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Biogeochemical processes governing chromium distribution in paddy rice rhizosphere

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Abstract

Heavy metals in agricultural soils pose significant risks to human and animal health by contaminating crops. Chromium (Cr) is particularly concerning, as its reduced state is relatively immobile and less harmful, whereas its oxidized state, Cr (VI), is toxic.

This study investigates Cr availability and solubility in paddy soils, focusing on the role of redox potential (Eh), pH, soil organic carbon, clay content, microbial activity, and ligands in iron plaque formation and metal translocation within rice plants. In addition, other metals such as iron (Fe), manganese (Mn), arsenic (As), and aluminium (Al) will be investigated as their presence affects Cr availability.

For this study, rice was grown on soils from agricultural paddy fields in Italy and India with varying levels of Cr contamination. A ligand in the form of citric acid was added to one group to assess its influence on metal availability. Pore water was analysed for metal content over time, root plaque was extracted to measure metal concentrations, and plant segments were digested using aqua regia and analysed to measure metal translocation. Additionally, an artificial root setup was performed to get an insight into iron plaque formation.

Results showed higher Fe and Mn levels in the pore water of the contaminated Indian soil compared to the Italian soil, but lower Cr levels, despite high Cr concentrations in the total soil digestion. Ligand addition increased Cr levels in Italian soils but decreased Fe and Mn levels. The highest plaque formation was observed in the Indian treatments, with slightly higher metal contents in the ligand group compared to the Mili-Q (MQ) group. Chromium contents in the plaque were higher in the ligand treatments for all three soils. Fe, Al, and Cr predominantly accumulated in the root biomass and were not translocated to other plant segments, whereas Mn concentrations were highest in the leaves.

This study provides insights into the complex processes that metals undergo in paddy soils and can help predict metal contamination in grains. However, to understand the specific effects of each factor, controlled experiments on individual factors are necessary.

Zusammenfassung

Schwermetalle in landwirtschaftlichen Böden können gesundheitsbeeinträchtigende Nachteile hervorrufen, indem die Feldfrüchte kontaminiert werden. Chrom (Cr) ist eines dieser Metalle, im reduzierter From (Cr III) relativ immobil und weniger toxisch als in der oxidierten Form (Cr VI). Diese Arbeit untersucht die Verfügbarkeit und Löslichkeit von Cr in gefluteten Reisböden, und belichtet welche Rolle das Redox Potential (Eh), pH, Kohlenstoff- und Tongehalt des Bodens, mikrobielle Aktivität und Liganden spielen, auch mit Auswirkungen auf die Bildung von Eisenplaques sowie den Metalltransport innerhalb von Reispflanzen. Zusätzlich werden die Konzentrationen anderer Metalle wie Eisen (Fe), Mangan (Mn), Arsen (As) und Aluminium (Al).

Für diese Studie wurde Reis auf Böden aus landwirtschaftlichen Reisfeldern in Italien und Indien mit unterschiedlicher Cr-Kontamination angebaut. Einer Gruppe wurde ein Ligand in Form von Zitronensäure hinzugefügt, um dessen Einfluss auf die Metallverfügbarkeit zu bewerten. Das Porenwasser wurde im Zeitverlauf auf Metallgehalte analysiert, Eisenplaques von den Wurzeln wurden extrahiert, um die Metallkonzentrationen zu messen, und Pflanzensegmente wurden mittels Aqua Regia aufgeschlossen und auf den Metalltransport innerhalb der Pflanzen hin untersucht. Zusätzlich wurde ein Aufbau mit "künstlichen" Wurzeln aus Silikonschläuchen erstellt, um Einblicke in die Bildung von Eisenplaques zu gewinnen.

Die Ergebnisse zeigten höhere Fe- und Mn-Gehalte im Porenwasser des kontaminierten indischen Bodens im Vergleich zum italienischen Boden, jedoch niedrigere Cr-Gehalte, obwohl die Gesamtbodenanalyse hohe Cr-Konzentrationen ergab. Die Zugabe von Liganden erhöhte die Cr-Werte in italienischen Böden, senkte jedoch dessen Fe- und Mn-Werte. Die höchste Plaquebildung wurde in der indischen Gruppe beobachtet, wobei die Metallgehalte in der Ligandengruppe leicht höher waren als in der Mili-Q (MQ)-Gruppe. Die Chromgehalte in den Plaques waren in den Töpfen, welche mit Liganden bewässert wurden, für alle drei Böden höher. Fe, Al und Cr akkumulierten überwiegend in der Wurzelbiomasse und wur-

den nicht in andere Pflanzensegmente transportiert, während die Mn-Konzentrationen in den Blättern am höchsten waren. Die Metallgehalte der Pflanzen waren deutlich höher als in den Eisenplaques.

Diese Studie liefert Einblicke in die komplexen Prozesse, die Metalle in Reisanbauböden durchlaufen, und kann helfen, Metallkontaminationen in Körnern zu verstehen. Um jedoch die spezifischen Auswirkungen der einzelnen Faktoren zu verstehen, sind kontrollierte Experimente zu den jeweiligen Faktoren notwendig.

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1. Introduction

"But man is a part of nature, and his war against nature is inevitably a war against himself." — Rachel Carson [1].

For centuries, in the pursuit of valuable minerals and resources, humankind has exploited the globe to fuel the growing industries and sustain an increasing population's standard of living. Unfortunately, this often occurs without sufficient regard for the environment or the well-being of local communities, leading to pollution and health issues. This seems to repeat itself over decades, as these impacts frequently arise without learning from past mistakes.

One of these valuable and demanded resources is Chromite ($FeCr_2O_4$). It is used in alloys to inhibit corrosion and oxidation and plays a key role in stainless steel production, developing pigments, leather processing, and more [2].

Chromite can be found in geological formations around the world. The biggest mines are situated in South Africa (18 million metric tons), Turkey (6.9 million metric tons), Kazakhstan (6.5 million metric tons), India (4.2 million metric tons) and Finland (2.2 million metric tons) [3].

In my master's thesis, I will focus on a chromite mine in the Sukinda Valley in Jaipur District in Orissa, India. It spreads over 420 km2 [4] and produces almost 8% of Indian chromite ore [5].

During the mining process, 11.73 tons of Cr(VI) are released into the environment annually, making it one of the most polluted places in the world [6]. Additionally, the agricultural soils in the vicinity have been heavily polluted, a problem especially for the rice paddy fields [6]. Heavy metal contamination of agricultural soils not only poses a threat to the biosphere but also to the microsphere, animals, and humans. Heavy metals can be very persistent in both aquatic and terrestrial environments [7].

The high metal contents found in the Sukinda Valley soil originate from the ultramafic complexes from the Precambrian age, which also contain the chromite minerals. These minerals

mainly consist of iron (Fe) and Cr-oxides [4]. The elevated Fe content found in the soil is attributed to the presence of hematite, goethite, and chromite minerals.

The soil samples that used in this study originate from an agricultural irrigation ditch in the vicinity of the mine, with total Cr concentrations in the soil of 499 mg/kg. These values are lower than those reported by Das et al. 2013, who sampled directly from the mine's location, which ranged from 1715 to 9548 mg/kg [4].

These values are a lot higher than the Cr concentrations under natural conditions in unpolluted sites, ranging between 10 and 50 mg/kg [8]. However, depending on the substrate, these values can increase dramatically, e.g. in basalt watersheds in China over 250 mg/kg Cr has been recorded [9].

To compare the findings from the Indian soil samples used in this study with unpolluted soils, samples were also collected from rice fields from the Po region in Northern Italy. Outcrops of mafic rocks are present in this region, leading to slightly higher Cr content due to this geological background [10] compared to the European average of around 70 mg Cr/kg [11]. Nevertheless, in my study, only around 14 to 18 mg/kg Cr were measured in the samples, which is also less than the numbers from Beone et al. 2018, who analysed metal concentrations in the Lombardy region, reporting 32 mg/kg Cr [10].

The Cr content in the Italian soils doesn't only originate from the natural degradation of the geological substrate, but also from the use of heavy metal-containing substances such as pesticides and fertilizers [12].

In both India and Italy, rice is an important carbohydrate in the diet. India, the largest rice-cultivating country, accounts for approximately 20% of the world's rice production [13]. The rice cultivation in the eastern region of India, where the Sukinda Valley is situated, has the highest intensity in the country, implicating how essential these soils are and how hazardous it can be polluting them [13].

As the primary food source for over two-thirds of India's population, rice is not only important for food security, but also plays an important role in the economy [14]. In Asian countries, it is also considered to be a major source of heavy metal intake [15, 16].

While Italy's rice production is smaller in comparison, it is the biggest rice producing coun-

try in Europe, with rice fields covering over 80,000 hectares as of 2015 [14]. In terms of global production, European milled rice amounts to just 0.4% [14].

Rice is mostly cultivated in paddy soils, which undergo periodical flooding and draining stages [17]. The flooding affects the pore oxygen content in the soil, depleting it rapidly, which leads to the respirational reduction of oxidized soil components by microorganisms in the order of nitrate (NO_3^-), Mn oxides, Fe oxides, and sulphate (SO_4^{2-}) [18]. These microbial-mediated reduction processes can lead to significant changes in soil redox potential (Eh) and pH, which itself can influence the metal concentration in the pore water [19].

Based on growth morphology and physiological characteristics, the development of rice is categorized into five stages: the tillering stage (Days 1–20), the jointing stage (Days 21–40), the flowering stage (Days 41–60), the filling stage (Days 61–75), and the maturing stage (Days 76–100) [20].

Heavy metals can exist in various forms with varying levels of solubility. They could be dissolved in the soil solution, exchangeable within organic and inorganic components, incorporated into the structural lattices of soil, and insolubly precipitated with other soil constituents [21]. Metals in soil undergo a series of different processes, resulting in precipitation, mobilization, redox reactions, adsorption, plant uptake and many more.

One of the important players governing metal transformation processes in the soil is organic matter (OM). It is mostly present as a solid phase and plays an important role in both the sorption and the mobility of many (heavy) metals. OM is the result of the decomposition of plants and animal remains, containing a "diverse and heterogeneous mixture of polyfunctional groups" [22].

Elevated OM content in the soil can lead to reducing conditions, as the increased decomposition consumes more oxygen, creating a more anoxic environment [23]. This can lead to a higher reduction rate of metals such as Fe, which has further implications for the soil's geochemistry. Reducing conditions on the other hand enhance the dissolution of OM [24].

Moreover, OM can bind and immobilize metals, which has been shown for Fe(III)[25]. For

instance, inorganic carbon (CO_3) can precipitate with Fe and immobilize it ($FeCO_3$), a typical process especially in wetlands [26].

Additionally, organic and inorganic carbon not only change abiotic factors but also affect the microbial activity in the soil. High levels of organic carbons in the soil encourage microbial activity and reductive dissolution of Fe-oxyhydroxides can take place faster, as does the Mn reduction [27]. This could be also depth-dependent, with increasing reduction with increasing depth [27]. It is important to mention that not only Fe and Mn are affected, but also other metals and their speciation.

Another important soil parameter is the clay content. Since clay is a very small particle it has a relatively high surface area, resulting in many possible adsorption sites which play a role in the availability of metals in the soil [28].

At high pH values, exposed edges of the soil particles can become increasingly negatively charged due to OH^- ions, and more positively charged at low pH values [29]. This could lead to metal interaction with soil particle surfaces at high pH values, but also affects the buffer capacity of the soil.

Among the processes affecting soil heavy metal availability discussed previously, pH plays a particularly crucial role. It significantly influences the solubility, speciation, and thus the movement and bioavailability of metals [21]. Lower pH levels are associated with higher metal content in pore water solutions, due to dissolution processes under these conditions [30].

Another important player in the biogeochemistry of soils is sulfur. While sulfur is relatively non-toxic, its redox cycle plays a crucial role in soil acidity and the mobilization of toxic metals [31, 32]. In addition to being a key nutrient [33], sulfur acts as an essential agent for biochemical electron transfer, especially under anaerobic conditions [34]. The redox state and distribution of sulfur can impact the mobility and therefore also the toxicity of trace metals [32].

Cr mainly exists in two oxidation forms; reduced as Cr(III) and oxidized as Cr(VI). Cr(III) is relatively immobile and therefore not as toxic as the more mobile hexavalent Cr. These oxidation states are influenced by many factors, some of them already mentioned, and ad-

ditionally also by the redox chemistry with other metals in the pore water such as Fe and Mn.

Chromate (CrO_4^{2-}), bichromate ($HCrO_4^-$), and dichromate ($Cr_2O_7^{2-}$) are the most common forms of Cr(VI) in soil. These can be weakly bound to soil particles and easily replaced by other anions, leading to high mobility in the subsurface, particularly in neutral pH environments with other oxyanions present [35, 36]. In contrast, Cr(III) is much less mobile, often precipitating as $Cr(OH)_3$ or forming Fe-Cr complexes.

Fe, being one of the most abundant elements in agricultural soils, with an average concentration ranging from 20 to 40 g/kg total Fe [37], is an important player in the soil's geochemistry. It primarily exists in two oxidation states: ferrous (Fe(II)) and ferric (Fe(III)) iron. Fe(II) is mainly found in primary minerals, whereas Fe(III) plays a significant role in pedogenetic processes and can be coordinated with water and hydroxyls [38]. In neutral pH conditions, ferrihydrite precipitation is promoted, while acidic pH conditions favour Fe mineral mobilization.

Most crystalline and poorly ordered Fe minerals can interact with other soil components, such as inorganic and organic colloids, to form complex aggregates with new surfaces [39]. Fe(II) has a higher solubility than Fe(III), making it more bioavailable and abundant [40]. The reduced sulphide (HS^-) can precipitate with Fe(II) to form Fe-sulphide such as pyrite. High concentrations of HS^- imply low Fe(II) contents in pore water, as Fe(II) precipitates out of the solution [41].

This process can also lead to the co-precipitation of other metals, such as Cr. However, sulphate can compete with chromate for adsorption sites on soil particles, potentially lowering the distribution coefficient (Kd) for Cr adsorption [42].

Kd is defined as the ratio of a compound content bound to the solid phase to the compound concentration in aqueous solution [43].

$$K_d = \frac{\text{Amount of solute adsorbed onto solid phase (mol/g)}}{\text{Concentration of solute in solution (mol/L)}} \tag{1.1}$$

Manganese (Mn), the twelfth most abundant element in the Earth's crust [44], is also preva-

lent in agricultural soils. It exists in various oxidation states ranging from 0 to +7, with Mn^{2+}, Mn^{3+}, and Mn^{4+} being the most common in natural systems. Of these, only $Mn^{2}+$ is soluble in aqueous solutions [45, 46]. The reduction of Mn is energetically more favourable than that of Fe, with the reduction of MnO_2 to Mn^{2+} yielding +578 mV, compared to the reduction of $Fe(OH)_3$ to Fe^{2+}, which yields only +59 mV [27].

An additional factor affecting the biogeochemistry in soils is the presence of plants which can exudate ligands and lead to oxygen input. Since in this experiment rice seedlings were planted into the soil, ligands will play a role in the distribution of metals.

Low molecular weight organic acids (LMWOAs) are the main active components of rice root exudates and significant sources of soil organic carbon [47]. These exudates are secreted by rice roots to maintain charge balance; plants release protons when they absorb more cations than anions and take up protons when more anions are absorbed [48]. The composition and types of these secretions depend on plant species, physiological conditions, and the soil environment [49].

LMWOAs play multiple roles in soil nutrient and metal interactions. They act as acidifying, bridging, complexing/chelating agents, compete with ions for adsorption sites, enhance the reduction and dissolution of compounds, and also affect the microbial community [50, 51]. These processes influence ion mobility, bioavailability, transformation, and cycling [52]. The carboxyl groups in LMWOAs can carry negative charges, displacing anions from the soil matrix [53]. Plant exudates mainly consist of anions, protons, organic compounds, and enzymes [54]. They have a significant capacity to reductively dissolve Fe-oxides, and therefore can impact the iron plaque (IP) formation [55].

Citric acid (CA), a representative LMWOA, has a high density of carboxyl groups and greater acid strength compared to other organic acids [56, 57]. It can solubilize phosphate at a high rate, which is crucial for plants [58]. In soil, CA degrades quickly, with degradation kinetics influenced by soil mineral phases, particularly Fe oxides that adsorb CA [59].

The CA concentration used in this experiment was 10 mM, which is within the natural range of rhizosphere organic acid concentrations (1-100 mM) [60]. However, unlike natural systems where exudates are only localized to the rhizosphere, the entire soil was irrigated

with CA in this experiment. Additionally, the citric acid was titrated to pH 7 using potassium hydroxide. An increase in pH following CA addition was observed for the Italian soils, similar to findings in other studies with delayed effects [61].

Since rice is a hydrophyte, a plant adapted to aquatic environments [62], rice roots exhibit radial oxygen loss (ROL), a process in which oxygen is actively transported from the leaves to the roots to support metabolic activities. While most of this oxygen is used up inside the plant [63], some diffuses through the aerenchyma and the outer root surface into the surrounding rhizosphere [64]. The quantity of oxygen diffusing into the rhizosphere depends on the rice species, root porosity, aerenchyma structure, and exodermis layer [65].

ROL can change the local chemistry by making the environment more oxic. This affects the Eh and pH [66], leading to the oxidation of metals and the formation of IP on root surfaces, primarily through the precipitation of crystalline and/or amorphous Fe minerals [67].

IPs are not uniformly distributed along the roots; immature, young roots show less IP formation than mature, thick roots [68]. Local physical and chemical soil properties can also impede plaque formation [67]. IPs can contain heavy metals like Mn, Arsenic (As), Cr, Nickel (Ni) or Aluminium (Al). Parameters such as sulphur and dissolved organic carbon content can enhance Fe-oxide precipitation on the root's surface by improving the surface characteristics and molecular structures of Fe-hydroxides in IPs, thereby increasing the binding affinity for heavy metals [67].

IPs can also alter the mobility of heavy metals and contaminants, affecting their uptake by plants [67]. This barrier can be both chemical and physical, depending on root proportions, Fe-hydroxide speciation, and new root development without IP formation [67]. The extent of IP formation can influence the uptake of potentially phytotoxic metals into the roots [69]. A heavy coating of IP may suppress the uptake and accumulation of Lead (Pb) [70], Zinc (Zn) [71], As [72], and Cr [73, 74] in rice.

IP formation is influenced by both abiotic and biotic factors. Abiotic factors include plant physiology and soil characteristics like organic matter composition, soluble Fe abundance, Fe-oxide binding capacities, soil sulphur content, and Fe/Mn availability [67, 74, 75].

High OM content can lead to higher IP formation since more OM decomposition creates

anoxic conditions, enhancing Fe reduction, which is then oxidized on the root surface. However, OM can also bind Fe(III) and immobilize it [25]. The ratio between OM and IP depends on the degree of OM decomposition and anoxic conditions around the root surface [76].

Sulphur exists in both inorganic and organic forms, with most of it being organic [77]. Elemental S can induce Fe and Mn plaque formation, while sulphate (SO_4^{2-}) influences IP formation [75]. This occurs since the S^{2-} content from the reduction of SO_4^{2-} by sulfur-reducing bacteria can increase, which might have been present at high concentrations in the ligand pots. S^{2-} reduces Fe(III) to Fe(II), which is then used in IP formation.

In my study, the plant species was the same for all setups, but Fe/Mn/S availability, OM content, pH, Eh, and soil composition differed among soil types and irrigation groups.

Plants take up metals from the soils, many needed as important nutrients, while others might be harmful to the plants, depending on their concentrations. Heavy metal uptake by agricultural plants and their accumulation might also endanger human health, especially in the grains of rice.

The uptake of a metal by plants primarily depends on its mobility and availability in the soil. These are governed by adsorption and desorption characteristics of the soil, which in turn depend on soil properties such as pH, OM content, cation exchange capacity (CEC), Eh value, as well as the presence of clay minerals, calcium carbonate, and Fe and Mn oxides [21].

Numerous factors influence metal translocation from soil through IP into plants, including plant and metal specificity, redox states, reactive surface area [69], metal concentrations, rhizosphere pH, Fe phases, and the development of new roots [63]. New roots can undermine IP advantages by taking up water and nutrients without IP [78].

The primary objective of this thesis is the evaluation of how Cr behaves under different geochemical surroundings (soil with high/low Cr content, ligand addition, plant addition). I will also analyze and discuss the IP formation and its composition, both from real plants and artificial roots, and how extensive ligand addition affects the IP.

Since IP might inhibit the uptake of heavy metals, its formation under ligand addition is an

important aspect to understand when wanting to make predictions about the heavy metal contamination of rice grains. Many factors play a role in these processes, and I will explain these regarding the data of the experiment that has been conducted over the last year.

I hypothesize that treatments with ligand addition will result in higher metal concentrations in the pore water, as ligands can enhance metal mobility depending on their speciation. However, citric acid may negatively impact plaque formation.

Furthermore, another Hypothesis is that rice plants grown in Indian soil will exhibit higher Fe and Mn concentrations in the pore water, plaque, and plant segments, given the significantly higher total concentrations of these metals in Indian soil compared to Italian soil. For Cr, despite its higher concentration in Indian soil, I anticipate lower mobility under the reducing conditions, making it less likely to follow the same trend.

To study the mentioned processes, rice was grown in both Italian and Indian soils, with the Italian soils having low total metal contents compared to the Indian soil which originated from the vicinity of the Sukinda Valley Cr mine. The Italian soils were collected directly from a rice field, while the Indian soil originated from an irrigation ditch next to a field.

For each soil, three treatment groups were set up:

1) Control group without plants, irrigated with MilliQ water

2) MilliQ group with rice plants, irrigated with MQ water

3) Ligand Group with rice plants, irrigated with ligand solution in the form of 10 mM citric acid.

For the Italian soil duplicates for the MilliQ and the ligand group were set up, this could not be done for the Indian soil since the amount of soil that was shipped to the University was limited.

2. Methods

All the experiments have been conducted at the Environmental Geochemistry Laboratory of the Centre for Microbiology and Environmental System Science (CMESS) at the University of Vienna.

2.1 Soil Origin and Sampling

The Indian sediments utilized in the experiment were sourced from Sukinda Valley, India (21° 3' 10.91" N, 85° 46' 6.85" E). The samples were collected from the upper 20 cm of an irrigation ditch close to a rice-growing agricultural site and were shipped to Vienna.
The Indian soil samples were already sieved, dried, and grounded.

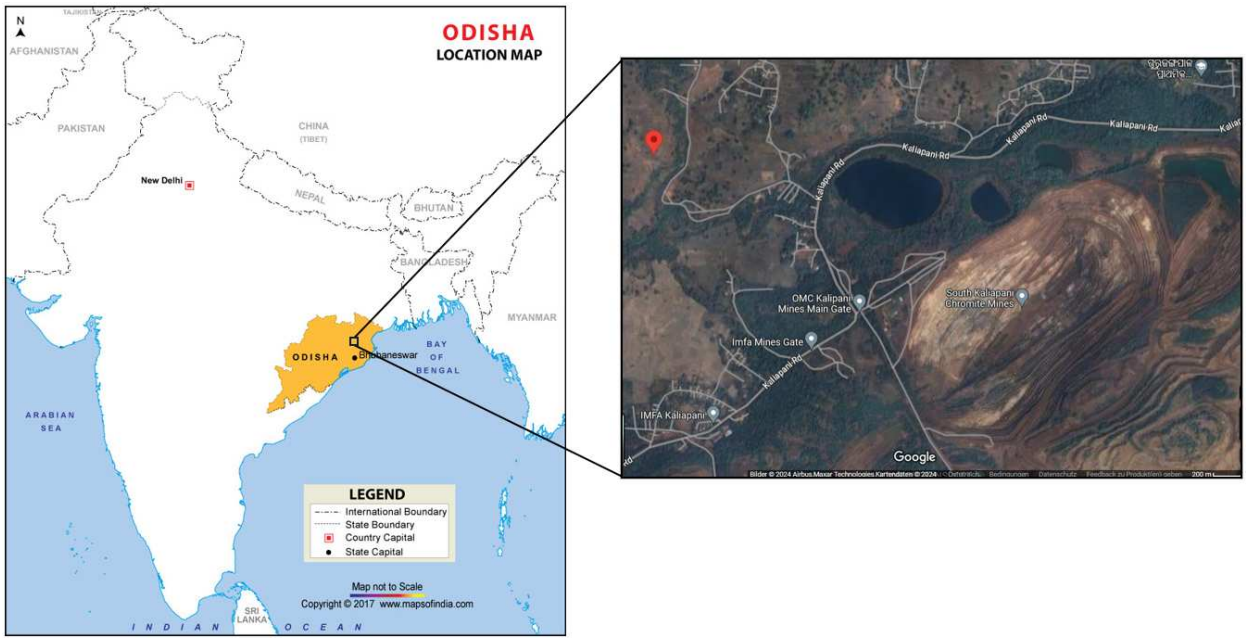

Figure 2.1: Indian soil sampling location at Sukinda Valley, ©www.mapsofindia.com, Google Maps

Additionally, soil samples were obtained with help from the "Ente Nazionale Risi-Centro Ricerche su Riso" in Castello D'Agogna, Italy. The samples were sourced from the "Veron-

ica" location at coordinates 45° 10' 38.2" N, 8° 53' 48.1" E, and the "Bozzole" location at coordinates 45° 13' 48.7" N, 8° 57' 48.3" E.

