



Research Article

Instrumental Characterization of Crop Residues and Coal Derived Humic Substances and their Impact on Soil Organic Matter

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Abstract | Post-harvest crop residues and coal have potential to be used for the extraction of humic substance, a macroorganic molecule. The humic substances were extracted, quantified and characterized using techniques such as spectrophotometer, High Performance Liquid chromatography, Fourier transform infrared spectroscopy and atomic absorption spectrophotometer. The analysis showed the presence of hydrophobic and hydrophilic sites. The Fourier infrared spectroscopy analysis showed spectrum bends at different wavenumbers while elemental analysis shows existence of different macro and micro nutrients which can be used to supplement the plant needs for nutrients by incorporation of humic substances along with commercial inorganic fertilizers. Though findings in this study showed KOH as cost effective extractant vis a vis NaOH. However, further characterization could not confirm it. The presence of hydrophobic and hydrophilic nature of sites could be utilized to develop slow release plant nutrients based on humic substances.

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Keywords | Humic substances, Extraction, Characterization, high pressure liquid chromatography, Fourier transmission infrared spectroscopy

Introduction

Soil organic matter (SOM) divided into three main pools: inert (humins), labile and stable. In broad sense it mainly consists of two fractions: Labile organic matter and humic substances (HSs). Humic substances further divided into humic acid (HAs), Fulvic acid (FAs) and humins. The humic substances are stable to variety of soils and act as a stable material (Stevenson, 1994).

The HSs primarily formed as a result of breakdown of flora and fauna in soil and structurally composed of polyelectrolyte organic compounds of condensed aromatic nature having different functional groups. Presence of hydrophilic and hydrophobic ends, and

functional groups such as -COOH, -OH, -C=O, suggesting that it is a pungent ring of di-hydroxyl phenyl and tri-hydroxyl phenyl type compound, linked together by-CH₂, -O, and -NH, other groups including aliphatic compounds, and yields negative charges in aqueous media (Cheng et al., 2002) such functional group, improve ion exchange properties of the soils. Recently there has been reported that HSs not only improve soil nutrients but crop productivity as well (Khan et al., 2018). The role of HSs in agricultural soils is established especially where soils are having low organic matter.

The percentages of HSs varies from 10–40%, (black peat) to 10–20%, (sapropel peat), 10–30%, (brown coal) 2–5% (compost materials) and 1–5% in soil

sludge (Fong et al., 2006). It has been shown that the amount of extractable HAs depend on several factors such as type and source of substrate materials, strength and kind of extracting agent and frequency of extraction etc. In Pakistan existence of substantial coal reserve (186 billion tons), ranking from lignitic to sub-bituminous (Akhtar et al., 2016; Mustafa et al., 2016), couples with ample post-harvest crop residues could provide economic option to be used for extraction of HSs, and utilized along with mineral fertilizers on soils which are inherently low in organic matter (OM<1%). There exists variation among different coal samples collected from various coal mines in Pakistan, while comparing three coal samples collected from Thar, Chakwal and Quetta there has been reported that Chakwal coal ranked second highest fixed C after Quetta coal (44% vs. 48%), and lowest gross calorific value (5204 kcal/kg against 5262 and 6136 in coal from Quetta and Thar area respectively (Mustafa et al., 2016) shown in Table 1.

Table 1: Elemental composition of Humic substances derived from different coal samples.

Lignitic coal	Mois- ture	Volatile matters	Ash %	Fixed carbon	Gross calorific value Kcal/kg
Thar	24.8	34.2	36.9	34.1	6136
Chakwal	15.9	14	25.8	44.3	5204
Quetta	9.9	11.9	29	48.2	5262

Several destructive and constructive methods such as ultra-violet (UV-VIS) spectroscopy, High Performance Liquid Chromatography (HPLC), Fourier transform Infrared (FTIR) spectroscopy, and atomic absorption spectroscopy (AAS) are employed aiming at understanding structure, composition and properties of HSs.

