

Single and Sequential Extraction of Copper by Different Extractants from Different Peat Soil Samples of Bangladesh

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Abstract

A comprehensive *in vitro* study on single and sequential extraction methods for Cu extraction by different extractants of distinct chemical nature from some peat soils of Bangladesh was conducted to assess the pattern of metal-extractability in drying and wetting sequences. Samples were collected from peat basins of two different districts of Bangladesh (Gopalganj and Bagerhat) and an incubation study was designed and conducted in which soils were incubated to three alternate drying and wetting cycles for 21 days. Single and sequentially extracted Cu ions in all three cycles and by all three selective extractants were compared. Maximum amount of Cu was extracted in drying days and 1M HCl extracted the largest proportion of Cu from all the soils in each incubation time. Conversely, 1M NH₄Cl was the least effective extractant for extracting Cu by both single and sequential process in either day of incubation. Considerable amount of Cu was extracted by 0.005 M DTPA in dry periods. In general, single extraction was found to be more effective in extracting Cu in the alternate drying and wetting cycles from the peat soil samples except for 1M HCl in initial drying days.

Keywords

Copper, Single and Sequential Extraction, NH₄Cl, DTPA, HCl, Peat Soil

1. Introduction

Peat soils generally consist of partially decomposed organic matter, derived mostly from plant material, which has accumulated under conditions of water logging and oxygen deficiency. These are organic soils containing a minimum of 20% organic matter with as much as 60% of the mineral matter is clay. There are ar-

guments about definitions of peat with organic matter content higher than 30% and thickness greater than 30 cm. Organic material occupies more than half of the upper 80 cm of the peat occurring at the surface or be buried under up to 15 inches of mineral soils or occupy one or more layers separately in layers of mineral matter [1]. In some cases, peat and mineral layers may occur at the top of the profile. They usually possess highly acidic environment with lower available nutrients, although reserved organic matter acts as total nutrient pool.

In Bangladesh, peat soils are found in Gopalganj, Bagerhat and adjoining parts of Khulna, Barishal and Jessore districts occupying an area of about 1,30,005 hectares which is about 1.06% of the total area of Bangladesh [2]. Peat soils are located mainly between the Ganges River Floodplain and the Ganges Tidal Floodplain regions of Bangladesh [2]. These soils are seasonally flooded, poorly to very poorly drained and have muck in an alternate emergence. Usually they contain a very high amount of organic matter which acts as reservoir of almost all the macro and micronutrients except Zn [3]. Such higher content of organic matter and heavy metal sometimes become major constraints for peat soils [2]. Most of the ions become available in oxidizing condition when water recedes from the soil and become unavailable again in reduced soil conditions.

To have a detail idea about the extractability of one of the native micronutrients, Copper (Cu), three different extractants of distinct chemical character; unbuffered salt (NH_4Cl), strong acid (HCl) and chelating agent (DTPA), were used in this study. These extractants were used both for single and sequential extraction of Cu in dry and wet condition to have a clear concept about the origin, mode of occurrence, mobility, biological and physicochemical availability of the trace elements as well as to provide a convenient way to determine the trace elements associated with soils and sediments [4]. Thus, the extractants were used in this study to figure out the status of Cu content and its extractability with these extractants during dry and wet cycles in some peat soils of Bangladesh.

2. Materials and Methods

2.1. Sample Collection and Preparation

Ten samples of peat soils were collected from Gopalganj and Bagerhat districts of Bangladesh to figure out Cu concentrations in those soils as well as to identify their extractability with different extractants. Following the recommendations of the Soil Survey Staff of the USDA [5] for composite sampling, soil samples were collected from 0 - 15 cm depth of the profiles. The geo-locations of the samples are shown in **Table 1**.

After collection, the peat samples were dried under the sunlight and noticeable non-soil materials including roots and debris were removed manually. The dried soils were then divided into two parts; one portion for incubation study and the other part for chemical analyses. For the incubation study, samples were ground with wooden hammer to crush the larger aggregates, screened through a 5 mm stainless steel sieve [6] and mixed thoroughly to make the composite sam-

ples. On the other hand, for the chemical analyses, another portion of the samples was ground to pass through a 0.5 mm wire sieve [6] and stored in plastic pots.

2.2. Chemical Analyses of Background Sample

2.2.1. Determination of Basic Chemical (Organic Matter and Organic Carbon) and Physico-Chemical Properties (pH) of Soils

Some basic properties of the peat samples were determined before doing extractions of soils with selected extractants. To know the initial condition of the soils, pH of the samples were determined by pH meter from soil-water suspensions of 1:2.5 ratio [7]. Total content of organic carbon was also measured using the Walkley and Black wet oxidation process [8]. Based on the assumption of containing 58% of organic carbon in organic matters of average soils [9], the calculated value of organic carbon was multiplied by the conventional Van Bemmelen's factor of 1.724 to get the total organic matter content of the samples [10].