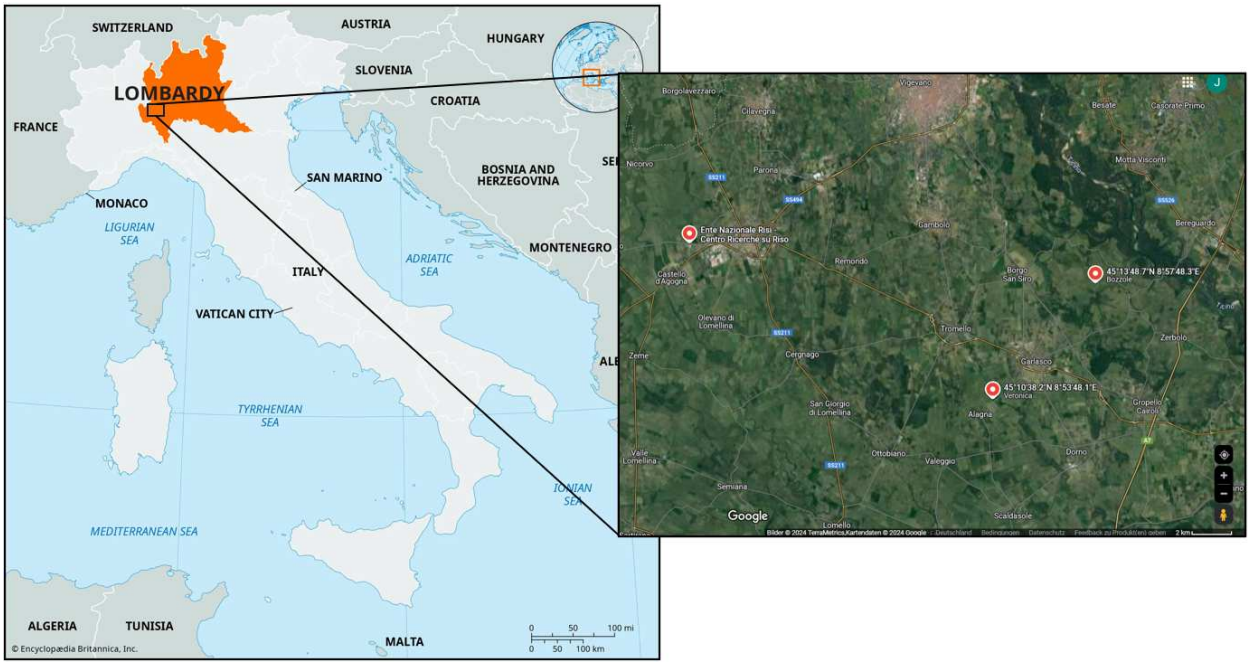

Figure 2.2: Centre Richerche su Rise, Veronica and Bozzole sampling locations ©Encyclopaedia Britannica, Inc., Google Maps

2.2 Soil Physico-chemical Analysis

The physico-chemical analysis of the soil samples was conducted by applying a variety of different methods. To estimate the soil composition, an estimation by hand has been performed according to the protocol DIN 19682-2 by "Geologischen Dienstes Nordrhein-Westfalen" [79]. The soils have been slightly wetted and the estimation of the composition according to the result path of the method was conducted.

Aqua regia digestion was conducted to analyze the total metal concentrations in the soil, according to the Script for the MES3 Laboratory and Field Course "Groundwater and Pollutants" from the "Department of Environmental Geosciences Center for Microbiology and Environmental Systems Science", University of Vienna. The soil samples underwent a preparatory process involving drying, sieving, and pulverization before digestion. Approximately 30 to 40 mg of each sample were weighed into digestion vessels, with three duplicates

prepared per sample. The empty weight of a 50 ml centrifuge tube was determined for each sample and each blank. For digestion, 1.5 ml of HCl and 0.5 ml of HNO_3 were subsequently added to each digestion vessel within a fume hood. The mixture was then heated incrementally on a hot plate to 80 °C, starting from 50 °C in 15 °C steps, on a hotplate. Following completion of the reaction, the vials were tightly sealed and heated to 120 °C for 90 minutes. Subsequently, the temperature was reduced to 80 °C, the vials were opened and the caps flushed using small amounts of MQ water. The open vials were gradually heated again to 140 °C until the samples were desiccated. After cooling, 1 ml of HNO_3 and 2 ml of MQ water were added to the samples. This solution was poured over the acid-resistant filters (Glass Microfiber filters, 934-AH Whatman GE Healthcare Life Sciences 47 mm diameter), and both the vessels and the filters were rinsed with MQ water into the 50 ml tubes, which were then filled precisely to 50 ml on a scale with MQ water and mixed.

For analysis, two sets of samples were prepared: undiluted (5 ml of sample into a 15 ml tube) and diluted (1 ml of sample and 4 ml of MQ water into a 15 ml tube). Additionally, three blanks were prepared. All samples underwent measurement using ICP-OES (CirusCCD, SPECTRO Analytical Instruments GmbH, Germany) to assess the total metal concentration.

The vessels used in the process were cleaned by rinsing with MQ water, followed by the addition of aqua regia in the fume hood and heating to 120 °C for 2 hours. The solution was then discarded, and MQ water was added, and heated to 100 °C for an additional 2 hours. Finally, the vessels were rinsed with MQ water.

X-ray diffraction analysis was conducted to assess the mineralogical characterization using the Benchtop Rigaku MiniFlex 600 diffractometer, with a Cu Kα radiation source (λ=1.54 Å, X-ray tube operated at 20 kV and 15 mA).

The dried soil samples were very finely ground and carefully placed on the sample holder. The measurement was conducted using a long scan setting with parameters set as follows: Start angle 5°, stop angle 80°, step size 0.04°, and scanning speed of 0.4° per minute. The collected data was analysed using Profex software, version 5.2.4.

The measurements of total carbon (TC), total organic carbon (TOC), and total inorganic carbon (TIC) were conducted using the LECO Carbon Analyzer. One sample from each soil was grounded, dried and 150 to 300 mg weighted into a ceramic boat. Using the Ramp-method, the samples were heated up to 1000 °C.

During the initial weeks, dissolved Fe speciation analysis was conducted using a plate reader (Infinite M Plex, Tecan, Switzerland). For the Italian soil samples, 500 µl of filtered sample were combined with an equal volume of 1 M HCl in 1.5 ml tubes. Subsequently, 50 µl of this solution was transferred to 100 µl of 1 M HCl in 96-well plates for Fe(II) analysis. For Fe(total) analysis, 100 µl of the sample was added to 50 µl of 1M HA-HCl in the well plate, resulting in a 1:2 dilution, followed by an additional 1:3 dilution, yielding a total dilution of 1:6.

For the Indian samples, higher dilutions were employed due to their elevated Fe content. Specifically, 375 µl of the sample was mixed with 1125 µl of HCl, resulting in a 1:4 dilution, followed by an additional 1:3 dilution, resulting in a total dilution of 1:12.

Ferrozine was utilized as a dye for the measurements. Each measurement included standards and two blanks for calibration and control purposes.

For the colorimetric dissolved sulphide quantification according to the protocol by Cline 1969 [80], samples at timepoint 129 (duplicate TP 84) and duplicate timepoint 97 were taken and conserved in 100 µl 0.1 M Zn-acetate. The 0.1 M Zn-acetate was prepared by adding 0.88192 g of $Zn(CH_3COO)_2$ (Merck KGaA) into 40 ml of MQ water.

The cline solution was prepared by adding 1 g N,N-Dimethyl-p-phenylenediamine, and 1.5 g of ferric chloride hexahydrate in 50 ml of 37 % HCl.

For the stock solution of 100 mM, 0.7804 g Na_2S was added into 99.7 ml anoxic MQ water inside the Glove box. This stock solution was diluted to 10000 µM and 1000 µM as further stock solutions, and then the actual standards were diluted from that: 100 µM (highest standard), 50 µM, 25 µM, 12.5 µM, 6.25 µM, 3.125 µM, and 0 µM Na_2S. The standards were diluted in 0.1 M Zn-acetate to a final volume of 1 ml each.

Then 180 µl of the standards and the samples were pipetted into a 96-well plate, and in each

well 20 µl of Cline solution was added as a colorant. The plate was incubated in the dark for 1 h before the absorbance was measured in the plate reader at 670 nm wavelength.

2.3 Rice Growing and Sample Analysis

The rice seeds from cultivar *Oryza Sativa japonica* were germinated following the "Ronald lab rice growing protocol (February 2020)" from the University of California, with some alterations. First, the husks were removed manually and immersed in a 1:3 bleach solution (DanKlorix) for 30 minutes. Following this, the seeds were rinsed with MQ water and placed on a kimwipe for several hours to dry. The Murashige and Skoog (MS, M5519-10L) media was prepared by dissolving 2.2 g of MS powder, 20 g of sucrose, and 8 g of agar in 1 liter of MQ water. The solution was thoroughly stirred and autoclaved at 121 °C for 15 minutes. Once sterilized, the hot solution was poured into 100 ml beakers, and after cooling, up to three seeds were carefully placed into each beaker, ensuring they were not buried too deeply. Before placement in the climate chamber, the chamber was thoroughly cleaned with a disinfectant (Virkon, LANXESS) to prevent mold growth.

The soils were prepared for the rice growing process by adding 1 liter of each sieved soil into a 2-liter beaker, to which 5 g of grass fertilizer (Bambus und Gräserdünger, Gardol) was added and mixed with MQ water. All beakers were filled with MQ water up to a 2 cm overlaying water line and left for two weeks to establish anoxic conditions. After 14 days, three germinated seeds were introduced into each of the pre-wetted, anoxic soils.

Two rhizon samplers (19.21.22F, Rhizosphere Research Products B.V., Netherlands) were deployed in each pot, one positioned at the surface at a depth of 0 to 5 cm between the three plants (referred to as the "Rhizosphere") and the other placed on the side at a depth of 5 to 10 cm (referred to as the "Bulk") throughout the experimental period. Pore water was extracted weekly using 5 ml syringes, with the initial 1-2 ml discarded and the subsequent 2-3 ml of sample was filtered (0.2 µm, Minisart Syringe Filter, sartorius) and collected in 15 ml tubes (or smaller centrifuge tubes). The collected samples were then acidified with 1 %

HNO_3 (22 µL for a 2 ml sample) for preservation at 4°C before metal analysis via ICP-MS. A watering system was employed to maintain a constant 2 cm overlying water level. After 30 days, a ligand solution consisting of 10 mM Citric Acid at pH 7 (titrated with 6 M KOH) was added following the removal of the overlying water layer.

Plant sampling was initially scheduled for 90 days, which corresponds to the rice maturing stage [20]. However, due to plant mortality in the ligand solution, the plants of this treatment were harvested earlier, with the Indian soil ligand treatment being harvested at TP 91. The MQ group was harvested at a later time point (day 129). Duplicate samples were harvested after 105 days.

The pH and Eh levels were periodically measured during the length of the experiment using a Mettler Toledo Five Easy ph Meter and an HQ40d Portable Dissolved Oxygen & Conductivity/TDS/Salinity Environmental Monitoring Package (HACH, Unites States) instrument, respectively, by direct insertion into the soil.

After the growing phase, the plants were carefully removed from the pots, and both roots and leaves were gently cleaned with MQ water to remove any soil particles or organic debris. Subsequently, the roots and seeds were separated from the plants, placed in aluminum foil, and dried in an oven at 75 °C for 48 hours. Following the drying process, the dry roots were weighed.

To extract the IP, the dithionite-citrate-bicarbonate (DCB) method was employed according to the protocol from Taylor et al. 1983 [81], with some alterations. Initially, a solution was prepared by dissolving 0.125 M $NaHCO_3$ (Carl Roth GmbH) and 0.03 M sodium citrate dihydrate (Merck KGaA) in MQ water. Subsequently, 30 ml of this solution was added to 50 ml centrifuge tubes along with the roots and silicon tubes and placed on a rotary shaker for 1 hour. Following this, 1.5 g of $Na_2S_2O_4$ (Sigma Aldrich) was added to each tube and shaken for 10 minutes. The resulting solution was transferred into 100 ml flasks, and the root rinse solution was added. The flasks were then filled to a volume of 100 ml with MQ water. Approximately 9 ml of this solution was filtered (0.2 µm) into 15 ml tubes and acidified to 1% HNO_3.

The conductivity of these solutions was measured to determine the total dissolved solids (TDS) content as a percentage, enabling the calculation of the necessary dilution rates for subsequent ICP-MS measurements (with a maximum TDS of 0.1 %).

An artificial root setup was constructed to simulate plaque formation on rice roots resulting from the radial oxygen loss (ROL) of the rice plants. Peristaltic pump silicon tubes (Tygon pump tubing LMT-55 1.52 ID, Saint-Gobain) and a wider silicon tube (3.0 x 0.75 mm, Fisherbrand) were submerged into pre-wetted Indian soil between 2 to 26 days. Oxygenated water was pumped through the tubes using a peristaltic pump (0.05 ml/min). The water was oxygenated by bubbling oxygen through it and cooling the bottle to maintain higher saturation levels.

After carefully removing the tubes from the soil, they were rinsed with MQ water to remove soil and dirt, dried at room temperature, and the plaque was extracted using the DCB-method. These extractions were acidified and analysed for metal concentrations using the same method as for the dried root analysis from the rice plants.

The pore water samples and the DCB extractions from the rice and artificial roots were analysed using an Ion Coupled Plasma Mass Spectrometer (ICP-MS) ((ICP-MS 7700, Agilent Technologies, United States) to determine the concentrations of Fe, Mn, Al, Cr, As, and Ni. The samples were diluted based on the specific trace metal, ranging from 1:10000 to 1:20. The limit of detection (LOD) was calculated using two different approaches [82, 83]:

- For counts per second (CPS), it was determined as the

$$\text{mean(Blanks)} + 1.65 \cdot \text{standard deviation(Blanks)} \tag{2.1}$$

- For concentrations (ppb, ppm) it was calculated as

$$\frac{3 \cdot \text{standard deviation(Blanks)}}{\text{slope of calibration curve}} \tag{2.2}$$

In most cases, both approaches yielded consistent results.

To assess the metal concentration in the rice biomass, the plants were divided into sections

of roots, stems, and grains (if present). These sections were then washed with MQ water and dried in the oven at 75 °C for 2 days. Subsequently, the dried sections were ground as finely as possible, placed into aluminum bags, and dried again at 75 °C for 2 days.

In the next stage, 30-40 mg of the ground plant material was weighed into digestion vessels in triplicate, if sufficient material was available, and underwent the same procedure as the soil samples described in section 2.2. Unfortunately, a third of the samples got lost.

3. Results and Discussion

3.1 Soil Properties

The soil samples were characterized based on their textural and chemical properties. The Bozzole soil was classified as soil type Su3/Sl2, indicating that loamy sand was the predominant fraction. The soil contained 10-40 % silt and less than 8 % clay, a relatively low clay composition.

The Veronica soil, sourced from a rice field close to the Bozzole site, exhibited a slightly different textural composition. Classified as a Sl3/St2 soil type, this soil also consisted primarily of loamy sand but had a lower silt content (0-40 %) and a higher clay content (5-17 %).

The Indian soil type was characterized as Lu/St3, indicative of a sandy loam texture. This soil contained 0-15 % silt and 17-25 % clay, a higher clay content compared to the Italian soils (Fig. 3.1). The clay content of the analysed soils in this experiment goes India > Veronica > Bozzole, from high to low.

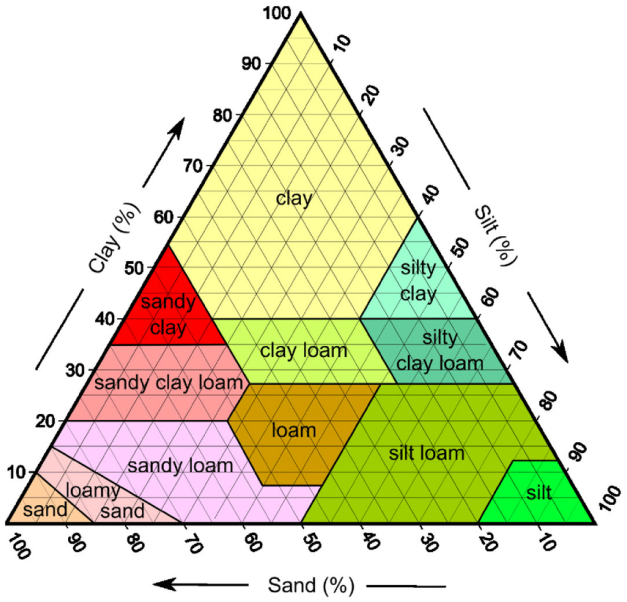

Figure 3.1: Soil texture triangle [84]

3.2 XRD Analysis

X-ray diffraction analysis revealed a high quartz content in all three soils (Fig. 3.2). Additionally, small amounts of plagioclase were detected in the Veronica soil, and possible clinochlore was identified in the Indian soil. Das et al. 2013 also conducted an XRD analysis on soils from the vicinity of the Sukinda Valley chromite mine, additionally found chromite, hematite and goethite additionally to the quartz in the soil [4].

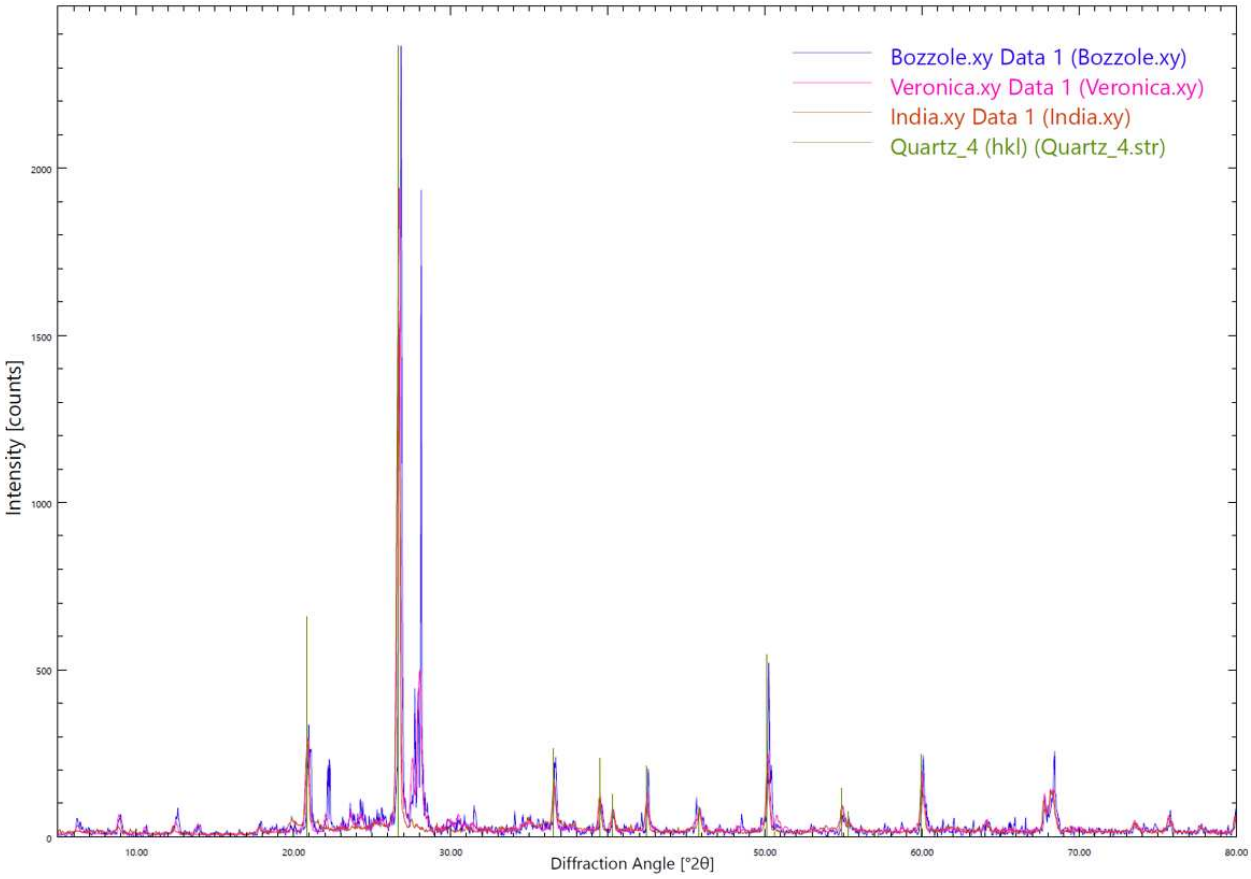

Figure 3.2: XRD of the Indian and Italian soils, paired with quartz

3.3 Total Carbon Measurement

The Bozzole soil had the highest organic carbon content, with 2.43 %, followed by the Indian soil with 1.7 %, and the Veronica soil with 1.03 % (Fig. 3.3). The inorganic carbon content was relative to the organic content very low, with 0.1 %, 0.02%, and 0.06% (for Bozzole, India and Veronica soil, respectively).

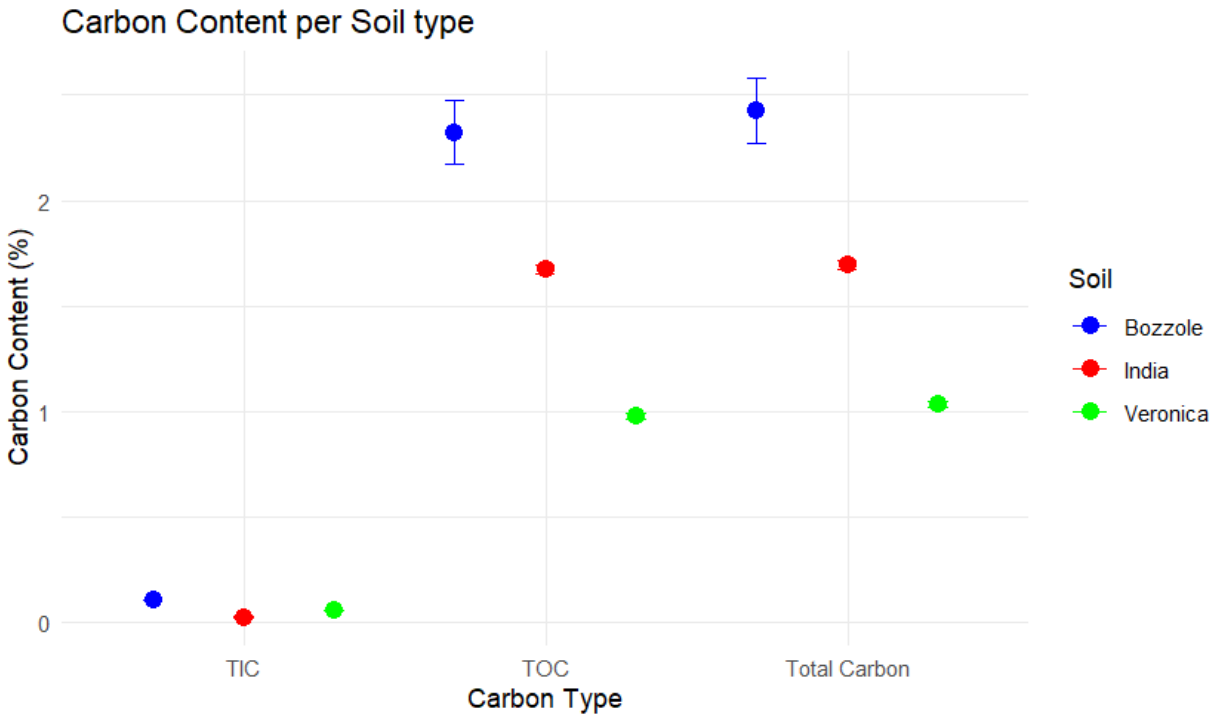

Figure 3.3: Inorganic, organic, and total carbon content for the three soils

3.4 Total Soil Digestion

The total soil digestion was performed using aqua regia, providing a measure of the total metal content.

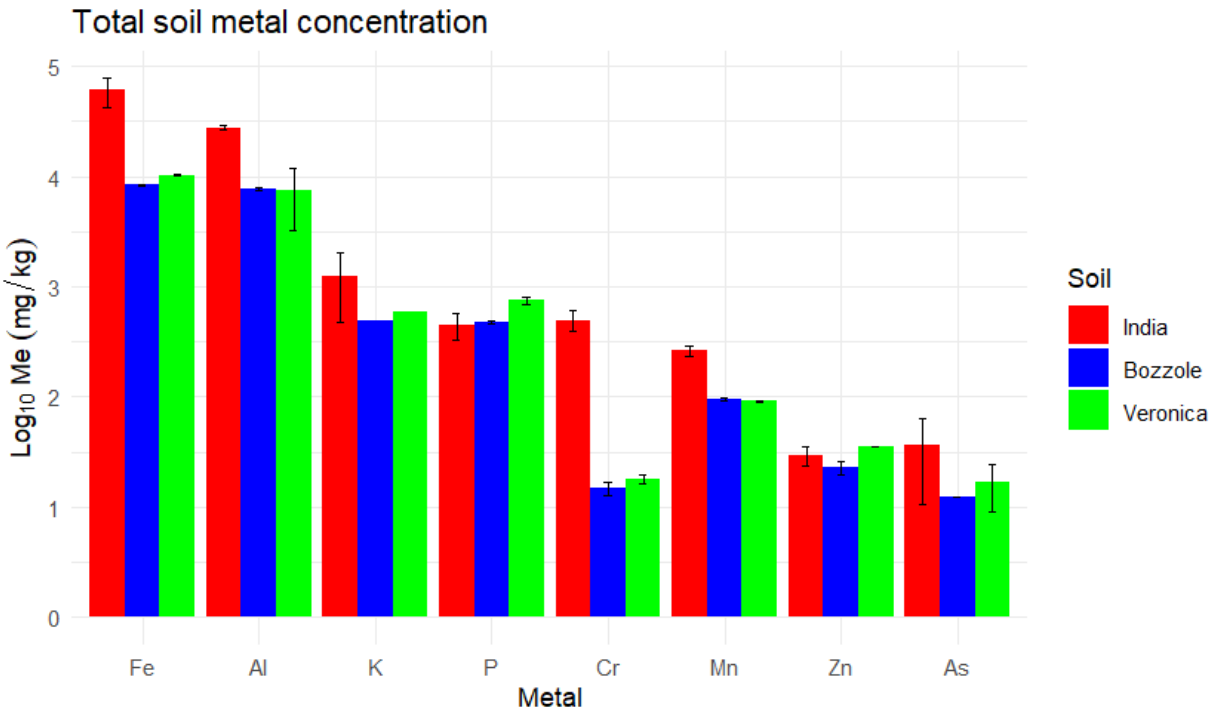

Figure 3.4: Total metal concentrations of aqua regia extraction from the three soils

Figure 3.4 illustrates the concentrations of Fe, Al, Potassium (K), Phosphate (P), Cr, Mn, Zink (Zn) and As in the soil samples following aqua regia digestion. The Fe and Al concentrations in the Indian soil (60.7 g Fe /kg and 27.6 g Al/kg) significantly exceed those in the Italian soils (9 and 7.5 g/kg). The K content is 1.3 g/kg in the Sukinda soil and around 0.5 g/kg for the Italian soils.

