed significant effects in Zn-3 between FA50 and Adest (p <0.001 (8.65*10-7) as well as FA100 (p <0.001 (0.000064).

K content in shoots increased from FA50 (884.2 ± 51.4) to Adest (1 317.5 ± 51.4) and Fa100 (1 214.1 ± 51.4) (Fig.129, Tab.42).

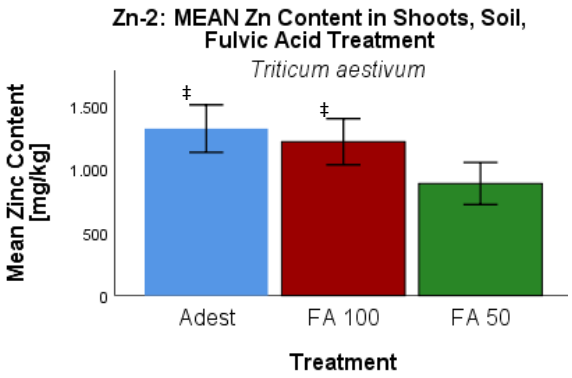


Fig. 129: Bar chart showing mean of Zn content in shoots of Triticum aestivum *in Zn-2, error bars: ± 1SD, significant differences to FA50 marked with ‡. FA50 = 2% FA, FA100 = 1% FA*

Descriptive Statistics

Zinc Content in Shoots

HM	Treatment	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Ni-3	Adest	178,633	51,385	74,315	282,950
	FA 100	69,657	62,934	-58,105	197,420
	FA 50	40,036	51,385	-64,282	144,353
Ni-4	Adest	99,506	51,385	-4,811	203,823
	FA 100	47,835	51,385	-56,482	152,152
	FA 50	31,480	51,385	-72,837	135,798
C	Adest	54,620	51,385	-49,697	158,938
	FA 100	56,468	51,385	-47,849	160,786
	FA 50	41,360	51,385	-62,957	145,677
Zn-4	Adest	61,647	51,385	-42,671	165,964
	FA 100	42,930	51,385	-61,387	147,247
	FA 50	47,167	51,385	-57,151	151,484
Zn-3	Adest	99,761	51,385	-4,556	204,079
	FA 100	79,975	51,385	-24,342	184,292
	FA 50	90,473	51,385	-13,844	194,791
Zn-2	Adest	1317,511	51,385	1213,194	1421,829
	FA 100	1214,093	51,385	1109,776	1318,410
	FA 50	884,225	51,385	779,907	988,542

Tab. 42: Descriptive statistics for Zn content in shoots (HM and FA treatment) of Triticum aestivum*, FA50 = 2% FA, FA100 = 1% FA*

Potassium

The average total K content of the shoots in the control is 50.8 ± 17.8 mg/dry weight, in Ni-4 it is 59.6 ± 45.8 mg/kg, in Ni-3 it is 99.4 ± 115.8 mg/kg, in Zn-4 it is 50.6 ± 21.8 mg/kg dry weight, in Zn-3 it is 90.1 ± 33.8 mg/kg and in Zn-2 the total K content in the shoots is 1 138.6 ± 249.7 mg/kg dry weight.

In the control the average total K content in the shoots is 54.6 ± 19.3 mg/kg dry weight, when treated with FA100 it is 56.5 ± 26.1 mg/kg dry weight and with FA50 it is 41.3 ± 3.7 mg/kg dry weight.

In Ni-4 the average total K content in the shoots is 99.5 ± 66.4 mg/kg dry weight, with FA100 treatment it is 47.8 ± 13.9 and with FA50 treatment it is 31.5 ± 2.7 mg/kg dry weight. In Ni-3 it is 178.6 ± 176.3 mg/kg dry weight, treated with FA100 total K content in the shoots is 69.7 ± 20.8 mg/kg dry weight and with FA50 it is 40.0 ± 2.4 mg/kg dry weight.

The average total K content in the shoots of Zn-4 is 61.6 ± 35.4 mg/kg dry weight, treated with FA100 the K content is 42.9 ± 6.9 mg/kg and with FA50 it is 47.2 ± 17.5 mg/kg dry weight. In Zn-3 it is 99.8 ± 62.8 mg/kg dry weight, with FA100 it is 80.0 ± 8.6 and with FA50 it is 90.5 ± 15.7 mg/kg dry weight. In Zn-2 the average total K content in the shoots is 1 317.5 ± 187.4 mg/kg dry weight, treated with FA100 it is 1 214.1 ± 181.9 mg/kg dry weight and with FA50 884.2 ± 166.0 mg/kg dry weight.

Statistical analysis of HMs, FA and different treatment combinations ((C, Ni, Zn) x (Adest, FA100, FA50)) showed significant decrease of total K content in shoots of FA100 (15 268.0 ± 2 271.7) and FA50 (11 657.7 ± 1 854.9) treated plants of Ni-3 group, compared to Adest (21 322.2 ± 1 854.9).

Following a detailed report of the statistical analysis.

Results for the Two Way ANOVA for mean K content (Tab.43) in correlation with HM treatment and FA treatment (Fig.130) was not significant for the corrected model on p <0.05 level ($F(17,35) = 123.358$, p <0.001 ($3.8364*10^{-26}$), $R^2 = 0.984$, $adj.R^2 = 0.976$),
Homogeneity criterion was not met
(Levene´s Test of Equality of Error Variances, based on mean: *Levene statistic* = 6.909, $df_1 = 17$, $df_2 = 35$, p <0.001 ($7.7502*10^{-7}$).

No significant main effect for HMs
($F(5,35) = 1.226$, $p = 0.318$, $\eta_p^2 = 0.149$) or for FA treatment
($F(2,35) = 3.263$, $p = 0.050$, $\eta_p^2 = 0.157$)
and no interactive effect
($F(10,35) = 1.235$, $p = 0.304$, $\eta_p^2 = 0.261$).