2.2.2. Determination of Extractable Cu

The samples were extracted with different extractants using single and sequential extraction procedures. Copper extractability of extractants by both the procedures was compared in the samples before and after incubation study. The list of extractants used for determining Cu availability is shown in **Table 2**.

Table 1. Location of the sampling sites.

(a) Gopalganj district						
Serial No.	Sample Name	Latitude	Longitude	Area Details		
				Village	Union	Upazila
1	S (G1)	23°01.891'N	89°55.891'E	Bajuni	Kajulia	Gopalganj Sador
2	S (G2)	23°05.512'N	89°59.261'E	Machh para	Kolabari	Kotalipara
3	S (G3)	23°02.611'N	90°01.050'E	Chhoto Digholia	Radhaganj	Gopalganj
4	S (G4)	22°55.206'N	89°54.460'E	Baladanga	Patgati	Tungipara
5	S (G5)	22°51.630'N	89°58.458'E	Karfa	Dumuria	Tungipara
(b) Bagerhat district						
Serial No.	Sample Name	Latitude	Longitude	Area Details		
				Village	Union	Upazila
6	S (B6)	22°49.566'N	89°50.594'E	Kaligati	Shibpur	Chitalmari
7	S (B7)	22°49.632'N	89°50.633'E	Kaligati	Shibpur	Chitalmari
8	S (B8)	22°50.605'N	89°50.115'E	Kachuria	Kodalia	Mollahat
9	S (B9)	22°51.656'N	89°49.334'E	Machuarkul	Barobaria	Chitalmari
10	S (B10)	22°49.133'N	89°50.544'E	Kendua	Gaola	Mollahat

Table 2. List of extractants used for single and sequential extraction of Cu.

Extractant type	Extractants used	Reason for using
1. Dilute Solutions of either weak acids (Acetic acid, 0.1 M HCl) or strong acids (1 M HCl, 1M HNO ₃)	1 M HCl	Releases elements from the non-residual metals and silicate mineral matrix.
2. Complexing extractants (0.005 M DTPA, EDTA)	0.005 M DTPA	Releases ions or nutrients that are bound organically
3. Weak replacement of non-buffered salts (MgCl ₂ , CaCl ₂ , NH ₄ Cl, NH ₄ NO ₃)	1M NH ₄ Cl	Releases ions from exchange sites on the soil solid-phase

1) Single Extraction

All the samples were extracted using each of the extractants (**Table 2**) in three replicates. Total amount of the extractants required for extracting the samples were calculated and prepared using standard procedure. The specific ratio of soil to extractant was followed for each extractant. For extractions, soil samples and extractants were shaken in 250 ml conical flasks with a mechanical shaker for certain periods to make a suspension. Prepared suspensions were then filtered through Whatman 44 filter papers.

2) Sequential Extraction

Three different chemical phases, viz., 1M NH₄Cl extractable (exchangeable), 0.005 M DTPA extractable (organically bound) and 1M HCl extractable fractions were tried in the sequential extraction procedure.

Four grams of 0.5 mm sieved soil sample with 40 ml of extractants (1:10) were placed in a 50 ml polycarbonate centrifuge tube to perform extractions sequentially. A brief summary of the sequential extraction procedures are given below in **Table 3**.

All the samples were centrifuged at a rate of 2000 rpm for 5 minutes; after each extraction procedure samples were filtered.

The basic difference between single and sequential extraction is the single extraction procedure involves one simple extraction scheme to extract an element from solid surface; whereas the sequential extraction procedure involves a number of extraction schemes where the extractants are applied sequentially to extract an element from solid surface by increasing reactivity.

2.2.3. Determination of Total Cu

The peat samples were digested with aqua-regia (HNO₃:HCl = 1:3) to determine the total concentration of Cu in soils. One gram of sample was taken for digestion with 20 ml aqua-regia and heated for approximately 3 hours on a sand bath at 150°C temperature. After digestion, samples were diluted to 100 ml volume with distilled water, stored and filtered before further analysis [11]. The concentration of Cu was determined from the diluted samples by using atomic absorption spectrophotometer (AAS).

2.3. Incubation Study

An *in vitro* incubation study was conducted in a seven days cycle of alternate wetting and drying in the laboratory under ambient temperature.

Table 3. Outline of the sequential extraction procedures.

Symbols	Particulars
F1	4 gm of soil samples was extracted with 40 ml of 1M NH ₄ Cl after shaking for 2 hours (h) at room temperature on a mechanical shaker.
F2	The residue from F1 was extracted with 40 ml of 0.005 M DTPA after shaking for 2 hours (h) at room temperature on a mechanical shaker.
F3	The residue from F2 was extracted with 40 ml of 1 M HCl after shaking for 2 hours (h) at room temperature on a mechanical shaker.

For the incubation experiment, 300 g of air dried, 5 mm-sieved soil sample was taken in plastic pots and incubated in alternate cycles of wetting and drying. In the first seven days of wetting cycle soils were saturated every alternate day to ensure field moisture condition. No water was added in the soils in the next seven days of drying cycle. Again, the wetting procedure was repeated for the next seven days of wetting cycle. Therefore, 1 - 7 days were wetting cycles, 8 - 14 days were the drying period and 15 - 21 days were the wetting cycle again.