The Indian soil also exhibits significantly higher concentrations of Cr and Mn, with 0.5 g Cr g/kg and 0.26 Mn g/kg, compared to the Italian soils, which both contain around 0.016 Cr g/kg and 0.093 Mn g/kg. The As concentrations in the soils are 0.037 g/kg in the Indian soil and around 0.014 in the Italian ones. The levels of Cr and As in the Indian soil exceed the threshold values set by ÖNORM L1075 (2004), which are 0.1 g/kg and 0.02 g/kg, respectively [85].

The higher metal content in the Indian soils could be derived from the mining activity and the high geological background in the area [4]. Aqua regia extracts metals up to the residual phase; however, not all phases are valuable to our study, which focuses on the bioavailable fraction of metals.

3.5 pH Data over Time

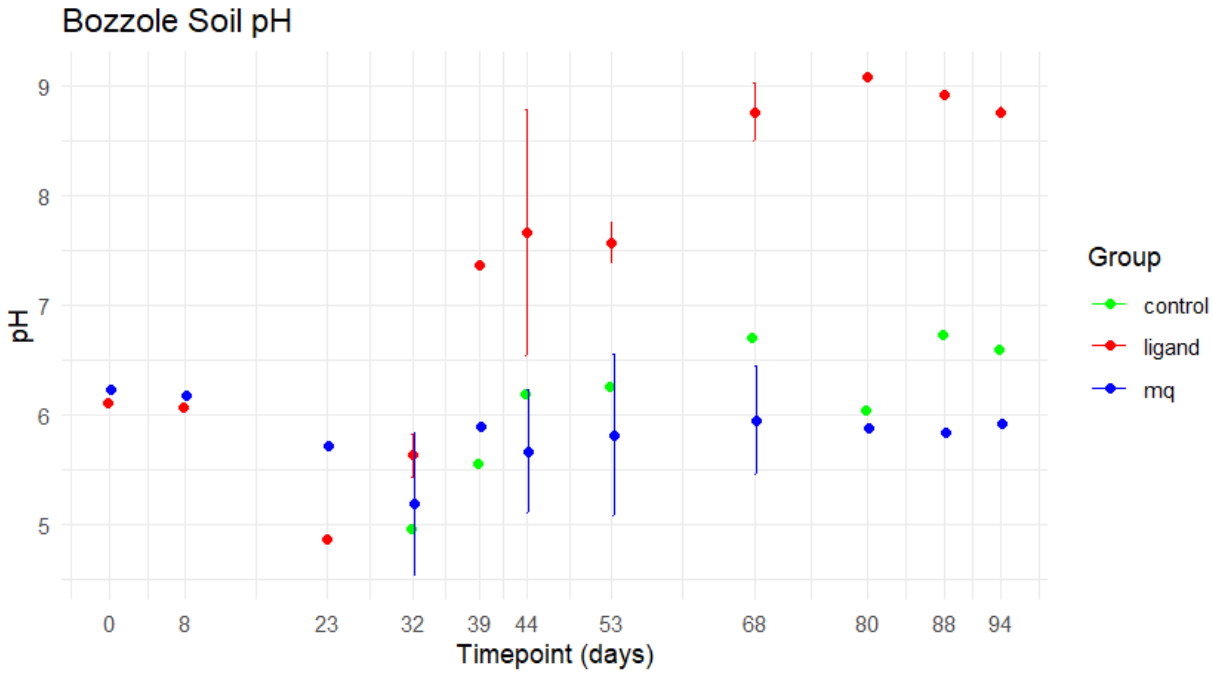

Figure 3.5: pH over time of the three groups control, ligand, and mq of the Bozzole soil

Figure 3.5 displays the pH values for the Bozzole soil across the three groups, including duplicates. Following the addition of citric acid at timepoint (TP) 31, which was normalized to pH 7 before the irrigation, a significant increase in pH was observed in the ligand group. It rose from 6.10 at TP 0 to 9.09 at TP 80. In contrast, the pH in the MQ group started at 6.23 at TP 0 and remained relatively stable throughout the experiment, with a variance of approximately 1 pH unit.

The control group lacked duplicates, and the first measurement of this treatment group was taken at TP 32. At this point the pH was recorded at 4.96, subsequently increasing to 6.73.

Figure 3.6 presents the pH values for the three groups of Veronica soil, including duplicates.

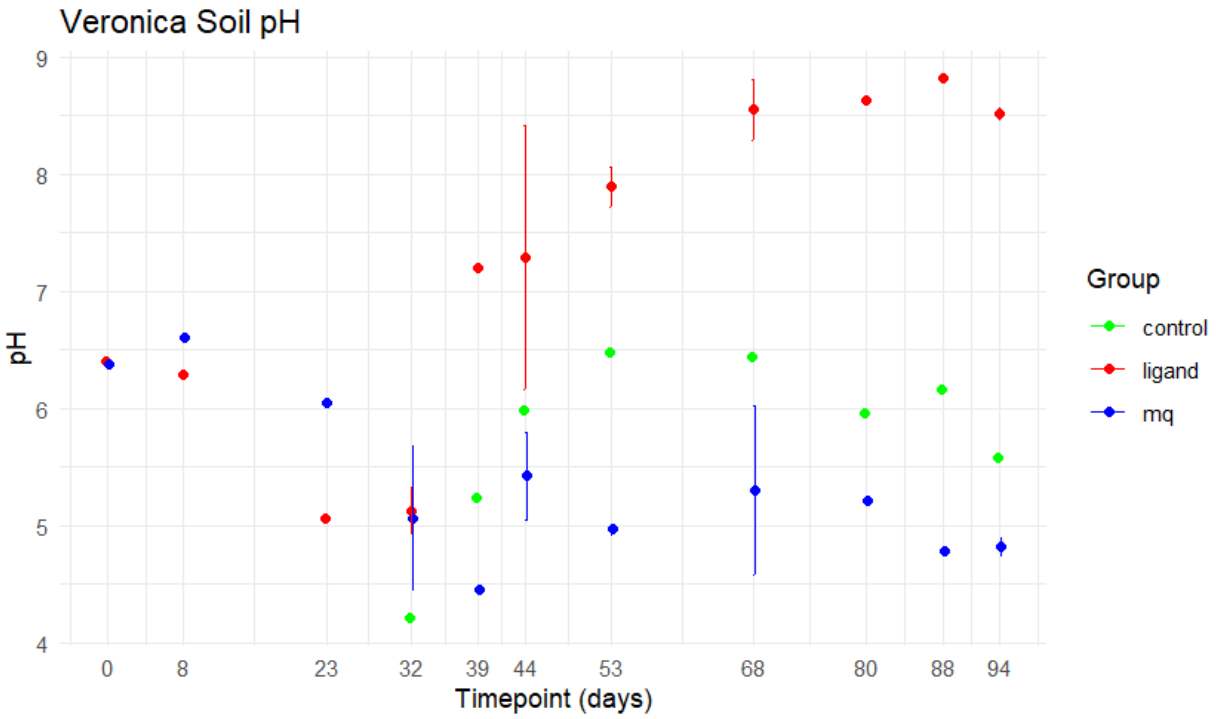

Figure 3.6: pH over time of the three groups control, ligand, and mq of the Veronica soil

Similar to the Bozzole soil pH trends, the pH in the ligand group starts at 6.40 and increases over time following the ligand addition, reaching a maximum of 8.82 at TP 88, after which it remains constant.

The pH in the MQ group starts at 6.38 and, despite some variation between duplicates, gradually decreases to 4.77 by TP 90. In the control group, the pH starts at a very low value of 4.21, rises rapidly to 6.48 by TP 53, and then slowly decreases to 5.58 towards the final measurement point.

The reduction of Fe(III)-oxides can increase pH, as this process consumes large amounts of protons. Fe(III)-oxides are quantitatively the most important electron acceptors in paddy soils [86]. This could explain why the pH in the Italian soils rose after the ligand addition, which can serve as a carbon source for Fe-reducing microorganisms, over the experimental period.

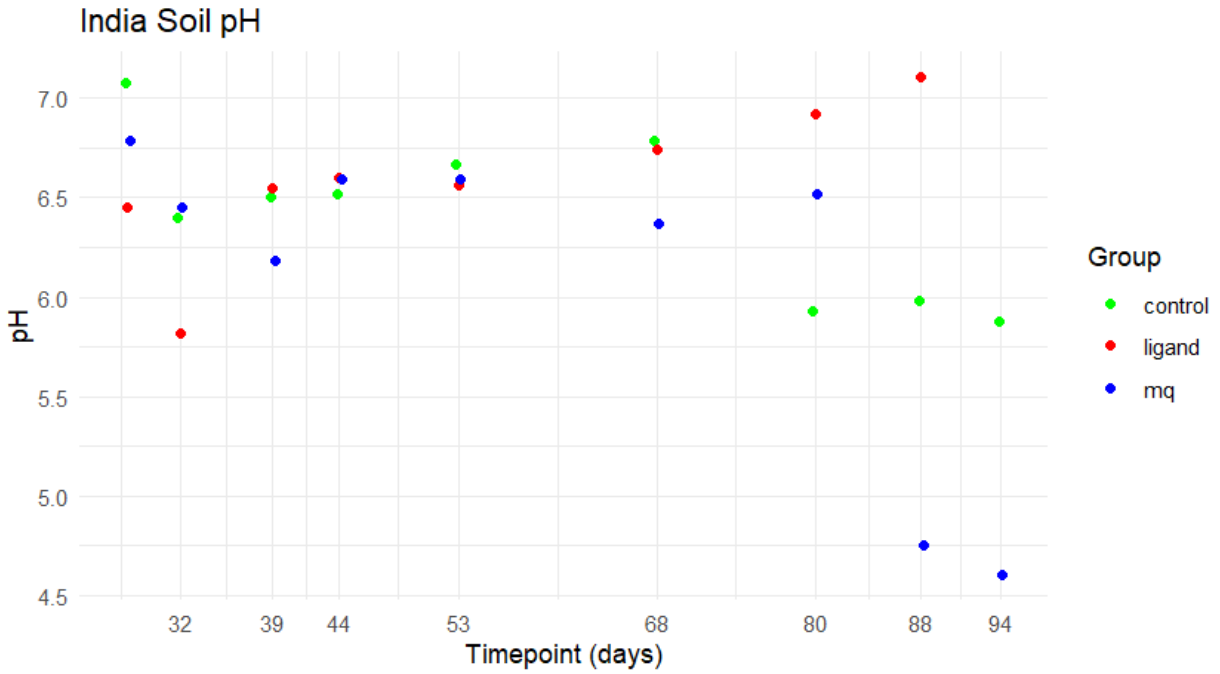

Figure 3.7: pH over time of the three groups control, ligand, and mq of the Indian soil

The pH values for the Indian soil are depicted in Figure 3.7. Duplicates were not performed. The pH values remain relatively stable around 6.5 across all groups, showing a small variation on day 68. After the addition of the ligand, which significantly affected the Italian soils, the pH in the Indian soil remained relatively constant, with a slight increase to pH 7.11 towards the later TP. In contrast, the pH in the MQ and control groups decreased to approximately 4.6 and 5.88, respectively, by TP 94.

As mentioned before, the Fe-oxide reduction can increase the pH. Since the Indian soil has the highest Fe content, and therefore also the most Fe-oxides, this could lead to a higher buffer capacity against pH-lowering processes, resulting in the relatively stable pH values in the Indian soils compared to the Italian ones.

Several other factors also contribute to soil buffer capacity, including the type of clay present and the organic matter content in the soil. Organic matter can act as a buffer since it has weakly acidic carboxylic and phenolic functional groups [87]. This effect might not be as strong as the buffering effect from the clay minerals, since no significant difference in total carbon content could be measured between the Italian and the Indian soils. Clay minerals have two buffering reactions, being able of protonation and deprotonation of edge

sites, additionally to the proton exchange reaction of their basal sites [88]. The higher clay content in the Indian soil could explain why in the ligand group the pH didn't increase as pronounced as in the Italian soils.

3.6 Iron Content in the Pore Water

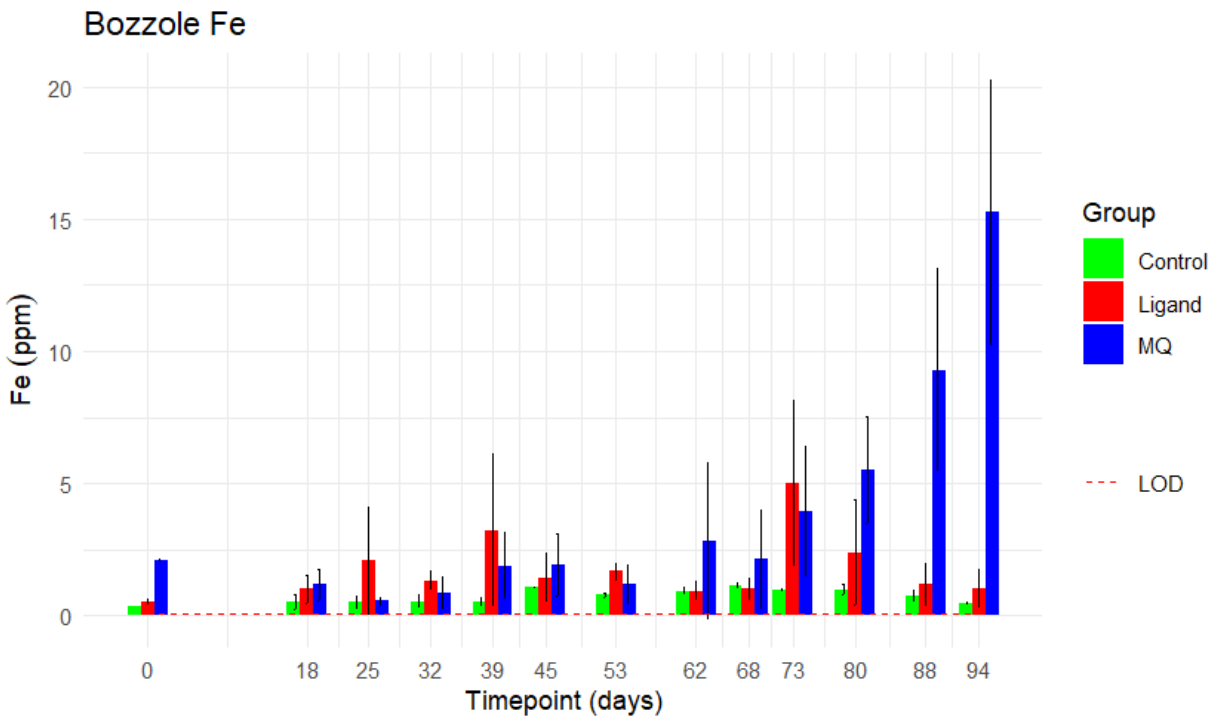

Figure 3.8: Bozzole pore water Fe concentrations of the three groups control, ligand, and mq over time

Figure 3.8 illustrates the Fe concentrations in pore water samples over time for the Bozzole soil. Error bars represent the variation between the two rhizon samplers placed at different depths in each pot.

In the MQ group, the Fe content is higher than in the other two groups from the first measurement point, despite the ligand group also being irrigated with water until TP 31 and no ligand addition during this period. Since plants had not yet developed, all three groups initially experienced identical conditions, yet the metal contents differed greatly. This shows the high fluctuation in natural systems and that the results of this study have to be taken

with caution.

The Fe concentration in the MQ group increases over time, reaching up to 19 ppm. In contrast, the control group's Fe level remained relatively stable at around 1 ppm. The Fe content in the ligand group shows fluctuations over time, starting at 500 ppb at TP 0, peaking at 5000 ppb at TP 73, and then decreasing to 727 ppb at the final measurement point. The high error bars reflect the differences between the rhizosphere and bulk soil samplers, with Fe content generally higher in the bulk soil, which was sampled from a deeper depth. Between TP 30 and TP 100, the correlation coefficient is greater than 0.05 (r=0.16), indicating no significant difference between the two samplers.

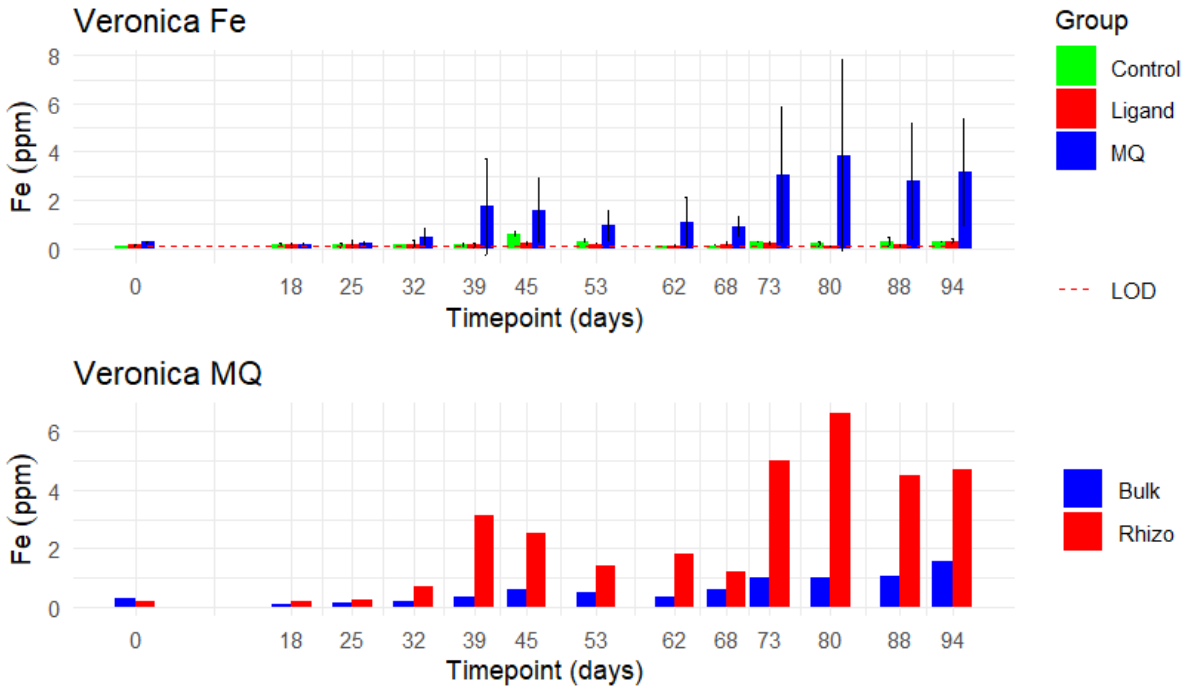

Figure 3.9: Dissolved Fe concentrations measured in pore water of Veronica soil of the three groups control, ligand and MQ over time (top plot), and Fe content of Veronica pore water Bulk and Rhizosphere sampler (bottom plot)

The Fe concentrations in the Veronica soil (Fig. 3.9), display a similar trend to those observed in the Bozzole soil, except with significantly lower Fe content in the ligand group. In the pore water of the Veronica soil, the Fe content in both the control and ligand groups remained stable over time, with around 200 ppb. Similar to the Bozzole soil, the group with plants irrigated with MQ water exhibited the highest Fe content, peaking at nearly 4000

ppb at TP 80. However, the error bars are quite substantial, often exceeding the mean value between the two samplers.

A closer examination of the Bulk and Rhizosphere samplers shows that the Fe content in the Rhizosphere sampler is significantly higher than the Bulk sampler (r value of 0.003 between TP 30 and TP 100, Fig. 3.9).

Overall, the Fe concentrations in the pore water of the Veronica soil were up to three times lower than those in the Bozzole soil. An increase in pH can lead to a decrease in Fe(II) in solution due to the rapid precipitation of dissolved Fe as Fe-oxyhydroxides [41]:

$$4Fe^{2+} + O_2 + 6H_2O \rightarrow 4FeOOH + 8H^+ \tag{3.1}$$

This could explain the lower Fe content measured especially in the ligand group of the Italian soils, which had high pH values (Fig. 3.8,3.9), but not the low values in the control group, since in this treatment the pH did not increase as significant as in the ligand treatment.

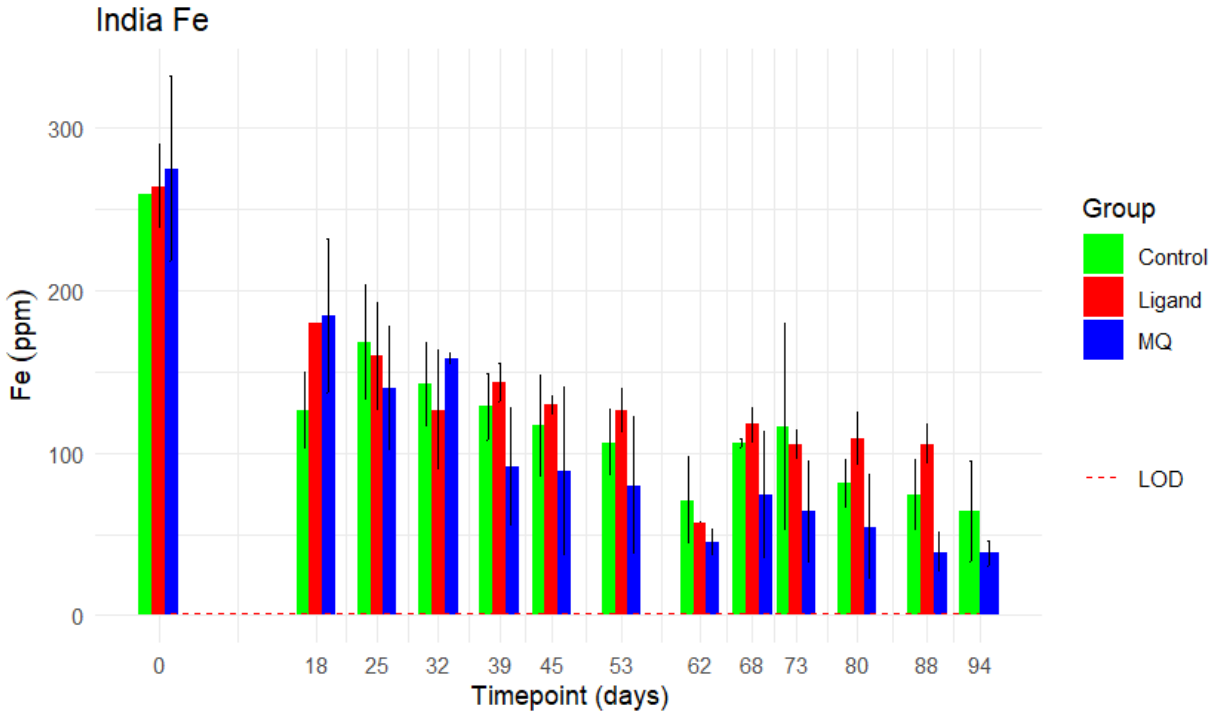

Figure 3.10: Dissolved Fe concentrations measured in pore water of Indian soil of the three groups control, ligand, and MQ over time

Figure 3.10 displays the Fe content (in ppm) for the Indian soil groups. The pore water

Fe concentrations are significantly higher than those observed in the Italian soils. All three groups (control, ligand, and MQ) exhibit a similar trend, starting at approximately 250 ppm at TP 0 and decreasing to 50-100 ppm by TP 94. The standard deviation between the two samplers is relatively low, indicating no significant difference between the rhizosphere and bulk samples.

Notably, in contrast to the Italian pore water concentrations, the ligand group and not the MQ group has the highest Fe content. The Fe concentrations of all treatment groups decline towards the end of the measurement period. These results will be discussed together with the Mn results in the following chapter.

3.7 Manganese Content in the Pore Water

The Mn concentration measurements were conducted by ICP-MS analysis from the pore water samples.

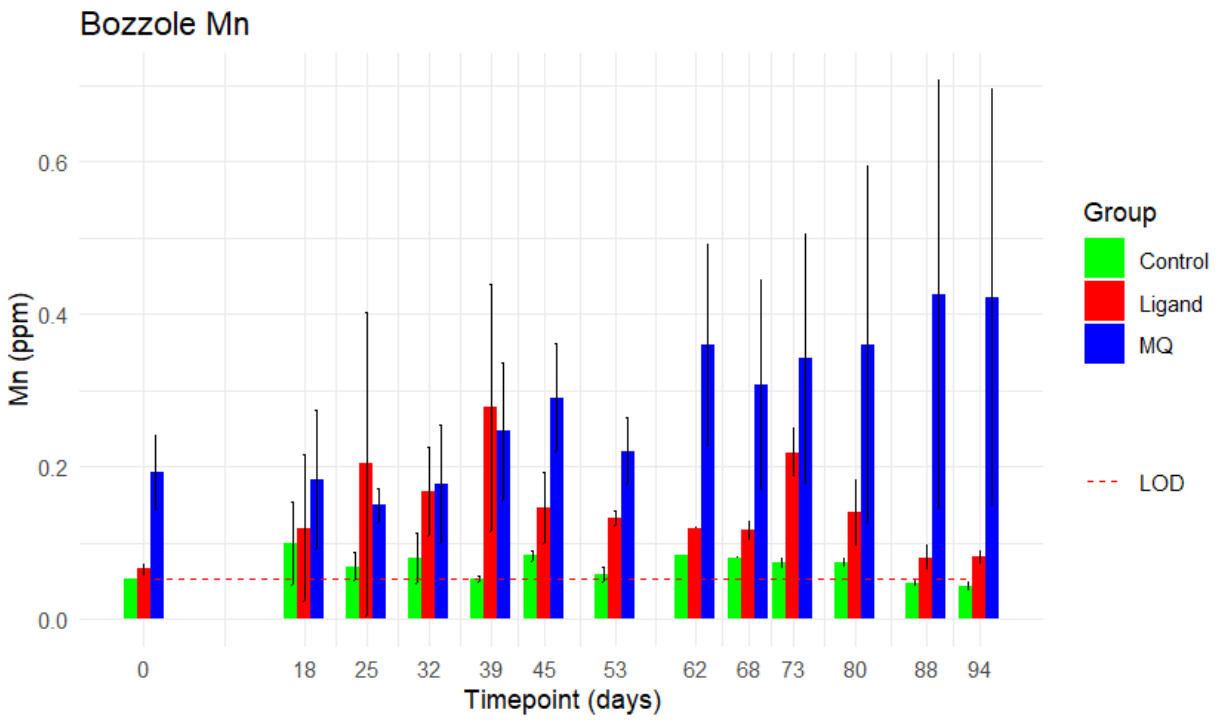

Figure 3.11: Dissolved Mn concentrations measured in pore water of Bozzole soil of the three groups control, ligand, and MQ over time

Figure 3.11 displays the Mn concentrations over time for the Bozzole soil. In the MQ group, the Mn content is higher than in the other two groups from the first measurement point. A similar trend to the Fe concentrations is observed, with the Mn content in the MQ group increasing until the end of the experiment, reaching 421 ppb at TP 94. The standard deviation between the two samplers in each pot is notable, indicating significant differences between rhizosphere and bulk Mn content (p-value of 0.01 between TP 30 and TP 100), with higher Mn concentrations in the bulk soil. Interestingly, the Mn content in the rhizosphere does not increase over time, while the bulk concentrations do.

