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Effects of Humic Acids Extracted from Mined Lignite or Composted Vegetable Residues on Plant Growth and Soil Microbial Populations

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Growing interest in the market for humic substances with agricultural applications has led to the development of new potential sources of these compounds other than fossil matrices (e.g. different kinds of lignite), which, until now, have represented the main raw material for the extraction of humus-like products. High quality compost (green compost) obtained through the aerobic biostabilization of selected organic residues, such as vegetable waste from source-collection at garden-produce markets, may be considered for this purpose. Beyond the primary need to develop technically and economically reliable procedures for the extraction of humic substances from compost at the industrial scale, importance must be placed on controlling the influence of such compounds on soil-plant systems. Humates from leonardite, representative of the active agents among humus-based commercial preparations, have been compared in pot trials with humic acids, potassium salts, from green compost in order to evaluate their respective effects on soil microbial activity and plant productivity. Differences between pot blocks amended with humic acids have suggested that humus-like substances extracted from compost seem to exert higher stimulative effects on microbial growth and vegetative biomass production than fossil humates.

Introduction

In agricultural systems, soil organic matter plays a crucial role in soil fertility (Kononova, 1966; Allison, 1973; Vaughan and Ord, 1985). Unfortunately, intensive land cultivation based on use of large quantities of chemical fertilizers and repeated tillage has created conditions of decline in organic matter levels in most agricultural soils (Mann, 1986; Schlesinger, 1986). In all cases, repeated cropping and a shortage of organic amendments or manuring will eventually deplete the humified fraction, that in a climax state represents a fairly stable component of the soil organic matter.

Humic substances are involved in many reactions, most of which are a consequence of their colloidal properties. They have high surface areas and show adsorptive capacities greater than those of the clay minerals. Humic materials are involved in binding and transporting of metal ions (Weber, 1988), and in sorptive interactions with biological molecules (e.g. extracellular enzymes) (Burns, 1986) or organic chemicals (e.g. pesticides) (Choudhry, 1984) in the soil. Humic compounds also are important in water retention, in amelioration of clay and sandy soils by promoting structure and aggregation, in increasing buffer capacity, and in supplying nutrients (Brady, 1974; Bohn *et al.*, 1985).

On the other hand, studies intended to elucidate the effects of humic substances on plant growth have recently generated increasing interest from both soil and crop biologists. Evidence of positive effects on plants can be summarized in a general stimulation of biomass production on a fresh or dry weight basis (Vaughan and Malcolm, 1985; Vaughan *et al.*, 1985). Increases in length of roots and shoots or in the number of leaves and flowers have been reported repeatedly (Rauthan and Schnitzer, 1981; Malik and Azam, 1985; Van de Venter *et al.*, 1991). Effects on membrane permeability have been suggested to explain the role of humic compounds in improving plant

nutrition on the basis of their surfactant-like behavior (Samson and Visser, 1989), that stimulates ion uptake in root tissues (Dell'Agnola and Nardi, 1987; Varanini *et al.*, 1993).

Humic colloids also may affect microbial growth and metabolism. Numerous investigations have shown that many soil microorganisms belonging to different taxonomic and functional groups positively react to the presence of humic substances in cultivation media (Visser, 1985*a*,*b*). Recent experiments (Schisler and Linderman, 1989) carried out in soil with humified organic amendants have demonstrated that microbial populations, including ectomycorrhizae, sometimes benefit from humic substances, while sometimes they do not. To explain the stimulatory effects of humic molecules on microbes, modification of cell membrane permeability to nutrients is supposed to be the prominent mechanism involved. Moreover, the humus-mediated increase of soil microorganisms may depress resident (root) pathogenic fungi. Drawing a global explanation of the microbial reactivity with humic substances meets with difficulty, given the variability of humic products used by the different investigators. Filling this knowledge gap is important because of the growing consideration of both fossil matrices and stabilized organic waste for the industrial development of humic products for direct use in agriculture.

