CURRENT STUDIES IN COMPARATIVE EDUCATION, SCIENCE AND TECHNOLOGY, VOLUME 3, NUMBER 1, 2016, PP. 239-248

Toxicity of Heavy Metals on the Mycoflora of an Old Agricultural Soil

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Abstract

Heavy metal contamination of the soil environment seriously affects soil quality by affecting the microbial community. The toxicity of various concentrations of heavy metals (Cadmium, Copper, Lead, Nickel and Zinc) on the mycoflora of an old agricultural soil was investigated. Changes in fungal population and diversity were determined using standard mycological techniques before the addition of each heavy metal and at the end of each incubation period. The total fungal count of the control soils ranged from 6.3×10^4 sfu/g soil to 1.1×10^4 sfu/g soil. While counts of the heavy metal treated soils for cadmium, copper, lead, nickel and zinc ranged from 2.8×10^4 sfu/g soil to 1.0×10^3 sfu/g soil; 1.7×10^4 to 1.9×10^2 sfu/g soil; 2.8×10^4 to 1.0×10^3 sfu/g soil; 6.4×10^4 to 2.0×10^4 sfu/g soil and from 7.8×10^4 to 1.0×10^3 sfu/g soil respectively. The order of decreasing total mean values of total fungal count of the control and treated soils was; Control > Zn > Cd > Pb > Ni > Cu. Copper had the greatest impact on the total fungal count haven recorded the least count. Generally, statistical analysis using ANOVA (F-test) showed that, with the exception of nickel, there was a significant difference in the effect of the concentrations of all the heavy metals on the total count of fungi at $P \leq 0.05$ irrespective of the incubation period. Thirteen (13) species of fungi were isolated from the control soil samples and the soils treated with heavy metals. The fungi were; *Aspergillus* sp, *Botryodiplodia* sp, *Botrytis cinerea*, *Candida tropicalis*, *Cladosporium* sp, *Fusarium moniliformes*, *Geotrichum candidum*, *Gliocladium* sp, *Mucor* sp, *Neurospora fructicola*, *Penicillium* sp, *Rhizopus nigricans* and *Torula kefyr*. All the thirteen species of fungi were isolated from the control soil samples throughout the investigating period. While five fungal species which were; *Candida tropicalis*, *Geotrichum candidum, Gliocladium sp, Neurospora fructicola*, and *Torula kefyr* were completely eliminated from soils treated with heavy metals. The effect of reduction in the population of soil fungi from the original level and the complete elimination of some fungi by heavy metals calls for immediate concern as this serves as limiting factors which affect soil quality.

Keywords: Heavy metals, soil quality, fungi, fungal count, fungicidal.

Introduction

Soil microorganisms serve as biogeochemical agents for the conversion of complex organic matter into simple inorganic compounds. Plant life and our basic food supply is dependent upon tremendous numbers of microorganisms that exist in the fertile soil, degrading organic matter, recycling nitrogen, carbon and producing

nutrient in the form that plants can use directly (Prescott *et al*., 2005).

The vast differences in the composition and physical characteristics of soil together with differences in the agricultural practices by which they are cultivated result in corresponding large differences in microbial population and in their kinds (Pelczar *et al.,* 1993).

Different species of fungi inhabit the soil and they are most abundant near the surface where an aerobic condition is likely to prevail. Fungi are active in decomposing the major constituents of plant tissue namely cellulose lignin and pectin. The physical structure of soil is improved by the accumulation of mold mycelium within it. Fungi bind soil particles to form water stable aggregate, this is one of the important characteristics of soil of agricultural importance (Prescott *et al.,* 2005).

Microbial transformations require a wide range of enzymes, including cellulases, lipases, amylases, proteases, amidases and oxygenase. Microorganisms serve as a rich nutrient source for grazing invertebrate including Protozoa and animals. Thus, if the microbial populations are disturbed or inhibited by a toxicant there will be a drastic change in community structure and secondary productivity could be greatly reduced. This disruption can have very obvious and dangerous environmental consequences (Alloway, 1990).

Soil microbial population is under tremendous pressure due to contamination of soil by a variety of toxic substances such as heavy metals from a variety of anthropogenic sources (Chaudhary *et al.,* 1996). Heavy metals at elevated concentrations are known to affect microbial population and their associated activities, which may directly influence the soil fertility (McGrath *et al.,* 1995; Smith and Paul, 1990). The concentration of a toxic metal that affect the growth and survival of different microorganisms vary greatly. It is expected that response of various soil microbial populations may possibly more accurately be assessed by studying the indigenous microbial populations in the soil amended with different concentration of heavy metals.

