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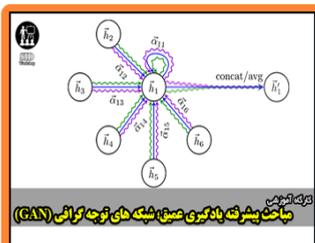## کارگاه های آموزشی مرکز اطلاعات علمی جهاد دانشگاهی

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کارگاه آنلاین مکالمه روزمره انگلیسی

Effects of heavy metal pollution on the soil microbial activity

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ABSTRACT: The effects of heavy metals on soil microbial processes were investigated over a period of six weeks. Analytical grade (Sigma) sulphate salts of copper, zinc and nickel were added individually and in combinations to soil samples and incubated in different plastic pots. Samples were taken from the pots forthnightly and the rates of microbial carbon and nitrogen mineralization, microbial biomass carbon and respiration were measured. The results showed the effect of metals on the measured parameters were significant ($P<0.05$.). By the 6th week postreatment, the rates of carbon accumulated were high in the copper (6.03 %) and copper:zinc (5.80 %) treatments but low in the nickel and zinc (4.93 % and 5.02 % respectively). The rates of nitrogen mineralization were 0.41 and 0.44 % in samples treated with copper and copper:zinc compared to 0.22 %-0.24 % obtained at the beginning of the experiments. Soil microbial biomass carbon declined from average value of 183.7 – 185.6 μg/g before treatment to as low as 100.8 and 124.6 μg/g in samples treated with copper:zinc and copper respectively.The rate of respiration of the soil microbial populations was equally inhibited by the metals. From an average rate of 2.51-2.56 μg of C/g respiration of the soil microbes declined to 0.98, 1.08 and 1.61 μg of C/g in the copper:zinc, copper and zinc treated soils by the end of the experiment. The results suggest additive or synergistic effects of the metals.

Key words: *Mineralization, microbial biomass carbon, additive, synergistic, heavy metals*

INTRODUCTION

The location of this work was Oshodi; a city within the Lagos metropolitan area in the South-West of Nigeria. It lies within latitude $6^0 33^I$ N and $3^0 20^I$ E and has a bimodal rainfall pattern with the rainy season lasting from March to November each year, and about a two week break in the month of August. The elevation is approximately 1500m above sea level. The soil in the area support tropical rainforest vegetation while Kaolinite is the main clay mineral present.

Many reports indicate that heavy metals interfere with the biochemistry of diverse group of microorganisms isolated from their natural environments (Alloway, 1995; Sani *et al.,* 2003; Utgikar *et al.,* 2004; Pennanem *et al.,* 1996).However, information relating to the sensitivity of whole soil bacterial communities to heavy metals is not common (Diaz Ravina *et al.*,1994; Chander and Brookes,1993). Microorganisms do not live in isolation but in complex biological communities within which exist complex interactions arising from biotic and abotic influences (Petersen and Klug, 1994; Chefetz *et al* .,1996).It is

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difficult to relate single species behaviours to the overall ecosystem. Since traditional methods of microbial ecology require that organisms from an environment be cultured in the laboratory before they can be identified and studied, monitoring the effect of pollutants on the activities of the overall communities poses a great challenge,because less than 10% of microorganisms from any environment is culturable (Baath,1992a; Diaz Ravina *et al.*, 1994; Frostegard and Baath 1996).

For ecotoxicological research to be capable of influencing policies and regulations pertaining to environmental sustainability and conservation it must be methodologically planned and ecosystem oriented (Baath *et al.*, 1998; Clarke,1999). This is because the existence of bacterial species that are tolerant to a pollutant is not indicative of the effect of selective pressure due to that pollutant on other species. Heavy metals come from a variety of sources but principally anthropogenic activities such as chemical manufacturing, electric power generation, coal and ore mining, melting and metal refining, metal plating and to

some extent domestic sewage (Gazso, 2001). Some of the metals such as copper, nickel and zinc are essential in very low concentrations serving as components of enzymes, structural proteins, pigments and in maintaining the ionic balance of cells (Kosolapov *et al.*, 2004).

