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ALLEVIATING SOIL ACIDITY, Al^{3+} AND/OR Fe^{2+} TOXICITY FOR SUSTAINABLE RICE PRODUCTION ON ACID SULFATE SOILS

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Abstract

It is believed that acid sulfate soils can be used for rice cultivation after undergoing a proper reclamation program. A field trial was therefore conducted on an acid sulfate soil in Malaysia to determine the effects of applying ground magnesium limestone (GML) with or without bio-fertilizer addition on the changes in soil chemical properties as well as the growth and yield of rice. Rice seedlings of MR219 variety were transplanted into each of the experimental plots. The treatments were GML (0, 2 and 4 t ha^{-1}) in combination with bio-fertilizer (0 and 0.25 t ha^{-1}). The initial soil pH (before treatment) was 3.78, while the exchangeable Al and extractable Fe were high, with values of 2.82 $cmol_c\ kg^{-1}$ and 211.01 mg kg^{-1}, respectively. As such, the untreated soil condition was unsuitable for rice cultivation. This was evidenced by the scanning electron microscopic study, which showed clearly serious rice root injury due to the presence of high concentration of Al^{3+} and Fe^{2+} in the untreated soil. Application of GML at 2 t ha^{-1} in combination with 0.25 t bio-fertilizer ha^{-1} increased soil pH to 5.25 from 3.78 (control plot). The treatment resulted in the reduction of Al^{3+} and Fe^{2+} concentration in the soil to the minimal level that eventually increased rice grain yield from 2.12 (control plot) to 3.99 t ha^{-1}. The increase in rice yield was due mainly to the significant enhancement of the soil fertility when GML was applied together with a bio-fertilizer, fortified with N_2-fixing bacteria and micronutrients. Thus, the combination of GML and bio-fertilizer is considered as an effective, sound and appropriate agro-tech to sustain rice production on acid sulfate soils found in Malaysia.

Key words: Acid sulfate soil; Toxic metals; Bio-fertilizer; Ground magnesium limestone; Rice

Introduction

Contemporary demographic trends and the estimated world population growth are envisioned to result in a 60% increase in demand for food and feed by 2050 (Lal *et al.*, 2021). Hence, to attain the sustainable goals of increased food production, it is essential to be aware of the areas in the globe that are still underutilized, but capable of producing food such as acid sulfate soils. The soils are sporadically distributed in the tropical and subtropical regions (Sade *et al.*, 2016). Acid sulfate soils are found abundantly along the coastal regions of Southeast Asia, especially in Malaysia, Thailand, Indonesia, Vietnam and the Philippines (Shamshuddin *et al.*, 2014). The environment in the region is conducive for pyrite (FeS_2) formation (Shamshuddin *et al.*, 2004a; Shamshuddin, 2017). Pyrite is the mineral responsible for the development of acid sulfate soils, endemic in the coastal regions of Malaysia. The soils are characterized by the presence of very high acidity, evidenced by their low pH of 4 or less, which in turn responsible for the release of high amount of Al and Fe ions into the soil environment. Crop production on acid sulfate soils is limited by the low pH stress and Al^{3+} toxicity or even Fe^{2+} toxicity (Shamshuddin *et al.*, 2014; Shamshuddin *et al.*, 2017). Notwithstanding, some of the acid sulfate soils in Peninsular Malaysia have been utilized for the cultivation of rice (Panhwar *et al.*, 2014a; Panhwar *et al.*, 2015; Panhwar *et al.*, 2016), oil palm (Auxtero & Shamshuddin, 1991) and cocoa (Chew *et al.*, 1984; Shamshuddin *et al.*, 2004b).

The consequence of pyrite oxidation is the generation of sulfuric acid and the associated toxic metal ions (Fe, Mn and Al) coupled with a deficiency of nutrients, especially available phosphorus due to fixation by Fe, which causes a very poor yield of agricultural crops. It is reported that crops grown in untreated acid sulfate soils produce very low yield due low pH stress (soil pH <3.5), nutrient deficiency and/or the presence of toxic metals at high concentration (Sarangi *et al.*, 2022).

Considering that acid sulfate soils are hydromorphic in nature, due to being located in areas with permanent high water table level, the choice of crops to be grown on the soils is limited. The best option is to utilize them for rice cultivation, instead of oil palm or cocoa. However, the problems of soil acidity and Al^{3+} and/or Fe^{2+} toxicity have to be alleviated first before rice is cultivated. Rice can only be grown successfully if the water pH in the rice field is about 6 (Alia *et al.*, 2015). However, the pH of water in acid sulfate soil areas without proper mitigation is 4 or less (Elisa *et al.*, 2014; Shamshuddin *et al.*, 2014). Furthermore, the Al content in the said water is very high (Shamshuddin, 2006). Alia *et al.*, (2017) stated that the critical Al activity in water for the healthy growth of MR 219 variety was 5.2 μM. According to Shamshuddin and Auxtero (1991) and Auxtero & Shamshuddin (1991), Al activities in the water of untreated acid sulfate soils found in Peninsular Malaysia were more than 10 times higher than the stated critical level mentioned above.

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Iron toxicity is another constraint that limits rice production on acid sulfate soils (Moore & Patrick, 1989). The critical Fe activities for Malaysian rice are 14.6 µM (Alia *et al*., 2017). In flooded soil, Fe^{3+} in the water will easily be reduced to Fe^{2+} within 2 weeks (Muhrizal *et al*., 2006). Since rice is mostly planted under anaerobic conditions, Fe^{2+} can easily be taken up by its roots, causing an excessive accumulation of Fe in the leaves of rice. Fe in rice cells catalyzes the active oxygen generation of hydroxylradical and H_2O_2 which results in toxicity to rice growing in the fields (Marschner, 1995).

