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Pools of Silicon in Soils and their Contribution to Rice

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Five major pools of silicon (Si) such as mobile Si, adsorbed Si, Si bound to organic matter, Si occluded in pedogenic oxides/hydroxides and amorphous Si were extracted from three soils namely one each from low, medium and high categories of plant available Si using a sequential extraction method. Different pools of Si extracted from soils were in the order of amorphous Si > occluded Si > organic Si > adsorbed Si and mobile Si. The mobile Si and adsorbed Si pools were the smallest pools of Si and ranged from 14.4 to 44.6 mg kg^{-1} and 4.9 to 89.4 mg kg^{-1}, respectively. The content of organic Si and occluded Si ranged from 234 to 619 mg kg^{-1} and 476 to 1989 mg kg^{-1}, respectively. Irrespective of the soils, amorphous Si was found to be the largest pool of Si ranging from 8019 to 16667 mg kg^{-1}. Among different pools of Si, organic Si pool showed significant positive correlation with plant available Si while other pools had non-significant. A pot culture experiment was conducted using bulk soil samples with rice as test crop for a period of 60 days, and Si content and its uptake by rice was determined. Typic Rhodustalfs which is medium in plant available Si showed higher Si content (7.0%) and uptake (106 mg pot^{-1}) compared to Chromic Haplusterts having high plant available Si. The correlation analysis to know the contribution of different Si pools to Si uptake by rice revealed that occluded, mobile, amorphous and adsorbed pools as the major contributors of Si to rice.

Key words: Plant available silicon, rice, silicon pools, silicon content, silicon uptake

Silicon (Si) is ubiquitous in the earth's crust. It is the second most abundant element after oxygen (~28%, w/w). The translocation as well as temporary or permanent immobilization processes operate at various scales, results in various Si pools in soils (McKeague and Cline 1963; Sommer *et al*. 2006) which includes mobile Si (soil solution Si/ dissolved Si), adsorbed Si forms (monosilicic and polysilicic acids adsorbed to clay colloidal surfaces), Si bound to organic matter, Si occluded in pedogenic oxides and hydroxides, amorphous Si (phytoliths and silica nodules).

In recent years, the quantification and study of various pools of Si in soil is gaining much importance. Cornellis *et al.* (2010) identified the components of the readily soluble Si pool which includes dissolved Si, adsorbed Si, amorphous silica (of biogenic and pedogenic origin) extracted by Na_2CO_3 (Si_{alk}), oxalate (Si_{ox}) and $CaCl_2$, respectively. According to Klotzbucher *et al.* (2015) Si forms that contribute to the pool of readily-soluble Si in soils are soluble and adsorbed Si, poorly crystalline aluminosilicates, amorphous Si. Study of different Si pools in soils helps in understanding biogeochemical Si-processes which governs Si dynamics in soils (Gerard *et al.* 2002) also Si-cycling between soil and plant (Danilova *et al.* 2010).

The $CaCl_2$ and acetic acid are the most commonly used extractants to estimate the plant available Si status of soils, which extracts dissolved Si (Fox *et al*. 1967; Haysom and Chapman 1975; Khalid *et al.* 1978) and adsorbed Si in soil (Snyder 2001; Korndorfer *et al.* 2001), respectively. However, these extractants fail to extract Si from other pools *viz.* Si bound to organic matter, Si occluded in pedogenic oxides/hydroxides and amorphous Si which may also contribute Si for crop uptake but, not clearly established. A sequential extraction method fractionating various pools of Si, from the most mobile to immobile pools in soils (Danilova *et al.* 2010) would help to understand different pools. The present study aims at quantifying different pools of Si in soils and also to assess their contribution to rice.

Materials and Methods

Three soils, one each from low (< 47 mg Si kg^{-1}), medium (47-102 mg Si kg^{-1}), and high (> 102

