Zinc deficiency and iron toxicity in rice soils of Office du Niger, Mali

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SUMMARY

Most countries in West Africa suffers from problems such as erosion, salinisation and spreading deserts. Mali is one of the poorest countries in this area with a surface that mostly consists of desert and semi-desert. Agriculture is the most important occupation for the people but is unfortunately endangered by poor water supply and over exploited soils. The river Niger that flows through the country is an important source for irrigation. By building the Markala dam and creating the Office du Niger, one of the largest irrigation schemes in West Africa, it has been possible to raise the groundwater level and increase the irrigation capacity in the area. This has ameliorated the conditions for rice production resulting in increased yields.

Iron and zinc are both essential elements for plants and humans. Zinc deficiency is a widespread problem in Sub-Saharan Africa. More than half of the soils in Mali are affected and approximately 80% of the population is considered to have an insufficient zinc intake. Zinc is the most critical micronutrient to rice growth and a zinc deficiency in rice cultivation causes reduced yields, some times to as much as 50%.

Iron toxicity is also a common problem in rice cultivation and zinc deficiency in rice is often linked to this phenomenon. Dissolved ferrous iron is taken up by the plant and accumulated in the leaves, especially at low pH and low oxygen levels. If the concentration of ferrous iron is high in the root zone iron plaques are formed, preventing the plant from taking up other nutrients such as Zn.

The aim of this study was to investigate how the zinc uptake in lowland rice is affected by different nutrient managements and intermittent irrigation. The project was carried out at Centre Régional de Recherche Agronomique (CRRA) in Sotuba, Mali. A pot experiment was set up with soils from Office du Niger using 6 different treatments and 3 replicates. Two differing soils were collected; one from the zone of Macina, rich in iron, and one from Kouroumari with less iron content. Both were deficient in zinc. The rice plants were grown for 21 days and then a visual observation were made. The soil and plant residues were analysed on zinc and iron content by extraction of DTPA and measuring the concentrations with AAS.

There were visual signs of zinc deficiency and iron toxicity in most plants. The plants being treated with intermittent irrigation and addition of extra nutrients were the strongest and most beautiful ones. It is difficult to do an interpretation of the results since the measured concentrations in the soil and plant analysis were very odd. It is advisable to do the experiment one more time, to do the soil analysis directly and to measure other parameters such as pH, Fe content in soil solution and CEC. Letting the plants grow for a longer period might also affect the results.

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INTRODUCTION

Mali is one of the poorest countries in the world with two thirds of its land area desert or semi-desert. It is a country rich in culture and different ethnic groups such as Bambara, Peul and Dogon. Most of the people live by farming but unfortunately the agriculture is endangered because of overexploited soils and poor water supply. Auspiciously, the river Niger flows through the country and is an important source for irrigation and fishing. Rice and cotton are the most common irrigated crops while millet and sorghum dominate rainfed cultivation.

Fig I Zinc deficiency in rice (www.knowledgebank.irri.org).

Iron toxicity and zinc deficiency are common problems in rice cultivation in Africa. More than half of the soils in Mali are deficient in zinc. This is not only due to a low zinc content but rather a result of its unavailability for the organism. The most important factor affecting the availability is the pH but also content of organic matter, cation exchange capacity and amounts of Al- and Fe-oxides are important. Zinc deficiency does not only result in reduced yield but also affects human health gravely.

Zinc deficiency in rice is often linked to iron toxicity. When the amount of dissolved ferrous iron is high in the root zone iron plaques are formed. They work as an effective adsorbent of zinc making it unavailable for the plant. Ferrous iron also cause physiological stress in the plant, especially under the reduced conditions of wetland rice. The soluble iron is absorbed by the roots and accumulated in the leaves resulting in brown spots and reduced growth.

The aim of this study was to investigate how the problem with iron toxicity and zinc deficiency in rice cultivation is affected by management of fertilizer application and by practising intermittent irrigation. A pot experiment was carried out with two soils from Office du Niger, one of the oldest and largest irrigation schemes in Sub-Saharan Africa, in central Mali. The experiment included 6 different treatments and 3 replications. Soil analyses were made concerning Zn and Fe content in the soil and in the plants.

1. MALI

Mali is situated in the very centre of West Africa. It is a large country with a surface of 1.24 million km^2 but with a fairly small population of only 12,3 million. The capital Bamako is situated in the south of the country. Two thirds of the surface is desert or semi-desert and the rest is mainly savannah and steppe. The river Niger flows in a great curve through the country and is an important source for the people. Agriculture occupies a large majority of the population and the Niger delta is where the most important agricultural area is found. The dominating export resources are gold and cotton. They account for about 80% of the export revenue.

Fig 1.1 The map of Mali (www.cmalliance.org).

Not many people have heard about Mali but most recognize the name Timbuktu, the mythical desert town. This is where the Tuaregs live, a nomad people that can be found in several countries in the south of the Saharan desert. They are famous by their customs and the men are recognized wearing a kind of veil in deep colours, mainly dark blue, winded around their heads. Mali is famous for its rich culture and warm and peaceful people. The dominating religion is Islam and mosques are found in every village. The country contains many ethnic groups that get along remarkably well. The largest ethnic group is the Bambara people, representing about one third of the population. They are mainly farmers living in the south-west of the country. Other ethnics are for exemple Peul, Dogon and Songhai.

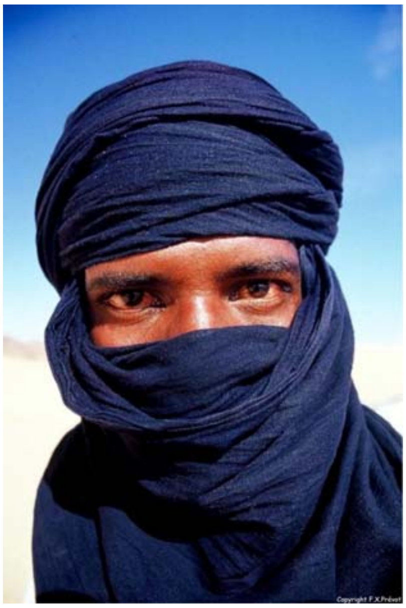

Fig 1.2 A Tuareg man (www.planet-dz.com).

The history of Mali is full of great empires and kingdoms. In the end of the 19th century, Mali was colonized by the French and became French Sudan. The Republic of Mali was created in 1960 and Modibo Keita became the first president. He was overthrown in 1968 by Lieutenant Moussa Traoré. The people were displeased with the new regime, partly because of corruption, increasing unemployment, raising prices on provision and severe conflicts with the Tuaregs, and in 1991 Amadou Toumani Touré became president. In 1992 a multi-party system was introduced and for the first time there were democratic elections in Mali, making Alpha Oumar Konaré president. Mali is today a constitutional democracy and since 2002 Amadou Toumani Touré is elected as president.

Fig 1.3 The mosque of Djenné.

Despite its “rich” people, Mali is one of the poorest countries in the world. It is ranked 172 in Human Development Report 2005, out of 175 countries. About 70% of the population is living with less than 1 dollar per day. The economy depends greatly on foreign assistance and the country is facing severe issues such as low literacy rate, health problems, poor infrastructure, corruption, desertification, polluted water and soil degradation. The status of women in the Malian society is low and discrimination is persistent in all domains. Domestic violence and female genital mutilation is widespread and there are limited economic and working opportunities for most women. Even though Mali is facing several difficulties the country is fairly democratic with a development in a positive and stable direction. There are none or few reports of torture, politically motivated disappearances, arbitrary arrests or denial of fair public trail. Civil liberties such as freedom of speech and press, freedom of religion, freedom of assembly and association are normally respected.

Table 1.1 Facts about Mali.

Population	12.3 millions
Area	1.24 millions km^2
Climate	Subtropical to arid
Government type	Republic
Religion	Muslim 90%, indigenous beliefs 9%, Christian 1%
Language	French (official), Bambara 80% and many other African languages
Age structure	0-14 years: 47% 15-64 years: 50% 65 years and over: 3%
Life expectancy at birth (total population)	48.7 years
Total fertility rate	7 children/woman
Population growth rate	3.1%
HIV/AIDS	1.65%
Literacy (total population)	46.4%
Population living with less than 1 dollar US/day	72.8%
Percentage of population with access to clean drinking water	65%
Percentage of women in the parliament	10.2%
Percentage of GDP spent on public health	2.2%
Percentage of GDP spent on education	2.8
Placement on corruption scale (1-10, 10 is the lowest)	3 (place 78 of 133 countries)
Placement of poverty scale out of 175 countries	172

(Sources: Ministry for Foreign Affairs – strategy for Swedish cooperation in West Africa (2004); www.cia.gov)

2. BACKGROUND

Iron and zinc are both essential elements for plants and humans. However, to little and too much is harmful. The soils in West Africa have a fairly low fertility but are rich in iron. Most of the soils are acidic, have low clay content and low organic matter levels. The parent material is quite low in nutrient reserves after a prolonged weathering and leaching. (Buri et al., 2000).

