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Effect of phosphogypsum amendment on soil physico-chemical properties, microbial load and enzyme activities

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Abstract

Phosphogypsum (PG) is produced as a solid waste from phosphatic fertilizer plants. The waste slurry is disposed off in settling ponds or in heaps .This solid waste is now increasingly being used as a calcium supplement in agriculture. This study reports the effect of PG amendment on soil physico chemical properties, bacterial and fungal count and activities of soil enzymes such as invertase, cellulase and amylase over an incubation period of 28 days. The highest mean percent carbon loss (55.98%) was recorded in 15% PG amended soil followed by (55.28%) in 10% PG amended soil and the minimum (1.68%) in control soil. The highest number of bacterial colonies (47.4 CFU g^{-1} soil), fungal count (17.8 CFU g^{-1} soil), highest amylase activity (38.4 µg g^{-1} soil hr^{-1}) and cellulase activity (38.37 µg g^{-1} soil hr^{-1}) were recorded in 10% amended soil . Statistically significant difference ($p<0.05$) has been recorded in the activities of amylase and cellulase over the period of incubation irrespective of amendments. Considering the bacterial and fungal growth and the activities of the three soil enzymes in the control and amended sets, it appears that 10% PG amendment is optimal for microbial growth and soil enzyme activities.

Key words

Phosphogypsum, Soil bacteria, Fungi, Soil enzyme

Introduction

Phosphogypsum (PG) is the major solid waste generated from phosphate fertilizer plants, at an approximate rate of 5 tons per ton of phosphoric acid produced. The waste is often disposed off as slurry to storage or settling pond or waste heap. Paradeep Phosphates Ltd. (PPL), a premier phosphatic fertilizer plant located near the coastal port city of Paradeep in the state of Orissa produces considerable quantity (2,700 tons d^{-1}) of phosphogypsum which is disposed off near the factory, thus posing the risk of soil and water contamination. For last few years, phosphogypsum is being used in agricultural soil as a calcium supplement to enhance crop production. Mullino and Mitchell (1990) have reported use of PG to increase yield and quality of forages in Florida, USA. Application of PG in agriculture and its radiological impact were studied (Papastefanou *et al.,* 2006). Soratto and Crusciol (2008) studied the effects of dolomite and PG surface applications on annual crops nutrition and yield. Bianeo *et al.* (2008) reported the effects of PG and potassium chloride on the nutritional status and production and orangoleptical quality of pine apple fruits. However, the chemical and biological response of soil to the waste amendment needs to be thoroughly investigated before recommending its large scale field application.

The microbial population in soil play significant role in organic matter decomposition and maintenance of soil nutrients. Soil microbial biomass and microbial activity have been proposed as indicators of soil maturity (Anderson and Domsch, 1989; Machulla *et al.,* 2005). Further, the biochemical functions in the soil are catalysed by soil enzymes (Burns, 1983; Sinsabaugh *et al.,* 1991).The measurement of soil enzymatic activities forms a part of the general biological study of soil and gives a comparative assessment of specific biochemical processes (Mishra *et al.*, 1979; Behera and Mishra, 1989).

The effect of PG on green house gas emissions during decomposition in soil have been reported (Hao *et al.,* 2005).Lee *et al.* (2009) observed a positive impact of alkalized PG amendment on some chemical and biological properties of soil and yield of cabbage in China. Respiration in wetland soil was affected by PG applications (Delaune *et al.,* 2006). Mercury immobilization by PG application along with sulphate reducing bacteria has been reported by Adams *et al.* (2007).

The objectives of this study were to assess the effect of phosphogypsum amendment on some major soil physicochemical properties, bacterial and fungal population and activities of some important soil enzymes, namely invertase, amylase and cellulase.

