Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research

ISSN No: 0975-7384
CODEN(USA): JCPRC5

J. Chem. Pharm. Res., 2011, 3(5):565-573

Sequential Extraction of Common Metals (Na, K, Ca and Mg) From Surface Soil

***Bhupen K Baruah; Bhanita Das[1]; Ansarul Haque ; Chitrani Medhi; Abani K Misra**

Department of Chemistry, Gauhati University, Guwahati, Assam, India
[1]Department of Statistics, Gauhati University, Guwahati, Assam, India

---

ABSTRACT

Soil contamination with metals is now a world wide environmental concern. Bio-accumulation of metals by plants and so into food chains imbalances the ecosystem and causes human health problem. Toxicity of an element in soil depends only on its mobility, transformation and potential bioavailability to plants. Water soluble, exchangeable and mild acid soluble fractions of metals are most mobile and bioavailable form present in soil. Sequential extraction of metals from soil by the use of different extractants provides information not only its bioavailability but also the amount bound to different soil fractions. A study was carried out to investigate the fraction distribution of common metals Na, K, Mg and Ca and the influence of extraction time and volume of different extractants on surface soils collected from tea garden belts of Golaghat district of Assam, India. The modified three step sequential extraction procedure was applied for speciation of these elements. The extractant solutions were deionized water, 1M ammonium acetate solution and 25 g/L acetic acid solution. Extraction was first performed for 5 gm soil samples with 30 ml extractant eluate and the soil extract were collected after 10, 15, 20, 60 and 120 minutes for analysis. Soil extracts were also collected after a fixed extraction time of one hour using 10, 30, 50 and 80 ml of different extracting eluate. The study revealed that extraction time more than one hour have no role in speciation of metals from soil and the soil solution ratio greater than 1:6 had no influence on sequential extraction. The amount of Na, K, Mg and Ca in different soil fraction was found to be highest in exchangeable fraction followed by water soluble and acetic acid soluble. A distinct difference was also seen in the estimated values of metal contents using HNO_3:$HClO_4$ digestion method and modified three steps sequential extraction method which can be attributed due to medium organic matter and high bulk density of soil.

Keywords: Bioavailability, sequential extraction, exchangeable fraction.

INTRODUCTION

Soil quality performs important role in health of human, animals and plants. The quality of soil within a region is governed by both natural processes such as rainfall, the underlying geology, weathering processes, the vegetation or organic matter decay, soil erosion and anthropogenic effects [1, 2]. The increasing anthropogenic activities have caused concern about the possibility of releasing hazardous chemicals and metals into the soils. Soil contamination with metals is a very sensitive issue world wide. Concentration of metals associated to chemical compounds is important to evaluate the environmental risks on soil particularly on their bioavailability. Bio-accumulation of metals by plants and so into food chains imbalances the ecosystem and causes human health problems [3]. Total metal content estimation is a very poor indicator to assess the extent of contamination for a particular metal in soil. It is also insufficient to assess the environmental behavior of contaminated soil [4]. Toxicity of a metal in soil depends not only on its mobility, transformation and potential bioavailability to plants but also on its specific forms or binding state [5, 6].. The factors controlling the bioavailability of metals in soil are of great importance for human toxicology and agricultural productivity [7]. Plant uptake as well as bioavailability of metals significantly related with soil conditions.

Successful crop production demands the optimum use of plant nutrients in addition to other management practices in soil. Although Na, K, Mg and Ca are not equally important metal for plant nutrition but all are necessary for plant growth and they are mainly responsible for maintaining acid-base equilibrium in soil skeleton. Furthermore, Ca improves K/Na selectivity of membranes and prevents the soil from invasion from toxic ions [8]. High Na concentration in soil interferes with the adsorption and translocation of K and Ca by plants [9]. The relative high Na/Ca ratio also increases pH which decreases the soil permeability to water and can also disturbed the availability of plant nutrients [10]. On the other hand bio-available form of Ca, K and Mg decreases with the increase of salinity and sodium adsorption ratio (SAR) of irrigation water or soil solution.

