ISSN 1009－3095 *Journal of Zhejiang University SCIENCE* V.3, No.4, P.467－474, Sep.－Oct., 2002
http://www.periodicals.com.cn; http://www.zju.edu.cn/English
http://www.zjupress.com; http://lib.zju.edu.cn/eindex.htm; jzu_s@mail.hz.zj.cn

Alteration of certain soil microbiological and biochemical indices of a paddy soil under anthropogenic stress

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Received May 31, 2001; revision accepted Aug. 21, 2001

Abstract: A 21-day laboratory incubation experiment was conducted to investigate the impact of pesticides (insecticide, herbicide, fungicide) on paddy field soil health under controlled moisture (flooded soil) and temperature (25 ℃) environment. The electron transport system (ETS)/Dehydrogenase activity showed negative correlation with pesticides concentrations, decreased with increase of pesticide concentration. The higher doses (5 to 10 times field rates) of pesticides significantly inhibited ETS activity, while lower rates failed to produce any significant reducing effect on the control. The toxicity of pesticides in decreasing the ETS activity was in the order: insecticide > fungicide > herbicide, irrespective of their rates of application. The pesticides increased the soil phenol content, which increased with increasing concentration of agrochemicals. The pesticide application did not produce any significant change in soil protein content. The response of biomass phospholipid content was nearly similar to that of ETS activity. The phospholipid content decreased with the addition of pesticides in the order insecticide > fungicide > herbicide and the toxicity was in the order: 10 FR (field rate) > 5 FR > 1.0 FR > 0.5 FR > control.

Key words: Electron transport system (ETS) activity, Phenol, Phospholipids, Protein, Pesticides, Submerged soil

Document code: A　　**CLC number:** S154.3

INTRODUCTION

The declining productivity of irrigated rice crops after years of continuous, intensive cultivation appears to be associated with a decreasing N supply capacity of the soil (Olk et al., 1998), which in turn may be due to the enrichment of phenolic compounds in the soil (Olk et al., 1996), so that rice soil microbial biomass may be considered as the key to better understanding of a decreasing nutrient supply capacity.

Microbial activities have important effects on crop productivity; and nutrient cycling can be altered by agricultural management practices (Doran et al., 1987), which were aimed primarily in the past to improve productivity (Lovell et al., 1995). The microbial biomass is a sensitive indicator of changes resulting from agronomic practices and other perturbations of the soil ecosystem (Gunapala and Scow, 1998). Free phenolic acids exist in the soil solution comprising < 0.01% of the total soil organic matter (Vaughan et al., 1980); have toxic or stimulatory effects on plant and microbial growth (Wang et al., 1967; Anderson and Domsch, 1974; Olk et al., 1996); and can be readily utilized by soil microbes (Sparling et al., 1981).

Modern agriculture and industry depend on a wide variety of synthetically produced chemicals, including insecticides, fungicides, herbicides and other pesticides. Continued widespread use and release of such synthetics have become an everyday occurrence, and resulted in environmental pollution. In this context, the influence of pesticides on the microbial activity of soil microorganisms were studied by some investigators (Omar

* Project (No. G1999011809) supported by the National Key Basic Research Foundation (973) of China
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et al., 1992; Sanchez et al., 1994). The fungicide captan significantly reduced the total culturable fungi in soils studied (Martinez-Toledo et al., 1998). Similar results had been reported for other fungicides (Pezo et al., 1994). Pesticides generally appear to have no adverse effects on the population of total bacteria in soil except at concentrations exceeding recommended rates (Lal et al., 1988).

The present study was designed to investigate the effects of pesticide including insecticide, herbicide and a fungicide applied at various concentrations on soil microbiological and biochemical properties in a paddy field soil.

MATERIALS AND METHODS

1. Soil

A laboratory incubation experiment was conducted to determine the effects of pesticides including insecticide, herbicide and fungicide on some soil microbiological and biochemical characteristics under flooded soil condition at constant temperature of 25 ℃. The soil used was sampled from the surface layer (0－15 cm) of an agricultural field used for paddy rice near Jinhua City, Zhejiang Province, after removal of surface water. The soil was dried to moist condition, passed through a 2-mm sieve and stored at 4 ℃ prior to analyses. A subsample of the soil was taken, air-dried, ground, and analyzed for various physico-chemical characteristics (Anderson et al., 1993) listed in Table 1.

