

Review: Humic and Fulvic Acids.

A Practical Approach.

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1 GENERAL DESCRIPTION AND BASIC CHEMISTRY

Waksman defined humus as "a complex aggregate of brown to dark colored amorphous substances, which have originated during the decomposition of plant and animal residues by microorganisms, under aerobic and anaerobic conditions, usually in soils, composts, peat bogs, and water basins".

Soil Organic Matter

Soil Organic Matter or humic substances (HS) which are the largest constituent of soil organic matter (~60%) and are considered as a key component of sustainable agricultural practices and terrestrial ecosystems since they are responsible for many complex chemical reactions in soil¹. HS are often divided into humic and non-humic substances. In soil, one of the most important characteristics of HS is their ability to interact with metal ions, oxides, hydroxides, mineral and organic compounds,² also toxic pollutants¹⁶⁻¹⁸ and to form water-soluble and insoluble complexes. Accumulation of such complexes in soil can contribute to a reduction of toxicity, e.g., of aluminium (Al),^{19,20} or removal of heavy metals like Cr from aqueous solutions.²⁵

HS can interact with xenobiotic organic molecules such as pesticides^{15,23,24} and enhance nutrient availability (N, S, P), especially of those nutrients present at a very low concentration.^{8,14,22} The latter being directly involved to negate various morphological, physiological and biochemical effects on higher plants²⁷⁻²⁹ that impacts on yield and quality.

Non-Humic Substances can be defined as organic chemical compounds that can be categorized in one of the known classified compounds such as amino acids (Fig 1), sugars (Fig 2), fats and organic acids like citric acid (Table 1).

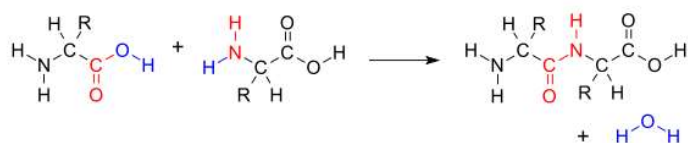


Fig 1: Peptide bond between amino acids

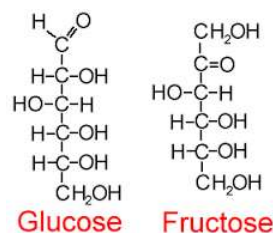


Fig 2: Typical sugar molecules showing carboxylic acid, hydroxyl and aliphatic groups

Table 1: Typical carboxylic acid groups associated with organic acids are shown in the following table.

Carbon atoms	Common name	Chemical formula	Common location or use
1	<u>Formic acid</u>	HCOOH	Insect stings
2	<u>Acetic acid</u>	CH ₃ COOH	Vinegar
3	<u>Propionic acid</u>	CH ₃ CH ₂ COOH	Preservative for stored grains
4	<u>Butyric acid</u>	CH ₃ (CH ₂) ₂ COOH	Butter

Humic Substances are the unknown and unidentifiable components that are found in extracts of organic sources like compost, peat, lignite etc. that usually are present as a “soup” of small to large organic molecules that will also differ in composition depending on the source.

HS from both the humic and fulvic acid categories are made up of C, H, O, N and S. The difference being the size of the molecules (Humic acids 1500 – 3000 Å size or >3,500 Da molecular weight, Fulvic acids 800 Å and <3,500 Da) (*Beckett et al*, 1987 and *Muscolo et al*, 2007)^{29,30}.

Compounds from all the above groups will have one or more active functional chemical group (Fig 3) like carboxylic acid (COOH), hydroxyl (-OH), carbonyl (C=O), phenolic rings and quinone groups that can and will react to charged positive or negative ions in solution. *Muscolo et al* (2007)²⁹ have shown (table 2), using ¹H NMR spectroscopy that high relative molecular mass HS (HMr >3,500 Da) (humic acids) contained 20.2% of the larger higher carbon/oxygen ratio molecules consisting of complex aromatic and phenolic structures. The low relative molecular mass HS (LMr <3,500 Da) (fulvic acids) did not contain any of these molecular structures in the molecules, with low carbon/oxygen ratio molecule carboxylic groups making up 34.4% of these more “simple” and reactive molecules.

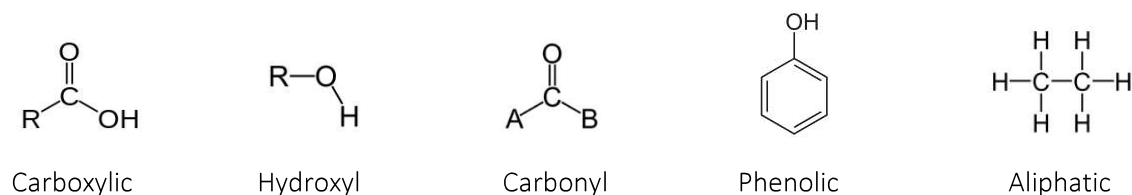


Fig 3: Functional groups associated with Humic Substances

Table 2: Distribution of C intensity in different regions (ppm) of ¹³C NMR spectra of high (HMr) and low (LMr) relative molecular mass humic fractions

Humic Fraction	Relative Intensity [Total Area Percentage (%)]				
	0–48	48–105	105–145	145–165	165–190
	Aliphatic C	Peptidic and carbohydrate C	Aromatic C	Phenolic C	Carboxylic C
LMr	26.7±0.2	38.9±0.4	Not detected	Not detected	34.4±0.2
HMr	24.7±0.3	41.0±0.3	16.0±0.2	4.2±0.1	14.1±0.3

Chemically, HS are **molecular aggregates** consisting of sugars (Fig 2), fatty acids, polypeptides (Fig 1), aliphatic and aromatic rings (Fig 3). HS are practically classified into humin and humic and fulvic acids in relation to different solubility at acid and alkaline pH ^{1,10} (Fig 4). This classification although based on superficial criteria, is of important

practical use in agriculture to separate different products with different applications. What is apparent is that HS consist of a heterogeneous mixture of compounds for which no single chemical structural formula will be fitting.

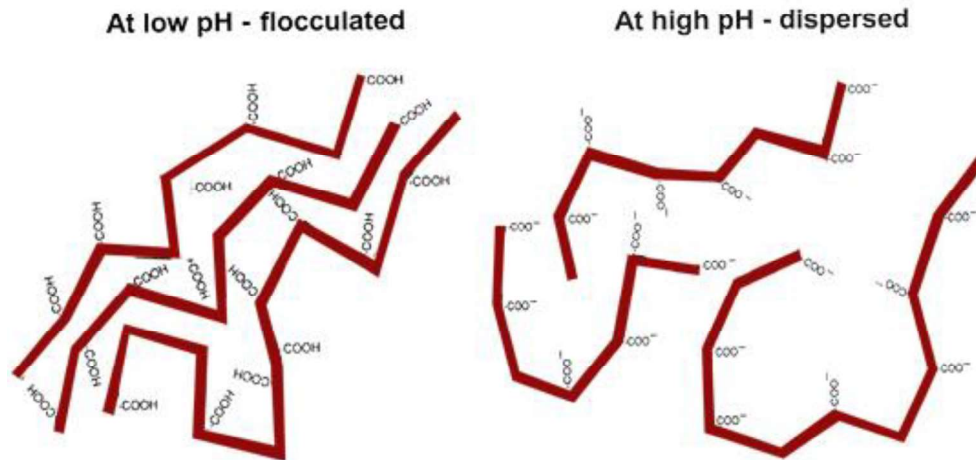


Fig 4: Flocculated or dispersed aggregate structure diagram as influenced by pH.

Therefore, HS that per definition does not fit into the separate classified organic acid, amino-acid or sugar groups as shown in Table 1 and Figs 1 and 2, **firstly** consist of plant and animal material resistant to decomposition (solid material or humin that is not soluble in water at any pH value or in alkaline solutions); **secondly**, substances in the process of decomposition (large molecules that are alkaline extractable, humic acids); **thirdly** a complex mixture of relatively smaller molecular size organic molecules resulting from humic acid decomposition mainly through microbial action and soluble in water at any pH (fulvic acids). Decomposition includes processes of hydrolysis or oxidation and reduction and microorganisms are always involved in these processes and will synthesize various compounds, usually of the fulvic acid type.

