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Addressing variations in status of a few nutritionally important micronutrients in wheat crop

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Abstract
The micronutrient density of seeds is important not only for human nutrition but also for the nutrition of the next generation seedling. Deficiencies of zinc (Zn), iron (Fe) and manganese (Mn) in soils and crops occur nearly in all countries, particularly in cereals growing areas. The deficiencies adversely affect both food production and human health in a major part of the world. Therefore, variations in micronutrient (Zn, Fe and Mn) accumulation were evaluated among 14 wheat varieties in a field containing DTPA extractable Zn, Fe and Mn 1.60, 17.55 and 3.50 mg/kg soil, respectively at the Research Farm, CCS HAU, Hisar, Haryana, India during 2008-09 The treatments for Zn, Fe and Mn consisted of control, 50% of the recommended dose, recommended dose, 150% of the recommended and recommended foliar spray. There is considerable genetic variation in concentration of Zn, Fe and Mn among all the wheat varieties. The accumulation of Zn, Fe and Mn were more in straw than seed, irrespective of the variety under study.

Key Words
Wheat genotypes, micronutrients levels, grain and straw yields, micronutrients concentration.

Introduction

Crops and varieties within a crop often differ in their yield potential, nutrient requirement and efficiency in nutrient uptake, making it difficult to formulate general fertilizer recommendations. During the last four decades under a global wheat improvement programme, a number of high yielding varieties of wheat have evolved and been introduced in the cropping system which has helped in increasing food production, thereby greatly reducing starvation, and protein malnutrition. However, this has caused greater depletion of micronutrient reserves in soil and thereby accentuated wide spread deficiencies of micronutrients. As much as 48, 12, 5, 4, 33, 13 and 41 percent soil in India are affected with deficiencies of Zn, Fe, Mn, Cu, B, Mo and S, respectively. These deficiencies cause not only hidden hunger but also lead to entire failure of crops and lower content of trace elements in plant parts. Moreover, standard micronutrient concentration values are not available to suggest suboptimal, optimal or excess levels of trace element content in seed in a specific field crop. Similarly, optimal relative micronutrient concentration or critical concentration values are not available for classifying seed into deficient or sufficient categories as an index for their fitness for supporting human health. This necessitated a proper evaluation of influence of genetic variability among the newly released wheat varieties for micronutrient accumulation in seed and straw. Where soils have enough Zn, Fe, Mn and Cu, identifying plant varieties or crop species capable of higher absorption and translocation into the seed is highly desirable. Therefore, in this experiment an attempt has been made to address the micronutrients enrichment of seed and straw by screening the most efficient varieties of wheat.

Methods

The influence of genotypes on Zn, Fe and Mn accumulation in seed and straw in fourteen recently evolved varieties of wheat was evaluated in a field experiment. The characteristics of the experimental field were pH-7.2, E.C.-0.37 dS/m, O.C.-0.69%, $CaCO_3$-Nil and DTPA-extractable Zn, Fe and Mn contents were 1.60, 17.55 and 3.50 mg/kg soil, respectively (Lindsay and Norvell, 1978). Three rows of each variety were sown in a plot size of 12.3m X 12.3m for each element and received a basal application of N, P and K @ 120, 60 and 60 kg/ha, respectively as urea, DAP and muriate of potash.

The details of the experimental treatments are:
Soil type: One
Genotypes**:** Fourteen (WH-1025, PBW- 502, WH-542, PBW- 550, HD- 2851, PBW- 343, WH-711, WH-896, WH- 147, C- 306, RAJ- 3765, PBW- 373 WH-1021, WH-1022)

Micronutrients Levels:
Zn = 0, 12.5, 25.0, 37.5 kg $ZnSO_4$/ha and 0.5% $ZnSO_4$ foliar application.
Fe = 0, 25.0, 50.0, 75.0 kg $FeSO_4$/ha and 1.0% $FeSO_4$ foliar application.
Mn = 0, 25.0, 50.0, 75.0 kg $MnSO_4$/ha and 0.5% $MnSO_4$ foliar application.

In the case of Zn and Fe foliar application, all the sprays were done after first irrigation at an interval of 10-12 days. . In the case of Mn first spray was done four weeks after of seeding and the next two sprays after irrigation at 7-10 day intervals. The crop was harvested at maturity and the grain and straw yields was recorded. The plant samples were ground in a stainless steel grinder and digested with diacid mixture (nitric and percholoric acid, 3:1). The Zn, Fe and Mn concentrations in plant digests were determined by atomic absorption spectrophotometer. Total uptake of Mn, Fe and Zn in different wheat varieties was computed. In order to correlate the nutrient content in plants, to biological parameters the number of tillers, ear length, number of grains/tiller and 1000 grain weight were also recorded.

Results
The increments in content of critical micronutrients can materially increase the vigour, ear length, number of grains/tiller and grain yield of the subsequent crop grown in the soil deficient in the treatment nutrient. Plant breeders can select seeds for higher micronutrient content but greater enhancement in most cases can be achieved through fertilization, both by soil applied or sprayed on the flowers, seed pods, or ears 1 to 3 times during seed development. In this study all the varieties responded both to soil and foliar applied Zn, Fe and Mn which significantly increased the tillering, ear length, number of grains/tiller, thousand grain weights, yield of grain and straw as well as their concentration in seed and straw (Grunes and Alloway 1985; Alloway 1986). In the case of Zn, the maximum increase in grain yield was found when the recommended doses of Zn (25 kg $ZnSO_4$/ha) was applied as a soil application and 0.5 % solution of $ZnSO_4$ as foliar spray (Table-1). However, the increase in biomass production with Zn application was much lower than the increase in grain indicating that Zn is of critical importance for seed setting than for vegetative growth.

Table 1. Effect of Zinc, Iron and Manganese application methods on grain yield of wheat.

Treatments	Grain Yield (q/ha)		
	Zn	Fe	Mn
Control	33.66-48.73	31.93-52.75	32.94-48.36
	(40.98)	(43.30)	(43.11)
Soil Application	34.35-50.90	36.55-57.38	34.42-52.60
	(42.51)	(49.06)	(48.62)
Foliar Application	33.82-48.20	34.24-57.84	33.49-60.32
	(42.22)	(47.85)	(47.93)

As Zn applied to soil has a significant effect for at least a couple of years, the soil application method has been considered as the most effective and economical for wheat in the long run. Both methods of Zn application clearly enhanced grain Zn concentration over control but the most effective method for increasing Zn in shoot and seed was foliar application that resulted in 1.0 to 5.5 and 1.0 to 2.1 fold increases in the concentration (Table 2). There was either no change or a slight increase in magnitude of Zn, Fe and Mn response when their levels were raised 50% higher than the recommended dose.

Table-2 Effect of Zinc, Iron and Manganese fertilization methods on metal concentrations in wheat grain.

Treatments	Micronutrients Content (mg/kg)		
	Zn	Fe	Mn
Control	26.5-39.5	29.7-42.5	22.9- 31.5
	(32.2)*	(34.4)	(27.6)
Soil Application	26.5- 46.5	32.4- 77.0	25.4- 36.2
	(37.9)	(49.2)	(31.06)
Foliar Application	32.5- 60.0	39.1- 98.4	25.4- 38.1
	(47.2)	(63.9)	(32.7)

*Figures in parenthesis are mean values

Soil application of Fe and Mn usually has no or only limited residual effects as Fe^{2+} and Mn^{2+} are rapidly converted to Fe^{3+} and Mn^{4+}. Foliar application of Fe^{2+} was more effective than soil application increasing concentrations from 1.0 to 2.3 and 1.0 to 3.54 mg/kg, respectively in seed and straw of wheat (Tables 2 and 3). However, because of its rapid oxidation in soil and low mobility in phloem, soluble Fe^{2+} fertilizer is ineffective in increasing the Fe concentration in plants especially in the grain that develops months after germination. In contrast to Zn content in seed, which is easily enhanced, foliar application of $FeSO_4$ was not much better for increasing Fe content in wheat. The Mn content in seed and straw can be increased significantly by applying 0.5% $MnSO_4$ solution to the generative tissues (Asher 1994). However, in the present study the corresponding increase in Mn concentration of seed and straw of wheat varieties varied from 1.0 to 1.3 and 1.0 to 2.54 fold (Tables 2 and 3). This might be due to the variations in uptake and use efficiency of Mn between genotypes (Marschner 1995).

Table 3. Effect of recommended doses of Zinc, Iron and Manganese fertilization methods on metal concentrations in straw of wheat

Treatments	Micronutrients Content (mg/kg)		
	Zn	Fe	Mn
Control	8.0- 17.0 (11.1)*	61.9- 169.1 (110.3)	11.3- 16.0 (13.6)
Soil Application	10.0- 23.5 (14.6)	85.2-241.6 (168.4)	13.2- 23.7 (17.4)
Foliar Application	20.5- 45.5 (32.4)	131.3- 306.1 (204.5)	16.8- 35.6 (22.8)

*Figures in parenthesis are mean values

Conclusion

The response to Zn, Fe and Mn fertilization of the enrichment of these metals in seed and straw may varies from variety to variety. When an increase in grain Zn, Fe or Mn concentration in grain is required, in addition to soil fertilization, foliar application should also be recommended.

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Biofortification of cereals with zinc and iron through fertilization strategy

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Abstract
Zinc (Zn) and iron (Fe) deficiencies are well-documented public health issue and an important soil constraint to crop production. Generally, there is a close geographical overlap between soil deficiency and human deficiency of Zn and Fe, indicating a high requirement for increasing concentrations of micronutrients in food crops. Breeding new plant genotypes for high grain concentrations of Fe and Zn (genetic biofortification) is the most cost-effective strategy to address the problem; but, this strategy is a long-term process. A rapid and complementary approach is therefore required for biofortification of food crops with Zn and Fe in the short term. In this regard, a fertilizer strategy (agronomic biofortification) represents an effective way for biofortification of food crops. In this paper, several examples are presented showing that application of Zn fertilizers greatly contribute to biofortification of cereal grains with Zn. By contrast, application of various inorganic and chelated Fe fertilizers remains ineffective for increasing grain Fe concentration. However, improving nitrogen (N) nutritional status of plants promoted accumulation of Fe (and also Zn) in grain. It appears that N nutritional status of plants plays a critical role in biofortification of cereal grains with Zn and Fe.

Key Words
Zinc deficiency, iron deficiency, agronomic biofortification, wheat, zinc fertilizers, iron fertilizers.

Introduction
Zinc and Fe deficiencies are a growing public health and socioeconomic issue, particularly in the developing world (Welch and Graham 2004). Recent reports indicate that nearly 500,000 children under 5 years of age die annually because of Zn and Fe deficiencies (Black *et al.* 2008). Zinc and Fe deficiencies together with vitamin A deficiency have been identified as the top priority global issue to be addressed to achieve a rapid and significant return for humanity and global stability (www.copenhagenconsensus.com). Low dietary intake of Fe and Zn appears to be the major reason for the widespread prevalence of Fe and Zn deficiencies in human populations. In countries with a high incidence of micronutrient deficiencies, cereal-based foods represent the largest proportion of the daily diet (Cakmak 2008). Cereal crops are inherently very low in grain Zn and Fe concentrations, and growing them on potentially Zn- and Fe-deficient soils further reduces Fe and Zn concentrations in grain (Cakmak *et al*. 2010). Thus, biofortification of cereal crops with Zn and Fe is a high-priority global issue. HarvestPlus (www.harvestplus.org) is the major international consortium to develop new plant genotypes with high concentrations of micronutrients by applying classical and modern breeding tools (i.e. genetic biofortification). Although plant breeding is the most sustainable solution to the problem, developing new micronutrient-rich plant genotypes is a protracted process and its effectiveness can be limited by the low amount of readily available pools of micronutrients in soil solution (Cakmak 2008). Application of Zn- and Fe-containing fertilizers (i.e. agronomic biofortification) is a short-term solution and represents a complementary approach to breeding.

Materials and Methods
The experiments described in this paper were conducted with wheat either under field or greenhouse conditions. Field trials were conducted in Central Anatolia, Turkey where soil Zn deficiency is well documented. In the case of soil application, the rate of $ZnSO_4$ application was 50 kg $ZnSO_4$ per ha, while foliar Zn was applied at the rate of 0.5 % $ZnSO_4.7H_20$ (approx. 4 kg $ZnSO_4.7H_20$ /ha) at 2 growth stages. In greenhouse studies, plants were grown with 0.05 mg Zn (low Zn plants) or 2 mg Zn (adequate Zn plants) or 10 mg Zn (high Zn plants) per kg soil, and Zn was applied in the form of $ZnSO_4{\cdot}7H_2O$. Nitrogen treatments were 50 mg N (for low N plants) or 200 mg N (for adequate N plants) in the form of $Ca(NO_3)_2{\cdot}4H_2O$. In the greenhouse experiments investigating the effectiveness of Fe fertilizers on increasing grain Fe, the following Fe compounds used : $FeSO_4$, Fe-EDTA, Fe-EDDHA and Fe-citrate, applied either into soil at the rates of 0, 5 and 10 mg Fe per kg soil or sprayed to foliar at the rate of 0.2 % Fe-EDTA at the booting and early milk stages. The rates of soil applied nitrogen ranged from 100 to 600 mg N per kg soil and applied in the form of $Ca(NO_3)_2$ (see Kutman *et al*. 2010 for further details).

Results

Field tests in Central Anatolia, where Zn deficiency is widespread, showed that soil- and foliar-applied $ZnSO_4$ significantly enhanced grain Zn concentration in wheat. The largest increases in grain Zn concentration were found in the case of combined application of soil and foliar Zn fertilizers that caused more than a 3-fold increase in grain Zn (Figure 1). Under certain conditions, Zn fertilizers were also highly effective in increasing grain yield of wheat. In contrast to Zn-deficient locations, soil application of $ZnSO_4$ in Zn-adequate soils had no or very little effect on grain Zn. However, irrespective of soil Zn status, foliar Zn applications resulted in significant increases in grain Zn, especially in the case of late-season foliar Zn application.

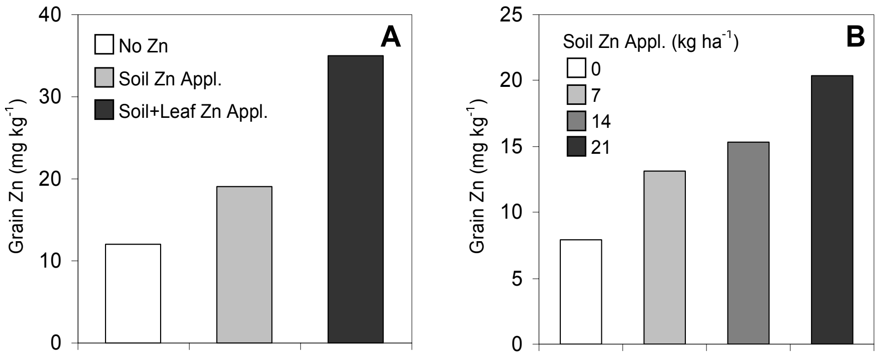

Figure 1. Grain Zn concentrations of durum wheat subjected to soil and foliar application of $ZnSO_4$ (A) and increasing rate of soil Zn fertilization (B). Plants were grown on a highly Zn-deficient calcareous soil under field conditions in Central Anatolia (Cakmak *et al.* 2010).

Enrichment of wheat grain with Zn was maximized when plants were supplied sufficiently with nitrogen (N) through soil and/or foliar (e.g., urea) application of N fertilizers. In a greenhouse study, effects of soil- and foliarly-applied N and Zn fertilizers on grain Zn concentration of durum wheat were studied when grown on a Zn-deficient soil. When Zn application was adequate, both soil and foliar N applications significantly increased grain Zn concentration. Nitrogen application remained ineffective on grain Zn when Zn supply was suboptimal (Figure 2). It seems that N and Zn act synergistically in improving the grain Zn concentration when N and Zn are present at sufficient amounts either in the growth medium or in the leaf tissues. Enrichment of commonly applied compound fertilizers with Zn is a further fertilizer practice useful for increasing Zn concentration of plants. In India, application of Zn-coated urea fertilizer significantly improved both grain yield and grain Zn concentrations (Shivay *et al*. 2008).

In contrast to Zn fertilizers (e.g. $ZnSO_4$), Fe fertilizers applied either in inorganic form (e.g. $FeSO_4$) or chelated form (e.g., Fe-EDTA, Fe-EDDHA or Fe-citrate) into soil or as a foliar were not effective in improving grain Fe concentrations of wheat grain (Aciksoz *et al.* unpublished results). Among Fe fertilizers applied, Fe-EDTA appeared to be the best Fe source for increasing grain Fe concentrations. However, increasing N applications promoted Fe accumulation in grain at each Fe application. As found with Zn, there was a close positive correlation between tissue concentrations of Fe and N.

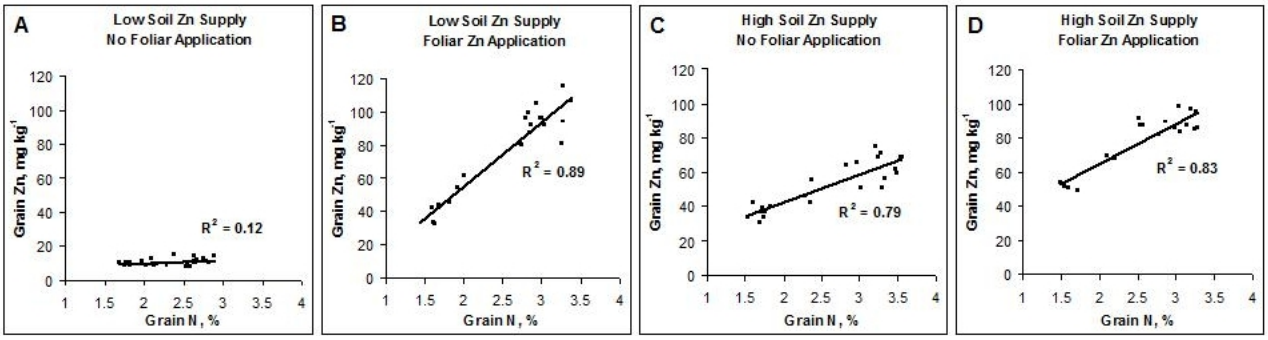

Figure 2. Correlation between grain concentrations of Zn and N in durum wheat (*Triticum durum* cv. Balcali 2000). Plants were grown at low (A and B) or high (C and D) Zn supply on a Zn-deficient calcareous soil under greenhouse conditions with (B and D) or without (A and C) foliar application of Zn (Kutman *et al.* 2010).

