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STUDIES ON THE SYNTHESIS AND BIOLOGICAL EVALUATION OF COPPER COMPLEXES OF SOME 4-AMINO-3-HYDRAZINO-5-THIO-1, 2, 4-TRIAZOLE DERIVATIVES

A . B. Tadros

National Institute of Oceanography and Fisheries, Kayet Bay,

Alex., Egypt.

Hendrik C.A. Van Beek

Laboratory of Chemical Technology , Delft University of

Technology Julianaan 136, Delft, The Netherlands.

ABSTRACT

4-Benzylideneamino-3-hydrazino-5-thio-1,2,4-triazole (H_2BAHTrz) and 4-(benzylideneamino)-3- (benzylidene-hydrazino)- 5-thio- 1,2,4-triazole (H_2BABHTtrz) were synthesized and their copper complexes have been prepared. The compounds are characterized by : chemical analyses. infrared. electronic spectra, magnetic moment, and NMR spectra. A mixed Cu (II) Cu (I) complexes have been prepared and isolated which suggest a possible mechanism for the reduction of Cu (II) to Cu (I) by sulfhydral group of the organic molecule.

H_2BAHTtrz behaves either as a monovalent or neutral bidentate ligand in which the coordination with copper ion occur normally through the amino hydrazino group and azomethine nitrogen or thiol sulphur atom and azomethine nitrogen. The coordination of copper ions are possible to be through sulphur atom, azomethine nitrogen and amino nitrogen with H_2BABHTtrz ligand.

The effective lethal concentration EC_{50} for each compound was tested using Chlamydomonas unicellular algae and marine bacterium phosphoreum species. EC_{50} is the concentration at which critical magnitudes such as

growth rate, yield numbers of viable cells are half those in the control (without test substance). The results had showed that, the compounds have low toxicity in comparison with the standard test substance, tributyltin oxide (TBTO). The bioactivity of the compounds against fouling organisms have been tested through 74% by volume technique, most of them showed high potential against the green macroalgae and differential activity against the other fouling organisms.

Key words : 1,2,4-triazole derivatives, copper complexes, structural studies, toxicity test and bifouling evaluation.

1. INTRODUCTION

This work is an extension of the previous investigations on 4-amino-3-hydrazino-5-thio-1,2,4 triazole and its metal complexes, which frequently exhibit unusual structural properties and have some antifouling potential[1]. As a part in the study of the complexes formed by metal (11) ions and the last ligand and some of its derivatives[2], the coordination chemistry and toxicity evaluation of 4-(benzylideneamino) -3-hydrazino-5-thio- 1, 2, 4-triazole and 4-(benzylideneamino) -3- (benzylidenehydrazino)-5- thio-1, 2, 4-triazole and their copper complexes have been investigated. It is found that, interesting change occurs in the copper complexes with the change in the method used for their preparation.

The best known screening test is a biological laboratory test, where EC_{50}3, values are determined by various organisms for various concentrations of the prepared compounds in sea water. The obvious selected test organisms were the flagellate Chlamydomonas reinhardii, and the bacterium Photobacterium phosphoreum as a test criterion.

2. EXPERIMENTAL

2.1. Preparation of Ligands

4-(Benzylideneamino)-3-hydrazino-5-thio-1,2,4- triazole L_I,4-(benzylideneamino)-3-(benzylidene-hydrazino)-5-thio- 1,2,4-triazole L_{II}, were prepared using the same procedure described by Ronald G. Dickinson[4].

2.2. Preparation of copper complexes

2.2.1. Copper complexes of H_2BAHTtrz L_I

Five different copper complexes were prepared with 4-(benzylideneamino) -3-hydrazino-5- thio-1,2,4-triazole. Aqueous solution of 2 mmole $CuCl_2$ was added to cold ethanolic solution of the ligand (2 mmole). The reaction mixture was refluxed for 15 minutes, the ppt. product filtered off and dried. This complex was called, Cu(H_2BAHTtrz). (HBAHTtrz) Cl (I). Analysis (Calc.), Found, Cu, (11.23), 11.00, Cl, (6.27), 6.70, S; (11.34), 11.72, C; (38.22), 37.75, H, (3.21), 3.10 and N; (29.72), 29.50. Cu_2(HBAHTtrz)$_2$Cl. $2H_2O$ (II), was prepared as in the above procedure, but with increasing the refluxing time to 90 min. The color of the reaction mixture became deeper and the complex was separated as above. Analysis (Calc.), Found, Cu, (28.28), 28.08, Cl, (15.78), 15.97, S; (7.14), 7.33, C; (23.13), (23.13), 23.00, H; (3.02), 3.00 and N; (17.99), 18.19.