Materials and Methods

The phosphogypsum (PG) slurry was collected from the heaps near the Phosphatic fertilizer plant (PPL), in Paradeep, Orissa. The slurry was sun dried and powdered for soil amendment. The soil was collected from an agricultural land without vegetation, sieved and amended with 5, 10, 15 and 20% PG. The experiment was conducted in laboratory of Department of Zoology and Biotechnology, OUAT, Bhubaneshwar during (July-August 2009). 2 kg of amended soil of each percentage was taken in polythene bags in triplicates for incubation. A control set without amendment with equal amount of soil was taken in triplicate. The control and experimental sets were incubated for 28 days at room temperature of 25 ± 2°C and controlled moisture at 20%. The soil physicochemical parameters such as pH, organic carbon, nitrogen, phosphorous, potassium and microbiological parameters such as total number of bacterial and fungal colonies along with activities of three soil enzymes, invertase, amylase and cellulase were studied at 7 days interval for a period of 28 days. Soil pH was measured by digital pH meter. Soil organic carbon was assessed as per the methods prescribed by Walkley and Black (1934). Potassium was measured by flamephotometer (Jackson, 1973). The method of Olsen *et al.* (1954) was used to measure phosphorous. Nitrogen was quantified by acid digestion method (Hach *et al.,* 1985). Dilution plate count technique was followed using nutrient agar and potato dextrose agar respectively for bacterial and fungal count (Parkinson *et al.,* 1971). Soil enzyme activities were determined as per the methods prescribed by Ross and Robert (1970). Amylase, invertase and cellulase activities were determined by using 3, 5 di-nitrosalicylic acid. Soil samples were incubated with substrates (starch for amylase, sucrose for invertase and carboxymethyl cellulose for cellulase) and Sorensen's buffer at 35°C for 24 hr and then centrifuged. A suitable aliquot of the supernatant was heated with 3, 5 di-nitrosalicylic acid and the colour was measured at 540 nm in a UV visible spectrophotometer (ELICO). The enzyme activities were recorded in µg glucose g^{-1} soil. Analysis of variance (ANOVA) test of the data was carried out by using M Stat C software (Michigan State University, USA). Values are presented as mean±standard deviation (SD).

Results and Discussion

Percent organic carbon declined in all the treatments up to 28th day of incubation. The highest mean percent carbon loss (55.96 and 55.28%) was recorded in 15 and 10% PG amended soil and the minimum percent carbon loss (1.6%) in control soil (Fig. 1). No significant variation in the level of pH, nitrogen, phosphorous and potassium were observed during the incubation. Lee *et al.* (2009) have reported decrease in soil pH with increasing application rate of unmodified PG. They have further noticed enhanced levels of available P, SO_4, exchangeable K,Ca and Mg with increased rate of PG application. Chung *et al.* (2001) observed that PG treatment of 2.5 and 5.0 g kg^{-1} soil lowered the pH by 0.7-0.8 units. Similar results have also been obtained by Jarak *et al.* (2003) after PG application in arenosol, a soil type with low fertility. Our results indicated that the soil pH progressively declined from 7.9 to 5.1 (Table 1) with increasing percent PG amendments which is in agreement with these previous results. However we contradict the reports of Smith *et al.* (1994) that there was no change in the pH of PG amended surface soil.

Our results also do not corroborate earlier reports by Lee *et al.* (2009) that levels of phosphorus (P) and potassium (K) increased with increasing dose of PG in soil.

The bacterial and fungal count exhibited an increasing trend from the 1st to 21st day and there after declined, irrespective of the treatments (Fig. 2 A and B). The maximum bacterial colonies were observed in 10% amendment (47.4 CFU g^{-1} soil) and the minimum in 20% amendment (39.6 CFU g^{-1} soil) on the 21st day. The fungal count too increased in all the treatments up to the 21st day and declined on the 28th day of observation.10% amended soil had the highest fungal count (17.8 CFU g^{-1} soil) with the least count in 20% (11.2 CFU g^{-1} soil). Lee *et al.* (2009) have reported significantly higher microbial activity with optimal application of alkalized PG in agricultural soil. Our results concur this earlier observation that PG amendment of 10% seems to be optimal for maximum microbial growth. Microbial growth and

Table - 1: Values of soil physicochemical parameters after phosphgypsum (PG) amendments at different intervals.