The aims and objectives of the present investigation are to determine the toxicity of some heavy metals on the population and diversity of soil fungi by the enumeration, isolation and characterization of soil fungi in unpolluted and in polluted soils an old agricultural soil using different concentrations of some heavy metals (lead, zinc, copper and cadmium and nickel). To subject the data obtained to the analysis of variance completely randomized design, using MINITAB for windows V 10. Means were compared at the 5% significance level using Duncan's multiple range text-DMRT and to explain the relationship between the result obtained and the associated health hazards since microbial populations disturbed or inhibited by a toxicant can result in drastic change in community structure and secondary productivity could be greatly reduced.

Materials and Methods

Study Area and Sampling sites

Soil samples were collected from an old agricultural soil located in Nkpolu - Oroworukwo Mile 3, Diobu, Port Harcourt, Nigeria. The old agricultural soil referred to in this study is one that was cultivated with annual crops and crops that

mature and are harvested before a year. Such farms when left for over a year after cultivation without harvesting the crops are termed "old".

The soil under has been cultivated on for over 2 years and there are matured plants/crops of *Manihot esculentus, Ananas comosa* and *Panicum maximum*. The distance between soils collected and plant is 15-25cm.

Source of Heavy Metals and Preparation of Heavy Metal Standards

Heavy metals with a specific gravity were bought from ANAL Concept Ltd, Environmental/Analytical Services Consultants in Port Harcourt. Each heavy metal was prepared according to ASTM references. Copper ASTM D1688; Lead ASTM D 3559-96; Zinc ASTM D 1691-95; Cadmium ASTM D 3557-95 and Nickel ASTM D 1886-94 (ASTM, 1999).

Experimental Design, Collection of Soil Samples and Preparation

The experimental design was randomized complete block design (RCBD). Each block unit or plot was 30cm × 30cm. Microbially influenced agricultural soil fertility is in the range 0-15cm depth, therefore, the volume of soil per plot was 30 × 30 × 15 = 13,500cm^3.

Each soil sample was collected at a depth of 0-15cm, within each plot using a sterile spatula. Three soil samples of about 500g were randomly collected in each site and then bulked together to form composite soil samples. The soils were placed in plastic bags, sent to the laboratory where soil samples were immediately prepared for microbiological analysis or stored at 4^0C until sample preparation.

The soil samples were mixed with salts, oxides of heavy metals (Cadmium, Lead, Zinc, Copper, and Nickel) at the concentration of 1ppm,10ppm, 100ppm 200ppm and 500ppm. Soil samples of 100g each were then packed in sterile polythene bags and maintained at 30% water holding capacity of each treatment solution and stored at 28 ± 2^0C for 28 days. A batch of untreated soil samples served as control for microbiological quality determination. All soil samples for future analysis were stored at 4°C according to ISO and OCED standards (Torstensson *et al.,* 1998).

Determination, Enumeration and Presumptive Identification of Soil Fungi

One gram of each soil sample amended with different concentration of the heavy metals (Cd, Zn, Pb, and Cu;- 1, 10, 100, 200) and (Ni - 10, 100, 200, 500) was collected after the required incubation period (1, 3,5, 7, 14, 21 and 28 days) and was serially diluted in normal saline solution and an aliquot of 0.1ml of 10^{-3} diluted sample was spread over the surface of sterile Potato dextrose agar (PDA) in Petri dishes. The composition of the medium was potato 200g, distilled water, 500ml glucose 15g and agar No. 1 20g. Ampicillin and streptomycin were added to inhibit bacteria growth (Institute of Pollution Studies, 1990).

Cultured plates were prepared in triplicates and were incubated for 5 to 7 days and the colonies which developed were counted as total viable fungi for each soil sample. Discrete fungal colonies were described and subcultured onto freshly prepared potato dextrose agar to obtain pure cultures for further characterization.

The pure isolates were identified using both cultural and microscopic examination in accordance with lactophenol method. This was done by needle mount method. A small portion of the growth was picked with a sterile needle and teased out in a drop of lactophenol cotton blue on a clean microscopic slide. This was covered with a clean cover ship, taking care to exclude air bubble. The prepared slides were examined under the microscope, starting with a low power objective (×10) to a higher power (×40) objective for a better field view and magnification (Harrigan and McCance, 1990). The fungal isolates were characterized based on macroscopic and microscopic appearances which comprised colonial morphology type of hyphal, presence of sterigma, shape and kind of spore/conidia, presence of special structure such as foot cell, and growth on glucose. Identification of the fungi was accomplished by comparison to Barnett and Hunter (1972).