If an aqueous solution of $CuCl_2$ (2 mmole) was added with stirring to hot suspended ethanolic solution of L_1 (2 mmole) , a light green complex was separated immediately. Analysis (Calc.), Found,

Cu, (11.24), 11.00, Cl, (6.27), 6.00, S; (11.34), 10.66, C; (39.00), 38.22, H; (3.20), 3.21 and N; (30.44), 29.72. This complex was called [(Cu(H_2BAHTtrz)$_2$)$_2$]. Cl_2 (III). In the last procedure when the refluxing time was increased to about 1 hr. the color of the separated complex became deep green and the complex called Cu (HBAHTtrz). 2H_2O. Cl. Analysis (Calc.), Found, Cu; (17.25), 17.00, Cl; (9.63), 9.22, S; (8.71), 8.00, C; (29.35), 30.00, H; (3.55), 3.50 and N; (22.82), 23.00. Cu(H_2BAHTtrz)$_2$Cl.2H_2O (V), was prepared by adding with stirring aqueous solution of $CuCl_2$ (2 mmole) to a hot suspended of the ligand (4 mmole) in ethanol. The reaction mixture then heated, filtered and dried. Analysis (Calc.), Found, Cu, (9.97), 9.90, Cl, (11.13), 11.10, S; (10.07), 10.07, C; (33.93), 34.50, H; (4.11). 4.00 and N; (29.39), 27.00.

2.2.2: Copper complexes of H_2BABHTtrz L_{II}

Three different copper complexes were isolated with this ligand based on the refluxing time of the reaction mixture and the metal / ligand ratio. When aqueous solution (2 mmole) of $CuCl_2$ was treated with (2 mmole) of L_{II} dissolved in a least amount of ethanol, olive green complex was formed fairly rapidly and separated by filtration. This complex was called (Cu(HBABHTtrz)$_2$. (HBABHTtrz)$_2$. Cl (VI). Analysis (Calc.), Found, Cu, (4.59), 4.66, Cl; (2.56), 2.53, S; (9.27), 9.00, C; (55.53), 56.00, H; (3.22), 3.00 and N; (24.29), 25.00. When the reaction mixture was refluxed for 1 hr. a deep green complex separated out. This complex was called Cu(HBAHTtrz). (H_2BAHTtrz). Cl (VII). Analysis (Calc.), Found, Cu; (8.75), 8.48, Cl; (4.78), 4.60, S; (8.65), 8.20, C; (51.83), 50.90, H; (3.53), 3.60 and N; (22.66), 22.42.

$Cu(HBABHTtrz)_2$. $(HBABHTtrz)_2$ (VIII), was prepared by adding with stirring (2 mmole) aqueous solution of $CuCl_2$ to (4 mmole) ethanolic solution of L_{II} and then heat the reaction mixture gently a yellow green complex was separated and filtered. Analysis (Calc.), Found, Cu; (4.71), 4.63, S; (9.51), 9.33, C; (56.99), 57.00, H; (4.19), 4.00 and N; (24.92), 25.00.

2.3 Analysis and physical measurements

The chemical analysis of carbon, hydrogen, sulphur, chlorine and nitrogen were done by Chemical Technology Department, Technical University Delft, Holland. The estimation of copper was carried out by standard complex -metric titration.

Infrared spectra of the ligands and their complexes were recorded on a Perkin Elmer 389 Infrared- spectrophotometer in the range 4000-400 cm^{-1} using potassium bromide pellets of the sample.

^{1}HNMR spectra of the ligands and diamagnetic complexes in deutrated DMSO were recorded at 35°C on 60 MHz Varian EM-360 spectrophotometer. ^{13}C NMR spectra were recorded on a 50 MHz Nicolet NT-200 WB spectrophotometer.

The magnetic susceptibility of the complexes was determined at room temperature by the Faraday method using Cohn balance with a Newport magnet in the Department of Inorganic and Physical Chemistry, Technical University, Delft, Holland.

The absorption spectra of the complexes as nujul mull were recorded on a PMQ Spectrophotometer in the range 250-1000 nm with M_4Q III monochromat.

2.4. Biological laboratory test

2.4.1. Test organisms

The water green flagellate Chlamydomonas reinhardii (CCAP 11/32 C) was produced from the culture collection of Algae and Protozoa (CCAP), 36 Story's way, Cambridge, CB3 DDT, England. A preculture containing exponentially growing cells was prepared by the method given in NPR 6505[5]. Freeze-dried cultures of the bacterium photobacterium phosphoreum supplied by Beckman Inc. They were activated by addition of saline water.

2.4.2. Preparation of test solutions

The concentrations to be tested were chosen on the basis of the solubility of the compounds in sea-water, which in some cases exceeds that in algal medium or DMSO. DMSO was used as solvent for the stock solution.

2.4.3. Growth inhibition test with C. reinhardii

The test was essentially the same as that described in NEN 6506[6] An algal suspension containing 10^4 cells per ml was prepared from a preculture. The test vessels were 180 ml culture bottles containing 100 ml of algal suspension, to which was added 100 µl of the DMSO solution (or suspension) of the test substance.