Table 1 Characteristics of the soil used

Parameter	Value	Parameter	Value
pH (H_2O)*	4.74	CEC (c mol/kg)[e]	7.328
WHC (g/kg)[a]	510	Sand (g/kg)	278
O.C. (g/kg)[b]	15.25	Silt (g/kg)	562
Av. N (mg/kg)[c]	106.40	Clay (g/kg)	160
Av. P (mg/kg)[d]	13.34	Soil texture[f]	Silt loam

* = Soil pH in 1: 2.5 soil: water suspension; a= water holding capacity (Anderson and Ingram, 1993); b= total organic carbon (Nelson and Sommers, 1982); c= Available nitrogen (Anderson and Ingram, 1993); d= Available phosphorus (Olsen and Sommers, 1982); e= Cation exchange capacity (Anderson and Ingram, 1993); f= Soil texture (Gee and Bauder, 1986)

2. Soil treatments and incubation conditions

The pretreated soil in portions equivalent to 150 g oven-dry weight were placed into 250 mL glass beakers. Three sets of 15 beakers each (5 treatments×3 replications), one beaker each for insecticide, herbicide and fungicide, were prepared to maintain various treatments. The soil samples were first adjusted to their required level of moisture contents (flooded soil, FS, the water level was 2 cm above the soil water interface) by adding distilled water and then preincubated at 25 ℃ for 7 days (conditioning period). Appropriate quantities of an insecticide (triazophos), herbicide (butachlor) and fungicide (streptomyces hygroscopicus var. jinggangensis yen) were added as 1.0 ml volumes maintaining the concentrations of 0 (control), 0.5 FR, 1 FR, 5 FR, and 10 FR. The 1 field rate (i.e., 1 FR) for insecticide, herbicide and fungicide was 1500 mL/ha, 2.5 kg/ha and 75 g/ha, respectively. All the soils including control were adjusted to the same moisture level of flooded condition. Then all three sets were covered with plastic sheets having small holes and incubated at 25 ℃ in the dark for 21 days, after which, soil samples were taken out and analyzed for various parameters. All results were taken as the mean of three replicate determinations and expressed on an oven-dry soil weight basis (105℃, 24h). The moisture was maintained constant by adding distilled water at regular intervals throughout the incubation period.

3. Soil assay

Electron transport system (ETS)/dehydrogenase activity was measured using Benefield et al. (1977)' s method of reducing 2-(*p*-iodophenyl-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride (INT) to iodonitrotetrazolium formazan (INT-formazan/INTF). The absorbance values obtained photometrically were

converted to nmoles INT-formazan/(min • g) (dry soil) using a standard curve of INT-formazan (INTF). The total soluble phenol compounds of the soil organic matter were determined with Folin Ciocalteau's phenol reagent method as described by Box (1983). Briefly, extracted 1 cm^3 soil (on oven-dried basis) with 5 ml 1 mol/L NaOH was shaken for 30 minutes, then centrifuged (6000 × g, 15 minutes, 5℃) to yield a clear supernatant. Then to 0.5 ml supernatant put into screw capped test tube to which was added 2.5 ml of 2 % Na_2CO_3 in 0.1 mol/L NaOH. The supernatant and added chemicals were then mixed on a vortex and then let to stand for 10 minutes; after which 0.25 ml of diluted (1 : 1) Folin-Ciocalteau reagent was rapidly added to them, and they were immediately mixed on a vortex; and than again allowed stand for 10 minutes. For full color development, after which absorbance at 750 nm was read on a spectrophotometer. Total protein was also determined by reaction of Folin Ciocalteau reagent with amino acids/proteins containing phenolic hydroxyl groups (Lerch et al., 1993). For this, the same clear supernatant liquid obtained for phenol determination after extraction with 1 mol/L NaOH was used. Then 0.25 ml of the supernatant soil extract was put into screw capped test tubes to which were added 0.25 ml H_2O (or 1 mol/L NaOH). This mixture was then hydrolyzed at 100 ℃ (on water bath) exactly for 5 minutes. Together with standards and then transferred into ice bath. Then to 0.50 ml digested soil extract was added 1.25 ml copper reagent. They were then mixed on a vortex; allowed to stand for 10 minutes. Then after 0.25 ml of diluted (1:1) 1 N Folin-Ciocalteau reagent was rapidly added to them, they were mixed immediately on a vortex; allowed to stand 15 minutes. For full color development and absorbance at 750 nm was read on spectrophotometer.