Microorganisms in the soil are responsible for nitrogen fixation, assimilation, and degradation of organic residues to release nutrients (Baath, 1989; Brookes, 1995). When heavy metals are retained in the soil by repeated and uncontrolled additions, they interfere with these key biochemical processes which alter ecological balance. They also endanger human health when the metals migrate through the food chain. Toxic effects of heavy metals on microorganisms manifests in numerous ways such as decrease in litter decomposition and nitrogen fixation, less efficient nutrient cycling (Baath,1989), impaired enzyme synthesis and activity in soil, sediments and water. In this work, the effects of metals on microbial processes of an agricultural soil artificially polluted with elevated levels of the metals were studied. The rates of carbon and nitrogen mineralization, respiration and biomass carbon were monitored.

MATERIALS AND METHODS

Agricultural clayey loamy soil samples were collected from a field plot within the premises of the Federal Institute of Industrial Research (FIIRO) Oshodi, Lagos, Nigeria. The soil was covered with green luxuriant vegetation at the time of sampling and was collected aseptically from a depth of 5 cm – 15 cm after carefully removing the surface vegetation. The samples were sieved (mesh size < 2 mm), sorted to remove stones, plant debris and any visible soil fauna and then thoroughly mixed with hand trowel. The soil was allowed to stabilize for 7days by incubating at 27 °C to permit the disturbance caused by sampling and sieving to subside (Baath *et al*., 1998). The pH of the soil used in the study was 7.23 (Table 1).

Approximately 1 kg each of the sieved and preincubated soil samples were distributed into eight 7cm diameter plastic pots labeled A – H. Solutions of analytical grade (Sigma) Sulphate (SO_4^{2-}) salts each of zinc, copper and nickel dissolved in 200 mL of distilled water were applied to the respective pots singly and in combinations (Table 2) and thoroughly mixed by a plastic miniature cement mixer. Soil sample, which received no metal amendment, served as control. All

samples were kept in the plastic pots at room temperature (27 - 28 °C). Distilled water was added periodically to maintain the soil moisture at constant level. Soil microbial biomass carbon was determined in triplicate samples by the fumigation – extraction procedure (Vance *et al.,*1987). Soil respiration (CO_2 measurement) was done repeatedly on 20 g portions of soil per treatment in 100 mL pyrex glass flasks. The flasks were covered with aluminium foil and secured with a septum. During sampling, the headspace of each flask was equilibrated with the soil atmosphere by pumping with a 10 mL syringe. There after, 100 µL of atmospheric air was injected and the same volume of gas removed for the CO_2 analysis using a Nitrogen carrier with a flow rate of 50 mL/min. CO_2 evolution was measured by a Beckman Model 865 infrared gas analyzer (Beckman, La Habra, California) (Petersen and Klug, 1994).

The moisture content of soil was measured gravimetrically by drying 50 g of soil sample at 105 ° C for 24 h. The total metal content of soil before incubation was determined after digestion by atomic absorption spectrophotometer (Table 3). The total soil carbon (%) was calculated by the dichromate digestion method (Kalembasa and Jenkinson, 1973) while the total soil Nitrogen (%) was determined by the Kjeldahl digestion (Bremner, 1965).All the analyses were done in triplicates.

The results were analyzed by two-way analysis of variance (ANOVA) at 95 % confidence interval. Differences between the control and other treatments were assessed using least significant difference (LSD).