Physicochemical parameters	Control	5%	10%	15%	20%
		DAY 1			
pH	7.9±1.6	7.5±1.3	6.7±1.7	6.2±1.2	5.3±1.3
Organic carbon (Gm %)	1.19±0.55	1.18±0.63	1.23±0.23	1.09±0.77	0.87±0.25
Nitrogen (Gm %)	0.15±0.02	0.12±0.01	0.15±0.03	0.11±0.02	0.08±0.02
Phosphorous (%)	0.005± 0.0001	0.005±0.0001	0.004±0.0003	0.003±0.0001	0.002±0.0002
Potassium (%)	0.02±0.01	0.01±0.01	0.014±0.01	0.012±0.01	0.01±0.01
		DAY 7			
pH	8.1±1.7	7.3±1.5	6.1±1.5	5.9±1,8	5.1±1.7
Organic carbon (Gm %)	1.18±0.9	1.12±0.8	1.20±0.4	0.9±0.3	0.75±0.3
Nitrogen (Gm %)	0.18±0.03	0.13±0.06	0.13±0.04	0.09±0.01	0.07±0.02
Phosphorous (%)	0.003±0.0001	0.002±0.0001	0.002±0.0001	0.002±0.0001	0.002±0.0002
Potassium (%)	0.02±0.01	0.02±0.01	0.03±0.01	0.02±0.01	0.02±0.02
		DAY 14			
pH	7.8±1.6	7.1±1.8	6.1±1.8	5.8±1.4	5.2±1.6
Organic carbon (Gm %)	1.18±0.45	0.92±0.33	0.87±0.23	0.55±0.13	0.51±0.23
Nitrogen (Gm %)	0.16±0.02	0.09±0.03	0.08±0.01	0.05±0.01	0.05±0.02
Phosphorous (%)	0.003±0.0001	0.003±0.0001	0.002±0.0001	0.002±0.0001	0.002±0.0001
Potassium (%)	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01
		DAY 21			
pH	7.4±1.6	7.2±1.4	6.2±1.3	5.4±1.6	5.2±1.3
Organic carbon (Gm %)	1.17±0.23	0.81±0.33	0.61±0.21	0.53±0.12	0.46±0.15
Nitrogen (Gm %)	0.11±0.02	0.08±0.04	0.06±0.04	0.05±0.02	0.04±0.02
Phosphorous (%)	0.003±0.0002	0.003±0.0001	0.002±0.0001	0.017±0.0001	0.014±0.0002
Potassium (%)	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.02
		DAY 28			
pH	7.3±1.3	7.2±1.6	6.1±1.3	5.3±1.4	5.1±1.3
Organic carbon (Gm %)	1.17±0.33	0.79±0.24	0.55±0.19	0.48±0.33	0.43±0.32
Nitrogen (Gm %)	0.12±0.02	0.07±0.02	0.05±0.01	0.04±0.02	0.04±0.01
Phosphorous (%)	0.003±0.0001	0.004±0.0002	0.006±0.0001	0.003±0.0001	0.003±0.0001
Potassium (%)	0.02±0.01	0.02±0.01	0.01±0.01	0.01±0.01	0.01±0.01

activities are invariably associated with utilization of organic carbon (Khan *et al*., 1999). In our study the high percent carbon loss recorded in 10 and 15% PG amended soil coincide with high bacterial and fungal counts.

Activities of all the three soil enzymes irrespective of treatments increased with days of incubation (Fig. 3A,B and C). The maximum activity has been recorded on the 21st day and minimum on the 28th day. The maximum amylase activity (38.4 µg g^{-1} soil hr^{-1}) was observed in 10% amended soil on the 21st day and the minimum (5.08 µg g^{-1} soil hr^{-1}) in control soil. ANOVA test indicates a significant difference in the enzyme activity ($p<0.05$, $F=6.65$) between days of incubation. The activity of amylase varied

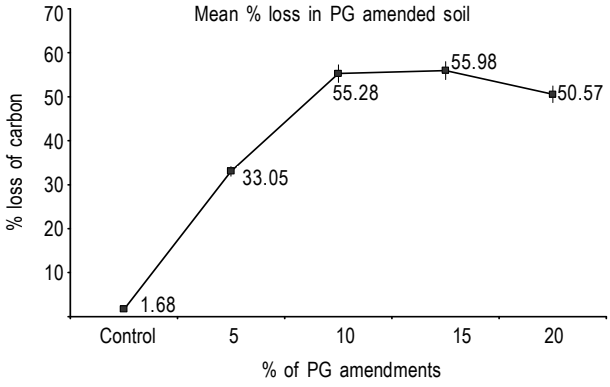

Fig. 1: Mean % carbon loss in phosphogypsum amended soil.

