Oxidized Lignites and Extracts from Oxidized Lignites in Agriculture

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PART I. EFFECTS OF OXIDIZED LIGNITES AND DERIVATIVES FROM HUMIC SUBSTANCES ON PLANTS AND SOILS.

Introduction

Most of the published research on the effects of humic substances on plants has been done in nutrient and sand cultures. Field research or soil pot studies on the response of agricultural crops to applications of oxidized lignite is less abundant. More work has been done using humic acid derivatives from oxidized lignite or peat than by using raw lignites. Because of this, the literature reviewed on the effect of these substances on plants and soils will come from all of these areas.

It is true that humic and fulvic acid fractions extracted from different terrestrial sources (soil, peat, compost, oxidized lignites, other coals, manure, etc.) and vegetation types do show differences in molecular size, chemical structure and functional groups. However, when highly humified extracts are purified and examined, the differences are fewer. For example, Amalfitano (1995) looked for major differences or similarities between the chemical structure of humic acids derived from the light fraction litter of soils with widely varying vegetation types, and found that the spectra from highly humified extracts were similar.

Reports by commercial enterprises on the beneficial effects oxidized lignite are often a series of side-by-side comparisons without statistical analyses, or are performed at a single location or over a single year, and thus have a narrow inference space. In addition, commercial enterprises have a vested interest in demonstrating positive outcomes from their experiments. Because of this, corporate research literature on the effects of oxidized lignites and derivatives on plants and soils will not be reviewed in this paper.

A note on terminology; often the term used in the literature for oxidized lignites is leonardite. Leonardite refers to a particular geologic deposit of oxidized lignite in North Dakota, but has often been misapplied to lignitic deposits found elsewhere. Humate is a common term meaning a source of humic and fulvic acids. The term humic acid, which is the alkali soluble but acid insoluble portion of a source of humic substances, often is applied to alkali extracts of these materials. These alkali extracts include the acid soluble portion (fulvic acid) component. Generally it is to be assumed that the term "humic acid" includes the fulvic acid component unless the author of the research literature has specified otherwise.

Effects Of Oxidized Lignites and Derivatives from Humic Substances on Plant Growth

Germination:

In the application of humic acid extracts to plants, Smidova (1962) found increased water absorption, respiration and germination rate in wheat, and Ishwaran and Chonker (1971) in soybeans (Glycine max L.). Dixit and Kishore (1967) found an increased germination rate in barley (Hordeum Vulgare L.), corn (Zea mays L.), and wheat (Triticum aesthivum L.). However Piccolo et al. (1993) observed no increase in the germination percentage or rate for either lettuce or tomato seeds treated in Petri dishes with unfractionated humic acids derived from an oxidized lignite. No evidence that humic substances increase the viability of seeds has been reported

Root growth - solution and sand culture:

Increases in root mass, length or number of initials were reported on the several crops grown in sand or nutrient solutions to which were added humic or fulvic acids, or extracts from oxidized lignites. Here are some examples:

beans - (Phaseolus vulgoris L.) Schnitzer and Piapst.1967.

corn - (Zea mays) Ivanova ,1965; Alexandrova ,1977;

cucumber - (Cucumis sativus L.) plants by Rauthan and Schnitzer,1981;

grapes - (Reynolds et al. 1995)

millet - (Pennin'tum sp. L.) Alexandrova, 1977;

pepper - (*Capsicum annuum* L.) Sanchez-Conde and Ortega,1968);

sugar beet - (*Beta vulgaris* L.) Sanchez-Conde et al., 1972;

tomato - (Lycopersicon esculentem L·) Sladky ,1959a; Lineham ,1976; Adani et al.,1998;

Root Growth - Soils

Lee and Bartlett (1976) investigated the response of corn to 8 mg L-1 Na-humate additions to a low organic matter soil, and found increased root proliferation.

Kelting et al., 1998a tested three types of humate (oxidized lignite) derived products on root growth and sapflow of balled and burlapped red maple (Acer rubrum L.) trees. Treatments included oxidized lignite as 1) an extract applied as a soil drench; 2) a liquid formulation to which various purported root growth -promoting additives had been added, also applied as a soil drench; 3) as a dry granular formulation, applied as a topdress. They found that no treated trees had more root length than non-treated controls, but all humate derived treatments increased sap flow.

Kelting et al. (1998b) also tested soil treatments of compost, peat and oxidized lignite on post-transplant growth of red maple (Acer rubrum L.) and Washington hawthorn (Crataegus phaenopyrum Hara) trees. Granular oxidized lignites increased total root length in Washington hawthorn but not in red maple.

Foliar applications of humic substances on root growth:

Sladky (1959b) applied humic materials as a foliar spray on begonia (*Begonia semperflorens* L.) plants grown in nutrient solutions and found increased root growth. Similar observations were obtained by Sladky (1965) with sugar beets grown in distilled water.

Shoot growth:

As is the case for root growth studies, most of the early publications on shoot growth enhancement are limited to young plants grown in pots or in nutrient solutions.

Piccolo et al. (1993) Treated lettuce and tomato seeds in Petri dishes with unfractionated humic acids (UHA) derived from an oxidized lignite at strengths ranging from 40 to 5000 mg/L. The fresh weight of total seedlings and per seedling increased in treatments with UHA and with increasing concentrations for both lettuce and tomato plants without showing signs of growth inhibition up to 5000 mg/L. The authors attributed this to cell elongation and more efficient water uptake.

