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Effect of Heavy Metals on Bacterial Population and Diversity of a Newly Cultivated Soil

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Abstract

Intensive human technology has resulted in increased heavy metal content in the soil thereby affecting soil fertility. The effect of various concentrations of heavy metals (Cadmium, Copper, Lead, Nickel and Zinc) on the bacterial population and diversity of a newly cultivated soil was investigated. The total culturable aerobic heterotrophic bacteria counts and the types of bacteria of the control soil and heavy metal treated soils were determined using standard bacteriological techniques. The total heterotrophic bacteria count of the control soil ranged from 2.04×10^7 cfu/g soil to 3.6×10^5 cfu/g soil. While the bacteria count of the heavy metal treated soils for cadmium, copper, lead, nickel and zinc ranged from 5.7×10^6 to 1.1×10^4 cfu/g soil; 8.0×10^6 to 1.8×10^4 cfu/g soil; 4.6×10^6 to 1.5×10^4 cfu/g soil; 5.0×10^7 to 2.1×10^4 cfu/g soil and from 8.8×10^6 to 2.3×10^3 cfu/g soil respectively.. The order of decreasing total mean values of bacteria count of the soils was Control > Ni > Zn > Cu > Pb > Cd. Generally, analysis of variance using F-Test showed that except for nickel, there was a significant difference at $P \leq 0.05$ between the total heterotrophic bacteria count of the control and the heavy metal treated soils. Twenty-one (21) bacteria genera were isolated from the control and the soils treated with heavy metals. The bacteria were; *Actinomycetes* sp, *Aeromonas* sp, *Arthrobacter* sp, *Bacillus* sp, *Clostridium* sp, *Corynebacterium* sp, *Enterobacter* sp, *Escherichia coli*, *Flavobacterium* sp, *Lactobacillus* sp, *Micrococcus* sp, *Nitrobacter* sp, *Nitrosomonas* sp, *Nocardia sp*, *Proteus* sp, *Pseudomonas* sp, *Serratia* sp, *Shigella* sp, *Streptococcus* sp, *Vibrio* sp, and *Xanthomonas* species. All the twenty-one genera were isolated from the control soils throughout the investigating period. Seven genera which were; *Aeromonas* sp, *Flavobacterium* sp, *Proteus* sp, *Serratia* sp, *Shigella* sp, *Streptococcus* sp, and *Vibrio* species were completely eliminated from soils treated with heavy metals. The effect of reduction in the population of soil bacteria from the original level and the complete elimination of some bacteria genera by heavy metals calls for immediate concern as this serves as limiting factors which affect soil fertility.

Keywords: Heavy metals, soil, bacteria, bactericidal effect, soil fertility.

Introduction

Maintenance of good soil quality is of prime importance for sustainable agriculture. Bacteria play vital roles in soil fertility and primary production through organic matter decomposition and nutrient cycling which makes minerals again

available for use as nutrients for plants and animals including humans. The bacteria population in the soil exceeds the population of all other group of microorganism in both number and variety. Bacteria are able to perform their role of nutrient recycling in nature because of their possession of an array of enzymes. A chemical that disrupts one of these enzymatic processes may ultimately alter the metabolic capability of the entire population (Alloway, 1990).

Soil microbial population is under tremendous pressure due to contamination of soil by a variety of toxic substances such as heavy metals (Chaudhary *et al.,* 1996). Heavy metals are dangerous contaminants of the environment. They persist in soil and it is very difficult to eliminate their effects in the soil-plant system (Podlesakova *et al*., 1999). 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 for each consuming species of the food chain. Heavy metals are toxic to humans as well as affect agricultural productivity. Soil quality depends also on agricultural practices by which they are cultivated which may result in corresponding large differences in microbial population both in total number and in kinds (Frankenberger *et al.,* 1995). Microorganisms are more sensitive to elevated metal concentration than other terrestrial organisms (Giller *et al.,* 1998). The concentration of a toxic metal that affect the growth and survival of different microorganisms vary greatly (McGrath *et al.,* 1995). Maximum permitted concentrations of contaminant in soil are often derived without considering soil microbial end points. It is argued that microorganisms should be protected because of their essential role in nutrient cycling (Smith and Paul, 1990).

The aims and objectives of the present investigation are to determine the effect of some heavy metals commonly released into the environment on the population and diversity of bacteria by the enumeration, isolation and characterization of soil bacteria in a newly cultivated farm soil using different concentrations of heavy metals (lead, zinc, copper and cadmium and nickel) and to subject the data obtained to statistical analysis. Results obtained will explain the the associated health hazards since soil bacteria can bioaccumulate heavy metals which are persistent in soil and can be transferred through the food chain to humans.