The form of iron causing toxicity to rice plant is likely to be Fe^{2+}, rather than Fe^{3+}. At soil pH >5, both Fe^{2+} and Al^{3+} are precipitated as their hydroxide's forms; thus, no longer toxic to the rice plants. Beneficial bacteria added into a bio-fertilizer used for rice cultivation help in producing growth hormones as well as organic acids that increase nutrient uptake by rice plants, resulting in crop growth enhancement. The organic acids secreted by rice roots with the help of microbes in the said bio-fertilizer can fix some Fe^{2+} and/or Al^{3+} via chelation processes (Panhwar *et al*., 2014b). This phenomenon further decreases their toxicity to rice plants. The use of GML in combination with bio-fertilizer enhances soil fertility that increases rice yields. In the end, the rice self-sufficiency level in the country is raised and sustains food security in the long run (Panhwar *et al*., 2023).

Applying ground magnesium limestone is among the most effective agronomic approach to raise soil or water pH in the rice fields and/or to enhance soil fertility (Elisa *et al*., 2014; Rosilawati *et al*., 2014; Muhrizal *et al*., 2003). The pKa of Al^{3+} is 5; hence, when water pH is raised to the level above 5, the toxic metal will be precipitated as inert Al-hydroxides (Shamshuddin *et al*., 1991). Likewise, Fe^{2+} (with pKa 4.58) will be precipitated as its hydroxide form when water pH reaches that level. Another approach to alleviate soil acidity is by applying bio-fertilizer, fortified with beneficial microbes.

Organic-based approach in rice production not only enhances soil fertility, but also results in C sequestration (Cooper *et al*., 2020). According to Sun *et al*., (2022), the improvement in soil properties via adding organic materials such as bio-fertilizers depends on soil environmental factors, predominantly soil pH and soil texture. Other agronomic practices to ameliorate high acidity problem in acid sulfate soils are submergence, leaching process and adding MnO_2 (Sobouti *et al*., 2020) and application of bio-fertilizers (Panhwar *et al*., 2014).

Low pH stress is a common problem in soils of the tropics, but extreme acidity (with soil pH < 3.5) is specific to acid sulfate soils only. This phenomenon requires special agronomic management to amicably resolve the problem. Specific procedures such as selective use of crops with the ability to grow under adverse conditions, adding macro- and micronutrients and soil improvements using green manures or bio-fertilizers fortified with beneficial microbes are slowly but surely help improve acid sulfate soil fertility to sustain crop production. There is therefore a need for suitable techniques and procedures to avoid the occurrence of adverse impact of acid sulfate soil acidity on the environment. It is hypothesized that application of the above-mentioned materials at the appropriate rates together with bio-fertilizer increase soil pH, add extra nutrients and reduce the toxic effects of Fe^{2+} and Al^{3+} are the way forward to enhance the fertility of acid sulfate soils to sustain rice production in the long run.

Material and Methods

Site description and soil type: The site of the agronomic trial was an abandoned paddy fields in the Kemasin-Semerak Integrated Agricultural Development Authority (IADA), Kelantan, Malaysia. It is located in the northeastern part of Peninsular Malaysia (5.86009 N, 102.44119 E). The site has been experiencing very low yield since the area was reclaimed for rice cultivation, averaging less than 2 t ha^{-1} $season^{-1}$. The experimental plots selected for soil sampling were abandoned by farmers. They were covered with Al-tolerant plant species locally known as purun (*Eleocharis dulcis*), indicating that the soil acidity was very high. The soil identified as the Jawa Series (Paramananthan, 1987) belonged to the clayey mixed family of Typic Sulfaquepts (Anon., 2014). For the purpose of characterization, soil samples were taken from the various soil depths using a soil auger.

Experimental design, treatments and field management: In this trial, MR 219 rice variety was transplanted in the experimental plots, with plot size measuring 2.0 m x 2.0 m. The treatments were: T1 - Control; T2 - GML (2 t ha^{-1}); T3 - GML (2 t ha^{-1}) + bio-fertilizer (0.25 t ha^{-1}); T4 - GML (4 t ha^{-1}); and T5 - GML (4 t ha^{-1}) + bio-fertilizer (0.25 t ha^{-1}). The bio-fertilizer and GML where thoroughly mixed 15 days before the rice seedlings were transplanted in the experimental plots. The fertilizers applied in the trial were based on the standard practice for rice cultivation in Peninsular Malaysia (Alias 2002). The fertilizer rates were (kg ha^{-1}): N-P-K @120-18-120 from urea and NPK Green (15:15:15 + TE). The experiment was laid out in randomized complete block design (RCBD), with 4 replications.

Soil and water analysis: Soil pH was determined in water (1:2.5 soil: water), using a PHM210 Standard pH meter (Benton, 2001). Electrical conductivity (EC) was measured by an EC meter (Benton, 2001). Exchangeable bases (Ca, Mg and K) and cation exchange capacity (CEC) were determined by the ammonium acetate buffered at pH 7 (Benton, 2001). Total carbon was analyzed by Carbon Analyzer Leco CR-412 (Leo Corporation, St. Joseph, MI). Exchangeable Al was extracted by 1 M KCl (at 1:10 ratio) by shaking for 30 minutes (Barnhisel and Bertsch, 1982), and the Al in the extract was determined by Optima 8300 ICP-OES Spectrometer (Perkin Elmer, Massachusetts, USA). Total N was determined by the Kjeldahl digestion method of Bremner and Mulvaney (1982). Available P was determined by the method of Bray and Kurtz (1945), with the extracted P measured by auto-analyzer (QuikChem 8000 Series FIA System, Lachat Instruments, Loveland, USA). Extractable Fe in the soil was analyzed by the double acid method. Iron in the soil was extracted using 0.05 M HCl in 0.0125 M H_2SO_4. A five-gram sample of the soil was mixed with 25 mL of the extracting solution and was shaken for 15 minutes. The solution was then filtered through a Whatman filter paper number 42 before determining the Fe it contained by Optima 8300 ICP-OES Spectrometer (Perkin Elmer, Massachusetts, USA). The results of the analyses are presented in (Table 1).

Table 1. Chemical properties of bio-fertilizer and GML used in the study.

Source	pH	N	P	K	Fe	Al	Ca	Mg	N_2 fixing bacterial population
		(%)							(CFU g^{-1})
Bio-fertilizer	7.35	5.02	0.25	0.35	< 0.01	0.01	< 0.01	< 0.01	1×10^{-8}
GML	9.75	na*	1.70	na	< 0.01	< 0.01	19.50	6.70	Na

*na = Not available

Water was taken 7 and 14 days after transplanting from each experimental plot for analysis. The pH of the water was immediately determined by a pH meter. The concentration of Al and Fe in the water was determined by Optima 8300 ICP-OES Spectrometer (Perkin Elmer, Massachusetts, USA).

