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Ratio of elements in humic acids extracted from restored mine soil as a measure of quality of carbon sequestered

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Abstract
Mining land loses large amounts of C (carbon) due to loss of topsoil and mechanical mixing of soil horizons. Biological reclamation of this soil stimulates SOC sequestration. A chronosequence study consisting of 8, 14 and 25years old reclaimed mine soils under *Azadirachta indica,Gmelina arborea*, *Dalbergia sissoo* at Gevra, Chhattisgarh, India was initiated to quantify the composition and ratios of humic acid. Carbon content of humic acid was highest in the case of *Azadirachta indica* followed by *Gmelina arborea* and *Dalbergia sissoo*. It had increased from 34.95% to 41.94%, 16.31% to 28.65% and 18.50% to 21.16% for *Azadirachta indica*, *Dalbergia sissoo* and *Gmelina arborea* with increase in year of reclamation for surface soil. Humic acid nitrogen content was found highest in the case of *Azadirachta indica* followed by *Dalbergia sissoo* and *Gmelina arborea.* N (%) in humic acid ranged from 1.29 for *Gmelina arborea* and 2.65 for *Azadirachta indica*. Highest C/N ratio was observed in case of *Gmelina arborea*. The C/N ratios of HA fractions ranged from 30.91 in *Gmelina arborea* to 15.27 in *Azadirachta indica* plantations. In case of C/H ratio, it ranged from 6.26 to 15.01 in restored mine soils. Thus, C/H ratio was significantly differing with slightly wider ratios in increasing depths. From elemental composition, it is evident that HAs contained more C than N and H. From the results of experiment we can say that humic acid C and N content improved with increase in year of reclamation due to enrichment in TOC and process of humification.

Keywords: Humic acid, humification, reclamation, C/N and C/H ratio

Introduction

Humic acids are one of the main components of humic substances of soils. Being a system, they reflect characteristics of the environment that forms them in their internal states, which affects the composition, structure, and properties of these acids. The provision stating that the latter correspond to bioclimatic conditions of their formation is not in doubt anymore, because, over a long history of study of humic acids of different types of soils, considerable data on their elemental composition and other characteristics have been accumulated, and their regular changes in the genetic and geographical aspects have been shown, as referred to in the original and summarized works of various authors (Kononova, 1963; Kononova *et al*., 1960; Dragunov *et al*., 1953) [8-9, 6]. The characteristics of humic acids, which are specific in relation to the environment that forms them, such as elemental composition, fractions of carbon of aliphatic and aromatic groups and their ratio, and optical and other properties, persist over time (Dergacheva, 2006, 2010, 2011), [2-3-4] and thus humic acids can be attributed to soil components that carry information on the natural conditions of the time of their formation (Dergacheva, 2008) [4]. This property of humic acids is the basis for the pedohumic diagnostic method and reconstruction of the paleoenvironment and its evolution (Dergacheva, 1997). One of the features of humic acids, which are specific in relation to the climate and natural environment as a whole and which adequately reflect the state of the environment during its formation period and persist in diagenesis, is the elemental composition and especially the ratio of main elements, which was pre viously shown on the limited data (Dergacheva,2011, 2008) [2-3]. In order to be able to use this indicator in the diagnosis and reconstruction of the degraded soil, a recent basis is needed—characteristics of the elemental composition of humic acids of restored soils that were formed in diverse environmental conditions.

Therefore, this study was conducted to assess the elemental composition and their ratios under three contrasting tree species, *viz.*,*Azadirachta indica*, *Dalbergia sisoo, Gmelina arborea* in three chronosequence (at 8, 14 and 25year after restoration) having similar soil-forming conditions.