Humic acids (Table 3) are considered to be the high molecular weight, less oxidized black to dark brown substances and which is the fraction that is not soluble in water under acidic conditions ($\text{pH} < 2$) but is soluble at higher pH values. Humic acids are the major extractable component of soil HS and are the primary organic compounds of soil (humus, peat, compost, manure and coal).¹ Commercial humic acid products are usually a salt of Potassium, Sodium or Ammonium and due to the fact that it is extracted with the hydroxides of these ions, the solutions are alkaline and at a pH above 10. In productive agricultural soils, where the pH ranges between 5 and 7, a large portion of the humic acids from these sources occurs as precipitated particles (Figs 4 and 5) that cannot be taken up by plant roots. It can then only assist in enhancing the uptake and utilization of nutrient minerals from the soil by binding the charged minerals on its ion exchange sites and prevent it from reacting with phosphate anions (Ca^{2+} , Mg^{2+} , Fe^{2+} , Cu^{2+} , Zn^{2+}) to form insoluble phosphate compounds or with sulphate anions to form poorly soluble gypsum (CaSO_4)²². It does however contribute towards cation exchange capacity (CEC) in the soil at sufficient concentrations.

Fulvic acids (Table 3) are soluble in water under all pH conditions and remain in solution after removal of humic acid by acidification. Fulvic acids can also be described as being “humic acids” of lower molecular weight and higher oxygen content. The colour of fulvic acids can vary from light yellow to brown in colour. In contrast with humic acids, fulvic acids that are always in solution, especially at the pH of productive agricultural soils, also contribute towards CEC of the soil with the main difference that mineral nutrient fulvate complexes are then in solution and both organic molecule and nutrient ion can be taken up by plant roots and therefore utilized by the plant. Water soluble nutrients and organic molecules do leach in soils but the rate of leaching as an organic-mineral complex, in relative terms, are much slower compared to the minerals alone in a water solution. Fulvic acids are poly-electrolytes and are unique colloids that diffuse easily through membranes and are therefore easily taken up by plant roots and leaves, whereas all other colloids like humic acid colloids do not.² Thus, fulvic acids in comparison to humic acids, contribute directly towards efficient nutrient uptake and utilization from soils.

Table 3: Stevenson (1982)³¹, summarized the chemical characteristics logically as depicted in the table. It can be seen that carbon and oxygen contents, acidity and degree of polymerization all change with increasing molecular weight. Therefore, it is apparent that no single structural formula will explain the heterogeneous nature of humic substance compounds.

Humic substances (pigmented polymers)				
Fulvic acid		Humic acid		Humin
Light yellow	Yellow brown	Dark brown	Grey-black	Black
<div> <div> <div>—————</div> <div>—————</div> <div>2 000</div> <div>45%</div> <div>48%</div> <div>1 400</div> </div> <div> <div>increase in intensity of colour</div> <div>increase in degree of polymerization</div> <div>increase in molecular weight</div> <div>increase in carbon content</div> <div>decrease in oxygen content</div> <div>decrease in exchange acidity</div> <div>decrease in degree of solubility</div> </div> <div> <div>—————></div> <div>—————></div> <div>—————></div> <div>—————></div> <div>—————></div> <div>—————></div> <div>—————></div> </div> <div> <div>300 000 ?</div> <div>>62%</div> <div>>30%</div> <div>>500</div> </div> </div>				
Chemical properties of humic substances. (Stevenson 1982)				

Humic and fulvic acids are mainly produced by biodegradation of lignin containing plant organic matter. They are not single acids but rather a complex mixture of many different acids containing functional groups that will react as a dibasic acid (2 replaceable hydrogen atoms like H_2SO_4) or as a tribasic acid (3 replaceable hydrogen atoms like H_3PO_4).

Electron microscope observations revealed the HS of different soils to have polymeric structures. The HS occurred in the form of rings, chains, and clusters as was observed under electron microscope and depicted in fig 5.

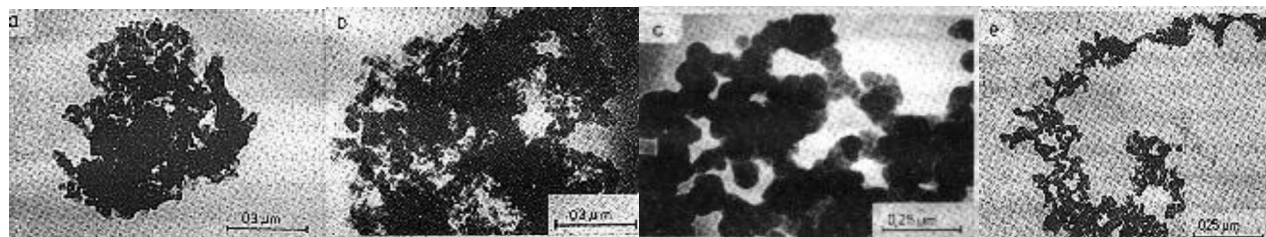


Fig 5: Electron micrographs of HS (Drozd 1978)³³

The sizes of these macromolecules can range from 60 - 5000 Å with Humic acids 1500 – 3000 Å and Fulvic acids < 800 Å (Beckett et al 1987). As mentioned earlier, humic acids are complex aromatic macromolecules with amino acids, amino sugars, peptides, aliphatic compounds involved in linkages between the aromatic groups. A hypothetical structure for humic acid shown in figure 6, contains free and bound phenolic OH groups, quinone structures, nitrogen and oxygen as bridge units and COOH and OH groups placed on aromatic rings. Buffle and Leppard, 1995³⁴(Fig 7) suggested a hypothetical model structure for a typical fulvic acid molecule which are much smaller and relatively to its size contains more functional groups.

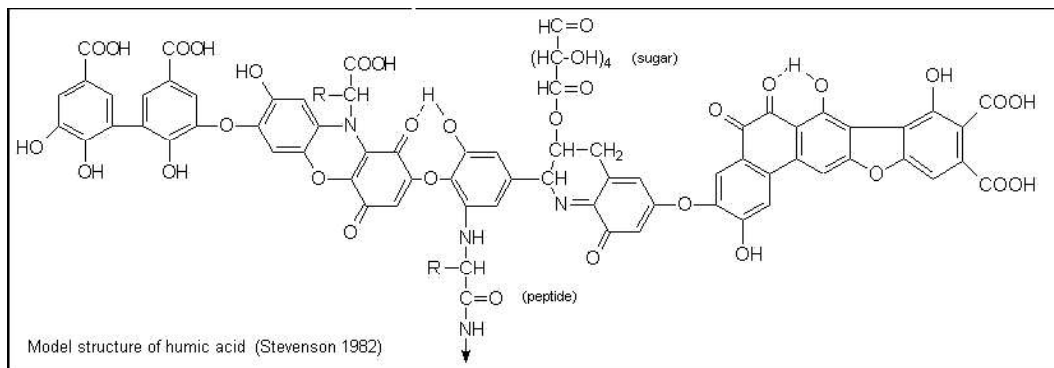


Fig 6: Hypothetical humic acid structure according to Stevenson (1982)³¹.

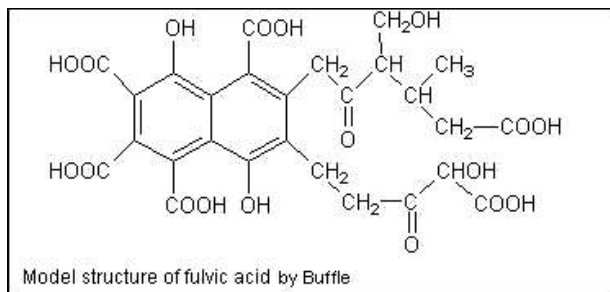
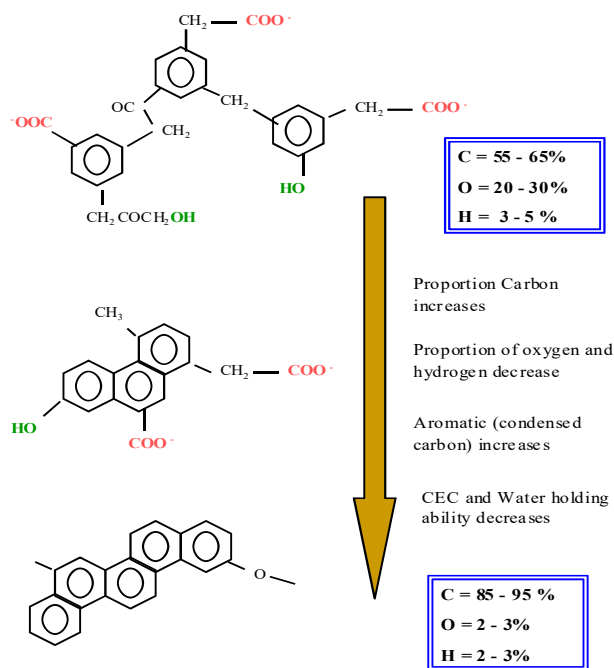


Fig 7: The hypothetical model structure of fulvic acid (Buffle's model)³⁴ contains both aromatic and aliphatic structures, both extensively substituted with oxygen containing functional groups.