Bottles containing algae only were used as controls, and a single series of bottles without algae, but with test substances was used for background counting.

The algae were incubated for 3 days on an algal mill[7]. at 20 ±

2°C under continous illumination with Philips TL 33 fluorescence tubes (6-7KLux). The cells were counted after 3 days with a Coulter Counter Model TA II, with a 100 μm aperture.

2.4.4. Microtox test

The microtox test was that described in the microtox manual[8]. All reagents were supplied by Beckmann.

A suspension of bacteria was prepared in saline poured into cuvettes, solution of test substances were prepared in saline by adding 200 μl of DMSO solutions or suspensions to 100 ml of saline in volumetric flask. On the basis of the results with algal species only a limited number of concentrations were tested .

2.4.5. Calculation of the EC_{50}- values and the NOEC

The effect of a chemical on the growth of the light emission by the bacteria is expressed as an EC_{50} values . This is the concentration at which critical magnitudes such as growth rate, yield, numbers of viable cells in the *inoculum* (algae), and light emission (bacteria) are half those in the control (without test substance).

EC_{50} value for the algae were calculated by means of parametric model[3]. The following equations were used :

$$N(t,c) = E_b - E(c) + \exp(tR(c))$$

or when no effect is expected on the inoculum, i.e. $E(c) = E_b$:

$$N(t,c) = E_b \exp(tR(c))$$

or when no effect is expected on the growth rate,. i.e. $R(c) = R_b$

$$N(t,c) = E_b - E(c) + E(c) \exp(tR_b)$$

where

$N(t,c)$ = number of cells. ml^{-1} at time t and concentration C of the test substance.

$E(c)$ = inoculum; number of cells. ml^{-1} in the culture containing concentration c of the test substance at t = 0.

$R(c)$ = the growth rate at concentration c of the test substance.

R_b and E_b = growth rate and inoculum, respectively, of the untreated cell.

in addition,

$$R(c) = R_b (1 + \exp (R_g (\ln c - R_o))^{-1}$$

and

$$E(c) = E_b (1 + \exp (R_g \ln c - E_o))^{-1}$$

where

R_o and E_o = natural logarithm of the respective EC_{50} values.

R_g and E_g = the gradient of the function for the growth rate and the inoculum, respectively.

The parameters E_b, E_o, E_g, R_b R_o and R_g were calculated by a weighed least square fitting of the model to the results.

Calculations and curve plottings were performed by computers, at IWIS-TNO, The Hague, The Netherlands.

The no observed effect concentration (NOEC) is the highest concentration tested that causes no effect in comparison with the control. NOEC were estimated from the calculated values.

For the microtox test only the EC_{50} values were calculated by the method given in Beckmann Microtox System Operating Manual[8] .

The relevant formulae are :

$$R(t) = \frac{I(t)\,(\text{blank})}{I(0)\,(\text{blank})} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

where R (t) = ratio after t minutes (mean value of measurements)

I (t) = Light reading after t minutes in the blank,

I (0) = Light reading after 0 time in the blank.

The effect. (t,T), of a chemical is then calculated from equation (2).

$$T(t, T, c) = \frac{R(t)\,.\,I(O,C)}{I(t,c)} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(2)$$

Where c is the concentration of test substance.

T is plotted on double-Logarithmic paper against the concentration of test substance.

EC_{50} corresponds to T = 1.

2.5. 74% By volume test

The composition of the dry paint in percent by volume is :

bioactive material 74 %, inert binder, 26%. The paints were prepared by dissolving the binder in suitable blend of solvents and the bio-active materials were dispersed in this binder by milling. The paints were applied on PVC panels with an average paint film thickness 65 μm and immersed in Den Helder harbour (Holland). The panels exposure are carried out in two ways : horizontally just below the water and vertically on a depth of 1.5 m. On the upper site of horizontally exposed panels mainly algae fouling will occur and on vertically exposed one mainly barnacles and tube worms.

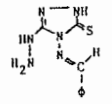
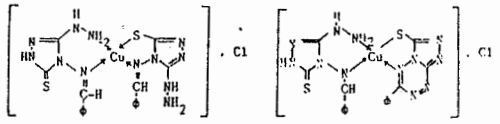
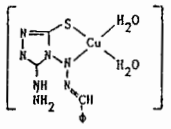
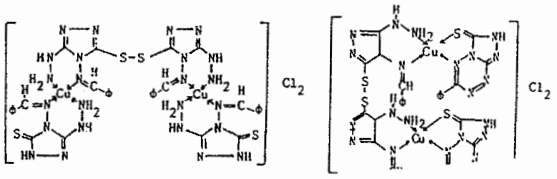
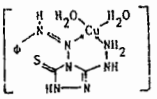
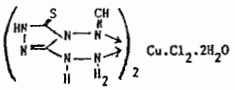
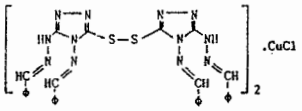
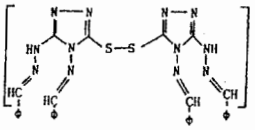
RESULTS AND DISCUSSION