Conclusion

The role of N nutrition in biofortification of wheat grain with Zn and Fe is a highly relevant issue in terms of designing new fertilizer programs for increasing grain Zn and Fe and selecting the most suitable parental lines in breeding programs aimed at improving grain Zn and Fe. Improving N nutrition of plants may contribute to grain Zn and Fe concentrations by affecting the levels of Zn- or Fe-chelating nitrogenous compounds required for transport of Zn and Fe within plants and/or the abundance of Zn or Fe transporters needed for root uptake and phloem loading of Zn and Fe. Finally, the results indicate that nitrogen management represents an effective agronomic tool to contribute to grain Zn and Fe concentrations.

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Effect of nitrogen and iron fertilizers on grain concentration of iron in wheat

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Abstract
Greenhouse experiments have been conducted to investigate the effectiveness of various soil- and foliarly-applied iron (Fe) fertilizers in increasing grain Fe concentrations of wheat plants under different nitrogen (N) nutrition regimes. The Fe fertilizers tested were FeSO4, Fe-EDTA, Fe-EDDHA and Fe-citrate and applied either into soil or foliar sprayed at the booting and early milk stages. The rates of soil applied nitrogen ranged between 100 to 600 mg N per kg soil. In an additional experiment, plants were also treated with 0.75 % urea during the booting and early milk stages. At a given N supply, shoot and grain concentrations of Fe were not affected by increasing application of Fe fertilizers. In contrast to soil Fe application, increasing soil application of N significantly enhanced Fe concentrations of shoot and grains. There was a close relationship between tissue concentrations of Fe and N. Foliarly-applied Fe fertilizers were either not effective or tended to improve Fe concentrations. However, at a given foliar application of Fe fertilizers, improving N nutrition of plants stimulated grain Fe accumulation. The results indicate an important role of N nutrition in enrichment of wheat grain with Fe. This N effect should be considered in breeding and fertilization programs focusing on enrichment of staple food crops with Fe.

Key Words
Iron deficiency, biofortification, wheat, iron fertilizers and nitrogen fertilization.

Introduction
Iron deficiency is a growing health concern in the developing world, and responsible for diverse of health complications including anemia and impairments in immune system (Welch and Graham 2004). It is estimated that nearly half of the world population is affected from Fe deficiency problem. Major reason for widespread occurrence of Fe deficiency in human populations is very little dietary diversity and high consumption of cereal-based foods with very low amount and poor availability of Fe (Bouis 2003; Welch and Graham 2004). Increasing concentration and bioavailability of Fe in commonly-eaten food crops is, therefore, a big global challenge and an important public health issue. Various strategies are available to alleviate Fe deficiency problem globally. Among these strategies, agricultural strategies like plant breeding (e.g., genetic biofortification) and agronomic approaches (e.g., fertilization) seem to be highly cost-effective and easily applicable in the developing world (Cakmak 2008). In case of agronomic approaches, there are little published data in literature dealing with the role of Fe fertilization on Fe concentrations in the edible parts of staple food crops. Most of the studies about Fe fertilization focused more on correction of Fe deficiency problem. In addition, in contrast to Zn, Fe fertilization seems to be not effective in increasing Fe concentrations of cereal grains (Rengel *et al*. 1999). Recent evidence in literature indicates that nitrogen nutritional status of plants has a positive influence on grain accumulation of Fe (Kutman *et al*. 2010), possibly by i) contributing to release of Fe-mobilizing compounds from roots (e.g., phytosiderophores, ii) enhancing root uptake and transport of Fe via increasing pool of transporter proteins (e.g., IRT proteins), iii) facilitating translocation and phloem transport of Fe via chelation with nitrogenous compounds (e.g., nicotianamine, peptides) and iv) improving seed deposition of Fe by increasing amount of proteins in seeds (Cakmak *et al*. 2010). In the present study, we investigated role of soil- and/or foliarly-applied various Fe fertilizers on grain accumulation of Fe in wheat under different N nutrition regimes.

Materials and Methods
Greenhouse experiments were conducted by using a calcareous soil containing 5.6 mg DTPA-extractable Fe per kg of soil. Durum wheat (*Triticum durum*, cv: Balcali-2000) plants were grown at different rates of Fe and N fertilizers. Following Fe fertilizers were examined for their effect on shoot and grain concentrations of Fe: $FeSO_4$, Fe-EDTA, Fe-EDDHA and Fe-citrate which were applied either into soil at the rates of 0, 5 and 10 mg Fe per kg soil or sprayed to foliar at the rate of 0.2 % Fe-EDTA at the booting and early milk stages. The same amount of Fe sprayed with 0.2 % Fe-EDTA has been also applied with other Fe fertilizers. The rates of soil applied nitrogen ranged between 100 to 600 mg N per kg soil and applied in form of $Ca(NO_3)_2$. In additional experiments, shoot parts of plants were sprayed with 0.75 % urea at the booting and early milk

stages. At harvest, shoot and grain parts of plants were analyzed for Fe concentrations by using ICP-OES and for determination of dry matter production.

Results

At a given N treatment, soil application of $FeSO_4$, Fe-EDTA, Fe-EDDHA and Fe-citrate did not affect Fe concentrations of shoot and grain, while increases in soil N application significantly elevated shoot and grain concentrations of Fe. Similarly, also foliar spray of urea at the booting and milk stages significantly enhanced grain concentrations of Fe. In the case of foliar application of Fe fertilizers, there were only slight increases in grain Fe. Among the foliarly-applied Fe fertilizers, Fe-EDTA appeared to be the best Fe sources in increasing grain Fe concentrations. There was a significant correlation between shoot or grain concentrations of N and Fe. Similar to Fe, also grain concentration of Zn was increased by improving N nutrition of plants either by soil or foliar application of N fertilizers.

Conclusion

The results indicate a critical role of N nutrition in enrichment of wheat grain with Fe (and Zn). The reason for this positive effect of N on grain Fe is not clear. As discussed recently by Cakmak *et al.* (2010), probably, a high N nutrition contributes to grain Fe concentration by i) affecting the pool of transporter proteins mediating Fe uptake and transport in plants, ii) increasing the amount of Fe-translocating nitrogenous compounds such as nicotianamine and certain peptides) or iii) elevating amount of Fe-binding proteins in grain.

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Effectiveness of borax and colemanite as boron sources for rice grown in flooded acidic soil

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Abstract
A field study was conducted to evaluate the effectiveness of boron fertilizers borax and colemanite (powder and granular) in supplying B to rice under flooded conditions. Boron application improved all the agronomic growth parameters and increased the yield. Both B fertilizers significantly increased the plant height, panicles/plant, number of grains/panicle and weight of 1000 grains. Both B sources were found equally effective in supplying B to rice crop. Borax gave significantly high yield at 2 kg B/ha and powder colemanite at 3 kg B/ha. Yield difference between borax and powder colemanite was not significant at all three levels. Powder colemanite applied plots had significantly high residual B in compare to borax at 0-15 and 15-30 cm and at 30-45 cm depth borax applied plots had high B content. Granular colemanite application did not significantly increase the crop growth and yield due to the large particle size B so that release was very slow.

Key Words
Boron, Rice, Borax, Colemanite.

Introduction

Rice production will need to increase upto 30% by 2025 in order to sustain the growing demand (IRRI 2008). Micronutrient deficiency is one of the major causes of the declining productivity trends (Alloway 2008). Boron is a micronutrient essential for normal healthy plant growth and development of reproductive tissues. Boron deficiency is wide spread in many regions and cropping systems (Shorrocks 1997). The common B sources are sodium tetra borate, ($Na_2B_4O_7 \cdot 5H_2O$), borax ($Na_2B_4O_7 \cdot 10H_2O$), sodium pentaborate ($Na_2B_{10}O_{16} \cdot 10H_2O$) and calcium borate colemanite ($Ca_2B_6O_{11} \cdot 5H_2O$). The sodium salts of B are reasonably soluble and therefore are widely used. Colemanite on the other hand is less soluble and therefore is applied to soils which have possibility of leaching. According to Shorrocks (1997) very few field studies on comparative efficiency of different B sources on crop yield have been conducted. Most studies of this nature have been carried out in pots often with B toxicity and leaching in mind rather than crop response. In this study we conducted field experiments to evaluate the effectiveness of borax, colemanite powder and colemanite granular on growth parameters and yield of rice under flooded acidic soil. The concentration of soil residual B after harvesting was also determined.

Materials and methods

A field experiment was conducted during 2008-2009 at traditional rice growing area in the northern part of Malaysia. The soil series was Kangkong (Typic Tropaquepts) deficient in B (0.3 mg/kg). Texture of the soil was silty clay loam with 4.8 pH and 2.2% organic carbon. Three B fertilizers borax, powder colemanite (PC) with particle size 75µm and granular colemanite (GC) particle size 0.3 mm were used. Boron levels were 0 kg B/ha, 1 kg B/ha, 2 kg B/ha and 3 kg B/ha; it was applied at the time of planting by broadcasting. Nitrogen (N), phosphorus (P) and potassium (K) fertilizers were given according to the recommendations of Malaysian Agriculture Development Authority (MADA). The crop parameters determined include number of tillers and panicles/plant, plant height, panicle height, fresh and dry weight of straw with and without grain, weight of panicle, grains/panicle, empty grains/panicle, weight of grain from each plot, weight of 1000 grains, B in plant straw and grain, B in three depths of soil.

Results and discussion

Boron application increased the number of tillers and panicles/plant at all three levels over the control. The number of grains/plant and the weight of the 1000 grains was increased with the increasing rate of B; the maximum number of grains were recorded in 3 kg B/ha (as borax) applied plots. Data regarding the empty grains/plant revealed that a steep reduction in empty grains was observed because of B application, minimum empty grains were at 3 kg B/ha. Boron also significantly increased the straw yield of the rice at 3 kg B/ha applied as borax and CP. Fertilizer application improved the B content of plant straw and grain at 2 and 3 kg B/ha. Yield of the rice crop significantly increased with the increasing level of CP and borax, it was 23% higher over the control at 3 kg B/ha, but in the case of borax yield difference between 2 kg and 3 kg B/ha was not significant. Borax and CP were equally effective as B sources. Borax and PC similarly affected on number of tillers, panicles, grains, plant height, weight of grains and B content of tissue over the control. At all three rates of B (1, 2, 3 kg B/ha) both fertilizers improved the plant growth parameters, only difference was noted at 1 kg B/ha rate, where borax gave significantly better results from colemanite by increasing number of tillers and panicles/plant, number of grains/panicle, B content in straw and grain. Boron fertilizer application significantly increased the residual B content in soil after harvesting at all three depths. The residual B in PC applied soil was significantly higher (0.88 mg/kg) in compared to borax (0.70 mg/kg) at 0-15 and 15 -30 cm depth while at lower depth (30-45 cm) borax applied soil had more B. Powder colemanite gave much better results in compared to GC because of finer particle size. Finer colemanite proved to be effective B source. Ashraf (2004) and Yu (2002) reported that plant height, number of productive tillers, grain weight and ultimately yield of paddy cultivars increased with B application.

Table 1. Effect of different levels of B sources on rice growth parameters

Fertilizers type	Boron rates Kg/ha	No. tillers/ plant	No. panicles/ plant	No. grains/ panicle	Weight 1000 grains (grams)	Empty Grains/ panicle	B in plant straw (mg/kg)
Borax	0	10.3* c	8.0 c	90.6 b	19.0 b	25.6 a	9.0 c
	1	13.0 c	10.0 c	96.0 c	19.6 b	23.0 a	14.0 b
	2	15.5 b	11.3 bc	129.0 a	22.83 a	20.3 b	16.0 a
	3	17.6 a	14.3 a	135.0 a	23.8 a	19 bc	17.0 a
Colem(P)	0	10.3 c	8.0 c	90.6 c	19.0 b	24.3 a	9.0 b
	1	13.3 b	10.6 b	96.0 c	19.8 b	23.0 a	12.7 b
	2	16.0 a	13.3 a	109.6 b	22.1 a	19.3 b	14.0 a
	3	17.1 a	14.6 a	133.0 a	22.9 a	17.6 b	15.7 a
Colem (G)	0	10.3 a	7.0 a	90.0 a	17.0 a	24.0 a	9.0 a
	1	11.0 a	7.3 a	93.3 a	17.7 a	23.6 a	9.7 a
	2	12.0 a	8.0 a	94.0 a	19.2 a	22.3 a	11.2 a
	3	12.5 a	9.0 a	100 a	20.3 a	21.6 a	11.7 a

*Means with same letter for each fertilizer and parameter are not significantly different at p=0.05

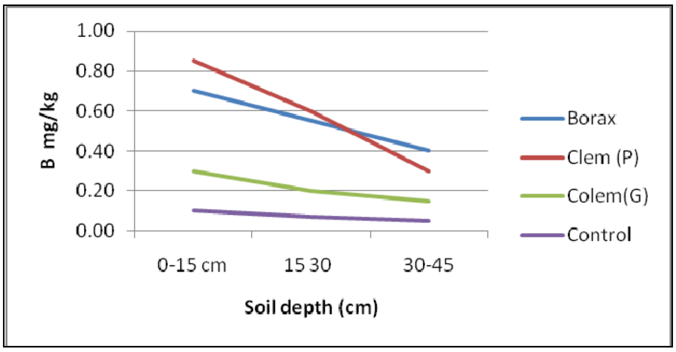
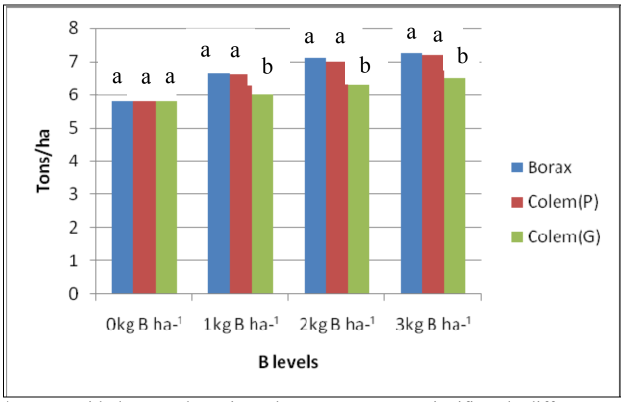


Figure 1. Residual B content remaining at different soil depths after harvest from plots receiving different sources of B fertilizer



* Means with the same letter in each treatment are not significantly different at p=0.05.

Figure 2. Yield of crop from field plots receiving different sources and rates of B

Conclusion

Boron application significantly increased the growth and yield of rice crop by improving number of tillers, panicles, grains and its weight at all three B levels. Fertilizer application also increased the B content in plant tissue. Both B sources borax and powder colemanite were equally effective for supplying B to rice crop. Yield difference between borax and PC applied soils was not significant at all three rates of B. After harvesting residual B from fertilizers was present in the soil. Powder colemanite applied soil had significantly high residual B content in upper layer in compare to borax applied soil. Powder colemanite prove to be much better source of B in compare to GC as GC did not significantly improve the growth parameters of the rice crop because of its particle size.

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HarvestPlus: Developing and delivering micronutrient-dense crops

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Abstract

Plant breeding for micronutrient density (biofortification) gained legitimacy when micronutrient deficiencies were recognized as global public health challenge of the 21st century. In response, HarvestPlus was established to add food nutritional quality to agricultural production research paradigms and reduce micronutrient malnutrition among poor at-risk populations by capitalizing on agricultural research as tool for public health interventions. HarvestPlus' applied and strategic research is driven by an impact/product pathway that integrates crop development, nutrition, socio-economic disciplines and country specific crop delivery plans. As for novel traits, biofortified product concepts must consider factors associated with probability of success in achieving: i) *technological goals* with trait discovery and expression in adapted genotypes, ii) *crop improvement goals* to generate a biofortified germplasm product without compromising agronomic performance, nutrition, or end-use quality; and iii) *commercial goals* to guide the design and delivery of the technology. HarvestPlus has three project phases: i) discovery (2004-2008), ii) development (2009-2013), and iii) delivery (2014-2018). An enhanced knowledge base allowed shifting from an emergent to a deliberate strategy with prioritizing 10 country/micronutrient crop profiles for six crops and projecting release dates in phase II. State-of-art and knowledge base is presented and multidisciplinary themes are explained with an emphasis on product development.

Key Words

HarvestPlus, biofortification, agriculture, plant breeding, micronutrients.

Introduction

HarvestPlus seeks to develop and distribute varieties of food staples (rice, wheat, maize, cassava, pearl millet, beans, and sweetpotato) which are high in iron, zinc, and provitamin A through an interdisciplinary, global alliance of scientific institutions and implementing agencies in developing and developed countries. Biofortified crops offer a rural-based intervention that, by design, initially reaches these more remote populations, which comprise a majority of the undernourished in many countries, and then penetrates to urban populations as production surpluses are marketed. In this way, biofortification complements other nutrition intervention programs such as fortification and supplementation.

A multidisciplinary approach

In broad terms, for biofortification to be successful first, breeding must be successful – high nutrient density must be combined with high yields and high profitability. Second, nutritional efficacy must be demonstrated – the micronutrient status of human subjects must be shown to improve when consuming the biofortified varieties as normally eaten. This includes evaluating that sufficient nutrients are retained during processing and cooking and that these nutrients are sufficiently bioavailable. Third, the biofortified crops must be adopted by farmers and consumed by those suffering from micronutrient malnutrition in significant numbers (Bouis *et al.* 2009).

Figure 1 reveals the incorporation of these key research issues in the impact pathway. The interdisciplinary nature of biofortification research necessitates collaboration between plant breeding and a range of disciplines including:

a) Socioeconomics: to accurately identify target populations that both consume the target crops and hold the largest share of the micronutrient burden (Figure 1, step 1) and, to measure the effectiveness of biofortified crops to improve human health as a cost-effective public health intervention (Figure 1, step 10).
b) Human nutrition: to determine micronutrient target levels that will have a measurable impact on human health (Figure 1, step 2); to estimated amount of the crop consumed by the target population; to evaluate the retention of the added micronutrient after storage, processing, and cooking (Figure 1, step 6) ; to test the bioconversion/ bioavailability of nutrients ingested from biofortified crops (Figure 1, step 7); and, to ultimately measure the biological impact of biofortified crops on human nutritional status (Figure 1, step 7).
c) Marketing Specialist: effectively delivering seed and generating demand for biofortified crops to farmers and dissemination partners.