Results

The result of the effect of various concentrations of heavy metals on the total fungal count of an old agricultural soil is shown in Figure 1. The results of the data obtained showed that, the total fungal count of the control soils ranged from 6.3 × 10^4 sfu/g soil to 1.1 ×10^4 sfu/g soil. The total fungal count of the cadmium treated soils ranged from 2.8 × 10^4 to 1.0 ×10^3 sfu/g soil. The highest value was observed at concentration 1ppm on the 1st day after pollution, while the lowest value was observed at concentration 100 and 200ppm on 28th day. The total fungal count of the copper treated soils ranged from 1.7 × 10^4 to 1.9 × 10^2 sfu/g soil. The highest value was observed at 1ppm concentration on the 1st day after pollution while the lowest value was observed at concentration 200ppm on the 28th day. The total fungal count of the lead treated soils ranged from 2.8 × 10^4 to 1.0 × 10^3 sfu/g soil. The highest value was observed at concentration of 1ppm on the 1st and 3rd day while the lowest value was observed on the 21st and 28th day at concentration of 200ppm.

The total fungal count of the nickel treated soils ranged from 6.4 × 10^4 to 2.0 × 10^2 sfu/g; the highest value was observed at 10ppm after 1st day of pollution while the lowest value was observed at 200ppm and 500ppm on the 28th day. The total fungal count of the zinc treated soils ranged from 7.8 × 10^4 to 1.0 × 10^3 sfu/g. The highest value was observed at the concentration of 1ppm on the 3rd day after pollution, while the lowest was observed at 200ppm on the 28th day.