The total phospholipids were determined from the phosphate content. Inorganic Ophosphate was released by digestion of the lipid extract with potassium persulfate, and color was developed by reaction of phosphate with ammonium molybdate and malachite green (Frostegard et al., 1991). Briefly, 3.0 cm^3 soil was put into chloroform resistant centrifuge tubes, to which was added 18.3 ml of a single-phase mixture consisting of $CHCl_3$: MeOH: citrate buffer (1:2:0.8) and after the soil and mixture were mixed in a vortex (this mixture consisted of 4.8 ml $CHCl_3$: 9.6 ml MeOH: 3.9 ml citrate buffer), they were shook on a mechanical shaker (90 rev/min) at room temperature for 2 hours; then centrifuged at 2500 rev/min for 10 minutes. at room temperature. The supernatant was separated from the residue and transferred to new chloroform resistant tubes. The residue was washed once with 5 ml of the single-phase mixture $CHCl_3$: MeOH: citrate buffer (1.3:2.6:1.1 ml) and vortexed, centrifuged again at 2500 rev/min for 10 minutes. at room temperature. To the transferred supernatants in their respective (same) tubes were added 6.2 ml $CHCl_3$ and 2 ml citrate buffer. Then the supernatant with mixture was let to stand overnight to separate into aqueous and chloroform layers. Then 1 ml of the lipid-containing phase ($CHCl_3$ lower layer) was transferred to new screw capped test tubes. After the cover was removed, the organic solvent in the digestion block (or water bath) at 95℃ dried up by evaporation. Then after 2 ml of saturated $K_2S_2O_8$ was added to the dry lipid extract, the tubes were sealed , incubated (i. e. put in water bath) at 95℃ for 48 hours. After 48 hours digestion, the volume of the liquid was checked and kept at 2 ml by adding enough saturated $K_2S_2O_8$. Four-tenths ml of 2.5% ammonium molybdate solution was added to the digested lipid extract before it was let to cool and stand for 10 minutes at room temperature; and then after 1.8 ml of malachite green solution was added to the digested lipid extract, it was incubated at room temperature for the next 30 minutes; after which, the absorbance at 610 nm was measured.

Data were examined by analysis of variance using Statistix 4.1 software.

RESULTS

1. Effect of pesticides addition on dehydrogenase/ETS activity

There was consistent decrease in ETS activity with increasing level of insecticide in the soil. A slight (non-significant) reduction of 3.1% and 4.3% in ETS activity occurred at 0.5 FR and 1.0 FR of insecticide addition, respectively,

compared with the control. A significant inhibition of ETS activity by 8.2 % was observed at insecticide level of 5 FR, as against the control. The highest insecticide concentration used (10 FR) resulted in a great reduction (by 14.2 %) in the ETS activity, relative to the control.

On the other hand, the herbicide butachlor applied at the same concentrations exhibited comparatively lesser but also consistent inhibitory effect on the ETS activity, which increased with increasing herbicide concentration (Fig. 1). The herbicide additions at 0.5 FR and 1.0 FR did not show any significant depression of the ETS activity compared with the control. Significant decreases (by 7.1 and 10.4 %) were evident only at herbicide concentrations of 5 FR and 10 FR, respectively, vs the control.

The application of fungicide also seeming had inhibitory effect on ETS activity, which increased with increasing concentrations (Fig. 1). The depressing effect of fungicide on ETS activity was found to be of lesser degree than insecticide but was more than that of herbicide. Here again, the lower rates of fungicide (0.5 FR and 1.0 FR) did not produce any significant change in ETS activity and only 1.7 % and 2.6 % reductions were recorded, respectively, compared with the control; while additions at 5 FR and 10 FR caused significant reductions (by 7.9 and 12.6 %) in ETS activity, respectively, vs the control.