The control Mn concentrations remain very stable throughout the experiment, at levels around 50 to 100 ppb, while the ligand Mn content rises to 277 ppb at TP 39 but decreases again to 81 ppb by the end of the experiment, similar to the initial values.

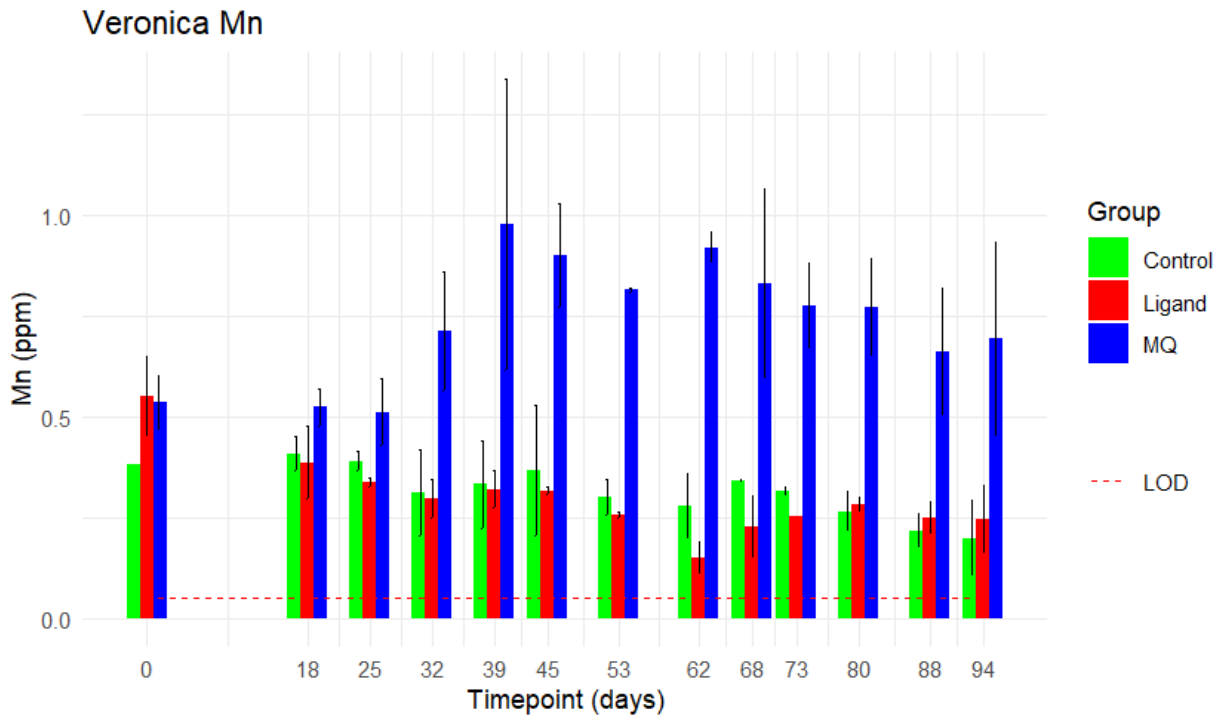

Figure 3.12: Dissolved Mn concentrations measured in pore water of Veronica soil of the three groups control, ligand, and MQ over time

The Mn concentrations in the Veronica soil are depicted in Figure 3.12. Initially, the Mn concentrations of the three groups range from approximately 384 (control) to 553 (MQ) ppb. Over time, the MQ group shows an increase in Mn content, reaching 693 ppb at TP

94, while the control and ligand groups exhibit a relatively stable decrease, reaching 200 ppb and 247 ppb, respectively, by TP 94. Overall, the Mn concentrations in the Veronica soil are higher than those in the Bozzole soil. Additionally, there is no significant difference between the bulk and rhizosphere Mn concentrations.

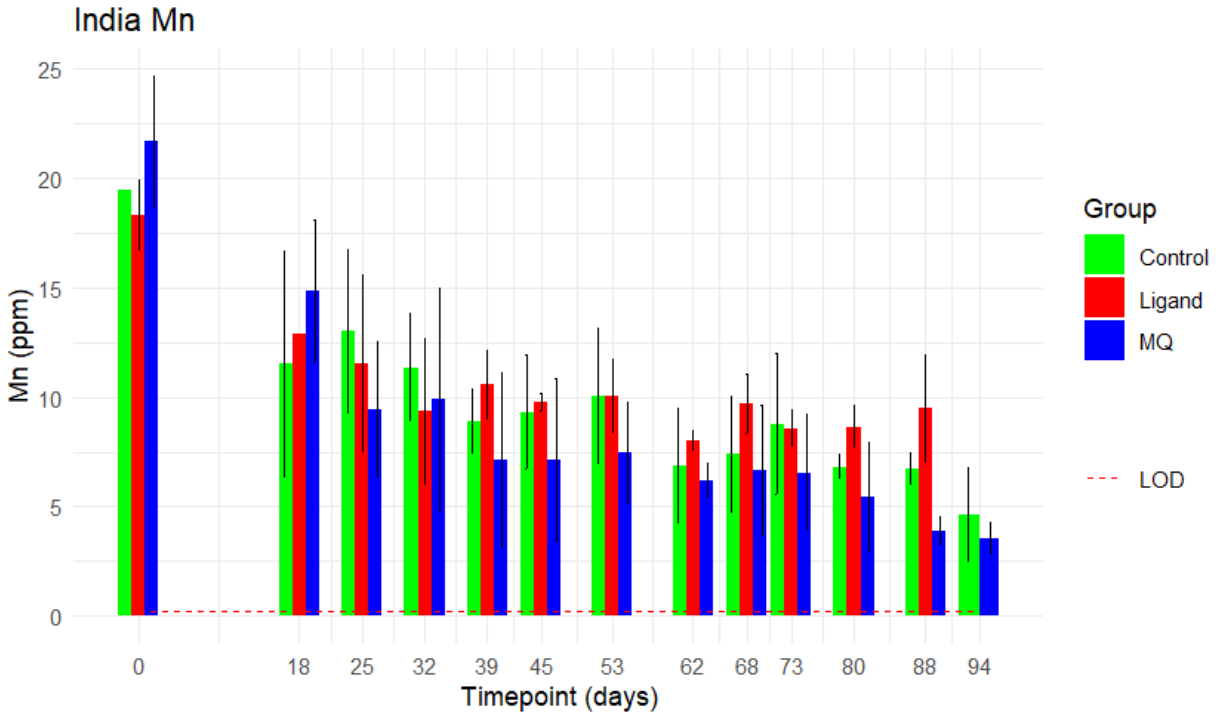

Figure 3.13: Dissolved Mn concentrations measured in pore water of Indian soil of the three groups control, ligand, and MQ over time

The Mn concentrations in the Indian soil, as shown in Figure 3.13, are significantly higher—more than ten times—than those in the Italian soils. Similar to the Fe content trend, the Mn concentrations for all groups start at comparable levels, around 20 ppm. Subsequently, all groups exhibit a decreasing trend. After the addition of the ligand at TP 32, the ligand group's Mn concentration stabilizes, while the MQ and control groups drop to 4.65 ppm and 3.5 ppm, respectively. There is no significant difference between the rhizosphere and bulk Mn concentrations.

The CA addition did not increase Fe or Mn concentrations in the Italian soils; in fact, the MQ group, which most probably has some ligand content from root exudation [89], exceeded the ligand group's Fe content towards the end (Fig. 3.8,3.9). This contrasts with other

findings where CA addition increased Fe concentrations significantly from 180 to over 900 ppm [61]. The unaltered and therefore low pH in those studies might explain the difference. In my study, the high pH of the ligand treatment could influence the microbial activity and therefore the Mn concentration in solution by enhancing oxidizing conditions. Also, the Mn concentrations in the pore water of Italian soils differ from those of Zou et al. 2024, where the total Mn content increased with CA addition to values between 17.1-105.3 ppm during the first 5 days, which are in the range of the Indian's pore water Mn content.

However, control treatments that were conducted in the Liu et al. 2020 paper with low pH HCl did not show increased soluble Fe and Mn, suggesting that chemical and microbial dissolution of Fe/Mn oxides, rather than low pH, is the main driver of metal mobilization [56]. On the other hand, the Indian ligand treatment had higher Fe and Mn levels towards the last timepoints compared to the control or MQ results. In the Zou et al. 2024 paper, the total Fe content was similar to the Fe(II) content, indicating that there was almost no Fe(III), aligning with my speciation analysis results [61].

Lower concentrations of organic ligands can result in reduced Mn availability since less fuel for the microbes is available [27]. This could explain the low Mn content in the Italian control groups. The ligand group is supposed to have the highest ligand concentration but also has a low Mn content. Here the high pH can influence microbial activity and therefore the Fe and Mn concentration in solution by enhancing oxidizing conditions.

3.8 Chromium Content in the Pore Water

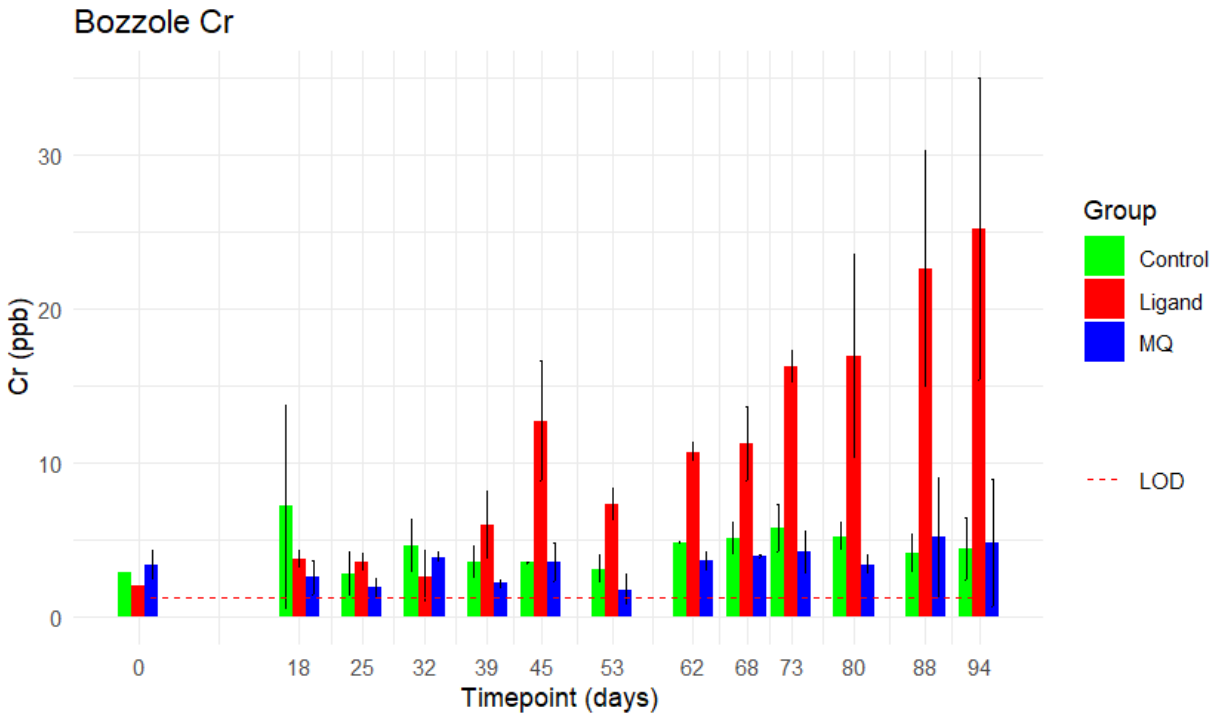

Figure 3.14: Dissolved Cr concentrations measured in pore water of Bozzole soil of the three groups control, ligand, and MQ over time

Figure 3.14 displays the Cr concentrations over time for the Bozzole soil. At Timepoint 0, the three groups start with similar Cr contents, ranging from approximately 2 to 3.4 ppb. Over time, Cr concentrations increase to around 4.4 ppb in the control group and 4.8 ppb in the MQ group. In the ligand-irrigated group, the pore water Cr content rises significantly after the addition of citric acid, reaching 25 ppb at TP 94.

The standard deviation of the later TP's are quite high, with more Cr content in the rhizosphere. However, the p-value between TP 30 and TP 100 (0.19) is too high to indicate statistical significance. The high standard deviation observed in the control group at TP 18 may be attributed to the addition of a new rhizon sampler that week, which had not yet stabilized in the environment and showed much lower Cr content.

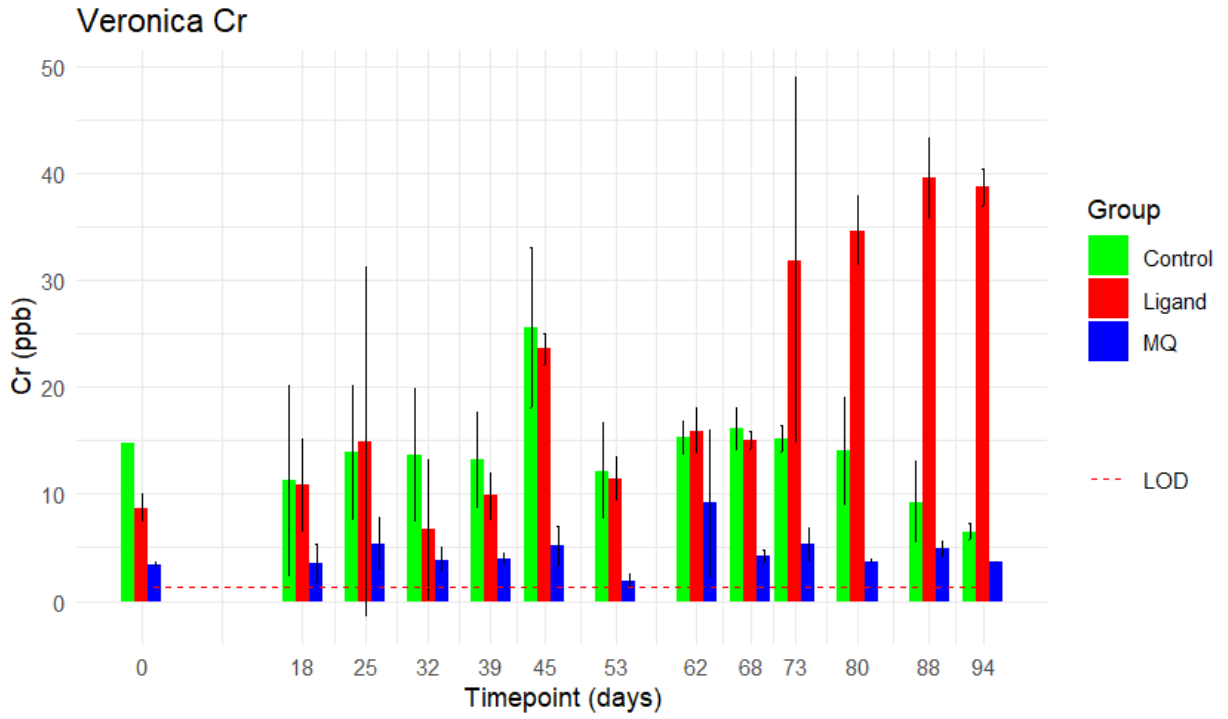

Figure 3.15: Dissolved Cr concentrations measured in pore water of Veronica soil of the three groups control, ligand, and MQ over time

The Cr concentrations differ among the various groups in the Veronica soil from the start, as shown in Figure 3.15. The control group exhibits the highest Cr concentration (14 ppb) in the pore water at TP 0, but also the highest fluctuation throughout the experiment. This variability is partly due to the addition of the second sampler. At TP 0, the ligand and MQ groups have Cr concentrations of 8.7 ppb and 3.4 ppb, respectively.

While the Cr content in the MQ group remains stable throughout the experiment, the control group concentrations increase until TP 45 before decreasing to 6.5 ppb at the end. The ligand group's Cr content increases significantly from TP 68 onwards, reaching almost 40 ppb by the end of the study.

Overall, the Cr concentrations in the pore water of the Veronica soil are higher in all three groups compared to the Bozzole soil. There was no significant trend observed between the rhizosphere and bulk samplers.

Only under high pH conditions (pH 8.0), Cr(III) oxidation by oxygen or MnO_2 is significant[90], which could explain the high Cr contents in the ligand group of the Italian soils, where lower

Mn content (roughly) corresponds to higher Cr concentrations (Fig. 3.113.12). Higher MnO_2 content also leads to higher mobility of Cr in the pore water by decreasing its adsorption tendency, resulting in lower Kd [20, 91]. At lower pH values (5-7), Cr(III) oxidation by MnO_2 is slower due to Cr(III) precipitation on the MnO_2 surface [92]. Cr oxidation can be catalysed by Mn-oxides, while with O_2 alone, it is very slow [43].

In the Veronica pore water samples, the lowest Cr content was found in the MQ pot, which also had the lowest pH values (pH of 4.5 to 5.5 between timepoints 39 and 94). In water-logged soils, with low pH values and under reducing conditions, Fe-oxides undergo reductive dissolution. This increases the hydroxide ion content which can form Cr-hydroxides, which have a high affinity for the soil colloids and are therefore scavenged out of the soluble phase [93, 94]. A similar trend, with lower Cr contents, was observed in the Bozzole samples, but with slightly higher pH values (5.2 to 5.9).

Additionally, Cr, in the opposite of Fe and Mn, is more mobile in its oxidized state [95]. This is consistent with the higher Cr concentrations found in the ligand groups towards later timepoints (characterized by higher pH) compared to the control and MQ groups (which had lower pH values) in the Italian soils (Fig. 3.14, 3.15), even though a higher pH doesn't necessarily mean the oxidation of species.

CA has multiple effects on the geochemistry of the soil, including solubilizing Cr(III) via chelation, competing for adsorption sites on mineral surfaces, and liberating other anions that compete with CrO_4^{2-}, resulting in higher chromate concentrations in the ligand-irrigated pore water [96]. It can also lead to the reductive dissolution of As-Fe oxide complexes, promoting the mobility of these metals [97]. Additionally, it might be complexed with Fe- and Mn-oxides and increase the desorption of metals from mineral surfaces [98]. This might explain the increase in Cr and As concentrations over time in the ligand treatment of the Italian soils, similar to the results of Liu et al. 2022 [56].

Other plant root exudates present in the soil may additionally enhance the reducing conditions in the soil, which can immobilize Cr [99]. This could explain the lower Cr content in the MQ groups of the Italian soils, where plants were grown, compared to the control groups without plants.

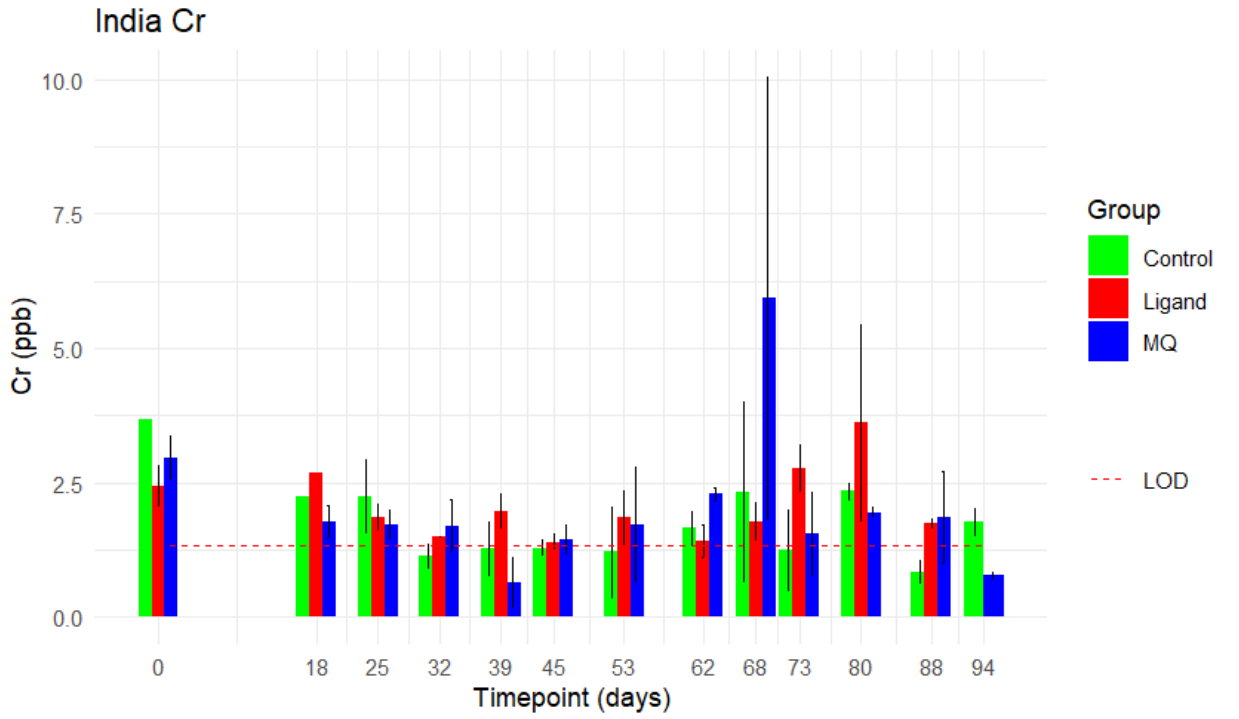

Figure 3.16: Dissolved Cr concentrations measured in pore water of Indian soil of the three groups control, ligand, and MQ over time

Interestingly, the Cr concentrations in the pore water of the Indian soil are very low, with a mean of 1.9 ppb (Fig. 3.16). Throughout the experiment, no clear trends are observed for any of the groups. Given that most values are below the LOD, drawing definitive conclusions is difficult.

The low Cr content could be due to the high Mn and Fe concentrations in the Indian pore water, which can lead to potential Cr(III) removal coupled with $Fe(OH)_3$ and $Mn(OH)_2$ precipitation [91]. Rai et al. 1984 also states that the majority of solids controlling Cr(III) solubility are either $Cr(OH)_3$ or Cr(III) co-precipitated with Fe-oxides [100]. Hem et al. 1977 reported that the total Cr concentration in their samples was close to the Cr_2O_3 solubility [101].

High geological background Cr content, such as present in the Sukinda Valley soil, does not necessarily mean high Cr mobilization in the pore water. This plays a crucial role in the prevention of Cr accumulation in rice grains [86] and could be due to the higher clay and Fe-oxide content provides more adsorption sites for Cr. Cr(III), being a positively charged

cation, can be more easily sorbed to negatively charged soil surfaces as a positively charged cation, while Cr(VI), being present as a negatively charged anion (CrO_4^{2-}), is less adsorptive. Other minerals like gibbsite and amorphous Fe-oxide, with high isoelectric points, can adsorb Cr(VI) anions at neutral and acidic pH [42].

In my experiment, higher concentrations of As, Cr, and Ni—all negatively charged in their oxidized forms—were measured in the ligand groups of the three soils. This might have likely been due to the process in which CA interacts with clay minerals, increasing their negative charge and decreasing the adsorption of negatively charged metal species, thus enhancing their mobility [102, 103]. The Italian soils had relatively low clay content, whereas the Indian soil had high clay content, potentially enhancing this effect.

Effect of Bacteria

CA can be utilized by microbes to mobilize insoluble nutrients and metals such as Fe, P, Zn, and others [104]. Microbes can also use CA as an electron donor for the reduction of Cr, Fe, Mn, and sulphate [105]. In one study, CA addition enriched the Fe-reducing bacteria such as Clostridium and Desulfitobacterium [56]. For Zou et al. 2024, this shift to metal-reducing bacteria led to increased Fe and As levels [61]. However, my data only showed an increase in As after ligand addition. This increment of As could be more pH-dependent, and the Fe liberated by microbial-induced dissolution (microbial presence was evident in my ligand groups, which had a thick bacterial mat) might have undergone other precipitation processes. This also occurred in the Geng et al. 2020 paper, where Fe-ions and Fe-complexes with organic ligands precipitated [106]. It could have also been that Fe has been taken out of the system by the removal of the mentioned bacterial mat, which has been done up to once a week during its peak growing phase.

3.9 Sulphide Analysis Data

Sulphide analysis was conducted to assess the concentration of soluble sulphide in the pore water solution. It was done towards the end of the experiment, where unfortunately the

ligand group already has been harvested, while the duplicates and India soils lack a control group. But from the data that is presented in Figure 3.17, a trend of higher sulphide content in the ligand treatment can be seen compared to the MQ content.

In the Bozzole duplicate soil, the sulphide contents in the ligand treatment is 15.3 µM and 13.2 µM for the timepoints 84 and 97, respectively, and therefore significantly higher than in the MQ treatment (with 1.6 and 1.4 µM). The amount of sulphide in the control groups seems to be similar to the MQ group, both around 0.9 µM sulphide.

