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Pedogenetic Activities of Soil Microbes as Influenced by Trivalent Cationic Chromium

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Abstract: We investigated the effects of different levels of chromium (111) (0, 10, 50, 100, 250, 500 and 1000 mg Cr kg^{-1} d.w.) on selected soil microbial parameters, such as total and active fungal mycelia, total microbial biomass carbon (C_{mic}) and soil potential activity (respiration) on an Isohyperthermic Arenic Kandiudult of Otamiri River floodplain, southeastern Nigeria. Field sampling was conducted in 2006 and collected soil samples were put in plastic containers before application of 7 treatments of Cr (111) as indicated. Soil samples were kept under laboratory conditions (water holding capacity = 30%; temperature = 25°C). Treatments were arranged using Completely Randomized Design (CRD) with 5 replicates. Routine and special analyses were conducted on investigated parameters. Results indicated significant ($p<0.001$) reduction in active and total fungal mycelia caused by additions of Cr (111) to the soil. A significant decrease ($p<0.01$) in C_{mic} was detected only in soils with 1000 mg Cr kg^{-1} d.w. Soil microbial activity declined to 17.07% in contaminated soils when compared with soils for control experiments. There were good relationships between soil microbial respiration and active fungal mycelium, suggesting their use in soil quality predictions.

Key words: Biotoxicity, chromium, fungi, microbial activity, pedogenesis, tropical soils

INTRODUCTION

Soil is a product of interactions of complex pedogenic processes (Birkeland, 1999), including the plant-rock-soil association (Darmody *et al*., 2001, 2004). Part of pedogenesis is the contribution of the microbial populations which play fundamental role in ecosystem functioning, especially in organic matter decomposition and nutrient cycling. Activity of soil microbes becomes a very sensitive and reliable indicator of changes in the pedosphere. But soil microbial responses are often altered by anthropogenic activities, most of which are deleterious (Aiyesanmi, 2006). Ekpo and Nwankpa (2006) reported that high levels of crude oil pollution caused significant depression in the growth of fungi possibly due to high heavy metals content. Apart from crude oil spillage and other exploration activities, heavy metals enter the soil system through the use of organic and inorganic fertilizers.

One of such biotoxic heavy metals is chromium, which among other forms can appear as a trivalent cation Cr (III) and stable in the soilsphere (Stepniewska *et al*., 2004). Chromium is both a mutagen and a carcinogen even at sub-ppm levels (Stewart *et al*., 2003). Chromium is highly soluble in an aquatic environment (Babel and Opiso, 2007) and readily adsorbed by living organisms. In humans, accumulation of Cr beyond permissible limits can cause severe health problems (Kurniawan, 2002), such as lung cancer, liver damage, kidney and reproductive problems.

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Chromium has no less important effects on microbial growth and activity. Negative correlations were reported between Cr and microbial performance (Kizilkaya *et al*., 2004) while no effect was also recorded among some authors (Majer *et al*., 2002). However contrasting effects among environmental factors make it difficult to pin down a single factor in a cause-effect relationship with another biological variable. It is in the light of the above fact that we studied the effects of different rates of Cr (III) on microbial activities related to soil formation and this is consistent with earlier suggestions (Dick, 1997; Gilier *et al*., 1998; Gigliotti and Farini, 2002). With increasing population and conflictive land uses in southeastern Nigeria coupled with the spate of land degradation, it becomes necessary to determine levels of Cr (III) content of soils considered biotoxic to the microbial community. Based on the above, the major objective of our study was to determine the minimum Cr (III) concentration in studied soils at which a significant decline in the activity of soil microbes was observed.