2.1 Zinc deficiency

Zinc is a micronutrient essential for the plant and the most critical limiting micronutrient to rice growth (Buri et al., 2000). It is an element necessary for the enzyme system and growth regulation. It promotes seed and grain formation, plant maturity and is essential for protein synthesis (Brady, 1999). A deficiency is expressed as chlorosis and necrosis on the leaves. The size of the leaves also diminishes since the production of auxines decreases and the distance between the internodes gets shorter. (Veldkamp et al., 1991). Zinc deficiency can decrease the yield of cereals to as much as 50 % (Asten et al., 2004).

Fig 2.1 Chlorosis in rice (www.knowledgebank.irri.org).

Zinc deficiency is a large problem in West African lowland soils. Approximately 66% are deficient in zinc which means that they have less than the critical level of 0.83 mg/kg soil required for rice production. (Buri et al., 2000 ; Mandal & Hazra, 1997). Zinc deficiency is not only a result of low zinc content in the soil but rather a result of its unavailability for the organism. Zinc is accessible to the plant as the exchangeable Zn^{2+}-ion. Most of the ions are bound to clay particles or inorganic constituents, like iron- and aluminum oxides, and thus inaccessible for the plant. Zinc also forms chelates, bound to organic matter, that can be decomposed and release ions for plant uptake. (Brady, 1999).

The most important factor affecting the zinc availability in the soil is the pH. The amount of available zinc for uptake decreases with an increasing pH since there is more negative charging in the soil that can adsorb zinc ions. pH also affects hydrolysis and an increasing pH helps the adsorption of easily hydrolysable metals in the soil. (Brady, 1999). Another important factor affecting the zinc availability for the plant is the amount of organic matter. Soils that contain less organic matter often hold less zinc. About 60% of the zinc in the soil is bound to soluble organic complexes and is released in decomposition. (Veldkamp et al., 1991). Also clay content, cation exchange capacity and the amounts of Fe and Al oxides determine Zn sorption. (Agbenin, 1998; Veldkamp et al., 1991).

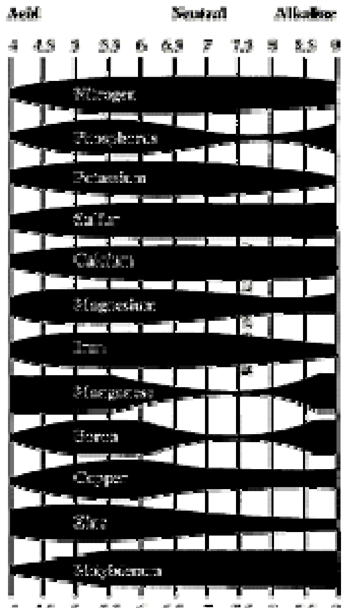

Fig 2.2 The effect of pH on plant nutrient availability.

Zinc deficiency often occurs in soils with bad drainage. Continues flooding may diminish the quantity of available zinc ions due to formation of sulphates and carbonates. Also low temperature, high soil moisture content and compact soil might induce zinc deficiency. The reason for this is the low rate of microorganisms that are able to break down the organic matter under theses circumstances. (Veldkamp et al., 1991).

2.1.1 Zinc deficiency due to iron toxicity

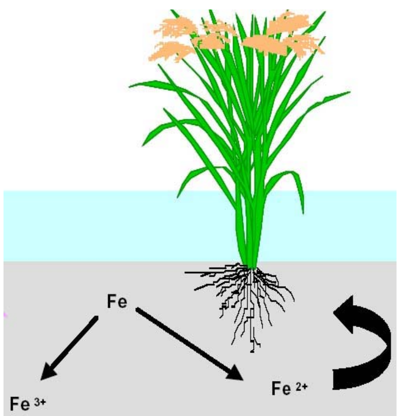

Fig 2.3 Plant uptake of ferrous iron.

Iron toxicity is a common problem in rice cultivation, especially in the lowlands, and zinc deficiency in rice is often linked to this phenomenon (Montás Ramírez et al., 2002). The topsoils of West Africa are fairly rich in available iron, reaching toxic levels in up to 20% of the soils (Buri et al., 2000). The amount of available iron is mainly affected by its oxidation state. It is the soluble Fe^{2+}-ion that is available for uptake. When the oxygen supply is low, or when the pH is low, the reduced Fe^{2+} is dominant. (Brady, 1999).

High amounts of ferrous iron, especially under the reduced conditions of wetland rice, cause physiological stress in the plant. A diminished ability of the rice roots to oxidize Fe^{2+} to Fe^{3+} results in a higher uptake of iron. (Sahrawat, 2000). The soluble iron is absorbed by the roots and accumulated in the leaves. The symptoms of Fe-toxicity often start with brown spots on the lower leaves of the rice plants, beginning on the tip and spreading toward the base of the leave. The shoots and the roots are most sensitive. One effect of iron toxicity is yield loss because of reduced growth, especially height, and reduced tilling. Iron toxicity can however cause negative effect on the yield without showing any visual symptoms and even low concentrations of Fe can be toxic. (Hua Li et al., 2001).

Fig 2.4 Symptoms of bronzing from iron toxicity in rice (www.agnet.org).

If dissolved ferrous iron is high in the root zone, iron plaques form at the root-soil interface and acts as an efficient adsorbent for zinc making it unavailable for the rice plant (Sajwan & Lindsay, 1986). These plaques are formed under anaerobic conditions with low redox values when the roots release oxygen into the rhizospere causing oxidation of Fe^{2+} to Fe^{3+} and therefore precipitation of Fe^{3+}-oxide or hydroxide. In general, the plaques are most extensive about 1 cm from the root tip and are detected as a thin orange-brown deposit on the roots. The plaques might protect the plant from toxic levels of elements such as Cu and Ni but also reduce the uptake of nutrients such as Zn. (Greipsson and Crowder, 1991). However, in a study made by Zhang et al. (1998) it has been shown that in iron deficient rice plants an iron plaque can increase the zinc uptake. This is because the rice plant under these conditions releases phytosiderophores, a type of amino acid, that mobilize zinc adsorbed to plaque and therefore enhancing zinc uptake.

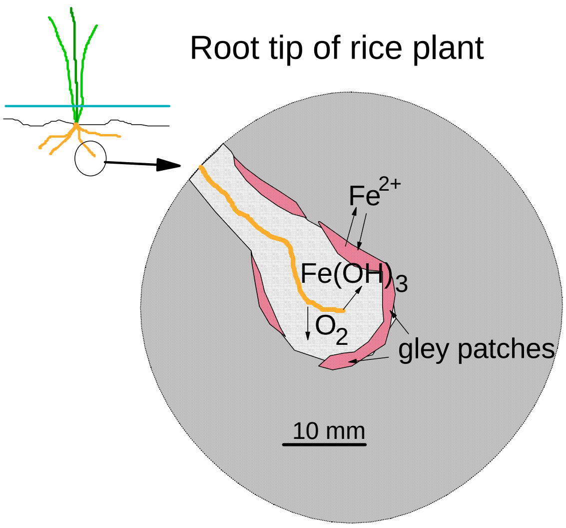

Fig 2.5 Formation of an iron plaque when ferrous iron is oxidized and precipitates as iron oxide.

In a study made by Fageria and Rabelo (1987), the uptake of almost all macro and micro nutrients were inhibited by high concentrations of iron in the soil. Increased concentrations of iron in solution, increases also the concentration in the plant, especially in the roots. Increased Fe concentration decreased Zn in the root but remained constant in the shoots. On the other hand, Qadar (2000) made the same observation but explains the higher concentrations of iron with the possibility of a dilution effect because of reduced growth.

When there is a nutrient deficiency in the soil, the roots of the rice plant has a decreased capacity of excluding iron which affects the tolerance to iron toxicity (Sahrawat, 2000). It has been shown that an effective way of reducing iron toxicity is application of nutrients such as P and Zn (Sahrawat et al., 1996; Audebert & Sahrawat, 2000; Hua Li et al., 2001). An enhancement of root oxidizing capacity, for example when applying potassium, makes the plant absorb less iron. (Hua Li et al., 2001). However, in a study of Sahrawat et al. (2000), it was shown that the iron toxicity was caused by high levels of iron in the soil and not by the deficiency of other nutrients. They claimed that iron toxicity is a “single element” problem. On the other hand, they have in this investigation studied the problem in a soil from Ivory Coast fairly rich in available zinc.