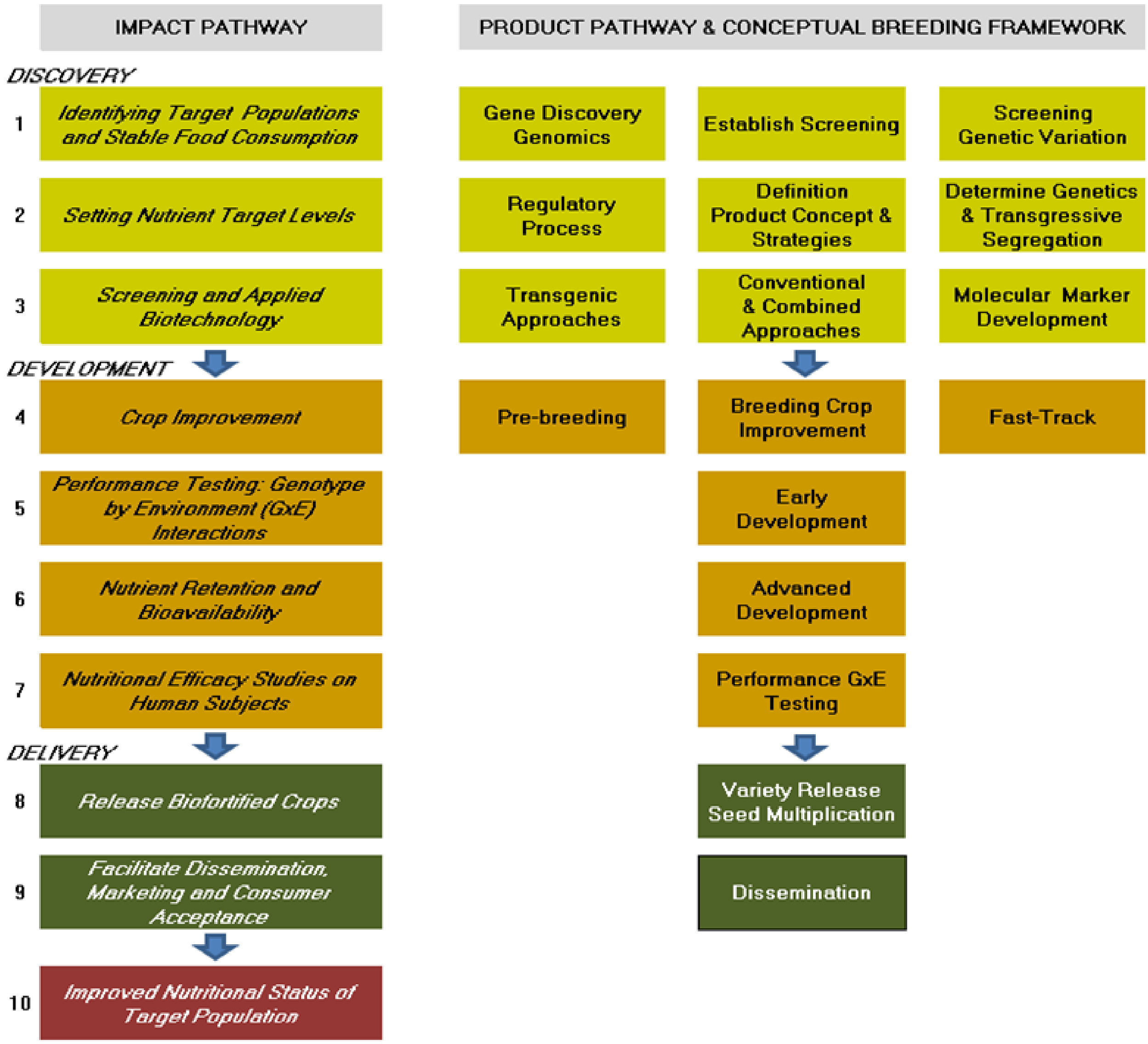

Figure 1. HarvestPlus Impact (left) and Product (right) Pathway.

Crop development elaborated

Figure 1 outlines the key biofortified germplasm development activities and product pathway (Pfeiffer and McClafferty 2007). Different research categories reflect sequentially arranged stages and milestones, and are superimposed upon a decision tree that allows monitoring progress and making strategic and go/no-go decisions when goals and targets cannot be achieved. Crop improvement activities under HarvestPlus focus, first, on exploring the available genetic diversity for iron, zinc, and provitamin A carotenoids. In parallel, agronomic and end-use features are characterized. In project phase I, establishing screening posed a major challenge as sampling/analytical protocols, high-throughput screening methods and in-house screening capabilities had to be developed, standardized and implemented, and environments characterized for their suitability for breeding and testing for micronutrients. Exploring the genetic diversity allowed identifying: (1) parental genotypes for use in crosses, genetic studies, molecular marker development, and parent-building, and (2) existing varieties, pre-varieties in the release pipeline, or finished germplasm products for "fast-tracking". Fast-tracking refers to releasing, commercializing, or introducing genotypes that combine the target micronutrient density with the required agronomic and end-use traits so they can be quickly delivered to producers and have an immediate impact on micronutrient-deficient populations. The micronutrient trait source is essential for the next breeding steps. If variation is present in unadapted trait sources, pre-breeding is necessary prior to using the trait in final product development; if variation is present in the tactical gene pool, the materials can be used directly to develop competitive varieties. The next breeding steps involve developing and testing micronutrient-dense germplasm, conducting genetic studies, and developing molecular markers to facilitate breeding. Then, genotype x environment interaction (GxE) – the influence of the growing environment on agronomic traits and micronutrient expression – needs to be determined in experiment stations and in farmers' fields in the target countries. Parallel to GxE trials, agronomy trials are conducted which include macro and micro fertilizer treatments to develop crop management recommendations for biofortified crops.

Beyond meeting *technological goals* of trait discovery and expression in adapted genotypes, and *crop improvement goals* that generate an agronomically and nutritionally superior biofortified germplasm product (both elaborated above), plant breeders must also address *commercial goals* that guide the design and delivery of this agricultural technology for public health. The commercial goal stage of HarvestPlus crop development invests in establishing productive research networks that link national research programs in target regions of the developing world with advanced agriculture and nutrition research institutes around the globe to help build sustainable research capacity in biofortification where it is most needed. Commercial goals insist breeders are keenly aware of consumer (not just farmer) acceptance and marketability of nutrient rich crops should those factors influence the breeding product concept. If organoleptic properties or grain color changes as a result of biofortification, can plant breeding mitigate these outcomes? The ultimate acceptance, and subsequent impact, of biofortified crops on producers and consumers will hinge on plant breeders not only developing attractive trait packages that do not compromise agronomic characteristics but also understand the value farmers and consumers place on the traits that will determine crop adoption and may require adjustments to the crop development strategy.

Results and conclusions

To date, HarvestPlus research has proven that added micronutrients have a measurable impact on human micronutrient status (Haas *et al*. 2005; van Jaarsveld *et al.* 2005; Low *et al*. 2007) but much work remains. The product development and release schedule given in Figure 2 reflects progress accomplished in developing micronutrient dense, competitive crops.

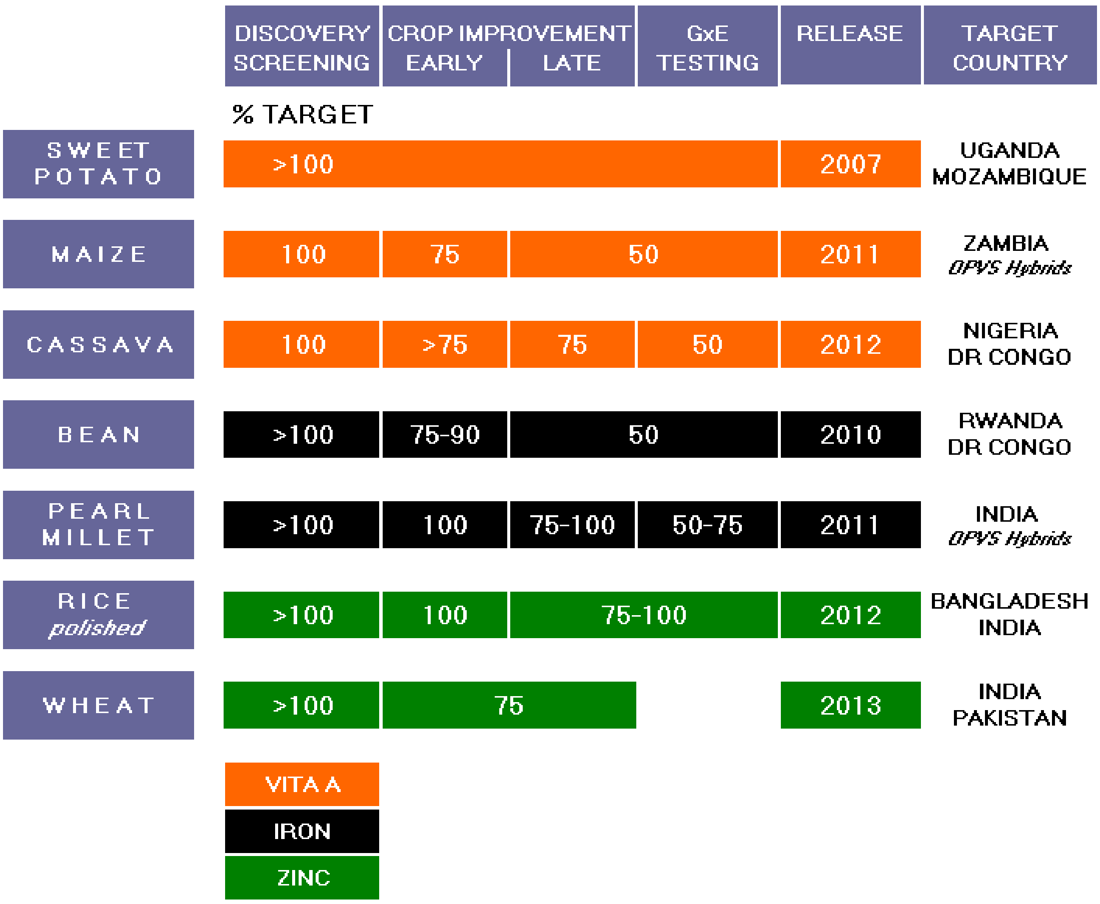

Figure 2. Product development and release schedule 2009 (iron, zinc, provitamin A expressed as percent of breeding target in lines at indicated stage of breeding).

As the program enters its second phase of full-scale crop development, planning is underway for effective delivery of these novel crops and the networks of expertise that makes up the multidisciplinary tapestry of biofortification continues to expand. Communication and marketing specialists are now becoming engaged with crop development and nutrition scientists to ensure adoption and sustainability or this agricultural innovation for public health.

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Influence of different K fertilizer sources on sunflower production

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Abstract
The use of chemical fertilizers is one of the ways for increasing agricultural productions. The objective of this study was to investigate the influence of different K sources (KCl and K_2SO_4) on sunflower yield. For this purpose, a randomized block experimental design with K_2SO_4 and KCl sources was established in the greenhouse. The treatments were consisted of 0 (standard), 50, 100, 150 and 200 kg/ha fertilizers each with four replicates. After development, plants were harvested. The heights and weights of each plant was obtained and dried in the oven. The potassium content of each plant was measured, using flame photometer apparatus. The obtained results indicated that by increasing potassium concentration in soil, its accumulation in plant tissues was also increased. So that the best yield was in 200kg/ha level of K2SO4 treatment and the lowest yield was in the 50kg/ha of the KCl treatment. Also, the sulphur content was increased by increasing $K2_SO_4$ fertilizer.

Key Words
fertilizer, K_2SO_4, KCl, Sunflower, yield

Introduction
Sunflower (*Helianthus annus*) is one of the most important oil plants in the world. Sunflower oil contains large amount of A, D, E, K vitamins and considerable proteins (20-40%) (Connor and Hall 1997). By fertilizing and increasing the soil fertility, the seed yield and its oil content are increased (Egli 1998). The objective of this study was to assess the influence of KCl and K_2SO_4 fertilizer levels on sunflower yield. Consequently, an experiment was conducted to recognize the efficiency of using KCl and K_2SO_4 fertilizer with different levels of K in soil.

Methods
A randomized block experimental design with two K sources (K_2SO_4 and KCl) and four replicates was established in the greenhouse under uncontrolled environmental conditions. The treatments were consisted of 0 (standard), 50, 100, 150 and 200 kg/ha fertilizer. First, the sunflower seeds were put in washing detergent 20% for 15 minutes for disinfectant and after that, seeds washed with water for acquiring the necessary wet for budding. The soils were carefully packed in the pots to obtain a uniform bulk density of 1.35 gr/cm^3.The soil fertilization process was performed until the target concentrations were achieved. In order to obtain a reliable set of data, four replicates for each treatment was established. Content of N and P that used were in arrangement 100 and 150 kg/ha and the amount of K in different level of K_2SO_4 and KCL fertilizers was assessed in this study. After 48 hours, the seeds were seeded in the pots. Three seeds were first seeded and after 20 days with comparing the plant growth, separated the weak shoots and thinned to 1. Having a standard treatment, the soil water content was always held at field capacity to prevent any water stress during the whole growth period. When plants were fully developed, after measuring plant height, sunflowers were harvested. Also, weight of each sample in different fertilizer levels was measured (Tables1 and 2) and (Figures 2 and 3).

Table 1. The average of plant weight and height in K_2SO_4 treatment.

Treatment	Average of height (cm)	Average of weight(g)
0	126	395
50	131	400
100	135	420
150	138	435
200	142	490

The plants were then washed with distilled water and dried in an oven for 48 hours (at 85 ^{oc}). The potassium content of each plant was measured, using flame photometer (Table 3) (Gupta 2000). Finally, the effect of two fertilizers of K_2SO_4 and KCl was assessed on sunflower yield.

Table 2. The average of plant weight and height in KCl treatment.

Treatment	Average of height (cm)	average of weight (g)
0	126	395
50	128	398
100	130	407
150	133	420
200	137	447

Table 3. Potassium concentration in the experimental plants.

Treatment	K (ppm)
standard	10
A1	14
A2	30
A3	41
A4	50
B1	11
B2	28
B3	39
B4	45

Results

To distinguish the influence of different soil fertilizer levels on sunflower yield, different amounts of potassium were measured. The results of these chemical analyses are given in Table 3. As can be seen in this table, the lowest potassium concentrations belong to the treatment with 50 kg/ha KCl fertilizer. Also, the highest potassium concentrations belong to the treatment with 200 kg/ha K_2SO_4 fertilizer. The relationship between soil potassium concentrations and amount of potassium in sunflower are presented in Figure 1. It was further observed that by increasing the potassium concentration in the soil, its concentration in plant tissues was also increased. The maximum yield obtained for the treatment with 200 kg/he K_2SO_4 and the minimum yield was in treatment with 50kg/he KCl (Figure 1).

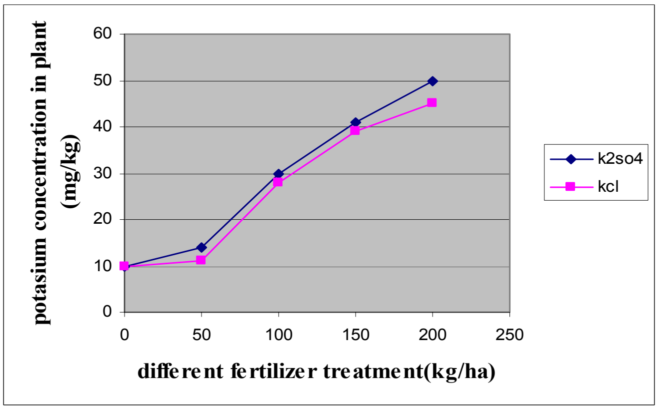

Figure 1. Comparing the concentration of potassium in sunflower after using KCL and K_2SO_4 fertilizers.

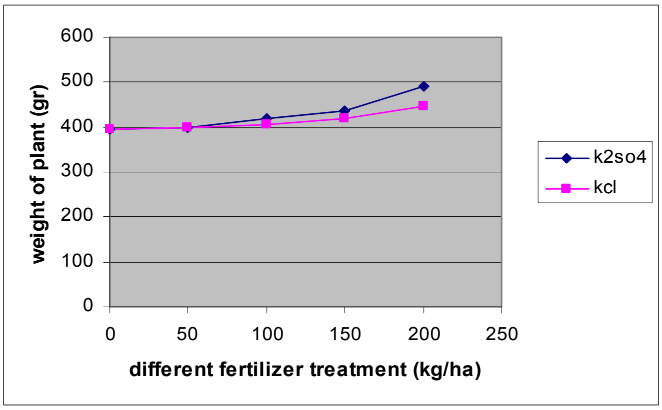

Figure 2. The influence of different KCl and K_2SO_4 fertilizer levels on sunflower weight

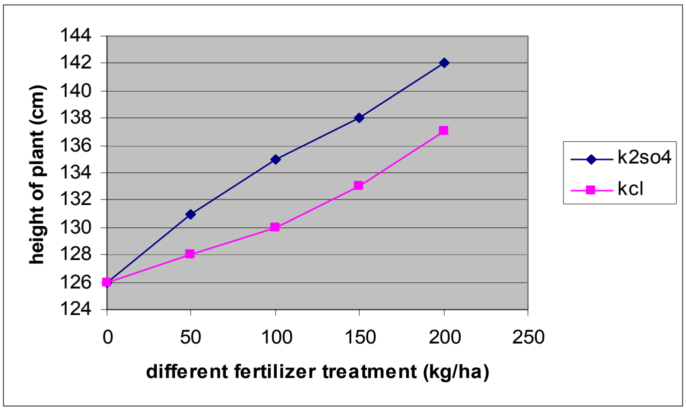

Figure 3. The influence of different KCl and K_2SO_4 fertilizer levels on sunflower height

Conclusion

Since the amount of sulphur is increasing with the K_2SO_4 fertilizer to the soil that has an important role on oil production, the influence of K_2SO_4 is better and more than the KCl fertilizer that have Cl. Thus, when both of them with attention to the yield increasing, we can use K_2SO_4 fertilizer with 200 kg/ha and if it doesn't exist, KCl fertilizer with 200 kg/ha has shown better results compared to the conditions that we don't use any fertilizers.