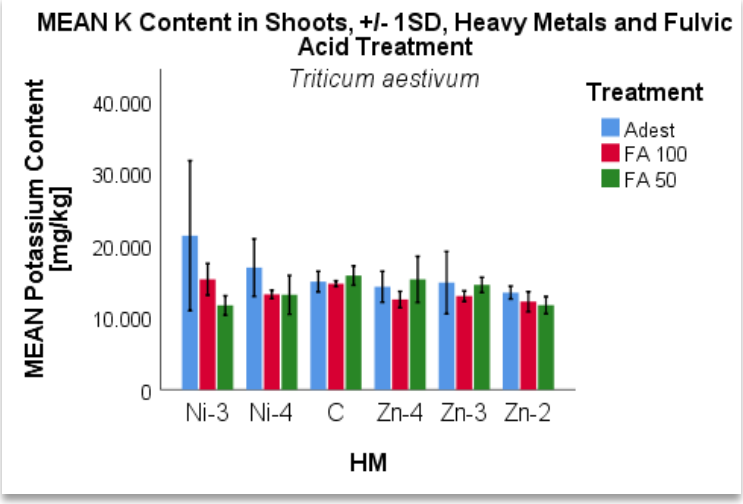


Fig. 130: Content of K (mg/kg dry weight) in shoots growing in different HM, no significant differences between treatment and HM groups.

LSD Test for Simple Effects showed significant effects in Ni-3 between Adest and FA100 (p = 0.046) as well as FA50 (p = 0.001). K content in shoots decreased from Adest (21 322.21 ± 1 854.85) to FA100 (15 267.99 ± 2 271.72) and FA50 (11 657.74 ± 1 854.85) (Fig.131, Tab.42).

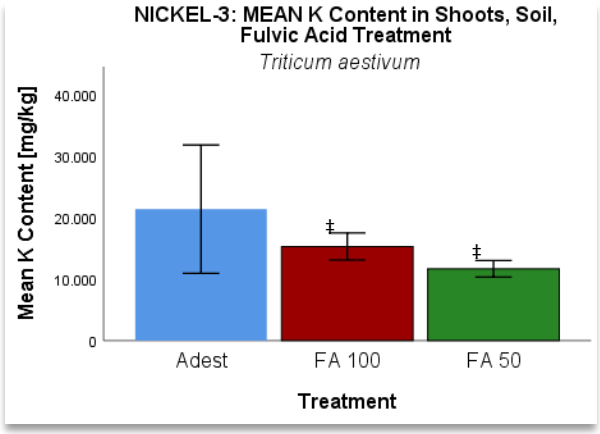


Fig. 131: mean of K content in shoots of Triticum aestivum *in Ni-3, error bars: ± 1SD, Significant differences to Adest marked with ‡.*

Descriptive Statistics

Potassium Content in Shoots

Treatment	HM	Mean	Std. Deviation	N
Adest	Ni-3	21322,21430	10406,52291	3
	Ni-4	16901,58201	3980,253403	3
	C	14964,21296	1442,122485	3
	Zn-4	14247,97277	2139,796960	3
	Zn-3	14815,37387	4326,552058	3
	Zn-2	13441,07761	892,8194510	3
	Total	15948,73892	4995,741160	18
FA 100	Ni-3	15267,98598	2194,145692	2
	Ni-4	13185,54819	591,1478422	3
	C	14669,07817	409,9882649	3
	Zn-4	12480,74967	1137,621276	3
	Zn-3	12947,53678	736,0963893	3
	Zn-2	12183,99218	1394,955230	3
	Total	13349,21688	1434,028719	17
FA 50	Ni-3	11657,73739	1341,086681	3
	Ni-4	13130,32645	2694,461284	3
	C	15802,74466	1328,429321	3
	Zn-4	15272,96028	3201,357156	3
	Zn-3	14500,74262	1050,229766	3
	Zn-2	11705,04412	1195,821687	3
	Total	13678,25925	2366,903596	18

Tab. 43: Descriptive statistics for K content in dry weight of shoots of Triticum aestivum *(soil) growing in different HM concentrations*

Descriptive Statistics

Potassium Content in Shoots

HM	Treatment	Mean	Std. Error
Ni-3	Adest	21322,214	1854,849
	FA 100	15267,986	2271,717
	FA 50	11657,737	1854,849
Ni-4	Adest	16901,582	1854,849
	FA 100	13185,548	1854,849
	FA 50	13130,326	1854,849
C	Adest	14964,213	1854,849
	FA 100	14669,078	1854,849
	FA 50	15802,745	1854,849
Zn-4	Adest	14247,973	1854,849
	FA 100	12480,750	1854,849
	FA 50	15272,960	1854,849
Zn-3	Adest	14815,374	1854,849
	FA 100	12947,537	1854,849
	FA 50	14500,743	1854,849
Zn-2	Adest	13441,078	1854,849
	FA 100	12183,992	1854,849
	FA 50	11705,044	1854,849

Tab. 44: Descriptive statistics for K content in shoots (HM and FA treatment) of Triticum aestivum, *FA50 = 2% FA, FA100 = 1% FA*

Soil, Translocation and Bioconcentration Factors

In the course of the soil experiments, pots without seeds were attended to, just like the other pots. The soil of these pots was analysed using ICP-OES to determine HM content. Measured HM content increased with increasing concentration of the Zn and Ni solutions.

Treatment with 10 mmol of Ni resulted in approximately 10 770 mg Ni in one kg soil, roughly 12-times higher than the amount in 1 mmol Ni treated soil. This factor decreases in lower concentrations. The same goes for treatment with 10 mmol Zn, resulting in approximately 5 300 mg Zn per kg soil, while treatment with 1 mmol Zn resulted in one tenth of this figure. Again, differences got smaller with lower concentrations of the HM solutions added to the pots (Tab.44).

[mg/kg]	Ni-2 (10mmol)	Ni-3 (1mmol)	Ni-4 (0.1mmol)	C	Zn-4 (0.1mmol)	Zn-3 (1mmol)	Zn-2 (10mmol)	Zn-1 (100mmol)
Ni	10 765.38	883.42	213.50	22.57	16.43	23.72	17.24	16.02
Zn	121.32	72.22	107.08	74.26	999.23	589.80	5 336.29	Too high

Tab. 45: Ni and Zn content (mg/kg on basis of dry weight) in soil treated with given HM concentrations

Total HM content in soil increases with rising HM concentration in pots. ÖNORM critical levels for agricultural soils in Austria (Zn = 300 mg/kg, Ni = 60 mg/kg) (Fig.132) are already exceeded in lowest concentrations of Zn and Ni respectively (0.1 mmol) (Fig.133, 134).