Chemical analysis of GML and bio-fertilizer: The bio-fertilizer (JITU™) used in the agronomic trial was obtained from a supplier in Malaysia. Its pH was determined in water (1:2.5). Nutrient concentration in the GML and bio-fertilizer was determined by the above-mentioned procedures.

Detection of nitrogen-fixing bacteria in bio-bertilizer: The sugarcane-based bio-fertilizer (JITU™) used in the trial was analyzed to detect and/or confirm the presence of special nitrogen-fixing bacteria. For this analysis, nitrogen-free semi-solid media containing 5g L^{-1} DL-Malic acid, 0.5 g L^{-1} K_2HPO_4, 0.1 g L^{-1} NaCl, 0.2 g L^{-1} $MgSO_4\cdot7H_2O$ and 0.02 g L^{-1} $CaCl_2.2H_2O$ with pH 6.8 (adjusted using NaOH) was prepared in the laboratory, using the method of Dobreiner & Day (1976). The plates were inoculated with test cultures and incubated at 37°C for 72 hours. The results of the analysis confirmed the presence of N_2-fixing bacteria in the bio-fertilizer applied in the experimental plots.

Scanning electron microscopy study on rice root: Selected rice roots were observed under scanning electron microscope (SEM). The roots of rice plants were dissected into small pieces, pre-fixed with 4% glutaraldehyde overnight and washed with 0.1M sodium cacodylate buffer three times for thirty minutes. Osmium tetraoxide buffer (1%) was used for post fixation. After a series of dehydration in acetone (35, 50, 75, 95 and 100%), the root samples were dried in a critical point dryer and mounted on aluminum stubs, sputter-coated in gold and observed under SEM (JEOL JSM-6400 attached with OXFORD INCA ENERGY 200 EDX).

Harvesting and yield component measurements: Rice grain yield parameters were determined - panicle number, spikelet number per panicle and percentage of filled spikelet, which were calculated using the formula 'filled spikelet per panicle/total spikelet per panicle × 100', and 1000 grain weight'. The parameters were determined from plants harvested in a 1.0 m^2 of each experimental plot.

Plant tissue analysis: The N, P and K in the plant tissues were determined by wet digestion method. The harvested plant was divided into 2 parts: rice straw and root. Half a gram of the plant tissue, which was dried in an oven at 50°C for 72 hours, was taken and placed in a digestion tube. Five mL of concentrated H_2SO_4 was added and the material was gently heated over burner in a fuming cupboard. When it started to boil, a few drops of 35% H_2O_2 were added. The addition of H_2O_2 was repeated occasionally with constant heating until clear and colorless solution was obtained. The solution was then diluted with distilled water. Potassium, Ca, Mg, Fe and Al in the solution were determined by ICP-OES. The amount of N and P in the solution was determined by an auto-analyzer (QuikChem 8000 Series FIA System, Lachat Instruments, Loveland, USA).

Statistical analysis

Data obtained from the study were analyzed by ANOVA for analysis of variance and Tukey's test for means comparison using SAS version 9.2 (SAS Institute, Inc., Cary, N.C., USA).

Results

Chemical properties of the experimental soil: The acidity of the untreated soil under study was very high, having the topsoil pH of 3.64, with lower level in the subsoil (pH 3.13). The occurrence of the diagnostic sulfuric horizon was confirmed by the presence of yellowish jarosite [$KFe_3(SO_4)_2(OH)_6$] mottles, which were observed within the 15-30 cm depth of the soil profile. This was proven beyond doubt that the soil was an acid sulfate soil as defined by the USDA Soil Taxonomy (Anon., 2014). Soil EC (<0.69 mS cm^{-1}), N (<0.004 %) and available P (<5.38 mg kg^{-1}) were very low. Likewise, exchangeable K (<0.04 $cmol_c$ kg^{-1}), Ca (<0.05 $cmol_c$ kg^{-1}) and Mg (<0.29 $cmol_c kg^{-1}$) were below the sufficiency level for the healthy growth of rice. On the contrary, exchangeable Al and extractable Fe in the soil were very high, with values >5 $cmol_c$ kg^{-1} and >135 mg kg^{-1}, respectively.

Chemical properties of the bio-fertilizer and GML: The bio-fertilizer (JITU™) used in the study contained high amounts of N (5.02%), P (0.25%) and K (0.35%), while its pH was 7.35. With the excellent chemical attributes, the bio-fertilizer would certainly help to enhance the fertility of the acidic soil under study. The content of Al and Fe in the bio-fertilizer was low, with values of about 0.01%. The bio-fertilizer was confirmed to contain N_2-fixing bacteria (1×10^{-8}CFU g^{-1}) (Table 1). As shown in Table 1, the pH of the GML was pH 9.74. It contained high amount of Ca (19.50 %) and Mg (6.70 %), with a small quantity of P (1.70%).

Effects of GML and bio-fertilizer application on soil solution Al and Fe: At low pH, the concentration of Al in the soil solution of acid sulfate soil was very high (Fig. 1a). The concentration of Al and Fe decreased with increasing water pH due to treatment with GML and/or bio-fertilizer. This is clearly depicted in Figure 1a, where Al concentration in the field plots is negatively correlated with water pH. Note that the figure was plotted using Al concentration and water pH sampled at 7 and 14 days (combined) after planting. It showed that when pH reached above 5, Al concentration in the water was at the minimum level. A similar trend was noted for Fe concentration that declined drastically when water pH was increased above 5. The application of various soil amendments into the soil increased soil pH (Table 2). Application of 2 t GML/ha alone was not enough to alleviate soil acidity for rice production as the soil pH was still low below 5 (4.14), while the exchangeable Al was high (2.56 cmol$_c$ kg^{-1}). GML applied at 2 t ha^{-1} increased soil pH to 4.14 (9.52 % increase) from 3.78. Applying GML at 4 t ha^{-1} increased pH from 3.78 to 5.25 (36.24 % increase), which was sufficient to alleviate the Al^{3+} toxicity. GML applied at 2 t ha^{-1} in combination with bio-fertilizer at 0.25 t ha^{-1} resulted in increased soil pH from 3.78 to 4.93 (30.42% increase). GML applied at 4 t ha^{-1} plus bio-fertilizer at 0.25 t ha^{-1} increased soil pH from 3.78 to 5.40 (42.86%).