Soils in the pots were mixed thoroughly for ensuring uniform sampling for further analysis at the end of each incubation cycle. Thereafter, the samples were collected randomly for further single and sequential analyses. For each of the extraction period, available concentration of Cu was analyzed following the same procedure as stated above in section 2.2.4.1 and 2.2.4.2. A brief detail of the extractants and the mode of extraction are shown in **Table 4**.

2.4. Statistical Analysis of Data

A general-purpose statistical software, STATA, (version 14) was used to calculate correlations among various pairs. Microsoft Excel package was also used for the statistical and graphical evaluations.

3. Results and Discussion

3.1. Initial Chemical and Physico-Chemical Conditions of the Soils before Incubation

3.1.1. pH, Organic Carbon and Organic Matter Content of the Collected Soil Samples

Some general but important chemical and physico-chemical properties of the soils like pH, organic carbon and organic matter percentage were analyzed from the collected peat samples in order to know the initial status of Cu content of the soils. Ten samples were collected from the peat soils of Gopalganj and Bagerhat districts and designated as S(G) and S(B) respectively. The background information about the pH, organic carbon and organic matter content of the selected soil samples are given below in **Table 5**.

Table 5 explains that the average pH of the Bagerhat peat basins S(B) were recorded in the neutral soil pH range (6.6 - 7.3) whereas the samples from Gopalganj peats S(G) were classified as "Slightly acidic (6.1 - 6.5)" as per the Soil Survey Manual [15].

Table 4. Summary of the extraction methods used in the present study.

Extractants	Ratio(w/v)/Time/Temperature	Pool	Reference
1 M NH ₄ Cl	1:6/16h/room temperature	Neutral salt soluble/exchange	[12]
0.005 M DTPA	1:2/2h/room temperature	Chelating, extractable	[13]
1 M HCl	1:33.3/2h/room temperature	Weak acid extractable	[14]

Table 5. Initial pH, organic carbon and organic matter content of the soil samples.

Sample	pH	Organic carbon (%)	Organic matter (%)
S(G1)	6.36	13.21	22.77
S(G2)	6.22	11.9	20.52
S(G3)	6.29	13.18	22.72
S(G4)	6.41	9.93	17.12
S(G5)	6.35	9.77	16.84
Average	6.33	11.60	19.99
S(B6)	6.71	8.57	14.8
S(B7)	6.79	7.94	13.7
S(B8)	7.13	6.38	11.0
S(B9)	6.85	6.99	12.1
S(B10)	6.92	7.24	12.5
Average	6.88	7.42	12.80

On the other hand, the mean organic carbon content of S(B) samples (7.42%) was relatively lower than the S(G) samples (11.60%). These values support the hypothesis that the content of higher organic carbons usually lowers the pH of the soil. Correspondingly, the organic matter content was assessed by multiplying the organic carbon value with the Van Bemmelen's factor (1.724) [10]. As a result, more organic matter contents were recorded in S(G) sites (average 19.99%) than S(B) sites (average 12.80%).

3.1.2. Total and Extractable Cu Content in Background Soil Samples

A portion of the peat samples were analyzed for determining initial total and extractable content of Cu in soils. It can be declared from **Table 6** that the peat basins of Bagerhat district, S(B) samples, contain more total Cu on an average (72.04 mg/kg) than the Gopalganj peats, S(G) (55.04 mg/kg). The two major factors that affect soil Cu content are pH and organic matter level of the soil. Copper level drops with the increase of organic matter in soils. As the S(G) samples contain more organic matter, their Cu level was lower than the S(B) samples. Moreover, comparatively lower pH of S(G) also lead to lower the total Cu content as acidic soils are most likely to be lower in Cu content [16]. However, the total concentration of metals usually has poor correlations with bio-availability of that metal to plants or organisms [17].

Table 6. Cu content in peat soil samples before incubation.

Sample	Total Cu (mg/kg)	Extraction of Cu by 1M NH ₄ Cl (mg/kg)		Extraction of Cu by 0.005M DTPA (mg/kg)		Extraction of Cu by 1M HCl (mg/kg)	
		Single	Sequential	Single	Sequential	Single	Sequential
S(G1)	50.1	0.391	2.879	2.852	0.001	17.931	21.3
S(G2)	54.3	0.712	0.09	4.404	0.003	20.564	36.06
S(G3)	47	1.435	0.061	4.782	0.002	18.431	49.46
S(G4)	64.6	4.039	0.634	4.926	0.004	29.93	50.56
S(G5)	59.2	1.069	0.325	4.136	0.001	20.231	32.73
Average	55.04	1.529	0.798	4.220	0.002	21.417	38.022
S(B6)	67.4	1.081	0.518	4.644	0.009	24.397	61.43
S(B7)	64.7	0.577	0.22	4.368	0.004	22.764	65.36
S(B8)	56.4	0.265	0.69	2.498	0.002	23.93	67.69
S(B9)	89.9	0.709	0.167	3.018	0.008	27.863	32.2
S(B10)	81.8	0.865	0.445	4.476	0.009	19.264	49.13
Average	72.04	0.699	0.408	3.801	0.006	23.644	55.162

In the case of determining extractable Cu concentration in both S(G) and S(B) samples, 1M HCl was found to be the most efficient extractants for both single and sequential extractions of the samples. On the other hand, 1M NH₄Cl was found to be least efficient for extracting Cu from both S(G) and S(B) samples in single extraction procedure; whereas 0.005M DTPA extracted minimum Cu from both the soil samples in sequential extraction process.