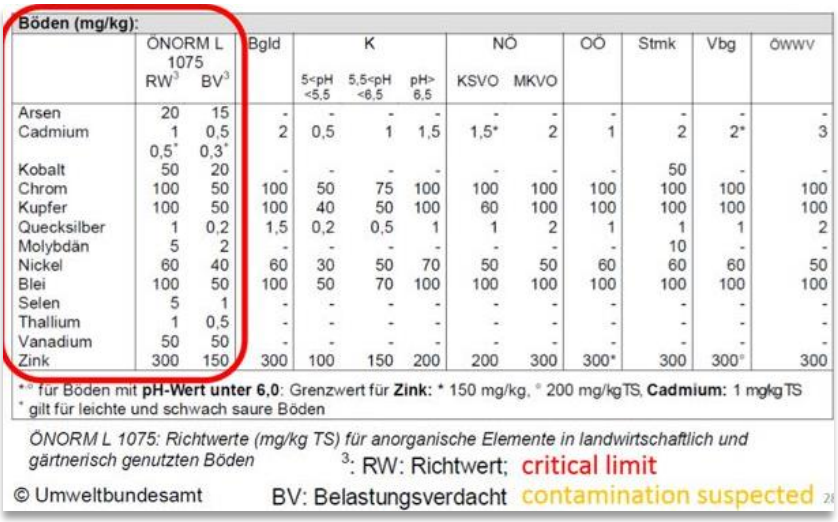


Fig. 132: ÖNORM Legal Limits for Heavy Metals in Agricultural Soils in Austria, Umweltbundesamt©. Critical Limit of Zn = 300 mg/kg and for Ni = 60 mg/kg.

		Nickel						Zinc					
	[mg/kg]	Ni-3	Ni-4	C	Zn-4	Zn-3	Zn-2	Ni-3	Ni-4	C	Zn-4	Zn-3	Zn-2
	Soil	883.42	213.5	22.57	16.43	23.72	17.24	72.22	107.08	74.26	999.23	589.8	5336.29
Adest	Root	54.26	10.14	6.85	34.16	5.98	5.52	181.116	215.34	77.69	118.88	257.38	6193.17
	Shoot	8.78	2.59	0.65	8.46	1.44	2.11	178.63	99.51	54.62	61.65	99.76	1317.51
FA100	Root	64.77	37.03	3.45	2.57	4.56	11.94	96.18	84.81	109.41	115.15	289.75	6723.01
	Shoot	25.48	3.71	2.68	5.44	1.15	1.93	69.57	47.84	56.47	42.93	79.98	1214.09
FA50	Root	75.63	20.1	4.45	26.35	6.13	7.12	84.81	124.17	109.71	98.08	262.29	5021.96
	Shoot	11.91	3.01	0.89	2.56	0.42	56.08	40.04	31.28	41.36	47.17	90.47	884.22

Tab. 46: MEAN Content of Ni and Zn in the different treated groups, plant organs of Triticum aestivum *and soil.*

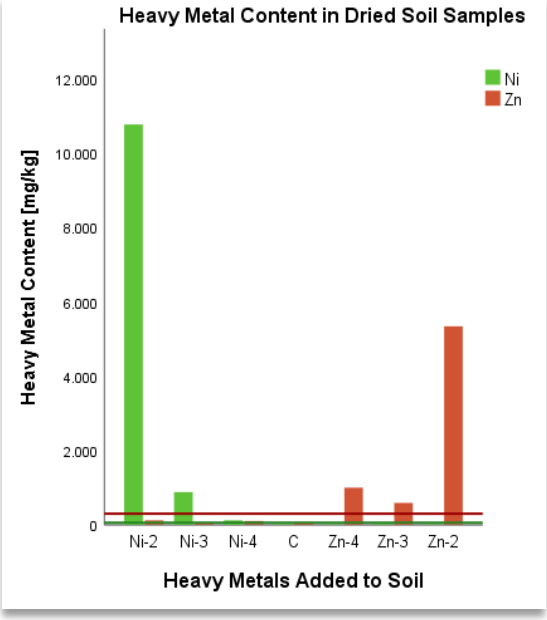


Fig. 133: Ni and Zn soil content in soil probes treated with different HM concentrations. Red line marks critical limit for Zn = 300 mg/kg, green line critical limit for Ni = 60 mg/kg.

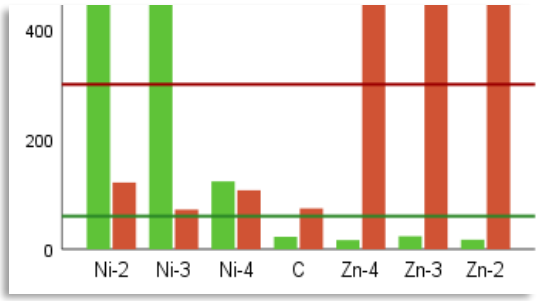


Fig. 134: Detail of Fig.113 giving a better view on the critical level marks. Even in the lowest HM concentrations of respective elements critical limit is exceeded.

Ni, Adest		
	BF	TF
Ni-3	0.06	0.16
Ni-4	0.05	0.26
C	0.30	0.09
Zn-4	2.08	0.25
Zn-3	0.25	0.24
Zn-2	0.32	0.38

Ni, FA100		
	BF	TF
Ni-3	0.07	0.39
Ni-4	0.17	0.10
C	0.15	0.78
Zn-4	0.16	2.12
Zn-3	0.19	0.25
Zn-2	0.69	0.16

Ni, FA50		
	BF	TF
Ni-3	0.09	0.16
Ni-4	0.09	0.15
C	0.20	0.20
Zn-4	1.60	0.10
Zn-3	0.26	0.07
Zn-2	0.41	7.88

Zn, Adest		
	BF	TF
Ni-3	2.51	0.99
Ni-4	2.01	0.46
C	1.05	0.70
Zn-4	0.12	0.52
Zn-3	0.44	0.39
Zn-2	1.16	0.21

(d) Zn, Adest

Zn, FA100		
	BF	TF
Ni-3	1.33	0.72
Ni-4	0.79	0.56
C	1.47	0.52
Zn-4	0.12	0.37
Zn-3	0.49	0.28
Zn-2	1.26	0.18