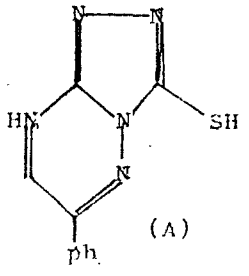
The analytical data are compatible with suggested stoichiometry (Table 1). The formulae of the copper complexes of both H_2BAHTtrz and H_2BABHTtrz which were isolated under refluxing time exceeding 15 min are suggested to be $Cu(L)_n.XCl$, where n is equal two or four, and X is 1 or 2, that is irrespective of the metal : ligand ratio added. Also, the disulphide structure of the ligand is proposed to be present which results from the oxidation of the ligands by copper (II) ions. Similar dimers were isolated from the reaction of Cu(II) with o-mercapto-N-methylbenzanilide (MMB), and o-mercapto- N-benzyl-N-methylbenzamide (MBB)[9].

On the other hand, if the refluxing time of the reaction mixture was extended to about 1 hr. the possible structures of the complexes are suggested to be XCu (L).XCl and $Cu(L)_2$.Cl with both L_I and L_{II} respectively. (X = no. of copper and chloride ions). Also, the rearrangement of H_2BAHTtrz ligand to the compound A is likely under oxidizing conditions[4] and proposed to be formed with some of its copper complexes.

TABLE I

List of Investigated Compounds

No.	Compound	Structure	Color, M.P., Yield, %
I	4-Benzylideneamino-3-hydrazino-5-thio-1,2,4-triazole $H_2BAHTtrz$		white, 145, 92
II	$Cu(HBAHTtrz).(H_2BAHTtrz).Cl$		dark green, 208, 62
III	$Cu(HBAHTtrz).2H_2O.CuCl$		black, 216, 78
IV	$(Cu(HBAHTtrz)_2)_2.Cl_2$		light grey, 211, 53
V	$Cu(HBAHTtrz).2H_2O.Cl$		yellow, 245, 84
VI	$Cu(HBAHTtrz)_2.Cl_2.2H_2O$		dark green, 206, 97
VII	$H_2BABHTtrz$		yellow, 245, 84
VIII	$Cu(HBABHTtrz)_2.Cl.(HBABHTtrz)_2$		olive green, 237, 64
IX	$Cu(HBABHTtrz)_2.Cl$		green, 200, 69
X	$Cu(HBABHTtrz)_2.(HBABHTtrz)_2$		yellowish green, 234, 61



(A)

^{1}H NMR spectra of both 4-(benzylideneamino)-3-hydrazino-5-thio-1,2,4-triazole and 4-(benzylideneamino)-3- (benzylidene-hydrazino) -5-thio-1,2,4-triazole have been measured previously in trifluoroacetic acid[10]. In d^{6}DMSO, H_2BAHTtrz has a signal at δ 5.5 ppm which is suggested to be due to the proton of the aminohydrazino group, it is slightly deshielded by benzene ring π-system[11]. Also, the thione from of the ligands is suggested form the presence of 4-NH signal at δ 10.4 ppm, Table (2). All copper complexes of the two ligands are diamagnetic except V δ II complexes. The proton of both azomethine nitrogen and aminohydrazino groups of H_2BAHTtrz ligand is shifted to lower field with all copper complexes,. (Table 2), by about 0.3 ± 0.1 and 0.7 ± 0.6 ppm respectively, suggesting the participation of the two groups in complex formation.

The two possible structures suggested for II and V copper complexes were based on the rearrangement of the ligand to compound A during complex formation , since there is no evidence to confirm one of them and exclude the other.

^{1}H NMR spectra of all copper complexes (VIII, IX and X) of H_2BABHTtrz (Table 2), demonstrate the shift of azomethine proton about 1.1 ppm upon complexation. Also, both imino proton signals are deshielded by complexation. Taking these observations into

TABLE 2

Characteristic ^{1}H NMR and ^{13}C NMR Signals of the Ligands and their Copper Complexes in DMSO.