Materials and Methods

Study Area and Sampling sites

Soil samples were collected during the months of November 2008 from a newly cultivated agricultural farm soil located in Nkpolu - Oroworukwo Mile 3, Diobu, Port Harcourt, Nigeria. Crop plants included *Dioscorea rotundata, Curcubita pepo and Manihot esculenta.* The distance between each plant, point of soil collection is 15 to 20cm. Houses and Canteens were located around the farm.

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^{0}C 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^{0}C 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).

Cultivation and Enumeration Soil Bacteria

At the end of each incubation period of 1 day, 3, 5, 7, 14, 21 and 28 days, one gram (1.0gm) of each soil sample amended with different concentration of the heavy metals was serially diluted in normal saline solution. One (1.0) gram of each treated soil was weighed and introduced into 9.0mls of the normal saline in the first test tubes labeled "stock sample", and a 10-fold serial dilution was carried out into six dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}).

The standard spread plate method with modification (Cuppucino and Sherman, 1983) was adopted and used for the determination and enumeration of the total viable count of heterotrophic aerobic bacteria. An aliquot (0.1ml) of 10^{-6} dilution was inoculated onto freshly prepared sterile and cooled Nutrient agar. The inoculum was spread with a sterile glass spreader. The inoculated plate was inverted and incubated at room temperature at 28 ± 2^{0}C for 24 hours, after which plates that grew between 30 and 300 colonies (Pelczar *et al.,* 1993) were counted and the average count for duplicate cultures was recorded as total viable count of bacteria. Counts obtained were calculated and expressed as colony forming unit per grams soil and recorded.

Counts were subjected to statistical analysis using the analysis of variance completely randomized design, using MINITAB for windows V 10 means were compared at the 5% significance level using Duncans multiple range text -DMRT analysis.

Isolation of Pure Cultures, Characterization and Identification of Soil Bacteria

Pure cultures of bacteria were obtained by plating discrete colonies onto nutrient agar and incubated at room temperature (28 ± 2^0C). Lawns of overnight cultures of the purified isolates were scrapped into 10% (v/v) glycerol thoroughly mixed and stored at -35^0C (Wellington and Williams, 1978). These glycerol suspensions served as a means for long term storage and a source for weekly working cultures. Weekly working cultures were 24 hours old cultures made from these frozen glycerol suspensions. To determine the types of bacteria isolated, the pure cultures of bacteria were obtained and subjected to the following characterization tests performed in duplicates. Gram staining and catalase test, coagulase test, sugar fermentation test, methyl red test, indole test and acid-gas test. The pure cultures were identified on the basis of their cultural, morphological and physiological characteristics in accordance with methods described by Cruikshank *et al.,* (1975) and with reference to Holt (1997).

Results

The result of the effect of various concentrations of heavy metals on the total heterotrophic bacteria count of a newly cultivated soil is shown in Figure 1. The results of the data obtained showed that, the total heterotrophic bacteria count of the control soil ranged from 2.04×10^7 cfu/g soil to 3.6×10^5 cfu/g soil. While the bacterial counts of the heavy metal treated soils ranged from 5.0×10^7 cfu/g soil to 2.3×10^3 cfu/g soil. However, for purposes of plotting the graphs, values of the bacteria counts were converted to $\times 10^5$. The bacteria count of cadmium treated soils ranged from 5.7×10^6 to 1.1×10^4 cfu/g soil. Both values were observed at concentration 200ppm on 1st and 28th day after pollution respectively. The bacteria count of copper treated soils ranged from 8.0×10^6 to 1.8×10^4 cfu/g soil. The highest value was recorded at 200ppm on day 1 and 1ppm on the 3rd day after pollution while the lowest value was on the day 28th day after pollution at concentration 100ppm.