(e) Zn, FA100

Zn, FA50		
	BF	TF
Ni-3	1.17	0.47
Ni-4	1.16	0.25
C	1.48	0.38
Zn-4	0.10	0.48
Zn-3	0.44	0.34
Zn-2	0.94	0.18

(f) Zn, FA50

TF, BF	<1	>1

Tab. 47: Translocation (TF) and bioconcentration (BF) factor for Triticum aestivum *for groups of different treatments (HM, FA)*

Translocation factor for Ni is with one exception (Zn-2, FA50) below 1, and for Zn below 1 in all groups. Bioconcentration factor for Zn in plants, which grew in Ni, the control and Zn-2, is generally above 1 and way lower for plants growing in Zn-3 and Zn-4. Uptake and mobility of Zn is higher than of Ni. Zn uptake is higher in the presence of elevated Ni levels in the soil. Furthermore, uptake increases with increasing Ni concentrations in Adest. This effect is not observed in FA treatments. Zn uptake is reduced, when soil is treated with lower Zn concentrations and increases again, when Zn is present in high concentrations. In slightly elevated Zn concentration in the soil, Ni uptake and translocation within plant were increased.

4.4. Fulvic Acid

FA in five different concentrations (100%, 50%, 2%, 1%, 0.2%) was measured in ICP-OES. Contents of K, P, Si, Zn, Al, Fe, Ni, Mn and As are listed in Fig.135.

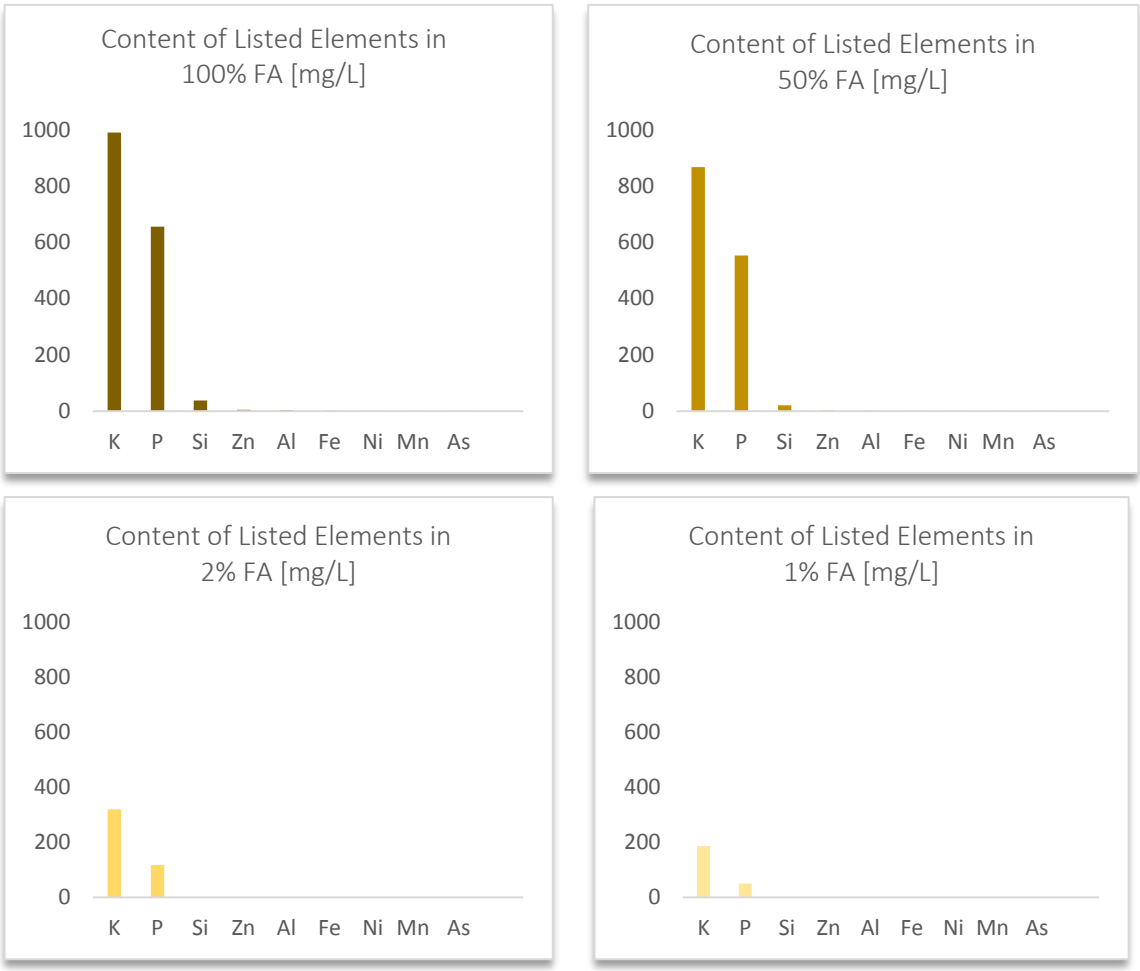


Fig. 135: Contents [mg/L] of K, P, Si, Zn, Al, Fe, Ni, Mn and As in different concentrations of FA

	100%	50%	1:50	1:100	1:500
Zn	4.78	2.65	0.2	0.07	0.04
Al	4.16	2.08	0.13	0.04	0.01
Fe	1.49	1.01	0.09	0.03	0
Ni	0.93	0.66	0.06	0.02	0
Mn	0.86	0.56	0.04	0.01	0
As	0.19	0.11	0.01	0	0

Tab. 48: Zn, Al, Fe, Ni, Mn and As in 100% FA [mg/L]

	100%	50%	1:50	1:100	1:500
K	991,04	867,23	320,04	186,54	72,38
P	655,99	553,62	117,6	49,53	11,15
Si	38,31	21,1	1,46	0,53	0,15

Tab. 49: K, P and Si in 100% FA [mg/L]

FA has a high content of K followed by P and followed by Si. Other elements like Zn, Al, Fe and Ni were measured as well, yet in comparatively negligible concentrations as can be seen in Tab.47.
Pure FA extract has concentrations of 991.04 mg/L Potassium, 4.8 mg/L Zn and 1.5 mg/L Fe (Tab.48).

5. Discussion

5.1. Effect of Ni and Zn on the Different Plant Species and Substrates

The effect of HM contamination on different growth and stress parameters of *Triticum aestivum*, *Thlaspi caerulescens* and *Amaranthus* (*caudatus* and *cruentus*), was studied in vitro, in hydroponics and in soil.