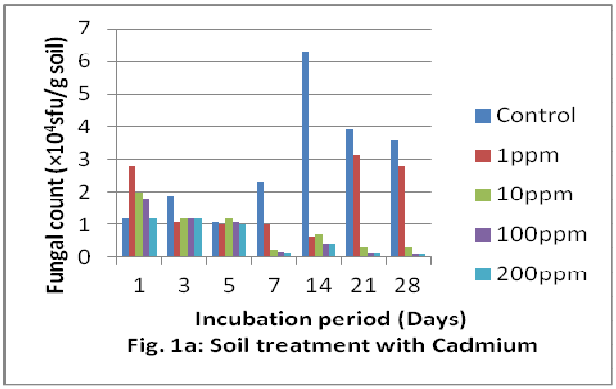

Fig. 1a: Soil treatment with Cadmium

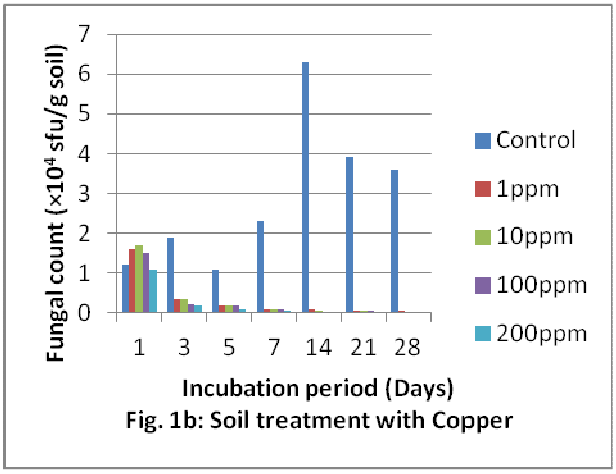

Fig. 1b: Soil treatment with Copper

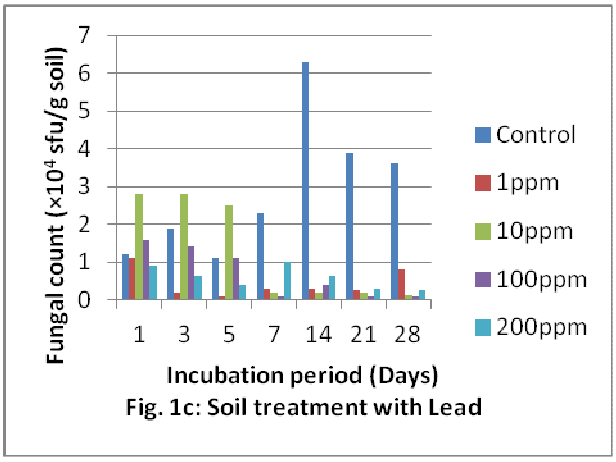

Fig. 1c: Soil treatment with Lead

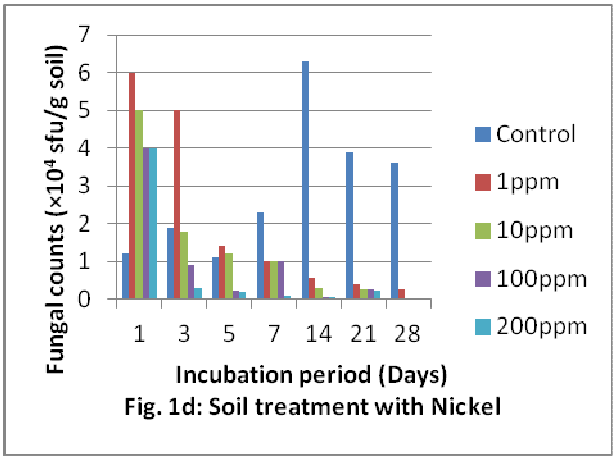

Fig. 1d: Soil treatment with Nickel

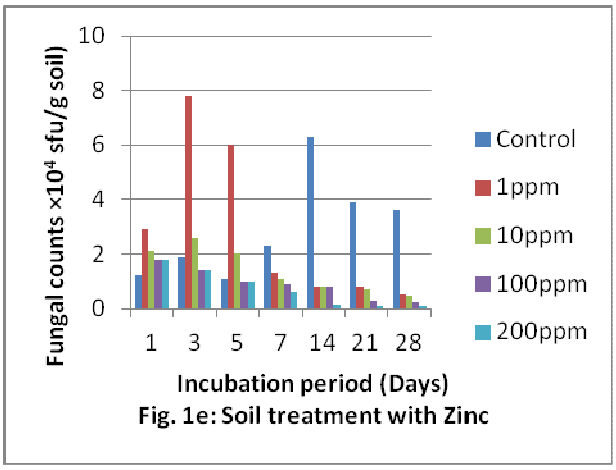

Fig. 1e: Soil treatment with Zinc

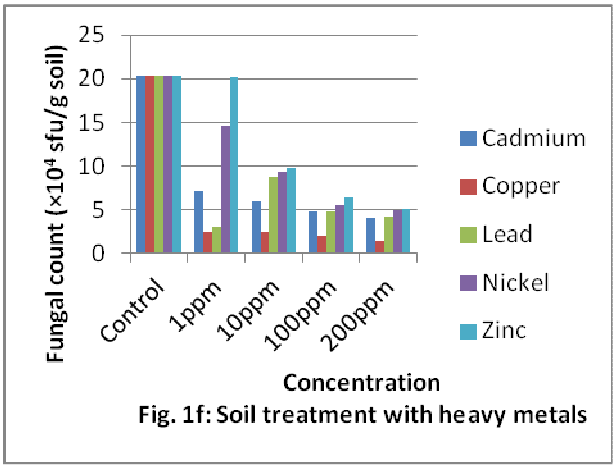

Fig. 1f: Soil treatment with heavy metals

Fig. 1: Effect of various concentrations of heavy metals on fungal count of an old agricultural soil

During the investigating period, species of thirteen (13) genera of fungi were isolated from the control soil samples and the soils treated with different concentrations of heavy metals. The fungi were; *Aspergillus* sp, *Botryodiplodia* sp, *Botrytis cinerea*, *Candida tropicalis*, *Cladosporium* sp, *Fusarium moniliformes*, *Geotrichum candidum*, *Gliocladium* sp, *Mucor* sp, *Neurospora fructicola*, *Penicillium* sp, *Rhizopus nigricans* and *Torula kefyr*. All the thirteen species of fungi were isolated from the control soil samples throughout the investigating period. While only species of six fungi which were; *Candida tropicalis*, *Cladosporium* sp, *Gliocladium* sp, *Mucor* sp, *Neurospora fructicola*, and *Penicillium* species occurred in the heavy metal treated soils throughout the investigation.

Discussion

The present study has demonstrated that the various concentrations of heavy metals had toxic effect on the population and diversity of fungal species in soil.

The order of decreasing total mean values of total fungal count of the control and treated soils was; Control > Zn > Cd > Pb > Ni > Cu. Copper had the greatest impact on the total fungal count haven recorded the least count.