Adani et al. (1998) Studied the effects of humic acids extracted from peat (CP-A) and from leonardite (CP-B) on the growth and mineral nutrition of tomato plants (Lycopersicon esculentum L.) in hydroponics culture were tested at concentrations of 20 and 50 mg/L. Both the humic acids tested stimulated plant growth. The peat derived humic acids stimulated only root growth, while the leonardite derived humic acids showed a positive effect on both shoots and roots, especially at 50 mg/L.

Lee and Bartlett (1976) studied stimulation of corn seedling growth in low organic matter soil with 8 mg/Kg Na-humate and found increases in seedling growth of 30 to 50%.

Tan and Tantiwiramanond (1983) applied humic and fulvic acids to sand cultures of soybeans (Glycine max L.), peanuts (Arachis hypogea L.) and clover (Trifolium sp.). Shoot, root, and nodule dry weights increased in response to treatments up to 400 to 800 mg/kg soil.

Reynolds et al. (1995) planted greenhouse-grown 'Chardonnay' vines (Vitis vinifera L.) in a sand medium to which was added one of five levels of granular Gro-Mate (GM), a commercial humate. Shoot length responded to increasing level of granular humates. Fresh and dry weights of leaves, shoots, and roots, as well as leaf count and area, exhibited increasing linear or quadratic trends in response to increased level of granular GM.

Reynolds et al. (1995) found that very high granular applications of oxidized lignite may result in leaf necrosis and retarded growth on grapes in sand culture.

Kelting et al. 1998b, tested several types of organic materials on post-transplant growth of red maple (Acer rubrum L) and Washington hawthorn (Crataegus phaenopyrum Hara) trees. Soil treatments applied at planting included additions of compost, peat and oxidized lignite. They found that all soil treatments did increase top dry mass for Washington hawthorn, with the oxidized lignite treated trees showing the greatest increase. No treatments significantly increased dry mass for red maple.

Foliar applications of humic substances and shoot growth:

Sladky and Tichy (1959) sprayed tomato plants with a solution of 300 mg/ L humic acid, and found that both fresh and dry weight of shoots was increased. They reported that higher application rates inhibited growth and deformed leaves.

Sugar beets (*Beta vulgaris* L.) also responded with increased shoot growth when foliar sprayed with humic acids A. (Sladky, 1965).

(Sladky, 1959b) sprayed begonia plants with either humic or fulvic acids and found increased shoot growth. The investigator also indicated that fulvic acid was slightly more effective than humic acid.

In a review of published reports, Chen & Aviad (1990) found that fulvic and humic acids may stimulate shoot growth of various plants when applied either as foliar spray at concentrations of 50 to 300 mg/L, or when applied in nutrient solutions at concentrations of 25 to 300 mg/ L. This stimulatory effect often extended to roots, regardless of the mode of application.

Crop yields in soil pots and field trials

Martin and Senn (1967) found that the use of humic acid derivatives (HAD) added to tomatoes grown in 3-gallon pots increased yields, especially during the latter stages of growth. Applications of HAD resulted in a greater number of fruits of comparable size to the check for the first 5 harvests. Quality and grade of fruit was superior to controls, with HAD treatments resulting in more than 200 percent increase in yield of number 1 tomatoes.

Brownell et al. (1987) conducted field trials on tomatoes, cotton and grapes after application of two commercially available extracts from leonardite (oxidized lignite). One extract was used as an early season soil treatment, while the other was used as a foliar spray. Results from both treatments on tomatoes produced average yield increases of 10% over controls; on cotton the average yield increase was 11%. Unreplicated, large field trials on various cultivars of grapes produced yield increases ranging from 3 to 70% over untreated controls.

Wang et al. (1995) added humic acids to an alkaline soil with P fertilizer and examined wheat yields in field trials. Humic acid treated plots increased both P uptake and yields by 25%.

Crowford et al. 1968 conducted a three-year test to determine if humic acids could effectively influence sprout production and yield of sweet potatoes. Treatments included either soaking the seed potatoes in a 10% humic acid derivatives (HAD) solution or by incorporating HAD into the soil beds at the rate of 2 grams/lb. soil. The results were averaged over three years. Soil treatment increased sprout production from 69 to 231, and potatoes showed a 10 - 20% yield increase over controls. For the 10% HAD potato seed treatment, three year averaged yields increased 30 - 40% over controls. All humic treatments resulted in a significantly higher percentage of number one grade potatoes.

Duval (1998) Applied varying rates of leonardite up to 400 lbs./acre on turnip (*Brassica rapa* L) and mustard greens (*Brassica hirta* L.) with 3 plantings over a one-year period, and found no differences in the plant growth parameters studied. However, they did report that excessive rain over a 6-week period (13.5 inches, and 6.5 inches in one day on a fine sandy loam soil) eroded the soil and caused a nitrogen deficiency in the crop. In addition, they could not find detectable quantities of humic acid in the soils after the experiment was concluded. They also reported an infestation of yellow margined black turnip beetles (*Microtheca ochrolma* Stal), which destroyed the stand of plants 4 weeks after the 2nd planting, and then was replanted after applying an additional 120 lbs. ammonium sulfate per acre.