TABLE 1.
Some chemical characteristics of humates used in the cultivation experiment (values expressed as percent are on a dry matter basis)

	GCHAs	LHAs
pH (H_2O)	13.0*	9.2*
Total N	4.27 %	0.53 %
Total P	0.17 %	0.01 %
Total K	2.67 %	0.04 %
Total Na	0.05 %	2.04 %

* This value was corrected to 7.5 with 3.0 M H_2SO_4 before adding to the pot blocks

TABLE 2.
Physico-chemical characteristics of soil used in the cultivation experiment (values expressed as percent are on a dry matter basis)

Texture	Sandy
Clay	5.2%
Silt	5.0%
Sand	89.5%
Limestone	0.0%
pH (H_2O)	7.6
Total N	0.11%
Organic matter	1.43%
Total P	0.04%
Total K	0.22%
Humic acids	0.06%

The purpose of the present study was to evaluate the relative effects of humic acids extracted from either mined lignite (i.e. leonardite) or composted vegetable residues (green compost) on plant (*Cichorium intybus*) productivity, total microbial populations (aerobic bacteria, actinomycetes, cellulolytic microorganisms, fungi) and autotrophic nitrifying bacteria. The latter, believed to be inhibited by organic matter in general for a long time, occupy an important position in the biogeochemical nitrogen cycle in the soil.

Materials and Methods

Humic Substances for Soil Amendments

Humic acids, sodium salts, from leonardite (LHAs) (Aldrich-Chemie, Steinheim, Germany) were used as representative of humates of fossil origin. On the other hand, humic acids from green compost (GCHAs) were prepared as follows. Composted vegetable residues (Vallini *et al.*, 1990) were digested with 0.1 KOH for 24 hours at room temperature, in the ratio of 1/10 (w/v). The solute fraction was then separated from the undigested residue by centrifuging at 8000 rpm for 20 minutes. Glass wool filtered supernatant was acidified at pH 2.0 with concentrated H_2SO_4. In these conditions, floc-

culation of humic acids was allowed to proceed for 24 hours. Humic acids were finally yielded through centrifugation at 8,000 rpm for 20 minutes, resuspended in 0.1 N KOH, and dried. KOH was used as an extractant instead of NaOH, since the sodium ion could negatively influence physiology of certain crops. Therefore, KOH seems to be more suitable for the industrial production of humic amendants for agricultural applications. Table 1 reports some chemical characteristics of humates used.

Soil and Plants Used in Pot Trials

Sandy soil was collected at the Experimental Farm of the Faculty of Agriculture, University of Pisa, in San Piero a Grado (near Pisa, Italy), from an area that had not been cultivated for 10 years. Description of the physical, chemical and microbiological features of this soil is given in Tables 2 and 3. Soil was drawn to a depth of 35 cm after the top 10 cm layer, containing plant debris, was discarded. The soil was then sieved through a 15 mm grid and stored with natural moistness at + 4°C in the dark until use.

The plant species for determination of vegetable biomass production in cultivation experiments was a variety of chicory (*Cichorium intybus* var. *catalogna*).

Experimental Set Up

Plastic pots, 2.0 l, were used to arrange cultivation blocks with soil and different humates (LHAs or GCHAs) at increasing rates. Each pot, with a polyethylene bag inside, received 1.8 kg of soil. Soils were amended with LHAs and GCHAs at 250, 500, 1,000, 2,000 and 4,000 $mg.kg^{-1}$, on a soil dry matter basis. Control pots of soil contained no humic amendments. Thus, finally, the cultivation scheme consisted of eleven blocks with 30 replicates (pots) per block. Humate amendments of LHAs and GCHAs were resuspended in aqueous solutions, and pH was adjusted to 7.5 with 3.0 M H_2SO_4. Suspensions were then added to different pot series to reach the fixed final concentration in each block. Five chicory seeds were sown in each pot. After germination, the seedlings were reduced to one per pot. Cultivation was conducted for 120 days in a glasshouse at constant temperature of 20 ± 1°C and relative humidity of 70 percent, with pots placed in a randomized block.

Harvests, Analyses and Measurements

Soil was characterized following the analytical procedures suggested by the Italian Society for Soil Science (1985). Humic acids in soil samples were determined as indicated by Riffaldi *et al.* (1986).