Effects of bio-fertilizer and GML on soil nutrients at harvest: Application of GML and/or bio-fertilizer changed soil properties in a positive way (Table 2). The application of GML alone improved the environment in the soil for rice growth. The enhancement of soil fertility was even better if GML was applied in combination with bio-fertilizer as the results on rice yield had shown. Application of GML increased the amount of macronutrients in the soil, i.e., Ca and Mg. Significant positive impact on the exchangeable Ca and Mg due to ground magnesium limestone application was noted. By applying 2 t GML ha^{-1} (T2) alone, the exchangeable Ca and Mg increased from 0.75 to 1.35 (80%) and from 0.68 to 1.08 (58.82%) cmol$_c$ kg^{-1} soil, respectively. When the GML was applied in combination with bio-fertilizer (T3), there was further increase in the nutrients 2.09 (178%) and 1.72 (153%) cmol$_c$ kg^{-1}, while exchangeable Al in the soil was about 1 cmol$_c$ kg^{-1} after the application of this treatment. The maximum (0.69 cmol$_c$ kg^{-1}) reduction in soil exchangeable Al was observed after applying 4 t ha^{-1} of GML with bio-fertilizer at the rate 0.25 t ha^{-1}.

The extractable Fe in the untreated soil was 211.01 mg kg^{-1} (Table 2), which was considered very high. By applying 2 t GML ha^{-1}, its content in the soil was reduced to 177.16 mg kg^{-1}. When 2 t GML ha^{-1} was applied together with bio-fertilizer, the extractable Fe was further reduced to 42.99 mg kg^{-1}. This study showed that treating the soil with 2 t GML ha^{-1} plus bio-fertilizer at 0.25 t ha^{-1} was enough to increase soil pH to >5, resulting in the alleviation of the Al^{3+} toxicity that improved rice production. When GML was applied in combination with bio-fertilizer, the other macronutrients in the soil were also increased. The total N (18.69%), available P (0.78 mg kg^{-1}) and exchangeable K (0.56 cmol$_c$ kg^{-1}) increased from 1.21 %, 0.50 mg kg^{-1} and 0.19 cmol$_c$kg^{-1} after applying GML at 4 t with bio-fertilizer at 0.25 t ha^{-1} respectively. Further, the CEC was increased with the application of GML alone (>10.50 cmol$_c$ kg^{-1}) or combined with bio-fertilizer (>12 cmol$_c$ kg^{-1}).

Effect of Al and Fe on plant nutrient concentrations and roots scanning: The various treatments applied in the experimental plots affected the plant nutrient uptake. The application of GML alone and with bio-fertilizer influenced the nutrient composition in straw and roots of rice plants. Application of GML at 2 or 4 t ha^{-1} with bio-fertilizer increased the nutrient composition N (0.82-1.74%), P (0.16-0.22 %), K (2.43-2.45%, Ca (0.16-18 % and Mg (0.17-0.24%), with concomitant decease of Al (0.23-0.31 from 0.89%) and Fe (0.25-0.34 from 1.93%) in the rice straw. Similarly, N (0.34-0.36%), P (0.26-0.37%), K (2.43-2.45%), Ca (0.16-18%) and Mg (0.17-0.24%) in the rice roots were increased. Al (0.41-0.49%) and Fe (0.57-0.77%) in the roots were lower compared to those of the control treatments (Table 3). High Al concentration in the solution results in more uptake by the rice plants. Fig. 2a shows that Al in the rice straw is linearly correlated with exchangeable Al.

Root scanning study showed the significant effects of Al or Fe on its growth. (Fig. 3a) showed the healthy state of roots at 0 µM of Fe or Al. However, with the high amount of available Al or Fe in the solution, rice plant suffered from their toxicity, causing serious damages to their cells. With the increased concentration of Al or Fe in the solution, the root cells disintegrated over time and their growth was significantly curtailed (Fig. 3b and 3c). The phenomenon disturbed the growth and functions of the roots that eventually reduced rice yield.

Relationship between relative rice yield and soil pH, exchangeable Al and extractable Fe: The critical exchangeable Al to sustain rice growth on the acid sulfate soil was estimated in similar way as that of the critical soil pH (Fig. 4). Using the information observed in Figure 4b, the critical exchangeable Al estimated (corresponding to 10% drop in relative rice yield) was about 0.5 cmol$_c$ kg^{-1}soil. Based on the data presented in Table 2, reduction of exchangeable Al to that level could be achieved by applying 4 t GML plus 0.25 t bio-fertilizer ha^{-1}. Rice yield in 4 t GML with 0.25 t bio-fertilizer ha^{-1} was not significantly different from that of the 2 t GML+0.25 t bio-fertilizer ha^{-1}. The latter was cheaper and therefore more affordable for the rice farming communities in the country.

The critical extractable Fe in the acid sulfate soil to sustain rice growth/production was 35 mg kg^{-1} (Fig. 4c). As shown in Figure 1b, Fe concentration in the water was at the minimal level when water pH was raised to > 5, which could have taken place in GML 2 t ha^{-1} applied together with bio-fertilizer at 0.25 t ha^{-1}. Consistent with the improvement in soil fertility, application of GML alone or in combination with bio-fertilizer enhanced rice growth that significantly increased its yield (Table 4). The application of GML alone or with bio-fertilizer enhanced the rice growth parameters. The maximum 1000 grain weight (31.67 g) filled spikelet (91.69%), panicle numbers (707) and grain yield (4.77 t ha^{-1}) were observed in GML at 4 t with 0.25 t ha^{-1} of bio-fertilizer applied treatments. However, no significant difference was found in 1000 grain weight, panicle numbers (702 and 707) and grain yield (3.99 and 4.77 t ha^{-1}) among the GML 2 or 4 t ha^{-1} with bio-fertilizer amendments. Applying GML alone increased rice yield, but the yield was higher if GML was applied together with bio-fertilizer.