For the Veronica soil, the differences between the treatments are not as pronounced as for the Bozzole soil. The ligand treatment still had higher values compared to the MQ one but with 2 and 1.7 µM compared to 0.9 and 0.9 µM (TP's 84 and 97) not as significant. Interestingly, in the last timepoint of measurement, the sulphide content of the control group (2.5 µM) exceeds the one of the MQ group (0.8 µM). The sulphide concentration in the MQ pots is similar to those measured in the Bozzole soil, while the ligand treatment shows less sulphide liberation than in the Bozzole soil.

There was no duplicate treatment done for the Indian soil. In the Indian control and MQ treatments, the sulphide contents are quite similar, with 0.8 and 0.78 µM S^{2-}.

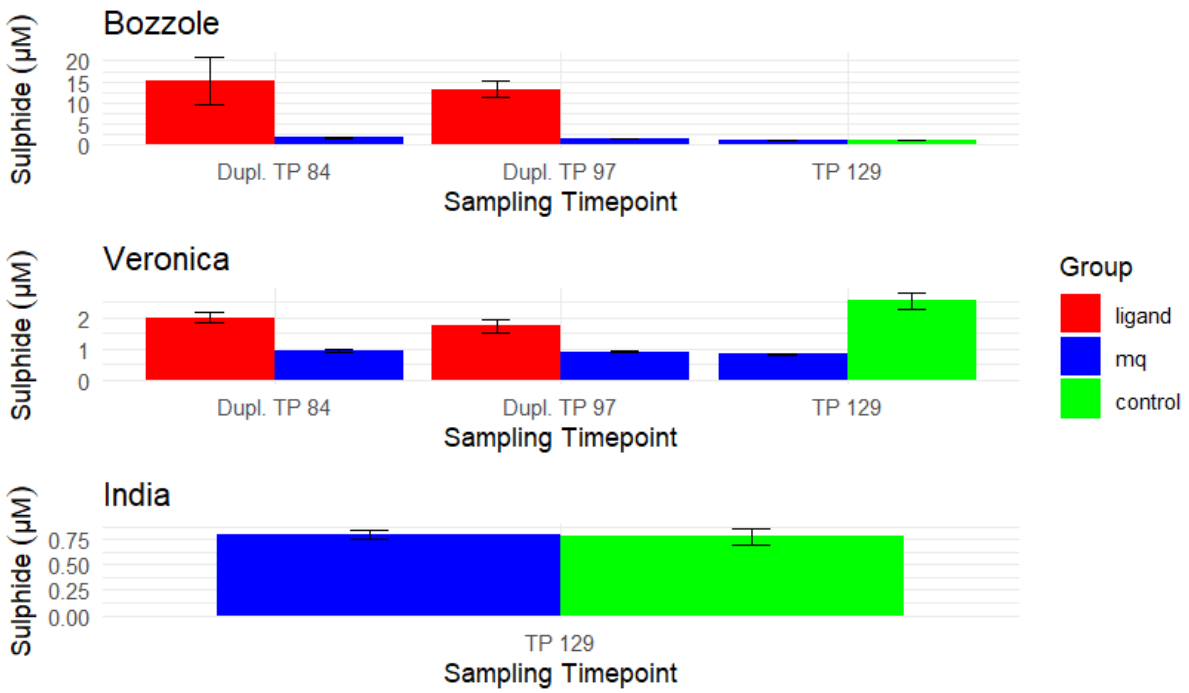

Figure 3.17: Dissolved sulphide content of the Veronica, Bozzole, and Indian pore water in µM

In the pore water of Veronica and Bozzole soils, sampled during later time points, sulphide concentrations were observed to be lower in the MQ treatment compared to the citric acid treatment. Concurrently, the Fe content in the pore water at these time points where higher in the MQ group than in the ligand group (Fig. 3.8,3.9)

This trend suggests the occurrence of ligand-induced sulphate reduction, an important process in anoxic soils which forms sulphide. As mentioned in the introduction, sulphide can precipitate with Fe(II) to form pyrite. Higher content of sulphide in the ligand groups could lead to enhanced pyrite precipitation, therefore decreasing the soluble Fe concentration in the pore water [41], an effect noticed in the Italian soil data.

3.10 Plaque Data

As seen in Figure A.2, the root weight differs greatly among the harvested plants. The roots of the plants irrigated with ligand solution show a lower mass, with 81 mg (Bozzole), 265

mg (Veronica), and 41 mg (India). These plants exhibited symptoms of decay and had to be harvested earlier than the MQ group. Despite the early harvest, the roots already showed signs of decomposition, contributing to the low root mass and potential loss of plaque, which will be discussed later. The roots were of a blackish color (A.5). The root mass of the MQ group was higher, with 877 mg (Bozzole), 1017 mg (Veronica), and 383 mg (India).

3.10.1 Iron Content in Plaque

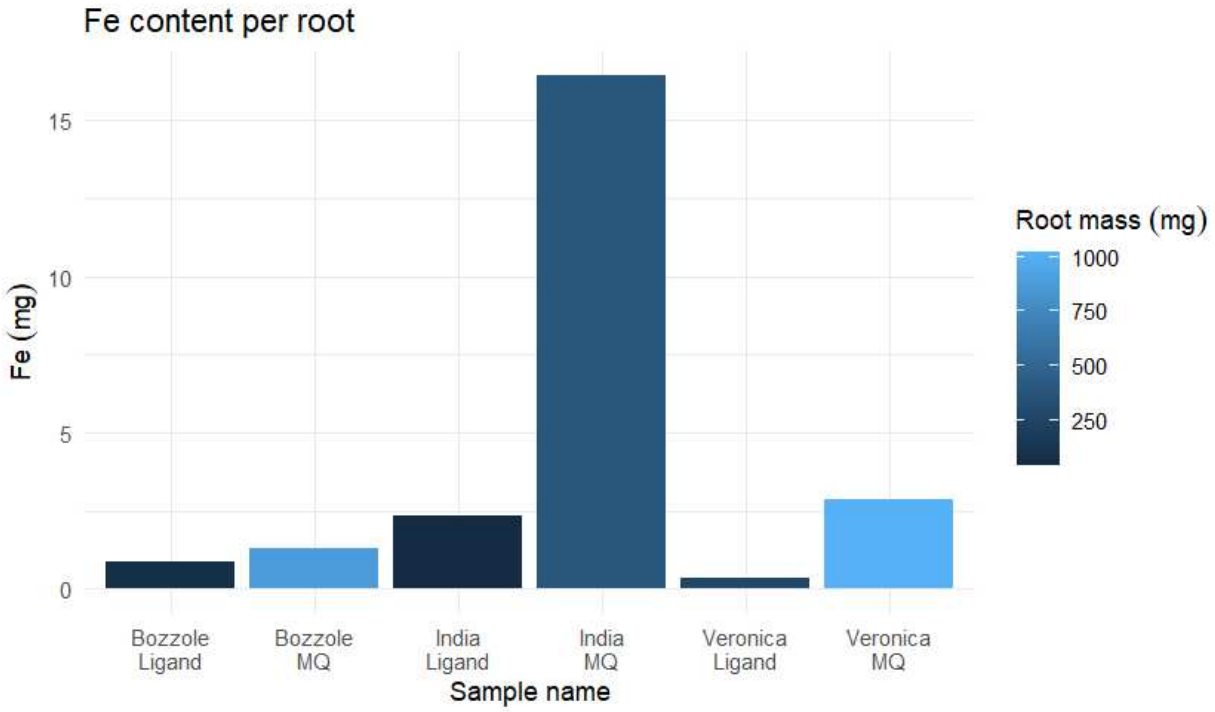

Figure 3.18: Fe content in the whole root of each plant

The Fe content of the whole root in the ligand group was lower compared to the MQ group of the corresponding soil (Fig. 3.18). Interestingly, the Indian MQ sample had the highest Fe concentration of 16.4 mg Fe, despite having a lower total root mass (383 mg) compared to the Veronica (1017 mg) and Bozzole (877 mg) planted rice plants. Even the roots of the Indian ligand group, which had the lowest weight in the setup (41 mg), yielded more Fe than the Bozzole samples and nearly as much Fe as the Veronica MQ group (2.3 mg Fe for India and 2.9 mg Fe for Veronica).

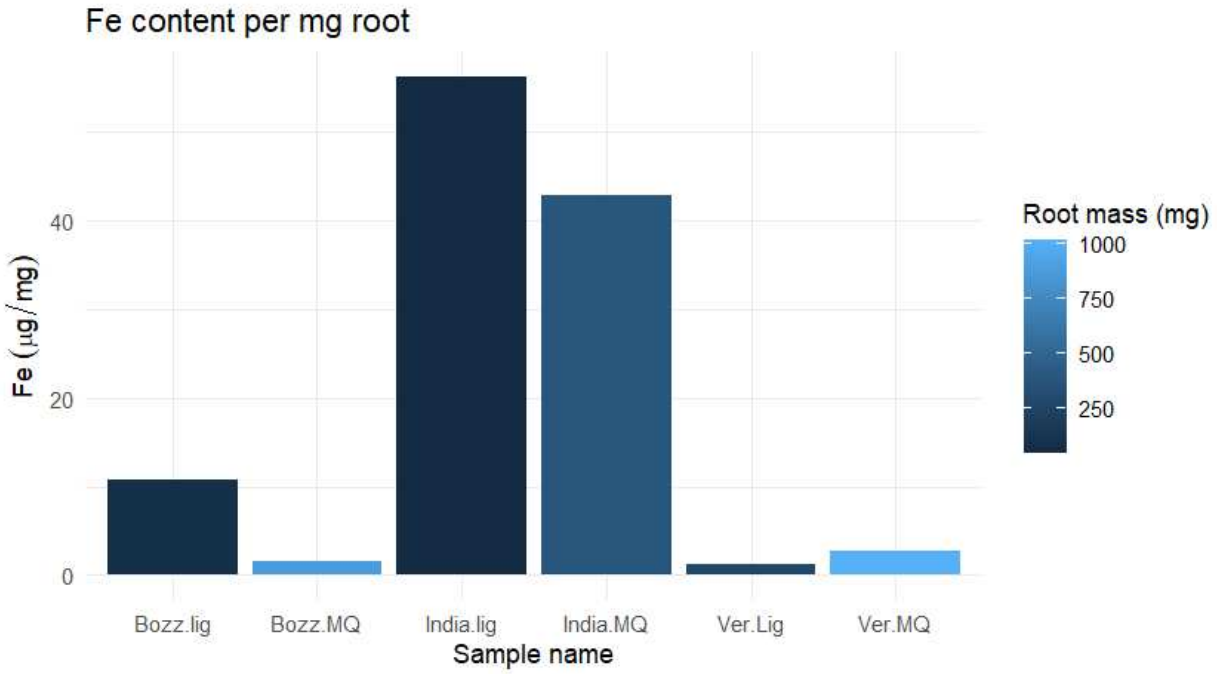

Figure 3.19: Fe concentrations in µg per mg root of each plant

Given the substantial variation in root masses, the Fe concentrations were normalized to each mg of root to allow for comparable Fe content in the root plaques. Figure 3.19 shows that the root solution from the Indian soils has a significantly higher Fe concentration (56 µg/mg for India ligand and 43 µg/mg for India MQ) root compared to the Italian soils (between 10 and 28 µg/mg).

Additionally, for both the Indian and Bozzole groups, the roots irrigated with ligand solutions have higher Fe yields. However, due to their low root mass, as indicated by the dark blue colour, the calculation error is high. The Veronica ligand group has a lower Fe yield than its corresponding MQ group.

3.10.2 Manganese Content in Plaque

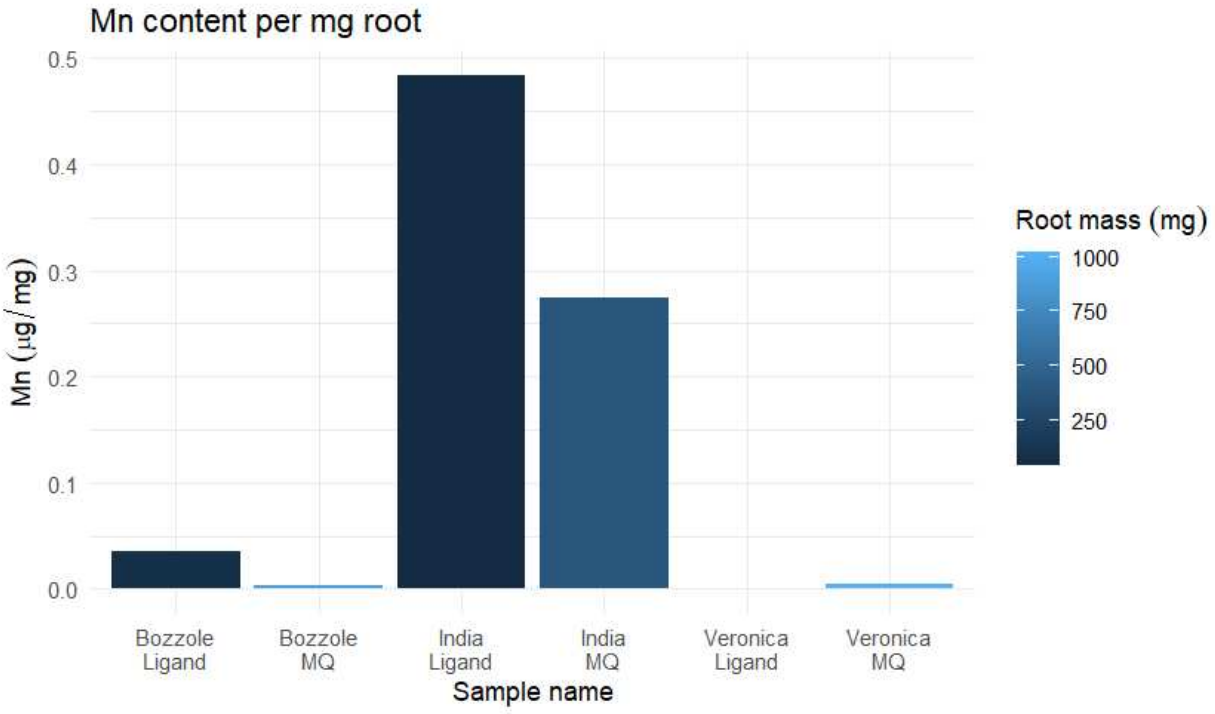

Figure 3.20: Mn concentrations in ng per mg root of each plant

The Mn concentrations per mg root in the DCB extraction solution are very low, as shown in Figure 3.20. Similar to the Fe concentrations, the highest Mn content was measured in the Indian ligand group with 0.48 µg/mg root, approximately double the content of the Indian MQ group (0.27 µg/mg root) and 14 times more than the Bozzole ligand group (0.035 µg/mg root). The Veronica ligand root's Mn content was below the LOD, while the Veronica MQ root had 0.0043 µg Mn/mg root.

Comparing the soluble Fe content in the pore water with the amount of Fe in the plaque shows a positive correlation: India > Bozzole > Veronica (high to low content in both plaque and pore water). The Mn content in pore water follows a different trend: India > Veronica > Bozzole (high to low). Although Veronica pore water has more than twice the Mn content compared to Bozzole, the Mn content in the plaque is similar (MQ group) or higher (ligand group) for Bozzole. Mn plaque formation correlates positively with Fe plaques and Mn^{2+} content in pore water, similar to Christensen et al. 1998's and Crowder et al. 1993's findings

[107, 76].

In the Indian soil, higher IP formation was observed in the ligand group compared to the MQ group, with double the Fe/Mn concentrations in pore water towards the end. Fe availability seems to be the leading factor in IP formation for this soil (Fig. 3.10,3.19).

Fe speciation, which is an important factor for IP formation, is often controlled by redox potential, making Eh a key variable affecting redox transformation during IP formation. The optimal Eh for Fe reduction is assumed to be less than around 200 mV [71]. Unfortunately, Eh measurements were not consistently taken throughout my experiment, but available data show Eh ranging from -157 mV (control Bozzole) to -235.2 mV (control Veronica). The range of these Eh values indicates Fe(II) as the favoured oxidation state according to the Eh-pH diagram (Fig. A.3), which could enhance IP formation.

The pH values of the Italian soils are similar to each other, showing high values for the ligand group and lower values for the MQ group. In contrast, the Indian soil exhibits better buffering capacity, as mentioned before. For both the Indian and Bozzole soils, the Fe concentration per milligram of root is higher in the ligand treatment (high pH) compared to the MQ treatment. This suggests that the role of citric acid as a Fe mobilizer might be more significant for the IP formation than the high pH's oxidizing effect, which can decrease Fe concentration in the soil solution [107]. Additionally, CA might influence the dissolution of pre-existing IP on the root and indirectly impact the root's oxidizing capacity [108].

For the Veronica soils, the ligand group shows less Fe per milligram of root than the MQ group. This indicates different soil processes and interactions, but could also be due to IP formation being promoted in lower pH ranges. According to Boone et al. 1983, IP formation is enhanced within a pH range of 3-5.3, which aligns with the Veronica MQ soil's pH range of 4.7-5 [109]. When the pH exceeds 5, the Fe(II) pool might be depleted due to oxidation [110].

This presents a dilemma: at low pH, the roots' oxidizing power is diminished, leading to low IP formation, while at higher pH, Fe(II) is oxidized and becomes insoluble. As noted by Mendelssohn et al. 1993, most plaques are found near the surface, where pH and Eh are optimal for Fe reduction—not too low (as they decline with depth) and not too high

[108]. However, this trend does not apply to the Bozzole soil, where higher plaque content is observed in the ligand group (Fig. 3.19) with a high pH of up to 9, where Fe oxidation occurs, but this treatment also had a significantly higher sulphide content (Fig. 3.17).

The ligand treatment in the Veronica soil yielded slightly lower Fe concentrations per mg root than the MQ group, with a higher sulphide content in the ligand group, but at a much lower concentration (2 µM, Fig. 3.17) compared to the Bozzole ligand group (exceeding 15 µM sulphide). As mentioned in the introduction, S^{2-} content influences the IP formation by reducing Fe(III), leading to a higher Fe(II) content in solution, which could then be used in IP formation, explaining these results.

A basic amount of S is essential for IP formation and can suppress toxic metals [75]. However, excessive S^{2-} can deplete the Fe(II) pool by precipitating as pyrite (FeS_2) [111], which can lead to co-precipitation of other metals, and also deplete oxygen and act as a toxin, reducing root plaque formation due to decreased ROL [111, 112]. The S speciation can also influence the barrier of IP against Cr uptake and its storage inside rice root tissues [74]. The decreased root mass observed in pots with CA addition could be a sign of sulphide toxicity.

Precipitation of FeS can be reversed when oxygen input into the rhizosphere via ROL oxidizes FeS precipitates near the roots, leading to "heavy" IP formation [63]. This could explain the high Fe content per mg root in the Indian and Bozzole treatments, but the roots' poor condition suggests that active ROL during the final growth stages was unlikely. Visually, less plaque was visible on the ligand group roots.

Unfortunately, data on the sulphide content in the Indian ligand treatment is unavailable; however, the higher Fe content in the ligand root plaque compared to the MQ root plaque suggests that CA irrigation likely enhanced the sulphide content, therefore enhancing the reduction of Fe(III) to more soluble Fe(II). This Fe(II) could again precipitate as pyrite once further away from the rhizosphere.

3.10.3 Chromium Content in Plaque

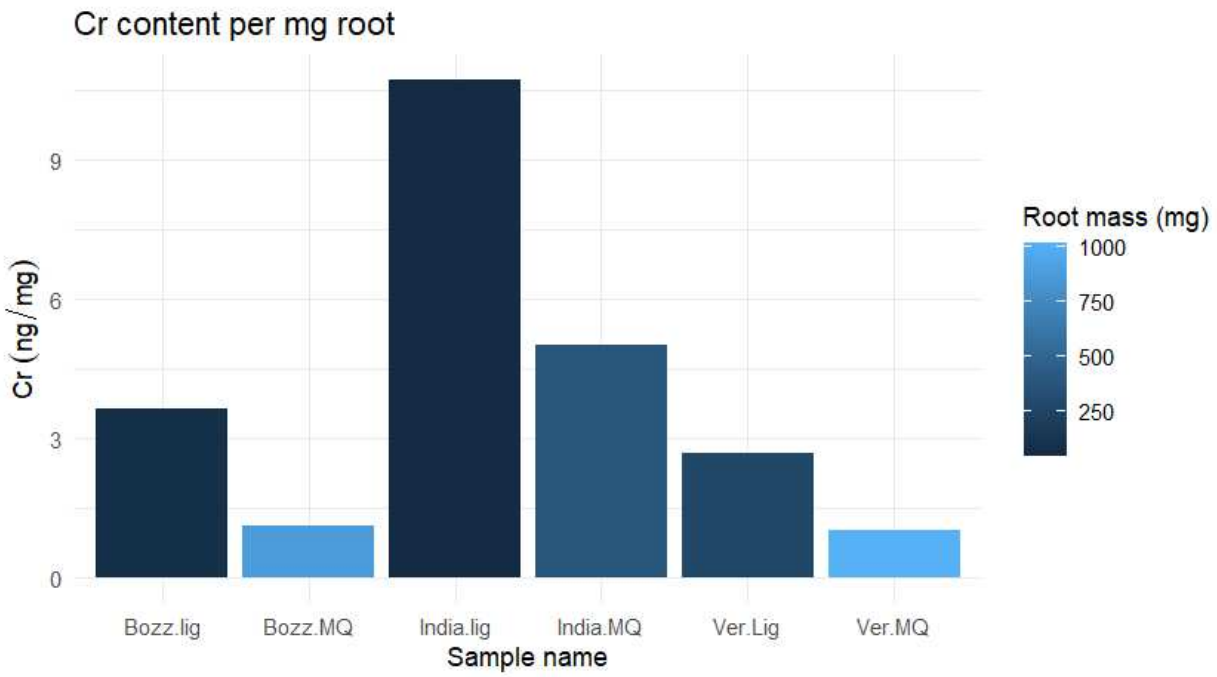

Figure 3.21: Cr concentrations in ng per mg root of each plant

Figure 3.21 displays the Cr concentrations on the root surfaces. Similar to the trends observed for Fe and Mn contents, plants grown in the Indian soil show the highest IP Cr content, with 10 ng/mg root in the ligand group, followed by 5 ng/mg root in the MQ group. Interestingly, despite the minimal Fe and Mn measured in the Veronica ligand sample, some Cr is present, with concentrations of 2.7 ng/mg root, which is more than double that of the Veronica MQ roots, which have 1 ng/mg root. The Bozzole ligand and MQ Cr contents are similar to those of the Veronica samples, with 3.7 and 1.1 ng/mg root, respectively. The Cr content in the plaque positively correlates with the Fe (p-value of 0.01, Fig. 3.22) and Mn (p-value of 0.003) contents per mg root.

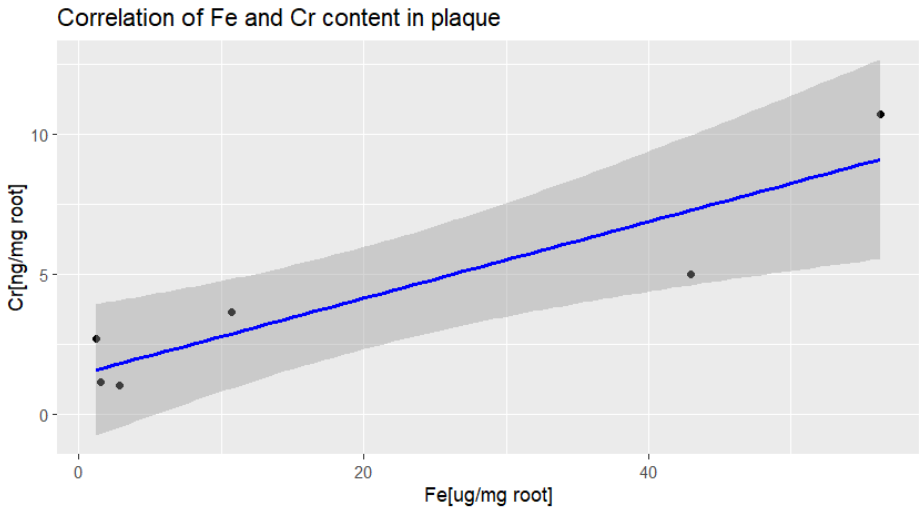

Figure 3.22: Correlation between the Fe and Cr content per mg root after DCB extraction

This could indicate that more Cr is adsorbed if more plaque is present, or that ligand addition liberates Cr, as more Cr was measured in the ligand root plaque of all soils, even though the Veronica ligand pot had less Fe than the Veronica MQ group (Fig. 3.19). This was not observed in the artificial root treatment (whose results will be shown shortly), where the ligand addition resulted in less IP formation on the tube surface compared to the MQ treatment.

3.11 Plaque on Artificial Roots

The artificial roots, composed of silicon tubes due to silicon's gas permeability, were submerged in the Indian soil for 2-26 days and analysed for the metal content of the plaque forming on their surface, using the DCB extraction. The metal contents of Ni, As, and Al were below the detection limit and will not be discussed further.

Fe in artificial Plaque

The Fe content in the IP extraction solution of the different tubes is displayed in the following Figure.