Effects Of Oxidized Lignites And Derivatives From Humic Substances On Nutrient Availability and Uptake

Uptake of macroelements in solution or sand culture:

Humic substances have been demonstrated to increase the uptake of plant nutrients. Many studies report increased growth, together with increasing uptake of plant nutrients. Studies that isolate the growth hormone-like response from growth resulting from increased uptake of limiting plant nutrients will be presented in the section on the biochemical effects of humic substances.

Bean (Phaseolus vulgaris) and rough fescue (Festuca scabrella Torr.).
Dormaar (1975) added humic acids at 1 to 50 mg L-1 to plants grown in nutrient solutions. Nitrogen uptake increased at 20 to 50 mg L-l, but uptake of P, K, Na, Ca, and Mg was not significantly affected.

Corn (Zea mays L.)
Lobartini et al. (1998) investigated the effect of humic (HA) and fulvic acid (FA) on the dissolution of aluminum phosphate (AlPO4) and iron phosphate (FePO4), and assessed their availability to plants. The results indicated that the amount of P released by HA or FA increased with time, with free orthophosphates present with small amounts of P-humic acid complexes. Humic acid was more effective than fulvic acid in dissolving the metal phosphates. The plant-availability of phosphate dissolution products was confirmed by growing corn plants in hydroponic solutions with AlPO4 or FePO4 as the source of P, and HA or FA at pH 5.0. Corn plants exhibited better P uptake and growth performance when HA or FA is present.

Cucumber (*Cucumis sativus*)
Rauthan and Schnitzer (1981) added up to 2000 mg L-l soil-derived fulvic acid to nutrient solutions. The uptake of N, P, K, Ca, and Mg increased to the shoots. Maximum uptake and maximum growth occurred at concentrations of 100 to 300 mg/ L FA.

Grapes
Reynolds et al. 1995, planted greenhouse-grown 'Chardonnay' vines (Vitis vinifera L.) in a sand medium to which was added one of five levels of granular commercial humate. They found that the granular humate increased petiole Fe and lamina P, K, and Fe.

Ryegrass (Lolium perenne L.)
Gaur (1964) found enhanced uptake of N, P, and K; and reduced uptake of Ca in ryegrass grown in pots in a soil with added humic acid extracted from compost.

Pepper
Sanchez-Conde and Ortega (1968) found increased uptake of N, P, and Mg, and reduced uptake of K, Ca, and Na on pepper plants irrigated with solutions containing 8 -100mg/ L humic acid.

Tomatoes (Lycopersicon esculentum L.)
Adani et al. (1998) studied the effects of humic acids extracted from peat (CP-A) and from leonardite (CP-B) on the growth and mineral nutrition of tomato in hydroponics culture. Both extract treatments showed increases in the uptake of N, P, and Fe.

Wheat (Triticum aestivium).
Vaughan et al. (1978) studied radioactively labeled ^{32}P uptake on excised roots and cell cultures of winter wheat. Concentrations of 5 to 50 mg/L humic acid enhanced ^{32}P uptake, but 500 mg/L reduced uptake.

More research has been done recently regarding the stimulation of nitrate uptake by humic substances:

Piccolo (1992) obtained humic extracts with distinct physical-chemical characteristics, by using various soil extractants and from different sources, in order to study their effect on nitrate uptake by barley seedlings. Results showed that the most effective humic fraction on plant nitrate uptake had the highest acidic functionality and the smallest molecular size, whereas the aliphatic and aromatic content of extracts did not appear to play a role.

The uptake of major anionic macronutrients like nitrate is substrate inducible and requires energy. Santi et. al., 1995 found that the activity and amount of plasma membrane -ATPase was increased in maize roots induced for nitrate uptake.

Pinton and Cesco (1999) studied the effect of the water extractable humic substances fraction (WEHS) on nitrate uptake of maize roots. They found significant increases in both nitrate uptake and plasma membrane H^+ATPase activity. Results supported the idea that the plasma membrane proton pump might be one of the primary targets of the action of humic substances on plant nutrient acquisition.

Nardi et. al. (2000) tested a low molecular weight humic fraction (LMW-HA) for its biological activity in maize seedlings. Results showed that LMW-HA increased nitrate uptake. The authors hypothesized that LMW-HA stimulated nitrate uptake by decreasing the pH at the surface of roots, thus facilitating the H+/NO3- symport. The nitrogen regulatory properties of LMW-HA appeared to depend on the combination of low molecular size, gibberellin-like activity and to the content of phenolic and carboxyl carbon.

Nutrient availability in soils:

Humic substances may influence the rate of release of nutrients from soil minerals. Tan (1978) has demonstrated that both humic and fulvic acids can enhance the release of fixed K from illite or montmorillonite.

Wang et al. (1995) studied the effect of humic acids on transformation of phosphorus fertilizer in an alkaline soil. Soil P was fractionated following 4 and 15 days incubation after humic acids were applied with phosphorus fertilizer to the soil. The availability of phosphate in the soil and in plants was determined at heading stage and at maturity in a pot experiment, and wheat yield was examined in a field trial. Addition of humic acids to soil with P fertilizer significantly increased the amount of water-soluble phosphate, strongly retarded the formation of occluded phosphate, and increased P uptake by 25%.

Nutrient uptake from soils:

Jelenic et al. (1966) added ^{32}P-labeled superphosphate plus Na-humic acids derivatives (HAD) extracted from lignite to two soils at rates of 2 to 12 mg HAD/kg soil. They found increased uptake of both soil-P and superphosphate-P by corn, with a maximum uptake observed at 3 to 8 mg HAD/kg of soil.