Table 2. Chemical characteristics of the soil in the field at harvest.

Treatments	pH	Exchangeable cations				Ex. Fe	Total N	Avail. P	CEC
		Ca	Mg	K	Al	(mg kg^{-1})	(%)	(mg kg^{-1})	(cmol$_c$kg^{-1})
		------------ (cmol$_c$kg^{-1}) ------------							
Control	3.78^{d}	0.75^{c}	0.68^{d}	0.19^{b}	2.83^{a}	211.01^{a}	1.21^{c}	0.50^{b}	10.50^{c}
GML 2 t ha^{-1}	4.14^{c}	1.35^{b}	1.08^{c}	0.22^{b}	2.56^{a}	177.16^{b}	9.37^{b}	0.57^{b}	10.62^{c}
GML 2 t ha^{-1}+BF 0.25 t ha^{-1}	5.25^{a}	2.09^{a}	1.72^{b}	0.35^{ab}	1.08^{c}	42.99^{c}	16.51^{a}	0.51^{b}	12.27^{a}
GML 4 t ha^{-1}	4.93^{ab}	1.43^{b}	1.30^{c}	0.28^{b}	2.12^{b}	76.22^{d}	11.73^{b}	0.72^{a}	11.56^{b}
GML 4 t ha^{-1}+BF 0.25 t ha^{-1}	5.40^{a}	2.31^{a}	2.77^{a}	0.56^{a}	0.69^{d}	28.51^{e}	18.69^{a}	0.78^{a}	12.59^{a}

Means followed by the same letter within a column is not significantly different (Tukey's test, at p>0.05)

Table 3. Elemental composition and uptake of rice straw and root.

Plant part	Treatment	N	P	K	Al	Fe	Ca	Mg
		---------- (%) ----------						
Rice straw	Control	0.82^{d}	0.06^{c}	0.13^{d}	0.89^{a}	1.93^{a}	0.14^{b}	0.14^{c}
	GML 2 t ha^{-1}	1.44^{c}	0.14^{b}	2.22^{c}	0.67^{b}	0.58^{b}	0.15^{ab}	0.16^{c}
	GML 2 t ha^{-1}+BF 0.25 t ha^{-1}	1.67^{ab}	0.16^{ab}	2.43^{ab}	0.31^{c}	0.34^{bc}	0.16^{ab}	0.17^{bc}
	GML 4 t ha^{-1}	1.54^{bc}	0.17^{ab}	2.24^{bc}	0.35^{c}	0.46^{bc}	0.16^{ab}	0.21^{ab}
	GML 4 t ha^{-1}+BF 0.25 t ha^{-1}	1.74^{a}	0.22^{a}	2.45^{a}	0.23^{c}	0.25^{c}	0.18^{a}	0.24^{a}
Root	Control	0.16^{c}	0.09^{c}	0.09^{c}	1.14^{a}	1.81^{a}	0.04^{d}	0.08^{c}
	GML 2 t ha^{-1}	0.25^{b}	0.21^{b}	0.15^{b}	0.64^{b}	0.97^{b}	0.08^{c}	0.10^{bc}
	GML 2 t ha^{-1}+BF 0.25 t ha^{-1}	0.34^{a}	0.26^{b}	0.19^{ab}	0.49^{c}	0.77^{bc}	0.10^{bc}	0.12^{ab}
	GML 4 t ha^{-1}	0.29^{b}	0.26^{b}	0.16^{b}	0.46^{c}	0.78^{bc}	0.11^{b}	0.14^{a}
	GML 4 t ha^{-1}+BF 0.25 t ha^{-1}	0.36^{a}	0.37^{a}	0.21^{a}	0.41^{c}	0.57^{c}	0.14^{a}	0.15^{a}

Means followed by the same letter within a column is not significantly different (Tukey's test, at p>0.05)

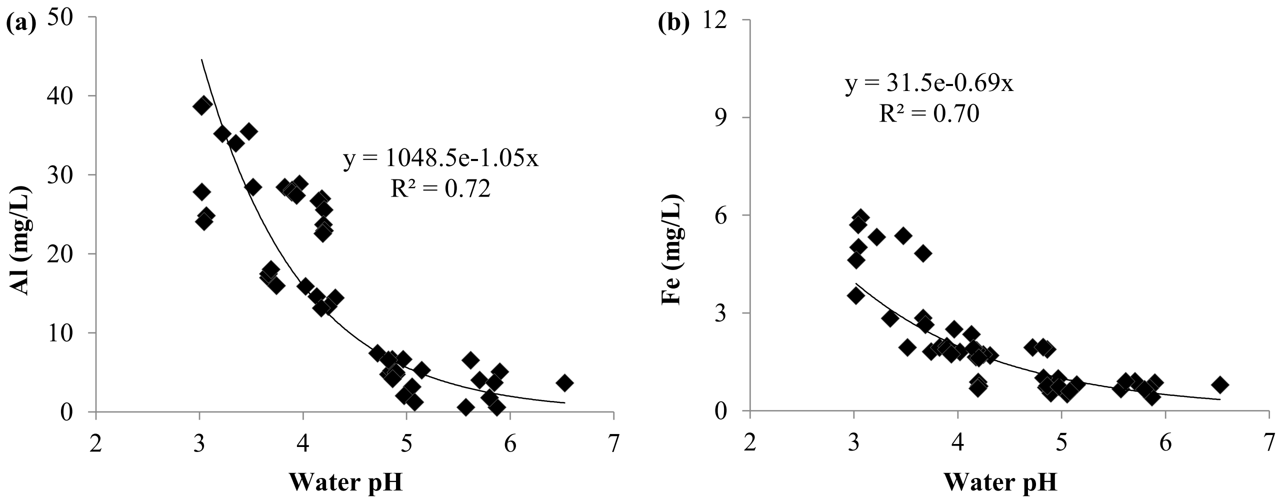

Fig. 1. Relationship between Al in water and pH (a) and between Fe in water and pH (b).