Generally, statistical analysis using ANOVA (F-test) showed that, with the exception of nickel, there was a significant difference in the effect of the concentrations of all the heavy metals on the total count of fungi at $P \leq 0.05$ irrespective of the incubation period.

Ayman *et al.,* (2000) demonstrated that copper contamination on soil microbial biomass seriously affects the recycling of nutrients in the soil and thus plant growth yield.

Generally, there was no clear cut variation in fungal counts of soils treated with the lower concentrations (1ppm and 10ppm) of heavy metals with increasing incubation periods. However, there was a general decrease in fungal counts of soils treated with the higher concentrations (100ppm and 200ppm) of heavy metals with increasing incubation periods.

Old agricultural soils contain organic matter, although the amount and type may vary considerably and have extensive roots which produces appreciable amount of organic materials including exudates, mucilage, sloughed off cells and their lysates (Hopkin and Shiel, 1996). These organic compounds give rise to intense microbiological and biochemical activity in the soil. However, statistically analysis showed that there was no significant difference at $P \leq 0.05$ between the effects of various concentrations of each of the heavy metals and between the incubation periods of the heavy metals on the total fungal count of the soil. This implied that all the heavy metals used in this study had the effect of reduction of fungal population from the original (control) level. McGrath *et al.,* (1995) reported that elevated concentration of metal compounds affect microbial population and or their associated activities.

During the investigating period, species of thirteen (13) genera of fungi were isolated from the control soil samples as compared to only six species of fungal genera isolated from the soils treated with different concentrations of heavy metals. The reduction in the types of fungal genera from thirteen to just six showed that there was a reduction in the diversity and occurrence of fungi in the soil samples treated with various heavy metals with increasing incubation period as compared with the control soil samples. The reduction in the types of fungi was observed on the 14^{th} day till the 28^{th} day of incubation of the soil samples with heavy metals. Two fungal genera (*Botryodiplodia* and *Gliocladium*) did not occur on the 14^{th} day for all the heavy metal treated soils. Six fungal species (*Botrytis cinerea*, *Candida tropicalis*, *Geotrichum candidum*, *Gliocladium* sp, *Neurospora fructicola*, and *Torula kefyr*) did not occur on the 21^{st} and 28^{th} days for all the heavy metals except that *Candida tropicalis* did not occur on the 21^{st} day in the 1ppm and 10ppm concentrations for copper and in the 1ppm concentration for zinc.

This showed that the reduction in the types of fungi species or diversity in-

creased with increasing incubation period with heavy metals and that the concentration of the heavy metals also had a reducing effect on fungi. The result also showed that except for *Botryodiplodia*, the heavy metals exhibited a fungicidal effect on the fungal species which did not occur between the 14th and 28th day during the investigation because these species did not reoccur or were not isolated or recovered from the heavy metal treated soils. The heavy metals also exhibited a bacteriostatic effect on some bacteria species. This is the case with *Botryodiplodia* species which reoccurred on the 21st and 28th day for all the heavy metal treated soils.

Konopka *et al*., (1999) also demonstrated that elevated metal loadings in metal contaminated soils can result in decreased microbial community size and decreases in activities such as organic matter mineralization and leaf litter decomposition.

The complete elimination of five fungal species which were; *Candida tropicalis*, *Geotrichum candidum, Gliocladium sp, Neurospora fructicola*, and *Torula kefyr* from soils treated with heavy metals during this present study calls for immediate concern. A soil is regarded as fertile when all the conditions – physical, chemical and biotic are satisfied. The absence of any one of them acts as a limiting factor and effects soil fertility.

This present study has shown that, heavy metal contamination of soil decreases fungal counts and therefore the biomass, and fungal types and diversity. Hattori (1992) reported that heavy metals influence the proliferation of soil microorganisms. When a new chemical is allowed to pollute the environment, some microbial processes could be disturbed and this could have an indirect catastrophic effect on the ecosystem (Hopkin *et al*., 1996).

Conclusion

The intensification of human technology has resulted in gross contamination of soils with heavy metals. Heavy metal contamination in soil can result in eradication of some primary food chain which in turn has major consequences for predator or consumer species. Alternatively, the lower level of the food chain may ingest heavy metals which normally become concentrated with each consuming species of the food chain. Heavy metals are toxic to humans as well as affect agricultural productivity.

Since heavy metals are dangerous and persist for years when they enter the soil environment, it is very difficult to eliminate their effects in the soil-plant system. It is therefore recommended that, emission or discharge of heavy metals into the environment by small, medium or large scale industries should be regulated by the relevant authorities.

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