Wang et al. (1995) added humic acids to an alkaline soil with P fertilizer with wheat grown in field trials, and observed increased P uptake and yield; both by 25%.

Xudan, (1986) in pot studies and field trials with wheat, found that spraying the leaves with fulvic acid resulted in greater uptake of ^{32}P by the roots.

Uptake of microelements:

Improved availability of micronutrients by solubilization from their inorganic forms in soils or in nutrient solutions plays an important role in the promotion of plant growth in soils by humic substances.

Studies by Varadachari et al. (1997) on the complexation of humic substances with oxides of iron and aluminum indicated two major modes of HA bonding - cation bridges forming oxide-M-HA links and direct bonding of HA to coordination centers at the oxide surface.

Dekock (1955) found that lignite-derived humic substances increased the solubility of Fe in solution, and increased Fe uptake and translocation from roots to shoots. This effect was observed even at high phosphate concentrations.

Lee and Bartlett (1976) found that 5 mg L-l Na-humate in a nutrient solution increased the Fe concentration in roots and shoots of corn.

Rauthan and Schnitzer, 1981 found that fulvic acid increased the uptake of Fe, Zn, Cu, and Mn by cucumber (Cucumis sativus) plants grown in Hoagland's solution.

Aso and Sakai (1963) found that the chlorosis exhibited by barley (Hordeum vulgare L.) and rice (Oryza sativa L.) was alleviated by additions of Fe (III)-humic substance complexes, while unferrated humic substances alone were ineffective.

Linehan and Shepherd (1979) observed that the addition of fulvic acid at concentrations up to 25 mg/L to nutrient solutions increased Fe uptake to shoots of wheat seedlings (Triticum aestivium).

Bar-Tal et al. (1988) demonstrated that solutions with fulvic acid added would maintain a zinc level of $10^{-3.5}$ mM in the presence of Ca-montmorillonite at pH 7.5, whereas solution Zn levels decreased to $10^{-5.5}$ mM without fulvic acid.

Plant Uptake of Humic Substances

If humic substances have direct effects on plant growth, then they must be absorbed and translocated by plants. Studies commonly use 14C-labeled humic substances to trace their uptake into and movement through plants.

Fuhr and Sauerbeck (1967b) reported that much of the absorbed radioactivity from 14C-humic acid was incorporated into the epidermis of sunflower (Helianthus annus L.), radish (Raphanus safivus L.) and carrot (Daunts carora L.) roots. In addition, the radioactivity that was observed in the stele originated from low molecular weight components of the humic materials.

Vaughan and Linehan (1976) found that 14C-labeled humic acid was taken up by wheat roots, and a small percentage (5%) was transported to the shoots.

Fuhr & Sauerbeck, (1967a) showed that fulvic acid is transported to the shoot to a greater extent than is humic acid.

Vaughan and McDonald (1976) also suggested that only low molecular weight fractions of the humic acids are biologically active. They examined the uptake of ^{14}C-humic acid by intracellular components of beet roots. The greatest amount of radioactivity was associated with cell walls and smaller levels with mitochondria and ribosomes.

Additional clarification of this issue resulted from an investigation on excised pea (Pisum safivum L.) roots (Vaughan and Ord 1981). Results showed greater uptake of the low molecular weight substances. They found that both low molecular weight humic acid and fulvic acid fractions are taken up both actively and passively, but humic substances with molecular weights above 50,000 daltons are absorbed up only passively.

Biochemical Effects of Humic Substances on Plants

Molecular size and activity:

Piccolo (1992) obtained humic extracts with distinct physical-chemical characteristics, by using various soil extractants and from different sources, in order to study their effect on growth regulation in watercress and lettuce. Results showed that the most effective humic fraction on hormone-like activity had the highest acidic functionality and the smallest molecular size, whereas the aliphatic and aromatic content of extracts did not appear to play a role.
Fulvic acids had a smaller molecular size, and tended to have higher acidic functionality than humic acids.

Membrane permeability:

Many investigators have proposed that these humic substances affect membrane permeability, increasing permeability to some ions and decreasing it to others. This could be due to the surface activity of humic substances on cell membranes.

Pinton and Cesco, (1999) studied the effect of the water extractable humic substances fraction (WEHS) on plasma membrane H^+ATPase activity of maize roots, and found significant increases in plasma membrane H^+ATPase activity. Results supported the idea that the plasma membrane proton pump might be one of the primary targets of the action of humic substances on plant nutrient acquisition

Respiration rate:

Sladky (1959a) grew tomato plants in nutrient solutions containing either humic acid (50 mg/L), or fulvic acid (50 mg/L)(Sladky 1959a). Oxygen consumption increased by 23% in HA treated plants, and by 34% in FA treated plants, compared to control plants.

Foliar applications of solutions of humic materials may also increase respiration rates. When leaves of begonia were sprayed with humic acid solution, a large increase in oxygen uptake was observed (Sladky, 1959b; Sladky & Tichy, 1959).

Chlorophyll density:

Sladky, (1959a) showed an increase in chlorophyll contents (HA =+63%, FA = +69%) resulting from applications of humic substances in nutrient solutions to tomatoes. Humic acid treatment increased chlorophyll density by 63%, and fulvic acid increased chlorophyll by 69%.