As DTPA extractant usually represents the plant-available fractions [13] [18] [19] it can be said that a little portion is available to plants. DTPA is evidenced to form soluble metal complexes thus reduces metal activity in the solutions. However, there was a wide difference in extracting Cu by the extractants for the samples of both the sites.

3.2. Copper Content of Soils by Different Extractants after Incubation

Single and sequential extraction of incubated soils in each of the drying and wetting cycles were performed by using three different extractants, 1M NH₄Cl, 0.005M DTPA and 1M HCl. Incubation was carried out for 1 - 7 days (dry cycle), 8 - 14 days (wet cycle) and 15 - 21 days (dry cycle).

3.2.1. Single and Sequential Extraction of Cu by 1M NH₄Cl after Incubation

The concentrations of 1M NH₄Cl extractable Cu (mg/kg) in the samples at different incubation days are presented in **Table 7**.

Table 7. 1M NH₄Cl extractable Cu contents (mg/kg) in peat soil samples at different Incubation days by single and sequential extraction.

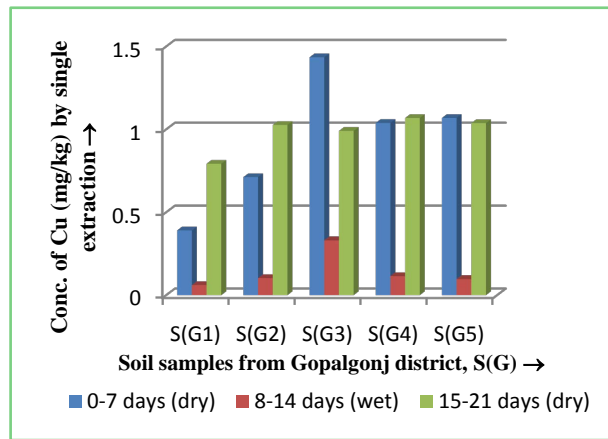
Incubation days →	0 - 7 days (dry)		8 - 14 days (wet)		15 - 21 days (dry)	
Sample↓	Single	Sequential	Single	Sequential	Single	Sequential
S(G1)	0.391	0.879	0.061	0.488	0.793	0.099
S(G2)	0.712	0.09	0.103	0.468	1.027	0.089
S(G3)	1.435	0.061	0.331	0.996	0.991	0.093
S(G4)	1.039	0.634	0.115	0.246	1.069	0.088
S(G5)	1.069	0.325	0.097	0.816	1.038	0.079
Average	0.929	0.398	0.141	0.603	0.984	0.090
S(B6)	1.081	0.518	0.229	0.624	0.577	0.099
S(B7)	0.577	0.22	0.055	0.666	1.837	0.067
S(B8)	0.265	0.69	0.445	0.594	1.519	1.199
S(B9)	0.709	0.167	0.463	0.468	1.597	1.629
S(B10)	0.865	0.445	0.523	1.074	2.257	2.709
Average	0.699	0.408	0.343	0.685	1.557	1.141

It is apparent from **Table 7** that the concentration of available Cu increases in drying days and decreases with wetting of the soil. The concentration increases further while drying the soils. For samples from both the sites, S(G) and S(B), extractable Cu content increase even more in the second drying cycle (0.984 and 0.090 mg/kg Cu for single and sequential extractions of S(G) samples; and 1.557 and 1.141 mg/kg Cu for single and sequential extractions of S(B) samples) than the first one (0.929 and 0.398 mg/kg Cu for single and sequential extractions of S(G) samples; and 0.699 and 0.408 mg/kg Cu for single and sequential extractions of S(B) samples). The organic carbons present in the soils become oxidized with the release of water from the soils. As a result, in the drying periods, the organo-mineral complexes are broken and Cu ions get released to the soil solution to become available for plants.