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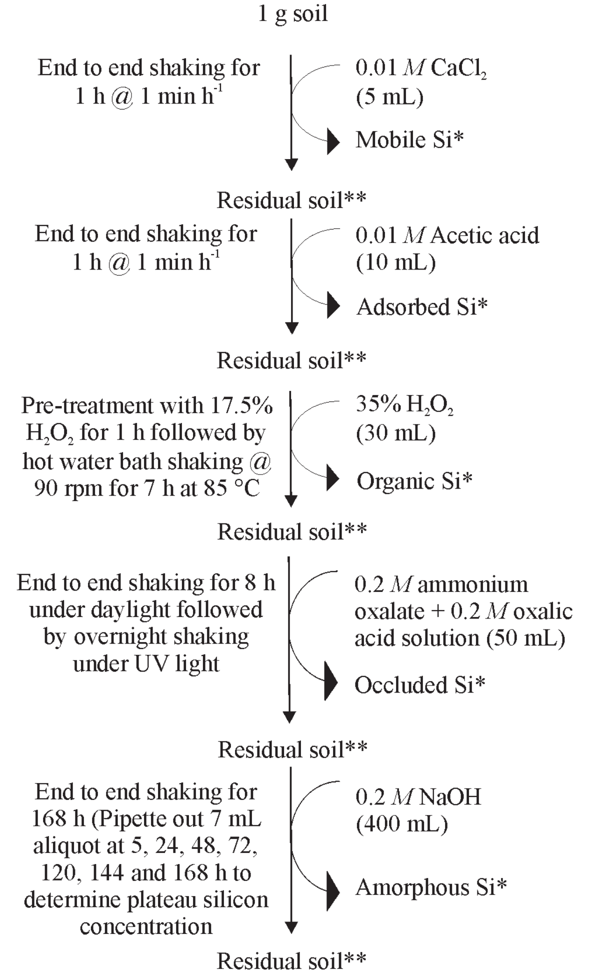
Fig. 1. Soil sampling locations in different soil types of Karnataka

mg Si kg^{-1}) in plant available Si content as estimated by acetic acid extractant method on the basis of Narayanaswamy and Prakash (2009) from Typic Kandiustults, Typic Rhodustalfs and Chromic Haplusterts locations, respectively representing coastal zone (CZ), southern dry zone (SDZ) and central dry zone (CDZ) of Karnataka, India (Fig.1) were collected from a depth of 0 to 15 cm. Soil samples were shade-dried, powdered and sieved through 2-mm mesh size sieve, then subjected for pH, electrical conductivity (EC), organic carbon (OC), cation exchange capacity (CEC), particle size analysis and plant available Si ($CaCl_2$ and acetic acid extractable Si) estimation (Table 1).

Extraction of Si pools from soil

The soil samples were subjected for extraction of different pools of Si using a sequential extraction method (Fig. 2) as outlined by Georgiadis *et al.* (2013). The sequentially extracted Si pools include: (1) mobile Si (extracted by $CaCl_2$ solution); (2) adsorbed Si (extracted by acetic acid); (3) Si bound to soil organic matter (extracted by H_2O_2); (4) Si occluded in pedogenic oxides and hydroxides (extracted by NH_4-oxalate under UV radiation); and (5) amorphous Si (extracted by NaOH).



*After shaking, the extracts of each step were centrifuged, filtered and analysed for respective Si pool with ICP-OES **The residual soil at each step was rinsed twice with distilled water to remove extractant of previous step.

Fig. 2. Flow chart for extraction of different pools of silicon (Georgiadis *et al.* 2013).

Step 1: Extraction of mobile Si pool

Air-dried soil (1.0 g) was taken in a plastic centrifuge tube and treated with 5 mL of 0.01 *M* $CaCl_2$

Table 1. Physicochemical properties of soils

Soil type	ACZ*	Physicochemical properties						
		pH (1:2.5)	EC (dS m^{-1})	OC (g kg^{-1})	CEC [cmol(p$^+$)kg^{-1}]	CCSi $^+$ (mg kg^{-1})	AASi $^{++}$ (mg kg^{-1})	Clay (%)
Typic Kandiustults	CZ	5.61 ± 0.01	0.18 ± 0.00	4.5 ± 0.03	27 ± 0.70	8.99 ± 1.34	2.38± 2.47	15.18 ± 0.13
Typic Rhodustalfs	SDZ	6.15 ± 0.10	0.31 ± 0.01	7.7 ± 0.09	37 ± 0.35	36.4 ± 3.75	82.2 ± 2.47	33.1 ± 0.21
Chromic Haplusterts	CDZ	6.67 ± 0.01	1.28 ± 0.09	7.1 ± 0.11	36 ± 1.06	40.7 ± 3.88	179.2 ± 4.15	47.81 ± 0.09

*ACZ - Agro climatic zone, CZ-coastal zone, SDZ- southern dry zone, CDZ-central dry zone

+CCSi- $CaCl_2$ extractable Si, ++AASi -Acetic acid extractable Si solution with a soil to solution ratio of 1:5 to extract mobile fraction of Si. The tubes were kept for shaking on a horizontal shaker for 24 h @ 1 min h^{-1}. The samples were centrifuged at 3000 rpm for 3 min. Then filtered and analyzed for Si concentration. The samples were rinsed twice with 5 mL of distilled water to remove the residual extractant of previous step.