Humic Substances



The diagram in fig 8 illustrates that the more oxidized the organic molecule, the higher the number of active groups on the molecule that can react with charged mineral ions, which at sufficient concentration in soils will have a direct effect on the CEC and water holding capacity of soils.

Fig 8: Diagram to illustrate the effect of C/O ratio on the chemical reactivity of HS.

2 METHODS OF EXTRACTION AND ANALYSIS OF HUMIC AND FULVIC ACIDS.

Humic acid can be extracted from humus by first mixing the humus with a dilute alkali (KOH, NaOH etc.) followed by a precipitation step of the humic acid out of solution by acidifying the mixture to a pH of 1 to 2. The fulvic acid, which is soluble at all pH levels, remains in the top acid solution. Recovering fulvic acid from land-based humus can be difficult with a low net yield of fulvic acid due to its soluble state because it is usually already leached out of the soil.

Determination of humic acid concentration is easy but standardization of the methodology used is crucial to obtain useful and comparable results the degree of acidification of the solution to precipitate humic acids will significantly influence the result obtained. The lower the pH used during acidification, the more humic acids will precipitate. Therefore, it is difficult to compare claims from different companies (particularly the USA, China, Russia and Germany where the largest manufacturers of humate products can be found), regarding the humic acid content of these products. It is also often claimed that Leonardite through its specific molecular structure, is more bio-active than other sources of humic acids but care must be taken when evaluating “results” since very often bio-activity can directly be related to the potassium (K) content used when extracting the humic acid (K-humate) and which usually makes up a significant amount of about 4.5-5%, of the total concentration of the product.

Practically in South Africa, BemLab reacted positively to requests to implement an internationally accepted chemical analysis procedure to analyse commercially available humic and fulvic acid products in order to better control and regulate the chemical quality of such products and also to assist in the registration of such products.

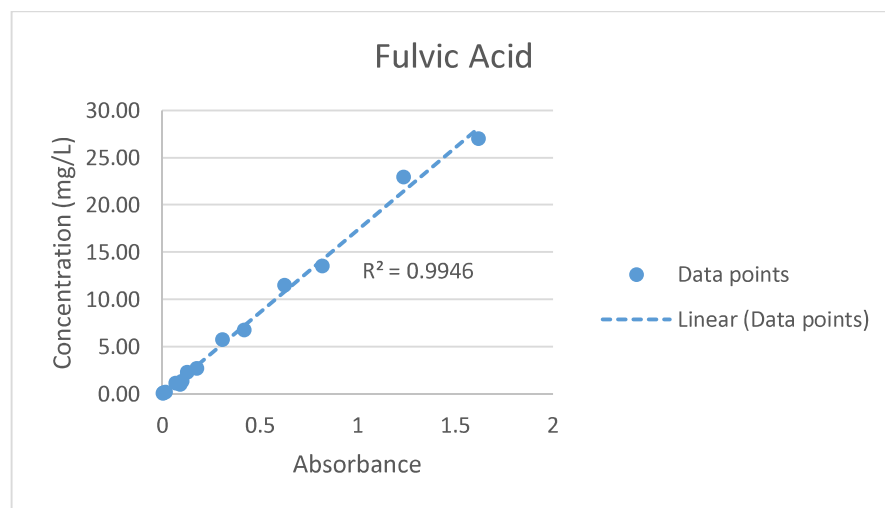
The method was developed using the procedure described in two publications, namely,

GAN, D., KOTOB, S.I. & WALIA, D.S., 2007. Evaluation of a Spectrophotometric Method for Practical and Cost Effective Quantification of Fulvic Acid. *Annals of Environmental Science*. 1, 11-15.

THURMAN, E.M & MALCOLM, R.I., 1981. Preparative isolation of aquatic HS. *Environ. Sci. Technol.* 15, 463-466.

The method, based on solubility at varying pH, while in this instance humic acid (HA) precipitation is standardized at a pH = 2. Both HA & FA is brought into solution at high pH. The solution is decanted and HA is precipitated by adding acid to reach a pH of 2.

HA concentration is gravimetrically determined, while the FA that remained in solution is determined spectrophotometrically. The optimal wavelength for determination of FA was experimentally determined using different concentrations of reference FA material obtained from the International Humic Substances Society (IHSS). A straight line calibration curve was obtained with an r^2 of 0.995 as shown in the graph below.



3 SOURCES AND CHARACTERISTICS OF PRODUCTS FROM DIFFERENT SOURCES.

Humus at the bottom of dams or lakes is mostly humic acid that precipitated. Fulvic acid on the other hand, can reach high concentrations in solution in poorly drained areas such as bogs and swamps and can sometimes be seen in streams where the water colour would be brown but clear. Humus from geological deposits such as black coal when oxidized, brown coal or lignite is composed of high humic acid but low fulvic acid contents due to leaching.

From a practical agricultural point of view, several types of humic/fulvic acid products with a wide range of active ingredient concentrations are offered to farmers. The sources of these products are extracts from brown coal (leonardite or lignite), peat, pecan nut shells, compost extracts, manure extracts, extracts from wood or fermented fresh plant material.

Humic acid sources.

Leonardite are considered to be the best source material for humic acid extractions. Leonardite is a soft, earthy, brown organic substance associated with lignite or soft fossilized plant material. It is organic matter which has not reached the state of coal and differs from soft brown coal by its high degree of oxidation. American leonardite are often claimed to be the benchmark standard for humic acids, however, some highly oxidized coal was discovered in Australia that is very similar to the American, but being younger, it has a higher content of humate compounds.

Fulvic acid sources.

Due to the solubility of fulvic acids in water and the fact that it easily leaches out of source material, it is usually only present in very low concentrations (0.2 – 1% w/v) in leonardite, peat, compost etc. sources. Some companies will dry fulvic acids to a powder, but drying is usually a costly practice that will reflect in the price of these products. Currently in South Africa, a high concentration fulvic acid product, from a sustainable renewable resource has shown to have high ion charge neutralizing and biological activities.

4 CHEMICAL CHARACTERISTICS

Humic and fulvic acids are complex aromatic polymeric molecules (*In polymer chemistry, polymerization is a process of reacting monomer molecules together in a chemical reaction to form polymer chains or three-dimensional networks*) that differs in size and molecular weight^{1,31}. The size of humic acids allow these macromolecules to “roll” up to form micro clusters or rings (Figs 4 and 5) that at a certain pH (acidic) will precipitate whilst fulvic acids are too small to go through a similar process and therefore remains in solution.

The presence of functional groups like carboxylic and phenol groups allows humic and fulvic acids to form complexes with ions such as Mg^{2+} , Ca^{2+} , Fe^{2+} and Fe^{3+} . Usually humic and fulvic acids have two or more of these groups arranged as to enable the formation of chelate complexes.^{14,22} The formation of (chelate) complexes is an important aspect of the biological role of humic acids in regulating bioavailability of metal ions as shown in examples below(fig 9) ²².

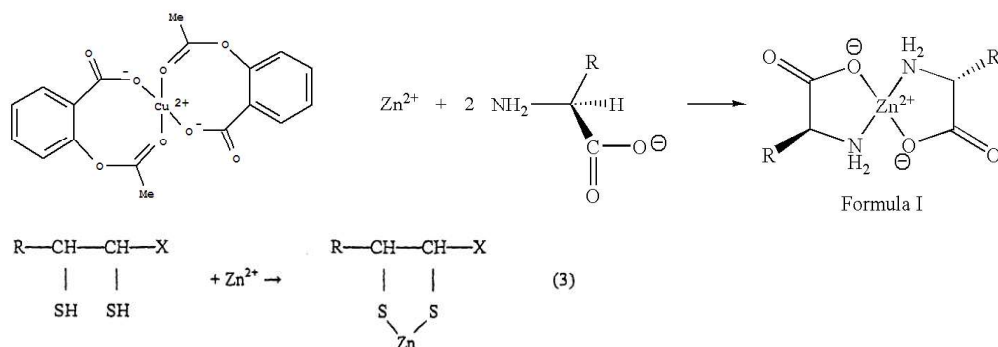


Fig 9: Chelation of Cu and Zn in top 2 examples with simple complexation of Zn by an amino-acid.