After 7, 60 and 120 cultivation days, microbial counts regarding each pot series were performed on 10 separate soil samples drawn from as many randomly taken pots belonging to a given block. Total number of aerobic bacteria, actinomycetes and fungi was determined on agar plates according to the techniques described by Pochon and Tardieux (1962). Cellulolytic microorganisms were counted on solid media Hudson (1972) without an antibiotic. Enumeration of autotrophic nitrifying bacteria (i.e. ammonia- and

TABLE 3.
Residual microbial populations in the soil before the addition of different humates (cells.g^{-1} soil, dry weight)

Total aerobic bacteria	$8.6 \cdot 10^6$
Actinomycetes	$2.4 \cdot 10^5$
Filamentous fungi	$4.1 \cdot 10^4$
Cellulolytic microorganisms	$2.3 \cdot 10^3$
Ammonia-oxidizing bacteria	$1.1 \cdot 10^4$
Nitrite-oxidizing bacteria	$4.9 \cdot 10^3$

nitrite-oxidizers) by the most probable number procedure (MPN) was carried out according to the methods proposed by Schmidt and Belser (1982).

For growth estimation of *C. intybus*, 10 plants from each block were harvested at 60 and 120 days. Vegetable biomass determinations were made on fresh and dry weight. Values were expressed as a mean of ten measurements per treatment. Average leaf and top heights of the plants belonging to a given block also were considered as parameters for growth evaluation.

Results and Discussion

Effects on Soil Microorganisms

Addition of humates to the soil at different rates influenced soil microbial populations depending on the origin of the amendment used.

Increasing concentrations of LHAs or GCHAs resulted in a progressive stimulation of bacterial growth (Table 4). However, population differences, in blocks where nothing or GCHA was added, were higher than those observed in LHA-amended soil. At the same LHA or GCHA rates, slight effects on soil actinomycetes were evidenced while filamentous fungi did not differ (Table 4). Moreover, cellulolytic microorganisms were positively affected by GCHA-amended soil (Table 5). This effect was particularly evident after 120 days growth. Results point out a marked influence of GCHA only on soil aerobic, heterotrophic bacteria. Such a response could be attributed to the nutritive value of humates from green compost versus humates from leonardite. Also, peculiar characteristics of humic molecules of GCHA may have resulted in higher biological activity (i.e. enzymatic activation of nutrient uptake and modification of bacterial cell permeability to nutrients) of this organic amendment. Nevertheless, evidence by the authors

TABLE 4.
Microbiological analyses of soil amended with increasing amounts of two humic acid sources, LHA or GCHA (cells.g^{-1} soil, dry weight)

HA Amendments ($mg.kg^{-1}$ d.s.)	Total Aerobic Bacteria ($\times 10^6$) sampling time (days) 7	60	120	Actinomycetes ($\times 10^5$) sampling time (days) 7	60	120	Filamentous Fungi ($\times 10^4$) sampling time (days) 7	60	120
0									
LHA	9.4a	18.3a	24.4a	3.1a	6.6a	12.4ab	4.5a	18.1a	13.5ab
GCHA	9.4A	18.3A	24.4A	3.1A	6.6A	12.4A	4.5A	18.1A	13.5A
250									
LHA	13.0a	10.6a	17.3a	6.0ab	7.9a	10.2ab	4.5a	16.1a	19.2ab
GCHA	33.8A	18.3A	42.0A	7.8AB	8.2A	12.1A	7.2A	15.1A	17.1A
500									
LHA	38.6ab	23.1a	15.5a	10.2b	10.3ab	15.3b	8.5a	19.2a	30.3b
GCHA	46.9A	38.0AB	21.3A	6.3AB	6.4A	15.6A	13.4B	12.3A	16.3A
1,000									
LHA	14.3a	18.0a	67.0a	10.9b	7.3a	5.0a	6.7a	12.9a	17.6ab
GCHA	36.9A	40.4AB	36.4A	9.3B	7.2A	9.3A	16.6B	8.8A	17.8A
2,000									
LHA	70.4b	18.6a	60.6a	6.0ab	7.3a	13.5b	6.4a	18.5a	8.6a
GCHA	94.5B	52.9B	35.5A	5.7AB	8.4A	12.2A	5.6A	17.1A	14.0A
4,000									
LHA	80.2b	43.1a	20.2a	8.5ab	11.5b	11.0ab	4.7a	17.1a	15.0ab
GCHA	91.2B	68.7B	114.9B	4.2AB	13.0B	17.3A	4.6A	24.0A	28.0A

