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Microbial populations and activity in two soils of Tanzania as influenced by mercury

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Introduction

Microorganisms play an important role in the cycling of nutrients in soils and the presence of toxic chemicals in soil may impair this function. Variable results have been reported on the response of different microbial groups to added mercury (Hg) compounds. Sinha *et al.* (1979) reported that 2-methoxyethylmercury chloride (Aretan), up to 20 mg/kg of soil, reduced populations of *Azotobacter*, *Rhizobium*, *Aspergillus* and *Trichoderma* but increased actinomycete and general bacterial populations. However, concentrations as high as 150 mg/kg of soil were observed to increase microbial numbers (van Faassen 1973).

Nitrification was found to be variably depressed or increased by Hg added to soil. Liang & Tabatabai (1977, 1978) reported inhibition of nitrification, of up to 98%, by 1000 mg Hg/kg soil. Results of van Faassen (1973) showed the inhibitory effect to vary between Hg compounds and among soil types, being more severe in sandy soil. van Faassen further observed that 10 mg Hg/kg soil (as mercuric chloride or phenylmercury acetate) slightly increased nitrification. Sinha *et al.* (1979) found Aretan at 2.5 mg Hg/kg soil to suppress nitrification for the first 30 days, after which it recovered. Information on effects of Hg on the nitrogenase enzyme is not available but observations by Sinha *et al.* (1979) of reduction of populations of *Azotobacter* and *Rhizobium* may suggest that the nitrogenase enzyme of these bacteria may also be affected.

The studies cited above did not cover wide Hg concentration ranges. Sinha *et al.* (1979) used a range of 0 to 20 mg Hg/kg of soil. Van Faassen (1973) used 10, 100 and 150 mg Hg/kg, while Liang & Tabatabai (1977, 1978) tested only 1000 mg Hg/kg soil. These high Hg levels are not common in normal soils, but may occur in isolated locations due to contamination. A wider range of Hg concentrations was simulated in the present studies to evaluate effects of Hg on viable microbial population, non-symbiotic nitrogenase activity and nitrification, in two soils.

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Materials and methods

Sampling and properties of the soils used. Surface (0 to 20 cm) soil samples from Arusha (Andept) and Morogoro (Oxisol) were collected and stored, without processing, at 4°C to arrest further microbial changes. The relevant physico-chemical properties of the soils are given in Table 1, but detailed properties are described elsewhere (Semu *et al.* 1986).

Table 1 Some physico-chemical properties of the soils used

Soil	pH (water)	Clay	Water-holding capacity	Organic carbon	Total N
Morogoro (Oxisol)	5.8	43%	56%	2.5%	0.2%
Arusha (Andept)	6.7	5%	32%	2.4%	0.2%

Viable microbial count. Moist soil (1 g) was weighed in 25-ml screw-cap glass bottles. Aretan or mercuric chloride solutions were applied in duplicate at 1 to 2000 mg Hg/kg soil calculated on oven-dry weight basis. The solutions were prepared such that on application, the soils attained a moisture tension of about 0.3 bar. This moisture tension was employed in subsequent (i.e. nitrogenase and nitrification) studies also. Duplicate samples were used for this and the subsequent experiments because earlier replicated determinations ($n = 4$) gave acceptable coefficient of variation values (Table 2).

Table 2 Coefficients of variation for the microbial parameters determined

Soil	Viable count	Nitrogenase activity		Nitrification
		Aerobic	Anaerobic	
Morogoro (Oxisol)	13%	24%	30%	14%
Arusha (Andept)	19%	28%	27%	8%

The samples were incubated aerobically (loosely capped) at 21°C for 7 d. The plate count procedure was then used for microbial counting. Sterile water (9 ml) was transferred from a 25-ml screw-cap bottle to the 1-g soil sample. The soil suspension was vigorously agitated (hand-shaken) five times, each shaking lasting 1 min and 1 ml of this suspension transferred to 9 ml of sterile water and shaken as above. In this way, serial dilutions were made up to the 10^{-6} dilution. Each of the serial dilutions (1 ml) was plated using nutrient agar (Oxoid). The plates were incubated at 21°C for 7 d, after which colonies were counted.