Generally, two extracting agent such NaOH and KOH have been used for extraction of HSs from coal and post-harvest crop residues following characterization through HPLC, FTIR and AAS so that its potential use as soil conditioners and plant growth promoter could be evaluated along with its potential role in developing slow released fertilizer. An incubation experiment was also conceded out to assess the role of HSs on soil organic matter (SOM). Overall aim of the experiment was to assess post-harvest crop residues and coal for HSs and characterize their chemical properties.

Materials and Methods

Chemical analysis of crop residues and coal samples

Crop residues were collected from crop experimental area in NARC, Islamabad and coal samples were collected from Chakwal coal reserves shown in Figure 1.

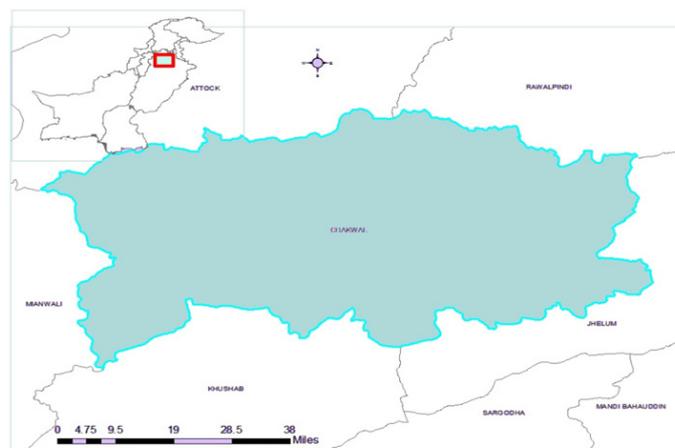


Figure 1: The map of Chakwal district.

Extraction of humic substances from crop residues and coal

Extraction of HA from coal samples were carried out as reported (Khan et al., 2014), briefly described by grinding, sieving (2 mm) and treated coal samples with KOH (0.5 N) and NaOH (0.5 N) solutions separately, filtered, centrifuged (5000 rpm; 5 min), sieved and dried to get HSs. From plant materials HSs was extracted following briefly described by soaking plant residues overnight in KOH (0.1 N), stirred; sieved (mesh No. 115) and diluted to 2 L with distilled water, washed again followed by sieving and stored. The pH of HSs extracted was adjusted to 7-8 using diluted H₂SO₄ (Khan et al., 2014).

Characterization of humic substances

Determination of humic substances through spectrophotometer: Humic substances were extracted from crop residues and read on spectrophotometer at 450 nm wavelength.

High performance liquid chromatography of crop derived humic substances

The HPLC chromatograms of PD HAs were separated by acetonitrile/water solution. For HPLC analysis, humic acid at rate of 0.05-0.2 gram per liter was poured to Machery Nagel RP C18 column (200 mm×4 mm), particle size of 7.00 micrometer along with 1000 Å pore size. Sample of the purified and dialysed HAs was dissolved in 5 ml NaOH (0.1 M) using ultrasonic bath for 15 min., diluted, and

neutralized with 0.1 M HCl (pH= 7). The wavelength UV-VIS of 254 and 280 nanometer were used to detect chromatograms and 470-475 nm wavelength were used to detect fluorescence emissions. The standard HA materials obtained from Aldrich were run in comparison with plant derived HSs

Fourier transforms infra-red spectroscopy of crop residues and coal derived humic substances

The Fourier infra-Red spectroscopy (FTIR) measurement of coal derived humic acid (CDHA), and plant derived humic substances (PDHSs) sample were carried out on for functional group description. For this purpose, finely powdered mixture of Potassium bromide (KBr) (300 mg) and HA sample (2 mg) was pressed at 10 tons pressure for 10 min to produce pellets. The FTIR spectra of pellets sample were recorded on Perkins Elmer Spectrum RX1 FTIR system on a spectral range of 4000-4000 cm^{-1} wave number.