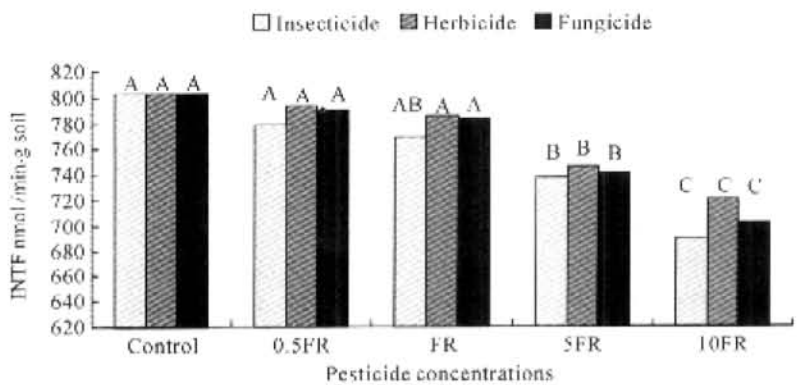

Fig. 1 Effect of pesticides concentrations on ETS activity. Bars with same letters, for a particular pesticide, are statistically non-significant at $P<0.01$

2. Effect of pesticides addition on soil phenol content

The addition of insecticide greatly increased the soil phenol content. There was a consistent increase in the phenol content with increasing level of insecticide in the soil (Fig. 2). Results revealed that insecticide addition at 0.5 FR did not enhance (1.3 %) the phenol content significantly, compared with the control. A significant increase in the phenol content by 2.3 % was noticed at FR level of insecticide addition, relative to the control after the end of 21-day incubation. The higher rates of 5 FR and 10 FR exhibited marked increase by 5.2 % and 6.5 % in the phenol status, respectively, as against the control.

In comparison to insecticide, herbicide application showed considerably less toxicity towards the soil phenol content. A consistent increase was also noticed here with increasing levels of herbicide (Fig. 2). The herbicide treatments of 0.5 FR and 1.0 FR did not have any significant stimulatory effect on the soil phenol content. A significant increase was only noticed at herbicide levels of 5 FR and 10 FR, where the phenol content were increased by 4.3 % and 5.4 %, respectively, relative to the untreated control soil.

The application of fungicide showed almost similar response, like insecticide addition, in increasing the soil phenol content. But the increase in phenol content with fungicide addition was comparatively lower than that with insecticide while it was higher than that with herbicide treatment at all levels of application (Fig. 2). The lower rate of 0.5 FR did not cause any significant change in phenol content, while 1.0 FR, 5 FR, and 10 FR resulted in significant in-

crease in the soil phenol content by 2.0 %, 5.1 % , and 6.0 % , respectively, with reference to the control.

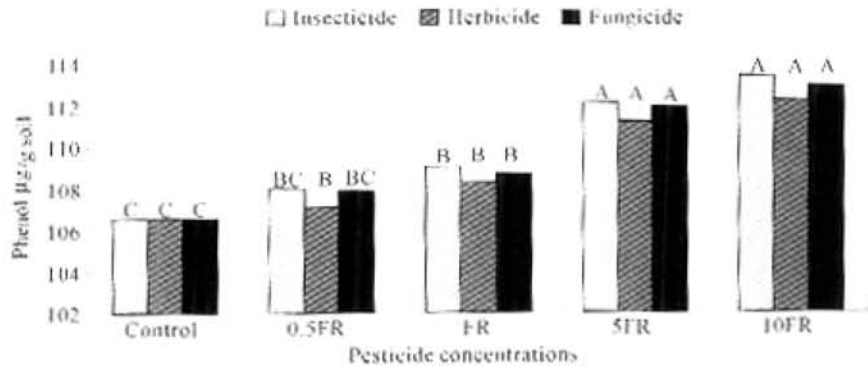

Fig. 2 Effect of pesticides concentrations on soil phenol contents. Bars with same letters, for a particular pesticide, are statistically non-significant at $P<0.01$

3. Effect of pesticides addition on soil protein content

The effect of pesticides concentration on soil protein content is shown in Fig. 3. The results revealed that the application of three pesticides i. e., insecticide, herbicide, and fungicide could not produce any significant change in the soil protein content at all application levels after 21-day incubation period under waterlogged conditions, although consistent but slight increases in the protein content were recorded with increasing pesticide concentrations, none of them resulted in any marked increase in protein level. The slightly higher protein contents found in insecticide treated soil were closely followed by those of fungicide and herbicide additions. The increase in protein content with all pesticide additions was found to be negligible (<1.0 %), although a positive correlation was observed between the concentrations of pesticides and soil protein content.