Step 2: Extraction of adsorbed Si pool

A 10 mL of 0.01 *M* acetic acid extractant was added to the residual soil with a soil to solution ratio of 1:10 in order to extract adsorbed Si fraction. The tubes were kept for shaking on a horizontal shaker @ 1 min h^{-1} for 24 h. The samples were centrifuged at 3000 rpm for 3 min, filtered and analyzed for Si concentration. The samples were rinsed twice with 10 mL of distilled water to remove the residual extractant of previous step.

Step 3: Extraction of organic matter bound Si

To extract organic matter bound Si, H_2O_2 was used as extractant with a soil to solution ratio of 1:30. First, the residual soil was treated with 20 mL of H_2O_2 (17.5%) and manually shaken for 4-6 times and kept for 1 h at room temperature. Then, 10 mL of H_2O_2 (35%) was added and the samples were kept in water bath at 85 °C, with 90 rpm for 7 h. Samples were then cooled in a cold-water bath for 15 min and centrifuged at 3000 rpm for 15 min and filtered. The samples were rinsed twice with 10 mL of distilled water to remove the residual extractant of previous step.

Step 4: Extraction of Si occluded in pedogenic oxides and hydroxides

This fraction was extracted with 0.2 *M* ammonium oxalate + 0.2 *M* oxalic acid solution under UV-light. First, the extractant was applied at daylight and room temperature (25 ± 5 °C) for 8 h, using a soil to solution ratio of 1:50. Then, the suspensions were irradiated with UV light (30W) overnight with a distance between the sample and the UV lamp of approx. 20 cm. During the whole time, the samples were shaken horizontally for 1 min h^{-1}. The samples were rinsed twice with 10 mL of distilled water to remove the extractant of previous step.

Step 5: Extraction of amorphous Si

The amorphous Si was extracted with 0.2 *M* sodium hydroxide solution for 168 h at room temperature, using a sample to solution ratio of 1:400, and preferably shaking slowly over the whole time. An aliquot of 7 mL was pipetted from the supernatant solution after 5, 24, 48, 72, 120, 144 and 168 h, filtered and analyzed for Si to determine the plateau concentration.

Analysis of extracted Si pools by ICP-OES

The filtered extracts at each step were analyzed for Si with ICP-OES (Thermofisher, 7000 series) at 250.690, 251.611 and 288.158 nm wavelength. The mean value of the three wavelengths was taken and expressed in mg kg^{-1} (Table 2). A separate set of standard prepared with the same matrix used in extraction of respective Si pools of each step and the standards were used for analysis of filtrates.

Greenhouse study

A greenhouse pot culture study was conducted using three soil samples one each from low, medium and high categories of available Si and used for analysis of different Si pools by filling each plastic pot with 2.5 kg of soil and replicated thrice. Two rice seedlings of variety IR-64 of 21-days-old were transplanted to each pot and submergence was maintained throughout the experimental period by adding distilled water. The plant biomass was recorded at the time of harvesting at 60 days after transplanting (DAT). The plant samples were washed with distilled water and oven dried at 65 °C for 72 h, powdered and samples were subjected for Si analysis by adopting standard procedures. The Si content and its uptake by rice in each pot were determined.

Plant analysis

The powdered grain and straw samples were dried in an oven at 70 °C for 2-3 h prior to analysis.

