INTERNATIONAL JOURNAL OF NATURAL AND APPLIED SCIENCES (IJNAS), VOL. 2, NO. 2(2007); P. 15 – 20, 2 TABLES, 3 FIGS.

The short term effect of heavy metal pollution on fungal diversity in sites at Cross River University of Technology, Calabar, Nigeria.

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ABSTRACT

This study evaluates the short term effect of heavy metal pollution of soil, on fungal populations. The heavy metals consisted of cadmium, copper and aluminium whose salt solutions were first spilled on randomly selected soil sites, followed by microbiological and physicochemical analyses of soil samples from the polluted sites, using standard procedures. It was observed that the overall fungal populations were not significantly reduced ($p>0.05$) except the cadmium-polluted site which showed significantly higher populations ($p<0.01$) than aluminium or copper-polluted sites. Species richness (S) and species diversity (D) in the control site were respectively significantly higher ($p<0.01$) and ($p<0.05$) than any of the polluted sites over the period of monitoring. It is concluded that cadmium promotes the growth of fungal population while aluminium and copper have no effect on their growth although there was reduced species richness and species diversity in all the polluted sites, unlike the control, probably resulting from the adjustment of the fungal populations to the pollutants. Further research in this area is recommended

INTRODUCTION

Industrial and domestic wastes often contain heavy metals, and these wastes find their way into the environment (Ajmal and Khan, 1985) and may contribute significantly to soil contamination through mismanagement. Toxicity of heavy metals can affect physiological, genetic or metabolic activity of microorganisms in the soil (Summers and Silver, 1972, Ehrlich, 1981).

Fungi are an important group of the soil microflora, and as saprophytes, they have the role of breaking down organic matter. Very little has been published on the effect of heavy metals on this group of organisms.

In Nigeria there are many workshops and cottage industries such as welding, mechanics, vulcanising, photographic establishments etc that generate waste that contain heavy metals. Mechanics for example produce waste from waste oil, fuel and used batteries, all containing heavy metals. There is no enforcement of legislation (if it exists) on dumping of toxic waste. As there are no separate disposal points for industrial or toxic waste most of this waste is discarded with domestic waste. In the case where the waste is in the liquid form it gets discarded wherever it is convenient for the operator. It may not always be discarded in the same place hence from time to time microorganisms are subjected to high levels of a particular pollutant for short periods. It is not really known what these short term pollution events have on soil microbial populations.

This study aims at investigating the short term effect of heavy metal pollution on fungal populations in the soil.

MATERIALS AND METHODS

Soil sampling

Four sampling sites S_1, S_2, S_3 and S_4 ($1m^2$ each) were chosen within the premises of Cross River University of Technology. The distance from one site to the next was 5m.

S_1, S_2 and S_3 were each contaminated with 10 litres of 1000mg/l solution of aluminium (in the form of chloride), cadmium (in the form of nitrate) and copper (in the form of sulphate) respectively, by pouring the liquid as evenly as possible over the $1m^2$ test area. Site S_4 was the control site and 10 litres of water was poured as evenly as possible over the test area.

Sampling was carried out 2 hours after the sites were polluted and then at intervals of 7 days for a period of two weeks. Two hours after contamination, soil samples were taken at 5 points on each site at about 4cm depth using a hand driven auger. The five samples on each site were pooled. All samples were conveyed to the laboratory in labelled polythene bags in a cool box containing ice to maintain a temperature of about 4^oC.

The fresh soil samples were treated immediately on arriving at the lab. The fresh samples were sieved using a mesh of 2.8mm (Kiikkala *et al.*, 2001).

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Physico-chemical analysis of soil

The moisture content of the soil samples was determined immediately, by noting the difference in weight before and after drying in an oven at 105^{o}C to constant weight.

method of Black *et al.*, (1965).