5.1.1. Germination Rate

Germination rate is similar in all HM treatment groups, except higher concentrations of Ni.

In the in vitro cultures mean germination rate of *Thlaspi caerulescens* is above 90% for the control, 1 mmol Zn and 1 mmol Ni alike.
Amaranthus cruentus has an average germination rate above 80% for the control and Zn, performing a little bit worse in 1 mmol Ni with 68%.
Triticum aestivum has an average of around 80% in the control, not enough data is available to draw conclusions for the two HM groups.

In hydroponics, again highest Ni concentration shows reduced germination rate.

In Amaranth (*Amaranthus caudatus*) the germination rate in 1 mmol Ni (36%) is significantly lower compared to all other groups (65%). No significant differences can be registered for *Triticum aestivum* (above 80% for all groups) and *Thlaspi caerulescens* (34–50%). Germination rate in the hydroponics is tendentially lower than in the in vitro cultures. This may have been caused by repeatedly watering hydroponics with HM solution, and therefore accumulating an overall increased amount of Ni and Zn, higher than the 1 mmol maximum in the in vitro cultures.

Similar germination rates are observed for all three plants in soils, with the exception that 1 mmol Ni shows no significant decrease in germination of Amaranth. The increased number of elements in soil compared to hydroponics, certainly leads to reduced mobility through complexation and adsorption with the HM in the solution, added by watering the pots. In the soil experiments wheat is treated with higher concentrations of Zn (100 mmol) and Ni (10 and 50 mmol), in addition to 1 mmol and 0.1 mmol, which leads to significantly decreased germination rates compared to all other groups, indorsing the threshold determined by Stankovic et al. (2010). (They tested germination of wheat in several Zn concentrations and showed, that approximately 100 mmol leads to highly decreased germination rates.)

5.1.2. Amaranth

An overview of *Amaranthus cruentus* growth performance in the in vitro cultures suggests severe degeneration, due to presence of 1 mmol Ni. Plants show stunted growth, seedlings have no coloration and develope no root hairs. Maximum root length decreases significantly from the control to Zn to Ni. Furthermore, significant decrease in distance between root tip and first root hair is observed from the control to Zn to Ni. Statistical analysis also confirms significant decrease of maximum root hair length from the control to Zn to Ni, suggesting higher toxicity of Ni than Zn, which also goes in line with the higher amount of Zn needed by plants (Alloway, 2009).
Using NewportGreen fluorescence dye, fluorescence at threshold-level of the control suggests accumulation of Zn in cell walls and cells of the calyptra. The same sequestration pattern is observed for Ni. Ni is also localized in vacuoles of the cortex. Seregin and Kozhevnikova (2009) reported similar results, using dimethylglyoxime to detect Ni in roots of *Amaranthus sp*. Red staining due to complexation with Ni was observed both in cell walls and protoplasts, weaker in cortex cells than in rhizodermis and central cylinder.

No observations can be made for the hydroponics, as *Amaranthus caudatus* seedlings did not survive, possibly due to harsh handling and because artificial light was too weak. In soil cultures *Amaranthus caudatus* shows no visible differences in growth performance parameters due to HMs.

5.1.3. Alpine Penny-grass (*Thlaspi caerulescens*)

An overview of *Thlaspi caerulescens´* growth performance in the in vitro cultures shows influence of Ni on root hair density in some seedlings, while others show no difference to the control.

Means show decrease of root length in HM groups compared to the control, but standard deviations are quite high, and no significant differences have been found. Seedlings in 1 mmol Zn agar have no root hairs yet mean is way above 0. When actual values are considered, most plants have no root hair, yet some show a maximum root hair length approximately twice as long, as in the control and Ni. These measurements come from root hairs outside the agar, without direct contact to the Zn in the medium. This suggests inhibition of root hair growth, only when in direct contact to Zn in high concentrations (1 mmol). The means for the distance between root tip and the first root hair are shorter in the Zn group, but standard deviations are high and differences statistically not significant.
NPG fluorescence dye localizes Ni in the cell walls. Zn is mainly sequestered in cell walls, calyptra and central cylinder. Cell walls show most intense fluorescence in the root elongation zone. Richau et al. (2008) found the same accumulation pattern, most intense in rhizodermis and cortex of the elongation zone, but for Ni. They also found Ni in the calyptra and rhizodermis of the meristematic zone. Küpper et al. (1999) on the other hand, observed storage of Zn in vacuoles and Krämer et al. (2002) reported Ni accumulation in both cell walls and vacuoles. Richau et al. (2008) also reported, that different populations show different sequestration patterns, which attests to the different findings described. When looking at sequestration patterns in leaves, it is observed that *Thlaspi caerulescens* mainly accumulates Zn in the vacuoles of epidermal leaf cells, and completely shuts Zn out of the vacuoles in the cells surrounding the stomata. It is also found in the cell walls of epidermal and mesophyll cells. Apoplastic compartmentation was another essential mechanism found in *Thlaspi caerulescens* (Rout and Das, 2003).

In hydroponics *Thlaspi caerulescens* did not survive, possibly due to harsh handling (strong mechanical stress when watering) and the artificial light being too weak. Similarly, *Thlaspi caerulescens* grew too slow and heterogeneously in soil, hence no further observations in the course of this work was possible.

5.1.4. Wheat (*Triticum aestivum*)

In *Triticum aestivum* of the in vitro cultures, roots in 1 mmol Ni are notably shorter than in 1 mmol Zn and the control. The same is observed for the shoots. No statistical analysis is possible due to the lack of data.

The same observation is made for *Triticum aestivum* in hydroponics. Overall wheat plants do not perform well in 1 mmol Ni compared to all other groups, which seem to be ± equally well off. This is also reflected in statistical analysis of maximum root length, stating the difference in root length between 1 mmol Ni treated plants to all other groups is significant. Roots of wheat in 1 mmol Ni are around 3.8-times shorter, than roots of plants in other treatment groups.
The same effect is observed for chlorophyll fluorescence. *Fv/Fm* ratio is significantly lower in Ni-3 (0.3) compared to all other groups (0.6–0.7). The mean for the *Fv/Fm* ratio is below 0.7 for all groups, which is low, indicating stress independent of HMs as well. This may be attributed to poor light conditions in this experimental design.
DAB and NBT staining solutions mark ROS in root tissue of *Triticum aestivum* brown and blue respectively. DAB prepared roots show more and darker staining in 1 mmol HM concentrations, while the control and 0.1 mmol treated plants show similar results. No differences between HM treatment can be observed in NBT staining of roots, except for the roots of plants growing in Ni-4, which show no discolouration at all.

