

Faktoren des Arsengehaltes in Reis (*Oryza sativa* L.)

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1. Zusammenfassung

Die As-Konzentrationen im Weißreis aus spanischem (südwestlich von Sevilla) Reisanbau zeigte im Durchschnitt deutlich geringere As-Konzentrationen als Reis aus italienischem (Po-Ebene) Anbau. Ziel dieser Arbeit war es, die Ursachen für die unterschiedlichen As-Konzentrationen im Reis zu finden und umfassende Kenntnisse über die As-Verfügbarkeit im Reisboden und die As-Aufnahme der Reispflanze zu erlangen, um dann Maßnahmen zur Verminderung der As-Konzentration im Reis vorschlagen zu können. Im Rahmen dieser Studie wurden Erhebungsuntersuchungen in zwei Jahren und Gewächshausversuche in 4 italienischen und 2 spanischen Böden sowie in Nährlösung durchgeführt. Folgende Ergebnisse wurden erzielt:

1. Die As-Konzentration im Weißreis kultiviert auf sechs verschiedenen Böden unter Feldbedingungen, variierte in gleichem Maße im Weißreis nach einer Kultur in diesen Böden im Gewächshaus. Dies zeigte, dass Bodenfaktoren entscheidend für die As-Konzentration im Weißreis waren.
2. In den Erhebungsuntersuchungen wurden Boden- und Pflanzenproben von 68 Feldern in Italien und Spanien untersucht. Eine multiple lineare Regressionsanalyse zeigte für die italienischen Böden einen signifikant positiven Einfluss des Gesamtarsengehaltes und pflanzenverfügbaren P-Gehaltes und einen negativen Einfluss des amorphen Fe-Gehaltes des Bodens auf die As-Konzentration im Reisstroh und Weißreis.
3. Im spanischen Boden führten hohe Konzentrationen an pflanzenverfügbaren Si sowie vermutlich die Ausfällung von Arsensulfiden zu niedrigen As-Konzentrationen im Reis.
4. Die As- und Fe-Konzentration in der Bodenlösung während einer Reiskultur in 6 verschiedenen Böden stiegen im Kulturverlauf in enger Beziehung zueinander an, was darauf hinwies, dass sich As nach Reduktion von amorphen Fe-(Hydr)Oxiden von diesen löste. Der überwiegende Teil der amorphen Fe-(hydr)Oxide blieb jedoch in oxidiert Form bestehen und konnte As binden.
5. Die mittlere Kieselsäurekonzentration in der Bodenlösung stand in enger negativer Beziehung zur As-Konzentration im Reisstroh und Weißreis,

während der Einfluss der As-Konzentration in der Bodenlösung auf die As-Konzentration im Weißreis und Reisstroh nicht beständig war.

6. Eine konstant hohe Konzentration an Kieselsäure während der Reiskultur konnte weder durch die Zugabe von Kaliumsilikat zum Boden noch zur Nährlösung erreicht werden aufgrund der Adsorption von Kieselsäure im Boden und der hohen Kieselsäureaufnahme der Pflanze. Die Zugabe von Kaliumsilikat reduzierte die As-Konzentration im Stroh, jedoch nicht im Korn.
7. Reispflanzen, denen As nur bis zur Blüte angeboten wurde, wiesen gleich hohe As-Konzentrationen im Weißreis auf, wie Weißreis von Pflanzen, denen kontinuierlich oder nur nach der Blüte As angeboten wurde. Dies zeigte, dass As gut aus der Wurzel und/oder dem vegetativen Teil ins Korn verlagert wurde.

Aufgrund der Ergebnisse kann eine Selektion von Flächen vorgeschlagen werden, die gleichzeitig einen niedrigen As-Gesamtgehalt, hohen Gehalt an amorphen Fe-(Hydr)Oxiden und geringen pflanzenverfügbaren P-Gehalt im Boden und konstant hohe Kieselsäurekonzentration in der Bodenlösung während der Kultur aufweisen. Die Möglichkeit einer Reduktion der As-Konzentration im Weißreis durch Si-Applikation muss näher untersucht werden.

Stichwörter: Reis, Arsen, Nassreisboden

Abstract

Rice cultivated in Spain (southwest from Seville) showed considerably lower As concentration in polished rice than rice cultivated in Italy (Po area). The aim of this study was to find factors causing the variation in As concentration and to improve knowledge of the plant availability of As in paddy soils and the As plant uptake in order to suggest measures for controlling As uptake. In the framework of this study two field surveys were conducted and experiments in the greenhouse in soil and nutrient solution revealing the following results:

1. The As concentration of polished rice cultivated on six soils differed highly between the soils; however, they were on the same level in both environments, field and greenhouse, and its variation between soils was in the same order indicating that soil was the decisive factor for As concentration in grain.

2. During two surveys soil and plant samples from 68 fields in Italy and Spain were investigated. A multiple linear regression analysis showed a significant positive influence of the total As, and plant available P content and a negative influence of the amorphous Fe content of the soil on the As content in polished rice and rice straw.
3. Reason for the low As concentration in polished rice cultivated in Spanish soils was probably caused by constantly high concentration of silicic acid in soil solution and due to precipitation of arsenic sulphides.
4. There was a close relationship between Fe and As concentration in soil solution during the cultivation period, suggesting that the major part of dissolved As originated from reduced iron-(hydr)oxide. A large part of amorphous iron-(hydr)oxides remained oxidized and had the potential to bind As.
5. With increasing silicic acid concentration in soil solution the As content in polished rice and straw decreased revealing an inhibitory effect of indigenous plant available Si in soil on As uptake. In contrast, the influence of As concentration in soil solution on the As content in grain was not consistent.
6. Silicon application did not result in constantly high silicic acid concentration in solution during cultivation in soil as well as in nutrient solution due to the high plant uptake and in the case of soil additionally due to adsorption of silicic acid in soil. Nevertheless, high Si application clearly reduced the As concentration of straw, however, not of grain.
7. In nutrient solution the As removal at flowering did not reduce As concentration in polished rice compared to plants supplied with As continuously or after flowering. The observations indicate that As was mobilised from the root to the shoot and that it was stored into the grain, although As was not available in nutrient solution.

To control the As concentration in paddy rice, results of this study suggest a selection of fields for low content of total As, plant available P and a high amorphous Fe content of the soil and a constantly high concentration of silicic acid in soil solution during cultivation period. Furthermore, the potential of Si fertilization to lower As uptake should be further investigated.

Key words: rice, arsenic, paddy soil

2. Einleitung

Bedeutung von Arsen für die menschliche Ernährung

Reis aus Nassreisanbau kann erhöhte As-Konzentrationen aufweisen aufgrund der erhöhten As-Pflanzenverfügbarkeit im befluteten Boden. Für die menschliche Ernährung in Europa und den USA stellt Reis eine der As-Hauptquellen dar (Schoof et al., 1999; Tao et al., 1998). Nur Fisch und Meeresfrüchte enthalten mehr As, allerdings in organischer Form, was im Allgemeinen gering toxisch ist. Im Gegensatz dazu kann im Reis bis zu 75% des Gesamtarsens in anorganischer Form vorliegen, welche toxisch ist (Schoof et al., 1999; Heitkemper et al., 2001; Zavala et al., 2008). Die durchschnittliche wöchentliche Aufnahme von anorganischem As wird in Deutschland auf 1 µg/kg Körpergewicht geschätzt, was deutlich geringer ist als die von der WHO vorläufig als tolerierbar festgesetzten As-Aufnahmemenge von 15 µg/kg Körpergewicht. Trotzdem kann für Menschen, die eine spezielle Diät einhalten, die As-Aufnahme von Bedeutung sein. So wies Reismilch, die eine wichtige Rolle spielen kann in der Ernährung von Vegetariern und Allergikern, Konzentrationen an anorganischem As von >10 µg/L auf, und überschritt damit den EU Grenzwert von As im Trinkwasser (Meharg et al., 2008a). Eine besondere Bedeutung kommt auch der auf Reis basierenden Babynahrung zu. Eine Erhebungsuntersuchung in Großbritannien deckte auf, dass die As-Konzentrationen im Babyreis im Mittel bei 0,11 mg/kg lagen und 35% der Proben den chinesischen Grenzwert für Babynahrung überschritten (Meharg et al., 2008b). In der EU und in den USA sind bisher keine Grenzwerte für Nahrungsmittel vorgegeben. Im Gegensatz dazu ist in Australien ein Grenzwert für Lebensmittel von 1 mg As/kg TS festgesetzt. Dieser hohe Grenzwert soll den großen Anteil von Fisch und Meeresfrüchten in der Ernährung der Australier berücksichtigen (Williams et al., 2005). In der europäischen Gesetzgebung ist eine Höchstkonzentration von 10 µg/L Gesamt-As im Trinkwasser zugelassen. In den USA bezieht sich der Grenzwert auf 10 µg anorganisches As/L. Die Trinkwasserrichtlinie in Bangladesch schreibt eine As-Konzentration von weniger als 50 µg/L vor. In vielen Regionen Asiens, wie in Teilen Bangladeshs, Vietnams und Indiens, sind die As-Konzentrationen im Trink- und Bewässerungswasser hoch; örtlich sind Konzentrationen von >1500 µg As/L nachgewiesen worden (Juhász et al., 2006). Das As gelangt aus arsenreichen Flusssedimenten ins Grundwasser und dann durch Brunnen an die Oberfläche. Da das arsenreiche Grundwasser aus

Brunnen auch als Bewässerungswasser im Nassreisanbau genutzt wird (Dittmar et al., 2007), kann dies zu erhöhten As-Konzentrationen im Reis und zu einer Akkumulation im Oberboden von bis zu 58 mg/kg führen (Imamul Huq et al., 2006).

Einfluss von As auf die menschliche Gesundheit

Anorganisches As ist akut toxisch und die Aufnahme von 0,1 g Arsenit kann tödlich sein (Umweltbundsamt, 2003). Eine hohe, nicht tödliche Aufnahme kann zu Magen-Darm-Symptomen, Beeinträchtigung des Herz-Kreislauf-Systems sowie des Nervensystems führen (WHO, 2001). Erfolgt eine langfristige Aufnahme, z. B. über das Trinkwasser, erhöht sich das Krebsrisiko an Haut, Lunge, Blase und Nieren. Des Weiteren können Veränderungen an der Haut auftreten, wie veränderte Pigmentierung und Hyperkeratosen (Umweltbundesamt, 2003). Organische As-Verbindungen, die vor allem über Fisch und Meeresfrüchte aufgenommen werden, wie z.B. Arsenbetain und Arsencholin, werden dagegen fast unverändert 2-3 Tage nach der Aufnahme wieder ausgeschieden (Umweltbundesamt, 2003). Im Gegensatz dazu zeigten neuere Studien, dass das organische MMAIII (monomethylarsonige Säure) toxischer wirkte als Arsenit (Petrick et al., 2000). Auch Dopp (2008) berichtete, dass das organische DMAIII (dimethylarsinige Säure) die As-Form mit der höchsten Membrandurchlässigkeit war und die stärksten cyto- und genotoxischen Effekte induzierte.

Aufgenommenes Arsenat wird größtenteils zu Arsenit reduziert. Beide As-Formen können dann in der Leber methyliert werden. Arsenat kann durch Konkurrenz mit Phosphat die zelluläre ATP-Bildung hemmen (Mandal and Suzuki, 2002). Arsenit bindet an Sulfohydrylgruppen von Enzymen, was zur Deaktivierung von z.B. Pyruvatoxidase, S-Aminosäure Oxidase, Cholinoxidase und Transaminase führen kann.

Arsen im Boden

Das As im Boden stammt vorwiegend aus dem Ausgangsgestein, aus dem der Boden entstanden ist (Yan Chu, 1994). Die durchschnittliche As-Konzentration in Gesteinen variiert deutlich und beträgt 1.0, 2.5, 7 und 10 mg As/kg in Sandstein, Kalkstein, Löss und Tonboden (Schachtschabel et al., 1998). Dementsprechend weisen fruchtbare Löss- und Tonböden im Allgemeinen höhere As-Konzentrationen auf als Sandböden. Der Gesamtarsengehalt von nicht kontaminierten Böden liegt

zwischen 1-20 mg/kg (Schachtschabel et al., 1998). In der Bodenlösung wurden vor allem die As-Formen Arsenat, Arsenit, DMA (Dimethylarsenat) und MMA (Monomethylarsenat) nachgewiesen. Unter mäßig reduzierenden Bedingungen bei einem Redoxpotential von +300 mV bei pH 4 bis -200 mV bei pH 9 ist Arsenit stabil, während Arsenat unter oxidierenden Bedingungen stabil ist (Inskeep et al., 2002). Arsenat kann an der Oberfläche von Tonmineralen, Fe-, Mn- und Al-(Hydr)oxiden adsorbieren. Eine wichtige Senke für Arsenit und Arsenat sind Fe-(Hydr)oxiden, wie z.B. Ferrihydrat und Goethit (Inskeep et al., 2002). Daneben kann Arsenat in festen Phasen als Fe-, Mn- und Ca-Arsenat auftreten, während Arsenit mit Sulfiden als z.B. Orpiment (As_2S_3), amorphes As_2S_3 oder Arsenpyrit (FeAsS) ausfallen kann. Diese Arsensulfide können für die Festlegung von Arsenit unter reduzierenden Bedingungen bedeutsam sein (Rittle et al., 1995). Da Phosphat und Arsenat sich chemisch ähnlich sind, konkurrieren sie um die gleichen Bindungsstellen im Boden (Sadiq, 1997). So zeigte Smith et al. (2002), dass eine hohe Phosphatkonzentration in der Bodenlösung die As-Konzentration erhöht.

Arsendynamik im Boden nach Beflutung

Die Beflutung des Bodens wirkt sich auf die Pflanzenverfügbarkeit des Arsens aus. Wenn der Sauerstoff im Boden verbraucht ist, nutzen fakultativ anaerobe Mikroorganismen oxidierte Verbindungen als Elektronenakzeptor. Dabei kommt es in einer bestimmten Reihenfolge zu aufeinander folgenden Reduktionsschritten im Boden. Das Verhältnis von oxidierten zu reduzierten Stoffen wird mithilfe des Redoxpotentials beschrieben, das zwischen +800 mV (in belüfteten Böden) und -300 mV (stark reduzierendes Milieu nach längerer Beflutung) schwankt (Schachtschabel et al., 1998). Bei einem Redoxpotential von etwa 400 mV beginnt der Prozess der Nitratreduktion, darauf folgt bei etwa 200 mV die Reduktion von Mn-(Hydr)Oxiden, gefolgt von der Reduktion von Fe-(Hydr)Oxiden ab etwa 150 mV. Ab einem Redoxpotential von etwa -50 mV beginnt die Reduktion von Sulfat zu Sulfid (Schachtschabel et al., 1998). Die Reduktion von Arsenat zu Arsenit beginnt ab 100 mV und ist am stärksten bei -50 mV. Unter diesen Bedingungen ist die Bildung von schwerlöslichen Arsensulfiden möglich.

Nach der Beflutung geht also das zunächst an (Hydr)Oxiden gebundene As zusammen mit den reduzierten Elementen in Lösung und wird damit pflanzenverfügbar. Dabei spielen vor allem die Fe-(Hydr)Oxide eine wichtige Rolle,

da diese im Boden im Allgemeinen in großen Mengen vorkommen (Masscheleyn et al., 1991; Onken and Hossner, 1996; McGeehan and Naylor, 1994). Nach O'Neill (1995) werden Arsensulfide vor allem bei hohem Vorkommen von Sulfat und niedrigem Redoxpotential von mindesten -150 mV gebildet. Das bedeutet, dass in den meisten Reisböden eine Bildung von Arsensulfiden wegen niedriger Sulfidgehalte unwahrscheinlich ist (O'Neill, 1995). Allerdings können in vom Meerwasser beeinflussten Gewässern und in marinen Sedimenten erhebliche Sulfid-Gehalte auftreten, was zur Bildung von Arsensulfiden führt (Moore et al., 1988).

In der Bodenlösung eines befluteten Reisbodens wurde überwiegend Arsenit nachgewiesen, neben Arsenat und geringeren Konzentrationen an methyliertem As (Takahashi et al., 2004; Xu et al., 2008; Abedin et al., 2002a). Es ist schwierig die möglichen Adsorptions-, Desorptions- und Fällungsprozesse, die die As-Konzentration in der Bodenlösung während einer Nassreiskultur beeinflussen, in einer Methode abzubilden. Dagegen könnte die Ermittlung von Bodeneigenschaften, die die As-Akkumulation maßgeblich beeinflussen, eine Selektion von Feldern ermöglichen, die zu einer geringeren As-Konzentration im Weißreis führen.

Arsen in der Rhizosphäre und Arsenmetabolismus in der Pflanze

Reispflanzen sind durch die Bildung des Aerenchymys an beflutete Böden angepasst. Dieses Gewebe besteht aus großen luftgefüllten Interzellularen und ermöglicht es der Pflanze, dass atmosphärischer Sauerstoff in die Wurzel gelangt. Dieser Prozess kann die Pflanzen vor toxischen H_2S - und Fe-Konzentrationen schützen. Ein Teil des Sauerstoffs tritt aus der Wurzel aus, was zu einer Erhöhung des Redoxpotentials von -100 auf +400 mV in der Rhizosphäre führen kann (Flessa und Fischer, 1992), so dass Fe oxidiert wird und an der Wurzeloberfläche einen Fe-Belag, sog. Fe-Plaque, bildet. Untersuchungen von Hansel et al. (2001) zeigten, dass die Fe-Plaque vorwiegend aus Ferrihydrit und geringeren Mengen an Goethit bestand. Diese haben eine hohe Affinität zu As, so dass die Fe-Plaque eine As-Barriere für die As-Aufnahme darstellen kann. Daneben ist denkbar, dass die Fe-Plaque als As-Pool dienen kann, weil die Pflanze organische Anionen ausscheiden kann, die As von den Bindungsstellen verdrängen und damit zur Aufnahme verfügbar machen. So wurden unterschiedliche Auswirkungen von Fe-Plaque auf die As-Aufnahme gezeigt (Chen et al., 2004; Liu et al., 2005).

Anorganisches As wird weitaus besser aufgenommen als organisches As (Abedin et al., 2002a). Arsenat wird über einen Phosphattransporter aufgenommen (Ullrich-Eberius et al., 1989), größtenteils rasch über eine Arsenat-Reduktase in der Wurzel zu Arsenit reduziert (Dhanker et al., 2006; Duan et al., 2007) und dann teilweise aus der Wurzel ausgeschieden möglicherweise über eine Effluxpumpe (Xu et al., 2007). Zudem kann ein Teil des Arsenits durch Glutathion und/oder Phytochelatine komplexiert werden und dann in die Vakuole abgeschieden werden (Pickering et al., 2000).

Neueste Studien zeigten, dass Arsenit von Reis über dieselben Transporter Lsi1 und Lsi2 aufgenommen wird wie Kieselsäure (Ma et al., 2008; Bienert et al., 2008). Lsi1 wurde als ein aquaporinähnliches Protein identifiziert, das in Exo- und Endodermis distal gelegen ist und eine passive Aufnahme vermittelt (Ma et al., 2006). Lsi2 ist dagegen proximal in der Exo- und Endodermis gelegen und vermutlich ein energieabhängiger Anionentransporter (Ma et al., 2007a). Über die Aufnahme hinaus scheinen Si-Transporter für die Verteilung des As in der Pflanze verantwortlich zu sein. So konnte gezeigt werden, dass die Beladung des Xylems über Lsi2 erfolgte und ein weiterer Si-Transporter Lsi6 an der Entladung aus dem Xylem und die Verteilung ins Blattgewebe beteiligt ist. Auch Lsi6 zeigte eine, wenn auch schwache, Transportfähigkeit für Arsenit (Bienert et al., 2008).

Es ist nicht bekannt, ob As in der Reispflanze mobilisiert werden kann, was die Auswirkung einer kurzfristigen Maßnahme zur Senkung der As-Aufnahme, wie z.B. eine kurzfristige Trockenlegung der Felder, verringern würde.

Wirkung des As auf die Pflanze

Die As-Wirkung bei Pflanzen hängt, wie beim Menschen, von der vorliegenden As-Spezies ab. Arsenat kann Phosphat ersetzen und damit die Bildung von ATP in der Zelle hemmen. Arsenit bindet an Sulhydrylgruppen, was zur Hemmung von einer Reihe von Enzymen führt. Das erste Anzeichen einer As-Toxizität ist Blattwelke gefolgt von Nekrosen an der Blattspitze und am Blattrand (WHO, 2001). Diese Symptome deuten eine Hemmung der Wasseraufnahme oder der Wasserbewegung innerhalb der Pflanze an. Bei Reispflanzen wurde zudem von einer stark reduzierten Bestockung bei As-Angebot berichtet (Chino, 1981). Des Weiteren wird die an Reispflanzen auftretende physiologische Funktionsstörung ‚Straighthead‘ mit der As-Aufnahme in Verbindung gebracht (Marin et al., 1992; Williams, 2003), jedoch ist der

genaue ursächliche Zusammenhang nicht bekannt. Diese Pflanzen weisen eine geringe Anzahl an ausgebildeten Körnern auf, was dazu führt, dass die Rispe bei Reife aufrecht steht. ‚Straighthead‘ tritt vorwiegend nach einer Baumwollkultur auf, die mit arsenhaltigen Pflanzenschutzmittel behandelt wurde. Daneben wurden die Symptome auch nach der Einarbeitung von frischer organischer Substanz in den Boden beobachtet (Williams, 2003).

Im Gegensatz zu den genannten Schadsymptomen wurde auch von einer positiven Wirkung von As auf Pflanzen berichtet. So förderte ein DMA-Angebot in der Nährlösung den Trockenmasseertrag von Reis (Marin et al., 1992). Die Ursache dafür ist nicht bekannt.

Der Einfluss von Kieselsäure auf die Arsenitaufnahme von Reis

Das Angebot von Kieselsäure zur Nährlösung einer Reiskultur verringerte deutlich die As-Aufnahme der Reispflanzen (Guo et al., 2005; Guo et al., 2007). Ursache dafür ist, dass Arsenit über die gleichen Transporter Lsi1 und Lsi2 aufgenommen wird wie Kieselsäure (Ma et al., 2008; Bienert et al., 2008). Reis gehört zu den Si-akkumulierenden Pflanzen und enthält doppelt soviel Si wie N (Ma und Takahashi, 2002). Daher erfolgt die Aufnahme über die Si-Transporter sehr effizient und deren Expression wird zudem stark reguliert. So verringerte ein Kieselsäureangebot von 1 mM Si über 3 Tage die Expression von Lsi1 und Lsi2 um 75%. Dies könnte auch die verringerte Arsenitaufnahme erklären. Über eine hemmende Arsenitaufnahme hinaus wurde auch von einer hemmenden Wirkung von Kieselsäure auf die Aufnahme von Cd (Shi et al., 2005; Zhang et al., 2008), Na (Gong et al., 2006; Yeo et al., 1999), P, Ca, Fe und Mn (Ma and Takahashi, 1989; Ma and Takahashi, 1993) berichtet. Dies wurde auf einen reduzierten apoplastischen Fluss zurückgeführt, verursacht durch Si-Ablagerungen im Apoplasten der Reisswurzel.