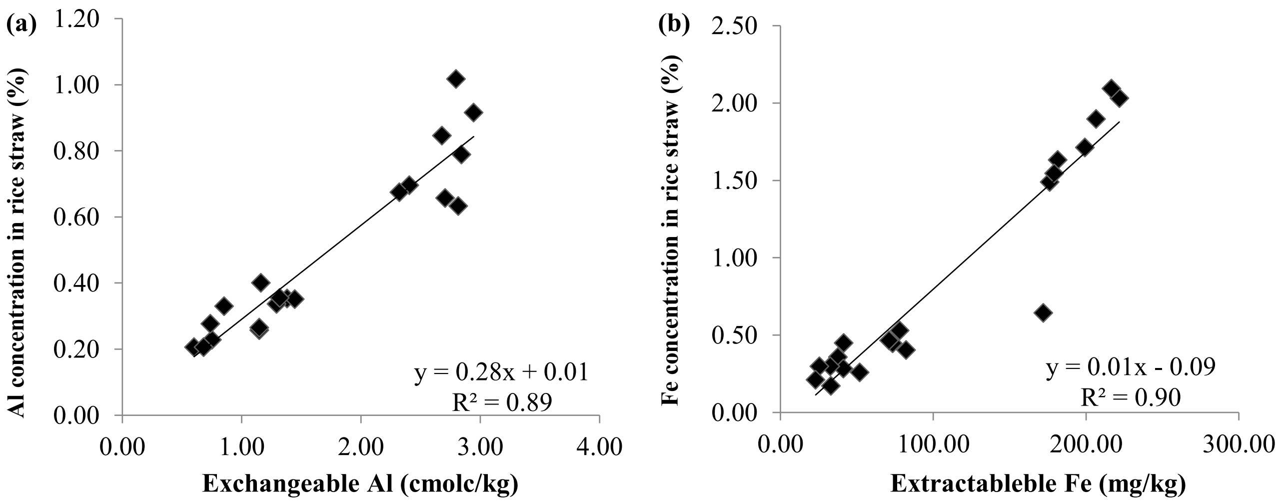

Fig. 2. Relationship between Al in rice straw and exchangeable Al (a) and between Fe in rice straw and extractable Fe (b).

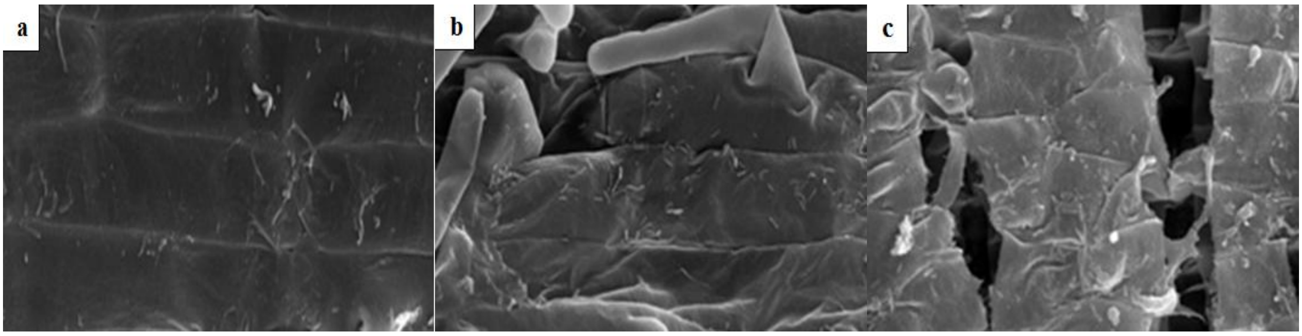

Fig. 3. SEM picture of rice root surface (a) at 0 μM of Fe/Al showing smooth surface b) at 100 μM of Fe showing shrinking surface c) at 100 μM of Al showing torn surface root tissue.

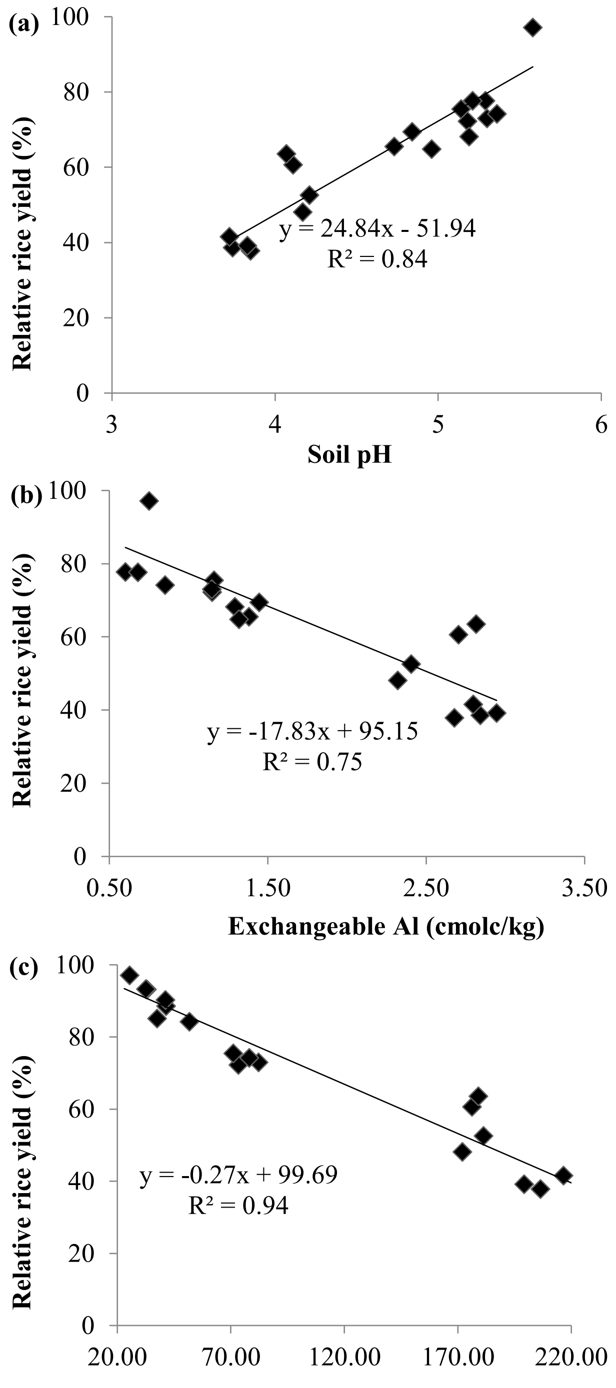

Fig. 4. Relationship between relative rice yield and soil pH (a), exchangeable Al (b) and extractable Fe (c).