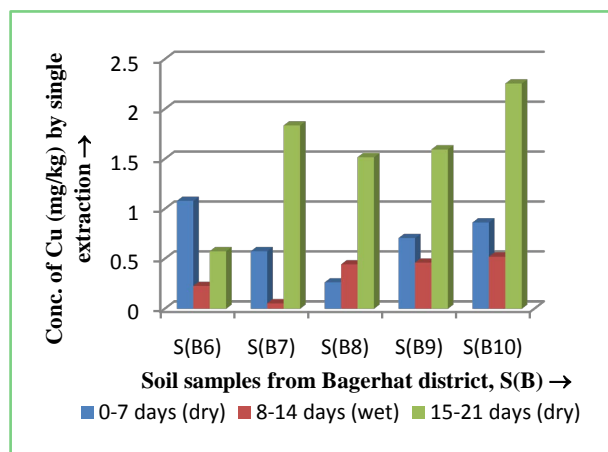
In the subsequent wet cycle, the extractable Cu concentration was found to be decreased. The accumulation and immobilization of remaining organic matters in the reduced soil condition might help Cu ions to form complex or to get fixed in colloid surfaces leading to lower extractability by single (0.141 mg/kg Cu for S(G) and 0.343 mg/kg Cu for S(B) samples) and sequential (0.603 mg/kg Cu for S(G) and 0.685 mg/kg Cu for S(B) samples) extraction processes.

The reason for the decrease of Cu content in waterlogged soils could be the increased concentrations of OM-bound Cu fraction as the lower values of Eh and higher values of pH in this system were propitious to the formation of metal-organic complexes [20] and microbial immobilization [21].

The graphical representation of Cu extractability trend by 1M NH₄Cl in single and sequential extraction process for Gopalgonj and Bagerhat soil samples are given below in **Figure 1(a)**, **Figure 1(b)** and **Figure 2(a)**, **Figure 2(b)**:

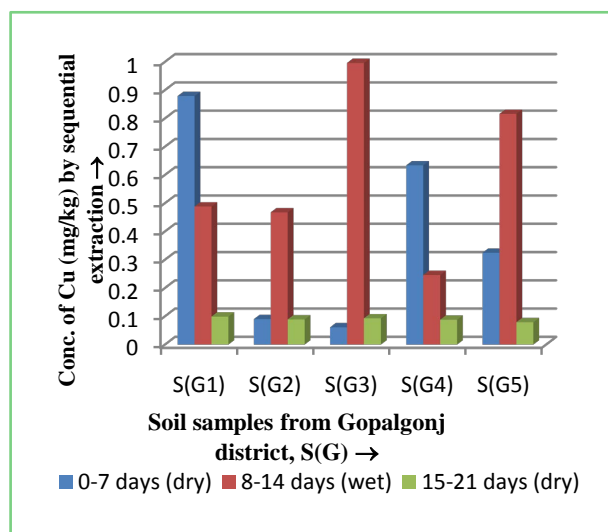


(a)



(b)

Figure 1. Single extraction of Cu by 1M NH₄Cl from samples of (a) Gopalganj's district, S(G) and (b) Bagerhat district, S(B) in 0 - 7 days (dry cycle), 8 - 14 days (wet cycle) and 15 - 21 days (dry cycle) of incubation.



(a)

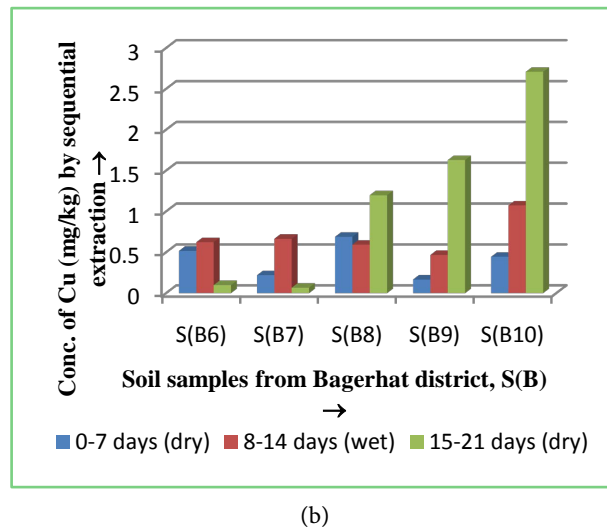


Figure 2. Sequential extraction of Cu by 1M NH₄Cl from samples of (a) Gopalganj's district, S(G) and (b) Bagerhat district, S(B) in 0 - 7 days (dry cycle), 8 - 14 days (wet cycle) and 15 - 21 days (dry cycle) of incubation.

The extractions in the mentioned three cycles carried no significant correlations except for the last drying cycle of S(B) samples (r values 0.046 and 0.009 for single and sequential extractions).

3.2.2. Single and Sequential Extraction of Cu by 0.005M DTPA after Incubation

The concentrations of 0.005M DTPA extractable Cu (mg/kg) in the soil samples at different incubation days are presented in **Table 8** where it is noticeable that, for the S(G) samples, the concentration of Cu was decreased from dry to wet conditions and increased again in the drying period in both the cases of single and sequential extractions. However, the S(B) samples showed quite a different pattern indicating the decrease of DTPA extractable Cu from drying to wetting period and further decrease in subsequent drying cycle.