Besonderer Hintergrund dieser Studie

Zu Beginn dieser Studie gab es Hinweise auf niedrige As-Konzentrationen im Weißreis aus spanischem Anbau (südwestlich von Sevilla) im Vergleich zu Weißreis aus italienischem Anbau (Po-Ebene). Zudem zeigten Erhebungsuntersuchungen, dass die As-Konzentration im Weißreis aus italienischem Anbau kleinräumig stark variieren konnte, z.B. im Weißreis von nebeneinander liegenden Feldern. Vor dem

Hintergrund dieser Hinweise und basierend auf dem Kenntnisstand aus der Literatur ergaben sich für die Studie folgende Aufgabenstellungen:

- Untersuchung des Einflusses von Kulturmaßnahmen auf die As-Akkumulation von Reis (s. Schlussbetrachtung).
- Ermittlung von Bodeneigenschaften, die die As-Akkumulation von Reis beeinflussen (Kapitel 3)
- Untersuchung der As-Dynamik und der Dynamik von mit der As-Verfügbarkeit in Verbindung gebrachten Elementen, wie Fe, Mn, P und Kieselsäure, in der Bodenlösung während der Nassreiskultur in Verbindung mit der As-Akkumulation im Reis (Kapitel 4).
- Untersuchung des Einflusses der Si-Applikation auf die As-Akkumulation im Reis (Kapitel 5).
- Untersuchung der Mobilisierung von As in der Reispflanze und des Zeitpunktes der As-Einlagerung ins Korn (Kapitel 6).

3. Evaluation of soil characteristics potentially affecting arsenic concentration in rice (*Oryza sativa* L.)¹

Abstract

Paddy rice may contain high As levels and contribute considerably to the human intake of As. The knowledge of soil characteristics affecting the As content of the rice plant enables the evaluation of the possible As transfer from soil to rice and the development of agricultural measures for controlling As uptake.

During field surveys in 2004 and 2006, soil and plant samples from 68 fields were analysed for As content revealing markedly differing As concentration in polished rice ranging from 50 to 322 $\mu\text{g As/kg d.m.}$ The soil factors total $\text{As}_{(\text{aqua regia})}$, pH, grain size fractions, organic C, plant available $\text{P}_{(\text{CAL})}$, amorphous $\text{Fe}_{(\text{oxal.})}$ and plant available $\text{Si}_{(\text{Na-acetate})}$ content (silicic acid) that potentially affect As content of rice were determined.

In simple linear regression analysis none of the determined soil factors significantly influenced the As concentration in straw and grain. However, a multiple linear regression analysis showed a significant positive influence of the total $\text{As}_{(\text{aqua regia})}$ and plant available $\text{P}_{(\text{CAL})}$ content and a negative influence of the amorphous $\text{Fe}_{(\text{oxal.})}$ content of the soil on the As content in polished rice and rice straw.

Soil silicic acid extracted with the Na-acetate buffer method did not reflect plant availability of silicic acid well, since the relationship to Si content in rice straw was weak. However, Si concentration in rice straw varied widely and was negatively related to As content in straw and polished rice.

Introduction

An applicable extraction method for determination of plant available As in flooded soils does not exist. However, to evaluate the potential of a soil resulting in low or high As content in rice is of great importance since As concentration in paddy rice may contribute considerably to human intake of As (Schoof et al., 1999). Soil parameters potentially affecting As accumulation in rice are the total As content, concentration of amorphous iron-(hydr)oxides, plant available phosphate, soil texture and the plant available Si. A high total arsenic content in the soil may affect the plant

¹ Accepted by Environmental Pollution, Elsevier

available arsenic. Besides that, it is well described in literature that arsenic is mainly bound to iron-(hydr)oxides in the soil (Inskeep et al., 2002). Due to flooding of paddy rice soil and the subsequent decrease of redox potential the bound arsenic is released. Thus, the concentration of iron-(hydr)oxides may have an influence on the As concentration in soil solution. In the literature a competition between phosphate and arsenate is often described because of chemical similarities. Phosphate may displace arsenate from binding sites in the soil and thus, increase arsenic availability and As uptake of plant (Sadiq, 1997). On the other hand plants take up phosphate and arsenate by the same transporter (Ullrich-Eberius et al., 1989). Therefore, phosphate may compete with arsenate for uptake which would result in a decrease of As content in the plant (Meharg and Macnair, 1990). A further characteristic affecting As concentration in rice could be the soil texture. Soils which contain a high percentage of clay minerals may contain more arsenic. Arsenic may be bound to hydrated surfaces and clay contains a high proportion of iron-, manganese- and aluminium-oxides at which surfaces arsenic could be bound (Sadiq, 1997). This could result in a higher mobilisation of As in clayey soils. In addition to this, previous studies showed that high silicic acid concentration in soil solution as well as in nutrient solution reduced the plant As uptake (Guo et al., 2005; Guo et al., 2007; chapter 4). Therefore, the plant available silicic acid content of the soil may affect the As content of rice. Besides the plant available silicic acid content in soil, the silicic acid concentration of irrigation water may influence the plant As uptake as it highly contributed to the silicic acid uptake of the Si accumulator rice (Ma and Takahashi, 2002; Desplanques et al., 2006). The aim of this study was to characterize the contribution of these soil characteristics on arsenic content of rice.

Materials and Methods

Soil and plant samples

Soil and plant samples were taken from 33 and 35 Italian fields located in the Po-area in 2004 and 2006, respectively. The sampling was conducted from 4 random points from each field and merged to a composite sample. From each point 20 plants were harvested and soil samples were taken to a depth of 30 cm. *Oryza sativa* L. cv. Selenio was sampled which was cultivated according to the common agricultural practice in the Po-area. Plants were harvested at maturity about 5 months after planting. Rice grains were separated from the straw which was cut 5 cm above the

soil. Rice straw was dried for three days at 60°C and milled afterwards. Rice grains were dried at 40°C over night to reduce moisture content in order to prevent fungi infestation, then grains were processed to white rice. Soil samples were air-dried and homogenized using a jaw crusher.

Chemical analysis

Soils were characterised for particle-size distribution by fractionation using sieving and sedimentation (DIN ISO 11 277, LUFA Hameln), pH in 0.01 M CaCl₂ measured with a pH-Electrode (Sen Tix 41, WTW) (Schlichting, 1995), total As by aqua regia extraction according to DIN 38414,S7 (VDLUFA 1991), oxalate soluble Fe according to Schwertmann (1964). The total C was analysed by a CNS-Autoanalyser (VarioEL, Elementar, Hanau, Germany). The carbonate content of the soil was determined with the aid of the Scheibler-equipment (DIN 19684 T5) (Schlichting, 1995). Organic C was calculated by subtraction of carbonate-C from total-C content. The plant available P was determined by the Ca-acetate-lactate method according to Schüller (1969). Plant available silicic acid was determined by the extraction with Na-acetate (Ma and Takahashi, 2002). Sulphur in soil saturation extract was determined by ICP-OES (Spectro flame-EOP, Kleve, Germany).

Dried and milled straw (350 µg) and polished rice (480 µg) were digested with 4 mL HNO₃ (65%) and 1.5 mL H₂O₂ (30%) in a microwave (ETHOSplus, MLS GmbH, Germany) for 20 and 15 minutes at 190°C, respectively, and total As content was measured with ICP-MS 7500c (Agilent Technologies, USA).

Statistical analysis

Analysis of variance, correlation analysis and multiple linear regressions were conducted by using SAS (SAS Institute INC, Cary, USA). Comparison of means was carried out according to Tukey test.

Results

In 2004 the total As_(aqua regia) content in the soil was within the range of commonly found As concentration in soils of 1 to 20 mg As/kg (Schachtschabel et al., 1998) (Table 1).

Table 1: Soil characteristics of sampled soils during the surveys in 2004 and 2006.

	2004		2006	
	mean	range	mean	range
total As_(aqua regia) [mg/kg]	8.3	3.2 – 16.4	10.1	2.6 – 35.7
pH	5.7	5.0 - 6.4	5.8	5.1 – 7.4
Clay [%]	17	5 – 28	18.8	5.1 – 37.1
Silt [%]	40	16 – 66	41.6	17.0 – 70.6
Sand [%]	43	13 – 67	39.7	7.2 – 77.0
Org. C [%]	1.19	0.69 – 1.81	-	-
Plant available P_(CAL) [mg/kg]	43	12 - 79	51.1	7.3 – 137.8
Amorphous Fe_(oxal.) [g/kg]	4.8	1.3 – 9.0	5.8	1.9 – 11.6
Si_(Na-acetate) [mg/kg]	35	7 – 95	31.9	6.6 – 79.0
Number of fields	33		35	

In 2006, one field had a considerably higher As content (35.7 mg/kg). Reasons for this are not known. Furthermore, some fields with higher P content were observed in 2006. The range and mean of all other characteristics were similar in both years. The Na-acetate extracted silicic acid content was only weakly related to the plant Si content (Figure 1).

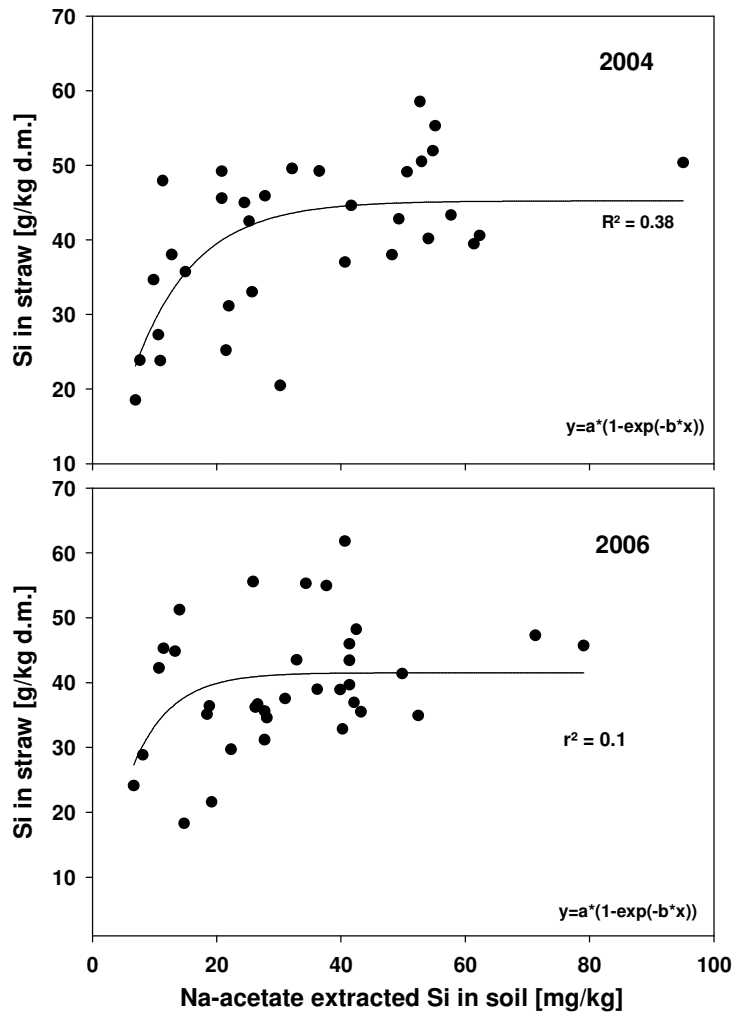


Figure 1: Relationship between Na-acetate extractable Si content in soil and Si concentration in straw during field survey 2004 and 2006.

However, the plant Si content was significantly negatively related to the As concentration in straw and polished rice (Figure 2 A and B). The As concentration in polished rice ranged from 50 to 322 $\mu\text{g}/\text{kg}$ with mean As concentration of 149 $\mu\text{g}/\text{kg}$ (Figure 3). The relationship between the As concentration in straw and polished rice was fairly close in both years (Figure 3).

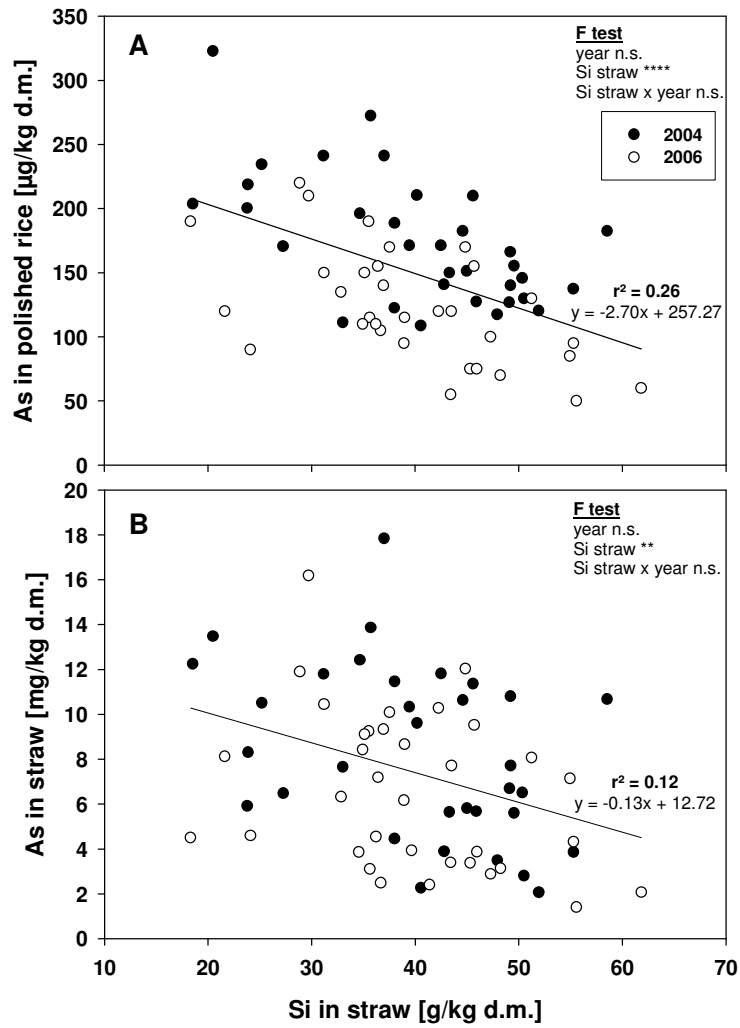


Figure 2: Relationship between Si concentration in straw and As concentration in polished rice (A) and straw (B). ****, ** and n.s. indicate significance at $p < 0.0001$, $p < 0.01$ and not significant, respectively.

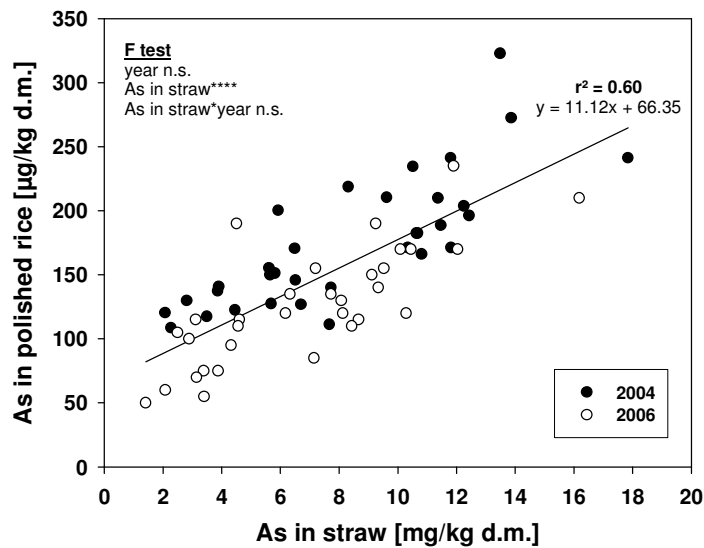


Figure 3: Relationship between As concentration in straw and polished rice from field survey 2004 and 2006. **** and n.s. indicate significance at $p < 0.0001$ and not significant, respectively.

Simple linear regression between the determined soil characteristics and As concentration in straw or grain gave no indication for a significantly influencing factor. As the soil of field samples differed in several characteristics potentially affecting As dynamics, data were subjected to a multiple linear regression analysis. The multiple linear regression analysis revealed that the $As_{(aqua\ regia)}$ content, amorphous $Fe_{(oxal.)}$ and plant available $P_{(CAL)}$ content of the soil significantly influenced the As content in polished rice and in straw in 2004 and 2006 (Table 2). In 2006, the contribution of the soil parameters to explain the As concentration in polished rice as well as in straw was considerably lower than in 2004. For visualisation, the data calculated by multiple linear regression model in 2004 and 2006 were related to the observed As concentration in polished rice and straw in both years (Figure 4).

Table 2: Results of the multiple linear regression analysis for polished rice and rice straw in 2004 and 2006

	2004	2006
Contribution of the model to explain As in polished rice (adjusted R ²)	53%	37%*
Significant influence	<ul style="list-style-type: none"> • total As_(aqua regia) • amorphous Fe_(oxal.) • plant available P_(CAL) 	<ul style="list-style-type: none"> • total As_(aqua regia) • amorphous Fe_(oxal.) • plant available P_(CAL)
Contribution of the model to explain As in rice straw (adjusted R ²)	77%	37%*
Significant influence	<ul style="list-style-type: none"> • total As_(aqua regia) • amorphous Fe_(oxal.) • plant available P_(CAL) 	<ul style="list-style-type: none"> • total As_(aqua regia) • amorphous Fe_(oxal.) • plant available P_(CAL)

*excluding one outlier

Equation of the linear multiple regression model: Arsenic in polished rice (y) = a₀ + a₁*x₁ + a₂*x₂ + a₃*x₃ + a₄*x₄.

		2004		2006	
		Polished rice	Straw	Polished rice	Straw
		Parameter estimate			
a ₀	constant	159.80448	3.98388	96.98692	3.87494
a ₁	Total As _(aqua regia)	8.86498	0.84462	3.97028	0.40875
a ₂	Amorphous Fe _(oxal.)	-17.88264	-1.32219	-7.42849	-0.52947
a ₃	Plant available P _(CAL)	0.67347	0.08871	0.62535	0.03802

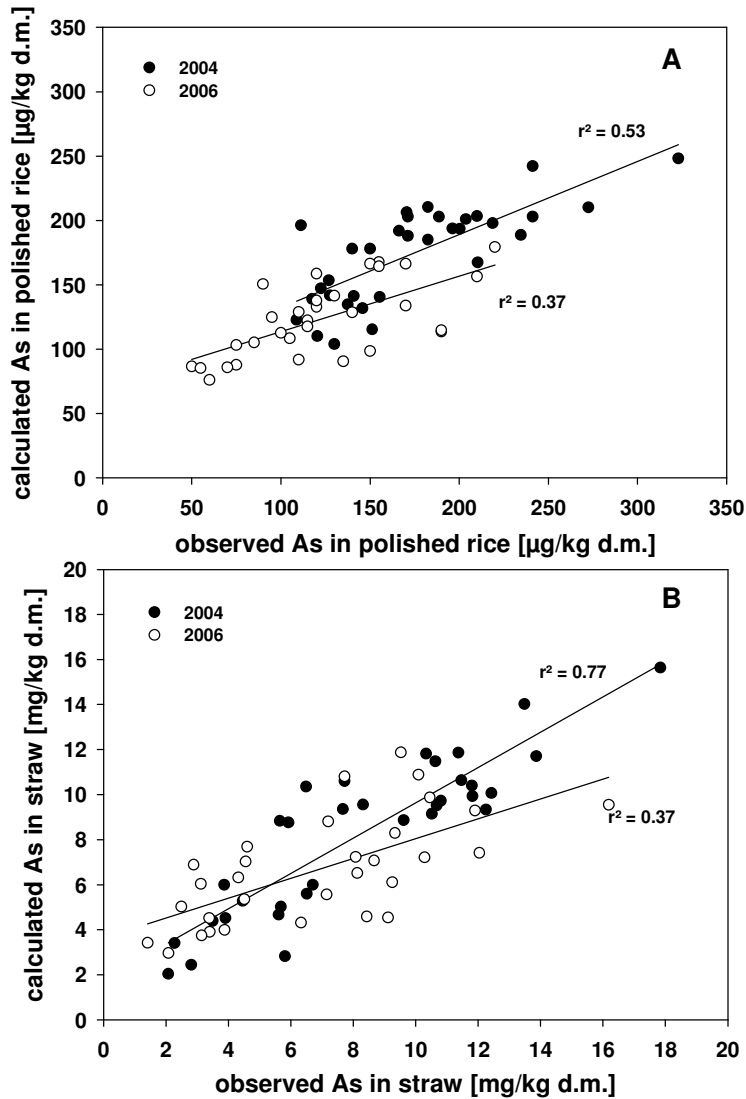


Figure 4: Relationship between the observed and calculated As concentration in polished rice (A) and straw (B) in the field survey in 2004 and 2006.

(*adjusted r^2 of the multiple linear regression analysis)

In order to demonstrate the effect of soil parameter variation the model of the year 2004 was used to calculate the As concentration in polished rice influenced by the total $As_{(aqua\ regia)}$ content of the soil, the amorphous $Fe_{(oxal.)}$ and plant available $P_{(CAL)}$ content of the soil (Figure 5, A and B). All soil parameters used for this calculation are in the range found in the field survey 2004. An increase of total $As_{(aqua\ regia)}$ from 4 to 14 mg/kg soil resulted in an increase of 86 µg As/kg d.m. in polished rice. An increase of amorphous $Fe_{(oxal.)}$ content from 2 to 8 g/kg soil decreased As content of

polished rice by 104 $\mu\text{g As/kg}$ (Figure 5 A), whereas the increase of plant available $\text{P}_{(\text{CAL})}$ enhanced the As concentration in polished rice (Figure 5 B).