Discussion

Soil pH in the field determined during the sampling time was very low (3.64), indicating high soil acidity. The sulfidic materials, which were mainly pyrite (FeS_2), were observed to occur immediately below the sulfuric horizon. Pyrite oxidation keeps generating and releasing acidity into the soil environment when the water table in the area drops to a level below the sulfuric horizon during the dry months of March-May every year (Shamshuddin, 2006). Consistent with the low topsoil pH of < 4, the exchangeable Al in that zone was very high (5.53 $cmol_c$ kg^{-1}), with the values higher in the subsoil. It can be assumed that Al concentration in the soil solution of the acid sulfate soils in the area was very high. According to Dobermann and Fairhust (2000), Al concentration in soil exceeding 2 mg kg^{-1} is not suitable for rice cultivation. The Al concentration higher than the above-mentioned level may result in damage to certain plant parts (Sarangi *et al*., 2022). It seems that soluble Al can be accumulated in the rice root tissues due to the presence of negatively charged pectic matrix in the apoplast cell walls (Alia *et al*., 2015). The binding between the pectic matrix and Al causes loosening of the enzyme in rice cell walls, preventing cell division and elongation. Thus, the root length is reduced and the root growth is curtailed due to less nutrients uptake by the rice plants.

Al is known to cross plasma membrane via Al ligand exchange, membrane bound protein or via stress lesion. When Al crosses the membrane, even at low amount of Al, many harmful interactions can occur. Once Al binds to phospholipid within the membrane, it can alter the function. Al will interact with the lipid in the plasma membrane that increases the highly toxic reactive oxygen free radicals, thus inducing lipid peroxidation. Lipid peroxidation is the early symptom of Al toxicity. According to Gupta & Toole (1986), at this point, the plasma membrane function is shifted to Al-induced depolarization and roots rapidly absorb Al, causing the root growth inhibition that results in the reduction of nutrient uptake.

The pKa of Fe^{3+} and Fe^{2+} are correspondingly 3.0 and 4.58, while that of the Al^{3+} is 5.0. Therefore, Fe in the water of the untreated acid sulfate soils under study with pH <4 was most probably existed as Fe^{3+} ions. Due to addition of GML in combination with bio-fertilizer, water pH in the experimental plots increased beyond 4.5. From then onwards, slowly but slowly, Fe^{2+} started to form in higher quantities. When the water pH moved to near 4.58, most of the Fe were in the form Fe^{2+}. As such, the most likely form of Fe causing toxicity to rice root is Fe^{2+} rather than Fe^{3+}. When water pH increased to a level >5.0, Fe^{2+} and Al^{3+} precipitated as inert hydroxides, which was no longer toxic to the rice plants (Panhwar *et al*., 2023).

Ferrous ions around rice root surface is oxidized to Fe^{3+}, forming ferric hydroxides which are precipitated as brown crust or coating. The coating may reduce the uptake of the essential nutrients from the soil for rice growth (Dobermann and Fairhurst, 2000). Since rice is planted under anaerobic conditions, it has a high tendency to take up more Fe^{2+} than any other plant. As Fe content in the soil was increased, more of it would be taken up by rice plant (shown by the rice straw). Iron is a micronutrient and therefore an essential element for plant growth, especially for electron-transport chains of photosynthesis and respiration. If Fe is accumulated within root cells, it results in the damage of the cells – it is toxic to the rice plant (Fig. 3b). Based on the data shown in Figure 1c, Fe concentration in the soil of the experimental plots was at the minimal level when the pH was raised to the level above 5.

Iron affects plant growth indirectly by fixing available P in the soil via the formation of insoluble ferric phosphate. Phosphorus is essential for root growth. When P is deficient, root growth is inhibited, making the root inefficient in taking up other essential nutrients such as K, Ca and Mg. In acid sulfate soil areas, which are mostly under anaerobic conditions, Fe content is very high (Shamshuddin, 2006; Shamshuddin, 2014). However, rice has an important adaptive feature in reducing Fe uptake where the root forms a ferric hydroxide coat, which can reduce the uptake of excess Fe. However, the coating reduces the uptake of other essential plant nutrients. With increasing in Fe^{2+} toxicity, the root of rice becomes scanty and blunted due to low uptake of essential nutrients, especially P. Tiny brown spots start to manifest on the leaves, followed by bronzing and drying of the leaves. It is a sign of Fe^{2+} toxicity (Dobermann and Fairhurst, 2000). This will eventually be translated into decreased rice yield.

The available P of <5 mg kg^{-1} in the soil was below the sufficiency level for normal rice growth/production (Dent, 1986). The low available P is mainly related to its fixation by the Fe and/or Al present in the soil. As shown in Table 1, the content of Fe and Al in the soil was very high. It seems that P deficiency is very common in soils with high Al and Fe concentration due to Al-Fe-phosphate interaction (Liao *et al*., 2006; Dent, 1986).

Table 4. Grain yield and yield contributing characteristics of MR 219 rice variety at harvest.