Materials and Method

Location of the Study Area

The study was conducted in the Gevra open cast coal mining project operated by South Eastern Coalfields Ltd., situated in Korba district of Chhattisgarh. Opened in 1981, the Gevra opencast project covers an area of about 19.03 sq.km and has been described as the largest open cast mine in Asia and is the second largest in the world. On the average, the project produced 0.66 million m^3 of overburden per 1 million tonnes of coal each year. The Gevra opencast project lies between 22° 18’00" and 82° 39’30" N latitude and is at an elevation ranging from 288 m to 328 m above mean sea level. The climate of the area is dry to moist tropical. The temperature rises to 48°C in May and drops to 7°C in December. The average rainfall is 1265 mm. Large areas of the mining project were used for dumping of overburden. To stabilize the dumps, different species of plants have been planted. Samples were collected from different overburden dumping areas of the Gevra mining project that were under various stages of restoration process (8, 14, 25yrs) under *Azadirachta indica, Dalbergia sisoo, Gmelina arborea* plantation.

Isolation of Soil Humic Acids

Humic acid was extracted by the method of IHSS (1981) [7]. Under this procedure sieved and dried soil sample was equilibrated to a pH value between 1 to 2 with 1 *M* HCl at room temperature. After that solution volume was adjusted with 0.1 *M*HCl to provide a final concentration that has a ratio of 10 mL liquid/1 g dry sample. Shaking and decantation was done. Neutralization of the soil residue with 1 *M* NaOH to pH = 7 was performed then added 0.1 *M* NaOH under an atmosphere of N_2 to give a final extractant to soil ratio of 10:1. After 4hr shaking of the suspension it was left overnight and supernatant was collected by means of decantation or centrifugation. The supernatant was acidified with 6 *M* HCl with constant stirring to pH = 1 and then allowed the suspension to stand for 12 to 16 h. Centrifuge to separate the humic acid (precipitate) and fulvic acid (supernatant – FA Extract 2) fractions.

We redissolved the humic acid fraction by adding a minimum volume of 0.1 *M* KOH under N_2. Added solid KCl to attain a concentration of 0.3 *M* [K^+] and then centrifuged at high speed to remove the suspended solids. Humic acid was reprecipitated by adding 6 *M* HCl with constant stirring to pH = 1 and allow the suspension to stand again for 12 to 16 h. Centrifuged and discarded the supernatant. Humic acid was suspended to precipitate in 0.1 *M* HCl/0.3 *M* HF solution in a plastic container and shaked overnight at room temperature. Humic acid was centrifuged and repeated the HCl/HF treatment until the ash content was below 1%. Precipitate was transferred to a Visking dialysis tube by slurrying with water and dialyzed against distilled water until the dialysis water gives a negative Cl^- test with silver nitrate $AgNO_3$. After that humic acid was freezed and dried.

Total elemental compositions of Humic Acid

The total Carbon, Nitrogen, Hydrogen and Sulphur content of the HA was determined by CHNS analyzer model Flash EA 1112 series. The C/N and C/H atomic ratios were calculated by determining the ratio of C to N and C to H contents, respectively.

Statistical analysis of data.

Mean and standard deviation of the values calculated from replicated sample. Data obtained was analysed using ANOVA procedure and mean separated by DMRT.ANOVA for factorial RBD and RBD was carried out using the Agricole package of R statistical software in R studio (R Studio, 2014).

Result and Discussion

Humic acid (HA) form an important part of the organic matter in soil. Natural organic matter (OM) is the prime attribute and centre for most of the chemical, physical and biological processes occurring in environment and soil. Hence, organic fraction can exert a profound influence on soil properties and ecosystem functioning (Tan, 2003) [16]. The term soil organic matter (SOM) has been used to encompass all organic materials found in soil (Stevenson, 1994) [15], ranging from as low as < 0.5% carbon in Aridisols to 33% in Histosols (Sombroek *et al*., 1993) [14]. Elemental composition provides worthwhile information on the reactivity of humic substances in soil. Nature of OM (leaf litter, plant residues, FYM, organic manures etc.) added to the soils and its decomposition under varied climates (dry and transitional heavy rainfall zones) and land use management (forests, cultivated, barren etc) will influence the elemental composition of humic substances. Afforestation had a positive influence on HA-C. Carbon content of humic acid was highest in the case of *Azadirachta indica* followed by *Gmelina arborea* and *Dalbergia sissoo*. It had increased from 34.95% to 41.94%, 16.31% to 28.65% and 18.50% to 21.16% for *Azadirachta indica*, *Dalbergia sissoo* and *Gmelina arborea* with increase in year of reclamation for surface soil (Table-1). Highest carbon in *Azadirachta indica* due to frequent biomass additions to soil surface through leaf fall and other plant residues might have contributed to higher organic carbon. The C content (%) in humic acid fraction ranged from 31.15 in *Dalbergia sissoo* soils to 33.41 in *Azadirachta indica* plantations. Abakumov *et al*., (2012) [2] also reported increase in carbon content of humic acid with increase in year of reclamation. This increase probably was due to increasing in TOC content with an increase in year of reclamation.