Chemical analysis of crop and coal derived humic substances

Macro nutrients (N, P and K) and micronutrients such as Zn, Fe, Cu and Mn were examined in coal derived HSs after double acid digestion (HNO_3 and HClO_4) in 2:1 ratio and were subsequently analyzed with AAS.

Incubation study on temporal impact of humic substances on soil organic matter content

Soil physio-chemical properties listed in Table 2 such as pH (1:1), Electrical conductivity (EC), $\text{NO}_3\text{-N}$, P and K contents were determined using standard procedure and listed in was amended with sunflower derived humic substances (SFDHS), maize derived humic substances (MZHS) and coal derived humic substances (CDHA), and distilled water only (DW) as control treatment. Amended soil samples were kept in incubator at $27\pm 1^\circ\text{C}$. Soil sampling were collected at prescribed interval time to analyses for total organic matter was measured by wet oxidation method (Walkley et al.,1934).

Results and Discussion

Determination of humic substances through spectrophotometer

The HSs extracted from various crop/plant residues materials and were quantified by UV-VIS spectrophotometer. Results showed that concentration ranged

from 1900-2600 mg L^{-1} . The highest concentration (2600 mg L^{-1}) was observed from Helianthus Annus L. followed by 2500 and 2400 mg L^{-1} in Vigna radiate and Morus nigra respectively (Figure 2).

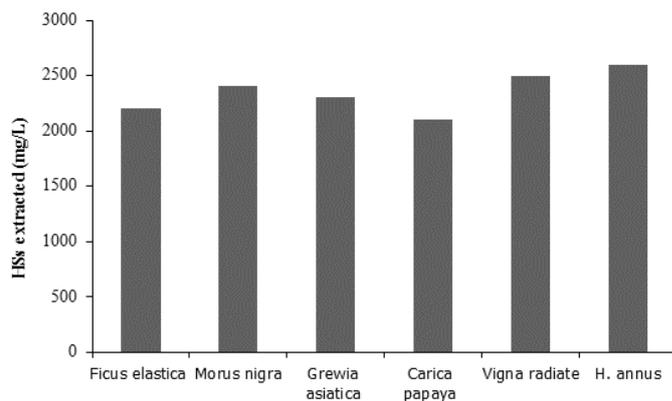


Figure 2: Relative proportion of HSs in different crop residues.

Humic substances recovery and chemical composition

Different low concentrated alkaline solutions had been used to extract HSs (Garcia et al., 1996). In the current study two extractants; KOH and NaOH were used to extract HSs from coal. Results show that 33% HSs recovery was obtained by using KOH as extractant against 20% using NaOH showing about 65% greater recovery efficiency by the use of KOH (Table 1). Earlier research also classified KOH as efficient extractant against NaOH. Earlier research found while comparing three different extractant for HSs, found that 0.25 M KOH was more effective in comparison to NaOH and $\text{Na}_4\text{P}_2\text{O}_7$ (Rocha et al., 1998). The response could be ascribed to lower ion radius (300×10^{-9} m) of hydrated K^+ against 450×10^{-9} m (hydrated Na^+) causing counterbalance, greater expansion and capacity of interaction of the inter and intramolecular charges in HSs (Chiang et al., 2000). The recovery of HSs from coal sample used in this study was 20-33% in both extractants greater than 2% as reported (without acid pre-treatment) and increased to an average of 80% by pre-treating the same coal sample with HNO_3 . Though NaOH is relatively low cost, it has found to have many limitations by adding as much as 7.5% Na content with the use of Na-humate (Garcia et al., 1996). The use of KOH as extracting agent have many benefits such as higher HAs recovery, and providing K as plant nutrient as well.