MATERIALS AND METHODS

Study Area

Soils of Otamiri River floodplain Southeastern Nigeria are located between latitudes $4°15^{1}00.220^{11}$ and $5°30^{1}10.310^{11}$N and longitudes $6°47^{1}21.310^{11}$ and $7°01^{1}05.410^{11}$E. Soils of the area were classified as lsohyperthermic Arenic Kandiudult (Onweremadu *et al*., 2006). Soils are derived from Coastal Plain Sands (Benin formation) of the Oligocene-Miocene geological era. The study site belongs to the lowland areas of southeastern Nigeria. It is humid tropical, having an average annual rainfall of 2400 mm and annual temperatures ranging from 20-29°C. The study area has a rainforest vegetation and farming is a major socioeconomic activity.

Soils Sampling

Surface soil samples were collected before rains in 2006 at the midslope of Otamiri River Slope. The site was considered part of the river valley with low chromium concentration (5.2 mg kg^{-1} d.w.) and uncultivated river vegetation Soil samples were air-dried and sieved using 2 mm sieve.

Experiment

Four kilograms of sieved fresh soil was incubated in plastic containers and added with chromium (III) sulphate solution at increasing concentrations of 0, 10, 50, 100, 250, 500 and 1000 mg Cr kg^{-1} dry weight). The soil condition was controlled by having a constant gravimetric moisture content (Water holding capacity = 30%) and temperature (Temperature = 25°C) and kept in the dark for 60 days. Soil water was recharged every two days to make up for evaporated moisture loss. The control soil samples were given the conditions as those treated with chromium sulphate solution but there was no chromium application on it. Five replicates were administered for each Cr concentration, giving a total of 35 samples for the study and arranged in a Completely Randomized Design (CRD).

Laboratory Analyses

Particle size analysis was determined by hydrometer method (Gee and Or, 2002). Soil pH was measured potentiometrically in I:1 soil/solution and a clear supernatant was estimated using a microprocessor ionanalyzer/901 (Orion Research, Beverly, M.A.), involving a combination of glass and Calomel electrode (Beckman Tullerton C.A.) Total Organic Carbon (TOC) and Total Nitrogen (TN) in air-dried soil samples (<180 μm) were analyzed in a Vario Max-ELEMENTAR CN-analyzer (D-63452 Hanau, Germany). Cation exchange capacity was estimated by summation of Mehlich -3 extracted cations (Darmody *et al*., 2000) while exchangeable cations were got through inductively coupled plasma spectroscopy (Mehlich, 1984). Silt-Clay Ratio (SCR) and Carbon-Nitrogen ratio (C/N) were calculated by dividing silt content by clay and carbon content by nitrogen, respectively.

During the first week after incubation, biological analyses were carried out on fresh soils stored at 4°C. Total and active fungal mycelia were measured by filter technique (Sundman and Sivela, 1978). One gram of fresh soil was mixed with 100 mL of K-phosphate buffer 60 mM at pH 7.5 and the suspensions were oscillated at 600 rpm for 2 min and 0.5 mL was transferred to a polypropylene prefilter, having 0.6 μm mesh size. A prefilter was treated with aniline blue in 80% lactic acid, to determine total fungal mycelium while another prefilter was treated with fluorescein diacetate to estimate active fungal mycelium as described by Soderstron (1977). Thereafter, prefilters were observed at a magnification of 400 x (Zeiss Azioskop MC 100 Spot Microscope, equipped with a mercury lamp). Length of fungal mycelium was determined by the intersection method and converted to fungal mass on the basis of average values of cross-section, density and dry mass of hyphae (Berg and Soderstrom, 1979). Total microbial biomass carbon was estimated by fumigation extraction method using 5 g oven-dry soil, calculating microbial biomass carbon as 2.64 Ec values (Vance *et al*., 1987), where Ec is the difference between extractable C from fumigated and non-fumigated samples. The potential activity of soils was measured as soil respiration determined by gas chromatograph as CO_2 evolved from fresh soil samples incubated for 60 min in standard conditions of 30% water holding capacity and 25°C temperature and kept in the dark. Values were expressed as mg CO_2 g^{-1} d.w. h^{-1}.