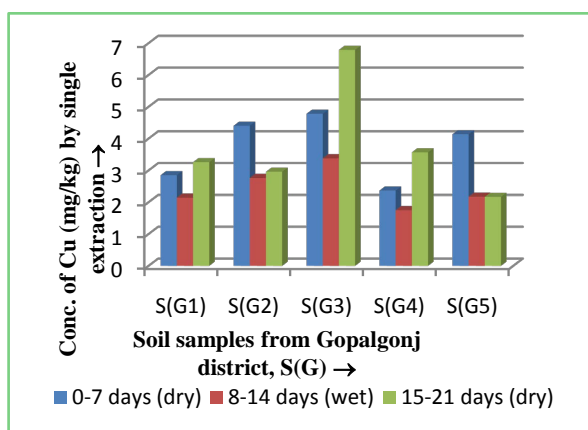
According to **Table 8**, 0.005M DTPA extracted maximum amount of Cu in drying period than the midst wetting days for S(G) samples. The result supports the finding that the extractability of Cu in soils increases by a factor of 1.3 to 1.5 from moist to dry condition [22]. Differing to the trend, DTPA extracted more Cu by both by single and sequential extraction (3.067 mg/kg and 2.572 mg/kg Cu respectively) in the wetting cycle than the last seven days of drying in S(B) samples. A dissimilar finding was reported expressing that increased solubility and extractability of Cu in the drying periods of soils is a common phenomenon [23].

The trend of Cu extractability by 0.005M DTPA in single and sequential process for both the peat soil samples are given below in **Figure 3(a)**, **Figure 3(b)** and **Figure 4(a)**, **Figure 4(b)**.

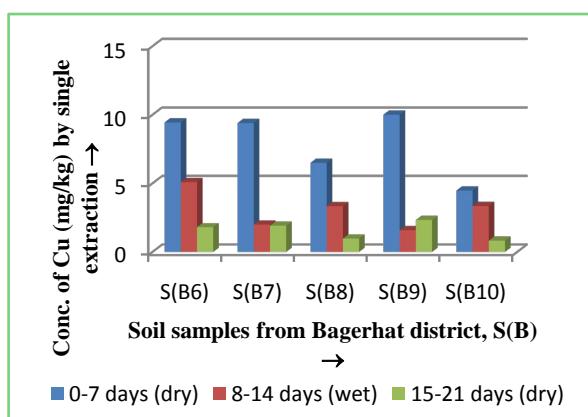
Statistically, the S(B) samples from Bagerhat district showed a significant value for 0 - 7 days of drying cycle only (0.8003, significant at 10%) among all the samples and in all three periods of alternate drying and wetting.

Table 8. 0.005M DTPA extractable Cu contents (mg/kg) in peat soil samples at different incubation days by single and sequential extraction.

Incubation days →	0 - 7 days (dry)		8 - 14 days (wet)		15 - 21 days (dry)	
	Single	Sequential	Single	Sequential	Single	Sequential
Sample↓						
S(G1)	2.852	0.914	2.146	4.04	3.26	0.498
S(G2)	4.404	1.083	2.763	1.19	2.96	0.987
S(G3)	4.782	1.046	3.38	3.78	6.79	1.456
S(G4)	2.368	0.778	1.751	0.67	3.57	0.578
S(G5)	4.136	1.002	2.174	0.63	2.17	0.578
Average	3.708	0.965	2.443	2.062	3.750	0.819
S(B6)	9.446	2.095	5.088	2.85	1.8	0.089
S(B7)	9.398	1.801	1.989	2.8	1.91	0.99
S(B8)	6.498	1.998	3.333	2.16	0.97	0.879
S(B9)	10.006	2.645	1.579	0.84	2.33	0.59
S(B10)	4.476	0.986	3.347	4.21	0.82	1.627
Average	7.965	1.905	3.067	2.572	1.566	0.835

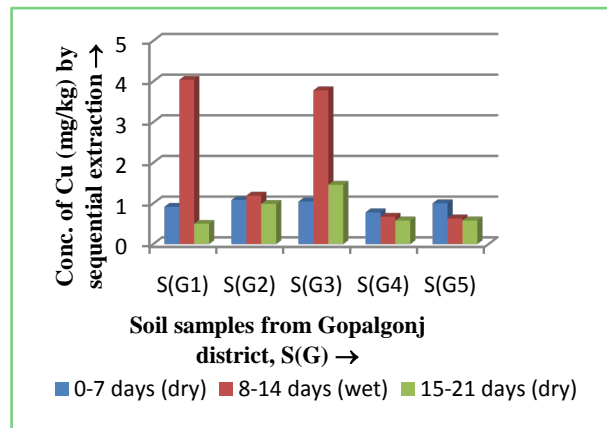


(a)

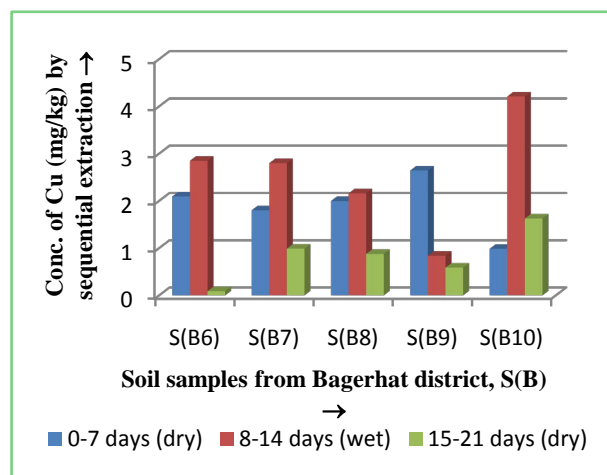


(b)

Figure 3. Single extraction of Cu by 0.005M DTPA from samples of (a) Gopalganj's district, S(G) and (b) Bagerhat district, S(B) in 0 - 7 days (dry cycle), 8 - 14 days (wet cycle) and 15 - 21 days (dry cycle) of incubation.