The higher amount of Na in soil plays an antagonistic role to the uptake of Ca, K and Mg in plants [11]. Excess Na could damage the structure of natural soil to the point that air and water infiltration are prevented, and root growth is also restricted. As the size of Na^+ is smaller, rate of adsorption of Na on soil surfaces and substitution of adsorption sites in soil by other cations (Ca and Mg) increases, hence soil become hard and compact results impermeable to water penetration. Moreover, HCO_3^- ions has higher tendency towards Ca and Mg to precipitate as $Ca(HCO_3)_2$ or $Mg(HCO_3)_2$ results in low soil porosity and clogging air and water movement through soil [9]. Acidity undoubtedly induces the mobility of K and Na in soils. Soil acidification and deficiency of available Na, K, Mg and Ca in the soil can affect nutrient uptake and root growth which can inhibit productivity [12, 13].

Long-term cultivation of tea (C*amellia sinensis var assamica*) using artificial agrochemicals and pesticides has resulted in soil quality degradation as well as poor quality tea production in Assam. Paddy fields around the tea gardens were subjected to wastewater irrigation disposal hence severely contaminated with chemical hazards particularly metals, and their long-term cumulative health effects are now become environmental concern. Concentrations of metals in soil can also be elevated due to high inputs from natural as well as anthropogenic sources. Injudicious use of chemical fertilizers increases the metal load in soil in tea garden belts as well as in surrounding paddy fields [14]. Therefore, understanding of transport and distribution of major cations in soil of the study area was important.

Sequential extraction procedure was developed to speed up, facilitate and improve the accuracy of the chemical fractionation of metals into several groups of different leach ability in soils which determines the distribution of metals in different phases [15]. The procedure is based on the principle that metals bounded to soil skeleton can be displaced from the geochemical phases by the use of appropriate chemical reagents [16]. Sequential extraction of metals from soil by the use of different extractants provides detail information about the mode of occurrence, origin, potential mobility and amount bound to different soil fractions and transport of the metals in natural environments. These information are highly worthy for the evaluation of potential risk of metal contamination and the feasibility of its remediation [17].

Many sequential extraction procedures for heavy metals are described in literature; nevertheless the method developed by Tessier *et al*. (1979) [18] is the most widely used and best known sequential extraction procedure at present. This procedure has reported satisfactory results in the distribution of metals in different phases owing to a careful selection of reagents in each steps and detail operating conditions. It was originally developed for sediment analysis modified for soil analysis later on [16]. Although this procedure is in general tedious and time consuming and also suffer from a number of limitations, such as the problem of achieving selective dissolution, readsorption and dependence of readsorption on the phase composition, poor selectivity of metals during the extraction process and the dependency of results on operating conditions, it is a widely used, accepted tool for metal fractionation [15]. To prevent various tiresome, a selective extraction procedure was developed by [19].to study the metal partitioning on contaminated forest soils and accuracy, efficiency and reproducibility were reported highly satisfactory. In all previous studies, a multi-element extraction method for heavy metals has been proposed no method for fractionation of Na, K, Ca and Mg from soil has developed. It was well reported that bioavailable (water soluble and exchangeable) form of Na, K, Ca and Mg is only a minor fraction compared to the total soil metal reserve. These metal containing minerals vary with the source of parent material and the degree of weathering. Moreover, release of these metals from the non-exchangeable pool into bioavailable pool depends upon particle size distribution, plant species, biological activity and soil pH [11].

The objective of our work was to apply modified three steps sequential extraction procedure for speciation of major elements Na, K, Mg and Ca for the assessment of bioavailability and also to determine the elements bound to different soil fractions. The three step sequential extraction scheme was developed to evaluate the Na, K, Mg and Ca metal content in three fractions viz., water soluble, exchangeable and acetic acid soluble by analyzing in soil samples collected from tea garden belts of Golaghat district of Assam, India. The influence of extraction time and volume of different extractants on fractionation of metals from soil was also investigated in this study.