2.1.2 Zinc retention induce by phosphorus application

Zinc deficiency is a growing problem in the Sahelian savanna and one reason is the heavy use of phosphorus fertilizers. High inputs of phosphorus as fertilizer is common due to a deficiency of P (Haefele et al., 2004; Friberg et al., 2002) and can be found in several of the lowland soils in West Africa (Agbenin, 1998; Buri et al., 2000). Application of phosphorus might limit the solubility and availability of zinc since Zn phosphate precipitates are formed. However P can increase, decrease or have no effect on Zn status since the effect depends on pH changes related with P sorption. The changes in pH have further on influence on surface charge and Zn hydrolysis. (Agbenin, 1998; Barrow, 1987; Saleque et al., 2001).

An addition of phosphate changes the pH in several ways. When reacting with P, the surface becomes more negative and by releasing hydroxide ions, and as a result raising the pH, the electrical neutrality is maintained. The pH might also decrease if the phosphate fertilizers are acid in themselves. Adding phosphorus in the field, will also stimulate plant growth and nitrogen fixation and therefore indirectly incite changes in pH. (Brady, 1999).

A study performed by Agbenin (1998) showed that at pH ≤ 5.0, the solubility of Zn and Zn^{2+} activities in soil solution were not affected by phosphorus application, but at pH 5, increasing addition of P decreased zinc solubility. The maximum adsorption was found around pH 7.3. One theory is that when applying phosphorus the net negative charge increases because of adsorption to the surface of $H_2PO_4^-$ and $HPO4^{2-}$. A more likely theory is that zinc retention is related to pH and an increasing pH means an increased Zn hydrolysis and more zinc is adsorbed in the soil.

It is suggested that zinc and phosphorus are sorbed at differing ends of the spectrum of electrostatic potentials. Zn reacts with the negative surfaces and phosphorus with the positive surfaces and there is only an overlap when the concentrations are very high of both elements. (Barrow, 1987). Further, Buerkert et al. (1997) and Bagayoko et al (2000) confirmed that a decrease of Zn concentration when applying phosphorus fertilizers is only due to the increase in biomass and thereby causing a dilution effect on zinc. These studies were made on millet and not on rice.

2.2 Management of the problems

There are different ways of handling the problem with iron toxicity and zinc deficiency. Cultivars have different sensitivity and there are ongoing research to develop tolerant genotypes. (Sahrawat et al., 1996; Gregorio et al., 2002; Qadar, 2002). However, it is also possible to handle the problem by management of fertilizer application and irrigation. Addition of the major nutrients N, K, P and trace metals such as zinc decreases the iron toxicity. (Montás Ramírez et al., 2002; Hua Li et al., 2001). But according to Sahrawat et al (1996), phosphorus may delay the appearance of the toxic symptoms by a couple of weeks but does not alleviate them

Fig 2.6 Flooded rice field in Mali.

Rice is normally grown under flooded conditions. However, different moisture regimes have different impact on the soils physico-chemical properties. A study made by Mandal & Hazra (1997) showed that different types of irrigation had diverse influence on zinc adsorption and thus its availability to rice. When letting the soil dry under flooded-drying conditions, the adsorption of Zn to the soil is decreased and more Zn can be taken up by the plant. After harvest, when the flooded soil is drained and dried, the changed properties return to their normal condition but not entirely. The soil is still enriched in the amorphous form of iron and manganese oxides even though it will decrease gradually. These have a larger surface area and therefore higher adsorption capacity. In recent years, intermittent irrigation of lowland rice (*Oryza sativa*) has been practiced with good results in yield (Keiser et al., 2002; Stoop et al., 2002) and an improved nitrogen economy (Chu et al., 2004). Intermittent irrigation should also decrease iron toxicity by allowing oxygen entry into the soils, decreasing the Fe^{2+} concentration by precipitating ferric hydroxide.

Addition of organic matter is also an important factor increasing the Zn adsorption. Normally there is no recycling of nutrients since all biomass, the primary source of Zn, is removed from the field after harvest. It is important to adopt practices that allow both organic matter recycling and accumulation. (Mandal & Hazra, 1997). The most cost-effective way of dealing with the problem is according to Abifarin (1989) the use of iron-toxicity tolerant varieties in combination with advanced soil and nutrient management.

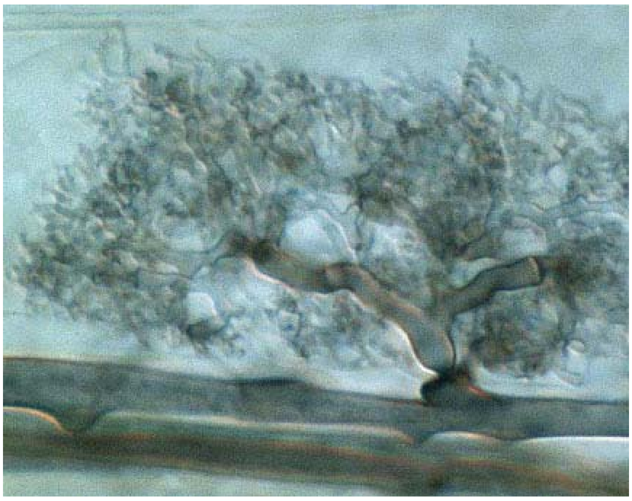

Fig 2.7 Vesicular arbuscular mycorrhizae (biology.kenyon.edu).

Mycorrhizae is also valuable for zinc uptake. Several reports of the effect of vesicular arbuscular mycorrhizae (VAM) on plant growth have been made. The hyphal mycelium of these fungi increases the total adsorption surface which improves the uptake of nutrients. Bagayoko et al.(2000) have showed large growth enhancing effects of VAM on crops grown on acid sandy soils of West Africa. This study was made on millet, cowpea and sorghum but the effect should be the same on rice.

2.3 Secondary effects of zinc deficiency

The low zinc content in soils and crops in Mali (Gårdestedt, 2004) may have serious effects on human health. Zinc deficiency in humans is common in Sub-Saharan Africa, about 80 % of the population is considered to have insufficient zinc intake (Brown et al., 2001). Zinc plays an important role in the immune system and a deficiency may increase the sensitivity to a wide range of pathogens. Studies on mice have shown that both primary and secondary antibody responses were depressed with a lack of zinc and also growth retardation and skin changes may appear. (Prasad, 1998). Zinc deficiency is believed to cause about 20 % of the child mortality in Africa, an equal portion as is caused by each of vitamin A deficiency and malaria (Ezzati et al., 2002). In a pilot project in Niafunké, a small community in the Niger inland delta, a zinc intake of about half the recommended was found (Jacks et al. 2005). In the community there is very high child mortality, 56 children of 131 born died before the age of five years (Jacks & Sall, 2003).

3. STUDY SITE

3.1 Office du Niger

Office du Niger was created by the French in 1932 and is the oldest irrigation project in West Africa. It is situated in the central Niger inland delta in the Sahel region of Mali. The area is divided into 5 major zones; Kouroumari in the north, N'Débougou, Molodo and Niono in the center and Macina in the southeast (fig 3.2). Kouroumari is the youngest and less exploited zone while Macina has been cultivated intensively during a long time.

Fig 3.1 Location of Office du Niger in Mali.

By creating the Markala dam in the south of the Office du Niger it has been possible to raise the groundwater level, from 50 m to as little as 50 cm in some areas. (Dicko M., 1994). Canals lead the water to "falas" where it is stored before being used for irrigation. All fields are irrigated by gravity. (H. N'D Ingénieurs, 1998). The potential irrigation capacity is 1 million hectare, but today less than 70 000 hectares are exploited. About 50 000 ha of rice is cultivated, 5 000 ha of sugarcane and the remaining 15 000 ha consists of vegetables. (Bouré et.al., 1999). There are plans to expand the Office du Niger but it will take a very long time before the maximum capacity is reached; approximately 25 years per 50 000 ha. (MDRE, 1998).