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Kinetics of DTPA extraction of zinc from calcareous soils from Iran

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Abstract
In recent years, our knowledge of Zn extraction from soil has been based mainly on the study of equilibrium condition using thermodynamic approaches. Thermodynamics can predict only the final state, while a knowledge of desorption kinetics may yield important information concerning the nature of reactions and also the rate of supply of Zn to plants via soil solution. The kinetics of Zn extraction by diethylenetriaminepentaacetic acid (DTPA) from the -2mm fraction of 12 calcareous soils was investigated using surface soil (0-30 cm) samples. Soils were equilibrated with 0.005 M, DTPA solution for 15 to 11520 minutes. Zero-, first-, second-, third order, parabolic diffusion and simple Elovich equations did not adequately describe Zn extraction kinetics. The best model for describing extraction data for all soils was the exponential rate equation (q=a tb). The magnitude constant, a, was significantly and positively correlated (r = 0.76) to organic carbon content and negatively correlated to pH (r = -0.67). The rate constant, b, was not significantly related to any of the measured soil properties (pH, CEC, CCE, ACCE, clay, OC, SSA) or to any combination of properties. It was concluded that the amount of Zn that can be extracted by DTPA solution from Iranian calcareous soils is highly variable.

Key Words
Aridisols, Entisols, carbonate, kinetic models.

Introduction
Considerable work has been carried out on the kinetics of extraction of P and K from soils and these findings have been related to soil properties and the availability of these elements to plants. However, investigations of the kinetic of Zn extraction from soil have been limited (Sparks *et al*. 1980; Sparks 1986; Dang *et al*. 1994) and there are no published reports on Zn extraction from calcareous soils of Iran where Zn deficiency in crops is widespread (Maftoun and Karimian 1989). Calcareous Aridisols and Entisols in Iran generally contain quite large quantities of total Zn, but have only very small quantities of Zn in soil solution. Most of these soils need supplemental Zn for optimum plant growth (Reyhanitabar *et al*. 2007) and the recovery of applied Zn by plants is low. Even where Zn chelate (Zn-EDTA) was applied only 5% or less was recovered by the first crop (Maftoun and Karimian 1989). This may reflect the rapid formation of Ca-EDTA in calcareous soils and loss of Zn from solution by sorption and precipitation.

The uptake of Zn by plants requires release of Zn sorbed on soil surfaces and possibly dissolution of Zn containing minerals (Uygur and Rimmer 2000). The rate of Zn release to soil solution is thus an important factor regulating its supply to plants. The chelating agent, DTPA is routinely used to estimate available Zn in calcareous soils (Lindsay and Cox 1985), but this standard method provides only a static or single measure of Zn availability to plants whereas dissolution of Zn in DTPA increases or decreases with time. Thus the kinetics of Zn dissolution in DTPA for different soil types need to be known so as to optimize extraction times for testing particular soil types. DTPA chelates Zn and to an extent simulates both Zn extractions from soil by plant exudates and Zn uptake by plants. The kinetic of Zn dissolution in DTPA may thus provide an indication of the rate of Zn supply to plants. This is especially important for Aridisols and Entisols, which contain considerable Zn but have very small amounts of Zn in soil solution (Maftoun and Karimian 1989). For these soils continuing Zn release from the soil over time will increase Zn availability to plants. Zinc extraction kinetics was investigated by Kuo and Mikkelsen (1980) and these data were described by an exponential rate equation. However other equations have been proposal for describing extraction kinetics and these may provide better descriptions of Zn extraction from soil (Sparks 1986). This study has determined the kinetics of Zn extraction by DTPA for 12 Aridisols and Entisols from Iran and identifies the equations that best describe this process.

Methods
Twenty composite soil samples (0-30cm) from the Gazvin Plains and Tehran province (Iran) were collected. Soil samples were air dried, ground in a stainless steel mill to pass a 2 mm sieve, stored in airtight polyethylene containers, and analyzed by the following procedures: Identification of carbonates and clay minerals was carried out by X- ray diffraction (XRD) using random powder and oriented clay on ceramic plates with a Philips PW 1830/40 instrument. Specific surface area (SSA) was measured using the N2-BET method with a Micrometrics Gemini III 2375 surface area analyzer.

To measure Zn extraction 50g oven-dried soil was extracted in duplicate with 100 ml of a solution consisting of 0.005 M Diethylentriaminepentacetic, 0.1 M triethanolamine, and 0.01 M $CaCl_2$ at pH 7.3 (Lindsay and Norvell 1978) at 25˚C ±1 in a constant temperature shaker, for periods of 0.25, 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 60, 72, 96, 120, 144, 168 and 192 hours. Seven drops of toluene were added to each flask to inhibit microbial activity. For each shaking period, a 5 mL subsample of the suspension was removed with a plastic syringe and the suspension was filtered through a 0.45 µm filter. Zinc concentration was determined by ICP-MS (Inductively Coupled Plasma Mass Spectroscopy) using a Perkin–Elmer Elan instrument.

Kinetic equations that have been used in soil extraction studies, namely zero-, first-, second- and third order rate equations (Dang *et al*. 1994), parabolic diffusion, exponential and Elovich (Khater and Zaghloul 2002) were evaluated by least- squares regression analysis for their suitability for describing Zn extraction data. A relatively high value of the coefficient of determination (r^2) was used as the criterion for the best fit.

Results and Discussion
Soil characteristics
In the studied soils, clay concentrations range from 106 to 410 g/kg, pH ($CaCl_2$) from 7.2 to 7.8, organic carbon from 6.3 to 24.7 g/kg, SSA from 8.5 to 39 m^2/g. Calcium carbonate equivalent ranged from 46.0 to 228.0 g/kg and active $CaCO_3$ equivalent ranged from 16.1 to 99.8 g/kg. DTPA extractable Zn values ranged from 1.2 to 4.9 mg/kg. X-ray diffraction (XRD) analysis of random powders identified abundant quartz and showed that calcite is the sole carbonate mineral. X-ray diffraction patterns of the clay fraction identified illite, chlorite and smectite as major clay minerals in all soil samples.

Kinetics of Zinc Extraction
Extraction of Zn in DTPA from 12 Aridisols and Entisols increased with time and the amount of Zn extracted differed greatly among soils (Figure1).

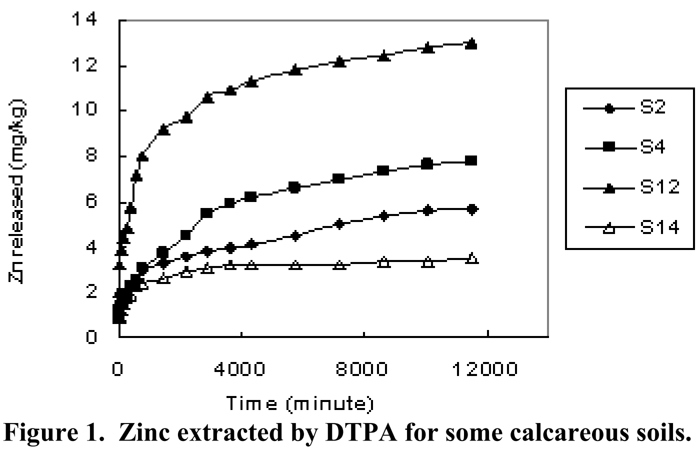

Figure 1. Zinc extracted by DTPA for some calcareous soils.

The amount of Zn extracted at 192h was approximately three fold higher for soil 2 (loam) compared to soil 14 (clay). Similar results have been reported by Dang *et al*. (1994) for Zn extraction by DTPA from vertisols. The zero-, first-, second-, and third- order, parabolic diffusion, Elovich and exponential equation were evaluated for describing Zn extraction kinetics up to 192h. The extraction kinetic was poorly described by the zero-, first-, second-, and third-order equations. With the increasing order of reaction from zero to third, the coefficient of determination (r^2) decreased for all soils which are consistent with the findings of Dang *et al*. (1994). These models fail to adequately describe Zn extraction kinetics and will not be discussed further. The parabolic diffusion equation provides an adequate description of extraction kinetics based on r^2, but there were systematic departures of data from the fitted curves .There was a discontinuity in the slope of the fitted line at about 24h suggesting that two different mechanisms with different rate constants are controlling the net rate of Zn extraction. For 9 of 12 soils, there are significant differences between the two linear slopes with the first slope (< 24h) being higher than the second slope. Parabolic diffusion equations have been fitted to the two sections of extraction data and provide an excellent fit to the data (r^2=0.98). As film diffusion was minimized by continuous shaking during the experiment (Sparks *et al*. 1980) it is likely that at least two particle diffusion mechanisms were involved. Possibly Zn was mostly desorbed from outer surfaces of micro-aggregates in the first 24h, with subsequent dissolved Zn being mostly provided by desorption and diffusion of Zn from inside aggregates (Figure2). This explanation is consistent with the Zn diffusion model of Bruemmer *et al*. (1988), the Zn sorption model of Barrow (1986) and the findings of Dang *et al*. (1994) for Vertisols, although Dang *et al.* (1994) reported a discontinuity in slope after very short extraction time. Extraction kinetics were well described by both exponential and Elovich equations although there were small systematic departures of data from the relationships in both cases (Figure3). The exponential equation provided higher values of r^2 than the Elovich equation. The values of constants "a" and "b" vary widely for different soil types. In this study "a" ranged from 0.35 to 1.32 with a mean of 0.69 and b from 0.19 to 0.33 with a mean of 0.25. The values of "a" for these calcareous Iranian Aridisols and Entisols are much higher than values for vertisols, reported by Dang *et al.* (1994) but the "b" values are similar. The constant "a" was positively correlated with organic carbon content, and negatively with pH. There was no significant relationship between rate constant b and measured soil properties either singly or in combination. Constant ,a, may indicate the number of surface sites available for desorption of Zn i.e. the total desorable Zn. Constant ,b, is a measure of the affinity of Zn for thses sites with increasing affinity of sites being indicated by the value of 1/b.

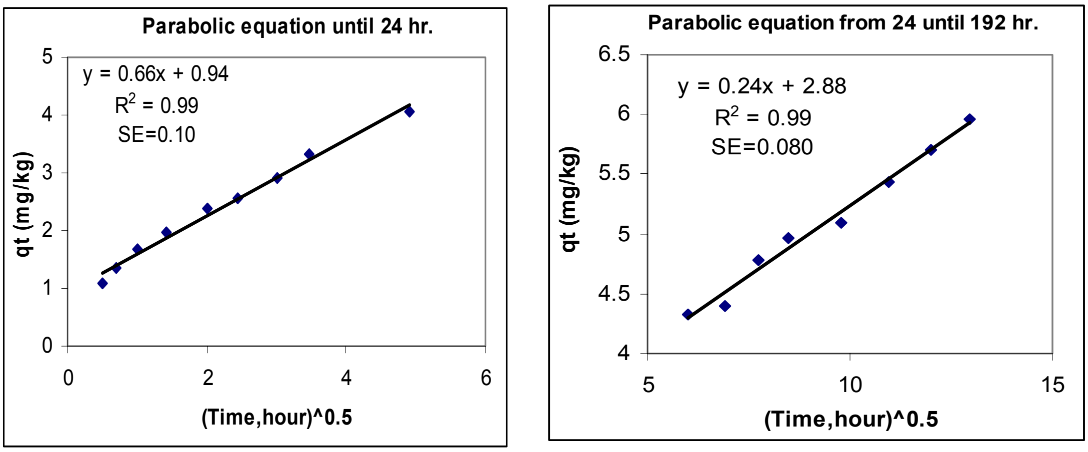

Figure 2. Zinc extracted data fitted to the parabolic equation for two periods for soil 5.

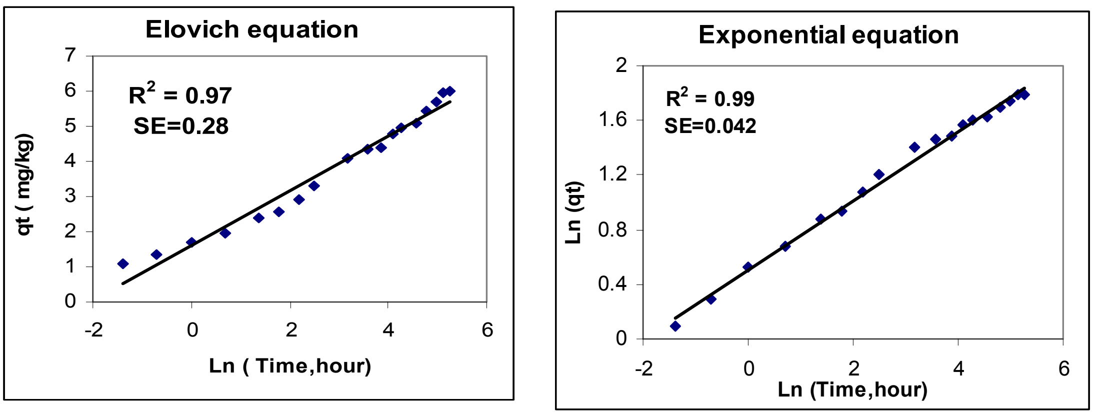

Figure 3. Fitted zinc extracted data for a calcareous soil (#5) according to the Elovich and exponential equations.

Conclusions

This research has shown that the amount of Zn that can be extracted by DTPA solution from Iranian calcareous soils is highly variable. The amount of extractable Zn for long extraction times (a constant) increases with the organic matter content of the soil and decreases as soil pH increases. This association with organic matter has also been observed by previous authors (Brennan 1992; Dabkowska 2003). The rate at which Zn was extracted from the soil (related to the b constant) was quite variable and is not systematically related to any of the measured soil properties. Consequently a soil test for available Zn that involved extraction for a single quite short time period may underestimate the amount of Zn that is eventually released to soil solution and plants. This research would have been fitted by inclusion of plant growth and Zn uptake measurements to calibrate the results of DTPA extraction and in particular to evaluate if the "a" and "b" constants can be utilized for predicting Zn uptake by plants. On this basis a soil type specific extraction procedure could be developed.

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Overview on Se use in soils of São Paulo state and its application to signal-grass

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Abstract
Soil fertilization with Selenium (Se) can enhance forages. The objective of this research was to evaluate the influence of Se on soil-plant systems based on a different analysis. It was observed Se content in the soil and forages (*Brachiaria decumbens*) in São Paulo state. Different levels of sodium selenate were applied to *Brachiaria brizantha* cv. Mandaru soil observed with Se deficiency. The micronutrient influences plant nutrition and directly affects animal nutrition. The experimental design used was randomized blocks in a factorial 3x3x2 with four repetitions. The treatments comprised of three soils (NITOSSOLO VERMELHO eutroférrico, LATOSSOLO VERMELHO Distroférrico and ARGISSOLO AMARELO Distrófico Abrúptico), three levels of Se (0, 10 and 20 g/ha) and two cuts (30 and 80 days after plant uniformity). The statistical analyses were performed with SAS (2004) system. As a result, low levels of Se were verified. Small contents of the microelement were observed in the soils and consequently in the forage analysis. Se soil levels correlate negatively with sand content. Se levels did not change the dry matter production and it did not reach cattle nutritional requirements. However, did interfere in calcium levels. More studies are necessary to recommend the optimum Se requirement level.

Key Words
Brachiaria brizantha, Brazil, forage, sodium selenate.

Introduction
Se essentiality in higher plants has been reported (Malavolta 2006). This microelement is essential for animals and its deficiency can result in the development of diseases (Terry *et al.* 2000). The geographic origin of the animals is a more important determinant of the Se concentration in beef than the presence or absence of supplemental Se (Hintze *et al.* 2001). Se levels in forages can vary from soil to soil and within the same soil because there are factors that can influence its absorption by plants. The United Kingdom showed that increasing the Na and Se levels in grass is better than using mineral supplements to increase Se in animal blood. Deficiency correction in grass increased milk production 9%, of protein level 9.6% and of fat in milk 15.6% (Selênio 2006). Se levels in dry matter of forages in 12 areas of São Paulo were 0.076 and 0.052 ppm Se in the wet and dry seasons respectively, showing general deficiency (Lucci *et al.* 1984). However, this research did not correlate the forage Se with the soil Se. There is little information about Se in Brazilian soils. Several factors in the soil may affect absorption of Se by plants. These factors are reflected in the necessity to find information on Brazilian soils and pasture grass under levels of Se application.

Methods
The overview on Se use in soils of São Paulo state in Brazil and in the grass (*Brachiaria decumbens*) cultivated in these soils during summer. The second stage of research was carried out in a greenhouse. It aimed to evaluate the behavior of the *Brachiaria brizantha* (Hochst. ex A. Rich.) Stapf cv. Marandu under application of Se levels from Sodium selenate in soils with deficient levels of Se. The experimental design used was randomized blocks in a factorial 3x3x2 with four repetitions. The treatments were three soils (typic Hapludalf, Oxisol and Red Dusky Podzol), three levels of Se (0, 10 and 20 g/ha) and two cuts (30 and 80 days after plant uniformity). The data were statistically analyzed (SAS 2004). Averages were compared using orthogonal contrasts and significance level of 10%.

Results
Small contents of Se were observed in the soils and consequently in the forage (Figure 1). When there is less than 500 µg of Se/kg in the soil, it is characterized as Se deficiency in the soil (Millar 1983). Se deficiency occurs in pastures containing less than 30 µg of Se per kg of dry matter (Malavolta 2006 and Millar 1983).

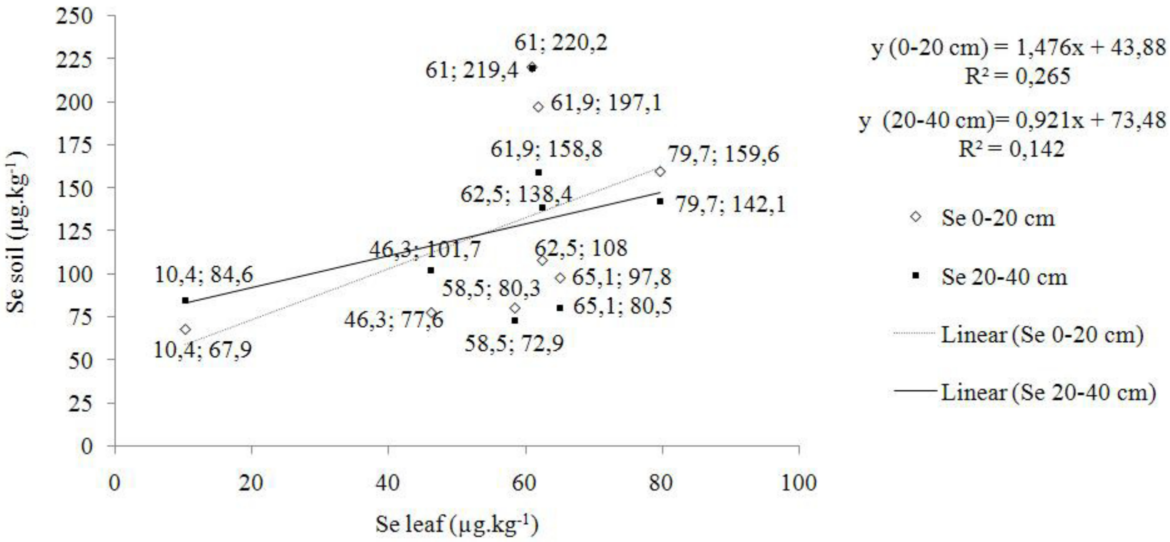

Figure 1. Correlation between Se levels in *Brachiaria decumbens* and soil (0-20 and 20-40 cm).