EXPERIMENTAL SECTION

Soil Sampling

Soil samples were collected from the paddy fields around the tea garden belts. The paddy fields around the tea garden belts in the present study were subjected to irrigation wastes disposal from the tea gardens, wastes from municipality and domestic sources. This possesses a health risk on local communities, growing food crops and vegetables in these paddy fields. A composite surface soil samples (8-10 samples per site from 0-15 cm depth) were collected from six sampling sites (four from contaminated and one from non-contaminated sites) of tea garden belt areas of Golaghat district of Assam during November, 2009 to March, 2010. The contaminated surface soils were collected from Negheriting TE east and west and Balijan TE north and south directions. One representative soil sample from paddy fields which was free from waste water and irrigation waste disposal also collected as controlled. Samples were properly packed in labeled bags and brought to the laboratory for analysis. Soil samples were air-dried, gently crushed and passed through a 2 mm pore size sieve, homogenized and properly stored in plastic containers until analysis.

Soil Analysis

Physico-chemical analyses of soil samples were done using analytical grade reagents. Soil pH was measured in a 1:2 soil / deionized water suspension after recirocral shaking for 30 minutes by digital pH meter (Eutech-356C). Electrical Conductivity (EC) of soil was measured using digital conductivity meter (ATC-975-C) [20]. Soil samples were analysed for soil organic matter (SOM%) using wet oxidation method [21]. After completion of mechanical analysis soil textural classification was done using the chart prescribed by [22]. Bulk density of soil was measured by standard procedure described by [23]. Total concentration of Ca and Mg in the soils was determined by EDTA titrimetric method and that of Na and K by flame photometry, after digestion with HNO_3:$HClO_4$ (3:1) acid mixture.

Sequential Extraction Procedure

Sequential extraction method is widely used method to study the forms, availability, mobility and transformation of metals in soils in which quantification of different fraction of metals in soil samples was done starting with the weakest and least aggressive chemical reagents to strongest to most aggressive chemicals at the end [6, 17]. In this method soil samples were leached for speciation of Na, K, Mg and Ca using a modified procedure (Table 1) obtained from methods described by [16, 17, 18, 19, 24].

For speciation of water soluble fraction a mixture of 5 gm of soil sample with 25 ml deionized water was placed in a 100 ml centrifuge tube and centrifuged at 4000 rpm for 30 minutes. Filtered the mixture (using Whatman No. 42) and finally residue was rinsed with 25 ml deionized water for complete speciation of the metals followed by shaking, centrifugation and filtration. After completion of the separation 25 ml of extracting reagents 1M CH_3COONH_4 (pH 7) was added to the same soil residue and centrifuged for 2 hours at 4000 rpm. Supernatant was collected by filtration and finally 25 ml of extracting reagent was added for complete speciation of the metals. After complete speciation of metals 25 ml of 25 gm/L CH_3COOH (Buffered to pH 2.6 with CH_3COONa) were added followed by 2 hour shaking and extractant was collected again as described as above.

Table 1. Modified procedure for extraction of Na, K, Ca and Mg from soil

Steps	Fractions	Reagents	Soil: Reagent	Conditions
I	Water soluble	Deionized water	1:5	20 ± 1^0C, 30 min shaking
II	Exchangeable	1M CH_3COONH_4 (pH 7)	1:5	20 ± 1^0C, 2 h shaking
III	Weak acid soluble	25 gm/L CH_3COOH (pH 2.6)	1:5	20 ± 1^0C, 2 h shaking

To study the effect of extraction time same procedure was applied for each extracting reagents except the extracting reagents were collected at 10, 15, 20, 60 and 120 minutes of centrifugation. To study the effects of eluate volume, 5 gm of soil samples with different volume of extracting reagents of 10, 30, 50 and 80 ml had placed in a 100 ml centrifuge tube and centrifuged at 4000 rpm; supernatants were collected after a fixed extraction time of one hour.

All extractions were performed in triplicate and the mean concentration of metals in extraction solutions was expressed. Total metal concentrations after sequential extraction was calculated as follows,

$$\text{Total bioavailable metal content} = \sum_{i=1}^{3}[M_i] = \text{Step I} + \text{Step II} + \text{Step III}.$$

RESULTS

Soil physico-chemical properties of soil samples determined in the present study are presented in Table 2. The pH values of soil suspension (1:2) of all representative soils were mostly acidic in reaction and contaminated soils were found to be less acidic in reaction compare with the non contaminated soils. The pH of contaminated and non-contaminated soils was found in the range of 4.98-5.80 and 5.88-5.99 respectively.