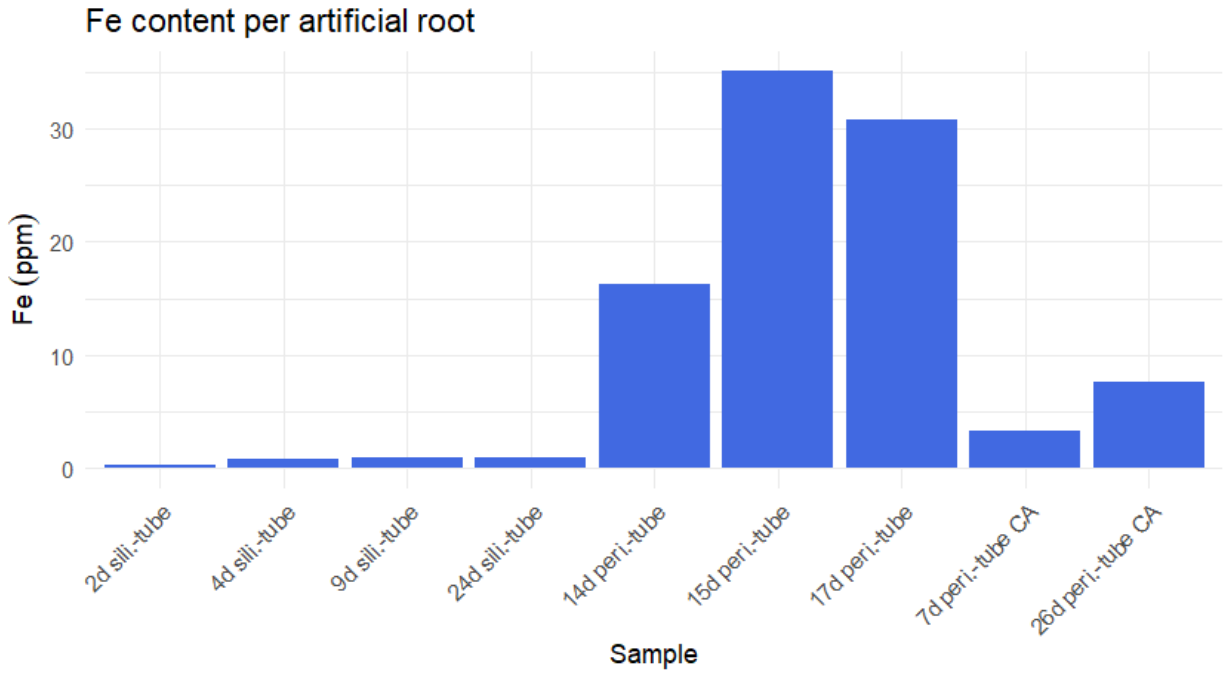

Figure 3.23: Fe concentrations in ppm in IP on the silicon and peristaltic pump tubes

As illustrated in Figure 3.23, the silicon tube did not appear to promote the formation of IP, as the Fe content in the DCB solution was only 0.9 ppm after 24 days. This is consistent with the visual observations, as no discoloration was noted on these tubes. Conversely, the peristaltic tubes exhibited some Fe content following DCB extraction, and visible reddish precipitates were observed on the tubes A.6. After 14, 15, and 17 days of immersion in the submerged, anoxic Indian soil, the Fe content in the plaque was 16, 35, and 30 ppm, respectively.

Interestingly, the two tubes subjected to CA irrigation had lower Fe contents of 3.3 and 7.6 ppm after 7 and 26 days of immersion, indicating lower IP formation compared to the group with MQ water irrigation. The low formation of IP on the silicon tube surfaces could be due to lower gas permeability or unsuitable surface structure for Fe-oxide precipitation. The peristaltic pump tubing also consisted of silicon but with substantial IP formation, indicates different chemical-physical parameters between the tubing types.

Manganese in artificial Plaque

The Mn content in the IP of the different tubes is shown in the following Figure.

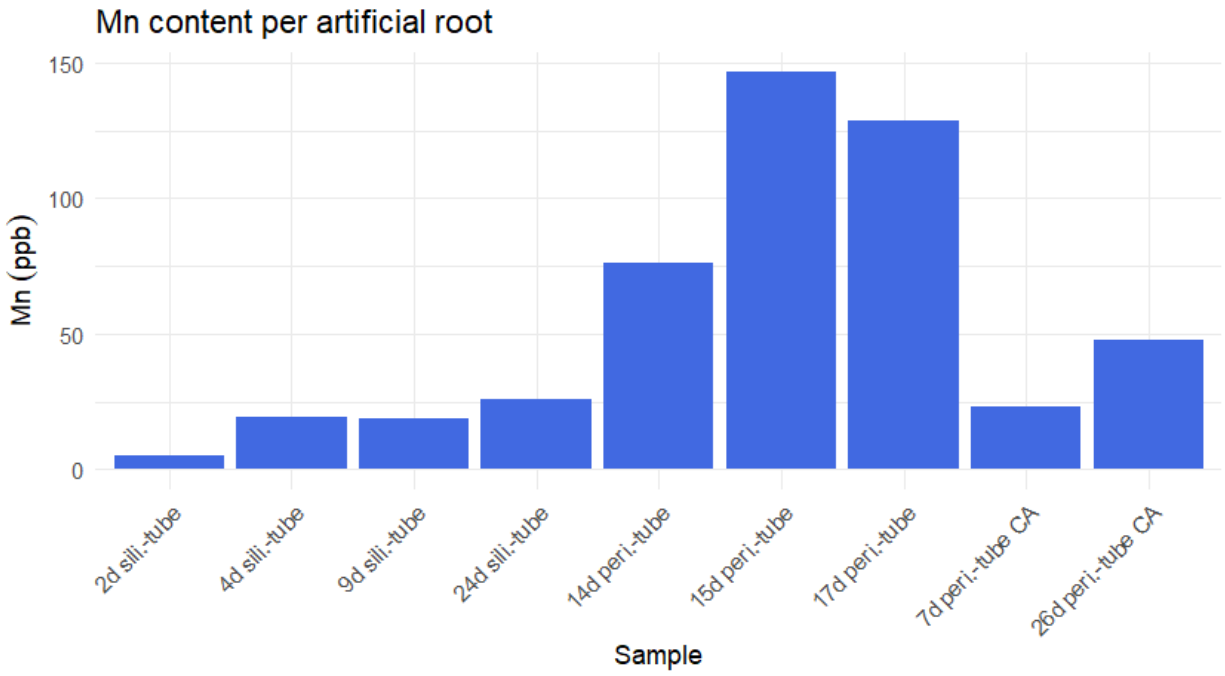

Figure 3.24: Mn concentrations in ppb in Fe plaque of the artificial roots

The Mn concentration per artificial root exhibits a similar trend to the Fe content, but with significantly lower concentrations. The silicon tubes again demonstrated lower Mn content than the peristaltic pump tubes, though the differences between the two types of tubing were not as pronounced as those observed for Fe content. Specifically, after 24 days, the Mn content was 25 ppb for the silicon tube compared to 128 ppb for the peristaltic pump tube after 17 days (Fig. 3.24).

The peristaltic pump tubes again showed higher Mn concentrations when irrigated with MQ water compared to those irrigated with CA solution. After 24 days in soil irrigated with the CA solution, the Mn content in the extraction solution was 47 ppb, which is still double that of the silicon tube sample with MQ irrigation. However, this decrease in metal concentrations from the MQ to the ligand group is not consistent with the findings from the real roots DCB extraction, where the Mn content was higher in the ligand than in the MQ group for the Indian soil. As previously mentioned, it is challenging to compare these

results directly due to several factors, including the low weight of the ligand roots and the resulting high error.

Additionally, the oxygen pumped through the tubes might have been consumed by enhanced bacterial sulfur reduction. Unlike real roots, artificial roots do not actively take up nutrients and metals, which would bring Fe(II) ions closer to the root surface, promoting IP formation. This process is absent in artificial roots, leading to Fe(II) being oxidized and immobilized before precipitating on the tube surface.

The fact that in the artificial root setup, the Fe and Mn concentrations in DCB extraction solutions were lower in the ligand group than in the MQ group suggests that CA might not be the main driver of IP formation. However, the total amount of plaque on the tubes could not be determined.

Chromium in artificial plaque

The Cr content in the IP of the two tube types is shown in the following graph:

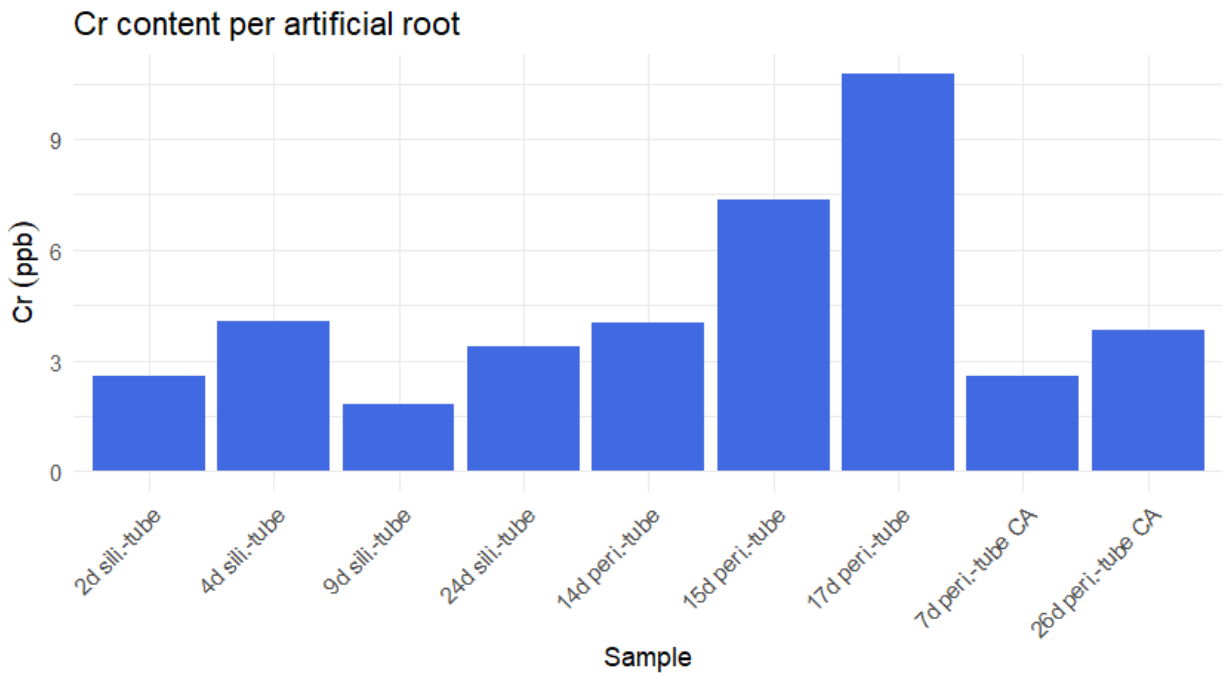

Figure 3.25: Cr concentrations in ppb in IP of the artificial roots

The Cr content in the IP of the silicon tubing after DCB extraction is 2.6, 4.1, 1.8, and 3.4

ppb after 2, 4, 9, and 24 days, respectively. The content in the peristaltic tubing is higher, though not as significantly as the Fe and Mn content, with values of 4, 7.4, and 10.8 ppb after 14, 15, and 17 days of settling in the soil. Again, the Cr content in the extraction solution of the peristaltic tubes submerged in citric acid-irrigated soils is lower than those in MQ-submerged soils, with 2.6 and 3.8 ppb after 7 and 26 days.

3.12 Metal Content of Plant Segments

The rice plant segments (roots, leaves/stems, and seeds) were digested in aqua regia to assess the translocation of different metals within the plants.

Fe content in the plant segments

In the Veronica soil, the measured Fe concentrations ranged from 232 to 2268 mg Fe/kg of plant biomass (Fig. 3.26). The highest Fe content was observed in the roots of the ligand duplicate treatment. However, due to the limited biomass available, only 3.8 mg (instead of the usual 30-40 mg) was used for digestion, leading to a high calculation error. A slight decrease from 325 to 232 mg Fe/kg from leaves to seeds was noted in the MQ group. Given the low concentrations and narrow range of values, no significant changes can be confirmed. For the Bozzole soil, the MQ treatment was the only one where all segments (roots, leaves, and seeds) could be measured. The highest Fe concentration was in the root biomass, with 3477 mg Fe/kg. In the leaves of both the MQ and MQ duplicate treatments, similar contents were measured (186 and 158 mg Fe/kg, respectively), while the seeds showed only 50 mg Fe/kg. The ligand treatment root biomass contained 953 mg Fe/kg.

In the Indian soil, no duplicates were performed, and the ligand group was harvested early due to plant mortality. The Fe content in the roots of the MQ treatment was exceedingly high, over 130,000 mg/kg. A reddish colour observed during sample grinding suggested the presence of residual plaque, likely explaining the unusually high Fe content. The Fe contents in the leaves of both the ligand and MQ treatments were similar (1873 and 1305 mg Fe/kg,

respectively). Overall, roots generally had higher Fe contents than other segments, with the rice plants in Indian soil exhibiting higher Fe levels than those in Italian soils, which had similar Fe contents to each other.

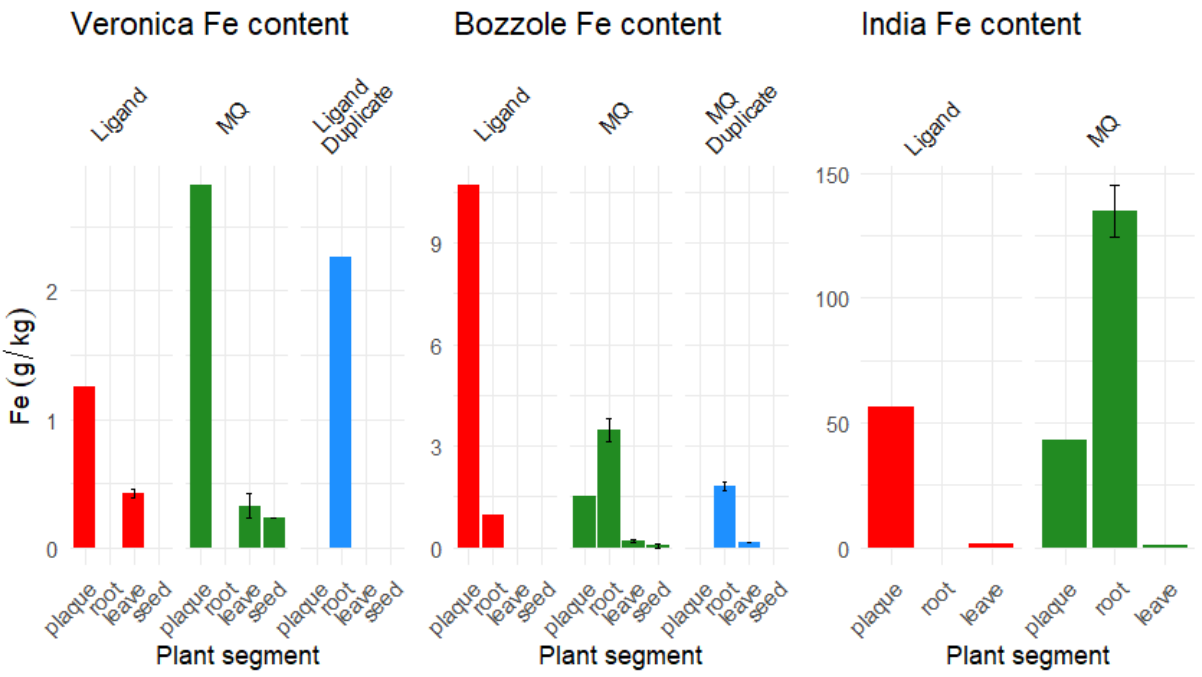

Figure 3.26: Fe concentrations in mg/kg biomass of the different plant segments and IP of the treatments

Mn content in the plant segments

In the Veronica soil, the Mn concentrations ranged from 165 to 374 mg Mn/kg plant biomass. Similar to Fe, the highest Mn content was found in the roots of the ligand duplicate treatment, but with a high error due to limited biomass. A slight increase from 165 to 236 mg Mn/kg from leaves to seeds was observed in the MQ group. Again, no significant changes can be confirmed due to the narrow range of values. The Mn concentrations in the ligand duplicate group roots were below the detection limit (Fig. 3.27). In the Bozzole soil, the highest Mn concentration was found in the leaves of the MQ duplicate treatment, with 1703 mg Mn/kg. The Mn content in the roots of all three measured groups was low: 3 mg/kg in the ligand, 11 mg/kg in the MQ, and 13 mg/kg in the MQ duplicate groups. The leaves and seeds of the MQ group had 777 and 210 mg Mn/kg, respectively.

In the Indian soil, the highest Mn content was found in the leaves of the ligand treatment, with over 1650 mg Mn/kg. In the MQ treatment, the Mn content in the roots was 792 mg Mn/kg, and 1055 mg Mn/kg in the leaves.

Overall, the Mn content trend differed from Fe, with leaves generally showing higher Mn content than roots. The Veronica soil had the lowest Mn contents, while Bozzole and Indian soils had similar concentrations.

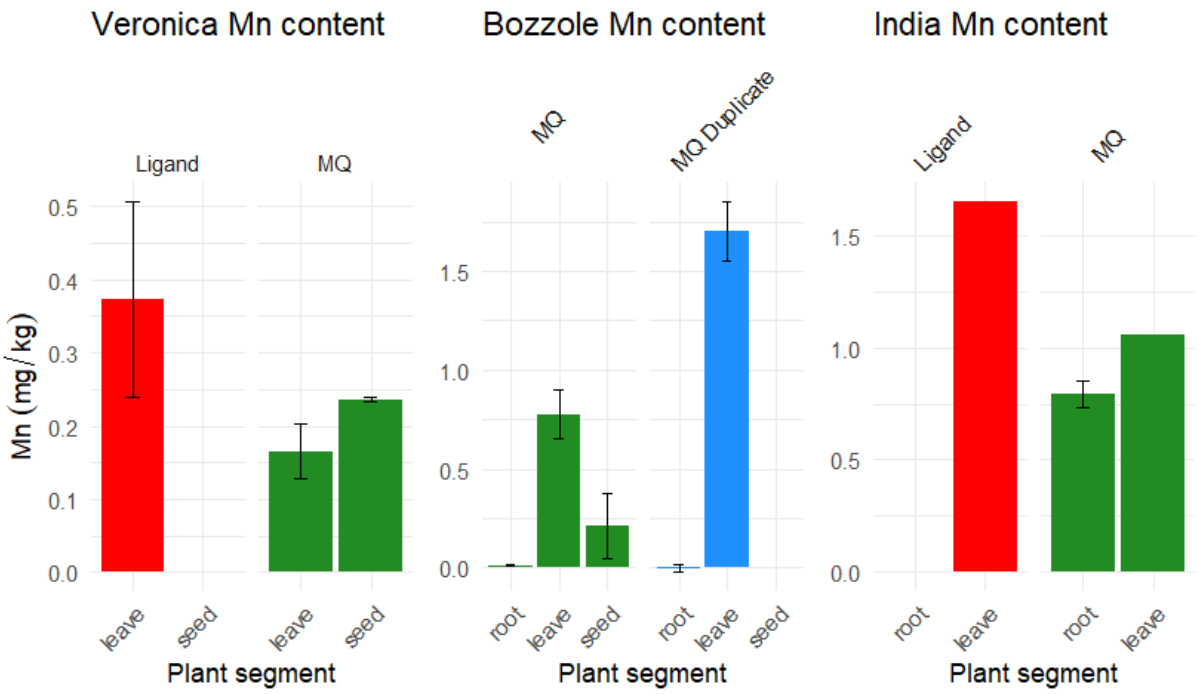

Figure 3.27: Mn concentrations in mg/kg biomass of the different plant segments of the treatments

Higher levels of Fe in the biomass were observed in treatments where higher concentrations of soluble Fe were detected in the pore water of the Italian soil. These findings are supported by the ones from Zeng et al. 2011 [21]. In the Bozzole MQ soil, only 5.35 % of Fe was translocated from the roots to the leaves, and an even smaller fraction, 1.44 %, to the seeds. This decrease in Fe from leaves to seeds was also reported by Zeng et al. 2011. For Mn, there was a slightly higher amount in the leaves than in the roots. In the seeds, the Mn content decreased again. It appears that Mn translocation in the plant is higher compared to Al, Fe, and Cr.

Cr and Al contents in the plant segments

Cr concentrations in the Italian rice segments were below the detection limit. In the Indian treatment group, the Cr content in the MQ group was over 40 mg/kg in the roots, and around 5 mg/kg in the leaves for both MQ and ligand groups. Unfortunately, no data on the Cr seed content is available. The difference between root and leave content suggests that most Cr is not translocated and remains in the roots. This is supported by the findings of Zeng et al. 2008, where Cr concentrations in plant segments were similar to those observed in my study for both roots and leaves [113].

A possible explanation for the higher accumulation in the roots could be that Cr is immobilized in the vacuoles of root cells, or that Cr(III) is reduced to the less mobile Cr(VI) by the cells, thus being retained in the root cortex cells [114].

Zeng et al 2011 also found that "the increase of rhizosphere pH and acid exudation would cause more Cr accumulation in rice plants" [21]. Unfortunately, no data on the ligand-treated root Cr content is available, but from the available data of the leaves of this treatment group, no significant differences were observed compared to the MQ group.

Al concentrations followed a similar trend to Fe, with significantly higher contents in roots compared to leaves and seeds. The Indian treatment showed higher Al content (up to 14,416 mg Al/kg in Indian MQ roots) compared to the Italian treatments (4,888 mg Al/kg in Veronica Ligand root and 1,944 mg Al/kg in Bozzole MQ roots).

4. Conclusion

This Master's thesis aimed to evaluate metal concentrations in the pore water of paddy soils, their interactions with plants and ligands, and how the composition of iron plaques (IP) are influenced by different soils and treatment groups.

Rice was grown in soils from different locations with varying metal contents, and pore water, IP, and plant segments were analysed for metal content. Three treatment groups were established for each soil: a control group with no plants and MQ irrigation, a group with plants and MQ irrigation, and a group with plants irrigated with a citric acid (CA) ligand solution. An additional artificial root experiment was conducted to further understand IP formation.

In the ligand treatment groups, a gradual increase in Cr concentration in the pore water was observed, potentially due to MnO_2-catalysed Cr(III) oxidation at higher pH or CA-mediated Cr mobilization via chelation or competition for adsorption sites on mineral surfaces. Significant differences were noted between the soils, particularly for the Indian soil, which originated near a Cr mine and exhibited the highest metal content. Surprisingly, the Indian soil showed lower pore water Cr concentrations compared to the uncontaminated Italian soils, possibly due to Cr precipitation onto Fe, Mn, and Al oxides or clays.

IP formation was highest in the Indian soil, especially in the ligand-treated group. A correlation between Cr and Fe content in the roots suggested that greater IP formation led to higher Cr retention. In the artificial root experiment (using gas-permeable silicon tubes), less IP formation was observed with CA irrigation, suggesting different processes influence IP formation on real versus artificial roots.

Within the plant, Fe was concentrated mainly in the roots, with little translocation to the stems and even less to the grains. Mn was more prevalent in the leaves, while Cr concentrations in plant segments were below the detection limit, likely due to retention by the iron plaques. The metal content in these plaques were significantly lower than the content in the

plants, suggesting that most of the metal translocate into the plants.

The findings from this thesis provide insights into the geochemical processes affecting metal behaviour in soils, with implications for understanding Cr contamination, particularly crucial in the Sukinda Valley region.

Limitations

Initially, all three groups of each soil experienced the same conditions, since no plant development took place and the switch from MQ to ligand irrigation started at a later timepoint. However, metal contents varied significantly in the early stages, reflecting the high variability of these complex paddy soil systems. Due to limited soil availability, duplicates could not be performed for all treatments, and plant mortality in the ligand treatment possibly compromised data comparison across groups. Therefore these results should be taken with caution.

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Bibliography

[1] R. Carson, "Silent spring. 1962," *New York*, 2009.

[2] J. A. Jacobs and S. M. Testa, "Overview of chromium (vi) in the environment: background and history," *Chromium (VI) handbook*, pp. 1–21, 2005.

[3] U. Staff, "Usgs mineral review," *Mineral Commodity Summaries*, 2024.

[4] S. Das, S. Ram, H. Sahu, D. Rao, A. Chakraborty, M. Sudarshan, and H. Thatoi, "A study on soil physico-chemical, microbial and metal content in sukinda chromite mine of odisha, india," *Environmental earth sciences*, vol. 69, pp. 2487–2497, 2013.

[5] V. Bolaños-Benítez, E. D. Van Hullebusch, P. N. Lens, C. Quantin, J. Van de Vossenberg, S. Subramanian, and Y. Sivry, "(bio) leaching behavior of chromite tailings," *Minerals*, vol. 8, no. 6, p. 261, 2018.

[6] S. Nayak, B. P, and P. Kale, "A review of chromite mining in sukinda valley of india: impact and potential remediation measures," *International Journal of Phytoremediation*, vol. 22, no. 8, pp. 804–818, 2020.

[7] J. Nouri, A. Karbassi, and S. Mirkia, "Environmental management of coastal regions in the caspian sea," *International Journal of Environmental Science & Technology*, vol. 5, pp. 43–52, 2008.

[8] A. Ertani, A. Mietto, M. Borin, and S. Nardi, "Chromium in agricultural soils and crops: a review," *Water, Air, & Soil Pollution*, vol. 228, pp. 1–12, 2017.

[9] W. Wu, S. Qu, W. Nel, and J. Ji, "The influence of natural weathering on the behavior of heavy metals in small basaltic watersheds: A comparative study from different regions in china," *Chemosphere*, vol. 262, p. 127897, 2021.

[10] G. M. Beone, F. Carini, L. Guidotti, R. Rossi, M. Gatti, M. C. Fontanella, and R. M. Cenci, "Potentially toxic elements in agricultural soils from the lombardia region of northern italy," *Journal of Geochemical Exploration*, vol. 190, pp. 436–452, 2018.

[11] C. I. APAT, "Agenzia per la protezione dell'ambiente e per i servizi tecnici," *Consiglio Nazionale delle Ricerche-Istituto di Ricerca sulle Acque*, vol. 2, 2006.

[12] C. I. APAT, "Agenzia per la protezione dell'ambiente e per i servizi tecnici," *Consiglio Nazionale delle Ricerche-Istituto di Ricerca sulle Acque*, vol. 2, 2003.

[13] P. Verma, D. Kaushik, and A. Pal, "Study of rice, its history and importance, with a review of nutritional values and rice growing regions in india," in *International Journal for Research Publication and Seminar*, vol. 7, pp. 112–118, 2016.

[14] H. Kraehmer, C. Thomas, and F. Vidotto, "Rice production in europe," *Rice production worldwide*, pp. 93–116, 2017.

[15] H. Liu, S. Hussain, S. Peng, J. Huang, K. Cui, and L. Nie, "Potentially toxic elements concentration in milled rice differ among various planting patterns," *Field Crops Research*, vol. 168, pp. 19–26, 2014.

[16] D. Mondal and D. A. Polya, "Rice is a major exposure route for arsenic in chakdaha block, nadia district, west bengal, india: A probabilistic risk assessment," *Applied Geochemistry*, vol. 23, no. 11, pp. 2987–2998, 2008. Arsenic in groundwaters of South-East Asia: With emphasis on Cambodia and Vietnam.