None of the soils evaluated showed Se levels high enough to supply desirable amounts for the grass, considering that the requirement for beef cattle is 100 µg/kg (NRC 2000). The levels in the plant were below the requirements for animals; however, the relations between Se in the plant and in the soils, both depths, were positive, in spite of the low correlation coefficients. The physico-chemical analysis on horizons in the soils presented the lowest Se levels for soils with the highest sand levels. The deficient areas correspond to soils with relatively high percentages of sand (Rosa 1991). The deficiency is usually found in sandy soils (Selênio 2006). The differences found between the horizons can be related to the occurrence of leaching with descending movement of the element, considering the fact that it is always in the form of anion selenite (SeO_3^{-2}). The Se distribution in the soil profile is a determinant of its concentration in the plant (Reid and Horvath 1980). Such fact would justify the behavior observed in the profiles analyzed in which the type of soil can greatly influence the magnitude of the leaching process (Sangoi 2003). In the plant, Se doses influenced the Ca level following a negative quadratic relationship ($y = -0.014x^2+0.25x+7.7$ ($R^2=1$)) with the highest point at 10 g/ha of Se and Se level of ($p=0.0012$), which presented a curve linear behavior (Table 1).

Table 1. Chemical analysis of *Brachiaria brizantha* submitted to Se treatment for versus periods (Averages followed by the same letter in the column did not statistically differ among one another by Tukey 10%)

		P	S	K	Ca	Mg	Cu	Fe	Mn	Zn	Se
		g.kg^{-1}					mg.kg^{-1}				µg.kg^{-1}
Levels of Se (g/ha)	0	0,8^a	0,9^a	32^a	8^{ab}	3,8^a	5^a	52^a	94,2^a	24^a	4,1^b
	10	0,9^a	1,1^a	31^a	9^a	3,7^a	5^a	44^a	94,6^a	24^a	27,4^{ab}
	20	0,8^a	1,0^a	32^a	7^b	3,9^a	5^a	47^a	93,5^a	24^a	49,0^a
Evaluation time (days)	30	0,8^b	1,0^a	39^a	6^b	3,1^b	6^a	53^a	56^b	24^a	41,1^a
	60	0,9^a	1,0^a	24^b	9^a	4,6^a	5^b	42^b	132^a	24^a	12,6^b

The Se level in the soil did not present a dose effect (Table 2) and were low considering the deficiency criterion (Millar 1983). Among the other chemical attributes concerning soil fertility, only P, Mn and H+Al showed effects. The doses presented positive effects with the reduction of H+Al levels, which followed a negative quadratic model, while the micronutrient Mn showed a positive quadratic relationship ($p=0.0003$).

There was difference for Se levels in the plant ($p=0.0370$) and in the soil ($p=0.0289$) for the soils evaluated (Figure 2). The inverse relationship between the Se level in the soil and in the plants for NITOSSOLO VERMELHO Eutroférrico can be justified by the possible unavailability of the form in which the Se is in the soil. The dry matter production (average 29g), the CP level (average 5.7%), the residual dry matter (average 42.3 g), the root length (average 36.8 cm) and the root weight (average 40 g) did not show a dose effect. The level of dry matter showed only time effect, increasing from 27 to 35%.

Table 2. Average levels of chemical attributes of soil fertility of applied to *Brachiaria brizantha* submitted to Se treatments (Averages followed by the same letter in the column did not statically differ among one another by Tukey 10%).

Doses of Se ($g.ha^{-1}$)	pH	M.O.	P	S	K	Ca	Mg	H + Al	Al	CTC	SB	V	m	B	Cu	Fe	Mn	Zn	Se
	$CaCl_2$	$g.dm^{-3}$	$mg.dm^{-3}$		$mmolc.dm^{-3}$							%		$mg.dm^{-3}$					$\mu g.kg^{-1}$
0	5,3^{a}	27^{a}	25^{a}	6^{a}	0,6^{a}	19^{a}	5^{a}	26^{a}	2^{a}	52^{a}	25^{a}	48^{a}	7^{a}	0,23^{a}	4,9^{a}	38^{a}	10,2^{a}	6,2^{a}	25,1^{a}
10	5,4^{a}	27^{a}	22^{b}	5^{a}	0,6^{a}	19^{a}	5^{a}	25^{a}	1^{a}	51^{a}	25^{a}	50^{a}	7^{a}	0,20^{a}	4,9^{a}	39^{a}	8,1^{b}	6,2^{a}	26,1^{a}
20	5,4^{a}	27^{a}	22^{b}	5^{a}	0,6^{a}	19^{a}	5^{a}	19^{b}	1^{a}	52^{a}	25^{a}	50^{a}	5^{a}	0,22^{a}	5,3^{a}	40^{a}	10,0^{a}	6,1^{a}	18,5^{a}

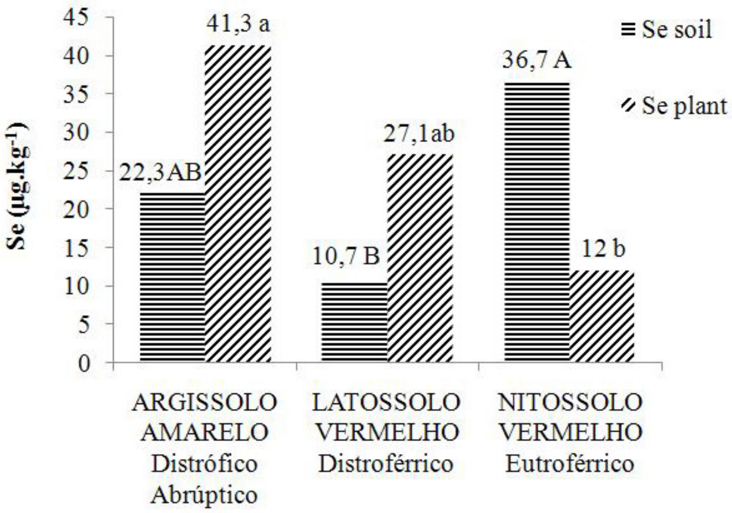

Figure 2. Average levels of Se in the soil and in the plant applied to *Brachiaria brizantha* submitted to Se treatments (Averages followed by the same letter in the column did not statically differ among one another for each parameter analyzed by Tukey 10%).

Conclusion

Some of the main soils in São Paulo state present low Se levels and therefore *Brachiaria decumbens* on them showed considerably deficient levels, the Se concentration in the soil and in the plant were related. The relation between Se levels in the soil and in the plant varies from soil to soil. The levels in the soil have a negative relationship with sand levels. The Se doses applied did alter the Se levels in the soil and were not enough for the grass to reach foliar levels necessary to supply the requirements for animals and did not modify the dry matter production; however, they changed the chemical composition of plants interfering with the Ca levels. Se fertilization for *Brachiaria brizantha* can be carried out through soil supply, but the doses were low, which requires more studies to ensure the efficient doses to be applied and preventing contamination for both animal and the plant.

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Relation of soil mineralogy and microbial communities based on micronutrient status

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Abstract
Micronutrients are trace elements which are needed by plants, animals and humans as well as microorganisms for healthy growth. The total amount and bioavailability of micronutrients is highly related to the composition as well as stability of major groups of rocks and minerals present in the soil, and can be released through various weathering mechanisms, among which bioweathering (mediated by soil microorganisms) has an important role. Meanwhile, microorganisms can also be influenced by the soil mineralogy. There is evidence that rocks and minerals with different composition support different microbial communities. It is, therefore, of interest to investigate to what extent the soil microbial communities are correlated with the mineral composition in terms of micronutrient content of soils, with focus on agriculturally important groups e.g. rhizobia and AM fungi. This objective will be achieved by setting up pot experiments with soils different in micronutrient content, amended with basaltic rockdust, and by a landscape study of soils with widely differing mineralogy.

Key Words
Bioweathering, microbial community composition, rockdust, trace elements.

Introduction
Trace elements are defined as elements that are present at low concentrations (mg/kg or less) in most soils, plants, and living organisms. Among these elements, boron (B), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), and zinc (Zn) are essential to the normal growth of higher plants (Alloway 2008) and cobalt (Co), Cu, Fe, iodine (I), Mo, Mn, selenium (Se) and Zn are essential to the growth and health of animals and humans (Welch 2008). These elements are collectively called micronutrients because even though some are present in large concentrations on earth, they are not required by organisms in large amounts. Micronutrient deficiencies can considerably reduce the yield as well as the nutritional quality of the crop products which are subsequently consumed by animals and/or humans (Alloway 2008), leading to nutrient deficiencies and imbalances. In addition to plants, animals and humans, microorganisms also require micronutrients. In relation to soils, microorganisms are important in the soil plant system because they are essential for the turnover and recycling of nutrients and some are important symbionts with plants that directly mediate uptake of nutrients e.g. arbuscular mycorrhiza (AM) fungi and others also help acquire nutrients e.g. by fixation of nitrogen. There is evidence of specific trace element deficiencies in rhizobia bacteria which are one of the most agriculturally important microorganisms and are known to require B, Co, Cu, Fe, Mn, Mo, Ni, Se and Zn for their survival as free-living soil saprophytes, as well as their symbiotic relationship with legumes (O'Hara *et al.* 1988). The response of rhizobia to nutrient deficiencies varies considerably between genera, species and strains (O'Hara 2001).

Whilst microorganisms have a significant effect on releasing the nutritional elements from minerals into soil environment through the mechanism of bioweathering, their community composition can also be affected by elemental constituents of different types of rocks and minerals. A literature review showed that there is evidence that mineral composition affects the structure of the associated microbial communities in some different environments (e.g. Boyd *et al.* 2007; Certini *et al.* 2004) including soil (Carson *et al.* 2009; Carson *et al.* 2007). However, all of the foregoing studies focused on the relation between microbial colonization and 'major' element constituents of different minerals, whilst trace elements (mainly micronutrients which are also needed by microorganisms) content of minerals may also be influential in this aspect.

In this project we are studying how minerals added to soils as well as differences in the inherent mineralogy of soils, may affect the microbial community composition. The study has a particular focus on agriculturally important crops and microorganisms as there is an important context in relation to adding minerals as nutrient sources in such systems. The aim is to answer the questions: how does variation in inherent mineral composition of soil explain microbial community composition, and how do soils varying from low to high availability of micronutrients (e.g. B, Mo) explain the diversity and abundance of rhizobia? Inherent mineralogical differences and associations with microbial community composition will be studied in a landscape scale study of Scottish soils using field samples of widely differing mineralogy while the addition of rockdust will be studied in pot experiments using Swedish soils.

Methods

Experimental set-up

A landscape study using the soil data and DNA archive of the National Soil Inventory of Scotland (NSIS) has been set up. In order to assess the functioning, composition and diversity of the microbial communities of soils, a set of physiological (MicroResp), biochemical (phospholipid fatty acids analysis, PLFA), and molecular (terminal restriction fragment length polymorphism, T-RFLP) techniques are used. Multivariate statistical methods will be used to analyse the data.

In two pot experiments we study if rockdust as a mineral amender improves the micronutrient content of the plants, as well as its effect on soil microbial communities using physiological, biochemical and molecular techniques. Plant biomass growth has been determined and plant samples are being analysed for micronutrient concentrations. In the first pot experiment three different soil types (sand, clay and peat) amended with two rates (high: 5 kg/m^2, low: 0.5 kg/m^2) of basaltic rockdust have been planted with a mixture of perennial ryegrass (*Lolium perenne*, L.) and red clover (*Trifolium pratense,* L.), and a treatment with silica dust used as control. The rockdust was mixed with the soils two year prior to our study and kept outdoors in a semi-natural growing area, enabling a study of more long-term effects of rockdust application. The second pot experiment was established 2009 using two soils poor in micronutrients and formed on different parent materials and a ryegrass and clover mixture. The same type and rates of rockdust and plants was used as described above.

Soil microbial methods

In MicroResp technique, substrate-induced respiration (SIR) through utilizing sole-carbon sources is used to determine and compare the physiological capacity of microbial community in contrasting soils (Leckie, 2005; Campbell *et al.* 2003). MicroResp has advantages over existing methods in that it does not rely on extraction of a soil suspension and subsequent growth of organisms (Biolog) and is a miniaturized system that is quicker and more sensitive than existing SIR methods (Lalor *et al.* 2007). Phenotypic fingerprinting of soil microbial communities can be done by the PLFA technique, which is based on the variability of fatty acids present in cell membranes of different organisms. After cell death, phospholipids degrades rapidly in soils and total PLFA content has been shown to correlate well with other measures of microbial biomass in soils (Bailey *et al.* 2002; Zelles *et al.* 1992). It has the additional advantage that the abundance of different microbial taxa can be estimated quantitatively. Molecular fingerprinting techniques are used for a rapid assessment of a microbial community, particularly for comparison or monitoring purposes. T-RFLP analysis (Singh *et al.* 2006) is based on restriction enzyme digestion of PCR-amplified DNA that has been fluorescently labelled at one end. As all molecular fingerprinting methods are likely to introduce biases during DNA extraction and PCR amplification but give a great deal more detailed information of which taxa, genus and species might be present depending on what genetic markers are used. TRFLP is being used widely due to its sensitivity and can give semi-quantitative information.

Soil mineralogical methods

In terms of mineralogical studies and sample characterisation, quantitative analysis of minerals using X-ray powder diffraction (XRPD) (Hillier 2003; Hillier, 1999) provides precise information about the mineral composition of soil. The chemical composition including the micro element concentration will be obtained by X-ray fluorescence (XRF) or total chemical dissolution and ICP-MS analyses of the digest. The partitioning of major elements between different mineral phases will be estimated by normative calculation as has been successfully demonstrated for K (Andrist-Rangel *et al.* 2006). This approach will now be further explored in relation to correlations between mineralogy and selected micronutrients of interest, e.g. Cu, Fe and Zn.

Progress in project

Two pot experiments were started in spring 2009, the first using soils that were mixed with rockdust 2007 and the second with two micronutrient deficient agricultural soils. Plants were harvested at the end of the growing season and are being analysed for micronutrients concentrations. Soils were sampled both before planting and after harvest and will be analysed using mineralogical, chemical and microbiological methods mentioned above during the winter and spring 2010. The landscape study has been started working on data sets and using DNA archive of NSIS and will be continued during spring 2010. This means that the project is in good progress and will have generated results both from the landscape study and the pot experiments that will be presented at the congress.

Conclusion

With the refined analytical methods available today, it is possible to study and get a better understanding of the relation between soil mineral composition and soil microbial communities as well as how soil microbial communities are affected by soil amendment with basaltic rockdust. This will have implications for what management approaches are options to improve micronutrient content of feed and food, e.g. within low-input agricultural systems.

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Selenium in soils of São Paulo state and its application to forage legume

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Abstract
The objective of this research was to evaluate Selenium (Se) levels in the form of Sodium Selenate in Selenium-deficient soils applied to *Stylosanthes capitata* cv. Campo Grande analyzing the effects of Se on mineral nutrition of the plant, which has a direct correlation with animal nutrition. The soils used were selected after sampling the main soils in the São Paulo state. The experimental design used was randomized blocks in a factorial 3x3x2 with four repetitions. The treatments comprised of three soils (NITOSSOLO VERMELHO eutroférrico, LATOSSOLO VERMELHO Distroférrico and ARGISSOLO AMARELO Distrófico Abrúptico), three levels of Se (0, 10 and 20 g/ha) and two cuts (30 and 80 days after plant uniformity). The analyses were performed by the proc mixed (SAS, 2004) system. The leguminous presented great capacity of Se absorption, being that the dose of 10 g/ha was enough for the plant to reach the required level for animal nutrition; nevertheless, this increase was followed by reductions of protein levels. The doses did not alter dry matter production; however, they affected the levels of Ca, S, Fe, Mn in the plant. It was observed that soil fertilization with doses of up to 20 g/ha of Se in pastures with legumes can favor Se consumption by animals. This was verified by the reduction of foliar levels as the maturation stage advanced.

Key Words
Brazil, *Stylosanthes capitata*, sodium selenate.

Introduction
Selenium essentiality in plants has been reported by Malavolta (2006), however Se is an essential micronutrient in human and animal nutrition (Terry *et al.* 2000). The geographic origin of the animals is a more important determinant of the Se concentration in beef than the presence or absence of supplemental Se (Hintze *et al.* 2001). Dry matter of forage plants in 12 regions of the São Paulo state, presented levels of 0.076 and 0.052 ppm of Se in the wet and dry seasons respectively, showing general deficiency (Lucci *et al.* 1984). However, data on the soil were not collected, which made it impossible to establish a correlation between Se in the soil and the plant. There is a need for more research to investigate the role of bacteria found in the rhizophore, which may facilitate selenate absorption through the root, possibly supplying key-protein, besides promoting the conversion of SeCys to SeMet, facilitating, therefore selenite volatility (Terry *et al.* 2000). The deficit of information on Se levels in the soil and the fact that there are factors related to soils which are able to affect its absorption by plants, require the need to verify the behavior of a leguminous forage grass applied to different soils submitted to doses of Se.

Methods
Based on a study on Se in soils of São Paulo state, we investigated the behavior of *Stylosanthes capitata* cv. Campo Grande in Se-deficient soils, submitting them to levels of Se application using the sodium selenate as a source in pots (7 kg) in a greenhouse. The experimental design used was randomized blocks with four repetitions. The treatments comprised of three soils (NITOSSOLO VERMELHO eutroférrico, LATOSSOLO VERMELHO Distroférrico and ARGISSOLO AMARELO Distrófico Abrúptico), three levels of Se (0, 10 and 20 g/ha) and two cuts (30 and 80 days after plant uniformity). The statistical analyses were performed by the proc mixed (SAS, 2004) system. The averages were compared utilizing orthogonal contrasts and significance level of 10%.

Results
The soils evaluated did not present Se levels high enough to supply desirable amounts to the forage plants (Table 1), considering the requirement of 100 µg/kg of Se in the dry matter for beef cattle (NRC 2000). The criterion of deficiency is defined whenever there is less than 500 µg/kg of Se in the soil (Millar 1983).

Table 1. Chemical analyses for soils from depths of 0-20 and 20-40 cm.