Treatment	1000 grain weight (g)	Spikelet (no/panicle)	Filled spikelet (%)	Panicle (no/m^2)	Yield (t/ha)
Control	21.78^d	78^c	74.84^c	553^c	2.12^e
GML 2 t ha^{-1}	22.34^c	79^b	84.71^b	585^b	3.04^d
GML 2 tha^{-1}+BF 0.25 t ha^{-1}	26.41^a	84^b	90.12^a	702^a	3.99^{ab}
GML 4 t ha^{-1}	23.21^b	101^a	88.81^{ab}	652^b	3.62^c
GML 4 t ha^{-1}+BF0.25 t ha^{-1}	31.67^a	81^b	91.69^a	707^a	4.77^a

Means followed by the same letter within a column is not significantly different (Tukey's Test, at p>0.05)

Soils in the upland areas of Peninsular Malaysia are characterized by the low basic cations, especially Ca and Mg. The soil of the current study was no different from that of the upland areas of the country. The untreated topsoil exchangeable Ca and Mg in the soil under investigation were very low, with values of 0.02 and 0.15 $cmol_c$ kg^{-1}, respectively. These values were too far below the critical level for rice requirement. For rice, Ca and Mg are very important for its growth. Calcium is required for cell wall and membrane stabilization, especially in root surface where all the nutrients is taken up via plasma membrane. On the other hand, Mg is one of the elements in the chlorophyll molecules, which is vital for photosynthesis. Thus, if rice lacks any of these nutrients, its grain quality and the rice productivity will be significantly affected (Marschner, 1995). In reality, Ca and Mg contents in the soil under investigation were insufficient to sustain rice growth; hence, the problem has to be alleviated via agronomic means, i.e., by GML application or other amendments (Shamshuddin, 2014).

GML and and/or bio-fertilizer application enhanced soil fertility via increased soil macronutrients. This was shown by the increase of total N, available P and exchangeable K due to the application of GML at 4 t with bio-fertilizer at 0.25 t ha^{-1}. The amount of the macronutrients in the soil had increased to the critical level required for rice production determined by Palhares (2000) for Ca and Dobermann and Fairhurst (2000) for Mg. GML produced in Malaysia contains varying amounts of dolomite [Ca, Mg $(CaCO_3)_2$] and calcite ($CaCO_3$) (Shamshuddin & Ismail, 1995). On dissolution, the GML releases Ca and Mg into the soil to be taken up by the growing rice plants in the fields.

The addition of organic material (such as compost) as a soil improves soil fertility, creating good soil conditions for rice grown in acid sulfate soil. Soil amendments or liming materials (such as dolomite) are elements supplementary to the soil that result in improvement of its capacity to support plant life in soil (Shazanan *et al.,* 2013; Nur Sa'adah *et al*., 2018). A study by Rendana *et al*., (2018) showed that application of organic materials on an acid sulfate soil enhanced its health via increasing soil organic matter from 3.19to 7.07% (Rendana *et al*., 2018).

When the GML was applied in combination with bio-fertilizer at the level proposed by the current study, the pH of the soil was significantly higher than that without. This was in part due to the action of the bio-fertilizer applied into the soil that had a pH of 7.35. Besides its high pH, the bio-fertilizer contained beneficial microbes (N_2-fixing bacteria) micronutrients, which were responsible for removing Al^{3+} and Fe^{2+} ions from the water of the plots as explained in detail by Panhwar *et al*., (2014a) and Panhwar *et al*., (2014b).

Application of bio-fertilizer would increase soil microbial activities and enrich the population of specific microorganisms that enhance plant growth (Mazolla, 2004). The bio-fertilizer tested in this study contained microbes that fixed some nitrogen from the air. Such being the case, total N in the bio-fertilizer treated plots were significantly higher compared to those without applying it. Nitrogen is a major component of chlorophyll, which rice use to capture sunlight as source of energy to produce carbohydrate. Good root development increases the translocation of carbohydrates from their sources to the growing points that enhances rice growth and eventually its production.

The grain yield of rice in the untreated plot was low because the plants were severely affected by Al^{3+} (0.89%) and/or Fe^{2+} (211 mg kg ^{1}) toxicity. For all we know, Al^{3+} toxicity inhibits root elongation, while Fe^{2+} toxicity forms a coating area on the root surface, preventing further nutrient uptake. The phenomena disrupted the rice plants from taking available nutrients from the soil solution. This explains why rice plants in the plots treated with the amendments contained higher nutrients in their tissues compared to those without application.

Furthermore, bio-fertilizer applied into the soil can inactivate some Al^{3+} by organic acids present in it via chelation process (Shamshuddin *et al*., 2014; Panhwar *et al*., 2023). In this process, the organic residual products of the bio-fertilizer decomposition would be bound to the hydroxyl-Al, forming hydroxyl-Al-OM which is non-toxic (Shamshuddin, 2014). Likewise, Fe^{2+} can be deactivated via the mechanism. When this happens, the Al and Fe activities in the soil solution can be reduced significantly, that would be translated into a better rice growth than otherwise is. When available Al and Fe decreases, the formation of insoluble Fe-Al-phosphate in the soil can be minimized, making P more available for rice uptake via its roots (Bolan *et al*., 1994; Straom *et al*., 2002), with the benefit reflected by the increased rice yield.

Conclusion

A field trial was conducted on acid sulfate soil to determine the effects of applying ground magnesium limestone with or without bio-fertilizer addition on changes in soil chemical properties as well as the growth and yield of rice. The initial soil pH was 3.78, while the exchangeable Al and extractable Fe were 2.82 $cmol_c\ kg^{-1}$ and 211.01 mg kg^{-1}, respectively. The condition of the soil was unsuitable to sustain production. Scanning electron microscopic investigations showed that the presence of high Al^{3+} and Fe^{2+} concentration in the untreated soil caused injury to the rice roots. Applying ground magnesium limestone at 2 t ha^{-1} in combination with 0.25 t bio-fertilizer ha^{-1} increased soil pH from 3.78 (control plot) to 5.25 (treated plot). The soil pH increase resulted in the reduction of Al^{3+} and Fe^{2+} concentration to the minimal level that eventually increased rice grain yield from 2.12 (control plot) to 3.99 t ha^{-1} (treated plot). The significant rice yield increase was due to the enhancement of the soil fertility (addition of Ca and Mg as well as the elimination of Al^{3+} and/or Fe^{2+} toxicities), resulting from ground magnesium limestone applied together with bio-fertilizer, which was fortified with N_2-fixing microbes and micronutrients. The newly introduced soil and agronomic management is considered to be among the best, sound or sustainable agro-tech for rice production on acid sulfate soils in the tropics. Thus, it is not only good for rice cultivation on acid sulfate soils in Malaysia, but also suitable agronomic option for the same soil type which are very widespread in the Southeast Asian region such as Thailand (Bangkok Plains), Vietnam (Mekong Delta) and Indonesia (South Kalimantan).

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