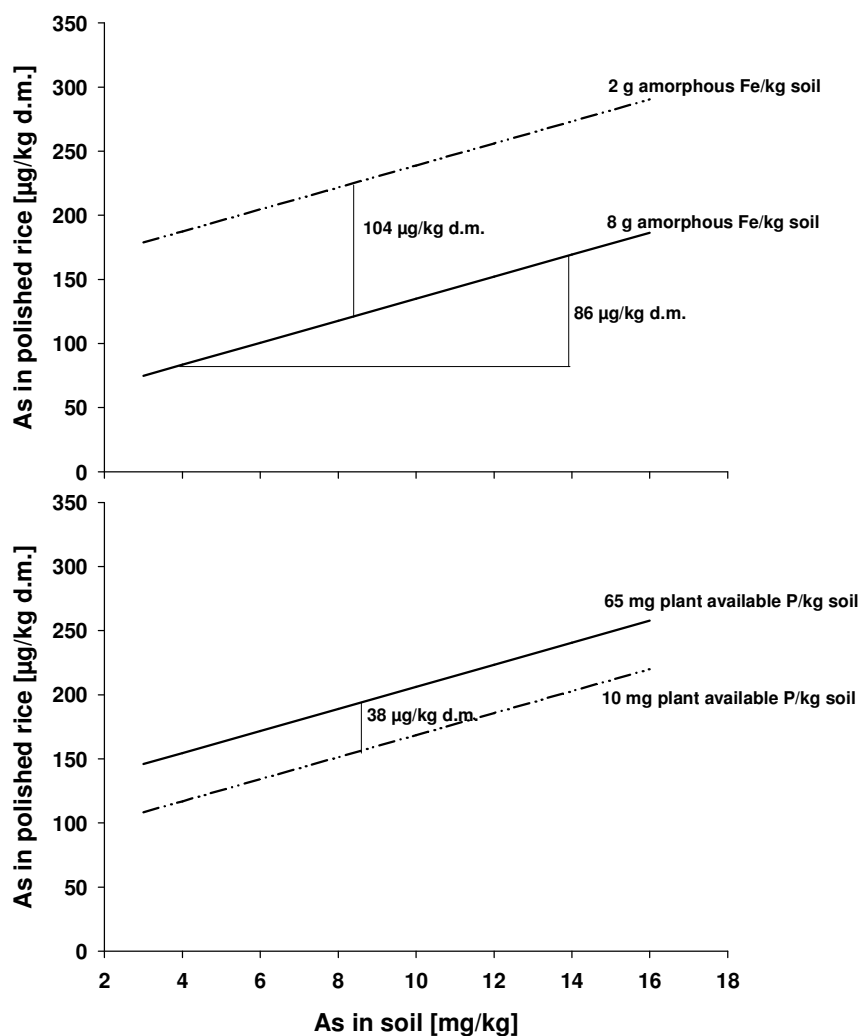


Figure 5: Calculated As content in polished rice as affected by total $\text{As}_{(\text{aqua regia})}$ in soil at two levels of amorphous $\text{Fe}_{(\text{oxal})}$ content (A) and two levels of plant available P content (B). Field survey 2004.

Discussion

Silicic acid in the soil and the Si and As content of the plant

To evaluate the plant availability of silicic acid, the Na-acetate buffer method was chosen, since a close linear relationship was found between the Si content in rice straw and the extracted Si up to 100 mg Si/kg soil (Ma and Takahashi, 2002).

However, in this study the relationship between the Na-acetate extractable silicic acid in soil and the Si concentration in straw was poor and best described by a saturation function (Figure 1). According to de Lima Rodrigues (2003), the critical value for plant available silicic acid extracted by Na-acetate is 60 mg/kg soil. In this study an effect of Na-acetate-extractable silicic acid on the Si content in rice straw was visible up to approximately 30 mg/kg soil. Furthermore, the relationship between silicic acid extracted by Na-acetate and plant Si content was not as close as observed by Ma and Takahashi (2002). The reason for this inconsistency might be that the silicic acid concentration of the irrigation water probably affects the critical value as it strongly contributes to the silicic acid plant uptake (Ma and Takahashi, 2002; Desplanques et al., 2006).

Information about the critical and optimum level of Si concentration in rice straw varies in literature. Most of the straw samples in this study contained below 50 g Si/kg d.m. which is considered as the critical level for Si deficiency in rice by Dobermann and Fairhurst (2000). The optimum Si concentration is considered to be 80-100 g Si/kg d.m. in rice straw at maturity by the same authors. Studies of Ma and Takahashi (2002) indicated no further increase of yield through Si fertilization at 60 g Si/kg in straw at harvest and a possible deficiency at Si concentration of lower than 35 g/kg in straw.

In chapter 4 it was clearly shown that a high silicic acid concentration in soil solution inhibited the As uptake of rice plants and decreased the As concentration in straw and grain. These findings are supported by the negative relationship between the Si content in straw and As content in straw and polished rice observed in this study (Figure 2A and B). Recently, Ma et al. (2008) revealed that the Si transporter Lsi1 and Lsi2 mediate arsenite uptake as well. Thus, the decreased As concentration in rice may be related to the reduced expression of the Si transporter caused by Si supply (Ma et al., 2006; Ma et al., 2007).

Amorphous Fe_(oxal.) content in soil and As content in rice

The data of the field survey showed that with increasing amorphous Fe_(oxal.) content in the soil the As content in polished rice decreased (Table 2, Figure 5 A). The oxalate solution of the extraction method dissolves mainly amorphous forms of Fe-oxide (Blakemore et al., 1987). It is well known that the mobility of As in soils strongly depends on the sorption of As on amorphous Fe-oxides (Inskeep et al., 2002).

Arsenate (AsV) as well as arsenite (AsIII) bind via ligand exchange reaction on Fe-oxides replacing OH⁻ groups. After flooding, amorphous Fe-oxides are reduced and dissolve allowing the release of As into the aqueous phase. However, only a small fraction of amorphous Fe-oxides is available for microbial reduction. Own results of previous greenhouse experiments indicated that less than 1% of the oxalate soluble Fe was reduced during cultivation (Bogdan and Schenk unpublished). This is in agreement with Van Bodegom et al. (2003) who observed that oxalate extracted more Fe than was dissolved during 2-3 months incubation of 18 paddy soils. Thus, a large part remains oxidized (Qu et al., 2004) and does not release As, it is even able to bind the As released from the reduced Fe-oxides. Therefore, even though Fe has a potential to release substantial amounts of As into the soil solution, its potential to bind As was predominant in the investigated soils. Thus, it is suggested that a high Fe_(oxal.) content in soil removes As from soil solution and reduces As plant availability. In addition, Fe(II) may also impair the phytoavailability of As, as recent laboratory studies showed the formation of innersphere complexes between As(III) and Fe(II) (Thoral et al., 2005). However, it has to be clarified under which circumstances and in which magnitude As(III)/Fe(II) complexes are formed in the field and their possible effect on the As uptake of the plant has to be investigated.

Plant available P_(CAL) and As content in rice

The multiple linear regression analysis indicated a positive influence of plant available P on the As content in rice straw and polished rice (Table 2, Figure 5 B). This is in agreement with Woolson et al. (1973) who observed an increase of As content in maize plant after P fertilization. In the literature the effect of P on As availability is widely discussed with respect to As mobilisation in the soil and As uptake. Phosphate and arsenate are chemically very similar and therefore occupy the same sorption sites in soils. In particular, both are strongly adsorbed on Fe-oxides via ligand exchange mechanism (Smith et al., 2002). Smith et al. (2002) demonstrated that phosphate was competing with arsenate as well as with arsenite for sorption sites leading to a decreased amount of adsorbed As and consequently to higher As concentration in soil solution. Therefore, also the plant availability of arsenite might be increased in the presence of phosphate. However, the effect depended on the sorption capacity of the soil. Besides competition in the soil, phosphate may also interact at binding sites at the Fe-plaque on the root which is formed due to the

release of oxygen from the roots. Geng et al. (2005) showed that increasing P concentration from 50-300 μM in nutrient solution decreased the As content in Fe-plaque indicating a competition of phosphate and arsenate for binding sites of the Fe-plaque. This resulted in an increased As content in root and shoot. Under field conditions the influence of P concentration in soil solution on the binding of As at Fe-plaque on the root surface has not yet been investigated. However, considering plant As uptake, phosphate has an inhibitory effect due to competition for the same transporter in the plasma membrane (Ullrich-Eberius et al., 1989). In several studies it was shown that increasing phosphate concentration in solution inhibited the arsenate but not arsenite uptake of the plant (Asher and Reay, 1979; Meharg and Macnair, 1990; Irtelli and Navari-Izzo, 2008). Although under paddy field condition As occurs to more than 75% in form of arsenite (Chapter 4, p. 34), it is not known if arsenite may be partly oxidized to arsenate in the root rhizosphere, as the rice root is known to develop an oxidized root zone (Armstrong, 1967).

According to the multiple linear regression analysis in the present study As-mobilization processes due to an increased P-soil level were stronger than the phosphate competition for arsenate uptake.

Total As content in the soil and As content in rice

The multiple linear regression analysis showed a significant positive influence of the total $\text{As}_{(\text{aqua regia})}$ content in the soil on the As content in rice (Table 2, Figure 5 A and B). However, this is due to the fact that the model considered the amorphous Fe and plant available P content in the soil at the same time. In the field surveys the simple linear correlation between the total As in soil and As in polished rice and rice straw was very weak with $r^2=0.04$ and $r^2=0.14$ in 2004 and $r^2=0.03$ and $r^2=0.12$ in 2006, respectively (data not shown). This is in agreement with literature which states that the uptake of As by rice plants mainly depends on the As availability rather than on the total As in soil (Sheppard, 1992; Xie and Naidu, 2006). However, the total As content in soil may affect the As availability by providing As that may become available depending on soil parameters, such as content of amorphous $\text{Fe}_{(\text{oxal.})}$ and plant available $\text{P}_{(\text{CAL})}$ as well as silicic acid availability.

Conclusion

To control the As concentration in paddy rice, results of this study suggest a selection of fields for low content of total $As_{(aqua\ regia)}$ and a high amorphous $Fe_{(oxal.)}$ content of the soil. Furthermore, fertilizer management such as low P application and Si fertilization may further decrease the As concentration in grain.

4. Arsenic in rice (*Oryza sativa* L.) related to dynamics of arsenic and silicic acid in paddy soils

Abstract

Paddy rice is a global staple food which in some circumstances can contain high levels of the toxic element arsenic (As). In order to elucidate factors influencing As dissolution in the soil solution during paddy rice cultivation, rice (*Oryza sativa* L. 'Selenio') was cultivated to maturity in six paddy soils in the greenhouse in 2005 and 2006. Concentrations of Mn, Fe, As, P and silicic acid in soil solution and As concentrations in rice straw and polished rice grain were determined. There was a close relationship between Fe and As concentrations in the soil solution, suggesting that the major part of dissolved As originated from reduced iron-(hydr)oxide. However, in addition to the factors causing As dissolution in the soil, other factors influenced the uptake of As by rice. The inhibitory effect of indigenous silicic acid in the soil solution on As uptake was clearly shown. This implied that soils with high plant available Si contents resulted in low plant As contents and that Si application to soils may decrease the As content of rice.

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5. Influence of silicic acid on the As concentration in paddy rice (*Oryza sativa* L.) cultivated in 6 soils and in nutrient solution

Abstract

Arsenic concentration in rice is considerably higher compared to other cereals and bears a potential risk to human health. Silicic acid was shown to reduce the As concentration in rice straw suggesting Si application as a tool for As reduction in grain. Rice (*Oryza sativa* L.) was cultivated in the greenhouse to maturity in four Italian and two Spanish paddy soils and in nutrient solution. The soil was fertilized with three different rates of potassium silicate. In nutrient solution, rice was cultured with three As levels (0, 0.05 and 0.1 mg/L) and three Si levels (0, 10 and 40 mg/L).

The silicic acid concentration in soil solution differed between soils. The fertilization of Si increased the silicic acid concentration in soil solution. Irrespective of Si fertilization, the silicic acid concentration in soil solution increased after flooding probably due to the release of silicic acid during reduction of iron-(hydr)oxides and the dissolution of bioopal. After six weeks of cultivation the silicic acid concentration in soil solution of Italian soils decreased most likely as a result of high Si uptake of the plant. The effect of Si application on the Si content of straw was markedly higher in soils having low Si concentrations in straw in the control. The Si content of straw was generally above the critical level of 35 g Si/kg d.m.. The As concentration was between 0.7 to 13.5 mg As/kg d.m. and 66 to 360 µg As/kg d.m. in straw and polished rice, respectively, and differed between soils. The soils having high silicic acid concentrations resulted in low As content in straw and grain. High Si application clearly reduced the As concentration of straw, however, not of grain.

The silicic acid concentration in nutrient solution could not be kept constantly high due to the high plant uptake. In the nutrient solution experiment, the As concentration in plant d.m. was in the order root > straw > polished rice. The supply of 0.05 mg As/L increased the As concentration in polished rice but doubling of As supply was not further reflected in the As concentration in polished rice. High Si application clearly reduced the As concentration of straw but not in polished rice.

Thus, in soil as well as in nutrient solution Si application reduced As concentration in straw but not in polished rice. Reason might be that the silicic acid concentration in soil solution and nutrient solution was not constantly high enough to affect Si translocation into the grain.

Introduction

Rice is one of the major staple food crops of the world and the potential risk to human health due to elevated As concentration in the grain is currently in discussion (Zavala et al., 2008; Torres-Escribano et al., 2008). The As concentration in rice is increased by a factor of up to 10 compared to other cereals (Williams et al., 2007), since As becomes plant available after reductive dissolution of e.g. iron-(hydr)oxides during flooding of the rice field. At the same time adsorbed arsenate is released into soil solution and is further reduced to arsenite. Besides the impact of dissolved As, silicic acid was shown to inhibit the As uptake of rice plants (Guo et al., 2005; Guo et al. 2007). Plants take up Si in form of silicic acid and in submerged fields both arsenite and silicic acid are present as uncharged molecules similar in size. Recent studies revealed two Si transporters Lsi1 and Lsi2 in rice that mediate the uptake of arsenite as well (Ma et al., 2008). Mutants either defective in Lsi1 or Lsi2 showed a strongly reduced As uptake indicating the important role of Si transporter for the As plant uptake (Ma et al., 2006; Ma et al., 2007). Bienert et al. (2008) could show that there was no competitive effect of silicic acid on the arsenite transport via Lsi1. For Lsi2 it is not known. However, the inhibiting effect of silicic acid on arsenite uptake can be explained by a markedly reduced expression of both Lsi1 and Lsi2 under silicic acid availability (Ma et al., 2006; Ma et al., 2007). A decreased expression of Si transporter decreases the arsenite uptake at the same time.

Besides, As uptake might be affected by Si depositions in the root reducing the apoplastic bypass flow. This was shown for elements such as Cd (Shi et al., 2005; Zhang et al., 2007), Na (Gong et al., 2006; Yeo et al., 1999), P, Ca, Fe and Mn (Ma and Takahashi, 1989; Ma and Takahashi, 1993).

Silicic acid concentration in soil solution differs considerably between soils. In general, after flooding of paddy soil the silicic acid concentration in soil solution increases caused by both the release from iron(hydr)-oxides (Beckwith and Reeve, 1963; McKeague and Cline, 1963) and the dissolution of bioopal that contributes considerably to the bioavailable silicic acid for rice (Desplanques et al., 2006).

The aim of the present study was to investigate the fate of silicic acid in soil solution after Si application and its influence on the As concentration in the rice plant. Furthermore, the interaction of As supply and silicic acid concentration was investigated in a nutrient solution experiment. In particular, influence of silicic acid on the As concentration in grain was investigated.

Materials and Methods

Soil

Four Italian and two Spanish paddy soils resulting in widely differing As concentration in polished rice, were selected from a survey of 33 fields in Italy and 11 fields in Spain for conducting pot experiments in the greenhouse. Italian and Spanish paddy soils originated from the Po-area and from the region south-west of Sevilla close to the Atlantic coast, respectively. The Spanish soils are characterized by a clay content of up to 60%, a high salt content and a pH of nearly 8. For the Italian soils the clay content and the pH varied between 12% and 19% and 5.2 and 6, respectively. The total As_(aqua regia) content were 6.5, 15.1, 5.0, 6.2, 6.9 and 9.8 mg/kg in the soils D, L, G, B, M and N, respectively. The soil characteristics of the selected soils are specified more closely in chapter 4 (p. 28). Soils were thoroughly mixed and 16 L soil was filled into 20 L pots.

Silicon treatments were 0, 5 and 10 g SiO₂ (hereafter named as 0, + and ++ Si treatment) per pot given as potassium silicate (K₂SiO₃) (VWR). The Spanish soils were only treated with 5 g SiO₂ due to lack of soil. Potassium was balanced in the + and ++ Si treatment with K₂SO₄.

Soil solution

A system was used which allowed sampling of soil solution without penetration of oxygen into the soil (description of the system in chapter 4, p. 29). Sampled soil solution (23 mL) was filled into a vessel which contained 400 µL HNO₃(65%) preventing the oxidation of ferrous iron. Soil solution was passed through a filter (quantitative, 2.5 µm, Carl Roth, Karlsruhe, Germany).

Nutrient solution

The nutrient solution according to Yoshida (1981) contained in mM: 1.43 NH₄NO₃, 0.32 NaH₂PO₄, 0.5 K₂SO₄, 1 CaCl₂, 1.6 MgSO₄, and in µM: 9 MnSO₄, 0.52 (NH₄)₆Mo₇O₂₄, 18 H₃BO₃, 0.15 ZnSO₄, 0.15 CuSO₄ and 35 Fe as EDDHA (Sequestren, Syngenta). The nutrient solution contained 3 levels of each As and silicic acid. Arsenic concentrations were 0, 0.05 and 0.1 mg/L supplied in form of Na₂HAsO₄*7H₂O (Sigma-Aldrich). Silicic acid treatments were 0, 4 and 16 mg Si/L supplied in form of K₂SiO₃ from the beginning of the experiment (4th of May) to 29th of July. Then the silicic acid concentration was increased to 0, 10 and 40 mg Si/L per

week. Furthermore, Si was supplied in 3 rates to ensure a continuous nutrition of plants because a fast depletion of silicic acid was observed. However, even this increased supply was depleted after 2 days. Potassium was balanced by addition of K_2SO_4 . Between the weekly change of nutrient solution, pots were filled up with deionised water. The pH was adjusted to pH 6 with KOH and H_2SO_4 .

Cultivation

For the experiment in soil, seeds of rice (*Oryza sativa* L. cv. Selenio) were soaked in tap water at room temperature for one week. Per pot, 60 of the germinated seeds were laid on the soil in water on 30th of April and later reduced to 40 plants. Water depth was increased with the growth of plants to 6 cm within 2 weeks. It was then kept constant with tap water during cultivation. Urea (Sigma-Aldrich) was fertilized 3 times (1g/pot). For this purpose the water table was lowered to 1 cm for 2 days.

For the experiment in nutrient solution rice seeds of the variety 'Selenio' were soaked in tap water at room temperature for 4 days. Germination was just visible when the seeds were laid between two layers of filter paper which was rolled up and put into a cup with 2 cm water table. After 12 days, on 4th of May, 33 seedlings per pot (18 L) were transferred into nutrient solution.

In both experiments plants received natural radiation and the day/night temperatures were in May, June, September, October 22°C/15°C and in July and August 26°C/18°C.

Harvest

Plants were harvested at maturity on September 21st. Rice grains were separated from the straw. Rice straw was dried for three days at 60°C and milled. Rice grains were dried at 40°C over night to reduce moisture content and prevent fungal infestation. Then grains were processed to polished rice by the Rice Research Institute Ente Risi in Castello d'Agogna, Italy.

In the nutrient solution experiment roots were washed 4 times with deionised water and harvested separately. After drying at 60°C for 3 days roots were milled.

Chemical analysis

Total As in the soil solution and in nutrient solution was measured with ICP-MS 7500c (Agilent Technologies). Silicic acid in soil solution and nutrient solution was

determined by the colorimetric molybdenum blue method at 811 nm with a spectrophotometer (van der Vorm, 1987). Dried and milled straw (350 µg) and polished rice (480 µg) were digested with 4 mL HNO₃ (65%) and 1.5 mL H₂O₂ (30%) in a microwave (ETHOSplus, MLS GmbH, Germany) for 20 and 15 minutes at 190 °C, respectively, and analysed for total As with ICP-MS 7500c (Agilent Technologies). Dried and milled straw was extracted for Si with a mixture of 1 M HCl and 2.3 M HF adapted from Novozamsky et al. (1984) and determined by a colorimetrically method (van der Vorm, 1987).

Experimental design and statistical analysis

There were four replicates in both experiments and all pots were arranged in a randomised block design.

Analyses of variance were conducted by using SAS (SAS Institute INC, Cary, USA). Mean comparisons were carried out according to the Tukey test.

Results

Experiment in soil

For Italian soils (D, L, G, B) the Si application increased the silicic acid concentration in soil solution at the first sampling 3 days after flooding (Figure 1). This was not observed with Spanish soils (N, M). Regardless of Si application the silicic acid concentration in soil solution increased continuously in all soils during the first 6 weeks. Then, for the Italian soils (D, L, G, B) the silicic acid concentration in soil solution declined and the difference between the 0 and + Si treatment became less distinctive. In contrast, for Spanish soils the silicic acid concentration in soil solution remained constantly high during the cultivation period. Without addition of Si the silicic acid concentration in soil solution was in the order D, G, L < B, M < N.