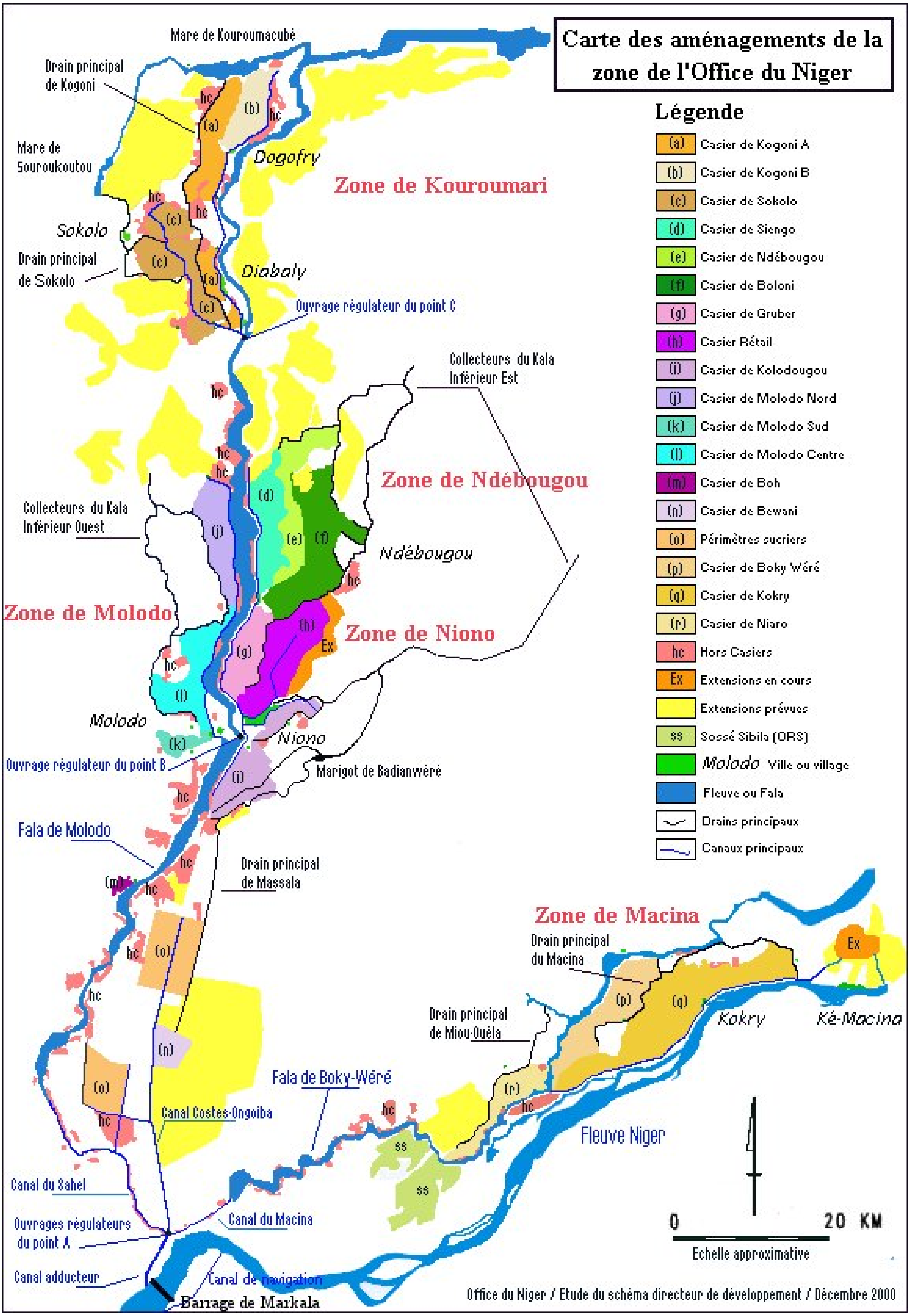

Fig 3.2 The different zones and *casiers* of Office du Niger.

3.2 The soils of Office du Niger

There is a large variation of soils inside the area with morphology from sandy soils to clay soils depending. The soils have a natural low fertility, characterised by a washout of nutrients, and are sensitive to soil degradation such as salinity and soil erosion by wind and water. The levels of organic matter is fairly low. (Doumbia, 2001).

There is a history of heavy fertilizer use in the area. The soils of Macina and Niono have been exploited for a long time compared to the zone of Kouroumari in the north which makes the soil properties different. In the older zones there is a larger accumulation of phosphorus because of heavy fertilizer application the past 30 years. There is also a lower amount of zinc in the soil since the nutrients have been removed with harvest[1].

3.2.1 Classification of the soils

The existing classification is in Bambara, one of the regional languages, and was made by Dabin (1951). It is based on the texture but also includes colour and structural aspect. Unfortunately it does not take in count the deep horizons or the granular composition. The different types of soils are often found from the north to the south in Mali, in the given order in table 3.1. Dian and Moursi are cambisols and the rest are fluvisols.

Table 3.1 Classification in Bambara of soils in Mali.

Classification	**Structure**	**Clay content**	**Characteristics**
Séno	Sandy soil		Forms sand hills
Danga	Loamy sand	5-20%	Very soft in wet season and hard in dry Season.
Danga blé	Sandy loam to clay loam	11-35%	Reddish, often crumbly. Ferruginous nodules in areas hit by wind erosion.
Danga fling	Loamy sand		Beige, blackish. Rich in silt and organic matter.
Dian	Silty clay	25-50%	Brownish, very compact. Cracks.
Dian péré	Silty clay		Cracks, high clay content.
Moursi	Clay soil		Blackish, crumbly on the surface. Cracks. Calcareous.
Boi	Loam		Bluish grey, crack, difficult drainage.
Boi blé	Loam		Red and yellow spots.
Boi fling	Clay loam	30-60%	Crumby on the surface, rich in humus and organic material, does not crack.

[1] Oral communication with Dr. Mamadou Doumbia at CRRA, October 2005.

3.3 The actors inside Office du Niger

The colonial state and the Malian state have developed Office du Niger. At the beginning, after building the barrage of Markala, only 457 ha were irrigated. The plan was to exploit the potential of 1 million hectares before 1992, but this has not yet been reached. During the 60s and the 70s, Office du Niger suffered some rigorous problems concerning the water management, the administration, small yields in rice production and illness among the people working there. Severe dry periods also affected the whole Sahel region as well as Office du Niger during this period. There was a great need of reforms to ameliorate the administration and the structure of the project and to reorganise the roles of the actors. (MDRE, 1998).

In 1994, a new law was created because of problems with decreasing physical resources (labour, water resources, land surface etc.) ending up with the abandonment of 30% of the cultivated land. This resulted in a production decline, financial problems and a very tense relation between the Office and the producers. The new law lead to the creation of several private companies that were in charge of the production while the Office was handling the public service. The organisation was decentralised by creating autonomic zones. (MDRE, 1998).

Today there are tree actors in the area that handle the social and economical activities; the state, the entity of Office du Niger and the producers. (MDRE, 1998). The land in the Office is mainly owned by the state and is rented by farmers that cultivate it. There are also some small family businesses that run market gardening with paid workers. Among the producers there is a strict hierarchy inside the family and the village. The chief is most of the time the oldest man in the family but the number of women running the market gardening is increasing. (Bouaré et al., 1999).

Fig 3.3 Market gardening in Mali (www.nicosia.org).

About 10% of the people in the area are considered to be wealthy. Some farmers that do not have enough money to pay for the land have been obliged to move to the surrounding *hors-casiers*. They are small and independent, they do not pay anything for the land and they have no access to the irrigation system. (Bouaré et al., 1999).

3.4 Rainfed and irrigated cultivation

There are two types of production; rainfed production with irrigation by natural rainfall and irrigated production by using gravity. Rice is the dominating crop in irrigated production while rainfed production mainly consists of sorghum and millet. The rainfed system is about to disappear in the region when it comes to rice production because of the disadvantage of small harvests: a non irrigated field produces 300-400 kg/ha of rice while an irrigated field can produce as much as 5100 kg/ha. (Bouaré et al.,1999). Crops are cultivated both in summer and winter. Normally rice is cultivated in both seasons but there is also a large production of vegetables in contra-season. Beside the crop production there are also some people living of breeding animals, mostly the transhumance, and a small population living of fishing. (MDRE, 1998).

Fig 3.4 Sorgho production.

Fig 3.5 Millet production.

The amount of fertilizers applied has not changed much in the last 30 years, there is only a small decrease in the usage of urea and NPK. In 1998 the average application rate in the Office du Niger were 81 NPK/ha and 154 kg urea/ha. (MDRE, 1998).

There are many issues in need of improvements; better use of fertilizers, adaptation to double culture, better techniques to divide the parcels and more respect of the agricultural calendar. Still the production has improved thanks to better tools and permanent cattle breeding. (MDRE, 1998). There is a big problem with the water management in the area and it is important to economise water to be able to cultivate during the dry period. Much more water than needed is directed into the system, the water system and irrigation pipes are in need of reparation and the irrigation is not optimal, which leads to a waste of water. The drainage is not either always optimal which leads to soil degradation such as salinisation. (H. N'D Ingénieurs, 1998).

3.5 Impacts on the environment and human health

3.5.1 Soil degradation

The most acute problem in the Office du Niger is soil degradation such as erosion, salinisation and alcalinisation. The irrigated areas have problems with decreasing porosity. The drainage systems and the infrastructure is badly maintained which leads to bad infiltration and oxygen deficiency. The water that is used in the area is rich in salts (sodium carbonate) which leads to alcalinisation and a disfavourable root development. Soil degradation is one reason for the decrease in yield, but it is also because there is a bad adaptation to the double culture and a bad fertilizer application. (Bouaré et al., 1999).