At each sampling time, values in the columns followed by different letters are significantly different at p = 0.05

TABLE 5.
Microbiological analyses of pot culture soil added with increasing amounts of two humic acid sources, LHA or GCHA (cells.g^{-1} soil, dry weight)

HA amendments (mg.kg^{-1} d.s.)	Cellulolytic Microorganisms (× 10^3) sampling time (days) 7	60	120	NH_4^+ Oxidizing Bacteria (× 10^3) sampling time (days) 7	60	120	NO_2^- Oxidizing Bacteria (× 10^3) sampling time (days) 7	60	120
0									
LHA	2.5a	4.6a	11.0a	13.0a	2.7a	0.04a	5.2a	4.8a	31.0a
GCHA	2.5A	4.6A	11.0A	13.0A	2.7A	0.04A	5.2A	4.8A	31.0A
250									
LHA	3.5a	4.9a	13.6a	12.5a	11.2a	0.4a	51.2a	28.0a	24.0a
GCHA	1.4A	3.5A	26.8BC	52.5A	7.0A	3.1AB	29.2A	11.0A	2,484.0B
500									
LHA	3.2a	4.6a	17.1a	18.0a	27.0a	1.1a	48.0a	82.0a	36.0a
GCHA	1.9A	6.1AB	18.8AB	11.4A	28.0A	0.2A	51.1A	165.0AB	2,296.0B
1,000									
LHA	2.8a	3.7a	16.5a	47.6a	4.4a	0.2a	30.0a	5.0a	24.0a
GCHA	2.6A	3.1A	17.0AB	53.5A	11.0A	5.4B	11.3A	10.0A	3,016.0B
2,000									
LHA	3.4a	2.5a	14.8a	52.4a	22.4a	1.6a	46.5a	11.2a	46.0a
GCHA	2.5A	7.0AB	37.1C	900.0B	105.0B	9.1B	1,140.0C	263.0B	30,377.0C
4,000									
LHA	2.8a	4.2a	18.5a	166.0a	1.8a	0.2a	29.6a	12.2a	11.0a
GCHA	1.9A	9.2B	51.4D	532.0B	117.0B	14.2C	532.0B	527.0C	30,826.0C

At each sampling time, values in the columns followed by different letters are significantly different at p = 0.05

(Vallini *et al.*, in preparation) suggests that potassium, added to GCHA, did not cause the stimulatory effects on microbial populations. On the other hand, although previous evidence has been restricted to mycorrhizal fungi (Vallini *et al.*, 1993), it seems likely that sodium supplemented LHA added to the soil would not exert any depressive effect.

Autotrophic nitrifying bacteria were shown to be stimulated by GCHA, especially at the highest rates (1,000 to 4,000 mg.kg^{-1}) introduced into the soil (Table 5). However, humates from green compost mainly affected nitrite oxidizers. In particular, nitrite oxidizing bacteria markedly increased in GCHA-amended soil at all rates supplied to the soil. No significant variation in the counts of both NH_4^+ - and NO_2^- - oxidizing bacteria was detected in LHA-amended soil. The positive effects of humic acids on the autotrophic nitrifiers in soil confirm previous observations that these bacteria can grow heterotrophically or can be stimulated by organic compounds (Bock, 1976; Focht and Verstraete, 1977; Matin, 1978). Therefore, the statement that organic matter inhibits the chemolitotrophic nitrifying bacteria should be reconsidered, especially when refractory organic compounds such as humic acids are involved (Abeliovich, 1992). Humic substances probably improve the metabolic activity of the autotrophic nitrifiers at either physiological or biochemical levels (Visser 1985*b*; Tan and Lopez-Falcon, 1987). Furthermore, humic substances might enhance nitrification by adsorbing compounds that inhibit nitrifiers (Hendrickson and Keeney, 1979; Sahrawat *et al.*, 1987). Although not yet understood, all these phenomena appear of great importance for the possible ecological significance in soil microbiology.