Total Cr on the soil was determined using a modification of EPA method 3052. The soil was digested in a CEM microwave model MDS-8ID, with hydrofluoric and nitric acid. Boric acid was added before sample analyses to facilitate the removal of hydrofluoric acid from solution though the formation of fluoroboric acid.

Statistical Analyses

Descriptive statistics, namely means and standard deviations were calculated on the 5 replicates for each treatment. Variability in soil incubated with different Cr (III) concentrations were assayed by one-way analysis of variance (ANOVA), followed by the SNK Student-Newman-Keuls Test ($p<0.05$, $N = 5$). Relationship between Cr (III) concentrations and biological parameters were analyzed by correlation coefficient using PC SAS version 8.2 (SAS, 2001).

RESULTS AND DISCUSSION

Soil Properties

Soils were sandy and highly weathered (SCR = 0.3) and strongly acidic which is attributable to parent material, climate, fluvial depositions and land use. Low CEC, is possibly due to low organic carbon and clay contents of the soil. Values of these soil properties are shown in Table 1. However, Oti (2007) reported that land degradation by soil erosion has altered soil properties negatively, resulting to soil productivity decline. Carbon-nitrogen ratio of soils before treatment, often used as an indicator of state of soil microbial biomass (Moore *et al*., 2000), was greater than 6 (C/N = 12), indicating higher soil fungi population compared with bacteria (Moore *et al*., 2000).

Table 1: Selected soil properties in the study site

Properties	Value
Sand (g kg^{-1})	840.0
Silt (g kg^{-1})	40.0
Clay (g kg^{-1})	120.0
SCR	0.3
Soil pH (water)	5.2
TOC (g kg^{-1})	15.6
TN (g kg^{-1})	1.3
C/N	12.0
CEC (cmol kg^{-1})	9.8

Cr = 5.2 mg kg^{-1} d.w., SCR = Silt-Clay Ratio, SOC = Soil Organic Carbon, TN = Total Nitrogen, CEC = Cation Exchange Capacity, d.w. = Dry weight

Table 2: Distribution of total and active fungal mycelium (mean±SD) in soil samples at different rates of Cr (III) concentrations

Rate (mg Cr kg^{-1} d.w.)	Total fungal mycelium (mg g^{-1} d.w.)	Active fungal mycelium (mg g^{-1} d.w.)
0	0.25±0.06	0.13±0.01
10	0.18±0.07	0.09±0.01
50	0.17±0.01	0.07±0.03
100	0.15±0.03	0.06±0.02
250	0.16±0.02	0.04±0.01
500	0.13±0.02	0.03±0.01
1000	0.11±0.03	0.02±0.01
Pr>t	<0.001	<0.001

SD = Standard Deviation, Cr = Chromium, d.w. = Dry weight

Table 3: Distribution of total microbial biomass carbon and soil potential activity (mean±SD) at different rate of Cr (III) concentrations

Rate (mg Cr kg^{-1} d.w.)	Total microbial biomass carbon (mg g^{-1} d.w.)	Respiration (CO_2 µg g^{-1} d.w. h^{-1})
0	0.61	41
10	0.58	40
50	0.65	39
100	0.60	40
250	0.61	38
500	0.62	37
1000	0.36	34
Pr > t	<0.001	<0.001

d.w. = Dry weight, Cr = Chromium

Chromium Concentrations and Microbial Performance

There were significant differences ($p<0.001$) in the distribution of these mycelial types given different rates of Cr (III) application (Table 2). There were significant ($p<0.001$) reductions in mycelia forms as Cr (III) concentrations were increased and these results are consistent with the findings of Ekpo and Nwankpa (2006) in crude oil polluted soils of Nigeria. Similar findings were reported by scholars in agroecologies other than the study site (Hiroki, 1992; Mainville *et al*., 2006), where marked effects of heavy metals pollution on fungal mycelium were found.