Elemental characterization of humic fractions on an atomic basis by Helal (2007) (Table 4) shows that fulvic acids contains 22% more hydrogen to carbon atoms, 21% more oxygen than carbon and 14% more carboxylic acid groups than humic acids. This implies that fulvic acids are more reactive towards reacting with cations or in other words fulvic acids contain more functional groups of an acidic nature, particularly COOH. The total acidity of fulvic acids (900 - 1400 meq/100g) are considerably higher than for humic acids (400 - 870 meq/100g) (Yamauchi *et al*, 1984)².

Table 4: Characterization results of humic fractions (Helal, 2007)¹¹.

Sample	Elemental composition (%)					Atomic H/C ratio	Atomic O/C ratio	COO ⁻ (meq OH/g)
	C	H	O	N	S			
FA	51.5	5.03	39.41	2.11	1.95	1.17 (+22%)	0.57(+21%)	5(+14%)
HA	56.2	4.48	34.9	2.6	1.82	0.96	0.47	4.4
Humic	57.6	4.57	33.74	2.34	1.75	0.95	0.35	4.2

Chemical functions in the soil.

Humic and fulvic acid cation salts or complexes (Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Zn^{2+} , Mn^{2+} , Fe^{2+} , Cu^{2+}) are present in soils, promoting available cations for plant utilization and preventing (specifically in the case of $2+$ cations) binding with phosphate ions that will render these elements inaccessible for root uptake. The alkaline cations are held primarily by simple cation exchange with COOH groups (RCOONa , RCOOK etc.)^{3,4,5}.

Low molecular weight compounds (fulvic acid complexes) bring about the solubilization of metal ions and assist in their transport to and uptake by plant roots. The high molecular weight compounds (humic acids) however, function as an exchange binding site for these polyvalent cations but due to the fact that these large molecules usually exist as micro-particles in the soil, it does not move through diffusion and/or it cannot be taken up by the roots.

Humic and fulvic acids will bind to clay mineral surfaces at the functional group sites. The physical, chemical and biological properties of the soil matrix is greatly influenced by its adsorption of these organic substances on its surface and on the interlamellar spaces of clay minerals. Modelling of the binding between hematite structures and different concentrations of fulvic acid molecules was done by the The Serge Stoll Group in Switzerland and is diagrammatically shown in fig 10. The same type of reactions can be expected from humic acids except that it is much larger molecules. Each of the fulvic acid molecules will have functional groups where nutrient ion exchange can take place.

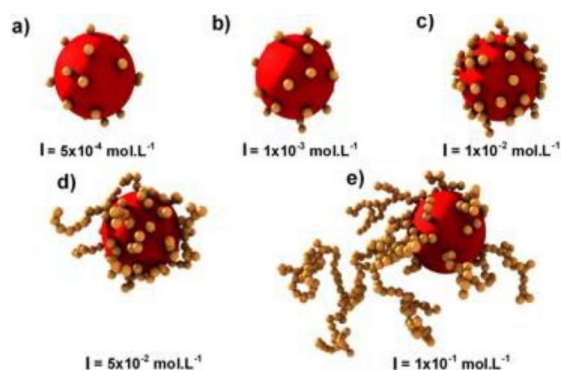


Fig 10: Fulvic Acid- hematite structures obtained at different ionic strengths: a) $5 \times 10^{-4} \text{ mol.L}^{-1}$, b) $1 \times 10^{-3} \text{ mol.L}^{-1}$, c) $1 \times 10^{-2} \text{ mol.L}^{-1}$, d) $5 \times 10^{-2} \text{ mol.L}^{-1}$, e) $1 \times 10^{-1} \text{ mol.L}^{-1}$. $f = 0.002$, $\text{pH} = 8$, $T = 25^\circ\text{C}$. The hematite particle is the central particle and fulvic acids are the small ones. At low ionic strength ($5 \times 10^{-4} \text{ mol.L}^{-1}$), only a monolayer of FA is observed at the hematite surface. By increasing the ionic strength, not only the number of adsorbed Fulvic Acid, but also the thickness of adsorbed FA increases. (Serge Stoll Group, University of Geneva, Switzerland)

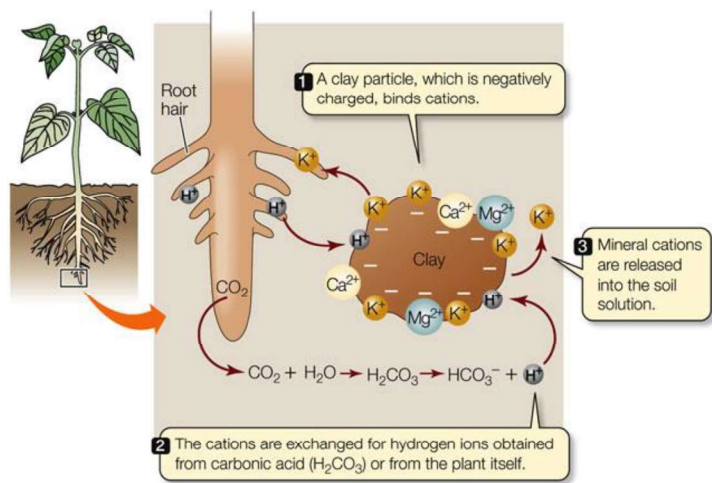
The chemical properties of HS, including hydrophilic and hydrophobic domains, facilitate interactions with a wide variety of soil constituents. Theoretically, these properties act to buffer biological susceptibility to nutritional extremes, such that high activities of salts, metals, and protons in the soil solution can be reduced, while low activities of nutrients are mobilized into plant-available forms.

5 PHYSICAL CHARACTERISTICS

Effect of humic and fulvic acids on soil physical condition, erosion and ion exchange capacity

HS profoundly influences the structure of soils. The deterioration of structure that accompanies intensive tillage is usually less severe in soils adequately supplied with humus. During intensive tillage of soils, humus is lost, resulting in hard and compact soils that are poorly aerated and with poor water-permeability and water holding capacity.

The addition of HS both as humic and fulvic acids will bind soil particles into structural units called aggregates that also plays a major role in managing soil erosion (Figures 4 and 5). These aggregates help to maintain a loose, open, granular condition of soils and enables the soil to hold more water. Consequently to the improvement of soil structure, water and oxygen will infiltrate and move downward through the soil to ensure proper aeration and supply of O^2 to the micro-organisms and roots that requires a continual supply of O^2 in order to during respiration produce CO_2 that converts to carbonic acid that has a pH lowering effect resulting in “freeing up” bound nutrients that will promote growth (Figures 11 and 12).



LIFE 8e, Figure 36.6

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Fig 11: Mechanism of ion exchange in the soil.

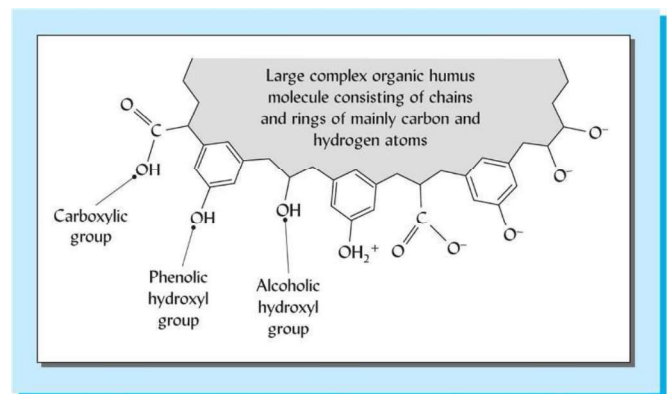


Fig 12: A simplified diagram showing the principal chemical groups responsible for the high amount of negative charge on humus colloids.

It is estimated that up to 70% of the exchange capacity of soils is the result of the colloidal HS aggregates. HS of which the major active portions will be humic and fulvic acids with a CEC range of 300 to 1400 meq/100g respectively, will improve the exchange capacity of soils (Fig 12).

6 BIOLOGICAL CHARACTERISTICS AND SOIL BIOLOGICAL ACTIVITY

Availability of nutrients for plant growth

In light of the importance of mineral nutrition for overall plant productivity, the effects of HS for ion uptake represent one of the topics which received a lot of attention by scientists.

Numerous studies have shown that HS enhance root, leaf and shoot growth but also stimulate the germination of crop species. These positive effects are explained as an interaction between HS and physiological and metabolic processes.^{7,26-29} The addition of HS was found to stimulate nutrient uptake, cell membrane permeability and it seems to regulate mechanisms involved in plant growth stimulation through hormonal action.^{26-29,35}

These organic molecules have both a direct and indirect effect on the availability of nutrients for plant growth (Figures 11 and 12). Firstly it serves as a source of N, P, K ¹⁴ and S³⁵ through mineralization by soil micro-organisms or secondly organic matter influences the supply of nutrients from other biological sources like serving as energy source for N-fixing bacteria to ultimately supply N to the crop.