N (%) in humic acid ranged from 1.29 for *Gmelina arborea* and 2.65 for *Azadirachta indica*. Intensive humification due to greater degree of condensation of aromatic rings in restored mine soil lead to greater N of humic materials than litter residues. Humic acid nitrogen content was found highest in the case of *Azadirachta indica* followed by *Dalbergia sissoo* and *Gmelina arborea* (Table 2). This may be due to presence of nitrogen-containing functional group in humic acid. In general, humus is enriched with nitrogen on the reclaimed sites (Abakumov *et al*., 2012) [1]. According to Orlov’s humus state classification scheme (Orlov *et al*., 2005) [11], enrichment of humus with nitrogen was intermediate in the reclaimed soils. Mean H (%) in humic acid ranged from 3.12% for *G. arborea* to 3.30 for *A. indica*. Mean H (%) was highest for subsurface soil (Table 3). In case of C/H ratio, it ranged from 6.26 to 15.01 in restored mine soils (Table 4). Thus, C/H ratio was significantly differing with slightly wider ratios in increasing depths. From elemental composition, it is evident that HAs contained more C than N and H. This could be explained by the fact that the formation of HA was accompanied by accumulation of carbon and nitrogen and loss of oxygen (Schnitzer, 2000) [13]. C/H ratios were found lowest for *Dalbergia sisso*o and according to Sartakov *et al*., 2017 the smaller this relationship; the greater role is played by the carbon atoms in the construction of molecular structures. The lower C/H ratio indicates a larger amount of saturated structures. In other words, an increase in the hydrogen content indicates a greater number of aliphatic carbons (CH_2) than aromatic carbons (C=C) (Traina *et al*., 1990) [17].

Highest C/N ratio was observed in case of *Gmelina arborea*. The C/N ratios of HA fractions ranged from 30.91 in *Gmelina arborea* to 15.27 in *Azadirachta indica* plantations (Table-5). The HA for *Gmelina arborea* restored soil recorded wider C/N ratios than *Azadirachta indica* restored soils and this may be due to N supplementation and its complexation with SOM (Martin *et al*., 1998). C/N ratio had decreased in 25 year of reclamation suggesting stability of carbon. Highest C/N ratio in case of *Gmelina arborea* indicated a high proportion of carbon than nitrogen for *Gmelina arborea*. According to Abakumov, (2012) [1] the stage of maximum changes in HA composition is from 7 to 10 years. This trend corresponded with the caloricity of HA, which tended to increase with increasing site age in the reclaimed chronosequence. In the revegetated sites, no trend was evident in the changing of HA composition, which can be explained by the heterogeneity of the ecosystem.

Table 1: Influence of tree species and years of reclamation on Humic acid N (%) at two depths

	Azadirachta indica				***Dalbergia sissoo***				***Gmelina arborea***			
Depth (cm)	**8yrs**	**14yrs**	**25yrs**	**Mean T*D**	**8yrs**	**14yrs**	**25yrs**	**Mean T*D**	**8yrs**	**14yrs**	**25yrs**	**Mean T*D**
0-15	2.13^{f}	3.67^{b}	3.14^{d}	2.98^{A}	0.85^{j}	0.74^{k}	0.96^{hi}	0.85^{E}	0.75^{k}	0.53^{m}	0.73^{kl}	0.67^{F}
15-30	3.15^{d}	0.87^{ij}	2.94^{e}	2.32^{B}	0.92^{hij}	0.97^{h}	3.33^{c}	1.74^{D}	0.64^{l}	1.64^{g}	4.36^{a}	2.21^{C}
Mean T*Y	2.64^{B}	2.27^{D}	3.04^{A}		0.89^{G}	0.85^{G}	2.15^{E}		0.70^{H}	1.09^{F}	2.54^{C}	
	Azadirachta indica				***Dalbergia sissoo***				***Gmelina arborea***			
Mean Tree	2.65^{A}				1.29^{C}				1.44^{B}			
	8 years				14 years				25 years			
Mean Year	1.41^{B}				1.40^{B}				2.58^{A}			
	0-15 cm				15-30 cm							
Mean Depth	1.50^{B}				2.09^{A}							