Table 2. Different pools of silicon in rice soils

Soil type	ACZ*	Pools of Si (mg kg^{-1})				
		Mobile	Adsorbed	Organic	Occluded	Amorphous
Typic Kandiustults	CZ	14.4 ± 0.08	4.90 ± 0.84	234 ± 3.4	476 ± 25	8019 ± 97
Typic Rhodustalfs	SDZ	42.4 ± 1.49	89.4 ± 0.90	478 ± 7.7	1989 ± 376	16667 ± 200
Chromic Haplusterts	CDZ	44.6 ± 2.16	28.4 ± 2.31	619 ± 8.3	1406 ± 146	11927 ± 76

*ACZ - Agro climatic zone, CZ-coastal zone SDZ- southern dry zone CDZ- central dry zone

The sample (0.1 g) was digested in a mixture of 7 mL of HNO_3 (70%), 2 mL of H_2O_2 (30%) and 1 mL of HF (40%) using microwave digestion system (Milestone-start D) at 150 °C with following steps: 1200 watt for 15 minutes, 1200 watt for 10 min and venting for 10 min. The digested samples were diluted to 50 mL with 4% boric acid. The Si concentration in the digested solution was determined as described below: 0.5 mL of digested aliquot was transferred to a plastic centrifuge tube, to which 3.75 mL of 0.2 *N* HCl, 0.5 mL of 10% ammonium molybdate ($(NH_4)_6Mo_7O_2$) and 0.5 mL of 20% tartaric acid and 0.5 mL of reducing agent (Amino naphthol sulphonic acid - ANSA) was added and the volume was made up to 12.5 mL with distilled water. After 1 h, the absorbance was measured at 600 nm with a UV-visible spectrophotometer. Similarly, standards (0, 0.2, 0.4, 0.8 and 1.2 ppm) were prepared by following the same procedure.

Statistical analysis

Pearson's correlation analysis was performed between the available Si content and different pools of Si, and also between different pools of Si in soil and Si content and its uptake by rice. The Si pools with significant positive correlation coefficients with plant Si uptake were identified as the major contributors of Si to rice.

Results and Discussion

Si pools in rice soils

Irrespective of soils under study, the pools of Si extracted were in the order of amorphous Si > occluded Si > organic Si > adsorbed and mobile Si. Among the different pools of Si, amorphous Si pool accounts to be the largest Si pool, while mobile and adsorbed Si pools constitute smallest pools of Si in the study (Table 2).

Mobile Si

This pool represents the readily soluble fraction of soil Si. The higher content of mobile Si was recorded in Chromic Haplusterts (high available Si) followed by Typic Rhodustalfs (medium available Si) and lower content in Typic Kandiustults (low available Si). The Chromic Haplusterts and Typic Rhodustalfs containing higher content of clay, recorded higher content of mobile Si pool. However, Typic Kandiustults having low clay content recorded lower mobile Si pool which suggests that clay mineral surfaces serve as major source of mobile Si. This is also found to be true with findings of Georgiadis *et al.* (2013) and Vandevenne *et al.* (2015) who reported a positive relationship between easily soluble Si and clay content. In the present study the mobile Si pool ranged from 14.4 to 44.6 mg kg^{-1} (0.01- 0.04 mg g^{-1}) which was in accordance with the range noticed by Cornelis *et al.* (2010). Vandevenne *et al.* (2015) reported 18 and 52 mg kg^{-1} of $CaCl_2$ extractable Si pool in the crop lands of central Belgium (temperate climate).

Adsorbed Si

The content of adsorbed Si pool ranged from 4.90 and 89.40 mg kg^{-1}. Higher adsorbed Si pool was recorded in Typic Rhodustalfs (medium available Si) followed by Chromic Haplusterts (high available Si) having higher clay content. Whereas, Typic Kandiustults (low available Si) having low clay content recorded lower adsorbed Si content. Dietzel (2002) reported that, adsorption of monosilicic acid takes place through a surface reaction between the monosilicic acid and the hydroxyl groups of the clay mineral surfaces. The high Si-adsorption capacity of soils might be attributed to the dominance of 2:1 clay minerals on which the monosilicic acid may polymerize and adsorb to the mineral surfaces as polysilicic acid or precipitate (Chadwick *et al.* 1987).

In the present investigation, the content of adsorbed Si pool was lower than the mobile Si pool. Our observation was in contrast to the hypothesis that acetic acid extracts higher Si content than $CaCl_2$ extractant (Haynes *et al.* 2013). In general, 0.5 *M* acetic acid extractant was used with soil to solution ratio of 1:2.5 while extracting adsorbed Si from soils. However, in the present investigation, comparatively lower concentration of acetic acid (0.01 *M*) was used in order to avoid extremely acidic conditions and thus to prevent a possible Si release from other soil compounds (Georgiadis *et al.* 2013) at a higher soil to solution ratio of 1:10 to extract adsorbed pool of Si. Thus, the lower concentration of the extractant and wider soil to solution ratio might be the reason for lower content of adsorbed Si.