Soils classification	Localization	Prof.	pH	M.O	P	S	K	Ca	Mg	H+Al	Al	CTC	SB	V	m	B	Cu	Fe	Mn	Zn	Se soil	Se plant
		(cm)	$CaCl_2$	$g.dm^{-3}$	$mg.dm^{-3}$		$mmolc.dm^{-3}$							%		$mg.dm^{-3}$					($\mu g.kg^{-1}$)	
ARGISSOLO AMARELO Distrófico Abrúptico	22°38,366'S 47°49,852'W	0-20	4,4	14	4	6	1,1	6	3	30	3	40	10	25	21	0,15	1,3	73	2,1	0,7	67,9	10,4
		20-40	4,5	15	3	8	0,4	7	3	24	1	34	10	30	12	0,13	1,1	60	2,9	0,3	84,6	
NITOSSOLO VERMELHO Eutroférrico	22°42,407'S 47°37,438'W	0-20	5,5	27	5	12	5,2	41	6	27	-	79	52	66	-	0,19	7,5	28	28,5	1,9	220,2	61
		20-40	5,2	21	4	13	3,5	24	5	29	1	62	33	53	4	0,16	6,2	15	21,1	0,9	219,4	
CAMBISSOLO HÁPLICO Tb Álico	22°38,404'S 47°49,024'W	0-20	5,5	31	7	17	1,7	58	8	35	-	103	68	66	-	0,24	1,9	75	50	3,5	108	62,5
		20-40	5,3	24	5	12	1,2	55	9	35	-	100	65	65	-	0,16	1,7	53	49,5	2,1	138,4	
NEOSSOLO QUARTZARÊNICO Distrófico	22°04,961'S 47°34,736'W	0-20	4,7	18	3	8	0,5	8	4	31	1	43	12	29	7	0,08	1,3	43	0,9	0,5	159,6	79,7
		20-40	4,4	19	3	7	0,3	4	3	41	4	48	7	15	36	0,16	1,4	51	0,6	0,9	142,1	
GLEISSOLO HÁPLICO Tb Distrófico	22° 15,054'S 47°52,044'W	0-20	4	65	6	6	0,4	3	2	240	5	245	5	2	49	0,36	1,9	76	0,6	0,7	80,3	58,5
		20-40	3,9	32	4	6	0,3	5	3	105	1	113	8	7	12	0,21	2,1	34	0,2	0,3	72,9	
LATOSSOLO AMARELO Distrófico textura média	21°56,630'S 47°28,506'W	0-20	5,5	24	4	8	1,1	39	6	23	-	69	46	67	-	0,16	1,8	39	2,9	3,4	77,6	46,3
		20-40	5,4	20	3	10	1,0	29	5	20	-	55	35	64	-	0,13	1,6	22	1,1	0,6	101,7	
LATOSSOLO VERMELHO Distroférrico	21° 57,768'S 47°26,866'W	0-20	5,3	28	33	9	1,2	40	8	29	1	78	49	63	2	0,16	8,3	40	7,1	5,7	197,1	61,9
		20-40	5,3	21	9	8	0,9	21	4	27	1	53	26	49	4	0,10	6,3	22	6	1,8	158,8	
ARGISSOLO AMARELO Latossólico Distrófico	21° 35,278'S 48°26,054'W	0-20	4,2	12	2	5	2,6	7	4	128	1	142	14	10	7	0,10	0,8	11	2,6	0,2	97,8	65,1
		20-40	4,2	12	2	5	2,3	6	3	151	2	162	11	7	17	0,07	0,6	10	1,3	0,1	80,5	

Table 2. Chemical analyses of *Stylosanthes capitata* submitted to Se treatments during the period (Averages followed by the same letter in the column did not statistically differ from one another by the Tukey (10%) test.

		P	S	K	Ca	Mg	Cu	Fe	Mn	Zn	Se
		$g.kg^{-1}$					$mg.kg^{-1}$				$\mu g.kg^{-1}$
Doses of Se (g/ha)	0	$1,2^{a}$	$1,0^{b}$	37^{a}	18^{b}	$1,8^{a}$	8^{a}	$56,4^{b}$	$60,9^{ab}$	96^{a}	$34,8^{b}$
	10	$1,3^{a}$	$1,2^{b}$	37^{a}	18^{b}	$1,8^{a}$	9^{a}	$52,8^{b}$	$58,6^{b}$	99^{a}	$113,6^{ab}$
	20	$1,3^{a}$	$1,4^{a}$	37^{a}	20^{a}	$1,8^{a}$	9^{a}	$68,6^{a}$	$70,2^{a}$	89^{a}	$135,9^{a}$
Evaluation time (days)	30	$1,1^{a}$	$1,1^{a}$	45^{a}	21^{a}	$1,9^{a}$	9^{a}	$66,0^{a}$	$46,7^{b}$	119^{a}	$159,8^{a}$
	60	$1,5^{a}$	$1,4^{a}$	29^{b}	17^{b}	$1,7^{b}$	9^{a}	$52,6^{b}$	$79,7^{a}$	71^{b}	$29,8^{a}$

The doses applied provided a curved linear response in foliar Se levels. There was reduction from 160 µg/g in the first cut to 30 µg/g in the second cut. Se levels decrease as the maturation stage advances (Correia 1986). The Se doses had an effect on levels of S, Ca, Fe and Mn in the plant (Table 2). The average S levels in the plant were low (Raij *et al.* 1996) as a consequence of the absence of S in the fertilizer. However the treatments with Se doses (p=0.0001) presented a positive linear effect in S concentration (Table 2) against the expectation of a reductions which due to competition for the same absorption paths as Se (Malavolta 1980). Selenate is the predominant form of Se in plant absorption and, unlike selenite, in the soil, it does not attach to Fe which may have allowed for higher absorption by Fe oxides due to selenate application. Among the chemical attributes related to soil fertility, only P, Mn and Zn showed response to the doses, while Se levels in the soil showed only a tendency to decrease (Table 3).

Table 3. Average levels of chemical attributes of soil fertility for the soils cultivated with *Stylosanthes capitata* and submitted to Se treatments (Averages followed by the same letter in the column did not statically differ from one another by Tukey 10% test).

Doses of Se ($g.ha^{-1}$)	pH	M.O.	P	S	K	Ca	Mg	H + Al	Al	CTC	SB	V	m	B	Cu	Fe	Mn	Zn	Se
	$CaCl_2$	$g.dm^{-3}$	$mg.dm^{-3}$		$mmolc.dm^{3}$							%		$mg.dm^{3}$					$\mu g.kg^{-1}$
0	$5,2^{a}$	26^{a}	24^{b}	6^{a}	$2,1^{a}$	21^{a}	7^{a}	27^{a}	1^{a}	57^{a}	30^{a}	51^{a}	4^{a}	$0,17^{a}$	$5,8^{a}$	39^{a}	$9,3^{a}$	$6,1^{b}$	$38,4^{a}$
10	$5,3^{a}$	27^{a}	29^{a}	5^{a}	$2,0^{a}$	20^{a}	7^{a}	25^{a}	1^{a}	52^{a}	28^{a}	55^{a}	6^{a}	$0,21^{a}$	$4,8^{a}$	40^{a}	$7,4^{a}$	$6,7^{a}$	$34,1^{a}$
20	$5,2^{a}$	26^{a}	24^{b}	6^{a}	$1,9^{a}$	19^{a}	7^{a}	25^{a}	1^{a}	54^{a}	29^{a}	53^{a}	5^{a}	$0,21^{a}$	$5,6^{a}$	43^{a}	$11,1^{a}$	$6,1^{b}$	$23,8^{a}$

Among the soils evaluated, there were differences in the Se levels in the plants (p=0.0010) and in the soil (p=0.0004), as shown in Figure 1. The levels obtained in the soils (Figure 1) are low, considering the deficiency criterion (Millar 1983) which suggests low levels whenever they are lower than 500 µg/kg. Despite the low levels in the soil, the foliar levels of the plants receiving Se doses were high enough to supply the minimum requirements for animal nutrition, considering the requirement established by NRC (1996) for beef cattle which is 100 µg/kg of Se in the dry matter.

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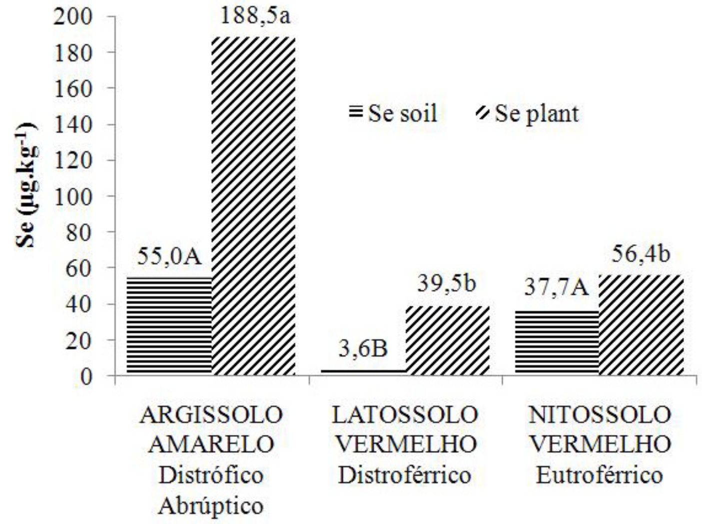

Figure 1. Average levels of Selenium in the soil and in *Stylosanthes capitata* submitted to treatments with Se (Averages followed by the same letter in the column did not statically differ from one another, Tukey 10% test).

There is a relationship between the Se in the soil and the Se in the plants (Robberecht *et al.* 1981). The relationship (y= 2.563x+12.51) has a correlation coefficient of 0.675. The results obtained for the plants demonstrated the possibility of supplementing Se to animal diets through Se application via fertilizer. There is a possibility to elevate Se levels in the blood of animals through the increase of Se levels in pasture with more efficiency than through mineral supplements to animals (Selênio 2002). The production of dry matter, the residual dry matter and evaluations of plant roots, such as length and weight, did not show effects from Se application. The crude protein level responded to the treatments (p=0.0195) presenting a decreasing linear behaviour with the averages 10.4; 9.4 and 8.8% for the control (0 g/ha of Se) and doses of 10 and 20 g/ha of Se respectively. The control value is significantly different from the highest dose, following the equation y=-0.8x+11.13 (R^2=0.979). The CP reduction in the plants due to the increase of Se level can be explained by the connection of this element with the UGA codon codification, suggested as an agent for ceasing protein synthesis. This interpretation requires further in-depth studies in the genetics field (Hatfield *et al.* 1992). We can not disregard any intervention of Se in the biological fixation process.

Conclusion

Some of the main soils in São Paulo state have low levels of Se. The Se dose applied did not alter the solubility of the element in the soils, but the dose of 10 g/ha was enough for plants to reach the required level for animal nutrition, but this increase was associated with a reduction in the level of protein. The doses did not affect dry matter production; however, they changed the chemical composition of the plant interfering in levels of Ca, S, Fe and Mn. Se supply through fertilization is effective in elevating foliar levels of Se in *Stylosanthes capitata* cv. Campo Grande. Soil fertilization with doses up to 20 g/ha of Se to pastures may increase Se consumption by animals, however, the reduction of foliar levels from the first to the second cut must be considered.

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Selenium in the rock-soil-plant system in the south-eastern part of Romania

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Abstract
Rocks and parental materials (loess, green schist, and limestone) samples, soil samples, plant samples, consisting of the aerial part of green wheat plants in the 5-6 stage on the Feeks scale and the wheat grains samples were analyzed. The two areas of investigated territory (the South-Eastern Romanian Plain and the Central and Southern Dobrogea) having contrasting content of selenium, about normal in rocks, soils and plants of the South-Eastern Romanian Plain up to low in rocks, soils and plants of Central and Southern Dobrogea. The grains of wheat plants results from the Romanian Plain selenium content was close to normal, while the grains of wheat plants grown in Central and South Dobrogea selenium content was almost zero, less than 0.5 μg/kg. The results lead to necessity to bio-fortify the wheat flour with selenium or mixing it with wheat obtained in other climatic zones.

Key Words
Total selenium content, mobile selenium content, loess, green schist, Chernozem, Kastanozems, wheat.

Introduction
Selenium is a trace essential for animal and human nutrition, having antiviral and anti-tumor effects (Rotruer and Pone 1993; Deélestra 1982). It was highlighted its role in plant nutrition (Läuchli 1993; Nowak *et al.* 2003), resulting in even crop increases by taking into soil, the plant or seed (Lăcătuşu *et al.* 2003). Romania is situated in an area of Europe with deficiency trends in this microelement. There have been recorded cases of selenium deficiency in calves, lambs, piglets and young buffaloes in the central part of the country (Salantiu 1970) and in lambs in Central Dobrogea. This paper brings contributions related to the abundance of selenium in rock-soil-plant system in the southeast part of the country, including the area in which there were frequent cases of myodystrophy in sheep, caused by selenium deficiency (Lăcătuşu *et al.* 2002).

Methods
Research has an expeditionary character, during which samples from rocks, soil from the upper horizon (0-20 cm) of major soil types of investigated areas were collected (Figure 1), and samples of wheat plants, both during the vegetation period, at the 5-6 stage on the Feeks scale (aerial part) and maturity (wheat grains), were collected. Harvesting of wheat plants and wheat grains was made in the same places where soil samples were collected. Wheat was chosen as test plant because it represents an important transmission vector of selenium, and not only, especially as grain in food of animals and humans. 17 rock samples, 81 soil samples and 81 samples of plants (green plants and grains) were collected. Soil samples belonging to the main soil types existing in studied areas: Typical Chernozem, Calcareous -Kastanic Chernozem in the South-Eastern Romanian Plain and Kastanozems, Calcareous-Brownish Chernozem, Regosols and Alluvial Soils in Central and Southern Dobrogea.

Total selenium and mobile content in rocks and soil, and total selenium content of plants in vegetation and in grain at maturity was determined in the laboratory. For determination of total selenium in rocks and soil, disaggregating of samples was made with a mixture of concentrated mineral acids (HNO_3 and $HClO_4$) and hydrogen peroxide (H_2O_2). Mobile selenium from rocks and soil was extracted with CH_3COONH_4-EDTA solution, at pH 7.0 (after Lăcătuşu *et al.* 1987). Total selenium in plant was dozen in hydrochloric solutions resulted after solubilization of the ash obtained at 475^oC. All dosages of selenium have been performed by atomic absorption spectrometry method, as a result of carrying away in air-acetylene flame of hydrogen selenate, formed as a result of reduction reaction of selenium with sodium boron hydride. Analytical results were processed statistically, for scattering parameters (standard deviation, minimum and maximum values) and grouping centre parameters (arithmetic mean, geometric mean, and median).

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Figure 1. Investigated area: 1. South-Eastern Romanian Plane; 2. Central and Southern Dobrogea.

Results

Selenium abundance in the rocks and parental materials

The main parental rocks and materials that were developed soils investigated are: loess (Romanian Plain), and loess, green schist and limestone (Central and Southern Dobrogea). Samples were taken from natural openings. Analytical data of the total selenium content determined in rocks and parental material presents low, lower value of 100 µg/kg. Although loess from southern Dobrogea has a slightly lower content of total selenium, however, values the values determined in the three areas of investigation are close (Table 1), which may prove genetic unity of loess in these areas.

Table 1. Medium total and mobile selenium content (µg/kg) in rocks and parental materials on which the soil formed in South-Eastern Romanian Plane and. Central and Southern Dobrogea

Areas	Rock type	total Se	mobile Se
South-Eastern Romanian Plane	loess	97 ± 22	9,4 ± 2,6
	loess	100 ± 15	4,6 ± 0,5
Central Dobrogea	green schist	22 ± 7	4,6 ± 1,0
	Jurassic limestone	20 ± 3	30 ± 0,8
Southern Dobrogea	loess	84 ± 18	4,8 ± 1,3

Total Se in clay (400-600 µg/kg), sandstone (50-80 µg/kg), limestone (30-100 µg/kg) in Kabata – Pendias and Pendias (2001).

If we compare these values with analytical data on total Se content in similar types rocks (clay, sandstone, in Kabata-Pendias and Pendias 2005), results that in the loess located in the south-eastern part of the country, including green schist and limestone from the Dobrogea, total Se content is much lower. The mobile Se content, soluble in CH_3COONH_4-EDTA solution at pH 7.0, represents about 10% of total Se. It is noted that mobile Se in loess of south-eastern Romanian Plate is double that those existing in the loess or green schist or Jurassic limestone from Dobrogea (Table 1). Considering both low total content of Se in green schist and limestone from Dobrogea, and low mobile selenium content of these Dobrogea rocks and loess, any soil that could not inherit in Dobrogea a satisfactory level of selenium for plant nutrition and transfer of this microelement in plant nutrition and hence on the food chain in animals and humans.

Selenium abundance in soils

The values of statistical parameters of total and mobile Se content in soils of two investigated areas are contrasting, for the purposes of reporting to higher values in soils of South-eastern Romanian Plain soils compared to those of Central and Southern Dobrogea (Table 2). Thus, the average of total Se content in Dobrogea soils is only 60% of the total Se content of soils in the South-Eastern Romanian Plain. Similarly, the mobile Se content of Dobrogea soils is only 28% of existing mobile Se content in soils of South-Eastern Romanian Plain. If we compare the average values of total Se content in soils of both areas (Table 2) with the mean of 383 ± 255 µg/kg, representing the total selenium in the upper horizons of many soils in different countries (Kabata-Pendias and Pendias, 2001), result that the total selenium in soils of both Romanian areas is only 62% (South-Eastern Romanian Plain), and 38% (Central and Southern Dobrogea) of this value. Therefore, it is confirmed that our country is located in an area with low selenium levels. Of the two investigated areas, Dobrogea is distinguished by much lower values, which could be related to the incidence of myodystrophy disease in sheep.