Soil pH was determined using the method of Walkley and Black (1934). Available phosphorus was determined by the method of Bray and Kurtz (1945). Total nitrogen was determined by the micro-Kjeldahl digestion method (Black *et al.*, 1965). Particle size measurement was carried out using the hydrometer method of Bouyoucos (1951). Potassium concentration was determined by the

Fungal counts and isolation of fungal species from soil samples

A sample of sieved soil (1gm) was suspended in 9ml sterile distilled water and thoroughly shaken for five minutes. Ten fold serial dilutions in the ranges 10^{-1} –10^{-8}

Were prepared using sterile distilled water (Atlas and Bartha 1992). Aliquots (0.1ml) of sample dilutions were each plated on malt extract agar made more acidic (pH 4.8), to suppress the growth of bacteria (Oxoid manual, 1976), and incubated at room temperature for four days. Plates with less than 200 colonies were used for counts. The number of visible colonies was multiplied by 10 times the reciprocal of the dilution factor, and recorded as colony forming units (CFU) per gram of soil.

Discrete colonies were sub-cultured to obtain pure cultures. The isolates were identified according to the methods of Beneke and Rogers (1970) and APHA (1998)

Statistical test

A two way analysis of variance test was carried out to determine significant differences, if any between fungal counts, species richness and species diversity in the soils contaminated with heavy metals and the control and between days of sampling.

Species richness and species diversity

Species richness and species diversity was calculated by the method described by Spellerberg (1981). In species richness (S) the number of different species are counted. Species diversity (D) is an index, which depends on the sample size thus:

$$D = \frac{s-1}{\log N}$$

where D = index of diversity, S= number of species (or species richness) and N = total number of individuals.

RESULTS

Total colony forming units (CFU) per gram of soil at the various sites are shown in Fig 1. The cadmium polluted site showed much higher CFUs/g of soil than any of the other sites and this was significant ($p<0.01$). However there was no significant difference between the control site and the sites polluted with aluminium and copper.

Fungal species richness of the various sites over the monitoring period are shown in Fig 2. The species richness (S) in the control site was significantly higher ($p<0.01$) than any of the polluted sites over the period of monitoring. There was no significant difference between sites polluted with heavy metals in respect of species richness.

Species diversity of the various sites is shown in Fig 3. The unpolluted control site showed a significantly higher ($p<0.05$) diversity than any of the polluted sites. There was no significant difference in species diversity between the metal polluted sites.

The soils polluted with heavy metals showed a reduction in number of species and thus a reduced species diversity index. The soil being slightly acidic (Table 1) would increase the availability of the heavy metals to the organisms and thereby increase the effect.

Some fungal genera seemed to be more susceptible to heavy metal pollution than others (Table 2). *Neurospora*, *Chysosporium* and slime moulds were absent from the heavy metal treated sites but were present in the unpolluted control site. Some other genera such as *Penicillium*, *Cladosporium*, *Aspergillus*, *Candida*, *Fusarium*, *Saccharomyces* and *Rhodutorula* seemed to be more tolerant to the heavy metals.

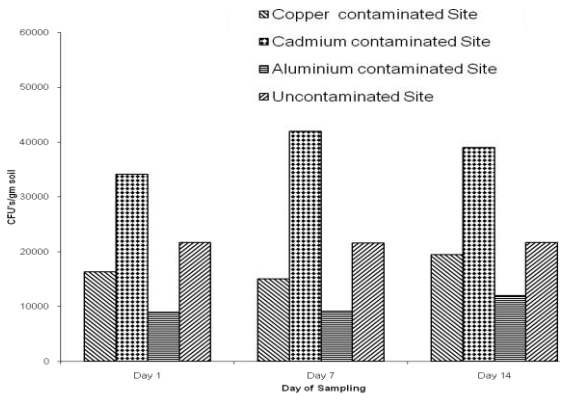

Fig 1. Colony forming units (CFU's) per gram of soil.

Table 1. Mean levels of some physico-chemical characteristics of soil samples from heavy metal contaminated sites (S1-S3) and control site (S4

Site	PH	Total Organic Carbon	Total Nitrogen %	Available Phosphorous mg/kg	Potassium cmol/kg	Particle Size %			Texture
						Clay	Silt	Sand	
S1	5.7	1.99±0.02	0.16±0.08	4.62±0.2	0.09±0.01	6.0±0.1	13.2±0.04	83.2±0.05	Sandy loam
S2	5.9	1.97±0.08	0.17±0.08	6.95±0.04	0.13±0.04	7.0±0.08	18.7±0.02	4.3±0.08	Loamy
S3	5.4	1.29±0.1	0.11±0.03	8.87±0.06	0.08±0.03	13.0±0.04	17.7±0.05	69.3±0.1	Sandy loam
S4	5.8	1.89±0.8	0.14±0.05	3.37±0.2	0.17±0.02	4.0±0.03	16.7±0.03	79.3±0.2	Sandy loam

Table 2. Fungi isolated at the various sites during sampling.