Means followed by the same letter(s) are not significantly different (*p<0.05*).

Table 2: Influence of tree species and years of reclamation on Humic acid C (%) at two depths

	Azadirachta indica				***Dalbergia sissoo***				***Gmelina arborea***			
Depth (cm)	**8yrs**	**14yrs**	**25yrs**	**Mean T*D**	**8yrs**	**14yrs**	**25yrs**	**Mean T*D**	**8yrs**	**14yrs**	**25yrs**	**Mean T*D**
0-15	28.34^{k}	34.95^{e}	41.94^{d}	35.08^{C}	28.39^{jk}	16.31^{p}	28.65^{hi}	24.45^{E}	28.56^{ij}	18.50^{o}	21.16^{n}	22.74^{F}
15-30	24.64^{m}	28.79^{h}	41.83^{d}	31.75^{D}	31.62^{g}	33.65^{f}	48.26^{b}	37.84^{B}	26.40^{l}	52.11^{a}	45.73^{c}	41.41^{A}
Mean T*Y	26.49^{H}	31.87^{E}	41.88^{A}		30.01^{F}	24.98^{I}	38.45^{B}		27.48^{G}	35.30^{C}	33.44^{D}	
	Azadirachta indica				***Dalbergia sissoo***				***Gmelina arborea***			
Mean Tree	33.41^{A}				31.15^{C}				32.08^{B}			
	8 years				14 years				25 years			
Mean Year	27.99^{C}				30.72^{B}				37.92^{A}			
	0-15 cm				15-30 cm							
Mean Depth	27.42^{B}				37.00^{A}							

Means followed by the same letter(s) are not significantly different (*p<0.05*).

Table 3: Influence of tree species and years of reclamation on Humic acid H(%) at two depths

	Azadirachta indica				***Dalbergia sissoo***				***Gmelina arborea***			
Depth (cm)	**8yrs**	**14yrs**	**25yrs**	**Mean T*D**	**8yrs**	**14yrs**	**25yrs**	**Mean T*D**	**8yrs**	**14yrs**	**25yrs**	**Mean T*D**
0-15	2.16^{o}	3.66^{f}	4.35^{d}	3.39^{B}	4.54^{c}	2.51^{k}	2.42^{l}	3.16^{E}	2.40^{lm}	2.79^{i}	2.07^{p}	2.42^{F}
15-30	3.14^{h}	2.25^{n}	4.25^{e}	3.21^{C}	2.38^{m}	2.38^{m}	4.79^{b}	3.19^{D}	2.66^{j}	3.47^{g}	5.30^{a}	3.81^{A}
Mean T*Y	2.65^{G}	2.95^{F}	4.30^{A}		3.46^{D}	2.45^{I}	3.61^{C}		2.53^{H}	3.13^{E}	3.68^{B}	
	Azadirachta indica				*Dalbergia sissoo*				*Gmelina arborea*			
Mean Tree	3.30^{A}				3.17^{B}				3.12^{C}			
	8 years				14 years				25 years			
Mean Year	2.88^{B}				2.84^{C}				3.86^{A}			
	0-15 cm				15-30 cm							
Mean Depth	2.99^{B}				3.40^{A}							

Means followed by the same letter(s) are not significantly different (*p<0.05*).