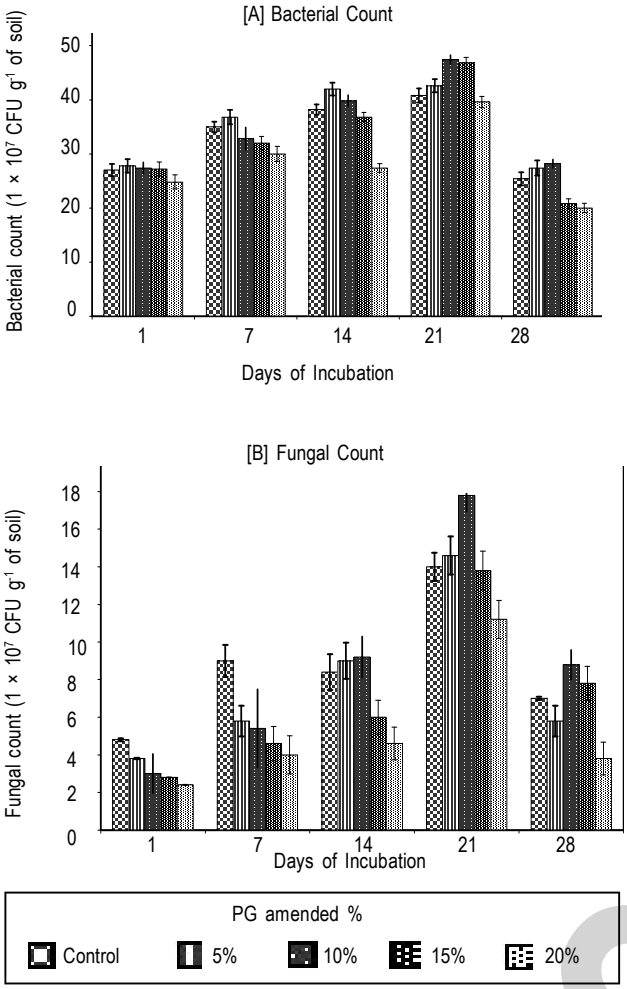

Fig. 2: Bacterial (A) and fungal (B) counts in phosphogypsum amended soul at different days of incubation