Non-symbiotic nitrogenase activity. Soil samples (3 g) were weighed in 7.5-ml screw-cap glass bottles and 0.5 ml of 20% glucose solution was added to each bottle. Aretan or mercuric chloride solutions were added to the bottles in duplicate at 12 to 2000 mg Hg/kg soil. These experiments were carried out in two sets, one incubated anaerobically and the other aerobically. In the anaerobic set the bottles were first tightly capped and the air inside flushed with nitrogen gas for 1.5 min to remove oxygen before incubation. The bottles of the aerobic set were loosely capped. Both sets were incubated at 21°C for 7 d.

After incubation, the bottles of the aerobic set were tightly capped. Acetylene gas was introduced into all bottles using a syringe, such that acetylene occupied 10% of the volume. The bottles were re-incubated at 21°C for 24 h and nitrogenase activity was assayed, from the amounts of ethylene produced, using gas chromatography.

Nitrification. Soil samples were first leached with distilled water to free them of nitrates and were then air-dried overnight. Samples of 10 g each were weighed in 160-ml screw-cap glass

bottles and spread uniformly over the bottom of the bottles to improve aeration. NH_4Cl was added to each sample at 50 mg N/kg soil. Mercuric chloride was added in duplicate at 1 to 1000 mg Hg/kg soil. The samples were incubated at 21°C for 7 d. They were then shaken with 20 ml of 1M KCl on a reciprocating shaker for 2 h to extract ammonium- and nitrate-N. The soil suspensions were filtered under vacuum. Untreated control samples were extracted in the same way. These extracts were analysed for ammonium-N by the automated indophenol method of Selmer-Olsen (1971) and for nitrate-N by the method of Henriksen & Selmer-Olsen (1970).

Results and discussion

Viable microbial count

The effect of Hg on microbial population of the Morogoro and Arusha soils is shown in Fig. 1. In the Morogoro soil, increasing concentrations of Aretan reduced the microbial population gradually, from 10^7 c.f.u./g in untreated soil or at 1 mg Hg/kg soil to about 10^6 c.f.u./g at 2000 mg Hg/kg. In the presence of mercuric chloride treatments, the population increased slightly above 8 mg Hg/kg soil, then decreased sharply above 100 mg Hg/kg, dropping ultimately to about 10^3 c.f.u./g at 2000 mg Hg/kg. In the Arusha soil the microbial response to Hg was the opposite of that in the Morogoro soil, in that it was under Aretan that the population increased slightly, with subsequent reduction above 100 mg Hg/kg soil, like in the Morogoro soil. The microbial population (about 10^7 c.f.u./g soil) under mercuric chloride incubation remained nearly constant up to 200 mg Hg/kg soil but dropped sharply thereafter, reaching about 10^5 c.f.u./g at 2000 mg Hg/kg soil.

The total microbial population of a soil comprises a very diverse group of species, some of which may, or may not, be affected equally by increasing Hg concentrations in soil. In the present study it is probable that increasing levels of Hg in soil inhibited or killed some microorganisms while others may have been resistant. Therefore the slight increase in the populations following increased additions of mercuric chloride in the Morogoro soil and Aretan in the Arusha soil may have been partially due to utilization by survivors of the presumably more readily available energy from dead microorganisms (Jenkinson 1966; Sinha *et al.* 1979). The slight increase may also be related to the substantial ability of these soils to adsorb Hg (Semu *et al.* 1986). This implies that the eventual effective concentration of Hg in the soil solution, after Hg adsorption, would be considerably lower than the amount originally applied, thereby allowing more microbes to survive and multiply. Smith & Long (1980) observed phenylmercury acetate to be more toxic to fungi *in vitro* than when applied to soil, where adsorption by soil would reduce its effective concentration. Thus the soils of the present study may have, through adsorption, protected microorganisms from Hg toxicity, as was also observed by van Faassen (1973). The ultimate, drastic decline in the populations, at still higher Hg concentrations in soil, may be due to susceptibility of those microorganisms which may have been resistant to the lower levels of Hg most of which would be adsorbed.