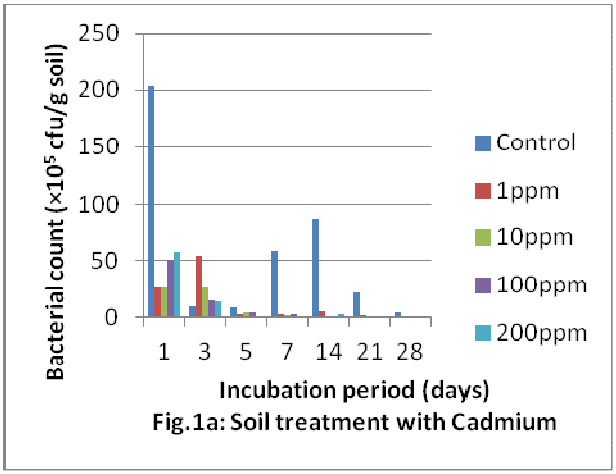

Fig.1a: Soil treatment with Cadmium

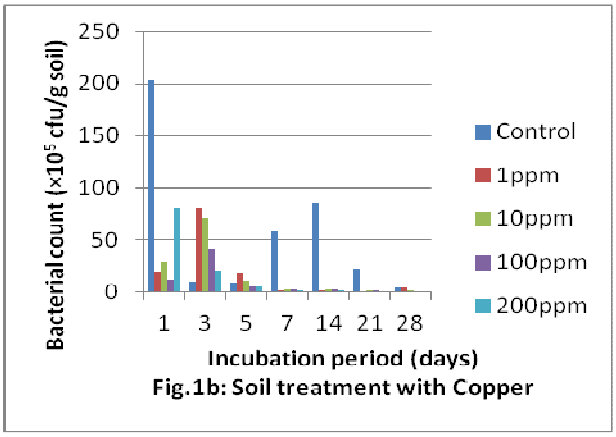

Fig.1b: Soil treatment with Copper

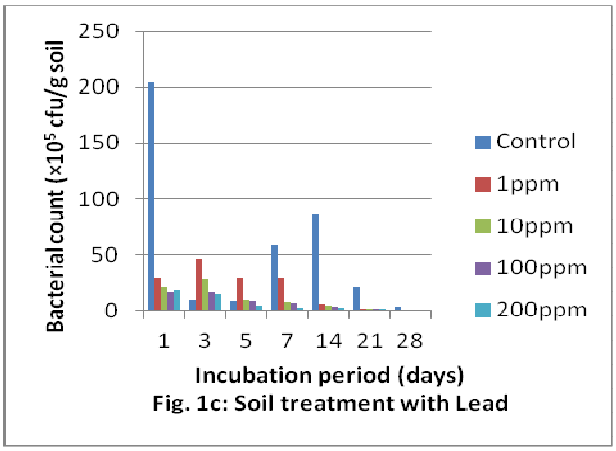

Fig. 1c: Soil treatment with Lead

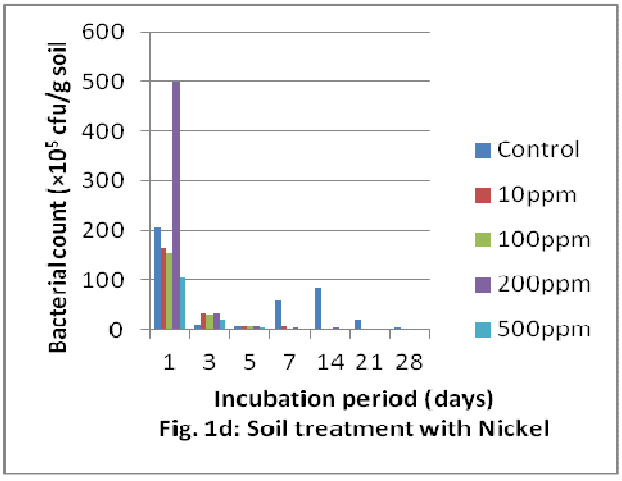

Fig. 1d: Soil treatment with Nickel

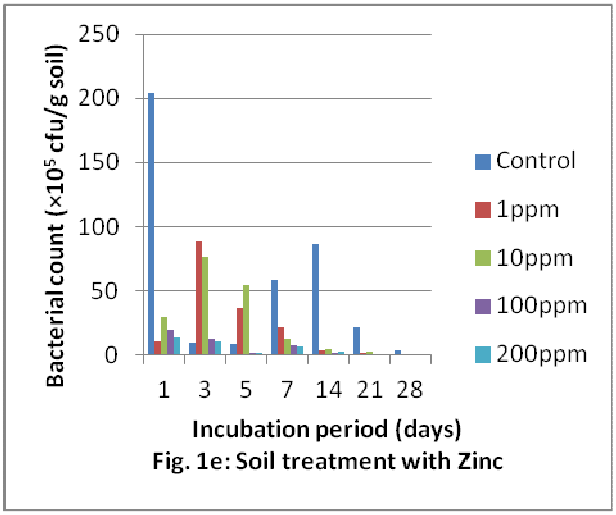

Fig. 1e: Soil treatment with Zinc

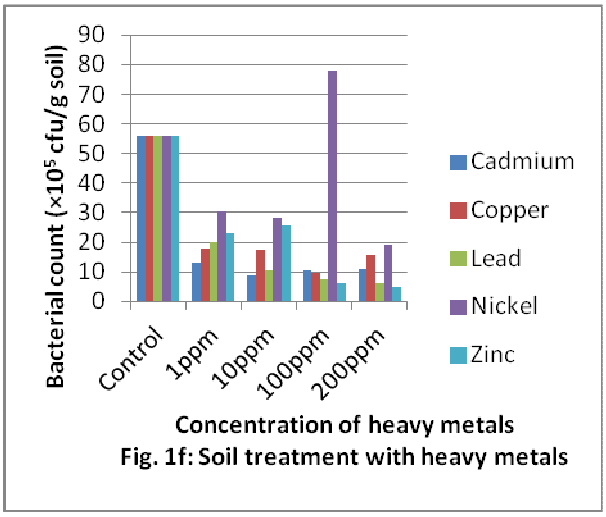

Fig. 1f: Soil treatment with heavy metals

Fig. 1: Effect of various concentrations of heavy metals on bacteria count of newly cultivated soil

The bacteria count of lead treated soils ranged from 4.6 x 10^6 to 1.5 x 10^4 cfu/ g soil. The highest value was recorded at 1ppm on the 3rd day after pollution while the lowest value was recorded on the day 28th day after pollution at both concentrations of 100ppm and 200ppm. The bacteria count of nickel treated soils ranged from 5.0 x 10^7 to 2.1 x 10^4 cfu/g soil. The highest value was observed on day I after pollution at 200ppm, while the lowest value was observed on day 28 after pollution at 500ppm. Generally, there was a decrease in bacteria count with increasing incubation period with nickel. The bacteria count of zinc treated soils ranged from 8.8 x 10^6 to 2.3 x 10^3 cfu/g soil. The highest value was recorded at 1ppm on the 3rd day while

the lowest value was on the day 28th day after pollution at concentration 200ppm.

During the investigating period, species of twenty-one (21) genera of bacteria were isolated from the control soil samples and the soils treated with different concentrations of heavy metals. The bacteria were; *Actinomycetes* sp, *Aeromonas* sp, *Arthrobacter* sp, *Bacillus* sp, *Clostridium* sp, *Corynebacterium* sp, *Enterobacter* sp, *Escherichia coli*, *Flavobacterium* sp, *Lactobacillus* sp, *Micrococcus* sp, *Nitrobacter* sp, *Nitrosomonas* sp, *Nocardia