No*	Compound	Assignment											
		$-NH_2$ # $-NH_2$	$-NH$ # $-NH$	H - ϕ	$-\overset{H}{\underset{	}{C}}=N$	H_2O	$-C_3$	$-C_5$	-CH	-C-ϕ	$-C_4$-ϕ	$-C_{2,3}$-ϕ
I	H_2BAHTtrz	5.5	12.7 10.4	7.3 8.0	8.0		164.2	149.4	143.9	134.5	128.6	126.3 126.0
II	Cu(HBAHTtrz).(H_2BAHTtrz).Cl	6.19	13.5 10.96	7.33 7.69	8.26	3.4	163.2	149.6	144.1	134.3	128.5 129.2	126.3
III	Cu(HBAHTtrz).Cl.2H_2O.CuCl	6.82	—— 10.02	7.33 7.93	8.4	3.4						
IV	$(Cu(HBAHTtrz)_2)_2.Cl_2 \cdot 2H_2O$	5.58	13.4 10.85	7.39 7.70	8.33	3.52						
V	Cu(HBAHTtrz).Cl.2H_2O	6.61	13.48 10.98	7.39 7.89	8.26	3.42			144.9	133.9 131.1	128.6 129.4	126.8 126.4
VII	H_2BABHTtrz		13.1 10.4	7.3 7.9 7.8 8.2	8.4 9.9		162.1	159.2	144.9 144.8	134.3 132.2	128.8	126.4
VIII	$Cu(HBABHTtrz)_2.(HBABHTtrz)_2.Cl$	— —	- 10.8	7.3 7.8 7.8 8.2	8.3 10.6		--	--	144.9 144.7	134.3 132.2	128.7	126.5 126.2
IX	$Cu(HBABHTtrz)_2.Cl$	—	13.37 10.75	7.8 7.4 8.2 8.0	8.34 10.12		162.65	—	144.9	134.2 132.1	128.9 128.7	126.5 126.2
X	$Cu(HBABHTtrz)_2.(HBABHTtrz)_2$	—	13.35 10.35	7.4 8.03	10.34 10.14							

* See Table 1

\# Signals due to the proton of the hydrazino group.

account, azomethine nitrogen and 2-N atom might be involved in chelation. ^{13}C NMR chemical shift data are presented also in Table 2. The spectra of some copper complexes of both ligands could not be measured due to solubility reason. It appears that the most important change in δ value with (Cu(HBAHTtrz). (H_2BAHTtrz). Cl and (Cu (HBAHTtrz). Cl. $2H_2O$ complexes generally affect -CH (arylidene CH of azomethine group) which is shifted to lower field in both complexes this leads us to consider that, coordination occurs through azomethine nitrogen atom[12].

The solid IR spectra of both H_2BAHTtrz indicate that, they are present in the thione form due to the presence of the band at 1030 ± 40 cm^{-1} and the absence of any absorption bands in the region of ν S-H group[13]. The bands lying in the region 3050-3260 (Table 3) are assigned to νNH vibrations for both imino and amino groups.

In spite of the complexity in locating and assigning the vibrations, the IR allow some conclusions to be drawn of deduced. Copper complexes of H_2BAHTtrz ligand are suggested to be bonded either through azomethine and amino nitrogens when the ligand is proposed to form the disulphide structure, or through the last two coordinate site and the thiol sulphur atom. The participation of amino group in complex formation is hardly to be detected from their νNH data, but it is quite clear to be bonded from the higher shift of its bonding vibration. Thioamide bands II and III undergo a small shift to lower wave number with all copper complexes, while the thio amide band I remains nearly unchanged. This result is also in keeping with their nmr spectra. With respect to copper complexes of 4-(benzylideneamino)-3- (benzylidene- hydrazino)-5-thio- 1,2,4-

TABLE 3

Characteristic IR Bands of the Ligands and Complexes cm^{-1}.

No.	Compound	Assignment								
						Thioamide band				
		νNH_2	νNH	δNH_2	$\nu C{=}N$	I	II	III	IV	νH_2O
I	$H_2BAHTtrz$	3240w	3160b 3000	1580s	1605	1490	1330	1090	790	
II	$Cu(H_2BAHTtrz).(HBAHTtrz).Cl$	3260	3160	1580	1610	1490	1330	1090		
III	$Cu(HBAHTtrz).2H_2O.CuCl$		3100b	1570sh	1605m	1505w	1320m	--		3440
IV	$(Cu(HBAHTtrz)_2)_2Cl_2$	3235w	3140b 3000w	1570s	1610m	1485m	1325m	1090w		
V	$Cu(HBAHTtrz)Cl.2H_2O$	3260w	3100b 3000w	1575sh	1610m	1490s	1320m	1070w		3420b
VI	$Cu(H_2BAHTtrz)_2Cl_2.2H_2O$	3180w	3100w	1590	1615b	1480b	1310m	1070w		3400b
VII	$H_2BABHTtrz$		3185m 3000w		1600w 1585s	1490s	1340m	1070m	820s	
VII	$Cu(HBABHTtrz)_2.(HBABHTtrz)_2Cl$		3180m 3050w		1605w 1585s	1480s	1340m	1070m	820w	
IX	$Cu(HBABHTtrz)_2Cl$		3180m 3050w		1605w 1590s	1490s	1340m	1070m	818m	
X	$Cu(HBABHTtrz)_2.(HBABHTtrz)_2$		3180b 3050w		1610w 1590s	1490s	1345	1070m	820m	

b: broad; w: weak; m: medium and s: strong

TABLE 4

Electronic Spectral Data of the Copper Complexes in Nujol Mull and their Magnetic Moment.