Elemental composition of coal derived HSs on ash basis using KOH and NaOH as extractants listed in Table 2 shows that N contents varies from 0.57%-1.53%, P contents varies from 0.04%-0.07% and K

contents varies from 0.30% to 0.44%. Micronutrients such as Zn, Cu, Mn and Fe was recorded as 35.2-59 mg L⁻¹ (means=47 mg L⁻¹), 5.4-19.2 mg L⁻¹ (mean=12.3 mg L⁻¹), 42-79 mg L (mean=60.5 mg L⁻¹) and 2434-8830 mg L⁻¹ (mean=5632 mg L⁻¹), respectively. Despite Fe coal extracted with either KOH, or NaOH show no difference in elemental composition. On average 34% more Fe was reported from coal derived humic acid (CDHA), where NaOH was used as extractant.

Chemical analysis of crop residues (Table 3) shows P ranges from 0.20% to 0.05%, K ranges from 0.74

to 0.90%. Among micronutrients Zn dominates in Ficus elastic as ~19 mg kg⁻¹ followed by ~16 mg kg⁻¹ in Vigna radiate.

Instrumental characterization of humic substances
High performance liquid chromatography of plant derived humic substances: The HPLC analysis in Figure 3 show that the first peak seemed between tR=1.5-3.5 relates the excluded fraction. In plant derived humic substances (PDHS) both sunflower and Maize chromatograms indicated sharp changes in the peak area. The fraction peak at tR=2-2.4 and tR=2 included the major contributions of hydrophilic end.

Table 2: Recovery and elemental composition of Humic substances extracted by different extractants.

Source	Recovery	Nitrogen	Phosphorus	Potassium	Zinc	Copper	Manganeze	Iron
			%				mg/kg	
Extracted with KOH	33±1	1.20	0.04	0.32	35.2	09.4	43	6068
Extracted with NaOH	22±1	1.34	0.07	0.44	34.2	11.4	42	8124

Table 3: Elemental composition of Humic substances derived from crop residues.

Sample ID	Phosphorus	Potassium	Calcium	Magnesium	Iron	Manganeze	Zinc
			%			mg/kg	
Helinathus annus	0.05	0.74	45.30	48.10	6.60	0.60	9.40
Ficus elastica	0.04	0.90	94.35	99.05	8.00	0.55	18.8
Vigna radiate	0.02	0.80	51.70	9.60	10.00	0.50	15.0

Table 4: Assignment of Absorption Peaks, bands in FTIR spectra, with associated funtional groups.

Wavelength (cm ⁻¹)	Assigned functional group	Reference
3300, 3400	H bonded OH group alcohol, phenol , organic acids	Chen <i>et al.</i> (2002)
2950	Methyl CH assymmetric CH ₃	Cheng <i>et al.</i> 2006
2830, 2855	Methlen assumetirc stretch -HC ₂	
1750	C=O stretching	
1620	Aromatic and Oleifinic, C=O of bonded congjugated Ketones, quinine	Duggan <i>et al.</i> , (1997)
1600	C=C, C=O stretching congjugated to the aromatic ring	Wang <i>et al.</i> , (2009)
1500, 1508	Aromatic ring vibrations combined with C-H in plane deformation	Smith and Chugtai. (1995)
1433, 1459	C-H deformation, asymmetric in CH ₃ and CH ₂	Sharma <i>et al.</i> , (2004)
1400, 1420	Aromatic ring vibration, combined with C, H in plan deformation	Smith and Chugtai (1995)
1329	Syringyl ring with C-O stretching	
1270	Guaiacyl ring with C-O stretching	
1223, 1220	C-C plus C-O , C=O stretching	Wang <i>et al.</i> , (2009)
1220	C-C or CO stretching	
1133, 1140, 1157	C-O stretching vibration of C-O-C group	Sharma <i>et al.</i> , (2004)
1100	C-O stretching vibration for C-CH ₃ and C-OH group	Pradhan and Sandle (1998); Sharma <i>et al.</i> , (2004)
1031	Aromatic CH deformation and CO stretching vibration	Wang <i>et al.</i> , (2009)
1010, 1000	C-O-C group	Arriagada <i>et al.</i> , (1997)
875, 878	CO ₃	
712, 715	Deformation vibration of planne CO ₃	

While, fractions in $tR=14-16.5$ endorsed to hydrophobic character of HSs. The changes in chromatograms of HSs could be ascribed to structural variation of PDHSs.