sp*, *Proteus* sp, *Pseudomonas* sp, *Serratia* sp, *Shigella* sp, *Streptococcus* sp, *Vibrio* sp, and *Xanthomonas* species. All the twenty-one species of bacteria were isolated from the control soil samples throughout the investigating period. On the other hand, only species of thirteen bacteria which were *Actinomycetes* sp, *Arthrobacter* sp, *Bacillus* sp, *Corynebacterium* sp, *Enterobacter* sp, *Escherichia coli*, *Lactobacillus* sp, *Micrococcus* sp, *Nitrobacter* sp, *Nitrosomonas* sp, *Nocardia sp*, *Pseudomonas* sp, and *Xanthomonas* species occurred in the heavy metal treated soils throughout the investigation.

Discussion

The present study has shown that the various concentrations of heavy metals had adverse effect on the population and diversity of bacteria species in soil. The order of decreasing total mean values of total heterotrophic bacteria count of the control and treated soils was; Control > Ni > Zn > Cu > Pb > Cd. Cadmium had the greatest impact on the total bacteria count haven recorded the least count. This agreed with the work of Hattori (1992) that the influence of Cd and other heavy metals influence the proliferation of soil microorganisms.

Statistically analysis (ANOVA) using F-test showed that there was a significant difference at $P \leq 0.05$ on the effect of the various concentrations of cadmium and of lead on the total heterotrophic bacteria count of the soil. On the other hand, there was no significant difference at $P \leq 0.05$ on the effect of the various concentrations of copper, nickel and of zinc on the total heterotrophic bacteria count of the soil. Generally except for nickel, there was a significant difference at $P<0.05$ between the total heterotrophic bacteria count of the control and the heavy metal treated soils.