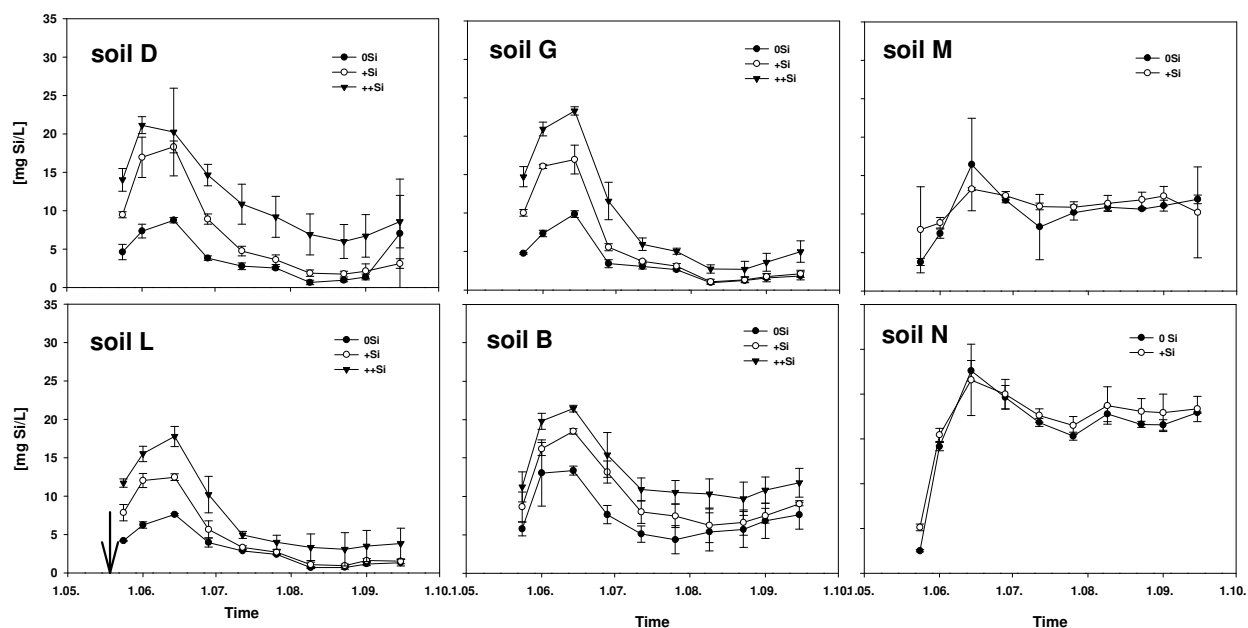


Figure 1: Silicic acid concentration in soil solution of Italian (D, G, L, B) and Spanish (M, N) soils in the course of the cultivation period of rice in pots as affected by Si treatment. (Arrow indicates start of experiment)

The different levels of silicic acid concentration in soil solution were reflected in the Si concentration of rice straw (Figure 2 A). The effect of Si application was considerably higher in soils which resulted in low Si concentration in straw in the control (D, L and G) than in soils that had high Si concentration in straw in the control (B, N and M). Plants of ++ Si treatment in Italian soils had a similar Si concentration in straw like those grown on Spanish soils. The + Si treatment in Spanish soils did not affect the Si concentration in straw which is in agreement with the observation that the silicic acid concentration in soil solution was not increased. The Si concentration of rice straw was positively related to the silicic acid concentration in soil solution following a saturation function (Figure 2B).

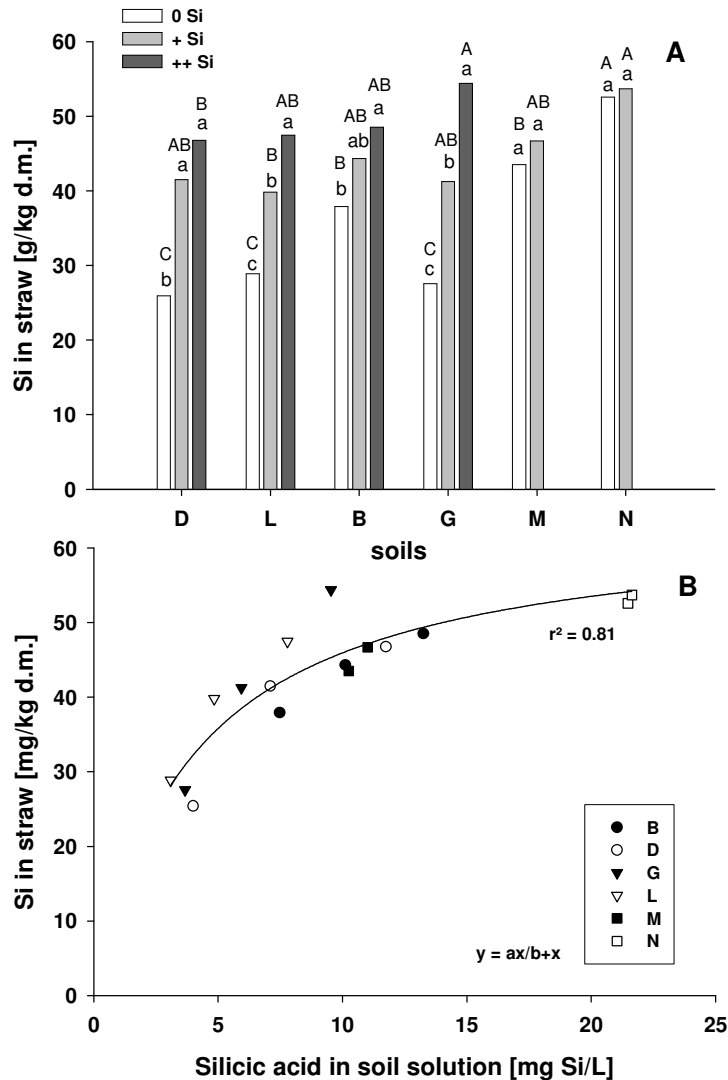


Figure 2: (A) Influence of Si application on Si concentration in rice straw grown in 4 Italian (D, L, B, G) and 2 Spanish soils (M, N). (B) Relationship between average¹⁾ Si concentration in soil solution and Si content in rice straw. *Small letters stand for comparison of Si treatments within the same soil. Capital letters stand for comparison of soils within the same Si treatment. Different letters indicate a significant difference at $\alpha = 0.05$ (Tukey test).*

¹⁾ Average of weekly measured concentrations until the end of grain filling in the 3rd week of August.

The yield of straw was influenced by the soils with soil N producing the highest yield and soil D the lowest (Table 1). However, Si application did not affect straw yield except for soil L. Paddy rice yield was influenced by the soils similar to yield of straw and was unaffected by Si application (data not shown).

Table 1: Influence of soil and Si application on straw yield grown in 4 Italian (D, L, B, G) and 2 Spanish soils (M, N).

Small letters stand for comparison of Si treatments within the same soil. Capital letters stand for comparison of soils within the same Si treatment. Different letters indicate a significant difference at $\alpha = 0.05$ (Tukey test).

Si application rate	Straw [g/pot]					
	D	L	G	B	M	N
0Si	77 <i>aD</i>	90 <i>bCD</i>	91 <i>aBC</i>	103 <i>aB</i>	92 <i>aBC</i>	123 <i>aA</i>
+Si	81 <i>aC</i>	92 <i>abBC</i>	101 <i>aB</i>	95 <i>aBC</i>	96 <i>aBC</i>	120 <i>aA</i>
++Si	70 <i>aB</i>	97 <i>aA</i>	95 <i>aA</i>	103 <i>aA</i>		

The Si application decreased the As concentration of straw in Italian but not in Spanish soils (Figure 3B). This was most pronounced on Italian soils that had high As concentration in straw in the 0 Si treatment. Accordingly, Figure 3 shows that the change of As concentration in rice straw was stronger with increasing change of Si concentration in straw due to Si application.

The As concentration of polished rice was influenced by the soils and was in the order $L > D, G > B, M > N$ (Figure 3A). However, no influence of Si application on the As concentration of polished rice was observed.

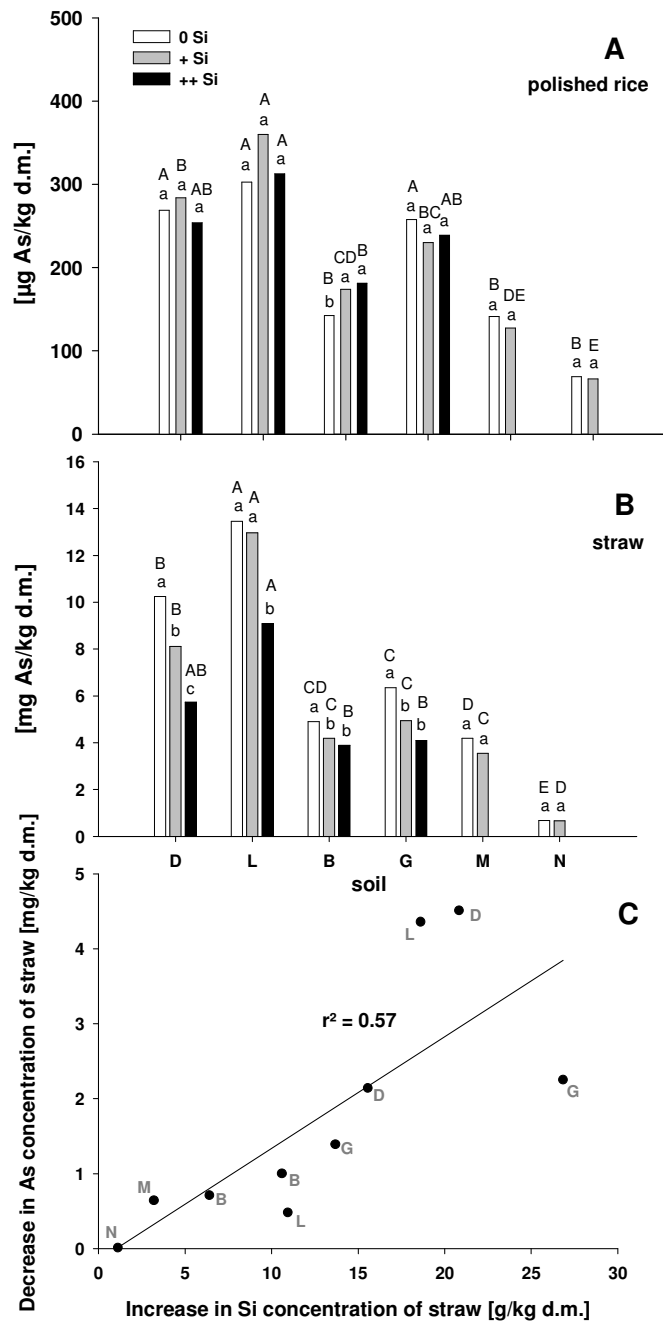


Figure 3: Influence of Si application on the (A) As concentration in polished rice and (B) straw grown in pots on 4 Italian (D, L, B, G) and 2 Spanish (M, N) soils. (C) Relationship between increase of Si concentration in straw due to Si application and decrease of As concentration in straw. Changes of Si and As concentration were computed as difference compared to 0 Si treatment. Small letters stand for comparison of Si treatments within the same soil. Capital letters stand for comparison of soils within the same Si treatment. Different letters indicate a significant difference at $\alpha = 0.05$ (Tukey test).

Experiment in nutrient solution

In general, Si application increased the straw and grain yield (Table 2) whereas root dry matter was not affected (data not shown). At 0 Si and +Si supply, straw yield of plants was reduced due to application of 0.05 mg As/L and 0.1 mg As/L, respectively (Table 2). Grain yield (Table 2) and root d.m. (data not shown) were not affected by As supply.

Table 2: Straw and grain yield of rice grown in nutrient solution as affected by Si and As application.

Small letters stand for comparison of As treatments within the same Si treatment.

Capital letters stand for comparison of Si treatments within the same As treatments.

Different letters indicate a significant difference at $\alpha = 0.05$ (Tukey test).

Si	As [mg/L]		
	Straw [g/ pot]		
	0	0.05	0.10
0	205 <i>a B</i>	166 <i>b B</i>	195 <i>ab B</i>
+	243 <i>a AB</i>	236 <i>a A</i>	209 <i>b B</i>
++	271 <i>a A</i>	262 <i>a A</i>	238 <i>a A</i>
	Grain [g/pot]		
0	98 <i>a A</i>	85 <i>a B</i>	99 <i>a B</i>
+	125 <i>a A</i>	122 <i>a A</i>	115 <i>a AB</i>
++	134 <i>a A</i>	146 <i>a A</i>	126 <i>a A</i>

The Si treatment was clearly reflected in the Si concentration in straw (Figure 4). The As concentration in plant d.m. was in the order root > straw > polished rice (Figure 5). In root and straw the As concentration in d.m. was continuously enhanced with increase of As supply. In polished rice the supply of 0.05 mg As/L nutrient solution enhanced As concentration in the grain but doubling of As supply in nutrient solution was not further reflected in As concentration in grain d.m.. With both As levels, the ++Si treatment decreased As concentration in straw but in root this Si effect was only observed in the highest As level. In contrast, Si application did not affect As concentration in polished rice.

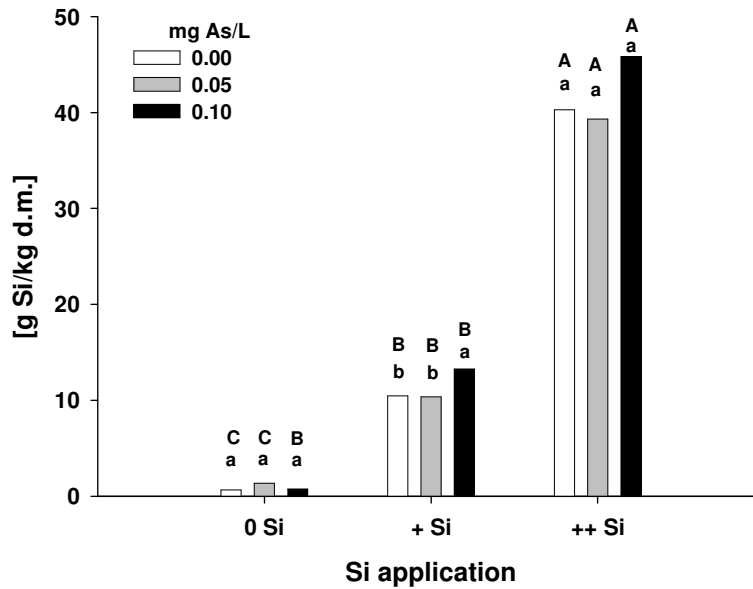


Figure 4: Influence of Si application on the Si concentration in straw of rice grown in nutrient solution as affected by As application. *Small letters stand for comparison of As treatments within the same Si treatment. Capital letters stand for comparison of Si treatments within the same As treatment. Bars having different letters are significantly different at $\alpha = 0.05$ (Tukey test).*

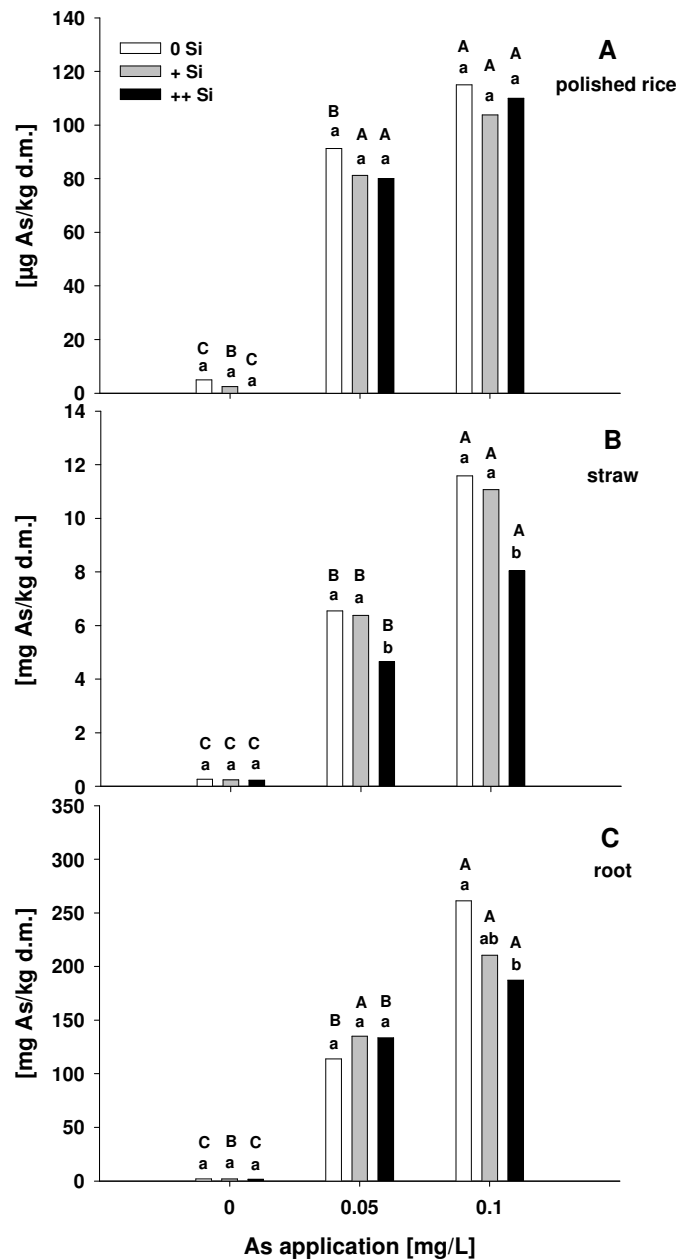


Figure 5 : Influence of As application on the As concentration in grain (A), straw (B) and root (C) of rice grown in nutrient solution as affected by Si application. *Small letters stand for comparison of Si treatment within the same As treatment. Capital letters stand for comparison of As treatments within the same Si treatment. Bars having different letters are significantly different at $\alpha = 0.05$ (Tukey test).*

Discussion

Yield

The yield of polished rice and rice straw (Table 1) cultivated in soil was not affected by Si application although the Si concentration in straw in the unfertilized treatment of Italian soils was clearly below the critical value of 50 g Si/kg d.m. (Figure 2B) (Dobermann and Fairhurst, 2000). This concentration was reached in the highest Si treatment and for Spanish soils. Reason for the absent effect on yield might be that the cultivar used in this study required less Si for optimal growth. Furthermore, a positive effect of Si on plant growth might be less pronounced in greenhouse cultivation.

However, in nutrient solution shoot dry matter and grain yield was increased by the ++Si treatment compared to 0Si treatment, which contained only traces of Si in straw d.m. (Table 2).

Silicic acid concentration in solution

The silicic acid concentration in soil solution was in the range as reported in literature (De Camargo, 2007; Schachtschabel et al., 1998). During the first 6 weeks after flooding it markedly increased in all soils regardless of Si application (Figure 1). Similar observations are reported by Ma and Takahashi (2002). This increase may be caused by the release of silicic acid during reduction of iron-(hydr)oxides (Beckwith and Reeve, 1963; McKeague and Cline, 1963) and the dissolution of bioopal, which contributes considerably to the bioavailable Si for rice (Desplanques et al., 2006).

The Italian soil B and the Spanish soils M and N had higher silicic acid concentration in soil solution than the Italian soils D, L and G. High silicic acid concentration in Spanish soils might be attributed to a high content of Si rich secondary minerals, mainly illite (70%) and smectite (20%). Additionally, the comparatively higher Si influx into the Spanish fields via irrigation water (data not shown) might have increased the content of dissolvable Si in Spanish soils. High silicic acid concentrations in soil B may be ascribed to a high content of iron-(hydr)oxides that released silicic acid during reduction. The oxalate-soluble Fe content of soil B was 2 to 3 times higher than in the other Italian soils (chapter 4, p. 28). From the first sampling on, Si treatments were clearly reflected in the silicic acid concentration in soil solution of Italian soils. However, at the first sampling, additional dissolved Si due to Si application was about 5 and 10 mg Si/L in the +Si and ++Si treatment, respectively. This was only about 1-

2% of the applied amount of Si. Since saturation of silicic acid was not reached (at about 56 mg Si/L), a silicic acid adsorption in soil possibly at iron-(hydr)oxides is suggested. Own results of previous studies indicated that only a small part of the reducible Fe in soil was reduced during cultivation (chapter 4). For the Spanish soils, the applied amount of silicon did not result in higher silicic acid concentration in soil solution than in the control possibly because the amount of applied Si was too low compared to the high amount of plant available Si already present in soil.

Beginning from the mid of June the silicic acid concentration in soil solution of Italian soils decreased probably as a result of high Si uptake of the plant. This was also observed by Kato and Owa (1997) and Liang et al. (1994). In Spanish soils the silicic acid concentration only slightly decreased, indicating a continuous release of silicic acid into soil solution which compensated for the silicic acid uptake by the rice plant. The high Si dissolution rates may be explained by the high content of Si rich secondary minerals. On average plants took up 22 mg Si per pot and day. This was about half of the amount of silicic acid contained in soil solution (5 mg/L and 8 L/pot), which had to be released from the solid phase in order to maintain the concentration in soil solution.

Si concentration in plant

In the soil experiment the Si treatments were well reflected in the Si concentration of straw grown on Italian soils (Figure 2A). The +Si treatment (5 g SiO₂/pot) corresponds to about 950 kg SiO₂/ha which is the average amount that rice plants take up during the cultivation period (Ma and Takahashi, 2002). Even though the Si concentration in straw barely reached the critical level of 50 g Si/kg d.m. (Doberman and Fairhurst, 2000), only about 50% in the +Si treatment and 30% in the ++Si treatment of the added amount of Si was taken up by plants. This might indicate that silicic acid was bound in soil unavailable for plant uptake (as previously discussed). The Si concentrations of straw grown on Spanish soils did not reflect the Si application confirming the data of silicic acid in soil solution.

In nutrient solution, the Si application rates were well reflected in straw (Figure 4). The highest Si application rate in nutrient solution did not result in Si concentration in straw as high as in the soil experiment (Figure 2A, Figure 4) indicating that plant demand was not met.

Arsenic in solution

In soil solution the mean As concentration differed between the soils and was within the range 28 and 76 µg/L (data not shown). As expected for a submerged soil, the major part of the total As was present as arsenite (76-100%), whereas the remaining was present as arsenate (chapter 4, p. 35).

The As concentration in nutrient solution was varied in a range as known for paddy soils. Arsenic was given in form of arsenate; however, As species were not controlled. Arsenate might have been partly reduced to arsenite by microbes in the nutrient solution. Besides, Xu et al. (2007) showed that arsenate was rapidly reduced to arsenite in plant roots after uptake and as such transported out of the root. Therefore, both arsenate and arsenite were probably present in nutrient solution.

Influence of silicic acid on the As uptake of rice plants

Increase of silicic acid concentration in soil solution as well as in nutrient solution significantly decreased the As concentration in straw (Figure 3B and 5B). Recent studies revealed that two Si transporters Lsi1 and Lsi2 mediate the uptake of arsenite as well. Arsenite has a size (4.11 Å) similar to that of Si(OH)₄ (4.38 Å), which is the form of Si taken up by the plant. Studies of Bienert et al. (2008) showed that there was no competitive effect of silicic acid on the arsenite transport via Lsi1. Whether this might be the case for Lsi2 remains unclear. However, the inhibiting effect of silicic acid on arsenite uptake can be explained by a reduced expression of both Lsi1 and Lsi2 under silicic acid availability (Ma et al., 2006; Ma et al., 2007). Ma et al. (2006 and 2007) showed that a silicon supply of 1 mM (28 mgSi/L) Si for three days decreased the expression of Lsi1 and Lsi2 to one fourth. A decreased expression of Si transporter reduces the arsenite uptake at the same time. In this study, silicic acid concentration in soil solution of soil N was close to 1 mM. Therefore, it may be speculated that in soil N the expression of Lsi1 and Lsi2 was markedly down regulated resulting in low As concentration in straw and polished rice (Figure 3A, 3B). In plants grown in the other soils the expression of Lsi1 and Lsi2 was possibly enhanced due to the lower silicic acid concentration, which was about 0.5 mM and 0.25 mM for soils M, B and D, G, L, respectively. This would have also increased the uptake of arsenite and might therefore be the reason for higher As concentration in straw and grain of rice grown on the soils D, G, L than that on soils M,B.