(a)



(b)

Figure 4. Sequential extraction of Cu by 0.005M DTPA from samples of (a) Gopalganj's district, S(G) and (b) Bagerhat district, S(B) in 0 - 7 days (dry cycle), 8 - 14 days (wet cycle) and 15 - 21 days (dry cycle) of incubation.

In this experiment, the 0.005M DTPA extractable Cu was found to increase upon drying for S(G) samples supporting the proposal of [22] explaining that increase in temperature during drying period can increase the extractability of Cu, but contradictory to the results found for S(B) samples. DTPA helps to release ions from insoluble organo-mineral complexes [17] and clays [24]. The existing microorganisms of that environment may influence Cu extractability of DTPA upon rewetting [25].

3.2.3. Single and Sequential Extraction of Cu by 1M HCl after Incubation

Single and sequentially extracted Cu (mg/kg) concentrations by 1M HCl in the peat soils from two different sites at different incubation days are presented in **Table 9**.

Samples showed an overall decrease of Cu concentration from drying to wetting sequence, although the amount of Cu extracted through sequential process

Table 9. 1M HCl extractable Cu contents (mg/kg) in peat soil samples at different incubation days by single and sequential extraction.

Incubation days →	0 - 7 days (dry)		8 - 14 days (wet)		15 - 21 days (dry)	
	Single	Sequential	Single	Sequential	Single	Sequential
Sample↓						
S(G1)	17.931	21.308	9.734	5.509	7.302	3.842
S(G2)	20.564	36.062	6.966	4.971	6.647	1.04
S(G3)	18.431	49.465	6.941	4.178	13.835	2.136
S(G4)	29.93	50.964	6.806	5.139	9.001	0.992
S(G5)	20.231	32.737	7.168	3.313	13.401	1.049
Average	21.417	38.107	7.523	4.622	10.037	1.812
S(B6)	24.397	61.73	11.203	13.688	24.733	3.45
S(B7)	22.764	65.366	15.787	9.512	18.44	1.89
S(B8)	23.93	67.691	10.967	7.106	8.773	6.33
S(B9)	27.863	32.222	21.649	10.55	10.897	6.933
S(B10)	19.264	49.134	10.925	8.099	8.909	1.908
Average	23.644	55.229	14.106	9.791	14.350	4.102

was somewhat higher than the single one in case of the first seven days of drying. Although the value was reduced a little in the next wetting cycle, yet was higher than the previous cycle of wetting.

The extractant extracted more Cu from both the peat samples sequentially (38.107 mg/kg Cu for S(G) samples and 55.229 mg/kg Cu for S(B) samples) than the single extraction process (21.417 mg/kg Cu for S(G) samples and 23.664 mg/kg Cu for S(B) samples) in first seven days of drying. However, in other cases of wetting and drying periods, single extraction process was evidenced to bring more Cu ions in solution than the sequential process in both the peat samples.

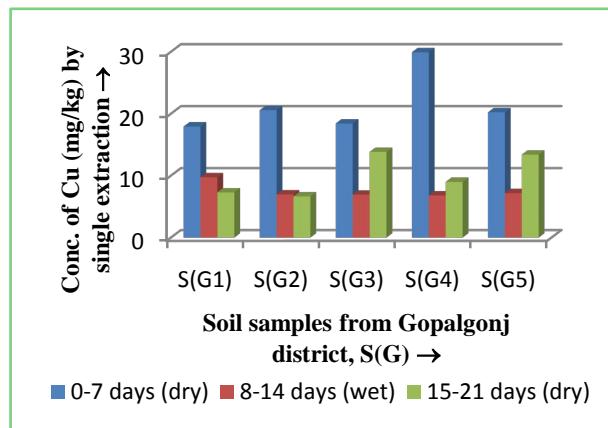
A strong acid like HCl usually enhances oxidation and subsequent mineralization of the organic matters leading to release of locked or trapped Cu ions from the complexes. Additionally, the release of organic acids can also increase Cu availability in solutions in dry days. However, the reserve of Cu content as organo-mineral complexes can be increased in the subsequent wetting period because of lower to zero rate of oxidation.

Likewise, the findings of lower extractability in wetting cycle, [14] mentioned the formation of metal-organic complex from the organic matter and the binding material of heavy metals as the reasons for such changes in Cu extractability due to wetting and drying.