When putting section of *Triticum aestivum* leaves in HM solutions of different concentrations to test tolerance, cells survive 10-times higher concentrations of Ni than Zn. Plants that grew in 0.1 mmol Ni survive 100-times higher concentrations of Ni and Zn respectively, than plants that previously grew in 1 mmol Zn or the control.
When comparing total Ni and Zn content in shoots and roots measuring digests with ICP, content of the HM respectively increase with increasing concentration in the HM solutions added to the pots. Translocation factor (TF) for Ni is below 1 in all concentrations and TF for Zn is mostly above 1, suggesting higher Zn mobility within the plant. The differences between the 0.1 mmol and 1 mmol HM solution, approximately corresponds to the difference between the HM content of these two concentrations within the plant, suggesting that *Triticum aestivum* is an indicator plant for these HMs.

In soil cultures of *Triticum aestivum* the highest HM concentrations Zn-1 and Ni-2 have the lowest germination rates and seedlings are very small and degenerated.
This is reflected in the dry weight of the Zn-1 and Ni-2 plants as well. Dry weight for Zn-1 is at least 10-times lower and for Ni-2 4-times lower than dry weight of plants in other concentrations (Ni-3, Ni-4, C, Zn-4, Zn-3, Zn-2), which is also reflected in the values of the other growth parameters. Zn-1 and Ni-2 are beyond the level of HMs, tolerated by wheat.
When comparing dry weight of shoots, Zn-2 has 4-times higher dry weight than Zn-3, suggesting a beneficial effect of Zn-2 on biomass accumulation. No significant differences in root dry weight is found in statistical analysis.
Maximum root length is significantly reduced in Zn-1, Zn-2 and Ni-2 compared to lower concentrations and the control. Roots of Ni-2 are 5-times shorter than roots in lower concentrations. Since Zn-1 has no roots at all, differences are self-explanatory in this case. Wheat seedlings in Zn-1 have no roots and only two brown, rolled-in leaves, embodying the symptoms as described by Rout and Das (2003).
The number of leaves significantly increases from Zn-2 to Zn-3.
Fm/Fv ratio as indication for chlorophyll fluorescence is below 0.7 in all groups, except in Zn-2 (0.81), being the only group above stress level.
When putting section of *Triticum aestivum* leaves, previously growing in Zn-2, in HM solutions of different concentrations to determine HM tolerance, cells survive in 10-times higher concentrations of Ni than in Zn.
Ni content in roots of *Triticum aestivum* in Ni-3 is 3.8-times higher than in Ni-4, 17-times higher than in C, 2.9-times higher than Zn-4, 12.3-times higher than in Zn-3 and 10.6-times higher than in Zn-2. Zn content of Zn-2 in roots is 22-times higher than in Zn-3, 54-times higher than in Zn-4, 52-times higher than in the control, 42-times higher than in Ni-4 and 48-times higher than in Ni-3. Presence of Ni seems to enhance Zn uptake in the plant.
Potassium content in roots is 1.3-times higher in Zn-2 than in Zn-4 and Ni-4 and 1.4-times higher than in Zn-3. K content in Ni-3 and C is 0.3-times higher than in Zn-3.
No significant differences for Ni and K contents in shoots between HM groups are found. Zn content of shoots in Zn-2 is 13-times higher than in Zn-3, 22-times higher than in Zn-4 and the control, 19-times higher than in Ni-4 and 12-times higher Ni-3.
Total HM content of the soil probes exceeds ÖNORM values already in lowest concentration (0.1 mmol). In all group's translocation factors for Ni and Zn are below unit. Bioconcentration factor for Zn in plants, which grew in Ni, the control and Zn-2, is generally above 1 and way lower for plants growing in Zn-3 and Zn-4. The overall uptake and mobility of Zn is higher than of Ni, reflecting the higher amounts needed by plants.

The effects of the HMs on the growth parameters reflect the symptoms of toxic HM concentrations in literature. The only differences are the actual concentrations estimated for toxicity threshold, which

depend not only on the concentrations of the added HM solutions, but on the type of medium, bioavailability etc. as well.
Generally, elevated concentrations of Ni in growth substrates, reaching phytotoxicity levels, cause reduction of germination, growth, biomass accumulation, cell division, nutrient absorption, reduction of Fe, Cu, Zn, Mg and Mn and replacement of Mg in chlorophyll, disturbing the PSII and leading to chlorosis and necrosis (Hassan et al., 2019). Symptoms of phytotoxic Zn concentrations are stunted growth of shoots, curling and rolling of young leaves, dead leaf tips, chlorosis, inhibition of root growth and reduction of Fe, Mg, K, P and Ca translocation within the plant (Rout and Das, 2003; Stankovic et al., 2010).

5.2. Effect of Fulvic Acid on the Different Plant Species in Zn and Ni Contaminated Substrates

The effect of foliar application of FA on different growth and stress parameters of *Triticum aestivum*, *Thlaspi caerulescens* and *Amaranthus* (*caudatus* and *cruentus*), was studied in vitro, in hydroponics and in soil.

5.2.1. Fulvic Acid

The FA used in the course of this thesis was said to contain high amounts of K and other micronutrients and trace elements. To get an idea of the composition, different concentrations (100%, 50%, 2%, 1%, 0.2%) were measured with ICP-OES. The FA used has a very high content of K, followed by P and Si and other elements like Zn, Al, Fe and Ni in comparably small concentrations. Pure FA extract has concentrations of 991.04 mg/L Potassium, 4.8 mg/L Zn and 1.5 mg/L Fe. In FA, extracted from soils of different agricultural sites in Pakistan, potassium concentration was between 4.88 and 8.8 mg/kg, Fe concentration was 0.105–0.720 mg/kg and Zn 0.010–0.051 mg/kg (Talpur et al., 2016). Due to great differences in FA composition, depending on extraction method, source, composition of soil, environmental factors etc., comparison of the total amount seems to be of minor importance. In both extracts K content is higher than other elements, but in FA used in this study, concentration of Fe and Zn are very close, while in the study of Talpur et al. (2016) amount of Fe was about tenfold the concentration of Zn.