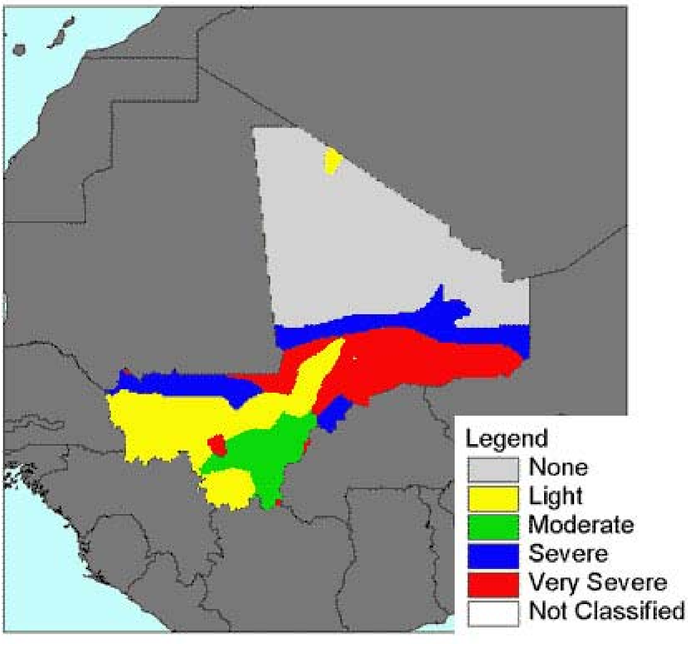

Fig 3.6 Severity of human induced soil degradation in Mali (www.fao.org).

3.5.2 The wild nature

There have been some observed positive effects on the nature when it comes to species composition. A more dense vegetation cover has occurred in for example Fala de Molodo, and several new floating leaved macrophytes have been detected because of the larger amounts of water. The land vegetation has also become thicker with larger bushes and more small trees. (Bouaré et al., 1999).

Unfortunately many areas have developed in the opposite direction because of the way of running agriculture. The main problem is overexploitation of trees and bushes for fuel and the raising of the ground water level, especially in the area west of Molodo. The nature has been destroyed by human activity; the herbaceous Sahelian vegetation has become swamps, the soil surface has changed and permeability and infiltration has diminished, making it difficult for the plant roots to develop. (Bouaré et al., 1999).

3.5.3 Pollution

There is a problem with pollution such as chemical products, pesticides and fertilizers. The chemical pollution comes first of all from sugar factories in Siribala and Dougabougou. No studies have been made of how the pollution might affect human health, even though an increase in dead birds and dead fish has been observed in the area. Human garbage and animal deposits is also a large health risk for the people in the area since it flows directly in the irrigation and drainage canals. (Bouaré et al., 1999).

3.5.4 Health problems and sanitary issues

The major health problems in the area are malaria and bilharzias. Almost 30% of the adults and 80% of the children in the age of 7-14 years old are affected by bilharzias. These are the highest numbers in Mali. About 50% of the children less than 10 years old are affected by malaria. Diarrhoea, especially among children, is also a widespread problem because of polluted wells. The distance between the well and for example the latrine is often very short. The wells are often very shallow, sometimes the depth does not exceed 3 m. The severities of these issues are escalating because of an increasing population. Approximately a fifth of all children are suffering from malnutrition even though there are good fishing recourses and lots of vegetables and rice. (MDRE, 1998).

Fig 3.7 Feeding malaria mosquito (www.swissinfo.org).

4. MATERIALS AND METHODS

4.1 Soil characterisation

To determine the status of Zn and Fe in the soils of Office du Niger, soil samples collected by Lassana Dioni and Abdou Ballo in spring 2005 were used. In total 54 samples from all five zones were analysed by extraction of DTPA to get the amount of elements accessible to the plants. The sampling sites were geo-referenced with GPS and only the top soils of 20 cm were collected.

5 g of soil from each sample site were put in small plastic bottles. There were also 2 blanks and 1 reference. An extraction solution of 2 l DTPA was prepared by mixing the following chemicals in the given order: 1 l distilled water, 26.6 ml TEA (Tri-ethanol-amine), 3.94 g DTPA and 2.94 g $CaCl_2 \cdot 2\ H_2O$. Then distilled water was added to get a total volume of 2 l and pH was adjusted to 7.3 with 1 M HCl (36-38%). Finally, 20 ml of the DTPA solution were added to the bottles which were put in a shaker for 2 h before filtration. Calibration solutions for the AAS analyses were prepared and the instrument was calibrated before making the measurements.

Other soil properties such as pH, EC, CEC and exchangeable bases were determined for the same 54 soil samples by two other students; Abdou Ballo and Souleymane Dambe.

4.2 Site selection and field sampling

Two contrasting soils were selected for the pot experiment on the basis of the performed soil analyses; both with a deficit in Zn but one with a low and one with a high Fe content. The first soil was collected in a flooded rice field at the village of Goursy in Macina (Coordinates: 13.8317348/5.63884761). This soil has a very high content of iron. The second soil, a soil with less iron content, was collected in a dry field in Sika, Kouroumari (Coordinates: 14.674297483/6.018654). The soil of Macina is a Boi blé, rich in clay, and the soil of Kouroumari, the Danga blé, is a sandy soil. Only the surface soil was collected.

4.3 Pot experiment

The collected soils were put on plastic to dry in open air for a few days. Afterwards, they were ground and sieved through a 2 mm screen. A water dynamic test was performed to estimate the amount of water needed in the experiment for the two different soils: 2 kg of each soil were used and one replication were made.

Each one of the two soils were prepared with 6 different treatments, each treatment were presented in triplicate:

- Flooded
- Flooded + phosphorus
- Flooded + phosphorus + zinc
- Intermittent irrigation
- Intermittent irrigation + phosphorus
- Intermittent irrigation + phosphorus + zinc

Fig 4.1 Growing rice plants in the pot experiment. 36 pots in total; 6 different treatments and 2 differing soils.

The pots were filled with 2 kg of soil and put in a randomised order. Zinc was applied at a rate of 15 kg/ha as $ZnSO_4 \cdot 7\ H_2O$ and phosphorus at a rate of 20 kg/ha as NPK. The soils were stirred and each pot was filled with water to obtain saturation; 915 ml for the Boi and 400 ml for the Danga. In each pot 4 seeds of rice were sown. The seedlings had been pre-germinated for 4 days and the rice variety used was Bouaké 189. It is an iron susceptible variety, fairly resistant to diseases and reacts well on fertilizers. Bouaké 189 is a rice type normally cultivated under irrigated conditions and has an average yield of 6 ton per hectare. (MDRE, 1998).

The plants were thinned to 2 plants/pot after 9 days. During growth, a general observation of the plants were performed as well as vigour, plant height and number of tillers. The plants were harvested after 21 days and dried for 48 h before measuring the weight.

Rice plants and soil samples from each pot were brought to KTH in Sweden for further analysis of Zn and Fe content. The plant residues were analysed by extraction of HNO_3 and the soil samples were analysed by extraction of DTPA. A student's t-test were performed to see if there were any statistically difference between the different treatments and the two irrigation types (appendix V).

5. RESULTS

5.1 Visual observation

At start the rice plants grew well in all 36 pots. After almost 2 weeks all plants were dead in the pots with the Danga-soil. There were black spots in the soil, probably sodium, and the water in the flooded pots were red because of oxidation of iron. The plants in the Boi were still living but had grown differently; some of them were bigger and more vital but most of them had brown spots on the lower leaves.

As shown in table 5.1 the plants being treated with intermittent irrigation were the biggest and most beautiful ones. The best results were obtained when also adding Zn and P, but the difference compared with only adding P were small. Several of the plants treated with intermittent irrigation had reddish leaves. The plants that were flooded during the whole experiment were smaller and had more dry and yellowish leaves. All flooded plants with no extra Zn or P were small and yellowish. Adding P and Zn in flooded irrigation ameliorated the size and appearance of the rice plants.

Table 5.1 Visual observation of growing plants. Height: small (>10 cm), medium (10-15 cm), large (<15 cm).