In the present investigation, adsorbed Si content of different soils varied between 0.005 and 0.008 mg g^{-1} which is very low when compared to the reports of Georgiadis *et al.* (2014) wherein, adsorbed Si ranged from 1.2 to 39 mg g^{1} in soils of humid temperate climate (SW-Germany). The mobile and adsorbed Si pools represent smallest fractions of Si among the different pools.

Organic Si

The organic Si is the fraction of Si in soil that is associated with organic matter. The organic Si pool was obtained through destruction of soil organic matter. The content of organic Si pool ranged from 234 to 619 mg kg^{-1}. The higher organic Si pool was observed in Chromic Haplusterts (high available Si) followed by Typic Rhodustalfs (medium available Si). These soils were rich in clay content (> 20%) and OC content (7.1 and 7.7 g kg^{-1}, respectively). While Typic Kandiustults (low available Si) characterized by comparatively higher sand content and low OC evidenced a lower organic Si pool. This showed clearly the involvement of clay and OC in retention of Si in the soils. However, Georgiadis *et al.* (2014) reported no correlation between organic Si pool and OC content. This was interpreted due to partial release of Si from clay minerals and pedogenic oxides-hydroxides in the hot concentrated H_2O_2 solution. The organic Si pool in our study ranged from 0.2 to 0.6 mg g^{-1} which is in accordance with results obtained by Georgiadis *et al.* (2014), who reported that Si in soil organic matter ranged between 0.04 and 0.9 mg g^{-1}.

Occluded Si

The content of the occluded Si ranged between 0.3 and 1.94 mg g^{-1}. The study on sequential Si extraction on soils of a temperate–humid climate by Georgiadis *et al*. (2014) revealed that amounts of oxalate-extractable Si (occluded Si) ranged between 0.01 and 1.7 mg g^{-1} in fine earth. The occluded Si pool was found to be higher in Typic Rhodustalfs (medium available Si) followed by Chromic Haplusterts (high available Si) while lower content of occluded Si was noticed in Typic Kandiustults (low available Si). The Si concentration in a soil is controlled by the dissolution of the siliceous materials and by the sorption reactions between soluble silica and reactive soil materials, particularly the pedogenic oxides and hydroxides, other anions and soil pH also influence the reactions (Obihara and Russell 1972).

The occlusion of dissolved Si depends upon the chemical composition of soil solution, the mineral surface and also the nature of dissolved silica (Dietzel 2002). Cornelis *et al.* (2010) reported that the oxalate extractable Si (occluded Si) represents 3.3-52.5% of total alkaline extractable Si (total amorphous Si). This finding holds good in the present investigation where, the occluded Si pool corresponds to 5-12% of amorphous Si pool.

Amorphous Si

The amorphous Si pool includes both minerogenic (non-biogenic silica) and biogenic silica. The minerogenic silica corresponds to non-crystalline inorganic fraction, such as opal formed at supersaturated soluble Si levels, volcanic glasses, opal coatings on secondary minerals while biogenic silica comprises phytolith, diatoms and sponge spicule (Sauer *et al.* 2006). This fraction is assumed to play a major role in Si availability and cycling since it is more soluble than crystalline minerals (Alexandre *et al.* 1997).

In the present investigation, the amorphous Si pool of different soils was in the range of 8 to 16 mg g^{-1}. Drees *et al.* (1989) reported that the amount of amorphous Si typically ranged from < 1 to 30 mg g^{-1} on a total soil basis. Vandevenne *et al.* (2015) reported that the biogenic Si or other reactive non-biogenic Si phases, defined as the alkaline-extractable Si concentrations in silt loam soils of temperate crop lands ranged from 4 to 8 mg g^{-1}.

Among the different pools, amorphous Si was found to be the largest Si pool irrespective of the soils under study. Danilova *et al.* (2010) reported that Si extracted by NaOH (amorphous Si) as the largest fraction of extractable Si in the soils of SW-Germany.

The higher amorphous Si pool was recorded in Typic Rhodustalfs (medium available Si) followed by Chromic Haplusterts (high available Si). The lower content of amorphous Si was observed in Typic Kandiustults with low available Si. The variation in amorphous Si content might be attributed to the texture and organic matter content of the soils. In general, clay and sandy clay loam soils with higher organic matter content recorded higher amorphous Si. The loamy sand soil with less organic matter content recorded lower amorphous Si content and thereby establishes the fact that, the amorphous Si pool is well associated with clay content of the soils.