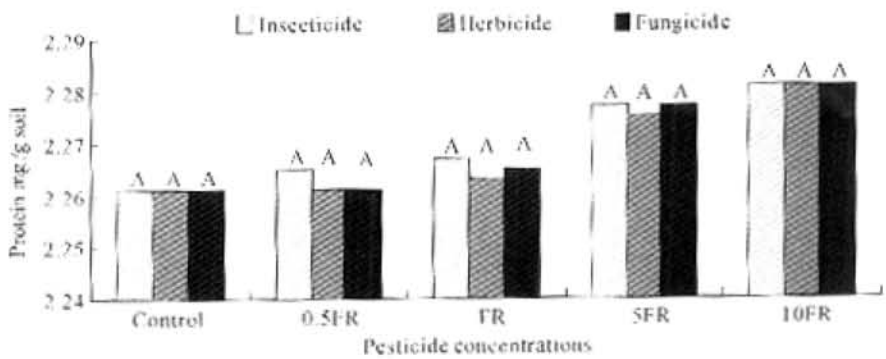

Fig. 3 Effect of pesticides concentrations on soil protein contents. Bars with same letters, for a particular pesticide, are statistically non-significant at $P<0.01$

4. Effect of pesticides addition on phospholipid content

The results given in Fig. 4 indicated that the insecticide application resulted in significant reduction of the microbial phospholipid content of the paddy field soil. A consistent decrease in phospholipid content was observed with the increasing levels of insecticide in the soil (Fig. 4). The addition of insecticide at the levels of 0.5 FR and 1.0 FR did not produce any significant reduction of the phospholipid content compared with the control. Significant decline by 1.9 % and 2.6 % were only evident at the higher insecticide additions of 5 FR and 10 FR, respectively, as against the control.

In contrast to insecticide, the magnitude of decrease in phospholipid content was smaller in soils spiked with herbicide butachlor (Fig. 4). The herbicide concentrations of 0.5 FR and 1.0 FR resulted in a non-significant slight decrease in the noted phospholipid content as compared with the control. A significant decline by 1.5 % and 2.3 % was recorded at herbicide levels of 5 FR and 10 FR, respectively, relative to the control.

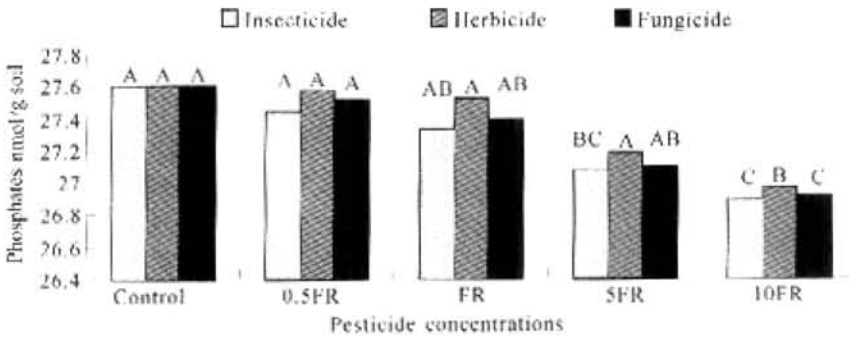

Fig.4 Effect of pesticides concentrations on phospholipid contents. Bars with same letters, for a particular pesticide, are statistically non-significant at $P<0.01$

Fungicide had similar effect as insecticide in inhibiting phospholipid content. However, the decrease in phospholipid content was comparatively lower than that caused by insecticide but more than that caused by herbicide (Fig. 4). There was a consistent decrease in phospholipid content with the increasing level of fungicide. The lower levels of fungicide (0.5 FR and 1.0 FR) did not produce any significant inhibition in phospholipid content, while higher levels (of 5 FR and 10 FR) caused significant reductions (by 1.8 % and 2.5 %, respectively), compared with the control. A highly negative correlation was noticed between the application rates of pesticides and phospholipid content.