No.	Compound	$\mu_{eff.}$ B.M.	LMCT			d — d assignment		
			σ_N | $Cu(II)d_{x^2-y^2}$	$\sigma(S)$ | $Cu(II)d_{x^2-y^2}$	πS | $Cu(II)d_{x^2-y^2}$	$^2B_{1g}$ | ^2E_g	$^2B_{1g}$ | $^2B_{2g}$	$^2B_{1g}$ | $^2A_{2g}$
I	Cu(HBAHTtrz).(H_2BAHTtrz).Cl	Diamg.	250sh	300, 340m		500sh		
II	Cu(HBAHTtrz).CuCl.$2H_2O$	1.13	240sh	300s,340m, 370sh		500sh		
III	$(Cu(HBAHTtrz)_2)_2Cl_2$	Diamg.	265,240	350,370	460s	580	670,630	
IV	Cu(HBAHTtrz).Cl.$2H_2O$	Diamg.	265	350	460s	520	630,790	
V	$Cu(H_2BAHTtrz)_2Cl_2.2H_2O$	0.86	290	350s	450w	580	650,750	810
VI	$Cu(HBABHTtrz)_2$.$(HBABHTtrz)_2$.Cl	Diamg.	250,275	320,350		500w		
VII	$Cu(HBABHTtrz)_2$.Cl	Diamg.	230,270	350s		500w		
VIII	$Cu(HBABHTtrz)_2$.$(HBABHTtrz)_2$	Diamg.	230,275	350		500w		

triazole, the stretching frequency of the two NH groups are still present after complexation, on the other hand there are no clear shifts in all thioamide bands, so, the coordination of the copper ions are proposed to be through azomethine nitrogen and 2-N atom as it is clear from their nmr data or these shifts might be due to the formation of the disulphide structure.

All copper complexes are diamagnetic except Cu (HBAHTtrz). $Cl.2H_2O.CuCl$ and $Cu(H_2BAHTtrz)$. (HBAHTtrz) . $Cl.H_2O$ complexes which have μ_{eff} 1.13 and 0.86 B. M. values respectively which is less than the spin only value (1.73 B.M.). This subnormal μ value may be attributed to significant spin exchange between the interacting Cu^{2+} ions or due to the mixed valency of Cu(II) and Cu(I)[14].

Most of the copper complexes exhibit characteristic charge transfer bands in the range 250-450 nm. The higher energy shoulder at about 270 ± 20 nm might be attributed to σ-Cu(II) LMCT as reported with copper complexes of thiamine derivatives[15]. Thiolate system and sulphhydral system (RSH) do appear to exhibit both σ_s-and π_s-cu (II) LMCT1[5]. Most exhibit moderately intense band near 350 nm to $\sigma_{(s)}$-$cu_{(II)}$, so the band appearing in the range 280-350, 350-400 nm with all copper complexes are assigned to the last two LMCT transitions. The intensity of $\sigma_{(s)}$-cu(II) indicate sulphur coordinate nearly to the square plane. The visible electronic spectra of most complexes are nearly identical and show weak or broad bands centered at 250 ± 10 nm. This could be assigned to the crystal field transition $^2S_{1g}$ - ^2E_g and is indicated of the plane geometry. In addition two bands or shoulder are found in the range (650-750) and

(800-900) nm. These may be attributed to the system $^2B_{1g}$ - $^2E_{2g}$ and $^2B_{1g}$ -$^2A_{1g}$ respectively. These spectral data are similar to those reported for square planar Cu(II) chelates. The slight solubility of the copper complexes in organic solvents in addition to their magnetic and spectral data suggest their polymeric nature.

C.T. bands also appear with the complexes probably due to the presence of Cu(II) oxidation state as in the case of Cu(HBAHTtrz). CuCl.2H_2O, Cu(HBABHTtrz)$_4$Cl and Cu(HBABHTtrz)$_2$Cl complexes. This may be due to the presence of some conversion of Cu(I) to Cu(II), in which such conversion in a mercapto complex is also shown by the reaction of Cu(I) with 8-mercapto- Quinoline[16]. Also, M-L charge transfer bands and transition between bonding and non bonding II levels might be possible

3.1. Algal tests

The algal tests were duplicated because some of the blanks in the first series developed excessively low pH's (Table 5), obviously, the algae produced an acid, for this reason the pH of the medium in series II was stabilized using sodium bicarbonate (150 mg.1^{-1}).