Even though the As concentration in straw was markedly reduced at high silicic acid supply in both experiments in soil and nutrient solution, the As concentration of grain was not affected (Figure 3A, Figure 5A). In contrast, the soils resulted in considerably different As concentration of grain which could be related to the silicic acid concentration in soil solution (chapter 4). Reason for the missing effect of Si application on As concentration in grain in this study is not known. There is evidence that arsenite is transported within the rice plant via Si transporter. Ma et al. (2008) reported an influence of Lsi2 on the As concentration in grain. Additionally, Bienert et al. (2008) show a weak transport of arsenite via Lsi6, a Si transporter responsible for the unloading of Si out of the xylem into the leaf tissue. The expression of Si transporter is down regulated at high silicic acid supply. It is possible that such down regulation of Si transporter requires a continuously high silicic acid concentration for affecting As concentration in the grain.

Besides the influence of silicic acid on the regulated arsenite uptake system, a decreased uptake of As could be the result of a reduced apoplastic bypass flow. An inhibiting effect of Si on the uptake of Cd (Shi et al., 2005; Zhang et al., 2007), Na (Gong et al., 2006; Yeo et al., 1999), P, Ca, Fe and Mn (Ma and Takahashi, 1989; Ma and Takahashi, 1993) was reported. This was ascribed to a reduced apoplastic bypass flow due to Si depositions in the root apoplast.

Conclusion

An influence of silicic acid on the As concentration in grain cultivated on Italian soils was not observed although the As concentration in straw was significantly reduced. Reason might be that silicic acid concentration has to be constantly high in order to reduce the expression of transporter that mediate the As influx into the grain. In future studies the influence of a constant high supply of silicic acid should be investigated.

6. Mobilisation of arsenic in rice plant (*Oryza sativa* L.) and its storage into the grain

Abstract

Paddy rice (*Oryza sativa* L.) may contain high arsenic (As) concentration compared to other grain crops. For development of measures to reduce As content of rice, knowledge about the mobility of As within the rice plant is required.

Therefore, to investigate the mobilisation of As within the plant, rice was grown in nutrient solution and exposed to As either before flowering or after flowering or continuously until maturity. Furthermore, rice was grown in 4 soils under greenhouse and field conditions and the time course of As storage into the grain during grain filling period was investigated.

In nutrient solution the As removal at flowering did not reduce As concentration in polished rice compared to plants supplied with As continuously or after flowering. Plants that received As only after flowering had the same As concentration in shoot d.m. and husk like plants receiving As only before flowering. However, continuous As supply resulted in doubling of As concentration in both plant parts. In contrast to grain and shoot, the As concentration in root d.m. decreased after As removal compared to the treatments receiving As only after flowering or continuously. The observations indicate that As was mobilised from the root to the shoot and that it was stored into the grain, although As was not available in nutrient solution.

Up to 2 weeks before harvest the 1000 grain-weight in soil experiments increased in the field as well as in the greenhouse. The As concentration in rice grain was constant during the whole grain filling period. It was on a similar level in both environments and its variation between soils was in the same order indicating that soil was the decisive factor for As concentration in grain.

Our results suggest that temporary cultural measures during the cultivation period, as e.g. drainage, might be ineffective because of mobilisation capacity within the rice plant. Moreover, harvest before final maturity of the grain would not reduce the As content, as the As concentration in grain remained constant during the grain filling period.

Introduction

Inorganic arsenic in rice is currently discussed as a possible health risk for humans (Zavala et al., 2008; Zavala and Duxbury, 2008; Zhu et al., 2008). Apart from As contaminated drinking water, rice is the largest food dietary source of inorganic arsenic (Schoof et al., 1999). Reason for elevated As content in paddy rice is the reducing environment of submerged soils which results in dissolution of As into soil solution and, therefore, an increased As phytoavailability. Since rice is a staple food for a major part of the world population, measures have to be found to reduce the As content of rice. For decreasing the As content of rice, knowledge about the mobility of the element in plant is necessary. However, details of As transport in xylem and phloem and form of bonding in the plant tissue are largely unknown.

In paddy soils mainly 4 As species have been found: arsenite (As(III)), arsenate (As(V)), dimethylarsenate (DMA) and monomethylarsenate (MMA) (chapter 4, p 35; Takamatsu et al., 1982; Abedin et al., 2002a). Inorganic As is taken up to a higher rate than methylated As species (Abedin et al., 2002a). Arsenate is taken up by a phosphate-transporter (Ullrich-Eberius et al., 1989), whereas arsenite is thought to be taken up by rice via aquaporins (Meharg and Jardine, 2003). In xylem sap of rice plants mainly As(III) was found (Xu et al., 2007) whereas the As concentration and species in the phloem sap are not known. However, the rice grain is mainly supplied with nutrients via the phloem (Oparka and Gates, 1981). Meharg et al. (2008b) observed a co-location of As with Zn and Fe in the aleurone layer which contained considerably higher As than the endosperm.

To reduce the As content of rice, a drainage period after flowering was proposed (Meharg et al., 2008), because As becomes unavailable for plant uptake under aerobic soil conditions. However, if As can be mobilised within the rice plant, it may be stored into the grain independently from the period of As availability in the soil.

A further possibility to decrease the As content of polished rice could be harvesting the grain before final maturity avoiding a possible late influx of As into the grain. However, the time course of As influx into the grain is not known.

Therefore, aim of this research was to investigate the mobilisation of As from the vegetative plant part and root to the grain and the course of As storage into the grain.

Materials and Methods

Experiment in nutrient solution: Mobilisation of As within the plant

Seeds of *Oryza sativa* L. cv Selenio were soaked in tap water at room temperature for 4 days. Germination was just visible when the seeds were laid between two layers of filter paper which were rolled up and put into a beaker with 2 cm water table. After 12 days the seedlings were transferred into nutrient solution clamped into foam for stabilisation. Pots contained 18 L nutrient solution and 33 plants per pot were cultivated in the greenhouse with temperatures adjusted to: in May, June, September, October 22°C and 15°C during day and night, respectively, and in July and August 26°C and 18°C during day and night, respectively. Plants received natural radiation. The treatments were: 1) no As supply (-/-); 2) As supply only before flowering (+/-); 3) As supply only after flowering (-/+); 4) As supply before and after flowering (+/+). The nutrient solution according to Yoshida (1981) contained in mM: 1.43 NH₄NO₃, 0.32 NaH₂PO₄, 0.5 K₂SO₄, 1 CaCl₂, 1.6 MgSO₄, and in µM: 9 MnSO₄, 0.52 (NH₄)₆Mo₇O₂₄, 18 H₃BO₃, 0.15 ZnSO₄, 0.15 CuSO₄ and 35 Fe as EDDHA (Sequestren, Syngenta). Arsenic treatment was 0.5 mg As/L in form of Na₂HAsO₄*7H₂O (Sigma-Aldrich). Nutrient solution was changed every two weeks. Within these two weeks the nutrient solution was alternately filled up with fresh nutrient solution or with deionised water. Nutrient solution pH was adjusted to pH 6 with KOH (10%). Plants were harvested immediately before flowering and at maturity and separated into root, straw and paddy rice. Roots were washed 4 times with deionised water. Afterwards the roots were dried at 60°C for 3 days and milled. Dehusking and milling of the rice grain was conducted by the Rice Research Institute in Castello d'Agogna, Italy, according to their practice resulting in the fractions husk, bran (pericarp and most of the aleurone layer) and polished rice.

Experiment in soil (field and greenhouse): As storage in the grain

Four fields were selected from a survey of 33 Italian fields which resulted in high (soil D and L) and low (soils B and G) As concentration in polished rice. The fields were located on 4 different farms south-west of Milan between the rivers Po and Ticino. Soil characteristics are described in chapter 4 (p 28). On all fields *Oryza sativa* L. cv Selenio was cultivated. The date of seeding and consequently flowering differed between the fields. Seeding was from the beginning to the middle of May and flowering around the beginning of August. The cultivation practices were conform to

good practice for Italian rice growers. Regarding irrigation practice this means seeding onto the irrigated field. After germination the field was drained for 3-4 days to improve rooting of seedlings. Then the water table was increased up to 10 cm adapted to the growth of seedlings. In general, fields are kept irrigated until 2-3 weeks before harvest. Rice plants were harvested four times during ripening (Table 1). In each field, 4 points were marked at the beginning of the cultivation period and 10 plants were sampled from each marked point. Samples were combined to a composite sample and separated in panicle and straw.

For the greenhouse trial soil was taken from the same fields close to the marked points. Soil from each field was mixed and 16L soil was filled into 20L pots. The pots were watered with tap water and covered by a 2 cm water layer. Seeds of *Oryza sativa* L. cv Selenio were germinated in a beaker with tap water covered with tissue for one week. Afterwards 60 germinated seeds per pot were laid on the soil in water and later reduced to 40 rice plants per pot. Greenhouse temperature was the same as for the experiment in nutrient solution. Plants received natural radiation. The water depth was increased with the growth of plants to 6 cm within 1 week. It was then kept constant with tap water during cultivation. Urea (Sigma-Aldrich) was fertilized 3 times (1g/pot). For this purpose the water table was lowered to 1 cm for 2 days. Plants were harvested four times during the cultivation period (Table 1).

Table 1: Harvest dates in the field and in the greenhouse of the experiment 'As storage in the grain'

Harvest	Date	
	field	greenhouse
1st	24. + 25. Aug	29. Aug
2nd	05. + 06. Sept	08. Sept
3rd	13. + 14. Sept	16. Sept
4th	26. Sept	29. Sept

After harvest the grain was dried for one day at 40°C and thereon dehusked by hand because the grain from the first harvest was small and milky. In order to treat the grain from each harvest in the same way all samples were dehusked by hand.

Sample analysis

Dried and milled straw (350µg), husk, bran, white and brown rice (480µg) were digested with 4mL HNO₃ (65%) and 1.5mL H₂O₂ (30%) in a microwave (ETHOSplus, MLS GmbH, Germany) for 20 and 15 minutes at 190°C, respectively. Arsenic was analysed by the ICP-MS (7500c Agilent Technologies).

Experimental design and statistical analysis

The experiment in nutrient solution included 4 replicates per treatment. All pots were arranged in a randomised block design. In the field 4 samples from each field were combined and composite samples were analysed. The greenhouse experiment in soil included 4 replicates per harvest date and pots were arranged in a randomised block design.

Analysis of variance was conducted by using SAS (SAS Institute INC, Cary, USA). Mean comparison was carried out according to T test and Tukey test.

Results

Experiment in nutrient solution: Mobilisation of As within the plant

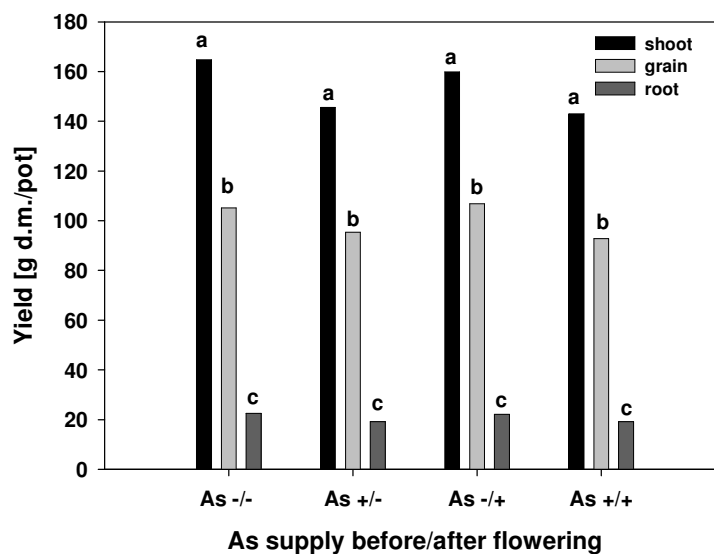


Figure 1: Yield of shoot, grain and root of rice plants grown in nutrient solution at maturity as affected by As supply during different growth stages. Letters stand for comparison within the same plant fraction. Bars having different letters are significantly different at $\alpha = 0.05$ (Tukey test).

Arsenic supply did not show any influence on the root and shoot d.m. yield at maturity (Figure 1). Plants which did not receive any As contained only traces of As in root and shoot d.m. (Figure 2, treatment -/-). Removal of As from nutrient solution after flowering did not affect As concentration in the shoot d.m. at maturity (treatment +/-), although shoot dry matter increased about 30% from flowering to maturity (data not shown). However, As concentration in the root d.m. decreased after cessation of As supply (treatment +/-). Continuous application of As after flowering resulted in twice as high As concentration in shoot and root d.m. at maturity (treatment +/+). Plants which received As only after flowering (treatment -/+) had the same As concentration in shoot d.m. as plants which received As only before flowering. The As concentration in the root was about 10 to 20 fold higher than that in the shoot.

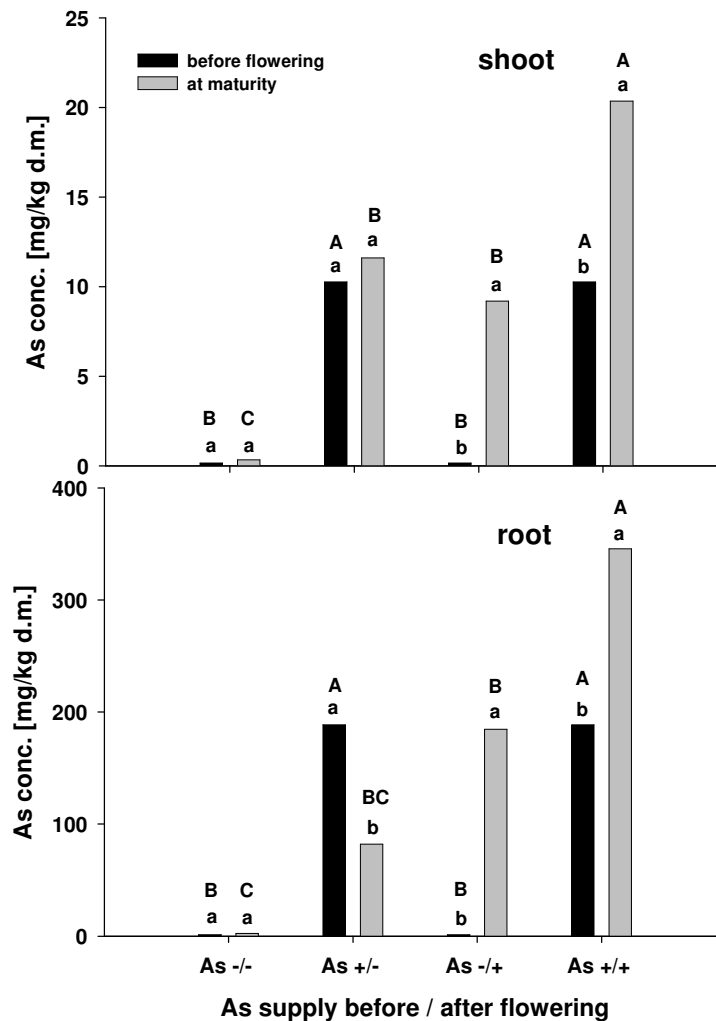


Figure 2: As concentration in shoot and root of rice plants grown in nutrient solution before flowering and at maturity as affected by As supply during different growth stages. Small letters stand for comparison of harvest dates within the same As treatment. Capital letters stand for comparison of As treatments within the same harvest date. Bars having different letters are significantly different at $\alpha = 0.05$ (T test and Tukey test).

A clear influence of the As supply schedule on the As concentration in husk and bran was also visible (Figure 3). Arsenic application only after flowering resulted in about 50% higher As concentration in the husk compared to the treatment which received As only before flowering, whereas the As concentration in husks of plants continuously supplied with As was doubled. The As concentration of bran of the

treatment continuously supplied with As was significantly higher compared to the plants which were treated with As only before or after flowering. However, the observed effects of As supply schedule on the As concentration in shoot, husk and bran were not reflected in polished rice (Figure 3). The treatments did not differ in As concentration in polished rice except for the (-/-) treatment.

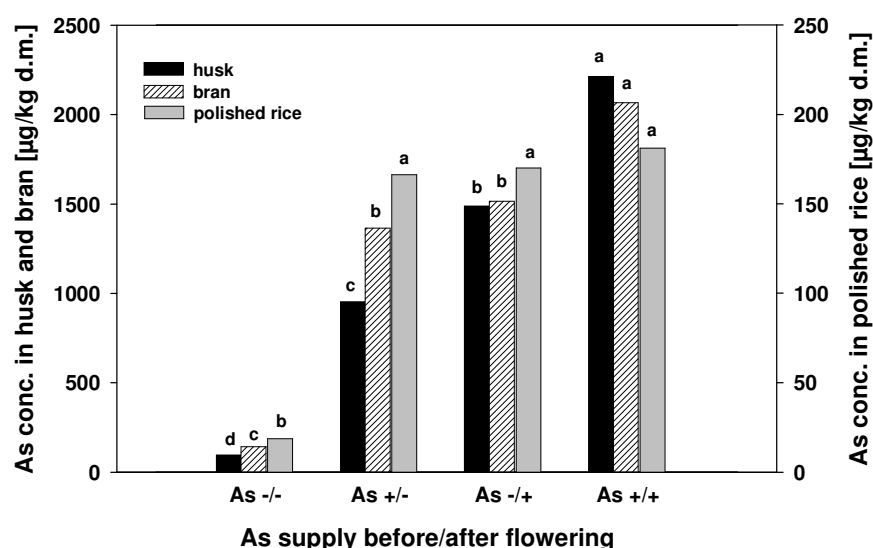


Figure 3: As concentration in husk, bran and polished rice as affected by As supply during different growth stages. Letters stand for comparison within the same rice grain fraction. Bars having different letters are significantly different at $\alpha = 0.05$ (Tukey test).

In order to quantify the As remobilisation from the root to the shoot, the quantity of As in the root, in the shoot and in the grain was calculated for the (+/-) treatment (Figure 4). Total As content in the plant d.m. was at the same level before flowering and at maturity, because plants did not receive As after flowering. However, the distribution changed. Before flowering only 29% of the As was contained in the shoot whereas at maturity more than half of the total As in plant was in the shoot. The As content in the grain amounted to less than 1% of total As content.

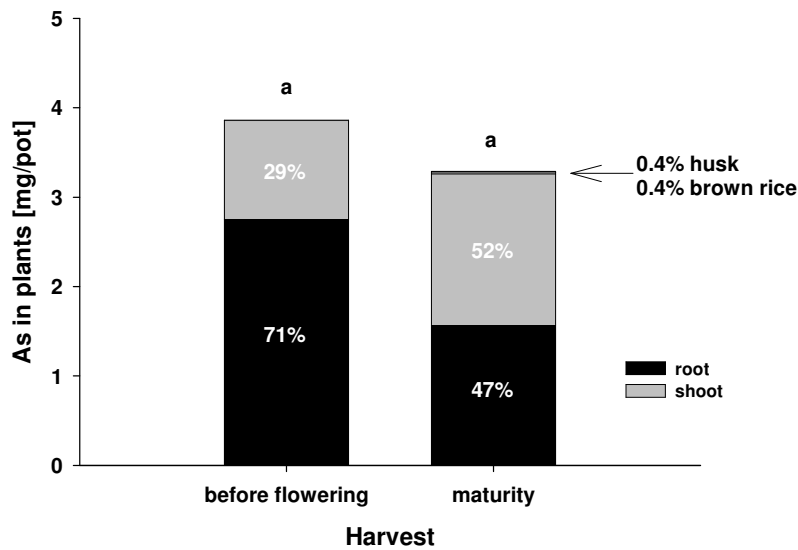
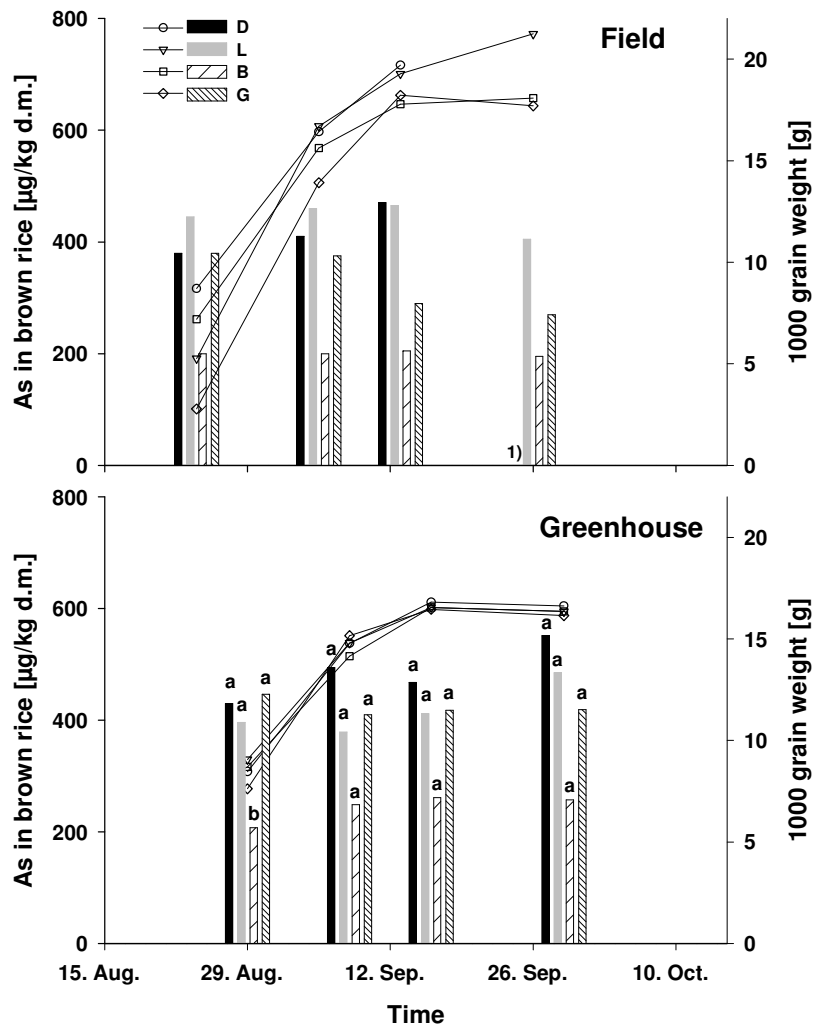


Figure 4: As content in different fractions of the rice plant in the treatment +As before/-As after flowering. Different letters indicate significant difference of total As in plant at $\alpha = 0.05$ (T test).