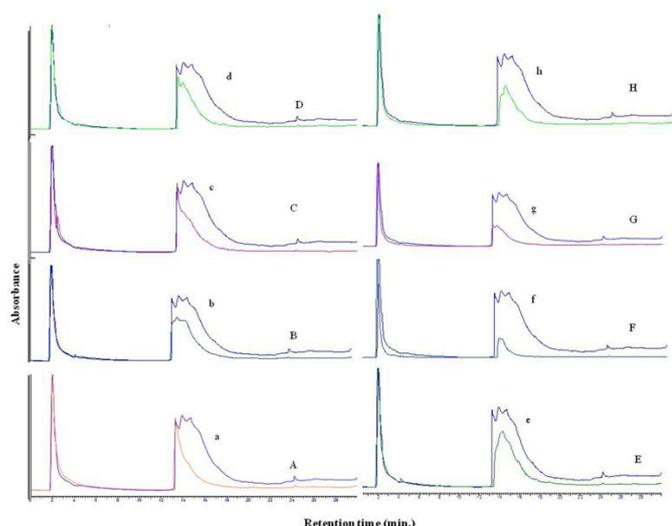


Figure 3: HPLC separations HA commercial and HAs derived from different plant materials; (A) Sunflower (B) Soybean (C) Rubber plant (D) Mulberry (E) Mung (F) Falsa (G) Rubber plant and (H) Papaya with corresponding HA standard material shown as a, b, c, d, e, f, g and h.

First peak at 2-3 minutes corresponded to the excluded fraction of all samples. Major difference among chromatogram of PDHA appear at $tR=13-18$ min., and at the $tR=13-18$ min. Separation of HSs are based on the differences in hydrophobicity, hence the fraction at $tR=3.8-4$ minutes comprises the largest contribution of hydrophilic feature, while at $tR=13-15$ minutes 3 hydrophobic fractions; F2, F3 and F4 were identified. The peak observed at $tR= 13$ minutes is more pronounced when plant residues were sunflower vis a vis plant residue of soyabean where though low peak was observed at $tR=13.8$ minutes preceded and proceeded by more peaks of relative lower height at $tR=13-13.8$ and $tR=14-15$ minutes.

Fourier transforms infra-red spectroscopy of coal derived humic substances

The FTIR analysis of HSs (Figure 4) extracted by showed that at around 2923.4 cm^{-1} sp^3 hybridization ($1s+3p$), forming C-H stretching. As aliphatic, it contains only C and H atoms. The functional group found at around $1558-1741\text{ cm}^{-1}$ linked with C=O section of-COOH. The RCOOH arises from the hydrolysis of ester group to -COOR linking gallo tannin to carbohydrate moieties. The spectrum identified at $1035-1108.8\text{ cm}^{-1}$ corresponded to Si-O vibration of clay minerals, as reported (Stevenson,

1994). Presence of Si-O at this wave number could be ascribed to the purification process hence showing the existence of clay minerals. Such behavior could be ascribed either hard to wash off the coal sample or the coal sample could not purify properly. Based on the Si-O it can be postulated that HSs co-precipitated with clay particles, a characteristic of organic metallic compounds Carboxylic acid group associated with 2 characteristic infrared stretch absorption changed with H bonding. The -OH stretch immersion for such dimmer is very resilient and broad extending from $2500-3300\text{ cm}^{-1}$ this immersion overlap the sharper C-H elongating peak which can be noticed at around $2990, 2950, 2870$ and 2851 cm^{-1} (Table 4). There were reported major peaks in Chakwal coal at 3433 cm^{-1} corresponded to alcohols, phenols, carboxylic acids stretching, peak at $1570-1577$ related to C = C ring and COO- stretching, peak at 1375 associated to C-H functional groups, and peak at 1090 ascribed to Si-O functional groups and metal oxygen stretching (Chen et al., 1997).