RESULTS AND DISCUSSION

The results showed that the microbial process of carbon mineralization was inhibited to varied extents by the metals (Table 4). In other words, carbon was accumulated. Among the treatments,Cu had the highest rate at 6.03 % followed by Cu:Zn which was 5.80 % by the end of the experiment. The least value (5.36 %) was obtained in the Cu:Ni treated soil. The other treatments except Ni showed subtle interference with the key microbial process but the level was insignificant (P>0.05) (Table 4). At the beginning of the experiment, Cu amendment gave carbon accumulation rate of 4.88 %.This rose steadily to 6.03 % by the last week of the experiment. The Ni:Zn treatment equally was 4.88 % at the beginning but peaked at 5. 29 % which did not vary from control. The

data obtained with respect to the rate of nitrogen mineralization showed that nitrogen was accumulated in the Cu and the Cu:Zn treated soils. Cu and Cu:Zn significantly (P<0.05) interfered with microbial nitrogen mineralization giving values of 0.41 % and 0.44 % respectively by the 6th week of experiment (Table 5). Results obtained from other treatments did not show any significant variation from the control .

The decline in the microbial biomass carbon in the soil with respect to the Cu and Cu:Zn amendments was significant (Table 6). The other treatments did not vary significantly from control. Among the treatments, the Cu:Zn amendment recorded the lowest

level of soil biomass carbon (100.8 µg/g). In the measurement made at the beginning of the experiment, the level of soil biomass carbon in the Cu:Zn amendment declined progressively from initial values of 184.6 (g/g) to 100.8 µg/g by the 6th week. The biomass carbon in the Cu treatment equally declined from 184.8 µg/g to 124.6 µg /g during the same period.

The soil respiration evidenced by CO_2 evolution declined significantly in samples taken from the Cu and Cu:Zn amendments (Table 6). The highest rate of decrease in respiration was recorded in the Cu:Zn treatment which was 0.98 µg of C/g by the 6th week. The rate of respiration in the Cu treated soil declined steadily to 1.08 µg of C/g during the same period. The relative toxicity of metal ions are known to be influenced by soil conditions. Important factors which influence microbe-metal interactions in soil include pH, the quantity and quality of clay minerals as well as other complex interaction involving the metal ions and other inorganic constituents.

Table 1: Characteristics of the soil

Chemical	Analysis
pH	7.23
Carbon (%)	4.87
Total nitrogen (%)	0.24
Biomass carbon (µg/g)	185.6
Respiration (µg of c/g)	2.56
*WHC (%)	51

*Water holding capacity

Table 2: Pattern of metal amendment in pre-incubated soil pots

Sample	Metal	Conc. (mg/kg)
A	Control	-
B	Cu	3000
C	Ni	3000
D	Zn	3000
E	Cu:Ni	1500:1500
F	Cu:Zn	1500:1500
G	Ni:Zn	1500:1500
H	Cu:Ni:Zn	l000:l000:1000

Table 3: Heavy metal profile of soil prior to amendment as determined by atomic absorption spectrometry

Concentration (mg/kg/soil)	Metal
6.65	Cu
12.0	Zn
1.63	Ni
ND*	Pb
ND	Cd
ND	Hg
ND	Cr

*ND: Not Detected

Table 4: Effect of treatment on rate of carbon mineralization in soil (%)

Sample	A	B	C	D	E	F	G	H
Treatment-week	Control	Cu	Ni	Zn	Cu:Ni	Cu:Zn	Ni:Zn	Cu:Ni:Zn
0	4.87	4.88	4.85	4.87	4.87	4.86	4.88	4.85
2	4.82	5.27*	4.79	4.92	5.01*	5.31*	4.89	4.86
4	4.79	5.57*	4.83	5.02	5.28*	5.64*	5.18	4.91
6	4.84	6.03*	4.93	5.02	5.36*	5.80*	5.29	5.12

*Asterisks indicate significant difference from the control treatment (N = 8, P < 0.05; mean SE from ANOVA.)