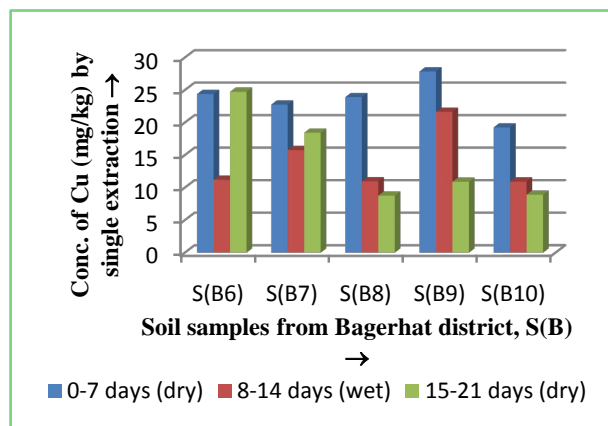
Graphically the trend of Cu extractability by 1M HCl in single and sequential extraction process for the peat soil samples can be shown as follows (**Figure 5(a)**, **Figure 5(b)** and **Figure 6(a)**, **Figure 6(b)**):

Statistically, the dry period Cu contents were statistically significant for both the S(G) and S(B) samples (r value 0.0031 and 0.0049 respectively). However, the

rest of the samples did not show any significant content of Cu concentration by extracting with 1M HCl in either day of incubations.

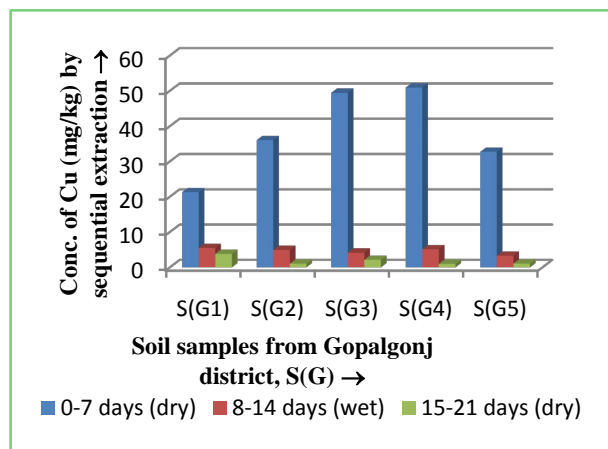


(a)

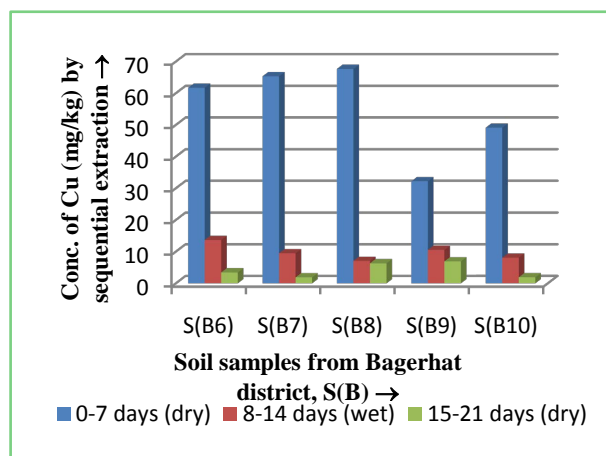


(b)

Figure 5. Single extraction of Cu by 1M HCl from samples (a) Gopalganj's district, S(G) and (b) Bagerhat district, S(B) in 0 - 7 days (dry cycle), 8 - 14 days (wet cycle) and 15 - 21 days (dry cycle) of incubation.



(a)



(b)

Figure 6. Sequential extraction of Cu by 1M HCl from samples (a) Gopalganj's district, S(G) and (b) Bagerhat district, S(B) in 0 - 7 days (dry cycle), 8 - 14 days (wet cycle) and 15 - 21 days (dry cycle) of incubation.

Considering **Tables 7-9** it can be postulated that single extraction process was found to be more efficient in extracting higher concentrations of Cu from the soil solutions than the sequential extraction procedure, which coincides with the findings of [26]. Although a disparate trend was figured out for HCl extractant in the first drying cycle of both the samples, however, it should be taken into consideration that single extraction signifies metal extractability than the phytoavailability of ions. Plant physiology and rhizosphere chemistry are two major factors that can alter the relation between extractants and available metal concentrations in tissues [27].

4. Conclusions

Among the three different extractants of three different chemical natures, acid extractants were found to extract available Cu ions more efficiently than others. HCl extracted maximum amount of Cu ions for both the samples from Gopalganj and Bagerhat peats. Conversely, extractant from neutral salt, NH_4Cl , extracted ions from the soils with least efficacy both by single and sequential processes. However, extraction of Cu ions by chelating agents like 0.005M DTPA was also mentionable.

The concentration of extractable Cu increases with drying of the soils and decreases gradually with subsequent wetting of the samples. Overall, single extraction was found to be more efficient in extracting Cu from the soil solutions than the sequential extractions for the extractants except for HCl in first drying cycle.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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