The concentration of FA, which worked best to decrease HM toxicity effects, seems to be quite specific. Wang et al (2019) compared FA treatment of the following concentrations 0, 0.1, 0.3, 0.5, 1.0 and 2.0 g/L. 0.5 g/L turned out to be most effective for decrease of Cd accumulation and improved translocation of Fe. The same, in terms of high sensitivity to concentrations, was found to be true for the concentrations used in the current work.

5.2.2. Amaranth

In *Amaranthus cruentus,* growing in the in vitro cultures, FA100 lead to significant increase in root length compared to the lower concentration, FA500 and Adest group both in 1 mmol Zn and the control. Significant decrease of root hair length in the control after treatment with FA100 is observed, compared to the Adest group. The lower concentration on the other hand leads to significant increase of the maximum root hair length compared to the Adest group. In previous studies increase of root hair length, due to application of certain concentrations of HS application, was reported as well (Du Jardin, 2015).

In soil cultures effect of FA on *Amaranthus caudatus* is seen as an undeniable increase of leaf-surface and diameter of the stem with increasing concentrations. Ability to recover faster after aphid invasion is observed for FA treated plants. Du Jardin (2015) also reports decrease of plant disease incidents and increase in ability to deal with abiotic and biotic stress. Furthermore, stability of roots and shoots

increased with increasing FA concentration and plants could be distinguished through these increased physical properties just by touch.

5.2.3. Alpine Penny-grass (*Thlaspi caerulescens*)

In *Thlaspi caerulescens*, which grew in vitro, FA (1% = FA100 and 0.2% = FA500) statistically shows no differences in various groups. But when considering actual values of root hair length in Zn, root hairs outside of agar (in agar no root hairs formed at all) are twice and even thrice as long in the FA500 group than in the non-treated group. This would be an increase of 100% and 200% respectively. This may be contributed to the fact, that root hairs outside the agar medium were exposed to FA application directly, while root hair within the agar would only access FA through transport within the plant. In previous studies increase of root hair length, due to application of certain concentrations of HS application, has been reported as well (Du Jardin, 2015).

5.2.4. Wheat (*Triticum aestivum*)

FA treatment shows no visual difference in *Triticum aestivum* plants, growing in different concentrations of Ni and Zn contaminated hydroponics, and no significant differences in maximum root length have been found as well.
Fv/Fm in Zn-4 is significantly higher in plants treated with FA50 compared to Adest.
When immersing the seedlings in DAB and NBT staining solutions different effect of FA is observed. Plants were put in centrifuge tubes and therefore were quite cramped, which could have led to signs of stress as well. In the control, Ni-3 and in Ni-4 NBT staining increases with FA treatment, while treatment with FA100 decreases NBT staining in Zn-4 and no effect is seen in Zn-3. This reflects the tendency observed for FA to increase Ni content in shoots and roots and decrease Zn content in shoots and roots.
In DAB staining on the other hand, FA reduces amount of staining in all groups. FA100 workes better in Zn-3 and Ni-3. FA50 workes better in Zn-4, control and Ni-4. Wang et al. (2019) looked at the effect of FA (0.5g/L) foliar application on leaves of lettuce in hydroponics under 20 µM Cd stress. FA reduced NBT and DAB staining noticeably in both the control and HM group.
Translocation factor of Ni in hydroponics, independent of FA treatment, is lower than 1.
TF of Zn on the other hand, is greatly influenced by FA50 treatment. Plants who received foliar application with FA50 have higher concentrations of Zn in roots than in shoots, while in Adest it is the other way around. In plants without FA, translocation factor iis (in most cases) above 1, reaching a maximum of 2.23. This indicates, FA treatment shows tendencies to decrease mobility of Zn from roots to above ground organs. Furthermore, increased Zn uptake in roots and decreased uptake of Ni are observed for FA50 treatment of plants.

In the soil FA has the same effect as observed in Amaranth, increasing mechanical properties of roots and shoots. FA50 roots can be detangled just by pulling on plants, in untreated plants this would cause rupture of the roots. FA has significant effects in other growth parameters as well. FA100 significantly increases dry weight in Zn-2, compared to Adest and FA50 treated plants. In the control FA50 leads to significant increase in dry weight as well. In Ni FA leads to reduction of root length. In Ni-4 FA50 leads to significant increase of root length compared to FA100, but both are shorter than in Adest treated plants. In Ni-3 both FA100 and FA50 lead to significant increase compared to Adest group.

In the control and in Zn-3 FA lead to significant decrease of root length compared to Adest. Number of leaves increased with FA treatment. In Zn-3, Zn-4 and the control FA50 leads to significant increase of number of leaves per plant and in Ni-3 FA100 leads to increase compared to FA50 and Adest. As reported by Drobek et al. (2019) number of leaves in cucumber increased with foliar application of FA as well.

Overall, translocation factor in wheat seems to be unaffected by FA treatment, but FA has significant effect on Ni, Zn and K uptake in roots and shoots. In Zn-3 and the control K content significantly increases with increasing FA concentration. In Ni-4 FA100 leads to significant increase of K content compared to Adest as well. This is supported in literature as well, as foliar application of FA was reported to enhance K levels in plants (Priya et al., 2014). In highest HM concentration Zn-2 and Ni-3, FA leads to significant decrease of K content. Ni content in roots of Ni-3 group is significantly higher in plants treated with FA100. FA50 treated plants of Zn-2 have significant higher Ni content in the shoots of the FA50 treated plants than FA100 and Adest group. In Zn-2 FA100 leads to significant increase of Zn in the roots and FA50 to significant decrease of Zn in shoots, compared to the other two groups respectively.