[17] H.-Y. Yu, F.-B. Li, C.-S. Liu, W. Huang, T.-X. Liu, and W.-M. Yu, "Iron redox cycling coupled to transformation and immobilization of heavy metals: implications for paddy rice safety in the red soil of south china," *Advances in agronomy*, vol. 137, pp. 279–317, 2016.

[18] I. Kögel-Knabner, W. Amelung, Z. Cao, S. Fiedler, P. Frenzel, R. Jahn, K. Kalbitz, A. Kölbl, and M. Schloter, "Biogeochemistry of paddy soils," *Geoderma*, vol. 157, no. 1-2, pp. 1–14, 2010.

[19] C. Ding, S. Du, Y. Ma, X. Li, T. Zhang, and X. Wang, "Changes in the ph of paddy soils after flooding and drainage: modeling and validation," *Geoderma*, vol. 337, pp. 511–513, 2019.

[20] M. Ao, T. Deng, S. Sun, M. Li, J. Li, T. Liu, B. Yan, W.-S. Liu, G. Wang, D. Jing, *et al.*, "Increasing soil mn abundance promotes the dissolution and oxidation of cr (iii) and increases the accumulation of cr in rice grains," *Environment International*, vol. 175, p. 107939, 2023.

[21] F. Zeng, S. Ali, H. Zhang, Y. Ouyang, B. Qiu, F. Wu, and G. Zhang, "The influence of ph and organic matter content in paddy soil on heavy metal availability and their uptake by rice plants," *Environmental Pollution*, vol. 159, pp. 84–91, 1 2011.

[22] A. Darma, J. Yang, E. Bloem, K. Możdżen, and P. Zandi, "Arsenic biotransformation and mobilization: The role of bacterial strains and other environmental variables," *Environmental Science and Pollution Research*, pp. 1–25, 2021.

[23] P. Zandi, J. Yang, A. Darma, E. Bloem, X. Xia, Y. Wang, Q. Li, and E. Schnug, "Iron plaque formation, characteristics, and its role as a barrier and/or facilitator to heavy metal uptake in hydrophyte rice (oryza sativa l.)," *Environmental Geochemistry and Health*, vol. 45, no. 3, pp. 525–559, 2023.

[24] M. Grybos, M. Davranche, G. Gruau, P. Petitjean, and M. Pédrot, "Increasing ph drives organic matter solubilization from wetland soils under reducing conditions," *Geoderma*, vol. 154, no. 1-2, pp. 13–19, 2009.

[25] D. Perret, J.-F. Gaillard, J. Dominik, and O. Atteia, "The diversity of natural hydrous iron oxides," *Environmental science & technology*, vol. 34, no. 17, pp. 3540–3546, 2000.

[26] S. Macfie and A. Crowder, "Soil factors influencing ferric hydroxide plaque formation on roots of typha latifolia l," *Plant and Soil*, vol. 102, pp. 177–184, 1987.

[27] S. C. Maguffin, L. Abu-Ali, R. V. Tappero, J. Pena, J. S. Rohila, A. M. McClung, and M. C. Reid, "Influence of manganese abundances on iron and arsenic solubility in rice paddy soils," *Geochimica et Cosmochimica Acta*, vol. 276, pp. 50–69, 5 2020.

[28] P. Akisanmi and P. Akisanmi, *Classification of clay minerals*. IntechOpen Rijeka, Croatia, 2022.

[29] J. A. Koupai, M. Fatahizadeh, and M. R. Mosaddeghi, "Effect of pore water ph on mechanical properties of clay soil," *Bulletin of Engineering Geology and the Environment*, vol. 79, pp. 1461–1469, 4 2020.

[30] T. W. Speir, A. P. V. Schaik, H. J. Percival, M. E. Close, and L. Pang, "Heavy metals in soil, plants and groundwater following high-rate sewage sludge application to land," *Water, Air, and Soil Pollution*, vol. 150, pp. 319–358, 11 2003.

[31] M. Hodson and S. Langan, "A long-term soil leaching column experiment investigating the effect of variable sulphate loads on soil solution and soil drainage chemistry," *Environmental Pollution*, vol. 104, no. 1, pp. 11–19, 1999.

[32] C. Yang, G. Lu, M. Chen, Y. Xie, C. Guo, J. Reinfelder, X. Yi, H. Wang, and Z. Dang, "Spatial and temporal distributions of sulfur species in paddy soils affected by acid mine drainage in dabaoshan sulfide mining area, south china," *Geoderma*, vol. 281, pp. 21–29, 2016.

[33] W. Cao, Z. Dang, X.-Q. Zhou, X.-Y. Yi, P.-X. Wu, N.-W. Zhu, and G.-N. Lu, "Removal of sulphate from aqueous solution using modified rice straw: Preparation, characterization and adsorption performance," *Carbohydrate polymers*, vol. 85, no. 3, pp. 571–577, 2011.

[34] Y. Sheng, G. Fu, F. Chen, and J. Chen, "Geochemical characteristics of inorganic sulfur in shijing river, south china," *Journal of Environmental Monitoring*, vol. 13, no. 4, pp. 807–812, 2011.

[35] M. K. Banks, A. P. Schwab, and C. Henderson, "Leaching and reduction of chromium in soil as affected by soil organic content and plants," *Chemosphere*, vol. 62, pp. 255–264, 2006.

[36] D. Rai, L. E. Eary, and J. M. Zachara, "Environmental chemistry of chromium," 1989.

[37] R. M. Cornell, U. Schwertmann, *et al.*, *The iron oxides: structure, properties, reactions, occurrences, and uses*, vol. 664. Wiley-vch Weinheim, 2003.

[38] C. Colombo, G. Palumbo, J.-Z. He, R. Pinton, and S. Cesco, "Review on iron availability in soil: interaction of fe minerals, plants, and microbes," *Journal of soils and sediments*, vol. 14, pp. 538–548, 2014.

[39] C. Colombo and J. Torrent, "Relationships between aggregation and iron oxides in terra rossa soils from southern italy," *Catena*, vol. 18, no. 1, pp. 51–59, 1991.

[40] J. Huang, A. Jones, T. D. Waite, Y. Chen, X. Huang, K. M. Rosso, A. Kappler, M. Mansor, P. G. Tratnyek, and H. Zhang, "Fe (ii) redox chemistry in the environment," *Chemical Reviews*, vol. 121, no. 13, pp. 8161–8233, 2021.

[41] X. L. Otero, T. O. Ferreira, M. A. Huerta-Díaz, C. S. Partiti, V. Souza, P. Vidal-Torrado, and F. Macías, "Geochemistry of iron and manganese in soils and sediments of a mangrove system, island of pai matos (cananeia - sp, brazil)," *Geoderma*, vol. 148, pp. 318–335, 1 2009.

[42] H. Golterman, W. Stumm, and J. Morgan, "Aquatic chemistry. an introduction emphasizing chemical equilibria in natural waters.," *Journal of Ecology*, vol. 70, no. 3, p. 924, 1982.

[43] K. PARTITION COEFFICIENT, "Understanding variation in partition coefficient, kd, values," 2004.

[44] J. B. Dixon and G. N. White, "Manganese oxides," *Soil mineralogy with environmental applications*, vol. 7, pp. 367–388, 2002.

[45] A. Das, L. Sukla, N. Pradhan, and S. Nayak, "Manganese biomining: a review," *Bioresource technology*, vol. 102, no. 16, pp. 7381–7387, 2011.

[46] A. A. Ismail, E. A. Ali, I. A. Ibrahim, and M. S. Ahmed, "A comparative study on acid leaching of low grade manganese ore using some industrial wastes as reductants," *The Canadian journal of chemical engineering*, vol. 82, no. 6, pp. 1296–1300, 2004.

[47] G. Duan, G. Shao, Z. Tang, H. Chen, B. Wang, Z. Tang, Y. Yang, Y. Liu, and F.-J. Zhao, "Genotypic and environmental variations in grain cadmium and arsenic concentrations among a panel of high yielding rice cultivars," *Rice*, vol. 10, pp. 1–13, 2017.

[48] P. Hinsinger, C. Plassard, C. Tang, and B. Jaillard, "Origins of root-mediated ph changes in the rhizosphere and their responses to environmental constraints: a review," *Plant and soil*, vol. 248, pp. 43–59, 2003.

[49] Y.-T. Chen, Y. Wang, and K.-C. Yeh, "Role of root exudates in metal acquisition and tolerance," *Current opinion in plant biology*, vol. 39, pp. 66–72, 2017.

[50] A. Schwab, D. Zhu, and M. Banks, "Influence of organic acids on the transport of heavy metals in soil," *Chemosphere*, vol. 72, no. 6, pp. 986–994, 2008.

[51] Y. Yin, X. Luo, X. Guan, J. Zhao, Y. Tan, X. Shi, M. Luo, and X. Han, "Arsenic release from soil induced by microorganisms and environmental factors," *International Journal of Environmental Research and Public Health*, vol. 19, no. 8, p. 4512, 2022.

[52] S. Zhao, J. Wang, S. Feng, Z. Xiao, and C. Chen, "Effects of ecohydrological interfaces on migrations and transformations of pollutants: A critical review," *Science of the Total Environment*, vol. 804, p. 150140, 2022.

[53] X. F. Zhu, C. Zheng, Y. T. Hu, T. Jiang, Y. Liu, N. Y. Dong, J. L. Yang, and S. J. Zheng, "Cadmium-induced oxalate secretion from root apex is associated with cadmium exclusion and resistance in lycopersicon esulentum," *Plant, cell & environment*, vol. 34, no. 7, pp. 1055–1064, 2011.

[54] M. Zhao, J. Zhao, J. Yuan, L. Hale, T. Wen, Q. Huang, J. M. Vivanco, J. Zhou, G. A. Kowalchuk, and Q. Shen, "Root exudates drive soil-microbe-nutrient feedbacks in response to plant growth," *Plant, Cell & Environment*, vol. 44, no. 2, pp. 613–628, 2021.

[55] S. O. Lee, T. Tran, B. H. Jung, S. J. Kim, and M. J. Kim, "Dissolution of iron oxide using oxalic acid," *Hydrometallurgy*, vol. 87, no. 3-4, pp. 91–99, 2007.

[56] L. Liu, Y.-P. Yang, G.-L. Duan, J. Wang, X.-J. Tang, and Y.-G. Zhu, "The chemical-microbial release and transformation of arsenic induced by citric acid in paddy soil," *Journal of Hazardous Materials*, vol. 421, p. 126731, 2022.

[57] O. O. Onireti, C. Lin, and J. Qin, "Combined effects of low-molecular-weight organic acids on mobilization of arsenic and lead from multi-contaminated soils," *Chemosphere*, vol. 170, pp. 161–168, 2017.

[58] H. Shen, X. Yan, M. Zhao, S. Zheng, and X. Wang, "Exudation of organic acids in common bean as related to mobilization of aluminum-and iron-bound phosphates," *Environmental and Experimental Botany*, vol. 48, no. 1, pp. 1–9, 2002.

[59] D. Jones and A. Edwards, "Influence of sorption on the biological utilization of two simple carbon substrates," *Soil Biology and Biochemistry*, vol. 30, no. 14, pp. 1895–1902, 1998.

[60] P. A. Van Hees, D. L. Jones, G. Jentschke, and D. L. Godbold, "Organic acid concentrations in soil solution: effects of young coniferous trees and ectomycorrhizal fungi," *Soil Biology and Biochemistry*, vol. 37, no. 4, pp. 771–776, 2005.

[61] L. Zou, O. Jiang, S. Zhang, G. Duan, W. Gustave, X. An, and X. Tang, "Effects of citric acid on arsenic transformation and microbial communities in different paddy soils," *Environmental Research*, p. 118421, 2024.

[62] Y. Hu, J.-H. Li, Y.-G. Zhu, Y.-Z. Huang, H.-Q. Hu, and P. Christie, "Sequestration of as by iron plaque on the roots of three rice (oryza sativa l.) cultivars in a low-p

soil with or without p fertilizer," *Environmental Geochemistry and Health*, vol. 27, pp. 169–176, 2005.

[63] N. Khan, B. Seshadri, N. Bolan, C. Saint, M. Kirkham, S. Chowdhury, N. Yamaguchi, D. Lee, G. Li, A. Kunhikrishnan, *et al.*, "Root iron plaque on wetland plants as a dynamic pool of nutrients and contaminants," *Advances in agronomy*, vol. 138, pp. 1–96, 2016.

[64] R. Joshi and P. Kumar, "Lysigenous aerenchyma formation involves non-apoptotic programmed cell death in rice (oryza sativa l.) roots," *Physiology and Molecular Biology of Plants*, vol. 18, pp. 1–9, 2012.

[65] C. Wu, Z. Ye, W. Shu, Y. Zhu, and M. Wong, "Arsenic accumulation and speciation in rice are affected by root aeration and variation of genotypes," *Journal of Experimental Botany*, vol. 62, no. 8, pp. 2889–2898, 2011.

[66] H. Zheng, Z. Fu, J. Zhong, and W. Long, "Low methane emission in rice cultivars with high radial oxygen loss," *Plant and soil*, vol. 431, pp. 119–128, 2018.

[67] P. Zandi, J. Yang, A. Darma, E. Bloem, X. Xia, Y. Wang, Q. Li, and E. Schnug, "Iron plaque formation, characteristics, and its role as a barrier and/or facilitator to heavy metal uptake in hydrophyte rice (oryza sativa l.)," 3 2022.

[68] A. L. Seyfferth, S. M. Webb, J. C. Andrews, and S. Fendorf, "Arsenic localization, speciation, and co-occurrence with iron on rice (oryza sativa l.) roots having variable fe coatings," *Environmental science & technology*, vol. 44, no. 21, pp. 8108–8113, 2010.

[69] R. D. Tripathi, P. Tripathi, S. Dwivedi, A. Kumar, A. Mishra, P. S. Chauhan, G. J. Norton, and C. S. Nautiyal, "Roles for root iron plaque in sequestration and uptake of heavy metals and metalloids in aquatic and wetland plants," *Metallomics*, vol. 6, no. 10, pp. 1789–1800, 2014.

[70] J. Liu, X. Leng, M. Wang, Z. Zhu, and Q. Dai, "Iron plaque formation on roots of different rice cultivars and the relation with lead uptake," *Ecotoxicology and environmental safety*, vol. 74, no. 5, pp. 1304–1309, 2011.

[71] C. Zhang, Y. Ge, H. Yao, X. Chen, and M. Hu, "Iron oxidation-reduction and its impacts on cadmium bioavailability in paddy soils: a review," *Frontiers of Environmental Science & Engineering*, vol. 6, pp. 509–517, 2012.

[72] W. Liu, Y. Zhu, Y. Hu, P. Williams, A. Gault, A. A. Meharg, J. Charnock, and F. Smith, "Arsenic sequestration in iron plaque, its accumulation and speciation in mature rice plants (oryza sativa l.)," *Environmental science & technology*, vol. 40, no. 18, pp. 5730–5736, 2006.

[73] X. Xia, J. Yang, Y. Yan, J. Wang, Y. Hu, and X. Zeng, "Molecular sorption mechanisms of cr (iii) to organo-ferrihydrite coprecipitates using synchrotron-based exafs and stxm techniques," *Environmental Science & Technology*, vol. 54, no. 20, pp. 12989–12997, 2020.

[74] P. Zandi, J. Yang, X. Xia, Y. Tian, Q. Li, K. Możdżeń, B. Barabasz-Krasny, and Y. Wang, "Do sulfur addition and rhizoplane iron plaque affect chromium uptake by rice (oryza sativa l.) seedlings in solution culture?," *Journal of Hazardous Materials*, vol. 388, 4 2020.

[75] Z.-Y. Hu, Y.-G. Zhu, M. Li, L.-G. Zhang, Z.-H. Cao, and F. A. Smith, "Sulfur (s)-induced enhancement of iron plaque formation in the rhizosphere reduces arsenic accumulation in rice (oryza sativa l.) seedlings," *Environmental Pollution*, vol. 147, no. 2, pp. 387–393, 2007.

[76] K. K. Christensen and K. Sand-Jensen, "Precipitated iron and manganese plaques restrict root uptake of phosphorus in lobelia dortmanna," *Canadian Journal of Botany*, vol. 76, no. 12, pp. 2158–2163, 1998.

[77] D. L. Sparks, A. L. Page, P. A. Helmke, and R. H. Loeppert, *Methods of soil analysis, part 3: Chemical methods*, vol. 14. John Wiley & Sons, 2020.

[78] P. Zandi, J. Yang, X. Xia, B. Barabasz-Krasny, K. Możdżeń, J. Puła, E. Bloem, Y. Wang, S. Hussain, S. M. Hashemi, *et al.*, "Sulphur nutrition and iron plaque formation on roots of rice seedlings and their consequences for immobilisation and uptake of chromium in solution culture," *Plant and Soil*, vol. 462, pp. 365–388, 2021.

[79] D. 19682-2:2007-11, "Soil quality - field tests - part 2: Determination of soil texture," 11 2007.

[80] J. D. Cline, "Spectrophotometric determination of hydrogen sulfide in natural waters 1," *Limnology and Oceanography*, vol. 14, no. 3, pp. 454–458, 1969.

[81] G. J. Taylor and A. Crowder, "Use of the dcb technique for extraction of hydrous iron oxides from roots of wetland plants," *American Journal of Botany*, vol. 70, no. 8, pp. 1254–1257, 1983.

[82] P. T. Armbruster DA, "Limit of blank, limit of detection and limit of quantitation.," *The Clinical biochemist*, vol. 29, pp. 49–52, 2008.

[83] I. H. T. Guideline *et al.*, "Validation of analytical procedures: text and methodology," *Q2 (R1)*, vol. 1, no. 20, p. 05, 2005.

[84] F. I. Zyserman, L. B. Monachesi, and L. Jouniaux, "Dependence of shear wave seismoelectrics on soil textures: a numerical study in the vadose zone," *Geophysical Journal International*, vol. 208, no. 2, pp. 918–935, 2017.

[85] S. Schwarz and A. Freudenschuß, *Referenzwerte für Schwermetalle in Oberböden: Auswertungen aus dem österreichweiten Bodeninformationssystem BORIS*. Umweltbundesamt, 2004.

[86] K. Zhang, Y. Yang, W. Chi, G. Chen, Y. Du, S. Hu, F. Li, and T. Liu, "Chromium transformation driven by iron redox cycling in basalt-derived paddy soil with high

geological background values," *Journal of Environmental Sciences*, vol. 125, pp. 470–479, 2023.

[87] H. G. J. van Rensburg, A. S. Claassens, and D. J. Beukes, "Relationships between soil buffer capacity and selected soil properties in a resource-poor farming area in the mpumalanga province of south africa," *South African Journal of Plant and Soil*, vol. 26, pp. 237–243, 2009.

[88] R. Aitken, P. Moody, and P. McKinley, "Lime requirement of acidic queensland soils. i. relationships between soil properties and ph buffer capacity," *Soil Research*, vol. 28, no. 5, pp. 695–701, 1990.

[89] Z.-J. Chen, Y.-H. Tian, Y. Zhang, B.-R. Song, H.-C. Li, and Z.-H. Chen, "Effects of root organic exudates on rhizosphere microbes and nutrient removal in the constructed wetlands," *Ecological Engineering*, vol. 92, pp. 243–250, 2016.

[90] I. Reijonen and H. Hartikainen, "Oxidation mechanisms and chemical bioavailability of chromium in agricultural soil–ph as the master variable," *Applied geochemistry*, vol. 74, pp. 84–93, 2016.

[91] E. Nakayama, T. Kuwamoto, S. Tsurubo, and T. Fujinaga, "Chemical speciation of chromium in sea water: Part 2. effects of manganese oxides and reducible organic materials on the redox processes of chromium," *Analytica Chimica Acta*, vol. 130, no. 2, pp. 401–404, 1981.

[92] C. Pan, H. Liu, J. G. Catalano, Z. Wang, A. Qian, and D. E. Giammar, "Understanding the roles of dissolution and diffusion in cr (oh) 3 oxidation by δ-mno2," *ACS Earth and Space Chemistry*, vol. 3, no. 3, pp. 357–365, 2019.

[93] H. B. Bradl, "Adsorption of heavy metal ions on soils and soils constituents," *Journal of colloid and interface science*, vol. 277, no. 1, pp. 1–18, 2004.

[94] J. Pan, J. Jiang, and R. Xu, "Adsorption of cr (iii) from acidic solutions by crop straw derived biochars," *Journal of environmental sciences*, vol. 25, no. 10, pp. 1957–1965, 2013.

[95] M. Stewart, P. M. Jardine, C. Brandt, M. Barnett, S. Fendorf, L. McKay, T. Mehlhorn, and K. Paul, "Effects of contaminant concentration, aging, and soil properties on the bioaccessibility of cr (iii) and cr (vi) in soil," *Soil and Sediment Contamination*, vol. 12, no. 1, pp. 1–21, 2003.

[96] J. M. Zachara, D. C. Girvin, R. L. Schmidt, and C. T. Resch, "Chromate adsorption on amorphous iron oxyhydroxide in the presence of major groundwater ions," *Environmental science & technology*, vol. 21, no. 6, pp. 589–594, 1987.

[97] J. Bosch, K. Heister, T. Hofmann, and R. U. Meckenstock, "Nanosized iron oxide colloids strongly enhance microbial iron reduction," *Applied and environmental microbiology*, vol. 76, no. 1, pp. 184–189, 2010.

[98] D. Menezes-Blackburn, C. Paredes, H. Zhang, C. D. Giles, T. Darch, M. Stutter, T. S. George, C. Shand, D. Lumsdon, P. Cooper, *et al.*, "Organic acids regulation of chemical–microbial phosphorus transformations in soils," *Environmental Science & Technology*, vol. 50, no. 21, pp. 11521–11531, 2016.

[99] S. Srivastava, S. Prakash, and M. Srivastava, "Chromium mobilization and plant availability—the impact of organic complexing ligands," *Plant and soil*, vol. 212, no. 2, pp. 201–206, 1999.

[100] D. Rai and J. Zachara, "Chemical attenuation rates, coefficients, and constants in leachate migration. volume 1. a critical review. final report," *U.S. Department of Energy Office of Scientific and Technical Information*, 1984.

[101] J. D. Hem, "Reactions of metal ions at surfaces of hydrous iron oxide," *Geochimica et Cosmochimica Acta*, vol. 41, no. 4, pp. 527–538, 1977.

[102] R. Xu, A. Zhao, and G. Ji, "Effect of low-molecular-weight organic anions on surface charge of variable charge soils," *Journal of Colloid and Interface Science*, vol. 264, no. 2, pp. 322–326, 2003.

[103] A. Signes-Pastor, F. Burló, K. Mitra, and A. Carbonell-Barrachina, "Arsenic biogeochemistry as affected by phosphorus fertilizer addition, redox potential and ph in a west bengal (india) soil," *Geoderma*, vol. 137, no. 3-4, pp. 504–510, 2007.

[104] A. Corsini, L. Cavalca, P. Zaccheo, L. Crippa, and V. Andreoni, "Influence of microorganisms on arsenic mobilization and speciation in a submerged contaminated soil: Effects of citrate," *Applied soil ecology*, vol. 49, pp. 99–106, 2011.

[105] K. Mei, J. Liu, J. Fan, X. Guo, J. Wu, Y. Zhou, H. Lu, and C. Yan, "Low-level arsenite boosts rhizospheric exudation of low-molecular-weight organic acids from mangrove seedlings (avicennia marina): Arsenic phytoextraction, removal, and detoxification," *Science of the Total Environment*, vol. 775, p. 145685, 2021.

[106] H. Geng, F. Wang, C. Yan, Z. Tian, H. Chen, B. Zhou, R. Yuan, and J. Yao, "Leaching behavior of metals from iron tailings under varying ph and low-molecular-weight organic acids," *Journal of hazardous materials*, vol. 383, p. 121136, 2020.

[107] A. Crowder and D. Coltman, "Formation of manganese oxide plaque on rice roots in solution culture under varying ph and manganese (mn2+) concentration conditions," *Journal of Plant Nutrition*, vol. 16, no. 4, pp. 589–599, 1993.

[108] I. A. Mendelssohn *et al.*, *Factors controlling the formation of oxidized root channels: A review and annotated bibliography*. US Army Engineer Waterways Experiment Station, 1993.

[109] C. Boone, J. Bristow, and G. VanLoon, "The relative efficiency of ionic iron (iii) and iron (ii) utilization by the rice plant," *Journal of plant nutrition*, vol. 6, no. 3, pp. 201–218, 1983.

[110] G. J. Taylor, A. Crowder, and R. Rodden, "Formation and morphology of an iron plaque on the roots of typha latifolia l. grown in solution culture," *American Journal of Botany*, vol. 71, no. 5, pp. 666–675, 1984.

[111] I. A. Mendelssohn, B. A. Kleiss, and J. S. Wakeley, "Factors controlling the formation of oxidized root channels: a review," *Wetlands*, vol. 15, pp. 37–46, 1995.

[112] J.-L. Fan, Z.-Y. Hu, N. Ziadi, X. Xia, *et al.*, "Excessive sulfur supply reduces cadmium accumulation in brown rice (oryza sativa l.)," *Environmental Pollution*, vol. 158, no. 2, pp. 409–415, 2010.

[113] F. Zeng, S. Chen, Y. Miao, F. Wu, and G. Zhang, "Changes of organic acid exudation and rhizosphere ph in rice plants under chromium stress," *Environmental Pollution*, vol. 155, no. 2, pp. 284–289, 2008.

[114] A. K. Shanker, M. Djanaguiraman, R. Sudhagar, C. Chandrashekar, and G. Pathmanabhan, "Differential antioxidative response of ascorbate glutathione pathway enzymes and metabolites to chromium speciation stress in green gram (vigna radiata (l.) r. wilczek. cv co 4) roots," *Plant Science*, vol. 166, no. 4, pp. 1035–1043, 2004.

[115] J. Monhemius, "The iron elephant: A brief history of hydrometallurgists' struggles with element no. 26," *CIM Journal*, vol. 8, pp. 197–206, 11 2017.