Table 5: Effect of treatment on rate of nitrogen mineralization in soil (%)

Sample	A	B	C	D	E	F	G	H
Treatment-week	Control	Cu	Ni	Zn	Cu:Ni	Cu:Zn	Ni:Zn	Cu:Ni:Zn
0	0.24	0.23	0.23	0.24	0.23	0.24	0.24	0.22
2	0.26	0.31*	0.27	0.29	0.26	0.32*	0.26	0.25
4	0.27	0.37*	0.29	0.29	0.27	0.39*	0.28	0.27
6	0.26	0.41*	0.30	0.31	0.34	0.44*	0.32	0.27

*Asterisks indicate significant difference from the control treatment (N = 8, P < 0.05; mean SE from ANOVA)

Heavy metals soil microbial activity

Table 6: Effect of treatment on biomass carbon of soil (µg/g)

Sample	A	B	C	D	E	F	G	H
Treatment-week	Control	Cu	Ni	Zn	Cu:Ni	Cu:Zn	Ni:Zn	Cu:Ni:Zn
0	185.6	184.8	183.9	185.2	185.1	184.6	185.0	183.7
2	184.7	176.5*	180.0	178.1	180.7	175.8*	181.5	182.1
4	183.5	147.3*	179.0	160.4	165.4	140.0*	175.8	180.9
6	184.4	124.6*	172.4	142.3	138.3	100.8*	170.4	174.2

*Asterisks indicate significant difference from the control treatment (N = 8, P < 0.05; mean SE from ANOVA)

Table 7: Effect of treatment on respiration responses of soil microbes (µg of C/g)

Sample	A	B	C	D	E	F	G	H
Treatment-week	Control	Cu	Ni	Zn	Cu:Ni	Cu:Zn	Ni:Zn	Cu:Ni:Zn
0	2.56	2.51	2.54	2.55	2.56	2.52	2.54	2.56
2	2.48	2.28*	2.34	2.45	2.30	1.98*	2.51	2.50
4	2.43	2.18*	2.32	2.42	2.17	1.76*	2.40	2.29
6	2.47	1.08*	1.78	1.61	1.90	0.98*	1.95	2.18

*Asterisks indicate significant difference from the control treatment (N = 8, P < 0.05; mean SE from ANOVA)

This probably explains the apparent toxicity experienced by the soil microbes as evidenced by the data on carbon and nitrogen mineralization, biomass carbon and respiration responses. In all, only the Cu and Cu:Zn treatments appeared to have significant (P<0.05) effects on microbial biochemical processes, in spite of the unusually very high levels of other metals (Table 1). Soil pH greatly influences the solubility, available and toxicity of metal elements in soil (Baath and Arnebrant, 1994). It has been reported that a neutral soil may contain high levels of Mn, Al or Pb without any sign of toxicity to microorganisms whereas toxicity may develop with certain organisms at much lower metal concentrations in acid soils (Marschner and Kalbitz, 2003; Utgikar *et al.*, 2003). This presupposes that toxicity is determined by solubility; while solubility is a function of pH. Therefore, no matter the metal, if the appropriate pH for maximum dissolution is not in place, the toxicity of such metal is seriously hindered; if not entirely diminished (Alloway, 1995; Baker and Senft 1995). It is most probable that the level of toxicity recorded could have been higher under lower pH values. The availability and toxicity of a given metal ion is always much lower in soil than in water solution. This can be attributed to competitive adsorption of the metal ions by soil organic and inorganic colloids. Under such conditions, the metal ions are entrapped and put 'out of circulation" to the overall effect that microorganisms are shielded from their adverse effects. Clayey – loamy agricultural soil was used in this study. This can partly explain the low toxicity observed. The clay mineral montmorillonite protects bacteria and fungi against

inhibition by metal species (Chen *et al.,* 1997; Baath 1992b).