		Flooded		Intermittent irrigation	
Treatment	Rep.	Height	Observation	Height	Observation
R	1	Medium	All leaves are dry and yellowish	Medium	Beautiful but with reddish tips
	2	Medium	Lower leaves dry and yellowish	Medium	Beautiful
	3	Small	All leaves are dry and yellowish	Large	Very big and beautiful
R+P	1	Small	Dry leaves	Large	Beautiful
	2	Medium	Thin plant, lower leaves dry	Small	Dry leaves
	3	Small	Dry leaves	Large	Beautiful
R+P+Zn	1	Small	Thin with some dry leaves	Medium	Beautiful
	2	Medium	Beautiful	Large	Beautiful but with lower leaves dry and reddish
	3	Medium	Beautiful, few dry leaves	Medium	Beautiful

5.2 Plant analyses

The results of Zn and Fe concentrations in plant residues varied between the different treatments and the different replications (appendix III). The pots that were flooded had higher concentrations of zinc but both irrigation practices shows a deficit of the nutrient since they were below the critical level of 20 mg Zn/kg (Veldkamp et al., 1991). As shown in figure 5.1

flooded irrigation and adding only phosphorus gave the highest Zn value (16.3 mg Zn/kg) followed by the treatment with P and Zn (12.4 mg Zn/kg). The best results when using intermittent irrigation was achieved by adding P (95.7 mg Zn/kg) but the difference between the treatments were small.

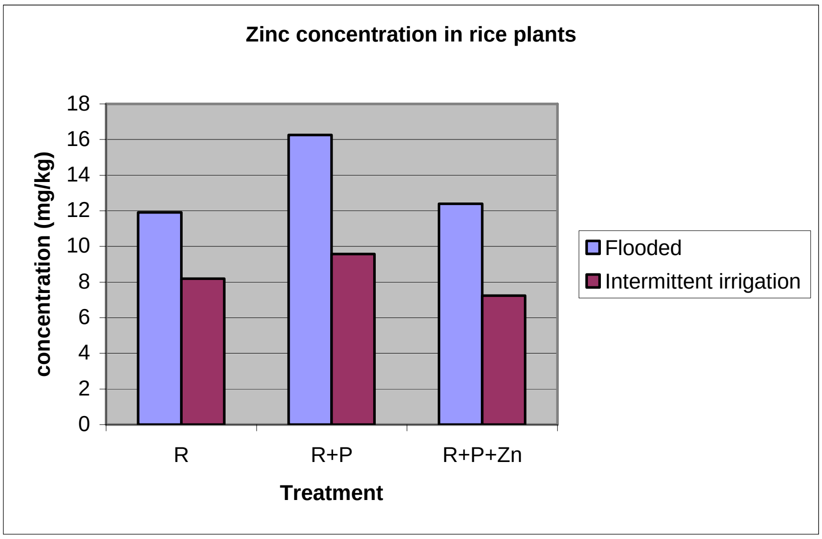

Figure 5.1 Zinc concentrations in the rice plants, measured with AAS after extraction with HNO_3.

The critical level for occurrence of iron toxicity in plants is 300 mg Fe/kg (Veldkamp et al., 1991). As shown in figure 5.2 all measured concentrations were below this level even though there were visual symptoms of iron toxicity. The iron concentration was highest in the plants that were flooded with also P added (234.5 mg Fe/kg). It was also high when using intermittent irrigation without adding any extra nutrients. The lowest concentrations were obtained with both intermittent irrigation and adding P and Zn or only P (121 and 110 mg Fe/kg). Some of the values were excluded since they were extremely large.

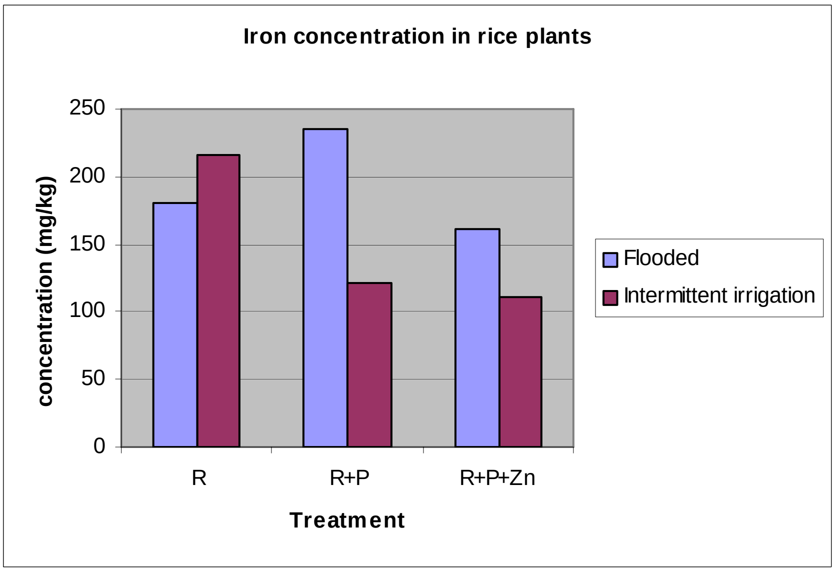

Figure 5.2 Iron concentration in rice plants, measured with AAS after extraction with HNO_3.

5.3 Soil analyses

The zinc deficiency in the Boi soil is very severe, all showing values below the critical level of 0.8 mg Zn/kg (Veldkamp et al., 1991). The highest concentrations were found in the pots where both P and Zn were added (figure 5.3). The other two treatments resulted in only half the amount of Zn. The flooded pots contained slightly higher concentrations of Zn than the ones with intermittent irrigation.

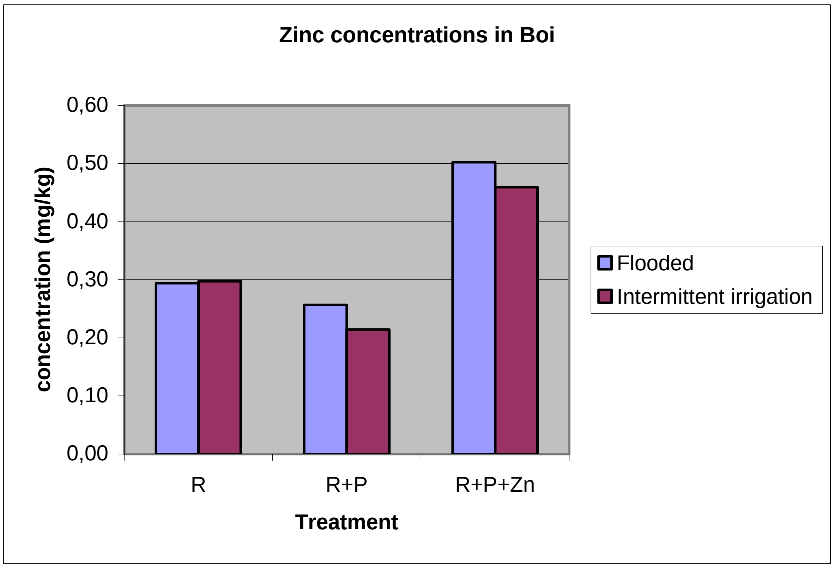

Fig 5.3 Zinc concentration in Boi.

All pots with the Boi soil contained low concentrations of iron and not one of the values reached over the critical level of 40 mg Fe/kg (Veldkamp et al., 1991). As shown in figure 5.4, the pots treated with intermittent irrigation contained more Fe than the ones that were flooded. Letting the soil dry and also adding P and Zn gave the highest iron concentrations. There were no big difference between adding nutrients or not when the soils were flooded. They all showed very low values.

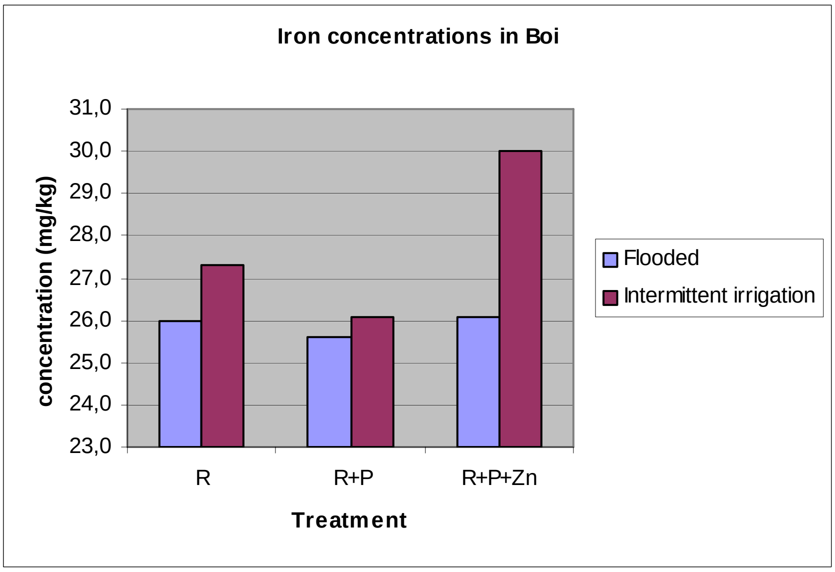

Fig 5.4 Iron concentration in the Boi.