Effects on Plant Growth

Results concerning production of vegetable biomass of chicory in soil treated with humic amendments are reported in Table 6. Humic amendments promoted plant

growth. This stimulatory effect was directly correlated with the amounts of humic acids added to the soil. However, response of chicory to GCHA additions was significantly more remarkable than that observed with LHA. As already observed for microorganisms, the higher contents of N and P in GCHA may have in part determined this major vegetative development. Such a difference also may confirm previous evidence that biological activity of humic substances depends on their molecular dimension, with the most active fraction in the low molecular weight portions of the extracts (Vaughan, 1967*a,b*; Mato *et al.*, 1972; Vaughan *et al.*, 1974). Humic acids from mature compost usually have a molecular size smaller than those extracted from fossil or soil samples. Composting does not allow the formation of humic acids of high molecular weight, due to short humification time and polycondensed molecules do not form (García *et al.*, 1991). Humic acids from green compost may have stimulated plant growth by increasing NO_3^- uptake, as reported elsewhere for low molecular size humic fractions (Piccolo *et al.*, 1992). More noticeably, a positive correlation appears to exist between population of nitrate-forming autotrophs and plant productivity in the different treatments amended with GCHAs (Fig. 1).

According to the evidence pointed out before for some microbial populations, the highest (4,000 $mg.kg^{-1}$) rate of GCHAs introduced into the soil has the strongest stimulatory effect on plant productivity.

Applying LHAs to the soil higher than 2,000 $mg.kg^{-1}$ inhibited chicory growth. These results are in agreement with previous reports on the depressive effects of sodium humates from different sources on plant growth (Mylonas and McCants, 1980; Vallini *et al.*, 1993). Nevertheless, concentrations higher than 2,000 $mg.l^{-1}$ recently were found to stimulate plant growth, although these doses did not represent the active frac-

TABLE 6.

Effects of increasing amounts of two sources of humic acid, LHA and GCHA, on the growth of chicory plants (values are the mathematical means of 10 replicates)

HA Amendments ($mg.kg^{-1}$ d.s.)	Plant Fresh weight (g) sampling time (days) 60	120	Plant Dry Weight (mg) sampling time (days) 60	120	Top Height (cm) sampling time (days) 60	120	Leaves (No) sampling time (days) 60	120
0								
LHA	0.2a	12.0ab	23.0a	850.0ab	8.1ab	33.8a	–	10.4*ab*
GCHA	0.2A	12.0A	23.0A	850.0A	8.1A	33.8A	–	10.4A
250								
LHA	0.4a	15.1bc	32.8a	1100.0b	9.8ab	35.4a	–	10.6*ab*
GCHA	0.7A	15.3AB	74.0A	1,140.0AB	13.0B	37.2AB	–	11.4A
500								
LHA	0.6a	18.8c	54.2a	1,330.0b	11.9bc	39.0a	–	9.2ab
GCHA	1.0AB	21.8B	136.6AB	1,800.0c	14.5BC	39.8B	–	13.0A
1,000								
LHA	0.6a	15.7bc	59.4a	1,090.0b	12.0bc	38.4a	–	12.6*b*
GCHA	3.9C	19.2B	418.0C	1,700.0BC	21.6C	41.4B	–	12.4A
2,000								
LHA	1.3a	18.6c	114.4a	1,300.0b	14.5c	39.0a	–	12.6*b*
GCHA	2.3B	33.5C	256.0B	2,830.0D	17.9C	42.2B	–	18.0B
4,000								
LHA	0.2a	7.0a	8.3a	410.0a	5.4c	28.0b	–	7.5*a*
GCHA	1.7AB	63.2D	169.8AB	6,180.0E	15.5BC	41.0B	–	19.8B

At each sampling time, values in the columns followed by different letters are significantly different at p = 0.05

tion of the oxicoal product used by Van de Venter *et al.*, (1991). It is indeed possible that different plant species react differently to the presence of humic substances (Vaughan and Malcom, 1985).

Conclusions

Experiments were conducted to compare the effects of two different humic acids on soil microflora and plant productivity. The origin of humic amendments, both used as humates, was either fossil lignite (leonardite) or compost-stabilized vegetable residues (green compost). Additions of humates at increasing rates to a sandy soil supporting chicory growth resulted in specific and differentiated stimulative effects on soil microbial groups and plant productivity. Nevertheless, the results clearly indicate that:

a) humic acids stimulate, generally, heterotrophic aerobic bacteria in soil, with humates from green compost being more active;

b) humates extracted from green compost had a positive influence on soil cellulolytic microorganisms;