Table 2. Physico-chemical characteristics of soil samples

Sampling Site	pH	EC (µScm^{-1})	SOM (%)	BD (gm/cc)
Negheriting (East)	5.42 ± 0.12	155.12 ± 2.45	5.02 ± 0.41	1.35 ± 0.40
Negheriting (West)	5.80 ± 0.09	164.23 ± 3.12	4.72 ± 0.04	1.67 ± 0.69
Balijan (South)	4.98 ± 0.74	179.29 ± 1.76	2.78 ± 0.16	1.42 ± 0.19
Balijan (North)	5.26 ± 0.19	182.43 ± 2.88	4.67 ± 0.06	1.22 ± 0.66
Controlled	6.10 ± 0.31	176.67 ± 2.09	5.52 ± 0.38	0.97 ± 0.42

EC of both contaminated and control soil samples were found within excellent range of irrigational use. It was found maximum at Balijan (North) and the minimum was recorded at Negheriting (East). Experimental data on soil organic matter (SOM) indicated that contaminated soils were found significantly different from non-contaminated soil sample except Balijan (South) sample. Bulk densities (BD) of all contaminated soil samples were found to be higher than the controlled sample. Maximum SOM and BD were recorded at sample collected from Negheriting (East) (5.02 ± 0.41) and Negheriting (West) (1.67 ± 0.69) respectively.

Na, K, Ca and Mg content in soil samples were analysed in two different ways viz. HNO_3:$HClO_4$ acid digestion method and sequential extraction method. Estimated results of HNO_3:$HClO_4$ acid digestion method is presented in Table 3. Estimated value of Na, K, Ca and Mg in the controlled sample was found to be 1.89, 1.45, 2.24 and 1.86 mg/kg respectively. Estimated values of all the metals in each soil samples were found to be higher than the controlled sample.

Table 3. Estimated Na, K, Ca and Mg content in soil in acid digestion method

Sampling Site	Na (mg/kg)	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)
Negheriting (E)	4.14	2.56	8.77	4.49
Negheriting (W)	4.56	3.29	10.33	5.28
Balijan (S)	4.29	3.11	8.45	4.63
Balijan (N)	3.99	2.09	9.67	4.78
Controlled	5.89	3.45	8.24	5.86

Table 4. Estimated values of Na, K, Ca and Mg in contaminated and controlled sample by sequential extraction and acid digestion method

Metals (mg/kg)	Contaminated soil sample		Controlled sample	
	Sequential extraction	Acid digestion	Sequential extraction	Acid digestion
Na	3.87 ± 0.93	4.56 ± 0.12	5.67 ± 0.08	5.89 ± 0.11
K	2.29 ± 0.11	3.29 ± 0.09	3.15 ± 0.10	3.45 ± 0.21
Ca	9.93 ± 0.85	10.33 ± 0.36	8.01 ± 0.11	8.24 ± 0.32
Mg	5.13 ± 0.14	5.28 ± 0.11	5.73 ± 0.07	5.86 ± 0.17

Soil samples collected from Negheriting (West) site showed maximum concentration of Na, K, Ca and Mg as 4.56, 3.29, 10.33 and 5.28 mg/kg respectively during the study. Therefore, soil samples collected from this site was used for modified sequential extraction method. Na, K, Ca and Mg content in Negheriting (West) site soil samples using modified sequential extraction method were found to be 3.87 ± 0.93, 2.29 ± 0.11, 9.93 ± 0.85 and 5.13 ± 0.14 mg/kg respectively. For controlled sample Na, K, Ca and Mg content were found to be 5.67 ± 0.08, 3.15 ± 0.10, 8.01 ± 0.11 and 5.73 ± 0.07 mg/kg respectively (Table 4).

The difference in Na, K, Ca and Mg content for contaminated soil sample estimated by acid digestion and sequential extraction method were 0.69, 1.00, 0.40 and 0.15 respectively. This difference in metal content for controlled samples was found to be 0.22, 0.30, 0.23 and 0.13 mg/kg. The small difference in metal content in controlled sample revealed that a small fraction of common metals were bounded to other soil fractions hence not available for plant uptake. Sequential extraction analysis results also showed that exchangeable form of Na, K, Ca and Mg was found in predominant in all soil samples during the study followed by water soluble fraction and acetic acid soluble fraction.