Table 2. Statistical parameters of total and mobile Se contents (µg.kg^{-1}) in the upper horizon (0-20cm) of soils from South-Eastern Romanian Plane and Central and Southern Dobrogea (cultivated with wheat - agricultural year 2007/2008)

Statistical parameter	South-Eastern Romanian Plate		Central and Southern Dobrogea	
	total Se	mobile Se	total Se	mobile Se
n (number of samples)	57	57	26	26
Xmin (Minimum)	5	3	6	2
Xmax (Maximum)	382	26	306	6
X (arithmetic mean)	237	14	143	4
σ (standard deviation)	83	7	76	1
Xg (geometric mean)	192	7	104	4
CV% (variation coefficient)	35	50	53	25
Me (Median)	256	20	166	4
Mo (Module)	273	5;21	177	4

Selenium in green wheat plants and wheat grain

As a consequence of contrasting content of selenium, especially mobile selenium, existing in soils of investigated areas, the selenium content of wheat plants was also different. Although green wheat plants grown on soils of Dobrogea has accumulated a certain amount of selenium, it was, on average, almost 50% less than that of green wheat grown on soils of South-Eastern Romanian Plain. Marked differentiation was achieved in grain (Table 3). Basically, wheat grown in Dobrogea not accumulated selenium, while the grains of wheat grown on soils of south-eastern Romanian Plain, selenium has been gained, however, at slightly below the average value (146 ± 189 µg/**kg**), characteristic for wheat grains harvested from 13 wheat-growing countries of the world (Kabat-Pendias, Pendias, 2001).

Table 2. Statistical parameters of total Se contents (µg/kg) in green wheat plants, at the 5- 6 stage on the Feeks scale, and in wheat grains cultivated on soils from South-Eastern Romanian Plane and Central and Southern Dobrogea (agricultural year 2007/2008).

Statistical parameter	South-Eastern Romanian Plate		Central and Southern Dobrogea	
	green plants	grain	green plants	grain
n	57		26	26
Xmin	10	0	6	under method detection limit (0.5 µg/kg)
X max	80	312	55	
X	39	130	22	
σ	18	111	14	
Xg	34	18	18	
CV%	46	85	64	
Me	40	126	18	
Mo	17; 45	39	13	

A comparative representation of the selenium content in rock-soil-plant system in the two investigated areas is shown in Figure 2.

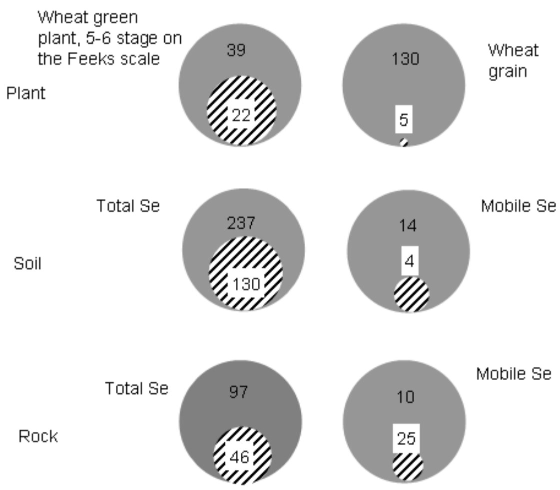

Figure 2. Comparative representation of the selenium contents (medium values, mg/kg) in the rock - soil - plant system of Central and Southern Dobrogea (▨) and of the South-Eastern Romanian Plain (●)

Conclusions

Average total selenium content of rocks and parental materials from South-Eastern Romanian Plain and Central and Southern Dobrogea is less than that quoted in the literature for rocks and similar materials. Compared with the average total selenium content in worlds soils (383 ± 255 µg/kg) non-affected by this micro-nutrient deficiency or excess, the total content of selenium in soils of South-Eastern Romanian Plain is 38% lower, and in the Central and Southern Dobrogea soils is 62% less. Average content of mobile selenium, soluble in CH_3COONH_4-EDTA solution at pH 7, in the Central and Southern Dobrogea soils was 3.5 times lower than in the South-Eastern Romanian Plain soils. Compared to the average of 146 µg/kg, considered representative value for selenium content of the grain of wheat cultivated in different world soils, selenium concentration in wheat grains grown in the South-Eastern Romanian Plain was by 11% lower, while grains wheat grown in Central and South Dobrogea, it was almost zero, however, lower than detection limit of the device (<0.5 µg/kg). It outlines the need to selenium biofortificate the flour produced by wheat cultivated in Central and Southern Dobrogea, or mix this wheat with grains obtained in other pedo-climatic areas.

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Soil fertility assessment in Tibetan villages in relation to the human Kashin-Beck disease

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Abstract
This research on the Tibetan Plateau near Lhassa was performed on request of the Kashin-Beck Disease Foundation (KBDF). Four Kashin-Beck disease (KBD) affected villages with and without KBD-affected families and one non-KBD-affected village were surveyed to assess if soil type and topsoil fertility are related to the KBD occurrence and more especially if a soil selenium (Se) deficiency is a predisposing factor. Fifty-seven fields were selected and a total of 114 soil samples were analysed for the following measurements: pH_{water} and pH_{KCl}, particle size distribution, total carbonates, total organic carbon, total nitrogen, cation exchange capacity, exchangeable (Na, K, Mg, Ca), available (K, Mg, Ca, P, Mn, Cu, Zn) and total (Na, K, Mg, Ca, Fe, Al, Mn, Cu, Zn, Ni, Cr, Cd and Se) elements. Soils are mainly Leptosols and Regosols. Comparing KBD and non-KBD family fields within the same KBD village, nothing can be deduced but the risk of Se deficiency seems higher in KBD villages than in the control village where the total Se content is four times higher. It is concluded that the diagnosis depends not only on soil types but also on their pedogeochemical backgrounds and that soil parent materials have to be more completely characterized.

Key Words
Human big bone disease, geomorphopedology, agropedology, vernacular soil qualification, WRB qualification.

Introduction
Kashin-Beck Disease is an endemic osteochondropathy primarily affecting children. More than 30 million people live in areas where the disease is endemic. The unknown aetiology of the disease represents a continuing challenge to medical and agro-environmental sciences. Currently, it is possible to identify a few predisposing factors, such as a mountain environment, Se deficiency, poor nutrition, a high concentration of organic matter in drinking water, and contamination of barley grain by fungi-producing mycotoxins (Malaisse *et al.* 2001). In a context where environmental factors and agricultural practices seem to affect the incidence of KBD, the aim of this contribution was in 2007 to identify the main soil characteristics and to assess the topsoil fertility of cultivated fields in southern Tibet (Lhassa region). Similar results were first presented in the 24th Triennal World Congress of the "Société internationale de chirurgie orthopédique et de traumatologie (SICOT)" – Hong Kong, 24-28 August 2008 – Kashin Beck Disease Symposium, 25 August 2008.

Methodology
At field level
In four KBDF-monitored villages, on the basis of a quick soil-relief survey by augering (down to 120 cm if possible) and from farmer's answers to a questionnaire, 2 soil samples, one composite in topsoil and one in subsoil, have been taken in 3 fields of 2 KBD-affected families (Randomised Clinical Trial list) and of 2 non-KBD-affected families. A similar strategy was conducted in a non-KBD-affected village but the sampling targeted only 3 families. In this way, 57 fields have been selected and a total of 114 samples collected.

Soil analysis
The following analyses and methods have been performed:
- pH_{water} and $pH_{KCl.1n}$ at 2/5,
- particle size analysis by chain hydrometer measurement after H_2O_2, HCl 0.2n, HCl 4n if needed and Na-hexametaphosphate treatments,
- total carbonates by 0,1n. sulfuric acid in double boiler and titration,

- total organic carbon by modified Springer Klee (controlled warming of the potassium dichromate – sulfuric acid reaction),
- total nitrogen by macro-Kjeldahl,
- cation exchange capacity and exchangeable Na, K, Mg and Ca with 1n. ammonium acetate at pH 7 by shaking, centrifugation and distillation,
- available K, Mg, Ca, Mn, Cu, Zn and P with 0.5n ammonium acetate + EDTA at pH 4.65,
- total Na, K, Mg, Ca, Fe, Al, Mn, Zn, Cu and Ni contents by tri-acid mineralization (HF 48 %, $HClO_4$ 70 % and HCl 10 %),
- and total Se, Cr and Cd contents by HNO_3 65 % and HCl 37 %.

Village location and physical context

The four villages monitored by KBDF are located north of the High Brahmaputra River (Yarlung Tsangpo in Tibetan) in the geological Tibetan block and the non-KBD-affected village is located south of the river in the geological Himalayan block. Effectively, the river flowing from west to east seems to underline relatively well the southern limit of the disease extension. The characteristics of the villages are as follows:

* Tsingda (4,200 m asl) and Wapuk (4,000 m) in Pondo county, ~ 60 km north east of Lhassa, along the Reting Tsangpo, as representative of a volcano-sedimentary geology and semi-nomad tradition (yak breeding). Fields are mainly distributed on old glacio-fluvial surfaces highly perched above the present riverbed, on foothill colluviums or on alluvial fan materials.

* Sheu (4,100 m) and Lume (3,850 m) in Nyemo county, ~ 90 km south west of Lhassa, along a tributary (flowing north-south inside a graben) of the Yarlung Tsangpo, as representative of a granitic context and a more sedentary farming tradition. Fields are mainly distributed on colluviums or partly on glacio-fluvial/alluvial deposits in valley plain.

* Targye (4,000 m) in Rinbung county, ~ 130 km south east of Lhassa, as representative of a sedimentary context and of a semi-nomad tradition but also as a non-KBD reference village. Fields are distributed on coarse alluvial fan materials, on colluviums or on alluviums (alluvial terraces and valley plain).

Results

Field observations

In this context of highlands, the cold semi-arid climatic conditions seem to be a little more severe in Tsingda where cracks and broken stones due to frost at the soil surface and cryoturbation features in a talus have been observed. Most fields present a slope < 10 % or even less, except some steep fields on colluvium (20-40 % slope). Soils are: (i) brownish black to dull yellow orange but frequently paler in topsoil than in subsoil, (ii) 15/20 cm (in Targye) to ~ 60 centimetres thick and (iii) slightly stony (< 15%) to stony (in Targye). As evidence of land improvement, removal and piling up of stones, man-made terraces, (old) irrigation channels (except in Tsingda) and levees along the main river (Targye) were observed. Calcrete mined out for wall whitewhashing (Wapuk), loamy soil mined out for bricks (Wapuk) and charcoal remains (Sheu, Lume) in soil are other anthropic features.

Soil analysis (0-20 cm)

The pH_{water} values are never below 6.1 and frequently between 6.9 and 8.2 but in any case, the total carbonate content was ≤ 1.8 %; the most "acid" conditions occurred in Tsingda and Wapuk. According to the FAO diagram, the soil texture corresponds to (i) loamy sand/sandy loam in Sheu, (ii) sandy loam/loam in Lume and Targye, and (iii) loam in Tsingda and Wapuk; the cumulative particle size curves of Wapuk showing a S shape (suggesting aeolian material) and of Targye, a rectilinear shape typical of an *in situ* weathering. The total organic carbon and total nitrogen contents ranged between 0.8-3.9 % and 0.07-0.34 %, respectively with the highest values in Tsingda. C/N ratios were between 8.0 and 13.5. The cation exchange capacity (CEC) measurements ranged between 8.5 and 20.8 $cmol_c$/kg; the highest values corresponding to the higher carbon contents (Tsingda) and the lowest to the loamy sand texture (Sheu). Considering the texture, the CEC values suggest that clays had a relatively high activity. Moreover according to the pH values, the base saturation ratios were frequently higher than 80 % or reaching 100 %. Exchangeable Ca and Mg represent frequently more than 70 and 8 %, respectively, of the CEC; on the other hand, the exchangeable K percentage was frequently too low inducing a too high Mg/K ratio. Available Cu, Zn or even K (for 3 villages) contents were low and available P content was relatively satisfactory in Tsingda and

Wapuk, higher in the 3 other villages, or even sometimes excessive. The potassium total content with ~ 2,500 mg/100g DM was always far higher than those of Na, Mg and Ca but Sheu and Lume showed the higher contents of Na and Ca. The lowest total contents of Fe, Al, Mn, Cu, Zn, Ni, Cr, Cd and Se were obtained for Sheu and its sandier texture, the highest ones of Fe and Cu for Lume and Targye and their loamier texture, and that of Cd with 0.2 mg/kg DM for Tsingda and its higher organic content. But if the total contents of Ni, Cr and Se were similar in Tsingda, Wapuk and Lume, the highest Ni and Se total contents were observed in Targye with 40,0 and 0.2 mg/kg DM respectively; this Se value in Targye being four times higher than in other villages.

Discussion

Pedological diagnosis

A range of indicators attest a prevalence of weakly developed soils in the study area, viz: (very) shallow and (very) stony in certain places (especially in Targye); more or less sandy (Sheu) to loamy (Wapuk); slightly acid (Tsingda and Wapuk) to neutral or even alkaline; mainly base saturated; relatively variable in their organic content but with a satisfactory index of mineralization, and consequently showing a certain capacity to adsorb and delivery nutrients to soil solution.

According to the FAO World Reference Base (WRB), they are mainly:

* Skeletic ((if not Hyper) Leptosols (Hypereutric) in Targye
* Colluvic Regosols (Eutric if not Hyper) in the other villages, (Skeletic) in some places, (Humic) namely in Tsingda and (Arenic) in Sheu.

Some Cambisols (in Wapuk) and Fluvisols could be also part of this context. However, soil depth and stoniness influence greatly the soil volume for roots and its fine earth percentage and thus the real soil element contents. Moreover, fertility is not only a question of element quantity but also of nutrient availability in well-balanced proportions (e.g. generally soils had an excessive exchangeable Mg/K ratio); availability depending also on pH (e.g. some Fe and P deficiency risks exist when pH is > 7.5. Thus, climatic conditions seem more severe in Tsingda (highest altitude, most northern location, at the confluence of two windy valleys), physical constraints including probably water supply dominate in Targye and chemical constraints, if any, would be most probable in Sheu.

Farmer's assessment and KBD versus non-KBD family field comparison

Farmers in each study area use vernacular expressions to qualify high, medium and low soil suitability. Except for K, the fertility estimation by farmers corresponds very well to the available element assessment in Tsingda, Wapuk and Lume. This comparison is not so conclusive in Sheu where soils were sandier and in Targye where soils were shallower and stonier. But due to the small size of the plots and unit conversion problems, information about yields is difficult to assess; that obtained for barley range between 0,77 t/ha in Lume and (the overestimated) 5,9 t/ha in Sheu… the average FAO reference being 2,7 t/ha. Comparing KBD and non-KBD family fields, nothing can be deduced from total element and available element measurements. Therefore, considering that ~ 0.10 mg/kg DM of available Se can represent a possible limit of excess (Dhillon and Dhillon 2003) and that the soluble form of Se can be very depleted in slightly acid to neutral soils, it is assumed that the risk of Se deficiency, with a total Se content < 0.05 mg/kg DM, is effectively higher in the four KBD-monitored villages located north of the High Brahmaputra river than in Targye located south of the river where the total Se content is ~ 0.20 mg/kg DM and pH frequently > 7.5. Moreover, if Deckers and Steinnes (2004) in their review suggest that KBD is more prevalent in the eroded hills where Regosols and Leptosols dominate the landscape, this appraisal with Master student contributions shows the importance to take into account the influence of the pedogeochemical background and the need to build local land information systems integrating the rock-relief-soil-land use relations in view to practice a more operational pedology (Bock 2002) in terms of land use planning, personalized advice, product quality, agro-environmental sustainability and finally of food chain security and human health.

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Technical aspects of zinc and iron analysis in biofortification of the staple food crops, wheat and rice

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Abstract
A major priority for any breeding initiative is to have in place effective tools for assessing the genetic variation of the trait of interest. Ideally, these technologies should be relatively low in cost and also rapid in their analysis to allow for high throughput. In its early years, the HarvestPlus program has relied heavily on ICP-OES for its micronutrient analysis as it has very good sensitivity, can have a throughput of around 500 samples per day and is able to detect Al and Ti which are seen as indicators of soil contamination in plant tissue samples. The down-side to the technology is the high cost of analysis and the degree of specialization needed to run the equipment. Colorimetric techniques such as Dithizone (for Zn) and Perl's Prussian Blue (for Fe) have been developed for high throughput screening and are currently in use within some breeding programs. Newer technologies are also being explored and they include NIRS and both hand-held and bench-top XRF. Results are promising and research in this area is continuing. Research into ways of minimizing harvest and post-harvest soil contamination of plant tissues has also lead to more robust protocols and development of "contaminant-free" equipment for use by all research disciplines working in biofortification.

Key Words
Biofortification, HarvestPlus, iron, zinc, analysis.

Introduction
It is estimated that over three billion people are afflicted with micronutrient malnutrition (Welch and Graham, 2004) and a new biofortification initiative is currently underway through the HarvestPlus challenge program (www.harvestplus.org) to provide better Fe, Zn and pro-Vitamin A carotenoid nutrition to many of those afflicted. Plant breeding programs working in biofortification of staple food crops such as rice and wheat aim to screen germplasm, varieties and elite lines for Fe and Zn-dense traits to identify genotypes that might be used as donor parents. Initial analysis from these research groups has shown that the micronutrient content, particularly Fe, is affected by production and post-harvest handling. Processing of grains plays a critical role in retaining, as well as losing micronutrients, especially during harvesting, threshing, drying, dehulling and milling. Technical strategies are needed to overcome the constraints of screening field harvested seed for analysis of micronutrients. This paper describes recent developments in this crucial area of post-harvest handling of seed, with a particular focus on rice and wheat, as well as more recent developments in rapid analysis techniques.

Materials and methods
Crop harvesting and processing methods for plant breeders are reported in Stangoulis and Sison (2008) while methods for rapid screening techniques are presented in Choi *et al.* (2007).
Micronutrient analyses were performed by ICP-OES through the Waite Analytical Service according to the method reported in Zarcinas *et al.* (1987).

Results and discussion
Crop sampling and processing methods
Crop sampling and processing methods have been developed and published (Stangoulis and Sison 2008) and are available for many staple food crops. These protocols will be updated as new research leads to improvements in sampling and handling methods. For example, in the wheat sampling protocols, there has been no method available to adequately remove soil contamination from field harvested seed, but new research is now available to show that a 5-10 second polishing using a modified commercially available Kett rice polisher, will remove Al and Ti from the surface of the grains, with these elements being indicators of soil contamination (Stangoulis *et al.* Data unpublished). This will now allow wheat breeders to harvest their biofortification plots in the same way as their other breeding plots.