Xudan (1986) also found that spraying wheat with fulvic acid in pot experiments and field trials resulted in a higher level of chlorophyll in the leaves

Hormonal effects:

Mato et al. (1971, 1972a, 1972b) have shown that humic acid and fulvic acid fractions of humic substances inhibit indoleacetic acid (IAA)-oxidase. Although unfractionated humic acid was more effective than humic or fulvic acid fractions at suppressing the destruction of the IAA plant hormone, the smallest molecular fraction (mol. Wt. < 700) showed the greatest inhibition of IAA-oxidase.

Nardi et al. (2000) tested a low molecular weight humic fraction (LMW-HA) for its biological activity in maize seedlings. Results showed that LMW-HA strongly inhibited K+ stimulated ATPase of maize microsomes and H+ extrusion in a manner similar to gibberellic acid (GA). Studies of changes in messenger RNA after the humic treatment was performed and an analysis of synthesized polypeptides demonstrated a positive post-transcriptional effect of HA on protein synthesis. The gibberellin-like activity of LMW-HA appeared to depend on the combination of low molecular size, and to the content of phenolic and carboxyl carbon.

Effects on enzyme activity:

Humic substances have been shown to inhibit the activity of the several other enzymes. They include:
carboxypeptidase A, (Ladd & Butler, 1971)
choline esterase (DeAlmeida et al., 1980)
chymotrypsin A, (Ladd & Butler, 1971)
invertase (Malcolm & Vaughan, 1979b),
peroxidase (Vaughan & Malcolm, 1979).
phosphatase (Malcolm & Vaughan, 1979a,c),
pronase, (Ladd & Butler, 1971)
trypsin (Ladd & Butler, 1971)

The following enzymes were stimulated by the presence of humic substances:

H^+ stimulated ATPase (Pinton and Cesco,1999)
K^+ stimulated ATPase (Nardi et al., 2000)
ficin (Ladd and Butler, 1971)
papin (Ladd and Butler, 1971)

Drought Tolerance and Water Use Efficiency

It has been widely claimed by commercial vendors of oxidized lignites that these materials increase drought tolerance or decrease water consumption. In a landmark study, Xudan (1986) investigated the effects of foliar application of fulvic acid on water use and yield of wheat in pot and field experiments. When subjected to a 9-day drying cycle, the stomatal conductance of control plants fell from 0.85 cm S^{-1} to nearly zero at the end of the cycle. Plants sprayed with fulvic acid at the start of the drying cycle maintained stomatal conductance of 0.30 cm S^{-1} for the entire interval. Fulvic acid applied to well-watered plants in pots also rapidly reduced stomatal conductance from 0.80 to a constant 0.25 cm $S^{-1.}$

When wheat plants were subjected to drought stress at head development stage, grain yield by control plants was depressed by 30% compared to the irrigated control. However, fulvic acid treated plants suffered only a 3% yield loss compared to the irrigated control.

Xudan (1986) also conducted field trials on wheat in north China. Fulvic acid was sprayed on plants just before head development, and allowed to grow to maturity over time when hot, dry winds are prevalent. He found that grain yield was increased by 7 to 18% over the untreated controls.

Piccolo et al. (1993) Treated lettuce and tomato seeds in Petri dishes with unfractionated humic acids derived from an oxidized lignite at strengths ranging from 40 to 5000 mg/L. They attributed the increase in fresh weight of the seedlings to cell elongation and more efficient water uptake.

It is clear that more research is needed to more firmly establish the effects of humic substances on water stress and water use efficiency.

Humic Substances and Soil Microbial Activity

Bkardwaj and Gaur (1972) found that humic acid as sodium humate and fulvic acid had a marked growth stimulating effect on Rhizobium trifolu. The maximum effect was at 500 mg/L. Humus extract dialyzed for fulvic acid exerted appreciable growth stimulating influence (over 200 percent greater growth rate than check) while undialysed sodium humate was less effective (52 percent over check). The growth promoting effect of farmyard manure containing an equivalent amount of humic acid was less than half as effective as that of sodium humate.

Vallini et al. (1997) investigated the effect of humic acids on activity and growth of Nitrosomonas europaea and Nitrobacter agilis in vitro under axenic conditions. Humates from compost-stabilized vegetable waste or leonardite were added to the chemolithotrophic-culturing medium. They found that both types of humic acids increased either NH4+ or NO2-oxidation and cell growth of nitrifying bacteria. By combining these results with data from a comparative growth evaluation of N. agilis, evidence was obtained that nitrifiers cannot use humic acids as an alternative carbon and energy source. They attributed the stimulating effect of humic acids on these bacteria to an increase in microbial membrane permeability favoring a better utilization of nutrients.

Effects of Humic Substances on Soil Physical Properties

Soil structure:

Piccolo and Mbagwu (1989) found a significant increase in water-stable aggregates in a sandy loam and a strong clay soil after treatment with humic substances derived from coal. If so, increased water infiltration and percolation, reduced runoff and resistance to erosion, and increased aeration are other beneficial effects that are indirectly supported by humic substances.

Piccolo et al. 1997 added humic substances from oxidized coal to two soils with severe structural problems and assessed their effect in reducing runoff erosion with simulated rainfall. They observed a reduction of soil loss of 36% on one soil treated with 100 kg/ha; and the same approximate magnitude of reduction on the other soil treated with 200 kg/ha. They found that the improvement in the water retention capacity more than aggregate stability accounted for the reduced runoff erosion. This delayed the onset of runoff and favored water entry through the stable interaggregate pore spaces within the soil beds. Percent moisture retained at field capacity increased from 26.3% to 29.3% at the 0.05g/kg rate for the Principina silt loam (Orthic Xerofluvent), and from 26.9% to 33.0% at the same rate for the Bovolone loam (Udic Ustochrept).