The Danga soil suffered from zinc deficiency in all pots (figure 5.5). The deficiency were less severe when adding both P and Zn, especially when using intermittent irrigation. The values for the first two treatments were exceptionally low but the difference between the two types of irrigation was small.

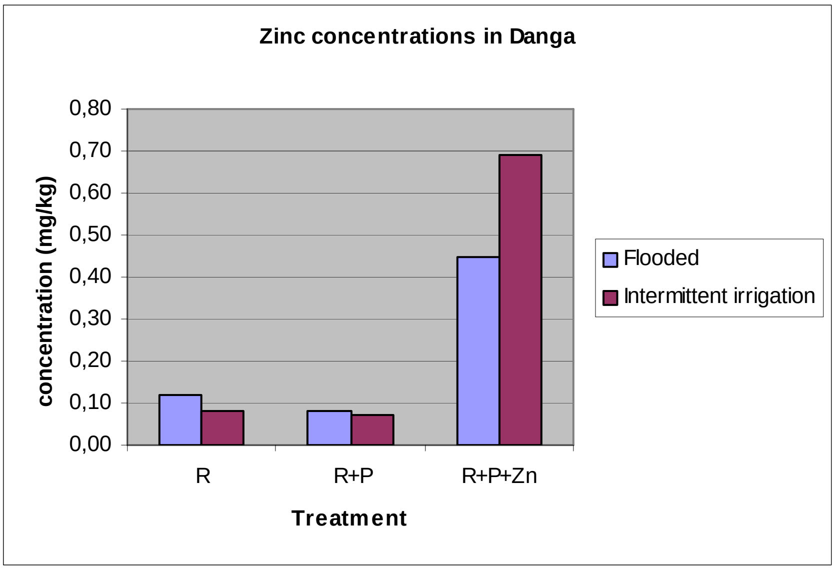

Fig 5.5 Zinc concentrations in the Danga.

The iron values were surprisingly low showing almost inexistent concentrations (figure 5.6). Using intermittent irrigation and also adding the two nutrients gave the lowest concentrations. The results from the pots with intermittent irrigation were a little bit higher than the flooded pots. Applying both nutrients gave lower values under flooded conditions.

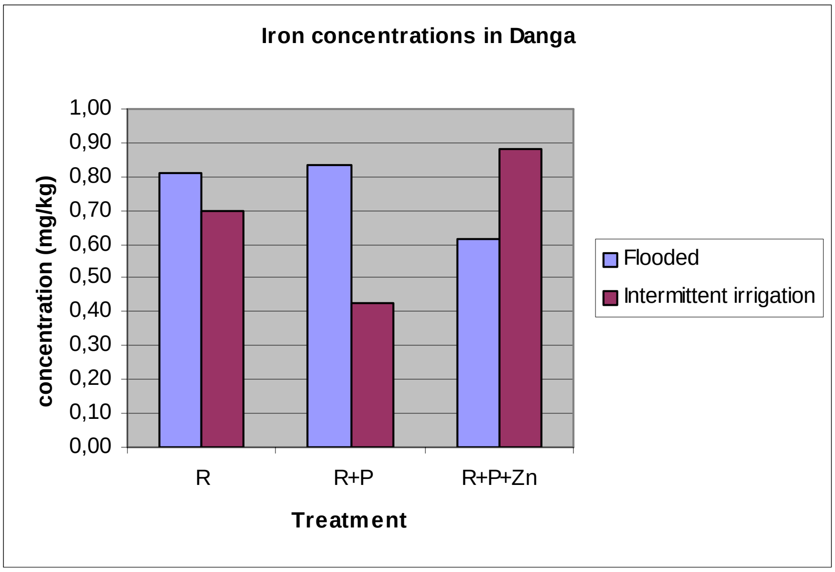

Fig 5.6 Iron concentrations in the Danga.

5.4 Statistical analyses

In the tables below the standard deviation (s) and the calculated t-values are shown (see the calculations in appendix V). The bold t-values are the ones that exceed the t-table value of 2.920 indicating that there is a difference between the compared groups. The standard deviation varies a lot between the different comparisons. High values indicate that there is a large difference between the values in the data set.

Table 5.2 and 5.3 shows if there is any difference between using intermittent irrigation or flooded. T-values and standard deviation have been calculated for each fertilizer treatment. According to the results, using different irrigation types does not affect the zinc and iron contents in the plants or the soils. Only two values exceed the t-table value of 2.920 showing that the different kinds of irrigation might have an effect on the concentrations of iron and zinc.

Table 5.2 Calculated standard deviation and t-values for zinc concentrations (mg/kg) when comparing intermittent irrigation and flooded irrigation.

ZINC		R		R+P		R+P+Zn	
		Flooded	IM	Flooded	IM	Flooded	IM
Plant	S	3,716	2,563	10,768	3,740	4,657	0,757
	t-value	*1,42*		*1,01*		*1,9*	
Boi	S	0,091	0,147	0,024	0,021	0,067	0,125
	t-value	*0,03*		*2,36*	*0,53*		
Danga	S	0,056	0,018	0,005	0,002	0,091	0,266
	t-value	*1,16*		***3,9***		*1,47*	

Table 5.3 Calculated standard deviation and t-values for iron concentrations (mg/kg) when comparing intermittent irrigation and flooded irrigation.

IRON		R		R+P		R+P+Zn	
		Flooded	IM	Flooded	IM	Flooded	IM
Plant	S	39,5	114,19	81,25	66,96	138,56	48,3
	t-value	*0,51*		*1,53*		*0,59*	
Boi	S	0,289	0,651	0,351	1,405	0,802	2,401
	t-value	***3,16***		*0,6*		*2,69*	
Danga	S	0,243	0,347	0,313	0,041	0,045	0,406
	t-value	*0,45*		*2,22*		*1,14*	

Table 5.4 and 5.5 examine if there is any difference between not adding any nutrients, adding P or adding both P and Zn. When looking at the plant residues the different fertilizer applications does not influence the iron and zinc concentrations. The different applications might however have an affect on the nutrient concentrations when using intermittent irrigation in the Danga and the Boi soils (bold figures in table 5.4). The standard deviation is in these cases low. The flooded soils in table 5.5 also shows that adding P and Zn affects the nutrient concentrations.

Table 5.4 Calculated standard deviation and t-values for iron and concentrations (mg/kg) when comparing the different fertilizer treatments and using intermittent irrigation.

IM			R	R+P	R	R+P+Zn	R+P	R+P+Zn
Plant	Zn	S	2,56	3,74	2,56	0,76	3,74	0,76
		t-value	*0,53*		*0,63*		*1,07*	
	Fe	S	114,19	66,96	114,19	48,3	66,96	48,3
		t-value	*1,16*		*1,42*		*0,18*	
Boi	Zn	S	0,15	0,02	0,15	0,13	0,02	0,13
		t-value	*0,97*		*1,45*		***3,34***	
	Fe	S	0,65	1,4	0,65	2,4	1,4	2,4
		t-value	*1,34*		*1,88*		*2,43*	
Danga	Zn	S	0,018	0,002	0,018	0,266	0,002	0,266
		t-value	*0,91*		***3,94***		***4,01***	
	Fe	S	0,35	0,04	0,35	0,41	0,04	0,041
		t-value	*1,36*		*0,6*		*1,95*	

Table 5.5 Calculated standard deviation and t-values for iron and concentrations (mg/kg) when comparing the different fertilizer treatments and using flooded irrigation.

FLOODED			R	R+P	R	R+P+Zn	R+P	R+P+Zn
Plant	Zn	S	3,72	10,77	3,72	4,66	10,77	4,66
		t-value	*0,66*		*0,145*		*0,571*	
	Fe	S	39,5	81,2	39,5	138,6	81,2	138,6
		t-value	*0,88*		*0,22*		*0,74*	
Boi	Zn	S	0,091	0,024	0,091	0,067	0,024	0,067
		t-value	*0,69*		***3,18***		***5,99***	
	Fe	S	0,29	0,35	0,29	0,8	0,35	0,8
		t-value	*1,52*		*0,14*		*0,92*	
Danga	Zn	S	0,057	0,005	0,057	0,091	0,005	0,091
		t-value	*1,15*		***5,31***		***6,97***	
	Fe	S	0,243	0,313	0,243	0,045	0,313	0,045
		t-value	*0,087*		*1,37*		*1,18*	

6. DISCUSSION AND CONCLUSION

Interpreting visual observation gives that intermittent irrigation with additional P and Zn improves the appearance of the rice plant. Using flooded irrigation gave smaller plants with dry and yellowish leaves but the results improved when adding nutrients. Intermittent irrigation probably ameliorates the zinc uptake and decreases the iron toxicity since more ferrous iron is oxidised and less zinc is adsorbed. Adding extra nutrients improves zinc uptake in the plant because of a higher resistance to iron toxicity.