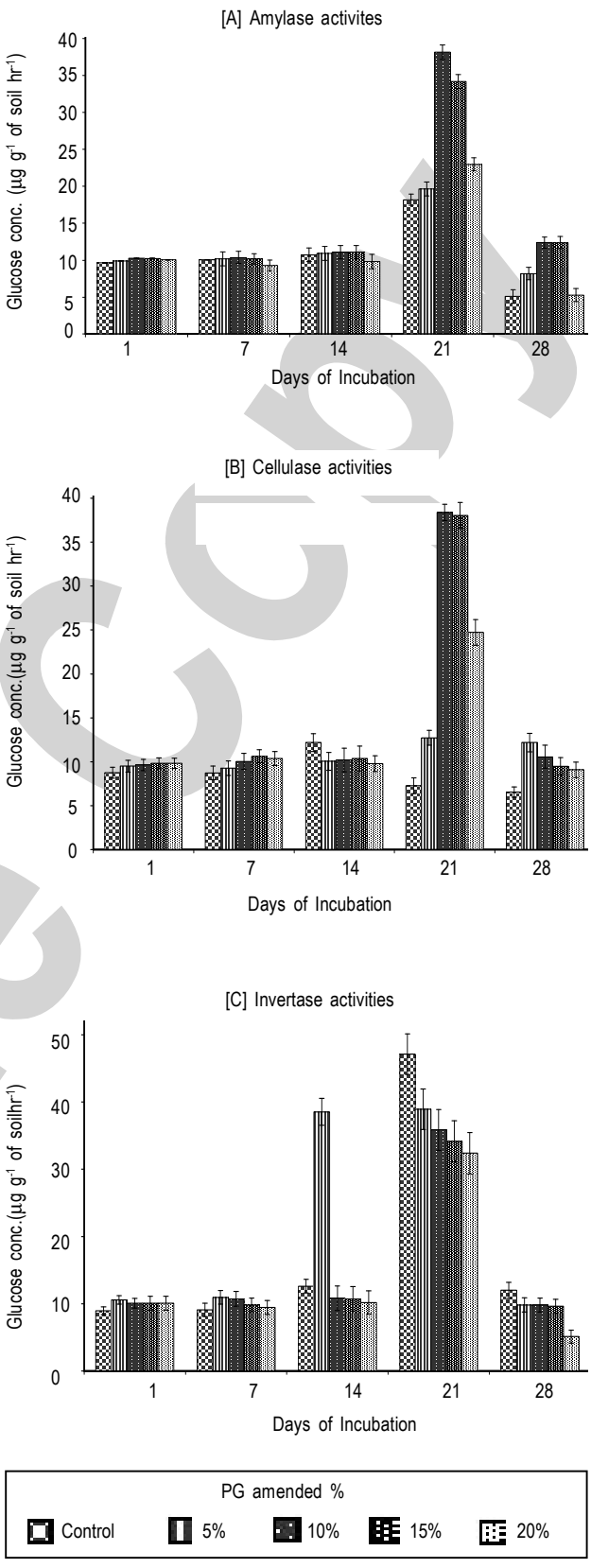

Fig. 3: Activity of (A) amylase, (B) cellulase and (C) invertase in phosphogypsum amended soil at different days of incubation

in the samples of the same soil collected at different periods and has a direct correlation with soil micro-flora (Kuprevich and Shcherbakova, 1971; Mishra *et al.*, 1979). In the present study the maximum numbers of bacterial and fungal colonies were observed on the 21st day coinciding with the maximum amylase activity, thus corroborating earlier findings.

Invertase activity showed the maximum value (47.09 µg g^{-1} soil hr^{-1}) on 21st day in control soil and minimum activity (5.12 µg g^{-1} soil hr^{-1}) was recorded in 20% amended soil on the 28th day. The difference in activity of invertase over the days of incubation was not statistically significant ($p<0.05$). Mishra *et al.* (1979) recorded very high invertase activity of 1437 µg g^{-1} soil hr^{-1} in pasture soil with adequate vegetation. The present soil samples were devoid of vegetation and in general indicated lower invertase activity irrespective of amendments. Cellulase activity showed its maximum value (38.37 µg g^{-1} soil hr^{-1}) on the 21st day in 10% amended soil and the minimum (6.54 µg g^{-1} soil hr^{-1}) in the control on the 28th day of incubation. The differences of cellulase activity over 28 days of

incubation was statistically significant (F=14.8, p<0.05). Cellulase acts on comparatively resistant organic matter i.e. cellulose and is mostly produced by bacteria and fungi. The high and low cellulase activity in 10% amended soil and control soil respectively is likely due to differential microbial activity. Previous studies (Saratto and Crusciol, 2008) have demonstrated that alkalized PG could nutrilize soil activity, enhance the levels of available sulphur (S) and phosphorous (P) and increase the yield of rice grain and bean.

A long term field experiment will help in successful utilization of PG in agriculture.

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References

Adams, E., A.G. Sanchez, S.E. Velazquez and A. Melendez: Phosphogypsum phosphate industry mercury sulphate reducing bacteria bioremediation. 1st Int. meeting on microbial phosphate solubilization/development in plant and soil sc.pp 121-134 (2007).

Anderson, T.H. and K.H. Domch: Ratios of microbial biomass carbon to total organic carbon in arable soils. *Soil Biol. Biochem*., **21**, 471-479 (1989).

Behera, P.K. and C.S.K. Mishra: Effect of paper mill wastewater and sludge amendments on enzyme activities in soil, Chapter 9 in "Soil pollution and Soil organisms" Ed. Mishra, Ashis Publ. House, New Delhi. pp. 139-147 (1989).

Bianeo, S., G. Ruggiero, G.C. Vitti and P.R. Santus: Effects of phosphogypsum and potassium chloride on the nutritional status, production and orangoleptical quality of pineapple fruits. Proc. 3rd Int. Symp. On phosphogypsum. Orlando, Florida, FIPR. Publ. pp. 348-361 (2008).

Burns, R.G.: Extracellular enzyme-substrate interactions in soil. *In*: Microbes in their natural environment (*Eds*.: J.H. Slater, R. Wittenbury and J.W.T. Wimpenny). Cambridge University Press, London. pp. 249-298 (1983).