	Penecillium	*Cladosporium*	*Mucor*	*Aspergillus niger*	*Aspergillus fumigatus*	*Aspergillus flavus*	Candida	*Rhizopus*	*Fusarium*	*Saccharomyces*	*Schizosaccharomyces*	Rhodutorulla	Slime mould	Neurospora	*.Chysosporium*	*Paecilomyces*	*Trichoderma*	*Histoplasma*
Day 1																		
Control	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Aluminium	√	√	√	√			√	√		√	√	√						√
Cadmium	√	√	√	√	√	√	√		√	√		√						
Copper		√	√	√	√		√		√	√	√							
Day 7																		
Control	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Aluminium	√	√	√	√	√	√		√	√							√		
Cadmium	√		√	√	√	√	√		√	√		√						
Copper	√	√	√	√		√	√	√	√	√	√	√						
Day 14																		
Control	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Aluminium	√	√	√	√	√	√					√						√	√
Cadmium	√	√		√	√	√	√		√	√								
Copper	√	√	√	√	√	√		√	√	√	√	√						

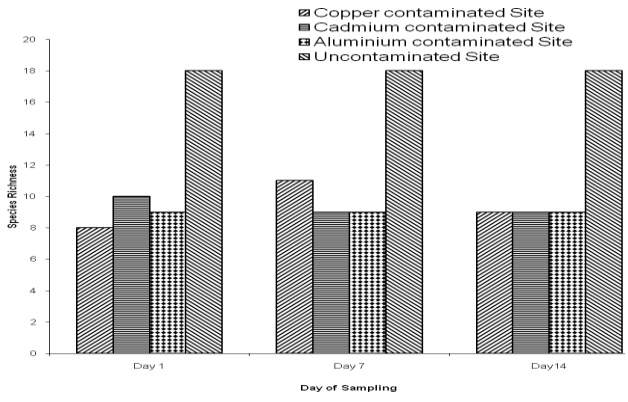

Fig. 2 Fungal Species Richness

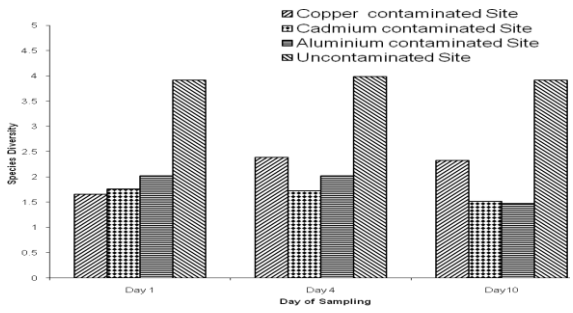

Fig 3. Fungal Species Diversity

DISCUSSION

Overall numbers of organisms as assessed by colony forming units are not reduced by pollution of heavy metals. This contradicted studies carried out on *Aspergillus niger* which showed that exposure to over 10 mg/l of cadmium, lead, mercury or zinc caused a reduction in the specific growth rate of the organism (Kuchari, 2002).

The cadmium treated site showed higher numbers and this may be expected considering that the metal was applied in the form of the nitrate salt. As soils are generally nitrogen limiting (Laegreid *et al*., 1999) an increase in nitrogen would result in increased activity of the resistant organisms.

The fungi are important to the soil ecosystem as decomposers and various fungi occupy different niches within that ecosystem as regards the type of organic matter they can decompose such as lignin, cellulose, etc. So the loss of even a few species may cause problems with the breakdown of certain organic matter. Aoyama and Nagumo (1997a, 1997b) and Kuperman and Carreiro (1997) found that low microbial biomass and activity may limit decomposition of soil organic matter and lead to their accumulation in heavy metal contaminated soils. Reduced microbial activity may originate from a change in microbial community structure after long term exposure to heavy metal. It has been observed that metal contaminated soil contained more metal resistant microbes with a restricted ability to degrade organic pollutants (Doelman and Haaustra 1989).

In conclusion, heavy metal pollution of soil results in reduction in both species number and species diversity. There is need for further research in this area.

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