Soil microbial biomass carbon (C_{mic}) was less affected by increasing doses of Cr (III) additions when compared with the effect of this heavy metal on total and active fungal mycelia (Table 3). Significant reductions (40.90%) for the total microbial biomass as shown in Table 3 was only found in soils with 100 mg Cr kg^{-1}, suggesting that higher concentrations of Cr (III) in soil is inhibitory to accumulation of C_{mic}. However, tillage practices like higher Cr (III) concentrations, could reduce soil microbial biomass C significantly (Acosta-Martinez *et al*., 2004), noting that C_{mic} in perennial pasture was greater than value of C_{mic} from continuously cultivated cotton soils. In other studies (Franzluebbers *et al*., 1995), C_{mic} was increased with conservation while the same tillage type modified soil microbial community (Pankhurst *et al*., 2002). Total soil activity represented by rate of respiration is shown in Table 3, indicating a significant reduction ($p<0.001$) in soils treated with 1000 mg kg^{-1}. There was 17.07% reduction in respiration in soils treated with trivalent cationic chromium when compared with control soils. Lower values of respiration (soil activity) could be a result of inhibition of intracellular enzymatic activities in fungi. Similar findings Klose and Tabatabai (2002) and Renella *et al*. (2002) reported that fumigation with chemicalsubstances inhibited proteases and consequently reduced intracellular activity (Acosta-Martinez *et al*., 2004) and microbial biomass while microbial biomass was positively affected by enzyme B-β-glucosidases activity at 0-7.5 cm depth (Ndiaye *et al*., 2002). The implication of reduced microbial performance following increasing applications of chromium is the inability of these soil organisms to optimally play their role in

Table 4: Relationships between Cr (III) concentration and soil biological parameters

Parameters correlated	R	R^2	1-R^2	Pr>t
Cr vs active fungal mycelium	-0.79	0.60	0.40	≤0.001
Cr vs soil potential respiration	0.91	0.83	0.17	≤0.001
Cr vs total microbial biomass	0.72	0.52	0.48	≤0.001
Soil potential activity vs active fungal mycelium	0.62	0.62	0.62	≤0.01
Soil potential activity vs total fungal mycelium	0.43	0.43	0.82	≤0.05
Soil potential activity vs microbial biomass carbon	0.36	0.36	0.88	≤0.05

Cr = Chromium, vs = Versus

sustaining environmental quality and agricultural productivity. Entry of chromium and other heavy metals alters soil system adversely, leading to either sub-optimal or total extinction of soil life (Brady and Weil,1999). It becomes necessary to assess microbial biomass in soils especially in soils vulnerable to contamination by heavy metals (Brookes *et al*., 1986).

Chromium and Biological Parameters

There was a significant positive correlation (R = 0.91; p<0.0001) with minimal coefficient of alienation between Cr (III) content in soils and potential respiration. Results also indicated that Cr (III) had high relationship with soil microbial respiration than microbial biomass, suggesting that the former has more predictive capacity than the latter. Also, there was a negative correlation between Cr (III) and active fungal mycelium, indicating that higher concentrations of Cr (III) certainly inhibit activity of fungal mycelium (Table 4).

Soil potential activity (respiration) in chromium-treated soils was related to some biological parameters (Table 4). There was a positive relationship between soil potential activity and active fungal mycelium (R = 0.62; p<0.01) when compared with total fungal mycelium (R = 0.43; p≤0.05), while microbial biomass carbon (R = 0.36; p≤0.05) had a very poor relationship with respiration. This result is consistent with the findings of Brookes (1995) that soil microbiological properties, such as respiration and microbial biomass carbon (C_{mic}) respond quickly to environmental conditions than inherent soil organic matter content.

CONCLUSION

This study revealed that Cr (III) concentrations had varying quantitative influences on both total and active fungal mycelia. Very low values of Cr (III) had significant (p<0.001) reductions in fungal performance while microbial biomass carbon showed least sensitivity to Cr (III) treatment. There were good relationship between soil potential respiration and active fungal mycelium when soils were treated with Cr (III). These results show that soil microbial respiration and activity of fungal mycelium can be very useful in modelling soil environmental quality and other soil toxicological tests.

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