Another very important but frequently less understood factor is the modifying influence of metal ions on metal toxicity. Some interactions may be explained as simple competition, antagonism, additive action or synergism (Diaz-Ravina and Baath,1996; Kosolapov *et al.,* 2004;). Inspite of working with a clayey neutral soil which shielded the microbes from a more direct and extensive contact, some apparent interactions could still be deduced from the results obtained from the metal treatments. Inhibition of soil microbial activities by a combination of Cu and Zn was greater at lower metal concentration than the effect of each metal separately at double the concentration. In the effect of treatment on respiration of soil microbes, for instance, (Table 7) the rate of inhibition in the Cu and Zn amended soils were 1.08 and 1.61 (µg of C/g) respectively. When combined ,i.e Cu:Zn, the rate of inhibition was greater at 0.98 (µg of C/g). This same relationship between Cu and Zn is deducible from the other Tables (4 - 6). It is noteworthy that the effect of Zn was not significant on the activities of the soil microbes all through the work; but in combination with Cu, it engenders a more toxicological effect than even Cu itself. Cu has long been reported to be very toxic to microorganisms in the free ionic form (Zevenhvizen *et al.,* 1979; Baker and Senft,1995). It is believed that an additive action or synergistic effect between Cu and Zn could have been responsible for the enhanced toxicity of Cu:Zn in the soil sample examined. Synergism was reported by Lighthart and Bond (1976) and Kelly

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and Tate (1998) as responsible for the inhibition of soil – litter respiration by a combination of Se and Cd. Also, according to Chander and Brookes (1993),additive effect in a combination of Zn and Cu caused larger decrease in soil microbial biomass compared to the sum of either metals singly.

Impeded litter decomposition and soil respiration are common features of heavy metal polluted soils (Marschner and Kalbitz, 2003; Illmer and Schinner, 1991). The degree of impedance however, are determined by the rate of carbon and nitrogen mineralization. Thus, under heavy metal pollution, the rate of such activities are impaired and carbon and nitrogen accumulate in the soil. It is clear from these results (Tables 4 and 5) that there was tendency towards accumulation of carbon and nitrogen in the Cu and Cu; Zn treated soils. It has been reported that the rates of mineralization of acid forest soils decrease inversely with the log of the heavy metal concentration (Baath and Arnebrant,1994; Marschner and Kalbitz,2003).

Assay of soil respiration also help to quantify the effects of metals on the total biological activity of soils. Addition of heavy metal salts to soils usually cause an immediate decrease in respiration rates, but responses are determined by the properties of both the metal and the soil. According to Brookes (1995), high levels of artificially added Pb may have no effect in clay soil but may decrease respiration rates in a sandy soil. In this work (Table 7) the respiration rates in the Cu and Cu:Zn treated soils decreased significantly. This could be due to the very high level of pollution as well as other interactions involving metals in combined ionic states. It can be seen,(Table 7) that the rate of inhibition of soil respiration was highest in the Cu:Zn (0. 98 µg of C/ g).

Microbial biomass carbon is a standard technique used to determine the effect of toxic substances on the soil microbial community (Chander and Brookes, 1993; Diaz Ravina *et al.,* 1994).The results in Table 6 show that the lowest measurement were obtained from the Cu:Zn and Cu amendments. The biomass measurement of Cu:Zn amendment was lower than the recorded values for either individual metals. Other authors (Chander and Brookes,1993; Kelly and Tate,1998)reported similar results, with respect to Zn and Cu and concluded that an additive effect was responsible for the enhanced effect noticed in the relationship between the metallic species in their report. It can be inferred therefore, that additive effect and not

synergism is the basis for interaction involving Cu and Zn metals in this work.

ACKNOWLEDGEMENTS

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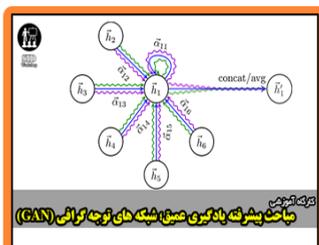## کارگاه های آموزشی مرکز اطلاعات علمی جهاد دانشگاهی

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