c) populations of autotrophic ammonia and nitrite oxidizers increased, surpris-

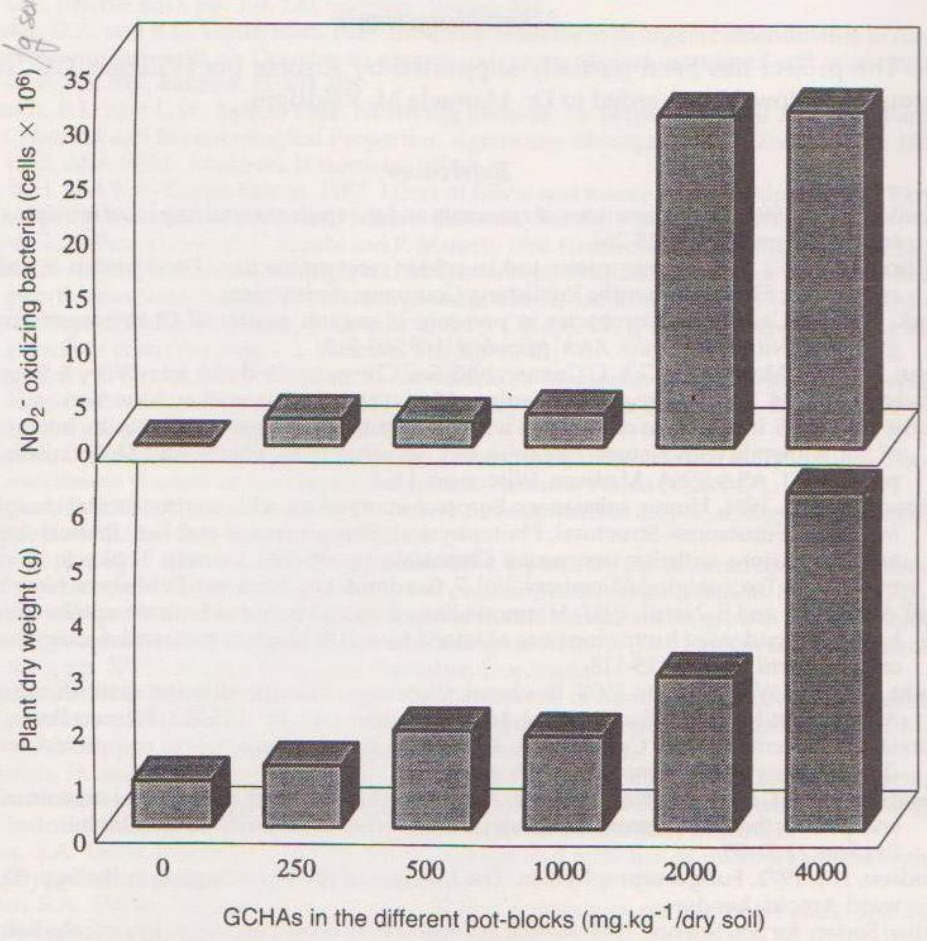

Figure 1. Comparison of vegetable biomass productions and counts of nitrite-oxidizing bacteria in the pot-blocks supporting the growth of *Cichorium intybus* amended with different rates of humates from green compost (GCHAs), after 120 days of plant cultivation

ingly, in soil amended with humates from composted vegetable waste;

d) although both humates from leonardite and compost added to the soil up to 2000 mg.kg^{-1} evidence a marked beneficial influence on chicory growth in terms of vegetative biomass production, greater effects were obtained with compost-derived humates. Compost-derived humates continued to improve plant growth up to the rate of 4,000 mg.kg^{-1}. On the other hand, humates from leonardite, at concentrations higher than 2000 mg.kg^{-1}, appeared quite toxic to the plants.

In the light of these first findings, it can be assumed that humic acids from organic matrices that have undergone compost stabilization possess characteristics which allow them to behave as stimulatory substances on soil-plant ecosystems and some of microorganisms related to the plant roots. Humates of fossil origin at high concentrations have confirmed some negative effects on biota as previously mentioned in the literature. Therefore, there are promising arguments to improve and enhance high-quality compost utilization for the extraction of humic substances. That would signify new interesting perspectives for the compost market. Nevertheless, much remains to be done in order to make this alternative sustainable from the technological and economical point of view.

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