IQbal *et al*., (2005) showed that aerobic heterotrophs are sensitive to metals such as Ni and Cd followed by Cu and Zn in heavy metal contaminated soils under laboratory conditions. 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 an increase and decrease in variation of bacteria 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 bacteria counts of soils treated with the higher concentrations (100ppm and 200ppm) of heavy metals with increasing incubation periods. Statistically analysis also showed that there was no significant difference at $P<0.05$ between the effect of the various concentrations of heavy metals and between the incubation period of the heavy metals on the total heterotrophic bacteria count of the soil. This implied that all the heavy metals used in this study had the effect of reduction of bacteria 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 twenty-one (21) genera of bacteria were isolated from the control soil samples in contrast to only thirteen bacteria species which occurred in the heavy metal treated soils throughout the investigating period. The thirteen bacteria species which occurred in the heavy metal treated soils throughout the investigation are chemoorganotrophs known to reproduce by budding e.g *Nitrobacter* or form mycelium which fragment into rods and coccoid forms or spores which are resistant to heat and desiccation e.g *Nocardia Arthrobacter* sp, and *Bacillus* sp. They are also known to possess transmissible plasmids or genes and gene transfer can occur by transduction and conjugation e.g *Actinomycetes* sp, *Bacillus* sp, *Enterobacter* sp, *E. coli*, and *Pseudomonas* species. These attributes must have contributed to the occurrence of these thirteen bacteria in the heavy metal treated soils throughout the investigation.

The reduction in the types of bacteria genera from twenty-one to thirteen showed that there was a reduction in the diversity and occurrence of bacteria 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 bacteria was observed on the 5th day till the 28th day of incubation of the soil samples with heavy metals. Two bacteria genera (*Proteus* sp and *Vibrio* sp) did not occur on the 5th and 7th day for cadmium, lead, and nickel on the 5th, 7th, and 14th day for copper and on the 5th, 7th, 14th and 21st day for zinc. Four bacteria (*Aeromonas* sp, *Proteus* sp, *Shigella* sp, and *Vibrio* sp) did not occur on the 14th day for nickel, and five (*Aeromonas* sp, *Clostridium* sp, *Proteus* sp, *Shigella* sp, and *Vibrio* sp) did not occur on the 14th day for cadmium and on the 14th and 21st day for lead. On the other hand, seven bacteria (*Aeromonas* sp, *Flavobacterium* sp, *Proteus* sp, *Serratia* sp, *Shigella* sp, *Streptococcus* sp, and *Vibrio* sp) did not occur on the 21st and 28th day for cadmium, copper and nickel, and on the 28th day for lead and zinc.

This showed that the reduction in the types of bacteria species or bacteria diversity increased with increasing incubation period with heavy metals. The result also showed that except for *Clostridium*, the heavy metals exhibited a bactericidal effect on the bacteria species which did not occur between the 5th 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 *Clostridium* species which reoccurred on the 21st and 28th day for cadmium, and on the 28th day for lead. *Clostridium* species are spore forming bacteria and some of the spores must have germinated which resulted in its proliferation and reoccurrence in the heavy metal soils treated with cadmium and lead.

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. IQbal *et al.,* 2005 in his study to investigate the effect of heavy metals on survival of certain groups of indigenous soil microbial population showed that Aerobic-heterotrophic bacteria populations were more sensitive to metal groups like nickel and cadmium. The complete elimination of seven bacteria genera which were; *Aeromonas* sp, *Flavobacterium* sp, *Proteus* sp, *Serratia* sp, *Shigella* sp, *Streptococcus*

sp, and *Vibrio* species 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 bacteria counts and therefore the biomass, and bacteria types and diversity. A chemical that is toxic to microbial communities alters the microbial ecology of the environment since soil microorganisms form an integral part of the nutrient cycling and energy flow processes of the ecosystem.

Conclusion

Contamination of soils with toxic metals is a frequent problem of industrialized areas along major roads. Risks to human, animals and plants are to be adequately considered if contaminated sites are to be redeveloped or in case of heavy metal pollution. Microorganisms have the ability to take up some concentrations of heavy metals and transfer to plants, and this is transferred to animals and man that feed directly or indirectly from the plants that get exposed to the metals and these metals tends to accumulate in animals and man that feed on them.

It is therefore recommended that, the soils that are to be used for cultivation of plants should be subjected to toxicity testing for the presence of heavy metals; that farms for cultivation should not be exposed to dumps sites or sites suspected to be contaminated with heavy metals and finally, that the government should set up agencies to regulate the emission or discharge of heavy metals into the environment by industries and automobiles.

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