Experiment in soil (field and greenhouse): As storage in the grain

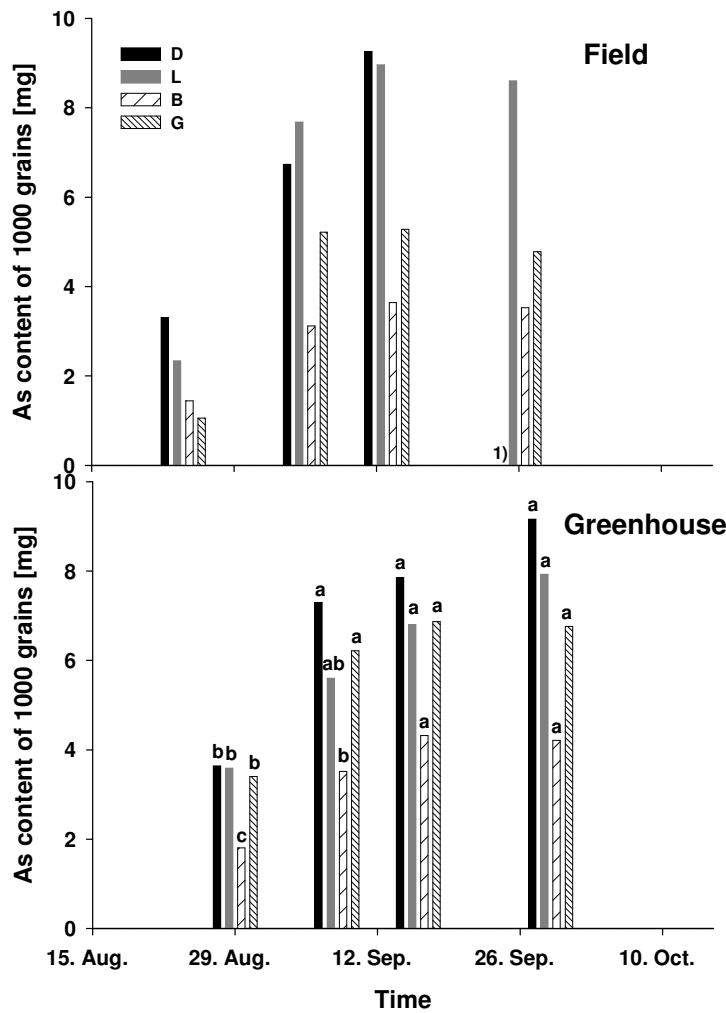
The 1000 grain weight increased until mid of September in the field as well as in the greenhouse (Figure 5). In the greenhouse, soils did not affect the 1000 grain weight whereas in the field considerable variation was observed at the first harvest. This might have been due to variation in flowering time as affected by sowing date. The As concentration in brown rice did not vary much during ripening in the field as well as in the greenhouse. However, the distinct lower As concentration in rice of soil B was shown in the field as well as in the greenhouse.



1) Harvested before the last sampling date

Figure 5: As concentration in brown rice (bars) and 1000 grain dry weight of rice (line) grown in the field and in the greenhouse on 4 different soils (D, L, B, G). Letters stand for comparison within the soil. Bars having different letters are significantly different at $\alpha = 0.05$ (Tukey test).

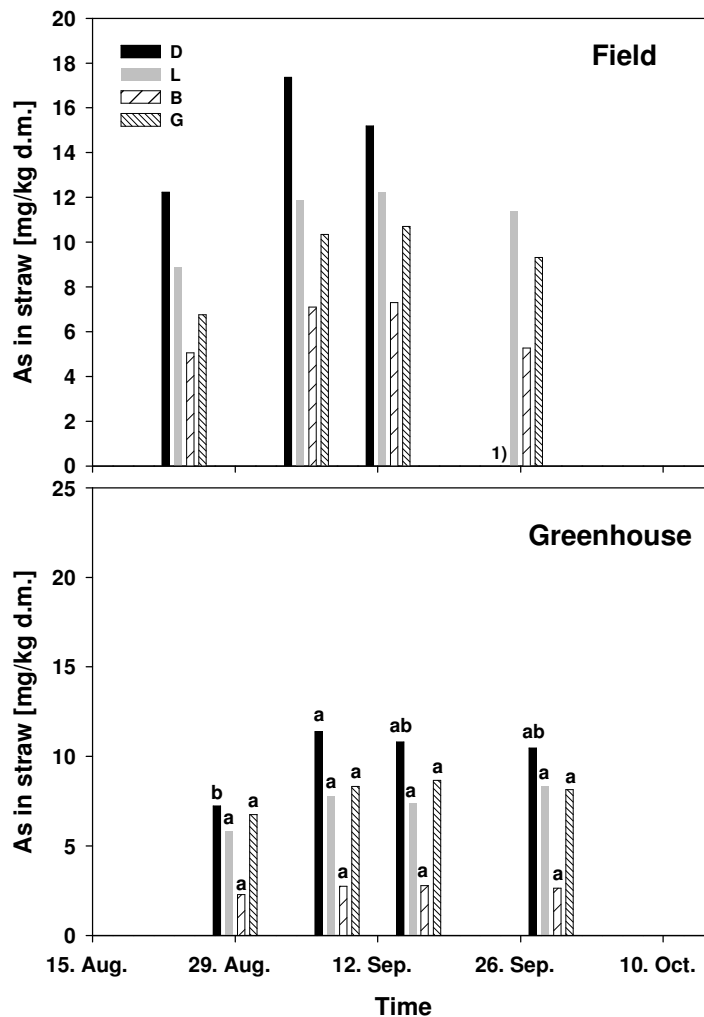
The amount of As contained in 1000 grains increased until mid of September in the field and in the greenhouse (Figure 6). This was caused by increase of grain weight since As concentration in brown rice was similar during the sampling period.



1) Harvested before the last sampling date

Figure 6: As content in 1000 grains of brown rice grown in the field and in the greenhouse on 4 different soils (D, L, B, G). Letters stand for comparison within the soil. Bars having different letters are significantly different at $\alpha = 0.05$ (Tukey test).

In contrast to brown rice (Figure 5) the As concentration in straw of plants cultivated in the greenhouse was about half of that observed in the field (Figure 7). However, the ranking of soils was similar in the field compared to the greenhouse. Like in brown rice plants growing on soil B had in straw the lowest As concentration.



1) Harvested before the last sampling date

Figure 7: As concentration in straw of rice grown in the field and in the greenhouse on 4 different soils (D, L, B, G). Letters stand for comparison within the soil. Bars having different letters are significantly different at $\alpha = 0.05$ (Tukey test).

Discussion

Arsenic species in nutrient solution

The As treatment was given as sodium arsenate and nutrient solution was changed every week. However, As species were not controlled. Since the oxygen content in nutrient solution was low ranging from 0 to 2 ppm one week after change of solution, a partial microbial reduction of As(V) to As(III) in nutrient solution was probable. Besides, Xu et al. (2007) showed that As(V) was rapidly reduced to As(III) in plant roots after uptake and as such transported out of the root. Both facts let assume that As(III) and As(V) were present in nutrient solution.

Influence of As on yield

The As treatment did not influence the dry matter yield (Figure 1). In addition, rice plants did not show any toxicity symptoms during cultivation. This is in agreement with Marin et al. (1992) who observed that rice plants treated with 0.8 mg As(V)/L (in nutrient solution containing the same P concentration like in the present study) were not significantly influenced in dry matter production. On the other hand 0.8 mg As(III)/L caused a decrease of plant dry matter of about 40% whereas 0.2 mg As(III)/L did not show any effect.

Tsutsumi et al. (1983) observed that the toxicity of As(V) strongly negatively depended on the P supply what is reasonable since P competes with As(V) for the same transporter (Ullrich-Eberius et al., 1989). It was estimated that an As(V):P ratio of 0.4-1.3:5 (in ppm) did not influence radicle elongation. In line with this, the ratio of 1 : 20 in the present study did not affect plant growth. However, Shaibur et al. (2006) observed a decrease of 23% and 15% for shoot and root dry matter yield of rice, respectively, by 0.5 mg As(V) at As : P ratio of 1:30. Reason for this inconsistency could be the use of different rice cultivars since Geng et al. (2006) observed cultivar dependent As toxicity. Additionally, cultivars might have differed in the capability to reduce As(V) to the more toxic form As(III).

Arsenic translocation and mobilisation within the rice plant

Arsenic concentration in root and shoot were similar to those found by Marin et al. (1992). Arsenic was easily taken up by the rice root and only a fractional amount was translocated to the shoot (Figure 2). Many other studies showed the high storage capacity of roots (Abedin et al., 2002b; Shaibur et al., 2006). Reason for this might be the formation of complexes with thiol-reactive peptides, e.g. phytochelatins (Pickering et al., 2000; Hartley-Whitaker et al., 2001). The As content in shoot treated with As only before flowering (+/-) was nearly the same before flowering and at maturity (Figure 1), although the shoot dry matter increased about 30% from flowering to maturity (data not shown). This indicates a translocation of stored As from root to shoot after flowering which is confirmed by the same As concentration in grain of plants treated with As only before flowering and in plants treated only after flowering or continuously (Figure 2). Figure 4 shows that, obviously, a large amount of As was transferred from the root to the shoot after flowering. Only a relative small amount of

As was transferred into the grain. These results are in contrast to results for *Helianthus* where no mobilisation from the root to the leaves could be observed (Raab et al., 2005). Our results suggest that a cultural measure reducing the plant As uptake (e.g. drainage) during grain filling, as it was suggested by Meharg et al. (2008c), would not reduce the As content in grain, since the As stored in root could be easily translocated to the grain.

The As concentration in straw of plants cultivated in the greenhouse was about half of that observed in the field (Figure 7). Reason might be that plants in pots grew in a smaller soil volume than in the field. Additionally, arsenic concentration in the tap water used in the greenhouse for irrigation might have been lower. The As concentration was in the order $D > L \approx G > B$ in the field as well as in the greenhouse, indicating that soil factors were decisive for As variation in straw between fields.

Arsenic pathway into the grain

The As concentration in husk and bran was about 10 times higher than in polished rice (Figure 3). High As concentrations in husk may be explained by As influx via transpiration stream in contrast to the grain (Oparka and Gates, 1984). The major part of nutrients and assimilates is transported to the grain via the phloem up to the aleurone layer (Oparka and Gates, 1981; Krishnan and Dayanandan, 2003). The uptake of As and its transport in xylem and phloem is partly known. Once As(V) enters the plant root via phosphate transporter it is reduced to As(III). Dhankher et al. (2006) reported that most plants show a high endogene As(V)-reductase activity that reduces >95% of the As that has been taken up to As(III). Also in rice a As(V) reductase was found (Duan et al., 2007). There is evidence that part of the reduced As(III) is excluded from the root by means of an efflux pump (Xu et al., 2007). The As(III) which is not excluded is rapidly complexed with soluble thiols, e.g. phytochelatins, and sequestered into vacuoles (Pickering et al., 2000). It seems possible that PC-As-complexes are transported via long-distance xylem- as well as phloemtransport, since this was shown for PC-Cd-complexes in Arabidopsis (Gong et al., 2003; Li et al., 2006). The dominant As species in xylem sap in rice and in rice roots was As(III) as shown by Xu et al. (2007). Small amounts of DMA were found in cucumber xylem sap although no DMA was detected in nutrient solution indicating that As may be methylated by plants (Mihucz et al., 2005). In line with this Norton et

al. (2008) observed an upregulation of genes in rice after As(V) exposure potentially involved in As methylation. Once As is methylated to DMA it seems to be translocated from root to shoot better than inorganic As, since DMA-supply resulted in relatively higher total As content in shoot compared to root than supply of As(V), As(III) and MMA (Carbonell-Barrachina et al., 1998; Marin et al., 1992; Raab et al., 2007). No information is available about As speciation in the phloem sap. However, similar to Cd arsenic might be transported via phloem as As(III) complex formed with thiol-peptides (Li et al., 2006). According to Ullrich-Eberius et al. (1989) after methylation As in plant might also be detoxified by formation of trimethylarsonium riboside.

Arsenic that has reached the aleurone layer may be stored in protein storage vacuoles in which minerals (e.g. Fe and Zn) are deposited and stored together with phytate (Brinch-Pedersen et al., 2007) because results of Meharg et al. (2008c) showed that As, Zn and Fe were co-located in brown rice. This would explain the high As concentration in bran. Details of transport of nutrients into the aleurone layer and endosperm is largely unknown (Oparka and Gates, 1981; Krishnan and Dayanandan, 2003).

The As pathway into the grain via phloem is also indicated by results given in Figure 5. In the field as well as in the greenhouse the As concentration in brown rice remained nearly constant during maturity. If As had entered the grain via xylem, the As concentration would have decreased towards the end of grain filling, since during this period no influx of water but still influx of assimilates occurs (Oparka and Gates, 1981).

However, next to the transport of nutrients via the phloem, a small portion of the total As in rice grain might be explained by As influx via xylem into the caryopses. In contrast to wheat, a xylem continuity between the pedicel and vascular bundle of rice exists, however, water enters the caryopses only under the specific conditions of >85% relative humidity (Oparka and Gates, 1984). Water influx via the xylem is especially imaginable through root pressure during night, when the xylem sap might be pressed into the caryopses. Additionally, results of Horigane et al. (2001) indicated a water influx into the endosperm during the first few days after anthesis.

Considering the As species found in polished and unpolished rice, may help to speculate about the As influx into the grain. In polished rice mainly DMA and As(III) was found (Zavala et al., 2008). As(III) might have been transported into the grain via xylem, since xylem sap contains a high percentage of As(III). Besides that, As(III) might be transported via the phloem as a complex with thiol peptides. Furthermore, inorganic As may be methylated within the plant and transported into the grain. Although the amount of methylated As in plant is small, it may be important for the As content in rice grain, since it was shown that methylated As was easily transported from root to shoot compared to inorganic As (Marin et al., 1992; Raab et al., 2007).

In conclusion, the observed continuous As influx into the grain during the grain filling period means that a harvest before final maturity would not reduce the As content in grain. Furthermore, our results indicate that As which was stored in roots was mobilised to the shoot. The As transfer into the endosperm was limited by a selective transfer resulting in As concentration in the order husk = bran > polished rice.

A strict regulation of As translocation within the plant also becomes obvious by comparing As- and Cd-concentration in rice straw and grain. The As concentration in straw was in the range 5-12 ppm, but 100 times less (150-250 ppb) in polished rice. With Cd, however, concentrations in straw (100-500 ppb) were in a similar range like in polished rice (100-200 ppb) (data not shown).

7. Schlussbetrachtung

Erhebungsuntersuchungen in Reisanbaugebieten in Italien/Po-Ebene (Kapitel 3) und Spanien/südwestlich von Sevilla (Ergebnisse nicht publiziert) und Gewächshausversuche (Kapitel 4) zeigten, dass die As-Konzentrationen im spanischen Weißreis im Mittel deutlich geringer waren als im italienischen Weißreis. Darüber hinaus variierten die As-Konzentrationen erheblich innerhalb Italiens. Die in dieser Studie beobachteten As-Konzentrationen im Weißreis lagen überwiegend im Bereich zwischen 70 und 220 $\mu\text{g}/\text{kg}$ d.m. (Kapitel 3, Abbildung 2) und damit in einem Bereich, der aus Erhebungsuntersuchungen in Europa und Amerika bekannt ist (Zavala und Duxdury, 2008). Arsen-Konzentrationen von 200 $\mu\text{g}/\text{kg}$ im Weißreis können nach einer Modellberechnung von Williams et al. (2005) bei einer auf Reis basierenden Ernährung 85% der maximal tolerierbaren täglichen Aufnahme an As ausmachen.

Ziel dieser Studie war es, die Ursachen zu finden, die zu den unterschiedlichen As-Konzentrationen führten, um dann daraus Maßnahmen zur Verminderung der As-Konzentration im Weißreis herleiten zu können.

Als Ursache für die unterschiedlichen As-Konzentrationen im Weißreis kommen Kulturmaßnahmen, Klima, Boden und Pflanzenfaktoren in Frage (Abbildung 1). Sowohl in der Erhebungsuntersuchung in Italien als auch in den Gewächshausversuchen wurde ausschließlich die Reissorte cv. Selenio untersucht, so dass ein Einfluss der Sorte auf die As-Akkumulation in diesem Fall ausgeschlossen werden kann.

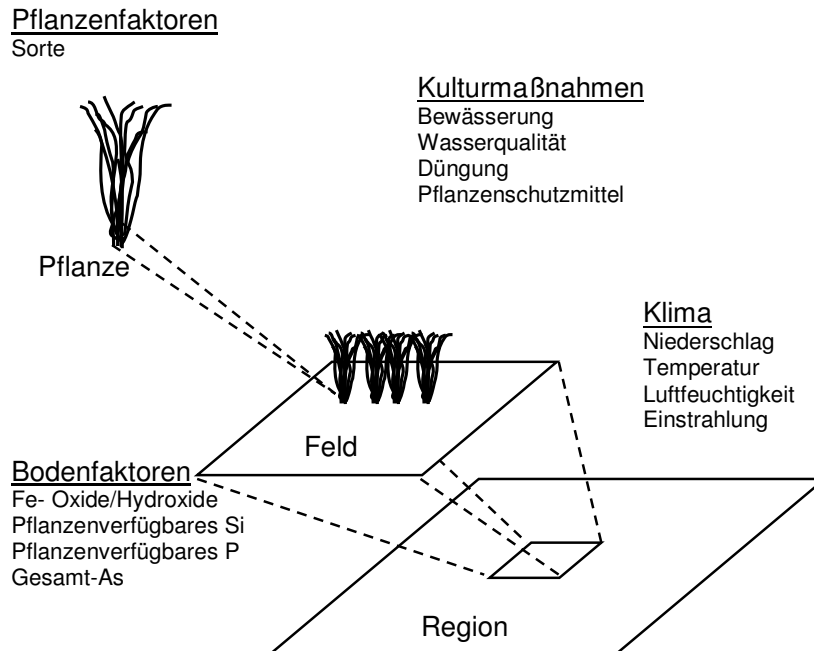


Abbildung 1: Faktoren, die die As Konzentration im Weißreis beeinflussen können

Kulturmaßnahmen

Im Zuge einer Befragung wurde die Kulturführung in den genannten Reisanbaugebieten in Italien und Spanien erfasst mit dem Ergebnis, dass sich diese zwischen den Ländern und innerhalb der Länder ähnelt und damit keine Erklärungsansätze bezüglich der Variation in As-Konzentrationen im Weißreis lieferten.

Während der Kulturführung ist ein As-Eintrag ins Feld durch Düngung, Bewässerungswasser und Pflanzenschutzmittel denkbar. Daher wurden im Verlauf der Befragung von italienischen und spanischen Anbauern Dünger- und Wasserproben genommen und auf As analysiert. Zum Vergleich wurden zudem bekannte As-Konzentrationen in einer Literaturstudie ermittelt.

Arsen in Düngemitteln

Arsen kann in phosphathaltigen Düngemitteln in erhöhter Konzentration vorliegen, da Phosphat als Rohphosphat abgebaut wird und im Boden häufig gemeinsam mit Arsenat vorliegt. Die genommene Probe Diammonphosphatdünger enthielt 1,57 mg As/kg während in der Literatur As-Konzentrationen von 3,2 bis 63,7 mg As/kg in verschiedenen Phosphatdüngern genannt werden (Boysen, 1992; Charter, 1995;

Sager, 1997; Raven et al., 1997; Merkel, 2004). Neben Phosphat ist auch Kalk ein Rohstoff, der für den Düngemittleinsatz abgebaut wird und der geogen bedingt erhöhte As-Konzentrationen aufweisen kann. So wurden Konzentrationen von 0-12.5 mg As/kg im Kalkstein nachgewiesen (Merkel, 2004). Mineralischen N- und K-Dünger enthielten nur sehr geringe As-Konzentrationen (Raven et al., 1997; Sager, 1979; Merkel, 2004).

Arsen im Bewässerungswasser

Die Analyse von je 8 spanischen Wasserproben aus zwei Jahren ergab im Mittel eine As-Konzentration von 6 µg/L, während 18 Wasserproben aus Italien durchschnittlich 1 µg As/L enthielten. Diese Ergebnisse stimmen überein mit Angaben der WHO (2001), die besagen, dass die As-Konzentration in den meisten Seen und Flüssen geringer als 10 µg/L ist.

Arsen in Pflanzenschutzmitteln

In Deutschland ist die Ausbringung von arsenhaltigen Pflanzenschutzmitteln seit 1974 verboten (Umweltbundesamt, 2003). Außerhalb Europas werden Herbizide, die Arsen enthalten, eingesetzt (WHO, 2001). Der Einsatz arsenhaltiger Pflanzenschutzmitteln im Reisanbau ist nicht bekannt.

Bewertung der Inputfaktoren

Der mögliche As-Eintrag in das Reisfeld wurde berechnet auf der Basis einer hohen und einer niedrigen As-Konzentration der Inputfaktoren. Die angenommenen Ausbringungsmengen sind den Angaben von italienischen und spanischen Reisanbauern angepasst. Die Ausbringungsmengen und Berechnungen befinden sich im Anhang. Die Tabelle 1 verdeutlicht, dass das Bewässerungswasser am meisten As einträgt.

Tabelle 1: Der berechnete As-Eintrag ins Feld über Inputfaktoren unter der Annahme von niedrigen und hohen As Konzentrationen in den Inputfaktoren

	Berechneter As-Eintrag (g As/ha)	
	Annahme von niedrigen As Konzentrationen in den Inputfaktoren	Annahme von hohen As Konzentrationen in den Inputfaktoren
Stickstoff	0.02	0.02
Phosphor	2.98	5.95
Kalium	0.13	0.13
Kalk	0.41	2.70
Bewässerungswasser	15.00	90.00
Atmosph. Deposition	0.50	4.00
Summe	19.04	102.80

Dem berechneten As-Eintrag steht die As-Abfuhr mit dem Erntegut gegenüber. Bei einem Ertrag von 7 t/ha und einer As-Konzentration von 0,7 mg As/kg Korn inklusive Spelze, erfolgt mit der Ernte eine As-Abfuhr von 4,5 g As/ha. Die geringere As-Abfuhr im Vergleich zum As-Eintrag führt zu einer As-Akkumulation im Boden. Um das Ausmaß der Akkumulation beurteilen zu können, muss die As-Menge im Oberboden (30 cm) berücksichtigt werden. Ein Boden in der Po-Ebene hat einen durchschnittlichen As-Gesamtgehalt von 0,008 g/kg (Daten aus Erhebungsuntersuchung 2004 und 2006). Wird der Oberboden mit einer Masse von 4 500 000 kg Boden/ha berücksichtigt, dann ergibt sich ein Gesamtgehalt von 36 000 g As/ha in den oberen 30 cm. Der jährliche As-Eintrag kann daher als gering eingestuft werden.