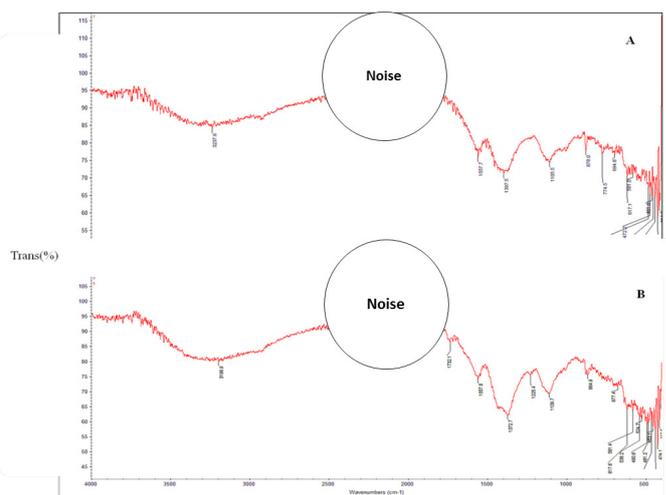


Figure 4: Comparison of CDHA extracted with NaOH (A) and KOH (B).

Band appeared at $834-874\text{ cm}^{-1}$, related to C-H vibration of syringyl units. The band evident at $1325-1380\text{ cm}^{-1}$ is indicating C-O stretching vibration attributed to syringyl ring; as reported by Wang et al. (2009). A band at $\sim 1750\text{ cm}^{-1}$ as observed in both H could be ascribed to C=O stretching (Chiang et al., 2000). The spectra of both KOH and NaOH extracted HA yield at $2925-2928\text{ cm}^{-1}$ mainly related to C-H stretching of alkyl structure (Galantini et al., 2006), and specifically to methyl (CH_3) and methylene (CH_2) asymmetric stretching. A band at $\sim 1400\text{ cm}^{-1}$ corresponding to the C-H bending (Smith et al., 1995) was found in both HSs sample (Figure 3). Band at $\sim 723\text{ cm}^{-1}$ could be attributed to the plane

deformation vibration of the planar CO₃ unit.

Temporal impact of humic substances on soil organic matter

Soil amended with HSs (Figure 5) showed that soil organic matter (SOM) were recorded as 1.05%, 1.04%, 0.78% and 0.42% in soil amended with coal derived humic acid (CDHA), sunflower derived humic substances (SFDHS), maize derived humic substances (MZDHS) and distilled water (DW) respectively during first fortnight followed by almost similar trend in second and third fortnight. On average there was observed two classes of impact on SOM *viz* an increase of 0.42% in MZDHS and DW, and more than double increase of 0.9-1% in soil amended with SFDHS and CDHA.

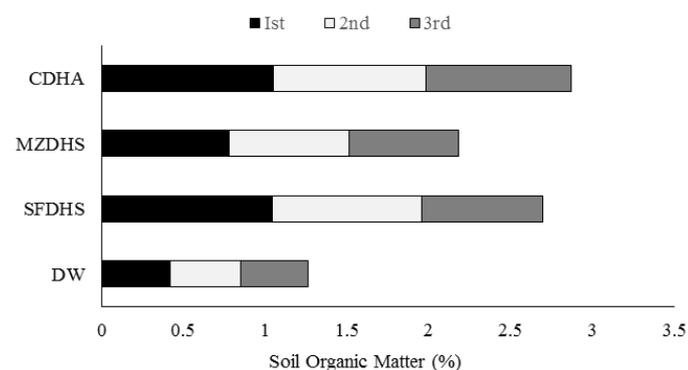


Figure 5: Response of SOM to HSs extracted from different sources.

Conclusions and Recommendations

This study identified and confirmed the existence of different functional groups in humic substances derived from various sources. The existence of hydrophobic and hydrophilic end sites show the potential capacity of such substances in developing slow released fertilizers. Increase in soil organic matter can have potential impact on its use in improving soil health.