To study the effect of extraction time on sequential extraction, experiment was performed for 5 gm soil samples with 1:5 soils to eluate ratio. Study revealed that increasing time of extraction over 1 hour had no influence on extraction of Na and K in sequential extraction steps I (Water soluble fraction) and step III (Weak acid soluble fraction). Trace amount of exchangeable Na (0.04 mg/kg) and K (0.03 mg/kg) were detected up to 120 minutes of extraction time in the step II (Exchangeable fraction) only (Table 5)

Table 5. Influence of sequential extraction time on Na and K extraction

Extraction time	**Metal fractions (mg/kg)**					
	Step I		**Step II**		**Step III**	
	Na	**K**	**Na**	**K**	**Na**	**K**
10 Min	0.08	0.07	0.08	0.04	0.03	0.05
15 Min	0.14	0.16	0.21	0.23	0.16	0.08
20 Min	0.33	0.65	1.17	0.55	0.26	0.00
60 Min	0.49	0.13	0.65	0.30	0.23	0.00
120 Min	0.00	0.00	0.04	0.03	0.00	0.00
Total	1.04	1.01	2.15	1.15	0.68	0.13

Table 6. Influence of sequential extraction time on Ca and Mg extraction

Extraction time	**Metal fractions (mg/kg)**					
	Step I		**Step II**		**Step III**	
	Ca	**Mg**	**Ca**	**Mg**	**Ca**	**Mg**
10 Min	0.87	0.51	1.09	0.39	0.17	0.00
15 Min	0.33	0.43	0.79	0.52	0.29	0.19
20 Min	1.09	0.88	1.95	0.74	1.74	0.31
60 Min	0.98	0.29	0.48	0.69	0.15	0.18
120 Min	0.00	0.00	0.00	0.00	0.00	0.00
Total	3.27	2.11	4.31	2.34	2.35	0.68

The results obtained for Ca and Mg extraction stated that extraction time up to 60 minutes was obligatory for complete speciation of metals for each sequential extraction steps (Table 6).

To study the effect of eluate volume on metal speciation, investigations were performed for 5 gm soil mixed with different volume of eluate volume ratio at a fixed extraction time one hour for each step (Table 7). The study revealed that eluate volume 50 ml and 80 ml (soil solution ratio more than 1:10) were found excess and no role in the speciation of metals from soil in each sequential step. Furthermore all metal content were found in below detection limit in acid extraction step with 10 ml of eluate volume. The Mg content in 10 ml of eluate was found higher compare with 30 ml eluate volume in water soluble and exchangeable fractions (Table 8). There was a large variability in metal extraction depending on extractant, metal type and nature of the soil. Weak extractants (deionized water and 1M CH_3COONH_4) showed larger variability among soil types with respect to metal content than did by CH_3COOH, and hence they might be used to assess metals bioavailability.

Table 7. Influence of eluate volume on Na and K extraction

Eluate volume	Metal fractions (mg/kg)					
	Step I		Step II		Step III	
	Na	K	Na	K	Na	K
10 ml	0.19	0.16	0.39	0.21	0.00	0.00
30 ml	0.85	0.85	1.76	0.94	0.68	0.13
50 ml	0.00	0.00	0.00	0.00	0.00	0.00
80 ml	0.00	0.00	0.00	0.00	0.00	0.00
Total	1.04	1.01	2.15	1.15	0.68	0.13

Table 8. Influence of eluate volume on Ca and Mg extraction

Eluate volume	Metal fractions (mg/kg)					
	Step I		Step II		Step III	
	Ca	Mg	Ca	Mg	Ca	Mg
10 ml	1.25	1.31	1.43	1.46	0.00	0.00
30 ml	2.02	0.80	2.88	0.88	2.35	0.68
50 ml	0.00	0.00	0.00	0.00	0.00	0.00
80 ml	0.00	0.00	0.00	0.00	0.00	0.00
Total	3.27	2.11	4.31	2.34	2.35	0.68