Ni		Hydroponics		Soil	
		Root	Shoot	Root	Shoot
Ni-3	Adest	4706.41	1328.91	54.26	8.78
	FA50	5699.70	2275.48	64.77	11.91
Ni-4	Adest	454.80	279.52	10.14	2.59
	FA50	458.44	247.12	37.03	3.01
C	Adest	54.71	12.85	6.85	0.65
	FA50	115.01	25.07	3.45	0.89
Zn-4	Adest	79.02	16.17	34.16	8.45
	FA50	27.26	9.93	2.57	2.56
Zn-3	Adest	62.45	20.43	5.98	1.44
	FA50	41.96	18.73	4.57	0.42

Zn		Hydroponics		Soil	
		Root	Shoot	Root	Shoot
Ni-3	Adest	389.96	58.24	181.12	178.63
	FA50	450	101.25	84.81	40.04
Ni-4	Adest	84.28	185.98	215.34	99.51
	FA50	138.04	109.36	124.17	32.48
C	Adest	150.08	186.34	127.33	54.62
	FA50	224.30	176.19	109.74	41.36
Zn-4	Adest	460.49	288.50	118.88	61.65
	FA50	521.52	189.85	98.08	47.17
Zn-3	Adest	4771.42	6006.10	275.38	99.76
	FA50	3963.67	3963.67	262.29	90.47

Tab. 50: Comparison of HM contents in roots and shoots of Adest and 2% FA = FA50 treated Triticum aestivum *in hydroponics and soil*

In Tab.49 means of Zn and Ni contents in different HM groups of both soil and hydroponics, comparing Adest and FA50, are given, showing that the average Zn content in roots and shoots of *Triticum aestivum* of all groups is never below 40 mg/kg (except for shoots of wheat plants growing on Ni-4 soil with FA50 foliar application) and HM treatment has greater impact in hydroponics. This comes as no surprise as HM complexes with elements in soil and bioavailability decreases.

The following tendencies in effect of FA on HM content in roots and shoots, hydroponics and soil cultures can be established:

- FA50 treatment increases Ni content in shoots and roots respectively when treating plants with 1 mmol Ni in hydroponics and soil.
- In 0.1 mmol Ni treatment and the control FA50 shows nearly no influence on Ni content in roots and shoots as well as hydroponics and soil.
- FA50 treatment decreases Ni content in shoots and roots respectively when treating plants with (1 mmol or 0.1 mmol) Zn.
- FA50 treatment shows tendencies to overall increases Zn content in hydroponics and decrease Zn content in soil.

6. Conclusion

The aim of this work was to determine the effect of FA on growth and stress parameters of *Triticum aestivum*, Amaranth (*Amaranthus caudatus* and *Amaranthus cruentus*) and on *Thlaspi caerulescens* in different growth mediums (in vitro, hydroponics, soil) under HM (Zn and Ni) stress.

Zn-1 (100 mmol) and Ni-2 (10 mmol) solutions, added to the soil pots, expose wheat plants to HM concentrations beyond toxicity threshold and cause stunted growth, short to no roots, rolled in and brown leaves, etc. Ni-3 shows toxicity effects in all substrates and plants, leading to shorter shoots and roots, decrease in root hair length, worse chlorophyll fluorescence and increased ROS staining. Ni is more toxic then Zn for plants, except in leaf cell tolerance test, where Zn causes cell death in lower concentrations than Ni. HM content in plants increases with increasing HM concentrations in added solutions and growth medium and is higher in hydroponics than in soil. The differences between the 0.1 mmol and 1 mmol HM solution, approximately corresponds to the difference between the HM content of these two concentrations within *Triticum aestivum* in hydroponics, suggesting that it is an indicator plant for these HMs. Wheat also shows higher Zn uptake and mobility within plant, than Ni and presence of Ni seems to enhance Zn uptake as well. Using NBT fluorescence dye, Ni is found in cell walls, calyptra and vacuoles of the cortex and Zn in cell walls and cells of calyptra from *Amaranthus cruentus*. In *Thlaspi caerulescens* Ni is localized in cell walls, while Zn is found in cell walls, calyptra and central cylinder, most intense in cell walls of root elongation zone.

The FA has a very high content of K, as well as P and Si. In the control FA increases K content in roots, maximum root length, maximum root hair length and dry weight.

In soil in the control as well as in the different HM concentrations FA leads to improvement of mechanical properties of Amaranth and wheat with increasing FA concentrations. In Amaranth it leads to increase of the leaf surface and diameter of the stem as well.

In HMs effect of FA is depending on the concentrations of the FA, the concentrations of the HMs, the type of HM, the plant species as well as the growth medium. Sometimes FA100, sometimes FA50 works better in increasing the number of leaves per wheat plant and the K content in the roots. Only in the highest HM concentrations – Zn-2 and Ni-3 – FA reduces K content in plants. FA reduces amount of DAB staining and sometimes increases NBT staining in roots of *Triticum aestivum* of hydroponics. In hydroponics FA50 has no effect on the transfer factor of Ni and decreases Zn translocation in plants but increases Zn uptake and decreases Ni uptake. In soil the translocation factor is not affected by FA treatment in general, but FA has a great influence on content and uptake of the HMs.

Overall FA increases Ni content in shoots and roots in 1 mmol Ni treated groups of both hydroponics and soil cultures and decreases Ni content in Zn treated groups. FA50 increases Zn content in plants of hydroponics and decreases Zn content in soil plants.

So overall, FA definitely influences different growth and stress parameters of the different plant species. As it was the case in this work and in many studies before, the effect of FA is very sensitive to the concentration applied, plant species, concentration and type of HM, growth medium (in vitro, hydroponics, soil) and their properties.

Data was also collected on other nutrients like P, Fe, S, Mg, Mn, Al and Si. Quick Two Way ANOVAS on the effect of FA and HM, showed significant differences for FA treatment in contents of P, Fe, S, Mg, Al and Si in roots of wheat, growing in soil, as well as for S and Mn in shoots. Due to limited time, these results were not reported in detail, but data is available, and these first findings recommend further research.

As FA treatment shows impressive effects on mechanical properties of wheat and Amaranth, as well as an increase of leaf surface and stem diameter in Amaranth, I suggest further experiments with enough replications for statistical analysis should be conducted. It would be of great interest to look further into physiological mechanisms and anatomical changes, which result in the improved mechanical properties of the plants in this study, to get a better understanding of the way FA works.

Especially outstanding is the ability of FA treated Amaranth to recover faster and better after aphid invasion, which is an observation that may be connected to the visible improvement of mechanical properties, and encourages further investigation, as overuse and abuse of pesticides pose an increasing threat to human and environmental well-being.

In this study plants were analysed before grain development, so no conclusion can be drawn in relation to biofortification and ecological intensification.

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