Table 4: Influence of tree species and years of reclamation on Humic acid C/N ratioat two depths

	Azadirachta indica				***Dalbergia sissoo***				***Gmelina arborea***			
Depth (cm)	**8yrs**	**14yrs**	**25yrs**	**Mean T*D**	**8yrs**	**14yrs**	**25yrs**	**Mean T*D**	**8yrs**	**14yrs**	**25yrs**	**Mean T*D**
0-15	13.29^{h}	29.95^{ef}	13.51^{h}	12.11^{D}	33.45^{cd}	22.06^{g}	29.95^{ef}	28.49^{B}	37.97^{b}	34.79^{c}	29.13^{f}	33.96^{A}
15-30	7.83^{j}	33.24^{cd}	14.23^{h}	18.43^{C}	34.25^{c}	34.70^{c}	14.48^{h}	27.81^{B}	41.37^{a}	31.71^{de}	10.49^{i}	27.86^{B}
Mean T*Y	10.56^{G}	21.39^{D}	13.87^{F}		33.85^{B}	28.38^{C}	22.21^{D}		39.67^{A}	33.25^{B}	19.81^{E}	
	Azadirachta indica				*Dalbergia sissoo*				*Gmelina arborea*			
Mean Tree	15.27^{B}				28.15^{B}				30.91^{A}			
	8 years				14 years				25 years			
Mean Year	28.03^{A}				27.67^{A}				18.63^{B}			
	0-15 cm				15-30 cm							
Mean Depth	24.85^{A}				24.70^{A}							

Means followed by the same letter(s) are not significantly different (*p<0.05*).

Table 5: Influence of tree species and years of reclamation on Humic acid C/H ratio at two depths

	Azadirachta indica				*Dalbergia sissoo*				*Gmelina arborea*			
Depth (cm)	**8yrs**	**14yrs**	**25yrs**	**Mean T*D**	**8yrs**	**14yrs**	**25yrs**	**Mean T*D**	**8yrs**	**14yrs**	**25yrs**	**Mean T*D**
0-15	13.10^{d}	9.56^{j}	9.62^{j}	10.76^{C}	6.26^{o}	6.49^{n}	11.82^{f}	8.19^{F}	11.88^{f}	6.62^{m}	10.20^{g}	9.57^{E}
15-30	7.84^{l}	12.81^{e}	9.83^{i}	10.16^{D}	13.26^{c}	14.12^{b}	10.08^{h}	12.49^{A}	9.91^{i}	15.01^{a}	8.64^{k}	11.18^{B}
Mean T*Y	10.47^{D}	11.18^{A}	9.73^{F}		9.76^{F}	10.31^{E}	10.95^{B}		10.89^{B}	10.82^{C}	9.42^{G}	
	Azadirachta indica				*Dalbergia sissoo*				*Gmelina arborea*			
Mean Tree	10.46^{A}				10.34^{B}				10.38^{B}			
	8 years				14 years				25 years			
Mean Year	10.37^{B}				10.77^{A}				10.03^{C}			
	0-15 cm				15-30 cm							
Mean Depth	9.51^{B}				11.28^{A}							

Means followed by the same letter(s) are not significantly different ($p<0.05$)

Conclusion

Carbon content of humic acid was highest in the case of *Azadirachta indica* followed by *Gmelina arborea* and *Dalbergia sissoo*. It had increased from 34.95% to 41.94%, 16.31% to 28.65% and 18.50% to 21.16% for *Azadirachta indica*, *Dalbergia sissoo* and *Gmelina arborea* with increase in year of reclamation for surface soil. Humic acid nitrogen content was found highest in the case of *Azadirachta indica* followed by *Dalbergia sissoo* and *Gmelina arborea*. N (%) in humic acid ranged from 1.29 for *Gmelina arborea* and 2.65 for *Azadirachta indica*. Highest C/N ratio was observed in case of *Gmelina arborea*. The C/N ratios of HA fractions ranged from 30.91 in *Gmelina arborea* to 15.27 in *Azadirachta indica* plantations. In case of C/H ratio, it ranged from 6.26 to 15.01 in restored mine soils. Thus, C/H ratio was significantly differing with slightly wider ratios in increasing depths. From elemental composition, it is evident that HAs contained more C than N and H. From the results of experiment we can say that humic acid C and N content improved with increase in year of reclamation due to enrichment in TOC and process of humification.

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