The results from the soil and plant analysis are difficult to interpret. There is an iron deficiency in all plants and also in both soils. This is contradictory to the visual observation and the analyses made in the beginning of the project. The low iron concentration in the rice plants treated with both intermittent irrigation and adding P and Zn might be due to oxidation of Fe^{2+} and higher capacity of excluding the element.

There are higher iron concentrations in the soils treated with intermittent irrigation than the flooded soils. This is odd since the results should be the opposite; the flooded soils should contain more iron since the oxygen level is lower and therefore the ability to oxidize ferrous iron. It would have been interesting to measure the iron concentration in the soil water since it is a crucial parameter affecting the Fe toxicity. The Fe^{2+}/Fe^{3+} system governs the redox conditions and measuring the redox potential in the pots would also have been motivating.

There was a zinc deficiency in both soils but the concentrations increased when adding nutrients. Adding extra zinc in the soil should have increased the Zn uptake in the plant but might have been counteracted by high Fe concentrations in the soil. The plants being treated with extra P showed high concentrations of both Zn and Fe. This might be due to the lower concentration of iron in the soil. But still, the Fe levels in the soil are not high enough to induce toxicity so it should not really have an effect on the Zn uptake.

It is difficult to come to a conclusion from the soil and plant analysis since many things might have gone wrong. Using dry soil samples does not give accurate results. The first 54 soil samples were 6 months old and the samples from the pot experiment were transported to Sweden before any analysis were made. The iron for example, might have been bound again to the soil and therefore giving low concentration values. Making conclusions from the visual observations are subjective since it is impossible to know the reason why some plants grew well and some not without reliable results from soil analysis. We do not know what would have happened if we would have let the plants grow for another month or even longer. Maybe the visual results would have been different. We do not know if the results from the soil analysis of the 54 samples were correct either. All plants and soils showed a deficiency of zinc and maybe the amounts of Zn added were not large enough to prevent a zinc deficiency.

There are several parameters, such as pH, CEC and Fe concentration in soil solution, that are important for the interpretation of the results but these parameters are unknown. To get a more certain outcome it is advisable to do the experiment one more time, to make the analysis directly and to make more measurements.

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APPENDIX I – Treatment and soil type in each pot

Pot	Treatment	Soil
101	R+IM	Boi
102	R	Boi
103	R+P+Zn	Danga
104	R+P+Zn	Danga
105	R+IM+P	Danga
106	R+IM	Danga
107	R+IM+P+Zn	Boi
108	R+P	Boi
109	R+IM+P+Zn	Danga
110	R+P	Boi
111	R	Boi
112	R	Boi
201	R+IM+P	Boi
202	R+IM+P	Danga
203	R+IM+P	Boi
204	R+P+Zn	Danga
205	R+IM+P+Zn	Boi
206	R+P	Boi
207	R+IM	Boi
208	R+IM+P+Zn	Danga
209	R+IM+P	Danga
210	R+IM+P+Zn	Danga
211	R+IM	Boi
212	R+P	Danga
301	R	Danga
302	R+IM+P+Zn	Boi
303	R+P+Zn	Boi
304	R+P	Danga
305	R+P+Zn	Boi
306	R+P	Danga
307	R+IM	Danga
308	R	Danga
309	R+P+Zn	Boi
310	R+IM	Danga
311	R+IM+P	Boi
312	R	Danga

APPENDIX II – Fe and Zn concentrations in plant residues and soil samples

PLANT RECIDUES		Flooded		Intermittent irrigation	
Treatment	Rep.	Zn (mg/kg)	Fe (mg/kg)	Zn (mg/kg)	Fe (mg/kg)
R	1	15,4	156,5	8,5	179,3
	2	12,3	158,3	10,6	343,4
	3	8	225,8	5,5	123,8
	Average	**11,9**	**180,2**	**8,2**	**215,5**
R+P	1	10,0	177,1	7,1	168,3
	2	10,1	292,0	13,9	~~744,9~~
	3	28,7	~~1770,2~~	7,8	73,6
	Average	**16,3**	**234,5**	**9,6**	**121,0**
R+P+Zn	1	16,9	315,6	6,9	~~459,3~~
	2	7,6	46,4	6,7	76,2
	3	12,7	124,0	8,1	144,5
	Average	**12,4**	**162,0**	**7,3**	**110,4**

BOI		Flooded		Intermittent irrigation	
Treatment	Rep.	Zn (mg/kg)	Fe (mg/kg)	Zn (mg/kg)	Fe (mg/kg)
R	1	0,397	25,8	0,467	26,6
	2	0,264	25,8	0,213	27,3
	3	0,222	26,3	0,212	27,9
	Average	**0,294**	**25,9**	**0,298**	**27,3**
R+P	1	0,284	25,6	0,223	24,6
	2	0,241	25,9	0,229	26,2
	3	0,246	25,2	0,190	27,4
	Average	**0,257**	**25,6**	**0,214**	**26,1**
R+P+Zn	1	0,58	25,2	0,604	27,6
	2	0,467	26,8	0,394	29,9
	3	0,461	26,1	0,38	32,4
	Average	**0,502**	**26,1**	**0,459**	**30**

DANGA		Flooded		Intermittent irrigation	
Treatment	Rep.	Zn (mg/kg)	Fe (mg/kg)	Zn (mg/kg)	Fe (mg/kg)
R	1	0,089	1,01	0,059	0,337
	2	0,184	0,54	0,086	0,729
	3	0,083	0,88	0,092	1,03
	Average	**0,12**	**0,81**	**0,079**	**0,7**
R+P	1	0,076	0,62	0,072	0,47
	2	0,082	0,68	0,068	0,389
	3	0,085	1,19	0,069	0,414
	Average	**0,081**	**0,83**	**0,07**	**0,42**
R+P+Zn	1	0,55	0,57	0,866	1,35
	2	0,41	0,615	0,38	0,61
	3	0,38	0,66	0,81	0,69
	Average	**0,45**	**0,615**	**0,69**	**0,88**

APPENDIX III – Number of plants and tillers and measured weight at harvest

Pot	Treatment	Weight (g)	Plant(s)	Tiller(s)
101	R+IM	0,166	2	1
102	R	0,051	2	
107	R+IM+P+Zn	0,139	2	1
108	R+P	0,033	1	
110	R+P	0,081	2	
111	R	0,048	2	
112	R	0,06	2	
201	R+IM+P	0,021	1	
203	R+IM+P	0,187	2	1
205	R+IM+P+Zn	0,029	1	
206	R+P	0,093	2	
207	R+IM	0,136	2	2
211	R+IM	0,321	2	3
302	R+IM+P+Zn	0,214	2	3
303	R+P+Zn	0,112	2	
305	R+P+Zn	0,029	2	
309	R+P+Zn	0,096	2	
311	R+IM+P	0,389	2	4

APPENDIX IV – Visual observation of plant vigour

Pot	2 days	7 days	9 days	20 days
101	7	5	5	5
102	5	5	3	7
103	5	9	9	-
104	7	9	9	-
105	7	9	9	-
106	9	9	9	-
107	3	3	3	3
108	3	3	5	9
109	7	9	9	-
110	3	3	3	7
111	3	3	3	7
112	3	3	3	7
201	7	7	6	9
202	7	9	9	-
203	7	3	3	3
204	7	9	9	-
205	7	5	5	7
206	3	3	3	7
207	3	3	3	3
208	5	7	9	-
209	7	7	9	-
210	7	7	9	-
211	3	3	3	3
212	5	7	9	-
301	5	7	9	-
302	5	5	3	3
303	3	3	3	5
304	5	5	9	-
305	5	5	5	7
306	7	7	9	-
307	5	7	9	-
308	5	7	9	-
309	3	3	3	5
310	3	5	9	-
311	3	3	3	3
312	5	7	9	-

APPENDIX V – Statistical analysis (student's t-test)

Formula for the t-test:

$$t = \frac{\tilde{x}_A - \tilde{x}_B}{SE_{A-B}}$$

where *t* is the calculated t-value, $\tilde{x}_A$ is the mean of the values in group A (same for group B) and SE_{A-B} is the standard error of the difference between the means of group A and B. SE_{A-B} is calculated as follows:

$$SE_{A-B} = \sqrt{\frac{s_A^2}{n_A} + \frac{s_B^2}{n_B}}$$

where s_A^2 is the variance for group A and n_A is the number of measurements. The variance is calculated as follows:

$$s_A^2 = \frac{\sum x_A^2 - \frac{\left(\sum x_A\right)^2}{n_A}}{n-1}$$

There is no difference between the two compared groups if the calculated *t* value is lower than the *t* value from table. When entering the t-table at 2 degrees of freedom (n-1) and a level of significance of 0.05, the tabulated t value is 2.920.