Der berechnete As-Eintrag ins Feld über Inputfaktoren zeigt, dass über das Bewässerungswasser am meisten As eingetragen wird und alle weiteren Faktoren eine untergeordnete Rolle spielen (Tabelle 1). Da das italienische Bewässerungswasser eine im Durchschnitt deutlich niedrigere As-Konzentration aufwies als das spanische Wasser, konnten die höheren As-Konzentrationen im italienischen Weißreis nicht durch Inputfaktoren erklärt werden. Darüber hinaus variierten die As-Konzentrationen im Wasser innerhalb der italienischen Proben nur geringfügig, so dass auch die Variation des As-Gehaltes im italienischen Weißreis nicht durch Inputfaktoren erklärt werden konnte.

Klima

In dieser Studie wurde ein Einfluss des Klimas auf die As-Konzentration im Weißreis nicht untersucht und Publikationen darüber liegen bisher nicht vor. Denkbar ist ein Einfluss der Luftfeuchte auf die Transpirationsrate und damit auf die Verlagerung von As in die oberen Pflanzenorgane, da Arsenit im Xylemsaft nachgewiesen wurde (Xu et al., 2007). Des Weiteren wirken sich Einstrahlung und Temperatur auf die Einlagerung von Assimilaten ins Korn aus (Yoshida, 1981) und könnten somit vermutlich auch die Einlagerung des As über das Phloem zur Aleuronschicht beeinflussen (Oparka and Gates, 1981; Krishnan and Dayanandan, 2003).

In der vorliegenden Studie können die As-Konzentrationsunterschiede im Weißreis innerhalb des italienischen Anbaugebietes nicht auf klimatische Faktoren zurückgeführt werden aufgrund der geringen räumlichen Ausdehnung und dem damit verbundenen einheitlichen Klima. Im spanischen Reisanbau herrschen im Durchschnitt höhere Temperaturen und eine höhere Einstrahlung als in Italien. Der Einfluss des Klimas ist nicht bekannt, jedoch lassen Ergebnisse aus Gewächshausversuchen (Kapitel 4) vermuten, dass das Klima eine untergeordnete Rolle bezüglich der As-Akkumulation im Weißreis spielt.

Bodenfaktoren

Die unterschiedlichen As-Konzentrationen im Weißreis gewachsen im italienischen und spanischen Boden konnten im Gewächshaus reproduziert werden (Kapitel 4, Kapitel 5). Da im Gewächshaus einheitliche Kulturmaßnahmen durchgeführt wurden und einheitliche Klimabedingungen vorhanden waren, bedeutet dies, dass der Boden einen ausschlaggebenden Einfluss auf die As-Akkumulation im Reis hat. Der Einfluss verschiedener Bodenfaktoren wurde in Kapitel 3 und 4 untersucht. Da die Eigenschaften der untersuchten italienischen Böden sich stark von denen der spanischen Böden unterscheiden, werden diese im Folgenden getrennt betrachtet.

Italienische Böden

In den italienischen Böden kommt den amorphen Fe-(Hydr)Oxiden eine zentrale Rolle zu. Ergebnisse aus dem Gewächshausversuch zeigten, dass eine positive Beziehung zwischen dem Anstieg der Fe- und As-Konzentration in der Bodenlösung bestand (Kapitel 4). Das bedeutet, dass mit der Reduktion von Fe-(Hydr)Oxiden das daran zunächst adsorbierte As in Lösung ging und damit pflanzenverfügbar wurde.

Gleichzeitig wirkte sich ein hoher Gehalt an amorphen Fe-(Hydr)Oxiden im Boden negativ auf die As-Konzentration im Reis aus, wie Daten aus zwei Erhebungsuntersuchungen nach Berechnung einer multiplen linearen Regression (MLR) zeigten (Kapitel 3). Dies lässt sich vermutlich dadurch erklären, dass der größte Teil der Fe-(Hydr)Oxide trotz der reduzierenden Bedingungen erhalten bleibt und somit weiterhin As adsorbiert. Die in Kapitel 4 beobachteten Fe- und As-Konzentrationen in der Bodenlösung während der Reiskultur spiegeln letztlich das Ergebnis der De- und Adsorptionsprozesse wider.

Im Weiteren zeigte die MLR, dass ein positiver Zusammenhang bestand zwischen dem As-Gesamtgehalt im Boden und der As-Konzentration im Stroh und Weißreis neben einem gleichzeitigen negativen Einfluss des Gehaltes an amorphen Fe-(Hydr)Oxiden und einem positiven Einfluss des pflanzenverfügbaren P (Kapitel 3). Dies bedeutet, dass sie bei hohem As-Gesamtgehalt und niedrigem Gehalt an amorphen Fe-(Hydr)Oxiden im Boden die Wahrscheinlichkeit für erhöhte As-Konzentrationen im Reis vergrößert.

Neben dem Einfluss des Gehaltes an Gesamtarsen und an amorphen Fe-(Hydr)Oxiden konnte ein positiver Einfluss des pflanzenverfügbaren P auf die As-Konzentration im Reiskorn und Stroh gezeigt werden (Kapitel 3). Da sich Phosphat und Arsenat chemisch ähneln, besteht im Boden Konkurrenz um Bindungsplätze. Dies führte vermutlich zur Verdrängung von Arsenat durch Phosphat und damit zu einer höheren As-Verfügbarkeit. In dem hier untersuchten Bereich war der Einfluss des pflanzenverfügbaren P deutlich geringer als der des Gehaltes an Gesamtarsen und amorphen Fe-(Hydr)Oxiden.

Spanische Böden

Wie in Kapitel 3 beschrieben unterschieden sich die spanischen Böden deutlich von den italienischen Böden hinsichtlich ihrer Eigenschaften. Die spanischen Böden wiesen einen hohen Tongehalt, einen hohen pH-Wert und einen hohen Gehalt an Schwefel gemessen im Bodensättigungsextrakt, auf. Des Weiteren zeigten die spanischen Böden hohe Kieselsäure-Konzentrationen im Verlauf der Reiskultur (Kapitel 4, Kapitel 5).

Der Gehalt an Gesamtarsen und oxalatlöslichem Fe befand sich in einem ähnlichen Bereich wie der der italienischen Böden. Trotzdem unterschieden sich die spanischen Böden, vor allem Boden N, in dem die As-Konzentration in der

Bodenlösung im Verlauf der Kultur abnahm, deutlich von den italienischen Böden, in denen die As-Konzentration anstieg (Kapitel 4). Dies lässt sich möglicherweise erklären durch die Bildung von FeAsS (Arsenpyrit) oder As₂S₃ (Orpiment) aufgrund hoher Sulfid-Konzentrationen in der Bodenlösung (Bostick et al., 2004).

Daneben beeinflusst das pflanzenverfügbare Si im Boden, also das Si, das während der Kultur als Kieselsäure in Lösung geht, die As-Aufnahme von Reis (Kapitel 4, Guo et al., 2005, Guo et al., 2007). Wie die Untersuchungen in Kapitel 5 zeigen, ist hierbei möglicherweise die Fähigkeit des Bodens ausschlaggebend, im Kulturverlauf ständig ausreichend Kieselsäure pflanzenverfügbar zu machen. Diese Eigenschaft könnte vor allem in Böden mit hohen Gehalten an siliziumreichen Tonmineralen zu finden sein.

Maßnahmen zur Verminderung der As-Akkumulation im Weißreis

Als bedeutsame Maßnahme zur Minderung der As-Konzentration im Weißreis ist zunächst die Selektion von Flächen, die gleichzeitig einen niedrigen As-Gesamtgehalt, hohen Gehalt an amorphen Fe-(Hydr)Oxiden und hohen pflanzenverfügbaren P-Gehalt im Boden und konstant hohe Kieselsäure-Konzentrationen in der Bodenlösung während der Kultur aufweisen. Letzteres kann möglicherweise durch eine Si-Düngung erreicht werden. In Kapitel 5 wurde gezeigt, dass die Applikation von Si zu geringeren As-Konzentrationen im Stroh führte. Es wird vermutet, dass eine konstant hohe Konzentration an Kieselsäure auch die As-Konzentration im Korn senkt. Wie die Versuche in Kapitel 5 jedoch auch zeigten, ist eine konstant hohe Kieselsäurekonzentration schwierig zu erreichen aufgrund der Adsorption von Kieselsäure im Boden und der hohen Kieselsäureaufnahme der Pflanze. Weitere Studien zur Applikationsmenge und Löslichkeiten von Si-Düngern sind notwendig.

Verschiedene Studien zeigten, dass die As-Konzentration im Reiskorn zwischen Sorten stark variieren kann (Xie und Huang et al., 1998). Daher ist vermutlich eine Sortenselektion eine weitere effektive Maßnahme zur Verringerung der As-Konzentration im Weißreis.

Laut Duxbury et al. (2007) führte der Anbau von Reis auf Dämmen mit einer Wasserführung zwischen den Dämmen zu konstant weniger reduzierenden Bedingungen und zu geringeren As-Konzentrationen in der Pflanze im Vergleich zum konventionellen Anbau. Dies wurde darauf zurückgeführt, dass vermutlich weniger

Fe-(Hydr)Oxide reduziert wurden und damit weniger As in Lösung ging. Nachweise dafür sollten in weiteren Studien aufgezeigt werden und die Effektivität dieser Methode auf verschiedenen Böden untersucht werden.

8. Literatur

Abedin, M.J., Feldmann, J., Meharg, A.A., 2002a: Uptake kinetics of arsenic species in rice plants. *Plant Physiol.* 128, 1120-1128.

Abedin, M.J., Cotter-Howells, J., Meharg, A.A., 2002b. Arsenic uptake and accumulation in rice (*Oryza sativa* L.) with contaminated water. *Plant Soil* 240, 311-319.

Armstrong, W., 1967. The use of polarography in the assay of oxygen diffusing from roots in anaerobic media. *Physiol. Plantarum* 20, 540-553.

Ascar, L., Ahumada, I., Richter, P., 2008. Influence of redox potential (Eh) on the availability of arsenic species in soils and soils amended with biosolid. *Chemosphere* doi:10.1016/j.chemosphere.2008.04.056.

Asher, C.J., Reay, P.F., 1979. Arsenic uptake by barley seedlings. *Austr. J. Plant Physiol.* 6, 459-466.

Beckwith, R. S., Reeve, R., 1963. Studies on soluble silica in soils I. The sorption of silicic acid by soils and minerals. *Austr. J. Soil Research* 1, 157-168.

Bienert, G.P., Thorsen, M., Schüssler, M.D., Nilsson, H.R., Wagner, A., Tamás, M.J., Jahn, T.P., 2008. A subgroup of plant aquaporins facilitate the bi-directional diffusion of As(OH)₃ and Sb(OH)₃ across membranes. *BMC Biology* 6 (26), doi: 10.1186/1741-7007-6-26.

Blakemore, L.C., Searle, P.L., Daly, B.K., 1987. Methods for chemical analysis of soils. New Zealand Soil Bureau Scientific Report 80, Department of Scientific and Industrial Research, Lower Hutt.

Bostick, B.C., Chen, C., Fendorf, S., 2004. Arsenite retention mechanism within estuarine sediments of Pescadero, CA. *Environ. Sci. Technol.* 38, 3299-3304.

Boysen P., 1992. Schwermetalle und andere Schadstoffe in Düngemitteln. Report 107 01 016/01. Berlin, Umweltbundesamt.

Brinch-Pedersen, H., Borg, S., Tauris, B., Holm, P.B., 2007. Molecular genetic approaches to increasing mineral availability and vitamin content of cereals. *J. Cereal Sci.* 46, 308-326.

Carbonell-Barrachina, A.A., Aarabi, M.A., DeLaune, R.D., Gambrell, R.P., Patrick Jr., W.H., 1998. The influence of arsenic chemical form and concentration on *Spartina patens* and *Spartina alterniflora* growth and tissue arsenic concentration. *Plant Soil* 198, 33-43.

Charter, R.A., Tabatabai, M.A., Schafer, J.W., 1995. Arsenic, Molybdenum, Selenium, and Tungsten Contents of Fertilizers and Phosphate Rocks. *Commun. Soil Sci. Plant Anal.* 26, 3051-3062.

Chen, Z., Zhu, Y.-G., Liu, W.-J., Meharg, A.A., 2004. Direct evidence showing the effect of root surface iron plaque on arsenite and arsenate uptake into rice (*Oryza sativa*) roots. *New Phytol.* 165, 91-97.

Chino, M., 1981. Uptake-Transport of Toxic Metals in Rice Plants, in: Yamane, I., Kitagishi, K.T. (Eds.), *Heavy Metal Pollution in Soils of Japan*. Japan Scientific Society Press, Tokyo.

Correll, R., Huq, S.M.I., Smith, E., Owens, G., Naidu, R., 2006. Dietary intake of arsenic from crops. Overview from the Bangladesh study, in: Naidu, R., Smith, E., Owens, G., Bhattacharya, P., Nadebaum, P. (Eds.), *Managing arsenic in the environment*. CSIRO, Collingwood, Australia, pp. 267.

De Camargo, M. S., Pereira, H. S., Korndörfer, G. S., Queiroz, A. A., Dos Reis, C. B., 2007. Soil reaction and absorption of silicon by rice. *Sci. Agric.* 64 (2), 176-180.

De Lima Rodrigues, L., Daroub, S.H., Rice, R.W., Snyder, G.H., 2003. Comparison of three soil test methods for estimating plant-available silicon. *Commun. Soil Sci. Plant Anal.* 34, 2059-2071.

Desplanques, V., Cary, L., Mouret, J.C., Trolard, F., Bourrié, G., Grauby, O., Meunier, J.D., 2006. Silicon transfers in a rice field in Camargue (France). *J. Geochem. Explor.* 88, 190-193.

Dhanker, O.P., Rosen, B.P., McKinney, E.C., Meagher, R.B., 2006. Hyperaccumulation of arsenic in the shoots of *Arabidopsis* silenced for arsenate reductase (ACR2). *PNAS* 103 (14), 5413-5418.

Dittmar, J., Voegelin, A., Roberts, L.C., Hug, S.J., Saha, G.C., Ashraf Ali, M., Badruzzaman, A.B.M., Kretzschmar, R., 2007. Spatial distribution and temporal variability of arsenic in irrigated rice fields in Bangladesh. 2. Paddy soil. *Environ. Sci. Technol.* 41, 5967-5972.

Dobermann, A., Fairhurst, T., 2000. Rice: Nutrient disorders and nutrient management. International Rice Research Institute, Philippines.

Dopp, E., 2008. Cellular uptake, subcellular distribution and toxicity of arsenic compounds. Book of abstracts, Arsenic in the environment, 2nd International Congress, Valencia, 21st -23rd of May 2008.

Duan, G.L., Zhou, Y, Tong, Y.P., Mudhopadhyay, Rosen, B.P., Zhu, Y.G., 2007. A CDC25 homologue from rice functions as an arsenate reductase. *New Phytol.* 174, 311-321.

Duxbuy, J.M., Panaullah, G., Koo-Oshima, S., 2007. Remediation of arsenic for agriculture sustainability, food security in health in Bangladesh. Working paper, FAO.

Ellis, J.R., Gates, P.J., Boulter, D., 1987. Storage-protein deposition in the developing rice caryopsis in relation to the transport tissues. *Annals of Botany* 60, 663-670.

EPA - United States Environmental Protection Agency. Background Report on Fertilizer Use, Contaminants and Regulations, 1999. Washington D. C.

Epstein, E., 1994. The anomaly of silicon in plant biology. *Proc. Natl. Acad. Sci.* 91, 11-17.

Flessa, H., Fischer, W.R., 1992. Plant-induced changes in the redox potentials of rice rhizospheres. *Plant and Soil* 143, 55-60.

Fuchs, S., Scherer, U., Hillenbrand, T., Marscheider-Weidemann, F., Behrendt, H., Opitz, D., 2002. Quantifizierung der Schwermetalleinträge aus Deutschland zur Umsetzung der Beschlüsse der Internationalen Nordseeschutzkonferenz. 200 22 233. Umweltbundesamt, Germany.

Geng, C.N., Zhu, Y.G., Liu, W.J., Smith, S.E., 2005. Arsenate uptake and translocation in seedlings of two genotypes of rice is affected by external phosphate concentrations. *Aquatic Botany* 83, 321-331.

Geng, C.N., Zhu, Y.G., Hu, Y., Williams, P., Meharg, A.A., 2006. Arsenate causes differential acute toxicity to two P-deprived genotypes of rice seedlings (*Oryza sativa* L.). *Plant Soil* 279, 297-306.

Gong, J.M., Lee, D.A., Schroeder, J.I., 2003. Long-distance root-to-shoot transport of phytochelatin and cadmium in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* 100, 10118-10123.

Gong, H.J., Randall, D. P., Flowers, T.J., 2006. Silicon deposition in the root reduces sodium uptake in rice (*Oryza sativa* L.) seedlings by reducing bypass flow. *Plant Cell Environ.* 29, 1970-1979.

Guo, W., Hou, Y.L., Wang, S.G., Zhu, Y.G., 2005. Effect of silicate on the growth and arsenate uptake by rice (*Oryza sativa* L.) seedlings in solution culture. *Plant Soil* 272, 173-181.

Guo, W., Zhu, Y.G., Liu, W.J., Liang, Y.C., Geng, C.N., Wang, S.G., 2007. Is the effect of silicon on rice uptake of arsenate (AsV) related to internal silicon concentrations, iron plaque and phosphate nutrition? *Environ. Pollut.* 148, 251-257.

Hansel, C.M., Fendorf, S., Sutton, S., Newville, M., 2001. Characterization of Fe plaque and associated metals on the roots of mine-waster impacted aquatic plants. *Environ. Sci. Technol.* 35, 3863-3868.

Hartley-Whitaker, J., Ainsworth, G., Vooijs, R., Bookum, W.T., Schat, H., Meharg, A.A., 2001. Phytochelatins are involved in differential arsenate tolerance in *Holcus lanatus*. *Plant Physiol.* 126, 299-306.

Heitkemper, D.T., Vela, N.P., Stewart, K.R., Westphal, C.S., 2001. Determination of total and speciated arsenic in rice by ion chromatography and inductively coupled plasma mass spectrometry. *J. Anal. At. Spectrom.* 16, 299-306.

Horigane, A.K., Engelaart, W.M.H.G., Maruyama, S., Yoshida, M., Okubo, A., Nagata, T., 2001. Visualisation of moisture distribution during development of rice caryopses (*Oryza sativa* L.) by nuclear magnetic resonance microimaging. *J. Cereal Sci.* 33, 105-114.

Imamul Huq, S.M., Shila, U.K., Joardar, J.C., 2006. Arsenic mitigation strategy for rice, using water regime management. *Land Contamination and Reclamation* 14 (4), 805-813.

Inskeep, W.P., McDermott, T.R., Fendorf, S., 2002. Arsenic (V)/(III) cycling in soils and natural waters: Chemical and microbiological processes, in: Frankenberger, W.T. Jr. (Ed.), *Environmental chemistry of arsenic*. Marcel Dekker, New York, pp. 183-215.

Irtelli, B., Navari-Izzo, F., 2008. Uptake kinetics of different arsenic species by *Brassica carinata*. *Plant Soil* 303, 105-113.

Liu, Z.G., 1985. Oxidation-reduction potential, in: Yu, T.R. (Ed.), *Physical chemistry of paddy soils*. Science Press, Beijing.

Islam, F.S., Pederick, R.L., Gault, A.G., Adams, L.K., Polya, D.A., Charnock, J.M., Lloyd, J.R., 2005. Interactions between the Fe(III)-reducing bacterium *Geobacter sulfurreducens* and arsenate, and capture of the metalloid by biogenic Fe(II). *Appl. Environ. Microbiol.* 71, 8642-8648.

Juhasz, A.L., Smith, E., Weber, J., Rees, M., Rofe, A., Kuchel, T., Sansom, L., Naidu, R., 2006. *In vivo* assessment of arsenic bioavailability in rice and its significance for human health risk assessment. *Environ. Health Persp.* 114 (12), 1826-1831.

Kato, N., Owa, N., 1997. Dissolution of slag fertilizers in a paddy soil and Si uptake by rice plant. *Soil Sci. Plant Nutr.* 43 (2), 329-341.

King, D.W., 1998. Role of carbonate speciation on the oxidation rate of Fe(II) in aquatic systems. *Environ. Sci. Technol.* 32, 2997-3003.

Krishnan, S., Dayanandan, P., 2003. Structural and histochemical studies on grain-filling in the caryopsis of rice (*Oryza sativa* L.). *J. Biosci.* 28 (4), 455-469.

Lemos, V. P., DaCosta, M. L., Lemos, R. L., 2007. Vivianite and siderite in lateritic iron crust: an example of bioreduction. *Quim. Nova* 30 (1), 36-40.

Li, Y., Dankher, O.P., Carreira, L., Smith, A.P., Meagher, R.B., 2006. The shoot-specific expression of γ -Glutamylcysteine synthetase directs the long-distance transport of thiol-peptides to roots conferring tolerance to mercury and arsenic. *Plant Physiology* 141, 288-298.

Liang, Y.C., Ma, T.S., Li, F.J., Feng, Y.J., 1994. Silicon availability and response of rice and wheat to silicon in calcareous soils. *Commun. Soil Sci. Plant Anal.* 25 (13&14), 2285-2297.

Liu, W.J., Zhu, Y.G., Smith, F.A., 2005. Effects of iron and manganese plaques on arsenic uptake by rice seedlings (*Oryza sativa* L.) grown in solution culture supplied with arsenate and arsenite. *Plant Soil* 277, 127-138.

Liu, Z.G., 1985. Oxidation-reduction potential, in: Yu, T.R. (Ed.), *Physical chemistry of paddy soils*. Science Press, Beijing.

Ma, J.F., Takahashi, E., 1989. Effect of silicic acid on phosphorus uptake by rice plant. *Soil Sci. Plant Nutr.* 35 (2), 227-234.

Ma, J.F., Takahashi, E., 1993. Interaction between calcium and silicon in water-cultured rice plants. *Plant Soil* 148, 107-113.

Ma, J.F., Takahashi, E., 2002. *Soil, fertilizer and plant silicon research in Japan*, Elsevier, Amsterdam.