Relationship between plant available Si and different pools of Si

In the present study, an attempt was made to know the relationship of different pools of Si with the plant available Si (CCSi and AASi). The correlation coefficient and regression equations showed that organic Si pool has significant positive correlation and regression with both CCSi and AASi. However, mobile and occluded pools showed significant relationship with CCSi but was non-significant with AASi. The Pearson's correlation and regression

Table 3. Pearson's correlation between different pools of Si and plant available Si content in soils (CCSi and AASi), Si content and uptake by rice

Parameter	Pools of Si				
	Mobile	Adsorbed	Organic	Occluded	Amorphous
CCSi^{+}	0.995**	0.62	0.969**	0.865*	0.749
AASi^{++}	0.809	0.109	0.950**	0.466	0.291
Si content	0.968**	0.483	0.987**	0.766	0.647
Si uptake	0.919**	0.854*	0.791	0.970**	0.895*

* Correlation is significant at 0.05 level, ** Correlation is significant at 0.01 level

$^{+}$CCSi - Calcium chloride extractable Si, $^{++}$AASi - Acetic acid extractable Si

coefficient between CCSi and AASi with different pools of Si was in the order of organic Si > occluded Si > mobile Si > adsorbed Si > amorphous Si (Table 3 and Fig. 4).

Irrespective of the pools of Si, there was a positive correlation and regression with CCSi and AASi content of the soils. This might be attributed to the dissolution kinetics of Si pools under submerged conditions. Koltzbucher *et al.* (2015) reported that the various Si forms were continuously regenerated under submerged conditions, due to the dissolution of pools to a certain extent. Upon submergence, the adsorbed Si on clay colloids releases Si to the soil solution due to exchange phenomenon and Si associated with poorly crystallized iron and aluminium oxides and hydroxides which undergo dissolution due to changes in pH, Eh and other factors (Kogel-Knabner *et al.* 2010) and a part of amorphous Si which include diatoms, plant bound biogenic Si (phytoliths) and products of various binding processes that undergo dissolution and become available to plants as dissolved Si.

Although mobile and organic Si pools increased with the available Si content of the soils, there was no definite trend for adsorbed, occluded and amorphous Si especially with soils of high available Si. However, there is no preliminary data to show such kind of relationships and requires further study in multiple locations with large samples.

Effect of available Si and Si pools on Si content and uptake by rice

Among different soils, higher Si content and uptake by rice was noticed in Typic Rhodustalfs with medium available Si whereas, Typic Kandiustults with low available Si recorded the least (Fig. 3). Though, Chromic Haplusterts characterized by higher available Si content than Typic Rhodustalfs (medium in available Si), not much significant contribution of Si for rice might be attributed to the lower contents of different pools of Si compared to Typic Rhodustalfs. The Typic Rhodustalfs, though had low status of available Si, showed higher contribution to the Si content and its uptake by rice because of higher content of different pools of Si. This clearly indicates that not only available Si as estimated by $CaCl_2$ and acetic acid extractants, but other pools of Si also play an important role in contributing Si to its content and uptake by rice.

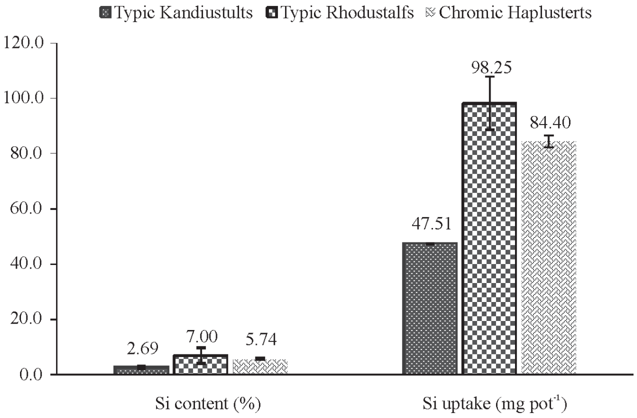


Fig. 3. Content (%) and uptake (mg pot^{-1}) of Si by rice in different soils

Relationship between different pools of Si and Si content and its uptake by rice

Irrespective of the soil type, there was a positive correlation between the Si pools and Si content and its uptake by rice (Fig. 5). All the pools of Si showed positive relationship with Si content of rice but, only mobile and organic pools were found to be significant. The relation between Si uptake by rice and pools of Si was in the order of occluded Si > mobile Si > amorphous Si > adsorbed Si > organic Si. Occluded, mobile, amorphous and adsorbed pools of Si had significant positive relationship with Si uptake by rice and hence considered as major contributors of Si to rice. However, evidence to suggest relationship