Acknowledgements

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Novelty Statement

The existence of hydrophobic ends in plant and coal derived humic substance can be used in development

of slow released fertilizer to counter the leaching as a result and improve nutrients use efficiency.

Author's Contribution

Raza Ullah Khan: Planned, analyzed, wrote the draft and technical input.

Ahmad Khan: Overall Planned, execution, correction and formatted.

Mohammad Khan, Fayyaz Hussain and Zafar Islalm: Reviewed and corrected.

Muhammad Asad Hameed: Drafted, reviewed and writeup.

Conflict of interest

The authors have declared no conflict of interest.

References

- Ahmad, K., R.U. Khan, M.Z. Khan, F. Hussain and M.E. Akhtar. 2013. Characterization and effects of plant derived humic acid on the growth of pepper under glasshouse conditions. *Pak. J. Chem.*, 3: 134-139. <https://doi.org/10.15228/2013.v03.i03.p07>
- Akhtar, J., S. Rehman, N. Sheikh and S. Munir. 2016. Agglomeration of Pakistani coal using diesel oil. *Energy Sour.*, 38: 3144-3149. <https://doi.org/10.1080/15567036.2015.1136977>
- Chen, X., S. Jeyaseelan and N. Graham. 2002. Physical and chemical properties study of the activated carbon made from sewage sludge. *Waste Manage.*, pp. 755-760. [https://doi.org/10.1016/S0956-053X\(02\)00057-0](https://doi.org/10.1016/S0956-053X(02)00057-0)
- Chen, Y., N. Senesi and M. Schnizer. 1997. Information provided on humic substance by E₄/E₆. *Soil Sci. Soc. Am. J.*, 41: 352-358. <https://doi.org/10.2136/sssaj1977.03615995004100020037x>
- Cheng, C.H., J. Lehman, J.E. Theies, S.D. Burton and M.H. Engelhard. 2006. Oxidation of black carbon by biotic and abiotic process. *Org. Geochem.*, 37: 1477-1488. <https://doi.org/10.1016/j.orggeochem.2006.06.022>
- Cheng, W.P. and F.H. Chi. 2002. A study of coagulation mechanisms of polyferric sulfate reacting with humic acid using a fluorescence quenching method. *Water Res.*, 36: 4583-4591. [https://doi.org/10.1016/S0043-1354\(02\)00189-6](https://doi.org/10.1016/S0043-1354(02)00189-6)
- Chiang, H.L., H. Tsai, C.L. Tsai and Y.C. Hsu.