Table 5 lists the results of the algal tests. The calculated EC_{50} values exhibit relatively wide (95%) confidence limits because the cells were counted only after 3 days. The results for the first and second series differed even where the test conditions were the same The results reveal that, all the copper complexes are less toxic than the corresponding organic ligands except complex III which is Cu(HBAHTtrz). 2H_2O.CuCl.

TABLE 5

Results of the Tests of the Compounds with Chlamydomonas reinhardii, and Bacterium photobacterium phosphorium:

Highest and Lowest pH Values in the Medium After Three Days of Incubation.

No*	Solubility $mg.L^{-1}$		Series I				Series II			
			Algal test	Microtox test			Algal test	Microtox test		
	Sea water	Algal medium	EC_{50} $mg.L^{-1}$	NoEC $mg.L^{-1}$	EC_{50} $mg.L^{-1}$	pH	EC_{50} $mg.L^{-1}$	NoEC $mg.L^{-1}$	EC_{50} $mg.L^{-1}$	pH
I	2.0	10.0	1.81	0.03	$\gg 1.8$	4.13-6.00	45	1.0		8.06-8.39
III	3.4	3.34	1.74	0.3	0.6	4.35-7.70	0.29	0.10	1.70	
V	11.0-10.3	0.56	3.93	0.3	$\ll 1.0$	3.75-4.40			0.45	
VI	24.5	20.1	11.1	0.1	0.1	8.30-8.40			0.60	8.09-8.40
VII	Insol.	2.0	>>1.0	>1.0	>1.0	8.50				
VIII	17.2	0.22	14.5	0.1	>1.8	8.65	6.2		1.15	
IX	37.0	2.66	2.17	1.8	>1.8	8.50		0.3	>3.2	
TBTO			0.018	0.001	>0.01	8.70			0.032	
Cu_2O							0.09	0.01		

* See Table 1

3.2. Microtox test

Generally the EC_{50} found in the microtox test were different to those of the algal test, with some exceptions (compounds I & VII, Table 5) complexation with copper of the first ligand (H_2BAHTtrz) increases its toxicity, on the other hand, copper complexes of the second ligand (H_2BABHTtrz) decreases its toxicity as it does in the algal test.

The standard test substance TBTO and copper oxide are well known algicides, they are considerably more toxic than the tested compounds in both tests.

3.3. 74% By Volume test results

During this test the bio-active material is present in its maximum obtainable concentration at the interface coating/sea dependent upon the solubility rate of the test product. In other words, if fouling can settle under these conditions, the bio-active material will possess insufficient antifouling agent for paint[17].

The usefulness of the test method has, however, been demonstrated by the results with tributyltin fluoride containing paint The result showed that, the paint containing the two ligands are less effective against both animal and plant fouling organisms. The general feature of the paints containing copper complexes is the attack of their surfaces with green algae over the first two weeks of immersion, afterwards when the bio-active material starts to leach out from the paint film the algal attachment decreases by time and showed good antialgal action up to 8 weeks, while the reference plate

TABLE 6

Rate of growth of fouling organisms on horizontal panels (H) and vertical panel (V)

Compound*	Period (days)							
	18		32		60		75	
	H	V	H	V	H	V	H	V
I	8G		10G	2P+8B		10P		
III	4G		2G	---	---	8P+2B	10G	10P
V	10G		6G	3B	1G	6P	10G	10P
VI	9G		7G	4B+3P	5G	6P	10G	10P
VII	9G		10G	7B+1P		10P		
VIII	9G		10G	5B+3P	1G	10P	10G	
IX	9G		10G	3B+3P		10P		
B#	---		---		3G		10G	
B†	8G		10G	7B+1P	6G	10P	10G	