Available water:

Humic acids are heterogeneous substances, which include in the same macromolecule, hydrophilic acidic functional groups (made up of the carboxylic and phenolic groups) and the hydrophobic groups (made up of the aliphatic and aromatic carbon groups) (Stevenson,1994). The humic acid hydrophilic groups (carboxyl and phenols) attract hydration water thus increasing the water retention capacity in soils.

Oxidized Lignite and Odor Control of Manure

Georgacakis, D. (1996) . found that ground lignite (humate), due to its excellent odor- and moisture-absorbing capacities, allowed for the successful incorporation of the wet and malodorous swine manure into the compost process. More work on the use of oxidized lignite for odor control and in the composting process is needed.

The Humin Fraction

Researchers have commonly overlooked the role of humins in soils. Humin is the alkali (and acid) insoluble portion of humic substances. The "humin fraction" includes humin, plus mineral impurities and other insoluble compounds. Humin benefits soils by holding water and by sorbing cations, polar and nonpolar compounds.

Kohl et al. (1998) studied the binding of 3 polycyclic aromatic hydrocarbons (PAHs) and 2 polychlorinated biphenyl (PCBs) contaminants to the humin fraction of organic matter from 3 different soil types. In all soils and contaminants, the humin fraction contained more than 50% of the bound residue and typically between 70 and 80%. Unfortunately, chemically extracted liquids from oxidized lignites leave the humin fraction behind.

Studies have shown that organic P compounds of from several sources, including manure, become bound to high molecular weight organic colloids (humin). The organic P associated with humic substances may exist, in part, as complexes with simple phosphate esters (e.g. inositol phosphates) Brannon and Sommers (1985a) have reported

Additional Research Needs

Additional research is needed to determine the effects of oxidized lignite and derived products in the following areas:

More field research conducted on soils with varying amounts of organic matter

Field research on broadcast applications of oxidized lignite, or banding of oxidized lignite with fertilizer, affects nutrient availability and uptake. This type of research needs to be conducted on soils that vary in pH, presence of free lime, available P and metal micronutrients.

Field research on nitrogen use efficiency using oxidized lignite.

Water use efficiency and abiotic stress tolerance.

Effects on microbial respiration and mineralization of organic matter.

Development of a reliable inexpensive fulvic acid test

Effects on ruminants, ruminant microbial diversity and numbers, efficiency of conversion of cellulose, disease incidence and severity. Although research in this area has been done by corporations, the results are almost always proprietary, and thus not available to the public. Research from public institutions is practically non-existent.

CONCLUSIONS

Solution and sand culture studies have demonstrated that soluble derivatives of humic substances will increase length and fresh and/or dry weights of shoots and roots, number of lateral roots, root initiation, seedling growth after germination, nutrient availability and nutrient uptake. These substances also affect a wide range of enzymatic processes.

Field trials and soil pot studies have also demonstrated these effects using oxidized lignite or derivatives of humic substances. The difference is that less of this type of research has been performed.

Additions of oxidized lignite to soils with low humic content may help to increase aggregate stability and available water capacity.

Recent research data has increased our understanding of the role of humic substances play in nitrate uptake by plants.

A limited amount of research exists on specific effects of oxidized lignites or derivatives of humic substances on plant drought tolerance, water use efficiency, and enhancement of soil microbial activity.

PART II. COMMERCIAL USE OF OXIDIZED LIGNITES AND EXTRACTS OF OXIDIZED LIGNITES

Uses of Oxidized Lignite Products

Vendors of oxidized lignite products commonly advise the following uses. Of course, the amount of research that supports each recommendation varies widely.

Soil treatment - broadcast for broad-spectrum benefits to soils and plants

Soil treatment - banded with micronutrient and phosphate fertilizers to increase availability

Foliar treatment for growth enhancement and stress tolerance

Applied to organic materials to increase the rate of the composting products.

Applied to manure for odor control

Extracts added to liquid fertilizers to help keep phosphates and micronutrient metals soluble.

Types of Oxidized Lignite Products Available

<u>Raw, ground oxidized lignites</u>. Cheapest cost of production per kilogram of humic substances

<u>Liquid extracts of oxidized lignites</u>. Generally base-treated, with a final concentration of 6 - 12% humic plus fulvic acids. More expensive to produce than oxidized lignites, due to the extraction process, low analysis, high transportation and storage costs per kilogram of humic substances.

<u>Dry water-soluble base extracts of oxidized lignites</u>. Most expensive to produce per kilogram humic substances. It is the dried down residue of liquid extracts of oxidized lignites. Drying costs are very high.

<u>Base treated raw oxidized lignites</u>. Addition of a base, generally KOH, sprayed on the oxidized lignite and dried. About the same cost to produce as liquid extracts per kilogram of humic substances

Raw oxidized lignite suspensions. Recent patent-pending process of micronizing and suspending oxidized lignite in water, without chemical alteration. Up to 37% oxidized lignite yielding 24% humic plus fulvic acids. Includes the humin component. Cost to produce per kilogram humic substances greater than raw oxidized lignite.