2000. Adsorption characteristics of alkaline activated carbon exemplified by water vapor, H₂S and CH₃SH Gas. Sep. Sci. Tech., 35: 903-918. <https://doi.org/10.1081/SS-100100200>
- Dugan, O. and S.J. Allen. 1997. Study of the physical and chemical characteristics of a range of chemically treated, lignite based carbon. Water Sci. Tech., 35(7): 21-27. <https://doi.org/10.2166/wst.1997.0256>
- Duggan, O. and S.J. Allen. 1997. Study of the physical and chemical characteristics of a range of chemically treated, lignite based carbons. Water Sci. Tech., pp. 35. [https://doi.org/10.1016/S0273-1223\(97\)00110-8](https://doi.org/10.1016/S0273-1223(97)00110-8)
- Fong, S.S., L. Seng, W.N. Chong, J. Asing. M. Faisal, M. Nor and A.S.B.T.M. Pauzan. 2006. Characterization of the coal derived humic acids from Mukah, Sarawak as soil conditioner. J. Braz. Chem. Soc., 17: 582-587. <https://doi.org/10.1590/S0103-50532006000300023>
- Galantini, J. and R.B. Ramon. 2006. Long term fertilization effects on soil organic matter quality and dynamics under different production system in semiarid Pampean. Soil Till. Res., pp. 72-87. <https://doi.org/10.1016/j.still.2005.02.032>
- Garcia, D., J. Cegarra and A. Abad, A. 1996. A comparison between alkaline and decomposing reagent for extraction of humic acid from low rank coals. Fuel Prod. Tech., 48: 51-60. [https://doi.org/10.1016/0378-3820\(96\)01025-9](https://doi.org/10.1016/0378-3820(96)01025-9)
- Gunzler, H. and H. Bock and I.R. Spektroskopie. 1990. Verlag Chemie. Weinheim, Germany.
- Gup, Y. and R.M. Bustin. 1998. FTIR spectroscopy and reflectance of modern charcoal and fungal decayed woods: Implication for studies of inertinite in coals. Int. J. Coal Geol., 37: 29-53. [https://doi.org/10.1016/S0166-5162\(98\)00019-6](https://doi.org/10.1016/S0166-5162(98)00019-6)
- Khan, M.Z., M.E. Akhtar, S. Ahmad, A. Khan and R.U. Khan. 2014. Chemical composition of lignitic humic acid and evaluating its positive impacts on nutrients uptake, growth and yield of maize. Pak. J. Chem., 4: 19-25. <https://doi.org/10.15228/2014.v04.i01.p04>
- Khan, R.U., M.Z. Khan, A. Khan, S. Saba, F. Hussain and I.U. Jan. 2018. Effect of humic acid on growth and crop nutrient status of wheat on two different soils. J. Plant Nutr., 41: 453-460. <https://doi.org/10.1080/01904167.2017.1385807>
- Koyo, Y. and H. Tomoo. 1988. Chemical and biological studies on environmental humic acids. J. Soil Sci. Plant Nutr., 34: 571-584. <https://doi.org/10.1080/00380768.1988.10416472>
- Kumada, K., 1987. Chemistry of soil organic matter. Tokyo: Japan Sci. Soc. Press, pp. 241.
- Maghoddloo, S., B. Noroozi, A.K. Haghi and G.A.J. Sorial. 2011. Hazard. Matt., 191: 380-387. <https://doi.org/10.1016/j.jhazmat.2011.04.096>
- Mustafa, A., T. Ahmad, J. Akhtar, K. Shahzad, N. Sheikh and S. Munir. 2016. Agglomeration of Makarwal coal using soybean oil as agglomerant. Eng. Sour., 38: 3733-3739. <https://doi.org/10.1080/15567036.2016.1141268>
- Rocha, J.C., A.H. Rosa and M. Furlan. 1998. An alternative methodology for the extraction of humic substances from organic soils. J. Braz. Chem. Soc., 9: 51-56. <https://doi.org/10.1590/S0103-50531998000100010>
- Smith, D.M. and A.R. Chughtai. 1995. The surface structure and reactivity of black carbon. Coll. Surf., 105: 47-77. [https://doi.org/10.1016/0927-7757\(95\)03337-1](https://doi.org/10.1016/0927-7757(95)03337-1)
- Stevenson, F.J., 1994. Genesis, composition and reaction in humas chemistry. 2nd Ed. John Wiley and Son, New York. pp. 340.
- Walkley, A and I.A. Black. 1934. An examination of Different method for determining soil organic matter and a proposed modification of the chromic acid in soil analysis. I. Exp. Soil Sci., 79: 459-465.
- Wang, S.K., K. Wang, Q. Lie, Y. Gu, Z. Luo, K. Cen and T. Fransson. 2009. Comparison of the pyrolysis behavior of lignins from different tree species. Biotech. Adv., pp. 562-563. <https://doi.org/10.1016/j.biotechadv.2009.04.010>
- Woelki, G., S. Friedrich, G. Hanschmann and R. Salzer. 1997. HPLC fractionation and structural dynamics of humic acid. Fresenius. J. Anal. Chem., 357: 548-552. <https://doi.org/10.1007/s002160050211>
- Woelki, G., S. Friedrich, G. Hanschmann and R. Salzer. 1997. HPLC fractionation and structural dynamics of humic acid. Fresenius. J. Anal. Chem., 357: 548-552. <https://doi.org/10.1007/s002160050211>