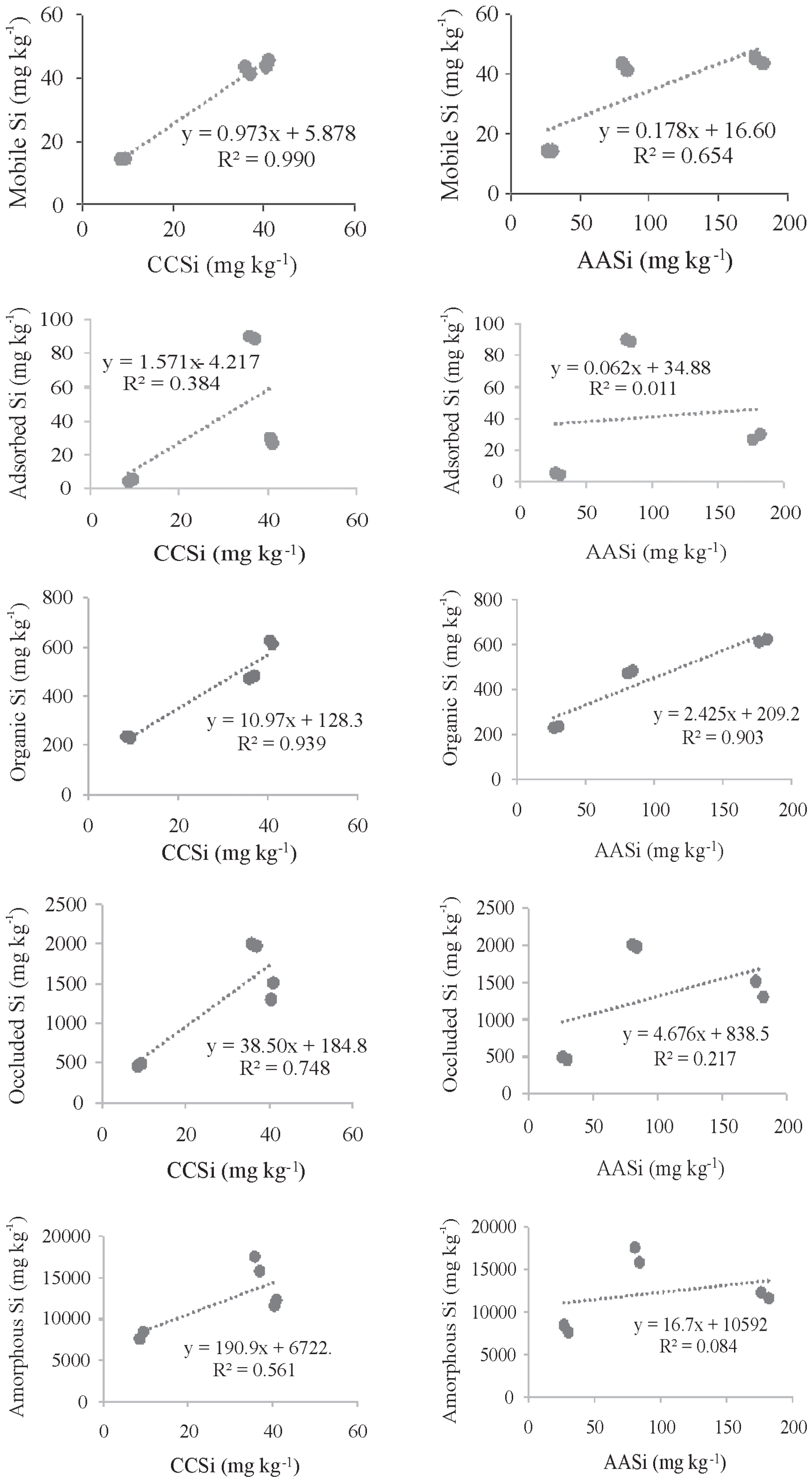


Fig. 4. Relationship between plant available Si (CCSi and AASi) and Si pools in soils