These results strongly support the hypothesis that beneficial effects of HS on plant development may, at least in part, depend on their capacity to improve nutrient availability for plant uptake under nutrient deficient conditions.

Several studies were conducted to establish how the presence of HS may interfere with the nitrate uptake and assimilation by plants. Results demonstrated a strong positive effect of the Low molecular weight (LMW) fractions (Fulvic acids) on nitrate uptake and assimilation, whereas High molecular weight (HMW) fractions (Humic acids) only contributed weakly in the same pathways, which corresponds with earlier data^{1,35}.

In practice it is important to take note that when soils are first placed under cultivation, the humus content generally declines over a period of 10 to 30 years and must be compensated for to effectively utilize applied mineral nutrients and to maintain microbial activity that plays an important role in soil mineralization and therefore releasing bound nutrients from the soil.

Soil biological condition

Organic matter serves as a source of energy for micro-organisms and micro-fauna in the soil. In general, numbers of bacteria, actinomycetes and fungi in the soil are directly related to humus content. Earthworms cannot survive in soils with low or depleted organic content. Organic substances in soil can have a **direct** physiological effect on plant growth through the supply of plant accessible nutrients or **indirectly** through the maintenance of large numbers of plant growth regulating bacteria that lives on the root surface in a synergistic association where the bacteria produce plant hormones which will stimulate plant root growth and improve nutrient utilization and in return the root exudates serves as a food source for these bacteria to live and increase their populations. It is also known that some of these synergistic bacteria also produces antibiotics and certain phenolic compounds that may enhance the ability of plants to tolerate certain pathogens and plant root parasites.

Uptake and mobility of fulvic acid in the plant.

Fulvic acid has a water bridging ability. It attracts water molecules, helping the soil to remain moist and aiding the movement of nutrients into plant roots. The small molecular size of fulvic acid allows it to enter the vascular system of plants. Fulvic acid easily binds or chelate minerals such as iron, calcium, copper, zinc and magnesium. As it moves through the roots, stem and leaves, it takes these minerals to metabolic sites within the plant². Sprayed on plants, fulvic acid is a non-toxic mineral chelating additive and water binder that maximizes uptake through leaves and stimulates plant productivity.

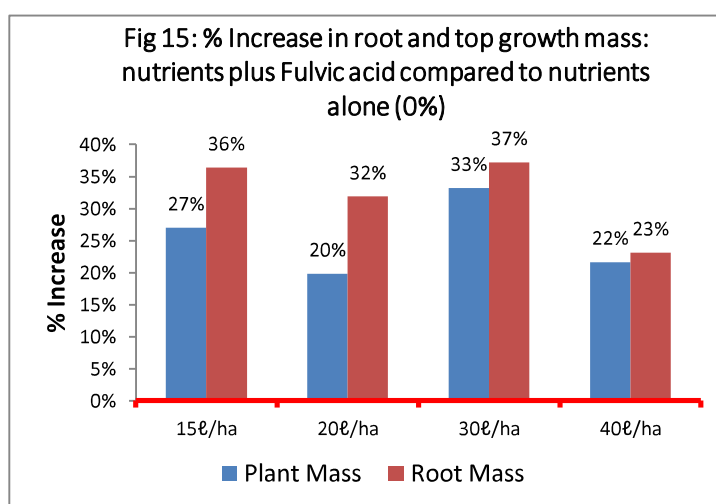
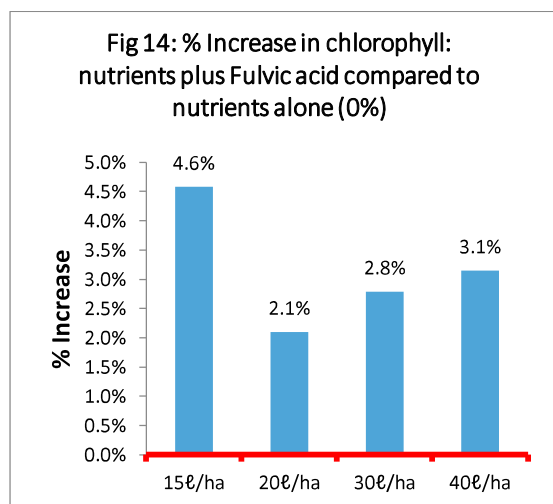
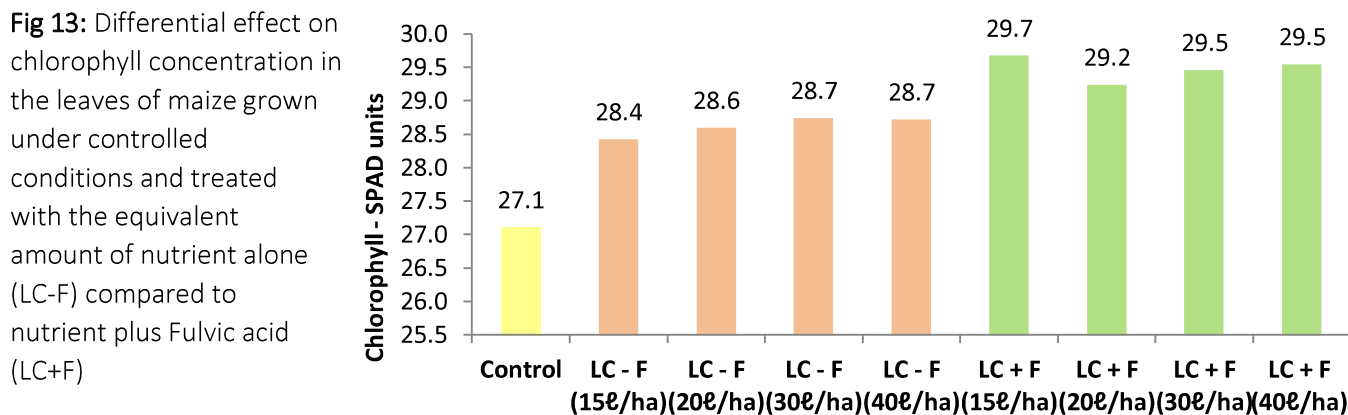
Direct impact on plant metabolism

Several reports claim that HS have a direct influence on the metabolism of plants acting like hormones or indirectly stimulate hormone production or catalyzes certain biochemical reactions, which all could result in growth stimulation. Some studies have shown that HS contain auxin or stimulate the activity of auxin-like molecules. This is probably an indirect production of hormones in the plant due to enhanced uptake of Zn from the soil and which is the catalyst for natural auxin formation in plants (Marschner, 1986)³⁶. Furthermore, the stimulatory effects have also been reported to increase CO₂ uptake, ATP synthesis, mitochondrial respiration and enhanced photosynthesis. Again certain reports fail to distinguish between direct and indirect effects because, if for example auxin production during Zn deficiency conditions, is stimulated through better uptake due to Zn-humic substance complexes used, then the increased hormonal activity will directly result an enhancement of all processes mentioned above.

However, the presence of hormone-like molecules were found in HS using ELISA methods and liquid phase analysis.

In certain cases and definitely regarding claims often made for commercial humic and fulvic acid products, these results can be questioned because,

- The effect of the nutrient portion e.g. K, of the products tested were not tested separately from the humic or fulvic acid portions. The real effect of a specific fulvic acid extracted from a renewable resource can clearly be seen in the results of a greenhouse trial where the nutrient portion of the product was tested separately from the fulvic acid containing the nutrients (Fig 13-15) (Malan & du Toit, 2014, personal communication).