Fulvic acids - extracted from highly oxidized lignites and peats by various methods. Actually the fulvic acid fraction is what is marketed. Very expensive per kilogram of humic acid fraction extracted.

Application Rates of Oxidized Lignites and Extracts

Based on the ranges in the concentrations of humic substances used by researchers in studies reviewed by Chen and Aviad (1990), they calculated the following rates for field applications. These rates are based on a midpoint average from which benefits were reported.

Assumptions for soil treatment are: 1) the plow layer weight was 2500 Mg/ha, 2) water content at field capacity is 30% by weight (quite high) and 3) the increase required in humic substances to be most effective is 100 mg/L. (range is 25 to 300 mg/L).

Soil Application: = 75 kg. humic substances per hectare (66 lbs./ac.). (range from about 20 to 225 kg/ha).

Assumptions for foliar treatment is a spray volume of 2000 liters per hectare and a midpoint concentration of 250 mg/kg soluble HA + FA. (range 50 to 300 mg/L).

Foliar Spray = 500 grams of HA + FA per 2000 liters. (range from about 100 to 600 g/2000 L).

General comments on application rates:

For soil applications at the rate of 75 kg/ha as suggested by Chen and Aviad (1990), using an oxidized lignite with 70% humic + fulvic acid content, the amount required would be about 110 kg/ha (97 lbs./ac.). The range would be from about 30 to 350 kg/ha. Vendors generally recommend from 40 to 750 kg/ha. Three assumptions here are that the entire humic and fulvic acid fraction will dissolve and remain in the soil solution without reacting with the soil mineral phase, and without leaching in the interval between applications. This rarely, if ever, is the case. Because of this, agronomic benefits probably decline below application rates of 100 kg/ha (88 lbs./ac.).

For foliar applications at the rate of 500g HA and FA per hectare, as suggested by Chen and Aviad (1990), using a 6% HA + FA extract of oxidized lignite, the amount required would be about 8.5 liters per hectare (about 1 gallon per acre). For 12% HA + FA liquid extracts, the rate would be half that. Vendors have suggested rates ranging from 1/2 gallon to 3 gallons (4 to 26 liters per hectare).

Usage on soils with high humus levels:

Soils have widely varying ranges of soluble humic substances in the organic fraction.
In fertile soils with high total humus levels, soluble organic matter may reach levels up to 400 mg/L (Chen & Schnitzer, 1978), while in soils of arid regions it may not exceed 20 mg/L (Chen & Katan, 1980). It would seem that the beneficial effects due to application of humic substances become diminished as native humus content increases. Because of this, the author does not recommend using broadcast applications of oxidized lignites on soils with more than 5% total humus by weight. On these soils, the banding of oxidized lignites or foliar applications of

oxidized lignite extracts should be tested for efficacy by the grower on small areas of his crop, before general use is adopted. More research on the effects of varying soil humus levels on the performance of oxidized lignites and extracts is clearly needed.

On calcareous soils of moderately high humus levels, where solubility of P or metal micronutrients is limiting plant growth, banding acidic oxidized lignite (pH 3.4 - 4.0) with fertilizer sources of these nutrients may result in increased availability to plants. Generally, 5 - 15 lbs. oxidized lignite per acre is applied in a band with fertilizer. Liquid humate extracts can also be banded with liquid fertilizers at the rate of 1 - 3 gallons per acre. Again, this approach should be tested in small areas by the grower, and again, more research data on the benefits of this strategy is needed.

Excessively low recommendations by vendors.

Occasionally vendors of humate products recommend rates so low that little practical benefit is realized. This happens more often with the more expensive oxidized lignite extracts.
Vendors do this for two reasons:

1. To support their claim that their product works better than their competitors and
2. To enjoy huge markups on their product, but still keep the product affordably priced at the usage rates recommended.

The damage done is twofold - the customer does not get the purported benefits he/she purchased and, researchers report little benefits from using humates at the rates recommended by the producer. For example:

In a study of humate-based biostimulants on Turkish hazelnut seedlings, Kelting (1997) found no significant differences in root or top growth compared to untreated controls. All treatments were applied at the manufacturers' recommended rates. In the dry-water soluble oxidized lignite treatment only 2.5 mg was applied to each 3.8 liter pot; a rate recommended by the producer. This amount would provide a HA concentration of just over 1ppm at 50% moisture by volume - if no leaching occurred over the course of the experiment. This is in comparison to the 25 - 300 ppm HA range where most investigators found significant growth effects Chen and Aviad (1990).

One vendor of a dry-water soluble product recommends a rate of 2 oz per acre, and sells the product to retailers at $16,000 per ton!

Conditions in which benefits from oxidized lignite products are reduced

High organic matter soils - especially over 5% humus.

Optimum fertility and growing conditions.

Long term compost or manure additions

Compost additions over 5 tons/acre in a given year

Severe limiting factors. Examples include severe deficiencies of N, extremely high or low pH, excessive wetness, excessive cold, severe compaction, heavy foliar disease pressure, etc.

Growth enhancement is decreased if oxidized lignite products are applied after other biostimulants like kelp extracts, yucca extracts, growth hormones, etc.