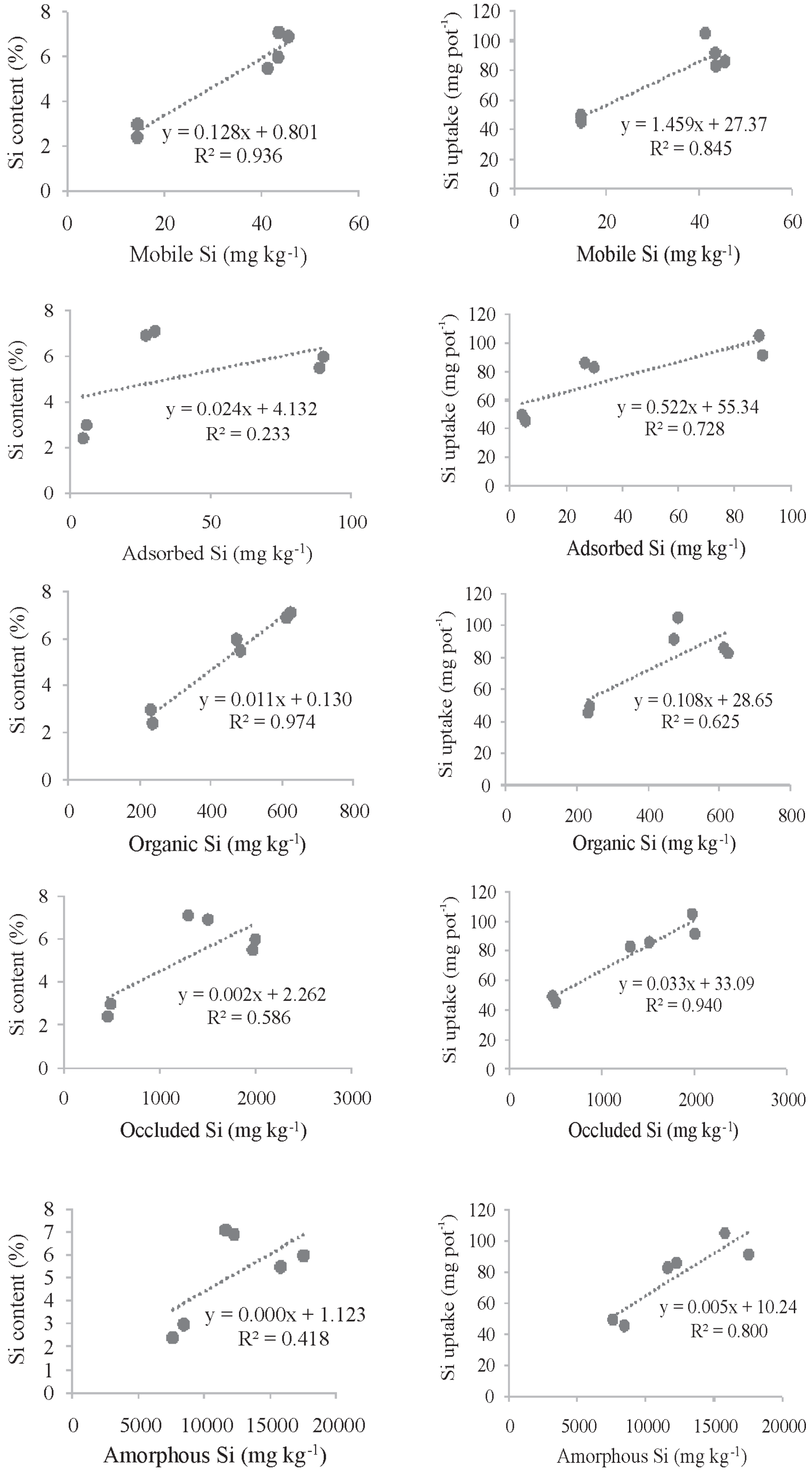


Fig. 5. Relationship between different pools of Si in soils and Si content and its uptake by rice

between Si pools and Si content / its uptake by rice is very much lacking.

In conclusion, the study provides information on content of different pools of Si in soils and their relative contribution to the crop uptake and revealed that occluded, mobile, amorphous and adsorbed pools of Si found to be the major contributors of Si to rice. Thus not only plant available Si, but other pools of Si also have contribution to the Si content and its uptake by rice, indicating the need for further investigation on different Si pools involving large number of samples.

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