- The possible effect of plant growth regulating hormones produced by bacteria in the root zone where HS products were used, is very difficult to distinguish from a possible direct hormonal effect which could be part of humic or fulvic acid compounds itself or residually being present in the soil ^{78,79,80}.
The effect of a tree extracted fulvic acid (ByoCarb50) on the growth and activity of bacteria and fungi in different soils are illustrated in Fig 16. Fulvic acid did not show any significant growth promoting effect on fungi or yeasts within the 96 hour incubation period, but bacterial growth stimulation was observed from 48 hours after incubation, especially at the 10 and 20 kg /ha equivalent dosages (Labuschagne, 2004, University of Pretoria, company research contract report)

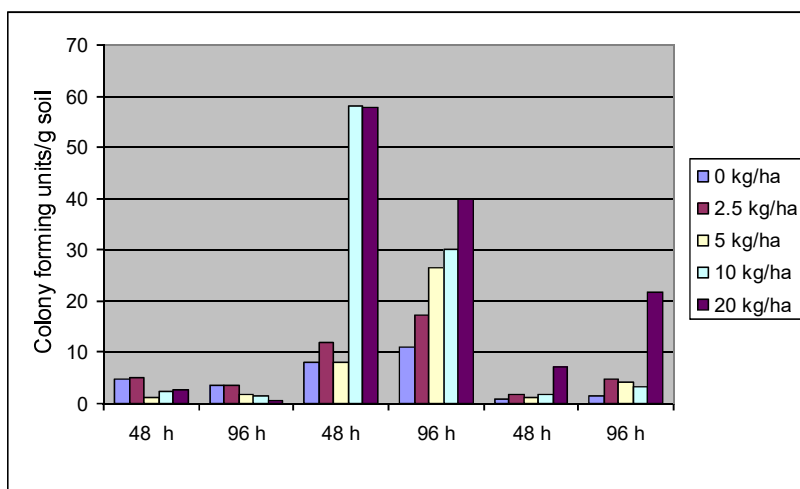


Fig 16: Fungi, bacteria and yeasts retrieved from soil HX (1) treated with ByoCarb50. [Mean of three replicate plates of two dilution series]

Auxin and gibberellin levels are usually higher in the rhizosphere than in the bulk soil, due to the fact that many of the *Bacillus* and *Lactobacillus spp* produce plant hormones in the root zone in their synergistic association with plant roots. Numerous soil and rhizosphere microorganisms, as well as the root systems of higher plants, have been reported as producing auxin⁸¹ and gibberellins⁸², but little information about their stability is known and only indirect conclusions have been made about their presence in amounts high enough to be biologically active.⁸³

- Both above situations without the presence of humic or fulvic acids can have an effect on the metabolism and growth of plants and specifically regarding increases CO₂ uptake, ATP synthesis, mitochondrial respiration and enhanced photosynthesis. This is especially relevant when certain nutrient deficiencies are being alleviated by the use of HS that will have an effect on the availability of nutrients that can be utilized by the plant.

It is therefore critically important to distinguish between direct humic and fulvic acid metabolic effects and secondary or indirect effects that has no bearing on the organic substances itself.

Worth noting however is that studies using HS marked with fluorescein have shown that the impact of HS on plants is mostly due to fulvic acids which can easily be explained by the fact that fulvic acids easily penetrate plants via the roots or leaves, while humic acids aggregates remains outside.

Literature reports.

Regarding photosynthesis and focusing on the chlorophyll content and electron transport, some reports are available. Schmidt et al. (2007)³⁷ investigated a hormone-like effect of water-soluble humic molecules (WEHS=fulvic acid) on the growth of *Arabidopsis* plants in sterile medium containing fulvic acid in concentrations ranging from 1–20 mg C. Application of WEHS were found to significantly increase the number and length of root hairs.

Garcia and Valdrighi (Garcia et al. 1991; Valdrighi et al. 1995/96)^{38,39}, reported extensively on stimulation of microbial activity through provision of carbon substrate HS.

It is not easy to distinguish between the direct and indirect effects of these substances. In fact, some of their positive effects may be ascribed to a general improvement of soil fertility, leading to a higher nutrient availability for plants. Whilst, in other cases, HS seem to positively influence metabolic pathways involved in the plant development, by acting directly on specific physiological targets^{26,28}. For this reason, understanding HS biological activity and the molecular mechanisms through which they exert their functions is an important agronomical and ecological task and a valid tool in facing increased and better quality agricultural produce as well as solving certain environmental problems.

7 PRODUCT CLAIMS AND FACTS

The most common and often exaggerated claims made by distributors and companies selling humic and fulvic acids are:

- The active ingredient content of products which are very often exaggerated to the higher end and could also be attributed to the fact that laboratories are not necessarily using the same analysis methodology since it is not internationally and sometimes not even standardized in the same country,
- Claims regarding soil chemical properties (chemically change the ion exchange properties of the soil), physical properties (modifying the structure and water holding capacity of the soil) and biological activity (stimulate the plant growth as well as the activities of soil micro-organisms) at application rates that are too low to have any of the above effects and without valid experimental results to prove it,
- Claims without dosage response data and curves per specific products to prove the generalized claim that for instance one liter of liquid concentrate is equivalent to 7-8 metric tons of organic manure. Similarly it is claimed that, one kilogram of concentrated powder is equivalent to about 30 metric tons of manure.
- Claims that humic acids are more bio-active than fulvic acid as a soil application. This in fact is dependent on what the concentration active ingredient is and how it is applied (single or split applications). Furthermore, the reason for using either one of these compounds must be clear namely: is it to supply CEC physical structure to a soil (long term at sufficient dosages that can be expensive and has to be compared to using compost or manure which are much cheaper and will achieve the same goal) or is it to neutralize charges of nutrient ions to improve uptake which is also dependent on concentration and application method.

The fact is that when studying the wealth of scientific literature on HS, it is not difficult to conclude that these compounds are still an enigma since there are many contradictory results and for every report of success of HS used in agriculture there are also reports of failure. Often failures can be ascribed to various factors like, products containing low or no active ingredient, application methods, concentration per area applied. Not all black or dark brown liquids/suspensions/powders are humic or fulvic acids and care must be taken that a valid analysis of the product is available. For example, black coal dust are sometimes sold to uninformed farmers as “slow release humic acid” whilst it is basically not oxidized at all and therefore are totally insoluble and inactive.

Product variety in the market.

Humic acids from various sources are available as cation (K, Na, Ammonium, Mg and B) humate salt powder, granules/crystals or liquid, usually at a high pH (>10) to keep the humic acid in solution. It will usually precipitate as granules in fertile soils where the pH is usually around 5 - 6 and is then less reactive.

Fulvic acid concentrates like potassium fulvates and sodium fulvates (often at relatively low active ingredient concentrations) and plant extracted fulvic acids are available. There are also high concentration products like spray dried material from China and the high concentration liquid fulvic extract from trees.

Analysis interpretation and confusing data on commercial product labels and specification documents.

All humic and fulvic acid products are made up of an organic portion and an inorganic mineral portion. When having such products analyzed the following minimum data is absolutely necessary:

- Total macro- and micronutrients including S.
- Humic and fulvic acid concentration (%) using standardized internationally accepted methodology.
- % Carbon
- Moisture %
- Specific density as kg/l

The main problem with evaluating effects of specific products such as HS lies in the variety of materials of various origins, and in the fact that the properties are often defined by extraction methods that vary among laboratories and product manufacturers.

8 DOSAGE RESPONSE AND OPTIMAL DOSAGES

In the most recent scientific publication “A Meta-Analysis and Review of Plant Growth Response to Humic Substances” by Rose et al (2014)⁴⁰, where 89 papers relevant to the question of, to what extent Humic substances will result in plant growth responses, were statistically analyzed to determine the main factors involved in the stimulatory effect when these compounds are used. The 89 papers provided over 700 points used in the analysis. The results showed that plant shoot growth response was not significantly influenced by either the growth media (soil) or the application site, but was significantly affected by the source of HS used, stressful growing conditions, the type of plant being treated, and the rate of HS applied (Table 5)

Table 5: Significance (p-value) of models containing a single moderator, according to plant shoot or root dry weight response to HS application

Moderator	Shoot growth	Root growth
None ^a	<0.001	<0.001
Media	0.336	0.016
Stress	0.015	0.144
Plant type	0.026	0.031
Application location	0.380	0.063
Application rate	0.002	0.261
Source	<0.001	<0.001

^aThe overall random-effect model without any moderators; significance indicates statistical difference between HS-treated plants and control (no HS treatment) plants.

Significant models are shown in bold (p<0.05).

The dosage response plot of the combined analysis data showed an initial sharp peak in growth response between 5 and 40 mg/kg (approximately 5-40 kg/ha) with a maximum around 20 mg/kg. These results were confirmed by Chen et al. (2004b)²⁷ and which was based on the extrapolation of a number of studies and his calculated amount of HS required for the optimum effective soil application was 22.5 mg/kg.

Data from field trials on wheat in Western Australia with a renewable resource extracted high concentration fulvic acid (55 – 55% dry matter, 18 – 22% pure fulvic acid) (C Malan, unpublished data), repeated on 12 farms within the same season, was combined and a dosage response curve was drawn to establish the *in situ* on farm optimum yield improvement (plant growth promoting) concentration. The data presented in Fig 17 shows that the optimum application dosage depending on the cost of the product will be between 7 and 21 liter/ha, which also corresponds well with the data presented by the previously mentioned authors. Based on assumptions for wheat price and the fulvic acid product price, the most cost effective dosage rate under these circumstances would be 7ℓ/ha. For higher value crops the dosage rate will then be in the order of approximately 20 ℓ/ha.