* See Table 1

B# Blank: paint contains TBTF

B† Blank: untreated panel

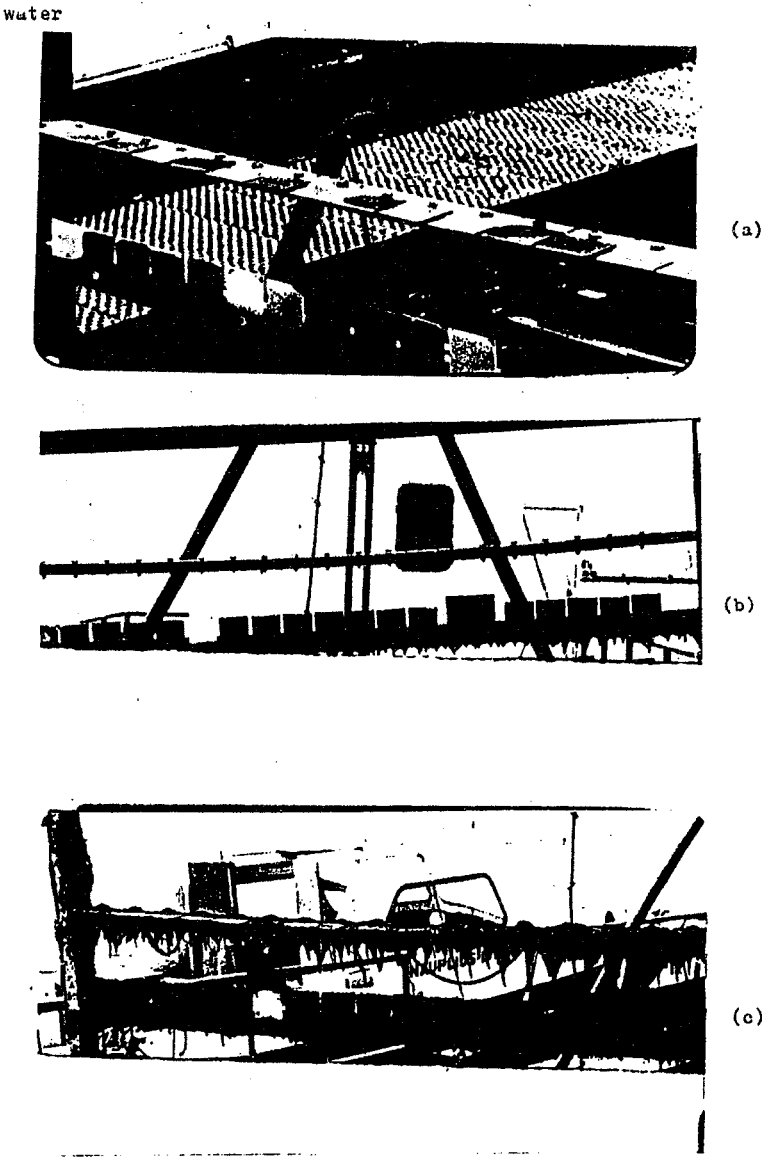
G : green algae observed

B : brown algae observed

P : barnacles observed

2(G) means: 10-20% of the surface is covered with algae.

Fig.(I) Effect on PVC coated panels of immersion in Holland Den Helder harbour.



(a) Immersion was 0 day (b) Immersion was 18days (c) Immersion was 55 days.

contains (TBTO) is started to be attached after nearly 55 days (Table 6). On the other hand, copper complexes of L_I (compounds III, V and VI) have differential activity against barnacles and brown algae.

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الملخص العربى

دراسة تخليق وتقييم بيولوجى لمتراكبات النحاس لبعض مشتقات ٤-أمنيو-٣-هيدرازينو-٥-ثيو- ٤,٢,١- ثلاثى آزول

د. عايدة بطرس تادرس ، د . هيندريك فان بيك

تم تخليق المركبين ٤-بنزيليدين أمينو-٣- هيدرازينو-٥-ثيو-١ ,٢ ,٤ ثلاثى آزول(١)، ٤- بنزيليدين أمينو-٣- بنزيليدين هيدرازينو-٥-ثيو-١ ,٢ ,٤ ثلاثى آزول(٢) وأيضا تم تحضير متراكبات النحاس لهم. وتم التعرف على تركيب المركبات باستخدام التحليل الكيماوى ، الأشعة تحت الحمراء، والطيف الألكترونى ، الحاسية المغناطيسية والطيف النووى المغناطيسى . وأعطى متراكبان النحاس للمركبين خليط من النحاسيك والنحاسوز فى كل متراكب.

والمركب الأول(١) كون متناسق مع أيون النحاس إما خلال مجموعة الأمينوهيدرازينو، الآزوميثين نيتروجين أو خلال ذرة ثيوكبريت ، الآزوميثين نتروجين. أما المركب الثانى(٢) فكون متناسق مع أيون النحاس خلال ذرة الكبريت ، الآزوميثين نتروجين، الأمينونتروجين. وقد اختبرت قيمة EC50 لكل مركب وهى القيمة التى يكون عندها معدل النمو يعطى عدد من الخلية الحية يكون نصف العدد الموجود فى الوسط الحكم (الوسط بدون المادة محل الإختبار) وقد استخدم فى هذا الإختبار طحلب وحيد الخلية وهوChlamydomonas ونوع من أنواع البكتيريا البحرية وهى Phosphoreum species وأظهرت النتائج أن المركبين منخفضين السمية بالمقارنة بمادة ثلاثى بيوتيل أكسيد القصدير.

أيضا تم إختبار فاعلية المركبات المحضرة تجاه كائنات حشف البحر باستخدام طريقة ٧٤٪ تركيز (حجم) وهى طريقة قياسية لمعرفة الكفاءة الحيوية للمركبات. ومعظم المركبات أظهرت كفاءة عالية تجاه مقاومة الطحالب الخضراء وكفاءات مختلفة تجاه أنواع الكائنات الحشفية الأخرى.