A. Appendix

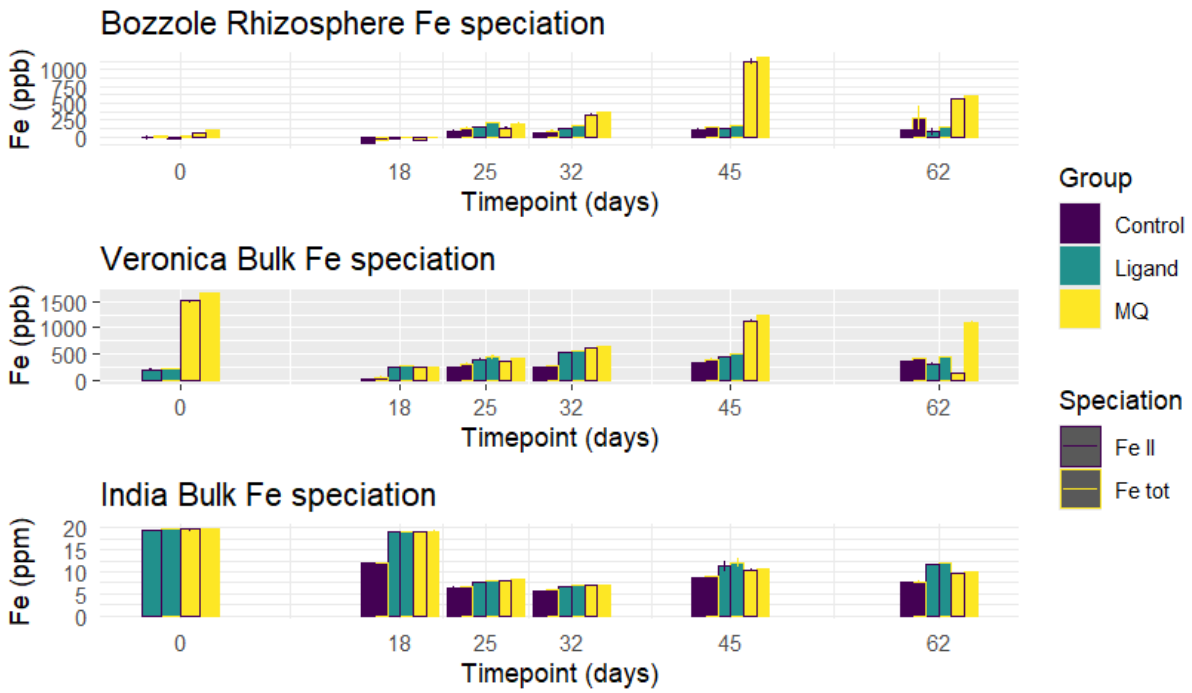


Figure A.1: Fe(II) and Fe(III) concentrations in the pore water samples of the Bozzole, Veronica and Indian soil

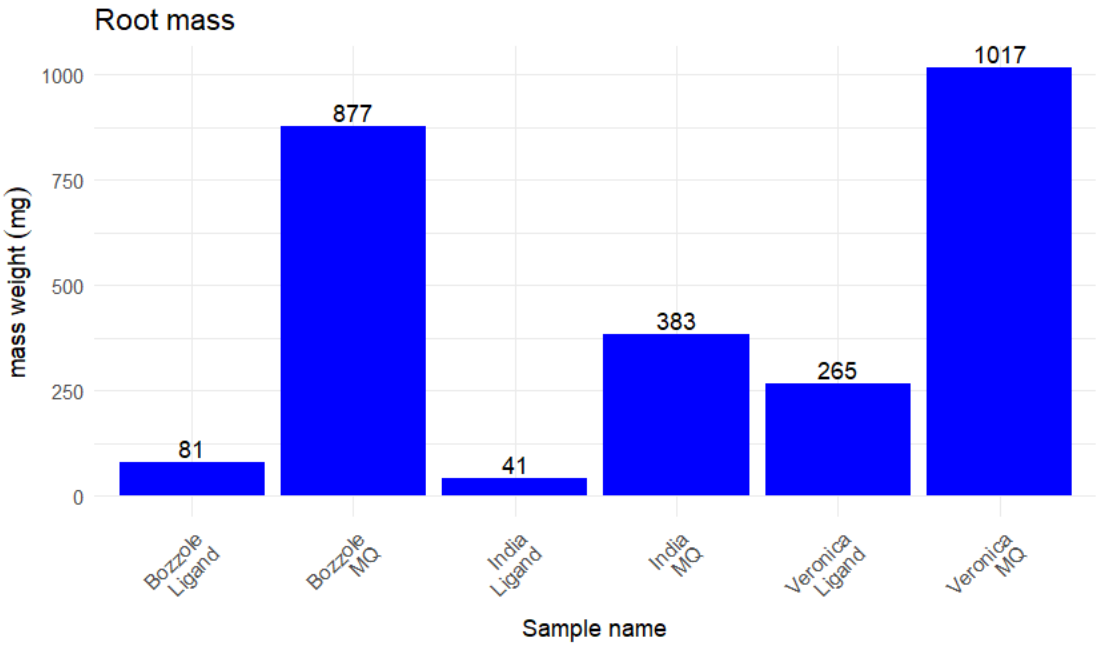


Figure A.2: Root mass of the harvested rice plants (mg)

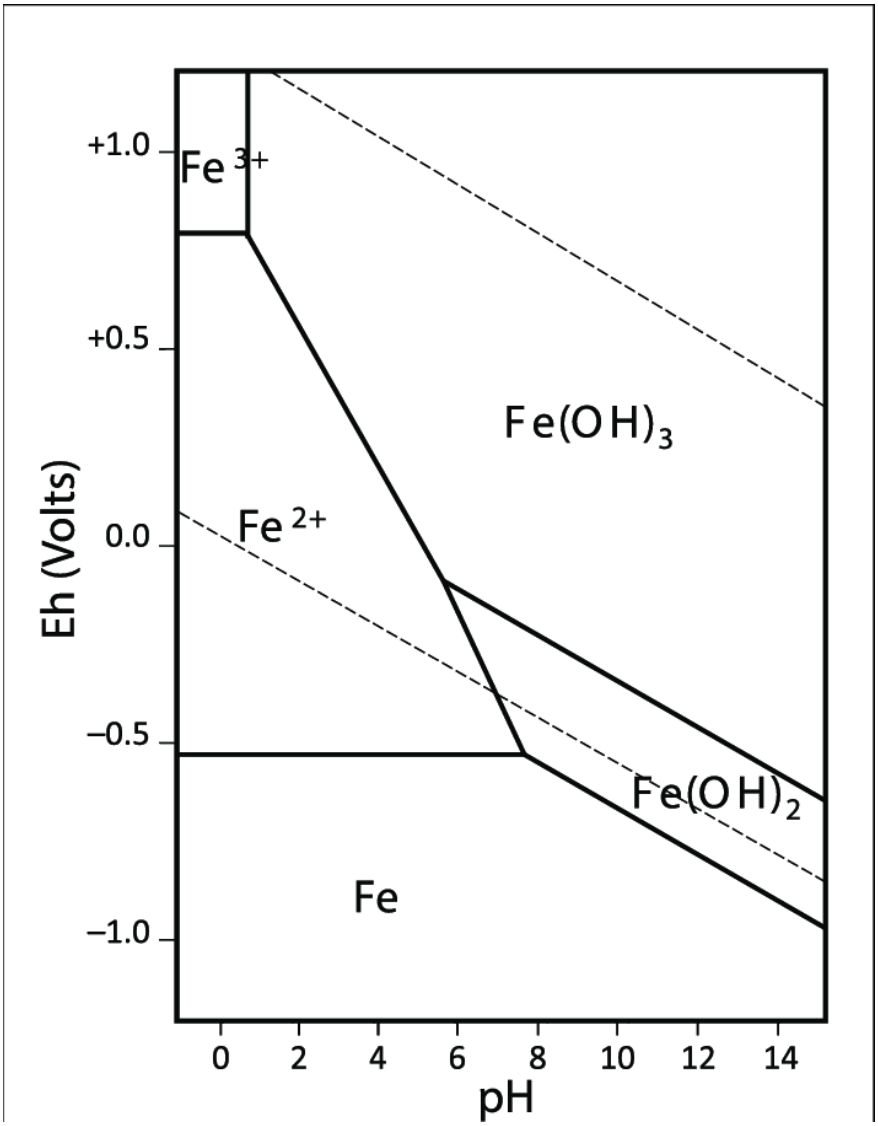
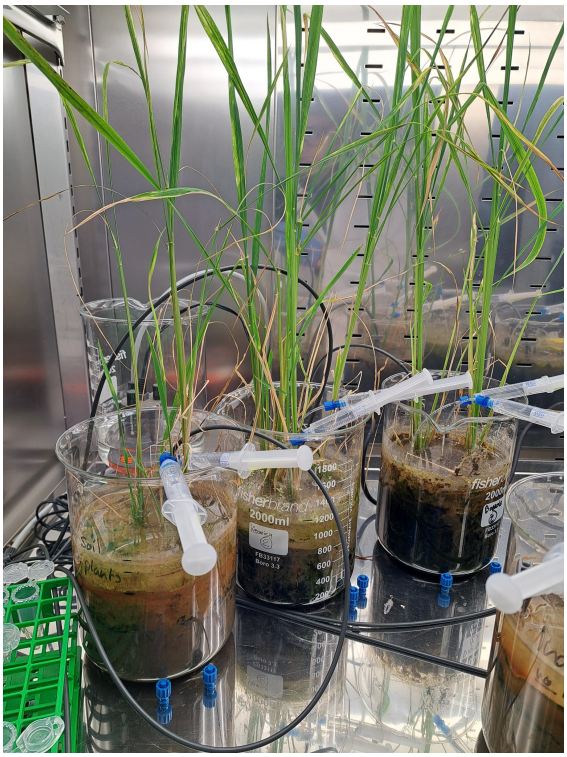
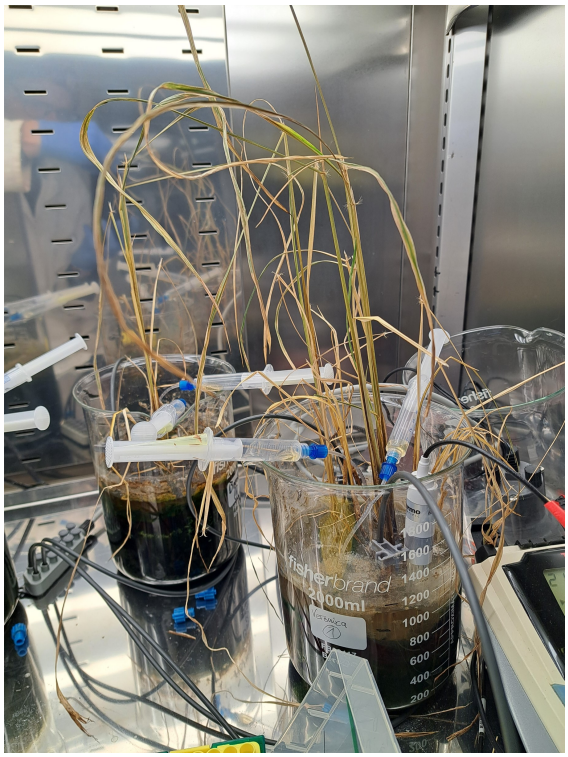


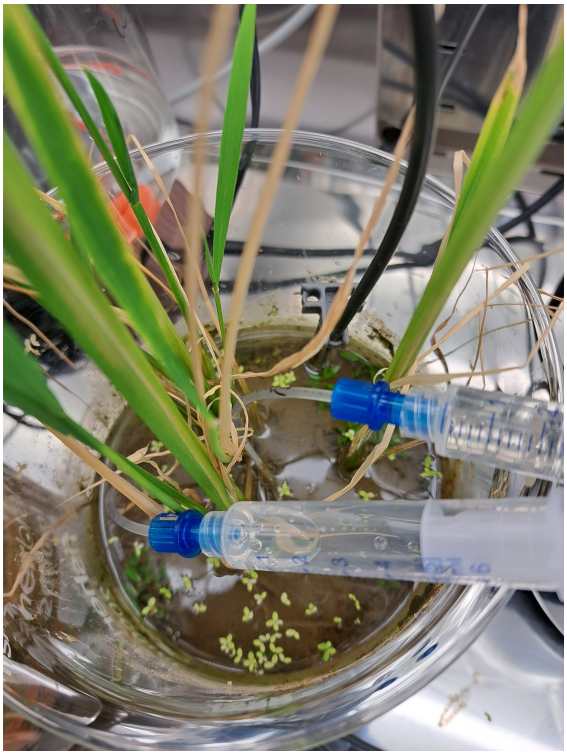
Figure A.3: Fe Eh-pH diagram [115]



(a) MQ setup



(b) Ligand setup



(c) MQ setup top view

(d) Ligand setup top view

Figure A.4: Rice growing setup, with the MQ treatments on the left side and the ligand treatments on the right side

(a) India MQ root

(b) India ligand root

(c) Veronica MQ root

(d) Veronica ligand root

Figure A.5: Roots of the Indian MQ and ligand treatment (top) and of the Veronica MQ and ligand treatment (bottom)

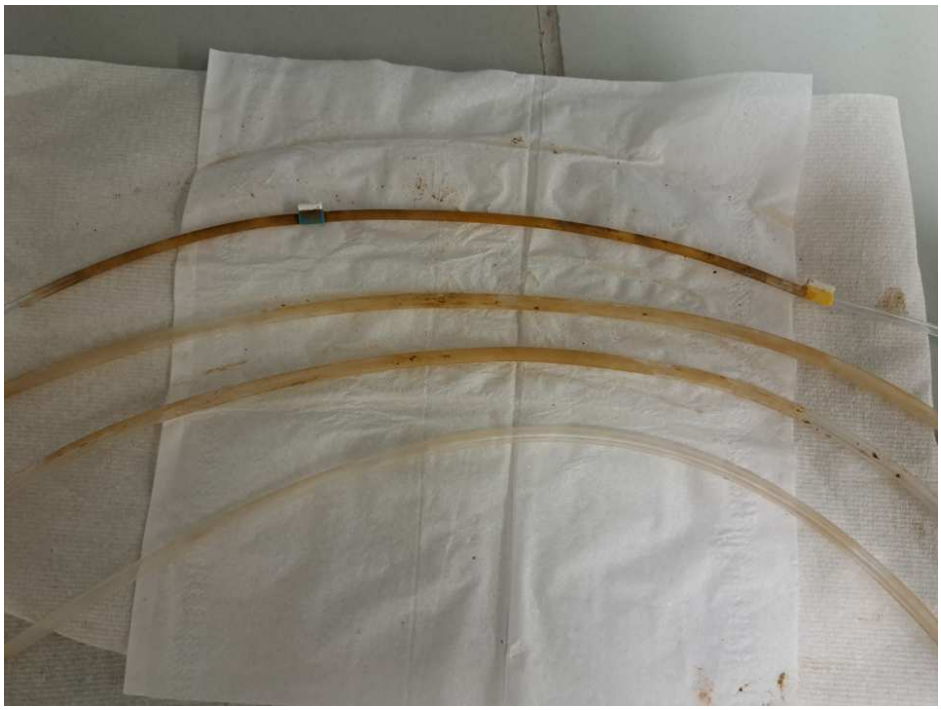


Figure A.6: Artificial roots after being submerged in anoxic soil for 14, 9, 4 and 2 days (from top to bottom) with the tube on the top being peristaltic, the others are silicon tubes, with visible reddish iron plaque

Table A.1: Total P, Cr, Mn, Zn and As concentrations of aqua regia extraction from the three soils

Metal(ppm)	India (ppm)	Bozzole (ppm)	Veronica (ppm)
P	0.4495637	0.4794323	0.7475762
Mn	0.2625957	0.0959287	0.0919595
Cr	0.4986721	0.0147028	0.0180663
As	0.0369120	0.0123324	0.0169433
Zn	0.0298240	0.0227407	0.0352511

Table A.2: Iron content in the pore water of the treatment groups over time, in ppb for Italian soils and ppm for the Indian soil

Timepoint	Group	Bozzole Fe (ppb)	Veronica Fe (ppb)	India Fe (ppm)
0	Ligand	519.7	145.6	264.2
18	Ligand	1009.8	139.9	180.4
25	Ligand	2088.1	170.2	159.5
32	Ligand	1316.1	180.1	126.6
39	Ligand	3235.4	134.2	143.4
45	Ligand	1431.8	197.9	129.5
53	Ligand	1677.2	162.8	126.3
62	Ligand	916.0	92.8	57.1
68	Ligand	1019.7	171.5	117.6
73	Ligand	5007.4	216.7	105
80	Ligand	2379.4	106.4	108.7
88	Ligand	1187.7	129.1	105.4
94	Ligand	1010.3	298.2	not measured
0	Control	339.6	107.6	259.6
18	Control	541.2	142.4	125.9
25	Control	524.3	143.2	168.1
32	Control	524.6	156.6	142.4
39	Control	526.9	133.3	128.6
45	Control	1053.2	579.3	116.7
53	Control	789.5	305.9	106.2
62	Control	924.6	114.0	71
68	Control	1139.3	119.1	105.8
73	Control	968.1	281.9	115.9
80	Control	976.7	201.5	81
88	Control	763.6	273.5	74.4
94	Control	461.1	278.7	64.1
0	MQ	2081.8	251.0	275.2
18	MQ	1175.7	150.3	184.8
25	MQ	558.1	189.3	140
32	MQ	851.3	452.7	158.3
39	MQ	1871.1	1731.4	91.9
45	MQ	1915.6	1572.7	88.5
53	MQ	1210.0	944.1	79.9
62	MQ	2808.5	1094.0	44.7
68	MQ	2121.1	895.7	74
73	MQ	3956.0	3024.5	63.7
80	MQ	5508.4	3820.4	54.3
88	MQ	9281.9	2778.8	39.1
94	MQ	15254.6	3143.7	38.3

Table A.3: Manganese content in the pore water of the treatment groups over time, in ppb for Italian soils and ppm for the Indian soil

Timepoint	Group	Bozzole Mn (ppb)	Veronica Mn (ppb)	India Mn (ppm)
0	Ligand	65.8	553.5	18.3
18	Ligand	119.8	388.0	12.9
25	Ligand	203.9	339.3	11.5
32	Ligand	167.1	299.0	9.3
39	Ligand	277.8	322.2	10.6
45	Ligand	146.2	317.6	not measured
53	Ligand	132.8	258.0	9.8
62	Ligand	119.4	150.6	10
68	Ligand	116.9	229.0	8
73	Ligand	218.2	254.8	9.7
80	Ligand	139.9	282.8	8.6
88	Ligand	81.1	251.0	8.6
94	Ligand	81.7	247.0	9.5
0	Control	52.7	384.3	19.4
18	Control	99.5	410.2	11.5
25	Control	69.2	392.0	13
32	Control	80.4	312.6	11.3
39	Control	52.9	333.9	8.9
45	Control	83.3	369.5	9.3
53	Control	58.8	302.4	10.1
62	Control	83.7	279.8	6.9
68	Control	81.3	341.3	7.4
73	Control	73.5	316.6	8.8
80	Control	74.3	267.1	6.8
88	Control	46.7	219.4	6.7
94	Control	42.9	200.9	4.6
0	MQ	192.1	538.1	21.7
18	MQ	183.1	524.3	14.9
25	MQ	149.8	512.8	9.4
32	MQ	176.6	712.4	9.9
39	MQ	246.5	978.1	7.1
45	MQ	290.5	899.6	7.1
53	MQ	220.9	814.7	7.4
62	MQ	359.0	920.4	6.2
68	MQ	307.5	832.3	6.7
73	MQ	342.3	774.6	6.5
80	MQ	360.0	771.3	5.4
88	MQ	425.6	660.9	3.9
94	MQ	421.6	693.3	3.5

Table A.4: Chromium content in ppb in the pore water of the treatment groups over time

Timepoint	Group	Bozzole Cr (ppb)	Veronica Cr (ppb)	India Cr (ppb)
0	Ligand	2.1	8.7	2.4
18	Ligand	3.8	10.9	2.7
25	Ligand	3.6	14.9	1.9
32	Ligand	2.7	6.7	1.5
39	Ligand	6.0	9.9	2
45	Ligand	12.7	23.6	1.4
53	Ligand	7.3	11.4	1.8
62	Ligand	10.7	15.9	1.4
68	Ligand	11.3	15.0	1.8
73	Ligand	16.3	31.9	2.8
80	Ligand	16.9	34.6	3.6
88	Ligand	22.6	39.6	1.7
94	Ligand	25.2	38.7	not measured
0	Control	2.9	14.8	3.7
18	Control	7.2	11.3	2.2
25	Control	2.8	13.9	2.2
32	Control	4.7	13.7	1.1
39	Control	3.6	13.2	1.3
45	Control	3.5	25.6	1.3
53	Control	3.1	12.2	1.2
62	Control	4.8	15.3	1.7
68	Control	5.2	16.1	2.3
73	Control	5.8	15.2	1.2
80	Control	5.2	14.0	2.3
88	Control	4.2	9.3	0.8
94	Control	4.4	6.5	1.8
0	MQ	3.4	3.4	3
18	MQ	2.6	3.6	1.8
25	MQ	1.9	5.3	1.7
32	MQ	3.9	3.9	1.7
39	MQ	2.2	4.0	0.6
45	MQ	3.6	5.2	1.4
53	MQ	1.8	1.8	1.7
62	MQ	3.7	9.2	2.3
68	MQ	3.9	4.2	5.9
73	MQ	4.2	5.3	1.5
80	MQ	3.4	3.7	2
88	MQ	5.2	4.9	1.8
94	MQ	4.8	3.7	0.8

Table A.5: Metal content in IP

Sample	**Root weight (mg)**	**Plaque weight (mg)**	**Cr [ppb]**	**Ni [ppb]**	**As [ppb]**	**Al [ppb]**	**Mn [ppb]**	**Fe [ppb]**
Veronica MQ	1178.18	161.57	10.55	41.65	161.79	1806.30	44.15	28701.33
Veronica Ligand	265.26	67.38	7.14	19.32	53.79	0.00	3.29	3333.87
Bozzole MQ	877.33	70.65	9.94	18.96	474.82	0.00	34.76	13222.70
Bozzole Ligand	80.70	11.20	2.95	2.49	9.74	0.00	28.18	8640.49
India MQ	382.83	96.85	19.21	2.96	69.46	1392.37	1048.12	164378.34
India Ligand	41.38	10.67	4.44	5.39	10.16	0.00	200.09	23291.22
Veronica MQ Duplicate	147.62	37.98	5.30	8.32	70.54	3866.29	31.20	44691.67
Veronica Ligand Duplicate	16.64	0.14	2.40	5.22	4.57	3073.12	23.41	4104.55
Bozzole MQ Duplicate	192.81	62.40	4.07	4.69	43.41	2676.76	503.12	80002.35
Bozzole Ligand Duplicate	9.28	3.06	2.36	3.79	1.71	2081.81	27.30	4214.21
2d sili.-tube MQ	-	-	2.59	0.00	1.27	0.00	5.17	285.30
4d sili.-tube MQ	-	-	4.08	0.00	1.27	0.00	19.26	777.36
9d sili.-tube MQ	-	-	1.81	0.00	0.85	0.00	18.79	903.62
24d sili.-tube MQ	-	-	3.37	0.00	1.27	0.00	25.83	924.33
14d peri.-tube MQ	-	-	4.01	0.30	3.81	0.00	76.09	16193.56
15d peri.-tube MQ	-	-	7.35	0.65	11.86	0.00	146.54	35050.76
17d peri.-tube MQ	-	-	10.76	0.00	5.08	564.45	128.70	30759.76
7d peri.-tube CA	-	-	2.59	0.00	3.81	0.00	23.01	3303.68
26d peri.-tube CA	-	-	3.83	0.00	5.08	0.00	47.44	7601.72

Table A.6: Concentration of metals in plant segments

Sample	**Al**	**Cr**	**Fe**	**Mn**	**Ni**
Veronica ligand leave	603.67	-1.14	398.86	385.32	0.00
Veronica ligand leave	633.37	-0.45	405.25	235.04	0.00
Veronica ligand leave	672.60	-0.78	465.10	500.89	0.00
Veronica MQ leave	343.63	0.00	280.45	176.06	0.00
Veronica MQ leave	514.69	1.30	433.29	195.02	0.00
Veronica MQ leave	331.81	0.43	261.89	122.78	0.00
Veronica MQ seed	295.87	-3.54	231.10	233.86	0.00
Veronica MQ seed	209.45	-1.47	232.84	238.72	0.00
Veronica ligand root duplicate	4888.16	-7.89	2268.42	-55.26	0.00
Bozzole ligand root	1439.29	1.79	953.08	3.08	0.00
Bozzole MQ root	1852.76	1.02	3224.27	8.43	0.00
Bozzole MQ root	2036.00	1.58	3729.01	13.54	0.00
Bozzole MQ leave	210.79	-2.58	165.05	686.32	0.00
Bozzole MQ leave	274.15	-1.28	153.84	727.27	0.00
Bozzole MQ leave	207.47	-1.93	239.52	918.07	0.00
Bozzole MQ seed	193.63	-1.39	70.50	317.59	0.00
Bozzole MQ seed	296.42	-1.04	94.63	292.24	0.00
Bozzole MQ seed	1.98	-2.09	-16.30	19.93	0.00
Bozzole MQ root duplicate	870.22	-0.77	1726.23	13.12	0.00
Bozzole MQ root duplicate	1714.20	-6.17	1890.12	-16.05	0.00
Bozzole MQ leave duplicate	309.11	-1.52	169.62	1616.46	0.00
Bozzole MQ leave duplicate	194.37	-1.61	142.07	1614.02	0.00
Bozzole MQ leave duplicate	290.50	-1.61	163.60	1876.90	0.00
India ligand leave	2021.48	5.42	1873.47	1651.62	0.00
India MQ root	14134.64	41.28	124751.43	743.10	0.01
India MQ root	15605.56	44.92	145590.95	856.83	0.01
India MQ root	13506.34	39.44	133832.92	775.53	0.01
India MQ leave	1442.56	4.13	1304.96	1054.96	0.00