Chung, Jong-Bae., Kang, Sun-chul and Park Shin: Short term effect of phosphogypsum on soil chemical properties. *J. Environ. Agri*., **20**, 317-324, (2001).

Delaune, R.D., J.D. Porthouse and W.H. Patrick: Effect of phosphogypsum on respiration and methane emissions in sediment. *Environ. Tech*., **27**, 575-584 (2006).

Hach, C.C., B.K. Bowden, A.B. Kopelove and S.V. Brayton: More powerful peroxide kjeldahl digestion method. *J. Assoc. off. Anal. Chem*., **70**, 783-787 (1985).

Hao Xiying, F.J. Lorney, Chang Chi, G.R. Travis, C.K. Nichol and E. Bremor: The effect of PG on green house gas emissions during cattle manure composting. *J. Environ. Qual.*, **34**, 774-781 (2005).

Jackson, M.L.: Soil chemical analysis. Prentice Hall of India, 1qq New Delhi. PP. 365 (1973).

Jarak, M., M. Belic, M. Govendarea, N. Milosevic, and S. Djuric: Effect of phosphogypsum and peat on microbiological and chemical properties of arenosol. *Acta Biologica Yugoslavica Senija* A. **52**, 1-6, (2003).

Khan, K.M., K., Rehman, U., Rafiq and H.S., Ghani: Comparative enzymatic studies on soil under cullivation on wheat and sugarcane. Int. *J. Agri. Biol.*, **1**, 132-134, (1999).

Kuprevich, V.F. and Shcherbakova: Soil enzymes. Publ. INSDOC, New Delhi (1971).

Lee Chang Hoon, B.Y. Ha, Y.B. Lee and P.J. Kim: Effect of alkalized PG on soil chemical and biological properties. *Comm. Soil Sci. Plant Anal.*, **40**, 2072-2086 (2009).

Machulla, G., M.A. Bruns and K.M. Scow: Microbial properties of mine spoil materials in the initial stages of soil development. *Soil Sci. Soc. Am. J.*, **69**, 1069-1077 (2005).

Mishra, P.C., R.K. Mohanty and M.C. Dash: Enzyme activity in subtropical surface soils under pasture. *Ind. J. Agric. Chem.,* **12**, 19-24 (1979).

Mullino, G.L. and C.C. Mitchell: Use of phosphogypsum to increase yield and quality of annual forages. Publ. of Florida Inst. Of Phosph. Research, Bartoe, Florida (1990).

Olsen, S.R., C.V. Cole, F.S. Watanabe and L.A. Dean: Estimation of phosphorous in soil by extracting with sodium bicarbonate USDA circular 939, Washington (1954).

Papastefanou, C., S. Stoulos, A. Loannidous and M. Monolopoulou: The application of phosphogypsum in agriculture and the radiological impact. *J. Environ. Radioact*., **89**, 188-198 (2006).

Parkinson, D., Gray, T.R.G. and S.T., William: Methods to study ecology of soil microorganisms. IBP Handbook No. 19, Blackwell Scientific Publ. oxford. PP. 116 (1971).

Ross, D.J. and H.S. Robert: Enzyme activities and oxygen uptake of soil under pasture in temperature and rainfall sequence. *J. Soil Sci*., **21**, 368-381 (1970).

Sinsabaugh, R.L., R.K. Antibus and A.E. Linkins: An enzymic approach to the analysis of microbial activity during plant litter decomposition. *Agric. Ecosys. Environ*., 34: 43-54 (1991).

Smith, C.J., M.B. Peoples, G. Kirthisinghe, T.R. James, D.J. Garden, and S.S. Tuomi: Effect of surface application of lime, gypsum and phosphogypsum on the alleviating of surface and sub surface acidity in soil under pasture. *Australian J. Soil Res*. 32, 995-1008, (1994).

Soratto, R.P. and C.A.C., Crusciol:Dolomite and phosphogypsum surface application of effects on annual crops nutrition and yield. *Agronomy J.*, **100**, 261-271 (2008).

Walkley, A. and I.A. Black: An examination of the Degtjareff method for determining organic carbon in soils: Effect of variations in digestion conditions and of inorganic soil constituents. *Soil Sci.*, **63**, 251-263 (1934).