The Fulvic Acid Fraction and Claims about Fulvic Acid Content

Oxidized lignites vary not only in the total amount of humic substances, they also vary in the relative proportion split between humic acids and fulvic acids. New Mexico oxidized lignites tend to be higher in the fulvic acid fraction than North Dakota lignites, for example. Reports on specific fulvic acid contents result from the confusion between the "fulvic acid fraction" and the "fulvic acid content". This relates to the fact that there is not currently a reliable and inexpensive fulvic acid test. The two chief ways in which oxidized lignite products are analyzed for humic acid are:

1. Colorimetric tests of a 0.5N NaOH extract of a humate source. The numbers are usually reported as "humic acid content", when it is more precisely the spectrally active humic acid plus fulvic acid content. Included but not reported are other water or base-soluble constituents that may show some absorbance at the 450nm analytical wavelength. These other soluble constituents make up a very small proportion of the total absorbance of the extract, and may vary among oxidized lignite products. Still, it is a good test for routine quantitative analysis of humic substances, and includes both humic and fulvic acids. It is also relatively inexpensive and easy for labs to do

2. A gravimetric test of humic acids precipitated from the 0.5N NaOH extract of a humic acid source, using 6N HCl to bring the pH of the extract at or below 2.0. The humic acid fraction then precipitates out, then washed, dried and weighed. What is being measured is chiefly the humic acids content. This value is always lower than the colorimetric test for humic plus fulvic acids, because what is not included or measured are the fulvic acids and other components still soluble at this acidic pH.

Since there is not currently a reliable fulvic acid test, companies report the "fulvic acid content" as being the difference between the colorimetric test and the gravimetric tests, attributing that difference entirely to fulvic acids. It is more accurate to represent that number as the "fulvic acid fraction", which contains fulvic acid plus other soluble organic components that usually are present. At this time most producers and customers do not know the difference between "fulvic acid" and "fulvic acid fraction".

Although we have recognized procedures, endorsed by the International Humic Substances Society, for extracting and purifying fulvic acid from soils, peats, oxidized lignites and aquatic sources, they are very expensive, time consuming and, most importantly, give variable yields.

Until a good fulvic acid test is developed, there are several ways for states to resolve this matter. Listed below are three options often considered by states:

1. Use both the colorimetric test for humic plus fulvic acids, and the gravimetric test for humic acids, and allow producers to report the difference as a "fulvic acid fraction".
2. Use only the colorimetric test for humic plus fulvic acids; and do not allow producers to report a "fulvic acid fraction"
3. Use only the gravimetric test for humic acids, do not include the fulvic acid fraction, and do not allow producers to report a "fulvic acid fraction"

The third option, which ignores the fulvic acid fraction entirely, as California currently does by only allowing the base extract - precipitation test for humic acids, is problematic for the following reasons:

1. Fulvic acids are the most biologically active fraction of humic substances, and have the highest amount of reactive functional groups.

2. Materials with a low ratio of "fulvic acid fraction to humic acid fraction" will enjoy a higher reported numerical humic acid content compared to materials with a higher ratio of "fulvic acid fraction to humic acid fraction". For example:

One oxidized lignite material with a relatively high proportion of low molecular weight humic substances, and may test at 70% HA + FA by the colorimetric test, and at 45% HA with the precipitation test, leaving a 25% fulvic acid fraction. If limited to just reporting humic acids by precipitation, they can only report 45% humic acids in their material.

Another oxidized lignite material with a relatively low proportion of low molecular weight humic substances, may test the same as the first material in the colorimetric test (70% HA + FA), but their precipitation test may give a result of 60% HA, leaving only a10% fulvic acid fraction. In this case they can report 60% humic acids in their material.

In other words, a producer of oxidized lignite with a relatively high proportion of biologically active small molecules is penalized relative to the producer of a material with a lower proportion of biologically active humic substances!

3. At present "fulvic acid" is a magic buzzword in agriculture, and is often promoted as having greatly enhanced effects on soils, microbes and plants; compared to other humic substances. Because of this, vendors can afford to go through the relatively expensive process of extracting the humic acid fraction for sale to the public at large markups.

The public needs to be educated about the proven benefits of fulvic acid and fulvic acid fraction products. Ignoring the existence of fulvic acid by restricting the reporting of humic substances to humic acid only, via the base extract-precipitation test, is a disservice to the public.

Chemical and Heat Treatment of Oxidized Lignites

Leonardite has been treated in a variety of ways to increase yield of humic and fulvic acids, their extracts, or the relative abundance of functional groups. Chemical treatment with oxidants, organic acids, and prolonged aeration of poorly oxidized coals, has been used with varying degrees of success. All add to the cost of production.

Heat treatment during base extraction for liquids has also been used frequently. Cegarra et al. (1994) used solutions of potassium hydroxide (0.1M and 0.25M) to extract humic substances from peats at temperatures ranging up to 80 degrees C. Although yields increased with temperature, the HA released from the extraction exhibited less oxidized molecules, a lower content of functional groups and larger molecular sizes than extracts performed at room temperature.

Oxidized Lignites and Extracts from Oxidized Lignites as Energy Sources

Humates have been touted as an energy source for microbes by vendors. Aside from small concentrations of readily oxidizable carbohydrates and organic acids that may be present via illuviation from overburden materials, oxidized lignites are, by definition, extremely resistant to further oxidation. The mean residence time for highly humified substances in temperate soils ranges from 250 to 1000 years or longer (Stevenson, 1994). Oxidized lignites and extracts from oxidized lignites should not be promoted as energy sources for soil microbes.

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