DISCUSSION AND CONCLUSION

The low value of pH may be due to presence of small amount of Na, K, Ca and Mg salt in soil. Soil samples found more acidic in wet seasons than the dry seasons by the leaching effect of rain water during summer which replaces basic cations (Ca^{2+}, Mg^{2+}, Na^+ and K^+) with H^+ ions [25]. Use of long term inorganic fertilizers instead of green manure, farmyard manure and rice-straw residues had also enhanced in increasing soil pH [26]. The availability of Na, K, Ca and Mg decreases with the decrease in pH below 5.5-6.6 [27]. The EC results suggested that soils were moderate to high in electrolyte concentration in both the controlled and contaminated samples. The increase in EC value may be due to agricultural run off from the tea gardens and domestic waste water disposal. Low soil EC may be due to higher leaching induced by heavy rainfall in the absence of adequate amount of soil organic matter or may be due to decomposition of organic matter at high temperature [28]. Moderate to high value of soil organic matter can be attributed due the exposure of agricultural run off from the tea gardens and domestic waste water disposal. Continuous use of agrochemicals increases the bulk density of surface soil and high temperature in summer season more organic mater decomposes which can be easily removed and washed away by surface runoff [29]. The soil BD result of the present study implied that the subsoil of the area could not hold sufficient amount of available nutrients and water [30].

The total metal analysis by acid digestion method (HNO_3:$HClO_4$) include all non-residual as well as metals present in silicate mineral matrix, the later fraction (silicate bound) is not considered as bioavailable [3]. The water soluble and exchangeable fraction is generally considered immediate nutrient reservoir for plants [11]. Metals associated with carbonates would be susceptible to pH changes and acetic acid soluble may be regarded as potentially phytoavailable [13]. Metals present in other chemical forms, such as oxides and organic matter binding have low solubility and high stability hence plant could not absorbed them easily [11, 31]. Detection of trace amount of exchangeable Na (0.04 mg/kg) and K (0.03 mg/kg) up to 120 minutes of extraction time in step II can be attributed that Na^+, K^+ exchange varies with both ionic strength and clay mineralogy [12]. For complete speciation of Ca and Mg for each sequential extraction step, extraction time up to 60 minutes was obligatory. It may be due to weak electrostatic force between the metal ions with surface of clay and organic matter present in the soil and increase in occupation of adsorption sites by smaller metal cations [13].

The study on influence of extraction time and eluate volume in major cation speciation from soil revealed that bioavailable form of metals in soil decreases gradually in each extraction step, because the strength of used extraction reagents increases in each extraction sequences [5].

Water soluble and exchangeable forms are the most available form for plant uptake. Acetic acid soluble form is not easy available for plant but become mobile and bio-available under conditions of lower soil pH. The optimum condition for complete speciation of Na, K, Ca and Mg from tea garden belt soils was 1 hour extraction with 1:5 soils to solution ratio. Metal content were found in below detection limit in acid extraction step using 10 ml of eluate. It may be due to insufficient eluate volume to dissolve the soil matrix.

Distribution of Na, K, Ca and Mg in soils in order to elucidate bioavailable form has been studied for contaminated soils of tea garden belts of Golaghat district of Assam. It was concluded from the findings of this study that contaminated soils were silty loam in nature with sand and silt being the dominant fraction. Soils were found in low organic matter content with high bulk density and slightly calcareous in nature. Common metal distribution by modified three steps sequential extraction method have emphasized on potential environmental pollution by them and quick identification of components in soil samples. It might be a proof of use as a fast screening method for the bioavailability of common metals in soil matrix.