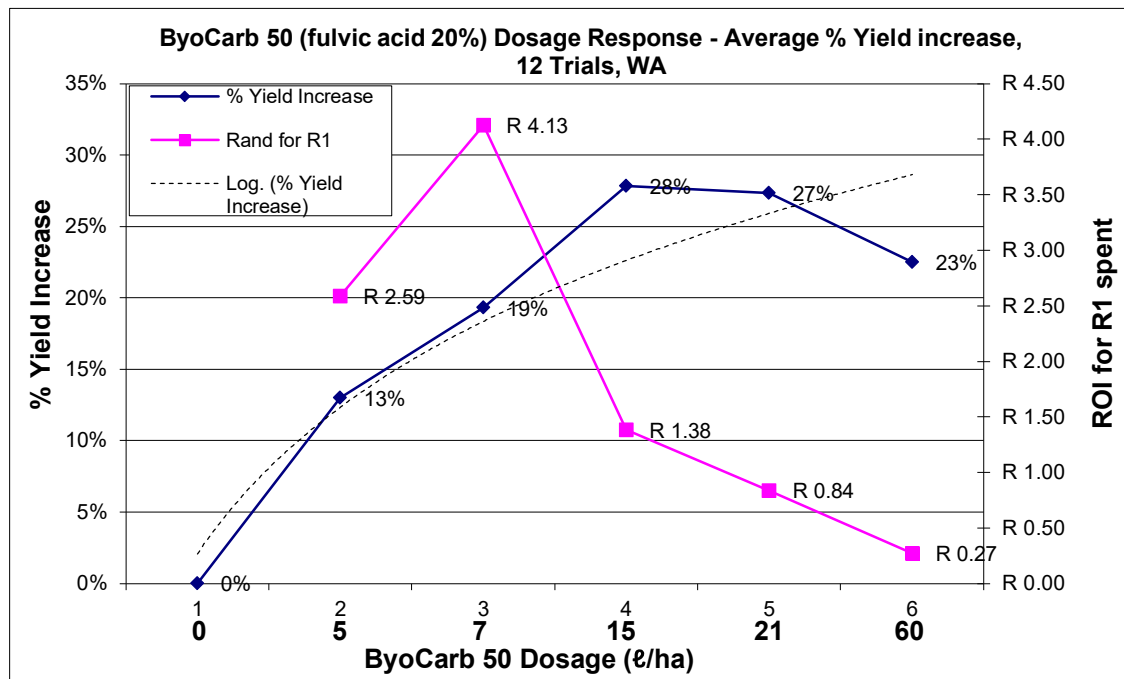


Fig 17: Plant growth promoting effect quantified as yield increase in wheat. Average of 12 Trials on different farms in Western Australia (2003). Assumptions: Wheat price/ton, R2 500; ByoCarb 50 price/£, R46.

Application of humic and fulvic acids (HS) as synergists.

The interaction of HS with plant essential elements, including N, P, K and micronutrients, is known to improve nutrient availability and also contributes to growth promotion by HS at low rates. This alone creates a substantial agronomic opportunity to improve the efficiency of fertilizer nutrient use over and above enhancing growth alone. Further evidence that HS can positively interact with beneficial microorganisms, offers the possibility of additional productivity gains if harnessed appropriately.

It is therefore clear that due to the chemical, physical and biological activity shown by HS, it can be used as synergists to harness all the above benefits. An example is to use a blend of both humic and fulvic acid, even in conjunction with compost or manure as soil treatments to really benefit from using HS products.

HS have been shown to significantly reduce urea hydrolysis from urea–ammonium nitrate and also retard the formation of nitrate, implying urease- and nitrification inhibition activity (Alkanani et al., 1990)⁴¹. There is also evidence that the association of soluble phosphate with HS reduces its binding (due to neutralization of ion charges) and precipitation in soil, allowing for greater plant uptake (Alvarez et al., 2004; Hua et al., 2008; Schefe et al., 2008)^{42,43,44}. These results points to a role for the use of HS in improving N- and P-use efficiency in cropping systems. The potential for using HS to improve micronutrient availability and absorption is also well recognized (Chen et al,1990²⁷, Michael T. Rose et al. et al., 2004a⁴⁰; Garcia-Mina et al., 2004^{45,46}), but there is a noticeable lack of experimental studies reporting the efficacy of micronutrient-HS fertilizer formulations.

Field trials on wine grapes were done in the Western Cape where a renewable resource fulvic acid product at a dosage of 30 £/ha (3x10 liter split application over a 2 month period) was used in 8 vineyard blocks (4 cultivars) with separate control and treatment blocks adjacent to each other. An average higher yield (2 cultivars, Chardonnay not determined, sold to outside buyer on vine) (Fig 18) and average higher wine mineral nutrient contents (Fig 19) were observed. During the season chlorophyll content as growth parameter was monitored (Table 6) showing varying higher chlorophyll content in different cultivars. Also, 4 months after treatment, soil was collected in all treatment and control blocks and a 1:2 water extract analysis was done to determine the available concentration (nutrients in solution that can be taken up by roots) of macro and micro-nutrients present (Fig 20). All elements except for Mn,

were present in higher concentrations varying between 8 and 153% higher than in the control soils, which is confirmed by the higher EC of 79%.

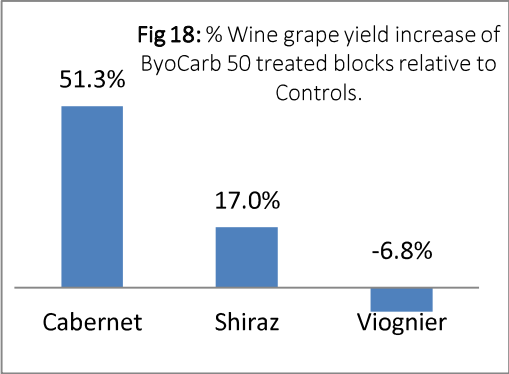
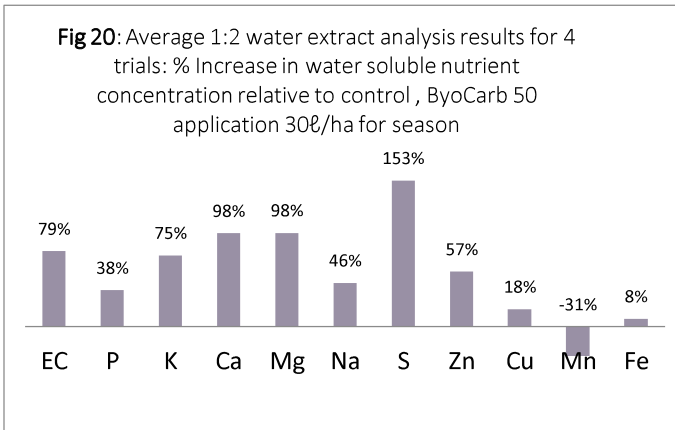
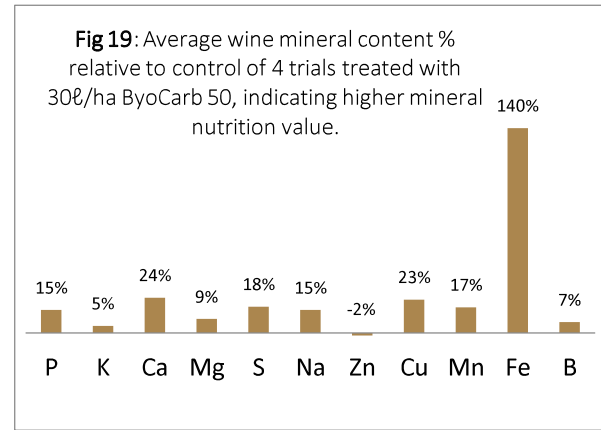


Table 6: Chlorophyll % relative to controls for 4 wine grape cultivars where ByoCarb 50 tree extracted fulvic acid was used at 30 ℓ/ha. Chlorophyll measured in SPAD units.

Vineyard blocks	Control	Treatment	% Difference relative to control
Cabernet	37.1	39.4	6.1%
Chardonnay	32.6	37.0	13.5%
Shiraz	32.4	36.3	12.0%
Viognier	32.5	32.9	1.2%



These results show the direct synergistic benefit in using the HS in conjunction with nutrients in the soil.

Another sustainable agronomy strategy that can be used is the combined use of HS and microbial inoculants in agriculture as plant-growth promoters (PGPs). PGPs can assist in nutrient acquisition, stress tolerance, and pathogen suppression through different biological actions.