DISCUSSION

This study revealed that ETS activity had negative correlation with pesticide concentrations, and decreased with increasing concentration of the pesticide in the soil. This can be explained. The half-life of the pesticide used was about 1 month or so, so after 21 days of incubation, this response is understandable; and as 0.5 FR and FR did not produce any significant inhibition of ETS activity, microorganisms might have used it as an energy source (El-Ghamry et al., 2000). However, higher doses resulted in high reductions. Lal and Lal (1988) reported similar observations. The fungicides folpet and captafol, at 1.0 mg kg^{-1}, inhibited dehydrogenases slightly. However, a 10-fold increase in their dosage reduced the activity significantly, but it recovered to the control level after 21 days (Atlas et al., 1978). The behavior of pesticides exhibited in soil is governed by various parameters. Of primary importance are the pesticide molecule's chemical, structural, and physical parameters, such as water solubility, dissociation constants, and vapor pressure (Briggs, 1990).

The addition of pesticides considerably strong effect on soil phenol content. An inverse relationship was observed between ETS activity and soil phenol contents. Stevenson (1994) reported that soil microorganisms can produce and decompose phenolic compounds. There are many soil fungi capable of utilizing phenolic acids as their sole source of carbon (Henderson and Farmer, 1955). Olk et al. (1996) also reported accumulation of phenolic compounds in soil organic matter, which he suggested is a characteristic of the anaerobic, or nearly anaerobic, soil conditions that exist at the initial stages of soil organic matter formation in submerged irrigated rice soils. It was found that phenolic acids can be readily utilized by soil microbes (Sparling et al., 1981) co-occurring with the free acids in the soil solution and so, should be readily available for microbial degradation (Wang et al., 1967). The present study revealed a marked increase in soil phenol

content with higher levels of pesticides addition. This increase in soil phenol content was found to maximize with insecticide addition followed by fungicide addition, and then herbicide addition; and it (phenol content) also increased with increasing pesticide concentration. This phenomenon might be due to decreased microbial activity with pesticide addition, particularly at higher rate of application, which might have resulted in lowering decomposition and utilization of phenol contents by soil microbial biomass (Sparling et al., 1981; Stevenson, 1994).

The application of pesticides produced a slight (non-significant) increase in protein content in the soil. This might be due to the comparatively more mineralization of organic N in control soils than in treated soil as the pesticide addition might have reduced the microbial population and activities (El-Ghamry et al., 2000). Consequently, as the microbial biomass decreased there was a decrease in the amount of enzyme activity and decreased organic C, N, and P mineralization (McLarchey and Reddy, 1998). The high resistance (stability) of organic N complexes in soil to microbial attack is of considerable importance to the N balance of the soil. Several explanations are often given for this phenomenon. For instance, proteinaceous constituents (e.g., amino acids, peptides, proteins) are stabilized through their reaction with other organic constituents; such as lignins, tannins, quinones; and reducing sugars and biologically resistant complexes are formed in soil by chemical reactions involving NH_3 or NO_2^- with lignins or humic substances. The complexes thus formed have been shown to be highly resistant to mineralization by soil microorganisms (Stevenson, 1994). The increased phenol contents in this study might be a strong reason for the increased protein contents (Olk et al., 1996).

The incorporation of pesticides caused marked changes, especially at their higher rates of application, compared to the control. Phospholipids occur in the cell membranes of all living cells and are not used as storage products (Petersen et al., 1991). It was reported that soil under aerobic condition contains larger amounts of phospholipids than under anaerobic (flooded) conditions (Reichardt et al., 1997). Frostegard et al. (1991) evaluated the use of total lipid phosphate ($L\text{-}PO_4$) as a measure of microbial biomass in soils with different organic matter content and suggested that the soil samples typically contained $\approx$ 30-50 nmol $L\text{-}PO_4$ (a range suitable for the method used) after persulfate digestion. The slightly lower values in the present study might be due to anaerobic conditions as anoxic conditions caused reduction in (aerobic) microbial biomass (Inubushi et al., 1991). Also, the lower quantities of phospholipids observed in agrochemicals treated soils than in the control soil, might be due to their toxicity as explained by Wingfield et al. (1977). Reichardt et al. (1997) also noticed a decline in phospholipid content, due to flooding in continuously cropped, irrigated rice fields.

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