For wheat, the post-harvest treatment of seed to reduce contamination is a little more simpler when compared to rice because there doesn't have to be a polishing step as the target populations for introduction of the biofortified wheat crop, mostly eat ground whole grained products like chapatti. But for rice, the target populations mostly eat milled, polished rice. This created an added complexity to the post harvest sampling method as all commercially available dehulling equipment give some form of contamination; mostly Zn due to the rice seed coming into contact with the black rubber-like compounds in the equipment. In the early days of biofortification research at IRRI, this problem was removed by manual hand-dissection of grains, where paddy rice was broken open by Teflon-coated forceps to extract the brown rice seed. It was imperative that new equipment was found to dehull the grains without contamination and various rubber-type commercially bought products were tested and a polyurethane product with 7 ppm Fe and 3 ppm Zn was found to have the lowest level of Fe and Zn respectively. The rollers in the dehuller were recoated with the new low nutrient compound and tested for contamination and were found to not contaminate samples (Figure 1). Rollers coated with the contaminant-free compound were sent to all NARES in South East Asia who were working on biofortifying rice and this eliminated the need for hand-dehulling. This was also taken one step further with the modification of the commercially bought Kett Mill, where the Zn contaminating black rubber compound in the polishing chamber was replaced with the new contaminant free compound and this greatly reduced Zn contamination. In 2007, over 10 new Kett Mills were converted and sent out to NARES programs to be used in their breeding programs. For rice, the research is still continuing to provide a contaminant-free polishing mill that is able to take very small sample sizes, with a focus on a small Chinese mill that will take under 5 g of sample. For wheat, the ability of the Kett rice polisher to remove soil contamination has lead to a satisfactory completion of protocols for this crop.

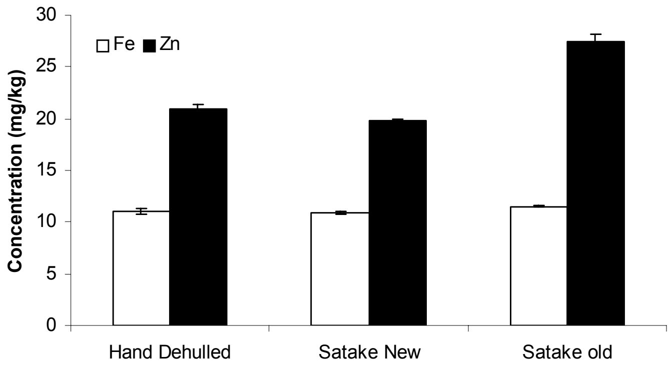

Figure 1. Effect of roller composition on Fe and Zn concentration (mg/kg) of Langi rice. For hand dehulled samples, values represent the mean of 4 replications ± SE, while for the remaining, values represent means of 10 replications ± SE. "Satake New" has modified rollers where the old black rubber was removed and polyurethane was recoated onto the metal rollers.

Micronutrient analysis

Pfeiffer and McClafferty (2007) summarised the various analytical methods and state of the art high throughput screening techniques available to plant breeders. They highlighted the importance of Inductively Coupled-Optical Emission Spectrometry (ICP-OES) over other accurate analytical tools (i.e. AAS) for the simple reason that it is sensitive enough to analyse for Al and Ti which are used as indicators of soil contamination. As the authors rightly point out, detecting contamination while assaying micronutrient concentration is complicated and references are not available to guide the research community but increases in Al and Ti in the analysis are the best indicators that we have at the moment as these elements don't readily enter the plant and their presence in analysis at significant levels is therefore an indication that the result may be inaccurate.

HarvestPlus collaborators have also been using high throughput screening techniques to try and reduce the throughput through the more expensive ICP-OES. High throughput techniques include colorimetric techniques such as modified Perl's Prussian Blue and 2,2 Dipyridal (Choi *et al.* 2007; Ozturk *et al.* 2006). Correlations between Fe determined by ICP and the 2,2 Dipyridal colorimetric method in rice, wheat, maize, sweet potato and cassava ranged between 0.88 and 0.98. The strategy is to eliminate up to 85% of screened material by using these techniques while those considered to have higher levels of Fe and Zn would then be evaluated more extensively by more accurate methods such as ICP-OES and AAS to identify the Fe and Zn-dense genotypes.

For future analysis, the indirect method of NIRS is being evaluated at CIP for its use in screening for Fe and Zn in various crops and the benefit of this technology is that the sample does not have to be digested before analysis and one can screen on whole grains. Also, given that many breeding programs are already using NIRS for traits such as protein, there is no extra work needed in sample preparation. XRF technology is also showing very promising results. The Thermo bench-top XRF has been evaluated and one can see from Figure 2 that the XRF gives comparable results to the ICP-OES for both rice and wheat. The benefit of using this technology is the ease of use which is of paramount importance to NARES in developing countries, the high throughput that one can achieve (analysis time is 3 min per sample) and there is no digestion of sample before analysis. For smaller grains such as rice, it is also possible to screen using whole grains which again reduces the time that one has to put into preparing the sample for analysis. Results so far are showing that XRF technology has potential to allow plant breeders to run their own high throughput analysis.

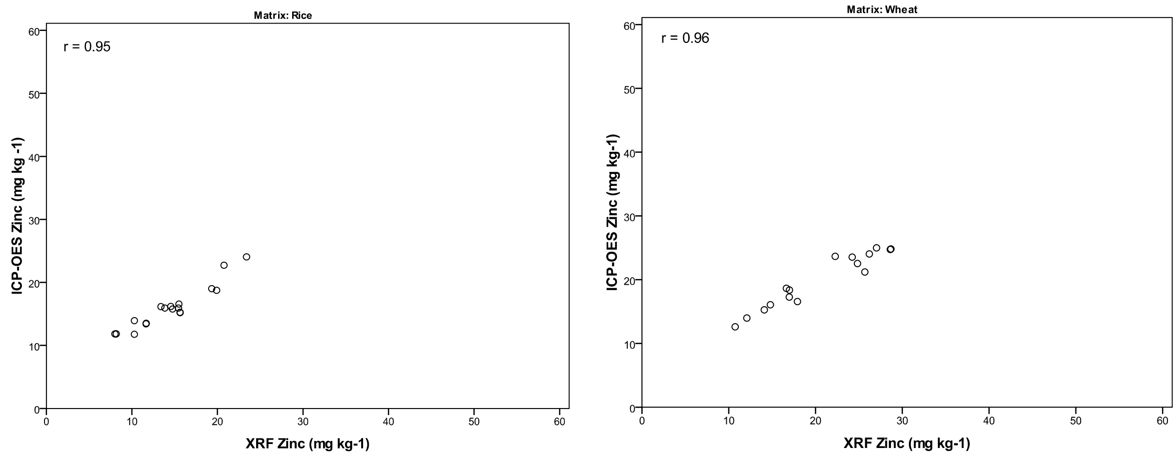

Figure 2. Correlation between XRF analysed Zn and ICP-OES analysed Zn for rice and wheat. XRF analysis time was three minutes.

Conclusion

The advent of biofortification has lead to extensive research programs to try and develop Fe and Zn-dense staple food crops. Plant breeders have had to rethink the way in which they screen for Fe and Zn-dense genotypes and this has required the elimination of soil and machinery contamination. New contaminant-free equipment has been identified and in some case, old types of equipment commonly used in plant breeding have had to be modified. Plant breeders now have protocols to follow to minimise contamination and they also have analytical tools to fasten the screening process. With technologies such as XRF, it is hoped that the complexity of selection in the breeding process will be reduced and further enhance the rapid release of new biofortified crops.

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Waterlogging effects on wheat yield, redox potential, manganese and iron in different soils of India

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Abstract
The effect of waterlogging tolerance in wheat genotypes and soil environments in micro plot (Lysimeter) experiment was investigated at the Central Soil Salinity Research Institute (CSSRI), Karnal, India. Redox potentials decreased sharply after waterlogging and were 343, 294,156, and 119 mV at 15 days after waterlogging in alkali soil at pH 7.5 (neutral soil), pH 8.2 (saline soil), pH 9.0 (sodic soil) and pH 9.4 (sodic soil) , respectively. Waterlogging caused a 4 and 9 fold increase in neutral soil (pH 7.5), 4 and 24 fold increase in saline soil (pH 8.2) and 8 and 12 fold increase in sodic soil (pH 9.0 and pH 9.4) in DTPA Fe and Mn, respectively at 15 days after waterlogging in comparison with drained conditions. These increases were higher, than reported critical concentrations for wheat. After 15 days waterlogging, all soils were drained, and the re-aeration resulted in an increase in redox potential and a decrease in DTPA-Fe and DTPA-Mn in soil solutions, but this occurred slowly taking 15- 25 days. Results support the working hypothesis that waterlogging tolerance is a product of tolerance to anoxia and microelement toxicities, and that these are both key factors limiting plant growth during and after waterlogging.

Key Words
Iron, manganese, redox potential, sodic soils, acidic sandy-duplex soil.

Introduction
Waterlogging is a widespread problem for wheat production, especially in the sodic/alkaline soils of India. The former includes 3.77 million ha of sodic soils and 2.96 million ha affected by seepage from irrigation canals (NRSA and Associates 1996). Such problems become more acute when the soils are not leveled or irrigation is followed by excess rain (Gill *et al.* 1992). Transient waterlogging also adversely affects crop production in the usually acidic sandy duplex soils in Australia. In Australia, transient waterlogging occurs primarily in sandy duplex soils, where rainfall rapidly penetrates a sandy topsoil and accumulates above a compacted clay subsoil with low hydraulic conductivity at 5 to >100 cm depth (Tennant *et al.* 1992). More recent estimates of waterlogging areas range from 1 to 2 million ha in Western Australia (Hamilton *et al.* 2000; Short and McConnel 2001), with about 3.8 million ha of crops affected in Victoria, Australia (Fried and Smith 1992).

The adverse effects of waterlogging on plants are often ascribed to decreased availability of O_2 and accumulation of phytotoxins (Armstrong and Armstrong 2001). Oxygen deficiency inhibits aerobic respiration, resulting in severe energy deficiency and eventually death (Greenway and Gibbs 2003). In addition, waterlogging can also reduce the availability of some essential nutrients, e.g. nitrogen, and increase the availability of nutrients, e.g. Fe and Mn (Ponnamperuma 1972). Such increases in micronutrients in soil and subsequently in shoots may affect plants both during waterlogging and also after waterlogging during recovery, as higher micronutrient concentrations in shoots have been reported during recovery period when soils have returned to fully aerated conditions (Setter and Waters 2003). While prolonged waterlogging is detrimental to plants, even short - term transient waterlogging can have long-lasting adverse consequences leading to poor growth and reduced grain yields, especially where temperatures are high and biological activities relating to soil redox processes can occur more rapidly. The objective of this investigation was to monitor changes in redox potential, iron and manganese concentration in different soils India under waterlogged and drained conditions, and evaluate these in relation to yield reductions of wheat in waterlogged soils.

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Methods and materials

A micro plot (Lysimeter) experiment for waterlogging (WL) tolerance was conducted at the Central Soil Salinity Research Institute (CSSRI), Karnal, India (N29°42'22.8"E76°57'10.6"), in 2004/05 and 2005/06 in field seasons (November to April). Eight genotypes of wheat (KRL 3-4, NW 1076, Brookton, PBW 343, , KRL 200, KRL 144 and HD 2009) were replicated four times and evaluated under neutral soil (pH 7.5), saline soil (pH 8.2), sodic soil (pH 9.0) and sodic soil (pH 9.4) condition. These micro-plots are highly homogeneous with respect to soil. Micro-plots are small plots of size 2mt x 2mt in which soil is made sodic artificially by adding required amount of sodium bicarbonate to make up the pH of the soil as 9.0 and 9.4 for sodic soil and sodium chloride to make up the pH of the soil as pH 8.2 for saline soil. These micro-plots are highly homogeneous with respect to sodicity as every year the equal amount of soil from each micro plots is taken out and than mixed thoroughly and filled again in each of the micro-plot. Seeds were sown in the third week of November in both the years. The waterlogged treatments were Non –waterlogged (drained), and waterlogged for 15 days. The WL treatment was imposed at 21 days after sowing (DAS). Plants were watered weekly prior to WL treatment. When WL commenced, water was maintained 2-5 cm above the soil surface during waterlogging periods. Samples of each soil were taken during WL and additional samples were taken after surface water was removed by gradual tilting of pots and draining out of water and the soils were allowed to dry.

Redox potential (Eh) were measured in the wet soils on 0, 5, 10, 15, 20 and 25 days after WL and during the recovery periods. Eh was determined in soil using (1) a millivolt meter (Cole-Parmer, Chicago, IL), (2) platinum redox electrodes made from platinum wire (0.75 mm dia; 10 mm long; AGR Matthey, Newburn, WA) manufactured according to Patrick *et al.* (1996). DTPA-Fe and DTPA-Mn were also determined in the wet soils. Fe and Mn were extracted by shaking 10 g of wet soil with 20 ml of diethylenetriaminepentaacetic acid (DTPA) solution for 2 hours (Lindsay and Norvell 1978). After filtering through a 0.42 µm filter, the solution was analyzed for Fe and Mn by atomic absorption spectrophotometry. The grain yield per plant was taken as a mean of five randomly selected plants from each genotype.

Results

Waterlogging treatment reduced grain yield in comparison to drained soils all the genotypes and higher reductions were observed in sodic soils. However, the percent reduction varied differently in different genotypes KRL 3-4 (12 %), NW 1076 (9.8 %), KRL 146 (9.0 %), Brookton (190 %), PBW 343 (162 %), KRL 200 (3.2 %), KRL 1.7 %), and HD 2009 (100 %) in sodic soil (Figure 1). This differential response of genotypes may be due to the operation of different tolerance mechanisms for waterlogging. Redox potential data presented here are corrected to pH 7 to enable comparison between different soils in India. Waterlogging significantly decreased the redox potential relative to initial values in all soil types; this occurred in soils waterlogged for 15d (Figure 2). In drained soil just prior to waterlogging, the Eh values of soil ranged from 450-500 mV; and after 15 d of waterlogging the soil redox potentials in pots had fallen to between 119 and 343 mV. Redox potential were ranged from initial to 15 days WL i.e. 453 to 343, 472 to 274, 485 to 156 and 490 to 119 mv in neutral soil (pH 7.5), saline soil (pH 8.2), sodic soil (pH 9.0) and sodic soil (pH 9.4), respectively. The sodic waterlogged soils (pH 9.0 and pH 9.4) become anoxic, i.e. have redox potentials

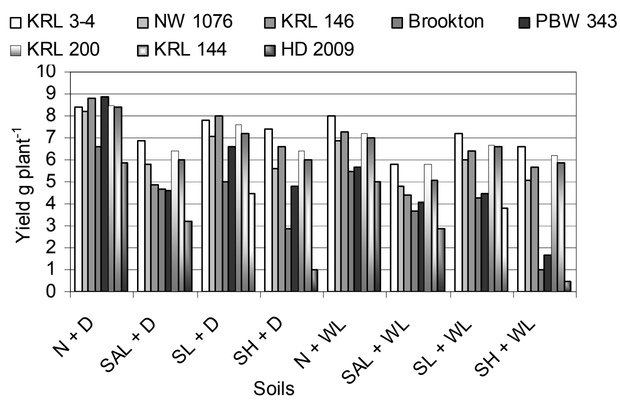

Figure 1. Effects of 15 days waterlogging (WL) on grain yield of different wheat genotypes in neutral soil (N, pH 7.5), saline soil (SAL, pH 8.2), sodic soil (SL, pH 9.0) and sodic soil (SH, pH 9.4) in Lysimeter.

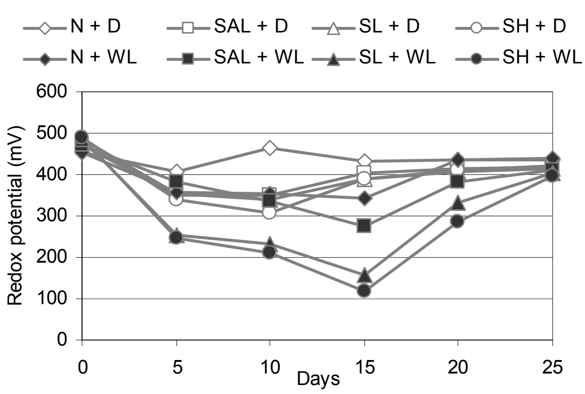

Figure 2. Effects of 15 days waterlogging (WL) on redox potential (mV) in neutral soil (N, pH 7.5), saline soil (SAL, pH 8.2), sodic soil (SL, pH 9.0) and sodic soil (SH, pH 9.4) in Lysimeter.

≤350mV, within 4-5 days after waterlogging while neutral soil (pH 7.5), saline soil (pH 8.2) become anoxic, after 10 days after waterlogging. Furthermore when these soils are allowed to drain after all water is removed from the soil surface at day 15, the natural soils in the field take at least an additional 14 days in sodic soil (pH 9.0 and pH 9.4 and 7 days in neutral soil (pH 7.5), saline soil (pH 8.2) to reach a redox potential similar to the original values in the drained soil as occurring prior to waterlogging . In summary, when waterlogging is visible at the soil surface for 15 days in sodic soils, this results in reduction in the soil for about 28 days, i.e. 14 days during the waterlogging treatment in addition to up to 14 days more after drainage. Rapid initial decrease in soil Eh in the waterlogged Indian soils is apparently due to removal of the oxygen and the release of reducing substances accompanying oxygen depletion before iron and manganese oxide hydrates can mobilize their buffer capacity (Ponnamperuma 1972).

Waterlogging significantly increased DTPA-Fe concentration in soil solutions at 15 days of waterlogging in comparison to drained treatments for all the soils. Waterlogging caused a 4 - fold increase DTPA-Fe in neutral soil (pH 7.5), saline soil (pH 8.2) and 8 - fold increase in sodic soil (pH 9.0) and sodic soil (pH 9.4) due to 15 days after waterlogging in comparison with drained conditions. However, the increase of DTPA-Fe at 15 days after waterlogging was half in neutral soil (pH 7.5), saline soil (pH 8.2) relative to sodic soil (pH 9.0) and sodic soil (pH 9.4). Patrick (1964) found that soluble iron begins to increase when the redox potential decreased to about 150 mV or less, and it continued to increase with further decreases in redox potential. This observation suggests that the transformation of iron is mainly caused by the reduction of ferric compounds to the more soluble ferrous forms.

Waterlogging significantly increased the Mn concentration in both the soils compared with drained conditions. After only 7 days waterlogging, DTPA-Mn had significantly increased about 3-fold in neutral soil (pH 7.5), sodic soil (pH 9.0) and sodic soil (pH 9.4) in relative to 7 - fold in saline soil (pH 8.2). In comparison with drained conditions, waterlogging caused a 24 - fold increase in DTPA-Mn in saline soil (pH 8.2) and 11- fold increase in sodic soil (pH 9.0 and pH 9.4) at 15 days after waterlogging. These increases in concentrations of DTPA-Mn in soil solution during waterlogging are 30 times higher than critical concentrations (DTPA-Mn 2.0 mg kg^{-1}) described by Gupta (2004).

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