9 REGISTRATION REQUIREMENTS AND REGISTRATION

The effective regulation of the use of HS and specifically humic and fulvic acids within certain guidelines and norms has become essential to control the quality of these compounds recommended and sold in the agricultural, animal feed and medical fields in terms of :

- What are the active ingredients in HS products and specifically humic and fulvic acids, mineral nutrient amendments and possible contamination with harmful chemicals like heavy metals.
- What is the concentration of all of the above and is it within the acceptable international norms.
- What is the source of the specific product?
- Biological activity and optimal active dosage (dosage response curves) must be verified with greenhouse and/or field trials. This includes testing the organic active ingredient separately without and with mineral nutrients if applicable.
- Any soil chemical or physical claims at specific recommended dosages must be verified with trial results.
- If possible, the determination of the percentage carbon and oxygen to be able to calculate the atomic C/O ratio would give a good indication of the CEC of a specific product.

Regulation and registration of these compounds is thus based on a simple principle that information regarding what the product is made up of (standardized analysis) and what it can do, and not claims what it is supposed to do, must be available.

South Africa is an important exporter of agricultural products like grain and fruit and the certification/registration of these compounds has become increasingly important especially where farm inputs in growing a crop for export are being monitored are being monitored and where residues are a concern, especially in the fruit export industry.

The recent implementation of a standard analysis procedure for humic and fulvic acids as a service to the industry by BemLab is an important step to regulate these type of products that are currently sold in South Africa. Furthermore, the feasibility of implementing the registration of the Group 3 fertilizers that will require testing as outlined above will hopefully be implemented soon.

10 PRACTICAL GUIDELINES FOR FARMERS

When a farm owner or manager needs to make a decision on what form and at what cost he wants to improve his soil to ensure sustainability and to comply with ever increasing demands, rules and requirements to produce quality and safe food for human and animal consumption, he has to understand the nature, quality and benefits that different organic sources will provide.

Bünemann et al (2006)⁴⁷, tabulated (see amended table) the possible reasons for applying a certain organic amendment and it highlights the specific benefits of the various soil ameliorating and improvement sources. This can help the decision maker on the farm to decide which source at what stage and with what crop will be the most beneficial to use for their specific circumstances.

Table 6: Intended benefits of organic amendments from Bünemann et al (2006)⁴⁷, amended.

Reason for organic amendment	Benefits
(a) Supply bulk nutrients for plant production	Animal manures, sewage sludge, and other composted organics supply N, P, K for plant uptake
(b) Increase availability of existing soil nutrients	Bacteria solubilize P and S from soil minerals (Grayston and Germida 1991; Gyaneshwar et al. 2002) ^{48,49} . Mycorrhizae act as an extension for roots to increase “root surface area” and improve effective nutrient utilization from the bulk soil solution (Dodd and Thomson 1994)
(c) Increase the availability of applied fertilizers	Humic and fulvic acid products increase fertilizer P availability by preventing precipitation of insoluble 2+ cation phosphate salts due to neutralization of charges before the inorganic reactions can occur (Delgado et al. 2002) ⁵⁰
(d) Fix N from air	Symbiotic and free-living N ₂ -fixing bacteria cyanobacteria (or blue-green algae) Anabaena and Nostoc and genera such as Azotobacter , Beijerinckia , and Clostridium ; and mutualistic (symbiotic) bacteria such as Rhizobium .
(e) Improve soil chemical fertility	Manure, sewage sludge, and compost can increase soil organic matter and cation exchange capacity. HS like humic and fulvic acids (at sufficient concentrations, active ingredient content and volume applied) can enhance micronutrient availability.
(f) Improve soil physical condition	Mulches prevent erosion and improve water infiltration and water storage. Manures and mycorrhizae enhance aggregate stability and pore structure (Tisdall and Oades 1982) ⁵¹ . HS like humic and fulvic acids (at sufficient concentrations, active ingredient content and volume applied).

(g) Improve soil biology	Manures and composts can add significant quantities of readily decomposable C substrate for microbes and adding selected beneficial microbes. Symbiosis between certain bacteria and mycorrhizae and rhizobia can improve by the addition of selected bacterial strains.
(h) Plant growth promoters	Rhizobacteria and specifically plant hormone producing rhizosphere bacteria in conjunction with humic and fulvic acids will supply plant growth-promoting hormones to stimulate root and general plant growth via improved water and nutrient utilization from the soil solution (Bowen and Rovira 1999) ⁵²
(i) Direct suppression of plant disease	Composted manure and brewed compost leachates may suppress plant diseases. Mycorrhizal fungi can control nematodes and root diseases which is enhanced by the addition of humic and fulvic acids (Siddiqui and Mahmood 1995) ⁵³
(j) Indirect suppression of plant disease	Selected beneficial Rhizobacteria can be added to seed or soil to enhance plant tolerance to root disease by out numbering existing pathogens. Organic substrates including humic and fulvic acids does stimulate plant-beneficial microbial populations (Labuschagne, unpublished data).
(k)Decontamination of polluted soils.	Microbially catalyzed reactions in soil can breakdown organic pollutants or precipitate metals making them unavailable for plant uptake or water transport or leaching.
(l) Break down crop residues and other compostable materials	Microbial inoculants enhances the breakdown of crop residues and waxes that cause water repellency (Roper 2004) ⁵⁴ . Produces natural sources of humic and fulvic acids.

11 WHAT QUESTIONS SHOULD FARMERS ASK

How to choose between different Humic and fulvic acid products.

The basic questions that must be asked and for which valid and logical answers should be given could be as follows:

- What is the active ingredient of the product? Humic or fulvic or both? Humic acids are used as soil applications to, at sufficient concentration, exert soil chemical and physical benefits but cannot be taken up by roots to transport mineral nutrients into the plant and must be further oxidized or broken down, usually by microbial action, to fulvic compounds. Contrary to this, fulvic acid can also, at sufficient concentrations, have a beneficial chemical/physical contribution to soils but in addition to that the molecules are small enough to bind mineral nutrients through neutralizing charges and can then be taken up by plant roots for utilization of both the nutrients and the organic molecule.
Fulvic acids, due to the small size of the molecules are very effective chelating and neutralizing molecules that are being used in foliar nutrient formulations, at sufficient concentration to be biologically active. Furthermore and specific to the tree extracted fulvic acid products, it has water dispersing (water surface breaking), wetting and re-wetting (hygroscopic) characteristics that both for soil and foliar applications, these characteristics will be beneficial for better infiltration, uptake and utilization of nutrients.
- What is the concentration as per analysis of the active ingredient/s?
- What is the source of the humic and /or fulvic acid extraction?
- Which mineral nutrients are in the product and at what concentrations as per analysis? Potassium, Sodium and Magnesium in particular important for humic acids since they are extracted with the hydroxides of these elements. Some products oxidized by acids like Nitric acid and will contain high amounts of Nitrogen but often low amounts of humic and/or fulvic acid and usually this will be an expensive nitrogen product where the same nutrition could have been supplied with conventional fertilizers and the small amount organic material in these products does not contribute to the intended goal.
- What is the pH of the product? Water soluble: humates pH>10 and fulvic below 6.

- What is the specific density of the product? Generally a good quality product with relatively low nutrient content and high organic active content will have a density higher than approximately 1.2 kg/ℓ.
- What is the carbon content of the product since it can be a good indication of concentration and general potential activity.

In conclusion it is important that it must be clear for what purpose HS are needed and intended and then the above information must be used to screen and decide on a particular product.

12 CONCLUSIONS

HS, as the major component of soil organic matter, have been widely studied in various areas of agriculture, such as soil chemistry, fertility and plant physiology. HS plays an important role in soil fertility and control of soil pollutants. It also contributes to the production of safe food and contributes to sustainable agricultural practices and has a significant impact on improving the environment and addressing certain environmental problems.

The amount of research data that proves the significant positive impact that these type of molecules has on a broad spectrum of agricultural applications, should be proof enough to consider the different types for their intended use, provided that attention is given to the quality of products and correct dosages being used per specific products, otherwise poor results will make the name “snake oil” stick and the true on-farm benefits will not realize.

The direct and compounding benefits where one benefit will lead to the next are:

- improvement of soil chemistry and structure
- improvement of biological status of soils
- improved utilization of nutrients supplied and obvious cost savings
- improved water utilization and possible cost savings
- improved yield – higher income/ha
- improved quality – higher income/ha

Because there are an ever increasing number of humic-coated or humic-containing fertilizers on the market, further research and validation of such products is urgently needed to provide farmers with reliable information for making agronomic decisions.

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