REFERENCES

[1] DL Karlen; SS Andrews; JW Doran. *Adv. Agron.,* **2001,** 74, 1-40.
[2] MA Arshad; B Lowery; B Grossman. Physical Tests for Monitoring Soil Quality. *In:* JW Doran; AJ Jones (Ed.); Methods for Assessing Soil Quality, Madison, WI, **1996**, 123-141.
[3] M Lei; Y Zang; S Khan; P Qin; B Liao. *Environ Monit Assess.* **2009,** DOI: 10.1007/s10661-009-1105-4.
[4] I Ahumada; J Mendoza; E Navarrete; L Ascar. *Communications in Soil Sc. & Plant Analysis*, **1999,** 30, 1507-1519.
[5] M Zemberyova; AA Zwaik; I Farkasovska. *J. Radioanalytical and Nuclear Chem.,* **1998,** 229(1-2), 67-71.
[6] MA Kashem; BR Singh; S Kawai. *Nutrient Cycling in Agro Ecosystem*, **2007,** 77, 187-198.
[7] BJ Alloway. Soil processes and the behaviour of heavy metals, *In*: BJ Alloway (ed.), *Heavy Metals in Soils*, Blackie Academic and Professional, London, U.K, 1995, 45-67.
[8] GR Cramer; RA Basset; JR Seemann. *Plant Nutrition*, **1990**, 13, 1453-1462.
[9] HM Raghunath. Groundwater, 2nd Ed. Wiley Eastern Limited, New Delhi, India, **1987,** 344-369.

[10] M Qadir; S Schubert; A Ghafoor; G Murtaza. *Land Degraded Development*, **2001,** 12, 357-387.

[11] C Palm; P Sanchez; S Ahamed; A Awiti. Soils: A Contemporary Perspective, ***In:*** Annual Review Environmental Resources, **2007,** 32, 99-129.

[12] PM Kopittke; HB So; NW Menzies. *European J. Soil Science*, **2006,** 57, 626-633.

[13] B Walna; S Drzymała; J Siepak. *Water, Air, and Soil Pollution*, **2000,** 121, 31-41.

[14] J Dutta; AK Mishra. *International J Applied Environ. Science*, **2010**, 5(1), 11-20.

[15] J Shiowatana; N Tantidanai; S Nookabkaew; D Nacapricha. *J. Environ. Qual*., **2001,** 30:1195-1205.

[16] A Probst; L Hernandez; JL Probst. *J. Phys. IV France*, **2003,** 107, 103-106.

[17] Z Chen; M He; K Sakurai; Y Kang; K Iwasaki. *Soil Sc. Plant Nutrition*, **2007,** 53, 517-529.

[18] A Tessier; PGC Campbell; M Bisson. *Anal. Chem*., **1979,** 51(7), 842-851.

[19] L Leleyter; JL Probst. *Int. J. Environ. Anal. Chem*. **1999,** 73, 109-128.

[20] ML Jackson. Soil Chemical Analysis; Prentice Hall of India Private Limited, New Delhi, India, **1973.**

[21] DW Nelson; LE Sommers. Total Carbon, Organic Carbon and Organic Matter. pIn: Methods of Soil Analysis, Part-II, 2nd Ed. A.L. Page, M.H. Miller and D.R. Keeny (eds.). American Society of Agronomy, Madison, W.I. USA, **1982**, 539-577.

[22] Soil and Plant Analysis Council, **(1992)**, Handbook on Reference Methods for Soil Analysis, Athens, GA, USA.

[23] SL Chopra; JS Kanwar. Analytical Agricultural Chemistry, Kalyani Publishers, Ludhiana, New Delhi, **1986.**

[24] MG Little; CTA Lee. *J. Afr. Earth Sci.* **2009,** doi:10.1016/j.jafrearsci.2009.12.001.

[25] PK Barua; PK Bora. *J. North Easter council*, **1975**), 2, 21-26.

[26] KN Tiwari. Fundamental of Soil Science, Indian Society of Soil Science, IARI, New Delhi, **2003.**

[27] PC Jaiswal. Soil, Plant and Water Analysis, (2nd Ed.), Kalyani Publishers, Guwahati, Assam, India, **2006.**

[28] WL Lindsay; Chemical equilibrium in soils, John Willey and Sons, New York, **1979.**

[29] KM Bujarbaruah. Why and How of Organic Farming?, *In*: KM Bujarbaruah; BK Sharma; N Prakash; R Kumar (eds) Organic Food Production in North East India: Prospect and Aspect), ICAR Research Complex for NEH Region, Shillong, Meghalaya, India, **2006,** 1-6.

[30] KK Borah; B Bhuyan; HP Sarma. *Archives of Applied Science Research,* **2009**, 1(2), 159-164

[31] X Xian. *Plants and Soil*, **1989,** 113, 257-264.