Ma, J.M., Tamai, K., Yamaji, N., Mitani, N., Konishi, S., Katsuhara, M., Ishiguro, M., Murata, Y., Yano, M., 2006. A silicon transporter in rice. *Nature* 440, 688-691.

Ma, J. F., Yamaji, N., Mitani, N., Tamai, K., Konishi, S., Fujiwara, T., Katsuhara, M., Yano, M., 2007a. An efflux transporter of silicon in rice. *Nature* 448, 209-212.

Ma, J.F., Yamaji, N., Tamai, K., Mitani, N., 2007. Genotypic differences in silicon uptake and expression of silicon transporter genes in rice. *Plant Physiol.* 145, 919-924.

Ma, F.J., Yamaji, N., Mitani, N., Xu, X.Y., Su, Y.H., McGrath, S.P., Zhao, F.J., 2008. Transporters of arsenite in rice and their role in arsenic accumulation in rice grain. *PNAS* 105 (29), 9931-9935.

Mandal, B.K., Suzuki, K.T., 2002. Arsenic round the world: a review. *Talanta* 58, 201-235.

Manning, B.A., Fendorf, S.E., Goldberg, S., 1998. Surface structures and stability of arsenic(III) on goethite: Spectroscopic evidence for inner-sphere complexes. *Environ. Sci. Technol.* 32, 2383-2388.

Marin, A.R., Masscheleyn, P.H., Patrick Jr, W.H., 1992. The influence of chemical form and concentration of arsenic on rice growth and tissue arsenic concentration. *Plant Soil* 139, 175-183.

Marin, A.R., Masscheleyn, P.H., Patrick Jr, W.H., 1993. Soil redox-PH stability of arsenic species and its influence on arsenic uptake by rice. *Plant Soil* 152, 245-253.

Masscheleyn, P. H., Delaune, R. D., Patrick, W.H., Jr., 1991. Effect of redox potential and pH on arsenic speciation and solubility in a contaminated soil. *Environ. Sci. Technol.* 25, 1414-1419.

Mattusch, J., Wennrich, R., Schmidt, A.-C., Reisser, W., 2000. Determination of arsenic species in water, soils and plants. *Fresenius J. Anal. Chem.* 366, 200-203.

McGeehan, S.L., Naylor, D.V., 1994. Sorption and redox transformation of arsenite and arsenate in two flooded soils. *Soil Sci. Soc. Am. J.* 58, 337-342.

McKeague, J.A., Cline, M.G., 1963. Silica in soil solutions II. The adsorption of monosilicic acid by soil and by other substances. *Can. J. Soil Sci.* 43, 83-96.

Meharg, A.A., Jardine, L., 2003. Arsenite transport into paddy rice (*Oryza sativa*) roots. *New Phytol.* 157, 39-44.

Meharg, A.A., Lombi, E., Williams, P.N., Scheckel, K.G., Feldmann, J., Raab, A., Zhu, Y., Islam, R., 2008. Speciation and localization of arsenic in white and brown rice grains. *Environ. Sci. Technol.* 42, 1051-1057.

Meharg, A.A., Macnair, M.R., 1990. An altered phosphate uptake system in arsenate-tolerant *Holcus lanatus* L.. *New Phytol.* 116, 29-35.

Meharg, A. A., Rahman, M. D. M., 2003. Arsenic contamination of Bangladesh paddy field soils: Implications for rice contribution to arsenic consumption. *Environ. Sci. Technol.* 37, 229-234.

Meharg, A.A., Deacon, C., Campbell, R.C.J., Carey, A.-M., Williams, P.N., Feldmann, J., Raab, A., 2008a. Inorganic levels in rice milk exceed EU and US drinking water standards. *J. Environ. Monit.* 10, 428-431.

Meharg, A.A., Sun, G., Williams, P.N., Adomako, E., Deacon, C., Zhu, Y.-G., Feldmann, J., Raab, A., 2008b. Inorganic arsenic levels in baby rice are of concern. *Environ. Pollut.* 152, 746-749.

Meharg, A.A., Lombi, E., Williams, P.N., Scheckel, K.G., Feldmann, J., Raab, A., Zhu, Y., Islam, R., 2008. Speciation and Localization of arsenic in white and brown rice grains. *Environ. Sci. Technol.* 42, 1051-1057.

Merkel, D., 2004. Personal Communication

Mihucz, V.G., Tatár, E., Virág, I., Cseh, E., Fodor, F., Zárny, G., 2005. Arsenic speciation in xylem sap of cucumber (*Cucumis sativus* L.). *Anal. Bioanal. Chem.* 383, 461-466.

Moore, J.N., Ficklin, W.H., Johns, C., 1988. Partitioning of arsenic and metals in reducing sulfidic sediments. *Environ. Sci. Technol.* 22, 432-437.

Norton, G.J., Lou-Hing, D.E., Meharg, A.A., Price, A.H., 2008. Rice-arsenate interactions in hydroponics: whole genome transcriptional analysis. *J. Exp. Botany* 59, 2267-2276.

Novozamsky, I., van Eck, R., Houba, V.J.G., 1984. Determination of Si in plants, extraction of Si with HF, atomic absorption, atomic emission. *Commun. Soil Sci. Plant Anal.* 15 (3), 205-211.

Ogawa, M., Tanaka, K., Kasai, Z., 1979. Accumulation of phosphorus, magnesium and potassium in developing rice grains: followed by electron microprobe X-ray analysis focusing on the aleurone layer. *Plant Cell Physiol.* 20(1), 19-27.

O'Neill, P., 1995. Arsenic, in: Alloway (Ed.), *Heavy Metals in Soils*. Blackie Academic and Professional, Glasgow, pp 105-121.

Onken, B.M., Hossner, L.R., 1996. Determination of arsenic species in soil solution under flooded conditions. *Soil Sci. Soc. Am. J.* 60, 1385-1392.

Oparka, K.J., Gates, P., 1981. Transport of assimilates in the developing caryopsis of rice (*Oryza sativa* L.). A pathway of water and assimilated carbon. *Planta* 152, 388-396.

Oparka, K.J., Gates, P., 1984. Sink anatomy in relation to solute movement in rice (*Oryza sativa* L.): A summary of findings. *Plant Growth Regulation* 2, 297-307.

Petrick, J.S., Ayala-Fierro, F., Cullen, W.R., Carter, D.E., Vasken Aposhian, H., 2000. Monomethylarsonous acid (MMA(III)) is more toxic than arsenite in Chang human hepatocytes. *Toxicol. Appl. Pharm.* 163 (2), 203-207.

Pickering, I.J., Prince, R.C., George, M.J., Smith, R.D., George, G.N. Salt, D.E., 2000. Reduction and coordination of arsenic in Indian mustard. *Plant Physiol.* 122, 1171-1177.

Qu, D., Ratering, S., Schnell, S., 2004. Microbial reduction of weakly crystalline iron (III) oxides and suppression of methanogenesis in paddy soil. Bull. Environ. Contam. Toxicol. 72, 1172-1181.

Raab, A., Ferreira, K., Meharg, A.A., Feldmann, J., 2005. Can arsenic-phytochelatin complex formation be used as an indicator for toxicity in *Helianthus annuus*? J. Exp. Botany 58(6), 1333-1338.

Raab, A., Williams, P.N., Meharg, A.A., Feldmann, J., 2007. Uptake and translocation of inorganic and methylated arsenic species by plants. Environ. Chem. 4, 197-203.

Raven, K.P., Loeppert, R.H., 1997. Heavy metals in the environment- Trace element composition of fertilizers and soil amendments. J. Environ. Quality 26, 551-557.

Raven, K.P., Jain, A., Loeppert, R.H., 1998. Arsenite and arsenate adsorption on ferrihydrite: kinetics, equilibrium, and adsorption envelopes. Environ. Sci. Technol. 32, 344-349.

Rittle, K.A., Drever, J.I., 1995. Precipitation of arsenic during bacterial sulphate reduction. Geomicrobiol. J. 13, 1-11.

Sadiq, M., 1997. Arsenic chemistry in soils: An overview of thermodynamic predictions and field observations. Water Air Soil Pollut. 93, 117-136.

Sager, M., 1997. Possible trace metal load from fertilizers. Die Bodenkultur 48, 217-223.

Savant, N.K., Datnoff, L.E., Snyder, G.H., 1997. Depletion of plant-available silicon in soils: a possible cause of declining rice yields. Commun. Soil Sci. Plant Anal. 28, 1245-1252.

Schachtschabel, P., Blume, H.-P., Brümmer, G., Hartge, K.H., Schwertmann, U. Lehrbuch der Bodenkunde. Scheffer, F.; Schachtschabel, P. (Eds.); Ferdinand Enke: Stuttgart, 1998.

Schlichting E., Blume H.-P., Stahr K., 1995. Bodenkundliches Praktikum, Blackwell, Berlin.

Schoof, R.A., Yost, L.J., Eickhoff, J., Crecelius, E.A., Cragin, D.W., Meacher, D.M., Menzel, D.B., 1999. A market basket survey of inorganic arsenic in food. Food Chem. Toxicol. 37, 839-846.

Schüller, H., 1969. Die CAL-Methode, eine neue Methode zur Bestimmung des pflanzenverfügbaren Phosphates in Böden. Zeitschrift für Pflanzenernährung und Bodenkunde 123, 48-63.

Schwertmann, U., 1964. Differenzierung der Eisenoxide des Bodens durch Extraktion mit Ammoniumoxalat-Lösung. Zeitschrift für Pflanzenernährung, Düngung, Bodenkunde 105, 194-202.

Shaibur, M.R., Kitajima, N., Sugawara, R., Kondo, T., Huq, S.M.I., Kawai, S., 2006. Physiological and mineralogical properties of arsenic-induced chlorosis in rice seedlings grown hydroponically. Soil Sci. Plant Nutr. 52, 691-700.

Sheppard, S.C., 1992. Summary of phytotoxic levels of soil arsenic. Water Air Soil Pollut. 64, 539-550.

Shi, X., Zhang, C., Wang, H., Zhang, F., 2005. Effect of Si on the distribution of Cd in rice seedlings. Plant Soil 272, 53-60.

Smith, E., Naidu, R., Alston, A.M., 2002. Chemistry of inorganic arsenic in soils: II. Effect of phosphorus, sodium, and calcium on arsenic sorption. J. Environ. Quality 31, 557-563

Snyder, G.H., 2001. Methods for silicon analysis in plants, soils, and fertilizers, in: Datnoff, L.E., Snyder, G.H., Korndörfer, G.H. (Eds.), Silicon in Agriculture. Elsevier, Amsterdam, pp. 185-196.

Takahashi, Y., Minamikawa, R., Hattori, K. H., Kurishima, K., Kihou, N., Yuita, K., 2004. Arsenic behaviour in paddy fields during the cycle of flooded and non-flooded periods. Environ. Sci. Technol. 38, 1038-1044.

Takamatsu, T., Aoki, H., Yoshida, T., 1982. Determination of arsenate, arsenite, monomethylarsonate, and dimethylarsonate in soil polluted with arsenic. *Soil Sci.* 133 (4), 239-246.

Tao, S. S. H., Bolger, P. M., 1998. Dietary arsenic intakes in the United States: FDA total diet study, September 1991-December 1996. *Food Addit. Contam.* 16, 465-472.

Thoral, S., Rose, J., Garnier, J.M., Van Geen, A., Refait, P., Traverse, A., Fonda, E., Nahon, D., Bottero, J.Y., 2005. XAS study of iron and arsenic speciation during Fe(II) oxidation in the presence of As(III). *Environ. Sci. Technol.* 39, 9478-9485.

Torres-Escribano, S, Leal, M., Vélez, D., Montoro, R., 2008. Total and inorganic arsenic concentrations in rice sold in Spain, effect of cooking, and risk assessments. *Environ. Sci. Technol.* 42, 3867-3872.

Tsutsumi, M., 1983. Comparative toxicity of arsenite and arsenate to rice seedling under various levels of phosphate supply. *Soil Sci. Plant Nutr.* 29, 63-69.

Ullrich-Eberius, C.I., Sanz, A., Novacky, A.J., 1989. Evaluation of arsenate- and vanadate-associated changes of electrical membrane potential and phosphate transport in *Lemna gibba* G1. *J. Exp. Botany* 40, 210, 119-128.

Umweltbundesamt, 2003. Stoffmonographie Arsen- Referenzwert für Urin. *Bundesgesundheitsbl* [46], 1098-1106.

Van Bodegom, P.M., Van Reeve, J., Van der Gon, A.C.D., 2003. Prediction of reducible soil iron content from iron extraction data. *Biogeochem.* 64, 231-245.

Van der Vorm, P. D. J., 1987. Dry ashing of plant material and dissolution of the ash in HF for the colorimetric determination of silicon. *Commun. Soil Sci. Plant Anal.* 18, 1181-1189.

Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA), 1991. Die Untersuchung von Böden, VDLUFA, Darmstadt.

Welp, G., Herms, U., Brümmer, G., 1983. Einfluss von Bodenreaktion, Redoxbedingungen und organischer Substanz auf die Phosphatgehalte der Bodenlösung. Z. Pflanzenernaehr. Bodenk. 146, 38-52.

Williams, P., 2003. Investigating links between minerals in rice grain and Straighthead. Final report to the Ricegrowers' Co-operative Limited, Leton, NSW, 2705, USA.

Williams, P.N., Price, A.H., Raab, A., Hossain, S.A., Feldmann, J., Meharg, A.A., 2005. Variation in arsenic speciation and concentration in paddy rice related to dietary exposure. Environ. Sci. Technol. 39, 5531-5540.

Williams, P.N., Villada, A., Deacon, C., Raab, A., Figuerola, J., Green, A.J., Meharg, A.A., 2007. Greatly enhanced arsenic shoot assimilation in rice leads to elevated grain levels compared to wheat and barley. Environ. Sci. Technol. 41, 6854-6859

World Health Organization (WHO), Arsenic and arsenic compounds; Environmental Health Criteria 224; Geneva, 2001.

Woolson, E.A., Axley, J.H., Kearney, P.C., 1971. The chemistry and phytotoxicity of arsenic in soils: I. Contaminated field soils. Soil Sci. Soc. Amer. Proc. 35, 938-943.

Woolson, E.A., Axley, J.H., Kearney, P.C., 1973. The chemistry and phytotoxicity of arsenic in soils: II. Effects of time and phosphorus. Soil Sci. Soc. Amer. Proc. 37, 254-259

Xie, Z.M., Huang, C.Y., 1998. Control of arsenic toxicity in rice plants grown on an arsenic-polluted paddy soil. Commun. Soil Sci. Plant Anal. 29, 2471-2477.

Xu, X.Y., McGrath, S.P., Zhao, F.J., 2007. Rapid reduction of arsenate in the medium mediated by plant roots. New Phytol. 176, 590-599.

Xu, X.Y., McGrath, S.P., Meharg, A.A., Zhao, F.J., 2008. Growing rice aerobically decreases arsenic accumulation. Environ. Sci. Technol. 42, 5574-5579.

Xie, Z.M., Naidu, R., 2006. Factors influencing bioavailability of arsenic to crops. In: Managing arsenic in the environment. Ed. Naidu, R. et al., pp. 223-234, Enfield, NH, Science Publishers.

Yamaji, N., Mitani, N., Ma, J.F., 2008. A transporter regulating silicon distribution in rice shoots. *Plant Cell* 20, 1381-1389.

Yan-Chu, H., 1994. Arsenic distribution in soils, in : Nriagu, J.O. (Ed.), *Arsenic in the environment, Pt 1: Cycling and characterization*; Wiley & Sons: New York.

Yeo, A.R., Flowers, S.A., Rao, G., Welfare, K., Senanayake, N., Flowers, T.J., 1999. Silicon reduces sodium uptake in rice (*Oryza sativa* L.) in saline conditions and this is accounted for by a reduction in the transpirational bypass flow. *Plant Cell Environ.* 22, 559-565.

Yoshida, S., 1981. *Fundamentals of rice crop science*. The International Rice Research Institute, Manily, Philippines.

Zavala, Y.J., Duxbury, J.M., 2008. Arsenic in rice: I. Estimating normal levels of total arsenic in rice grain. *Environ. Sci. Technol.* 42, 3856-3860.

Zavala, Y.J., Gerads, R., Gürleyük, H., Duxbury, J.M., 2008. Arsenic in rice: II. Arsenic speciation in USA grain and implications for human health. *Environ. Sci. Technol.* 42, 3861-3866.

Zhang, C., Wang, L., Nie, Q., Zhang, W., Zhang, F., 2008. Long-term effects of exogenous silicon on cadmium translocation and toxicity in rice (*Oryza sativa* L.). *Environ. Exp. Botany* 62, 300-307.

Zhu, Y.-G., Williams, P.N., Meharg, A.A., 2008. Exposure to inorganic arsenic from rice: A global health issue? *Environ. Pollut.* 154 (2), 169-171.

9. Anhang

Im Folgenden sind die Ausbringungsmengen und Berechnungen zum As-Eintrag dargestellt.

Mineraldünger

Nach Grignani et al. (2004) beträgt die durchschnittlich applizierte Mineraldüngermenge in Piemont, Norditalien 127 kg N*Jahr, 67 kg P₂O₅*Jahr, and 161 kg K₂O*Jahr. Zusätzlich wird ein Ausbringung von 270 kg Kalk berücksichtigt.

Die Ausbringungsmenge von 127 kg N/ha*Jahr und einer angenommenen As-Konzentration von 0,15 mg/kg N beträgt die jährlich ausgebrachte As-Menge 0,02 g/ha.

- $0,00015 \text{ g As/kg N} * 127 \text{ kg/ha N} = 0,02 \text{ g As/ha}$

Im Folgenden werden zwei unterschiedliche As-Konzentrationen im P-Dünger (Superphosphat 18% P₂O₅) angenommen, um den Bereich in der Literatur genannten Bereich abzudecken.

- $8 \text{ mg As/kg Superphosphate} * 372 \text{ kg/ha} = 2,98 \text{ g As/ha}$
- $16 \text{ mg As/kg Superphosphate} * 372 \text{ kg/ha} = 5,95 \text{ g As/ha}$

Für die K-Düngung wird eine Ausbringung mit KCl (50% K₂O) mit einer As-Konzentration von 0,4 mg As/kg KCl angenommen.

- $0,0004 \text{ g As} * 322 \text{ kg KCl/ha} = 0,13 \text{ g As/ha}$

Da die As-Konzentration im Kalk stark variiert, wurde für die Berechnung eine hohe und eine niedrige As-Konzentration angenommen.

- $0,0015 \text{ g As/kg} * 270 \text{ kg/ha} = 0,41 \text{ g As/ha}$
- $0,01 \text{ g As/kg} * 270 \text{ kg/ha} = 2,70 \text{ g As/ha}$

Bewässerungswasser

Der Wasserbedarf im Nassreisanbau ist abhängig von der Evapotranspiration und der Versickerung. Ein typischer durchschnittlicher Wasserverbrauch liegt bei 1500 mm in einer Saison, was einem Verbrauch von 15.000 m³/ha gleichkommt (DITBERT 2004). Die As-Konzentration betrug im Mittel 1 µg/L im italienischen und 6 µg/L im

spanischen Bewässerungswasser. Beide As-Konzentrationen wurden für eine Berechnung des As-Eintrages eingesetzt.

- $0,001 \text{ mg As/L} * 15.000.000 \text{ L/ha} = 15 \text{ g As/ha}$
- $0,006 \text{ mg As/L} * 15.000.000 \text{ L/ha} = 90 \text{ g As/ha}$

Atmosphärische Deposition

Zur Berücksichtigung des As-Eintrages über die atmosphärische Deposition wurden zwei As-Konzentrationen, 0,5 und 4 g As/ha*Jahr, eingesetzt, die den in der Literatur genannten Bereich abdecken (EMEP, 2001).

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Lebenslauf

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Veröffentlichungen

Publikationen

Bogdan, K. and Schenk, M.K. 2009: Mobilisation of arsenic in rice plant (*Oryza sativa* L.) and its storage into the grain.

Eingereicht.

Bogdan, K. and Schenk, M.K. 2008: Arsenic in rice (*Oryza sativa* L.) related to dynamics of arsenic and silicic acid in paddy soils.

Environmental Science and Technology, 42 (21), 7885-7890.

Bogdan, K. and Schenk, M.K. 2008: Evaluation of soil characteristics potentially affecting As concentration in rice (*Oryza sativa* L.).

Environmental Pollution. Akzeptiert.

Daum, D., Bogdan, K., Schenk, M.K., and Merkel, D. 2001: Influence of the field water management on accumulation of arsenic and cadmium in paddy rice. *In*: Horst et al. (Eds.) Plant nutrition – Food security and sustainability of agro-ecosystems through basic and applied research, Kluwer Academic Publisher, Dodrecht, The Netherlands, pp 290-291.

Poster

Bogdan, K. and Schenk, M.K. 2008: Mobilisation of arsenic in rice plant (*Oryza sativa* L.). *In*: Proceedings of the Jahrestagung der Deutschen Gesellschaft für Pflanzenernährung, Limburgerhof/Speyer, Germany.

Bogdan, K. and Schenk, M.K. 2008: As content of rice ass affected by As dynamics in soil solution of six soils during paddy rice (*Oryza sativa* L.) cultivation. *In*: Proceedings of the 2nd International Congress on "Arsenic in the environment: Arsenic from nature to humans.", Valencia, Spain.

Bogdan, K., Keiwtsch, L. and M.K. Schenk 2007: Influence of Fe-plaque on the As(V) and As(III) uptake of rice plants (*Oryza sativa* L.). Arsen 2007 – Verhalten von Arsen in geologischen, hydrologischen und biologischen Systemen, Leipzig, Germany.

Bogdan, K. and Schenk, M.K. 2006: Influence of the redox potential on the Fe and As concentration of soil solution and As content of rice. *In*: Proceedings of the first joint conference of the German Society of Plant Nutrition – DGP (Annual Meeting) and the Research Centre Biotechnology & Plant Breeding Uni Hohenheim – FSP (21st Colloquium)., University of Hohenheim, Stuttgart, Germany.

Bogdan, K., Schenk, M.K., Ruschinski, O., and Daum, D. 2003: Einfluss der Bewässerung auf den Gehalt an Arsen und Cadmium in Nassreis. *In*: Proceedings of the Jahrestagung der Deutschen Gesellschaft für Pflanzenernährung, Giessen, Germany. Auszeichnung mit dem 3. Posterpreis.

Vortrag

Bogdan, K. and Schenk, M.K. 2006: Arsenic dynamic in the soil-plant system of paddy rice. Workshop 'Bioavailability of Arsenic', Halle, Germany.