The gradual decreases in microbial numbers with increasing concentrations of Aretan in the Morogoro soil and, to a lesser extent, mercuric chloride in the Arusha soil may imply that most of the bacterial groups could withstand those amounts of added Hg. This may be so because the effective Hg concentrations in the soil solution may have been lowered due to adsorption. The different responses in the two soils, namely one mercury compound eliciting a gradual decrease of microbial numbers in one soil but resulting in a slight increase followed by a sharp decrease in the other soil,

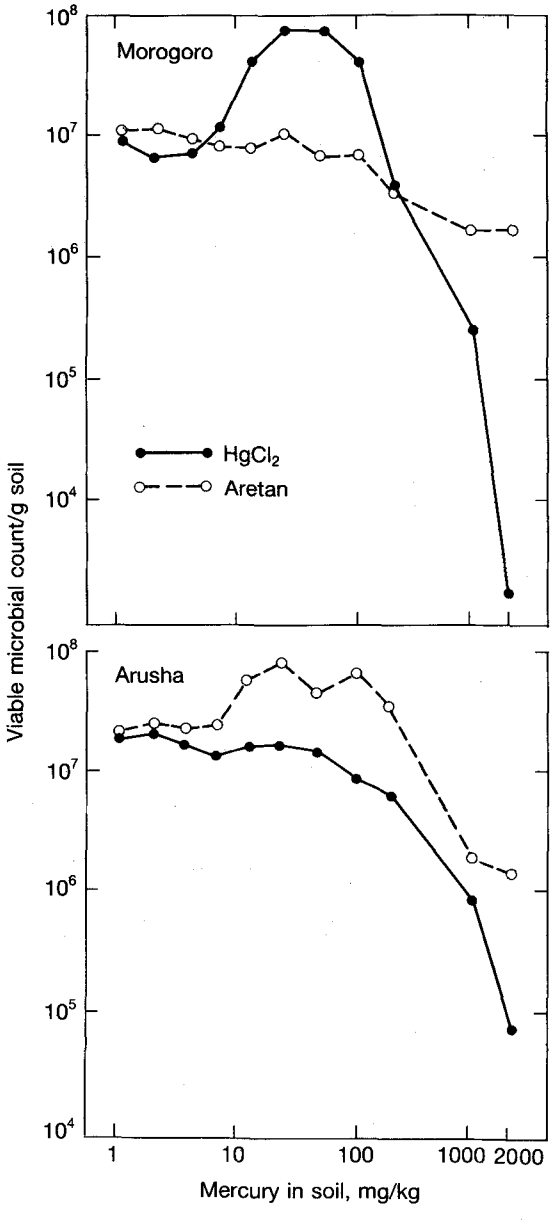

Fig. 1 Effect of mercury on microbial populations in Morogoro and Arusha soils.

is consistent with Hg adsorption patterns in the two soils. Adsorption studies (Semu *et al.* 1986) showed that the Morogoro soil had greater capacity to adsorb Aretan than did the Arusha soil. This may have resulted in more Aretan Hg in soil solution in the Arusha soil, and less in the Morogoro soil, leading to less drastic influences in the latter. On the other hand, the Arusha soil exhibited slightly greater capacity for mercuric chloride adsorption (Semu *et al.* 1986), resulting in less mercuric chloride in soil solution and, consequently, a less severe effect on microorganisms up to

200 mg Hg/kg soil. These trends are contrary to those observed by Duxbury (1981) whereby the decrease of bacterial numbers was very sharp. An explanation to this contrast may lie in the inherent differences between the soils, and in the composition of the microbial populations, of the present and Duxbury's study.

Non-symbiotic nitrogenase activity

In the Morogoro soil, Aretan decreased the nitrogenase activity of aerobically-incubated samples gradually up to 24 mg Hg/kg soil (Fig. 2). The reduction was sharp between 24 and 50 mg Hg/kg levels and remained low thereafter. The effect of

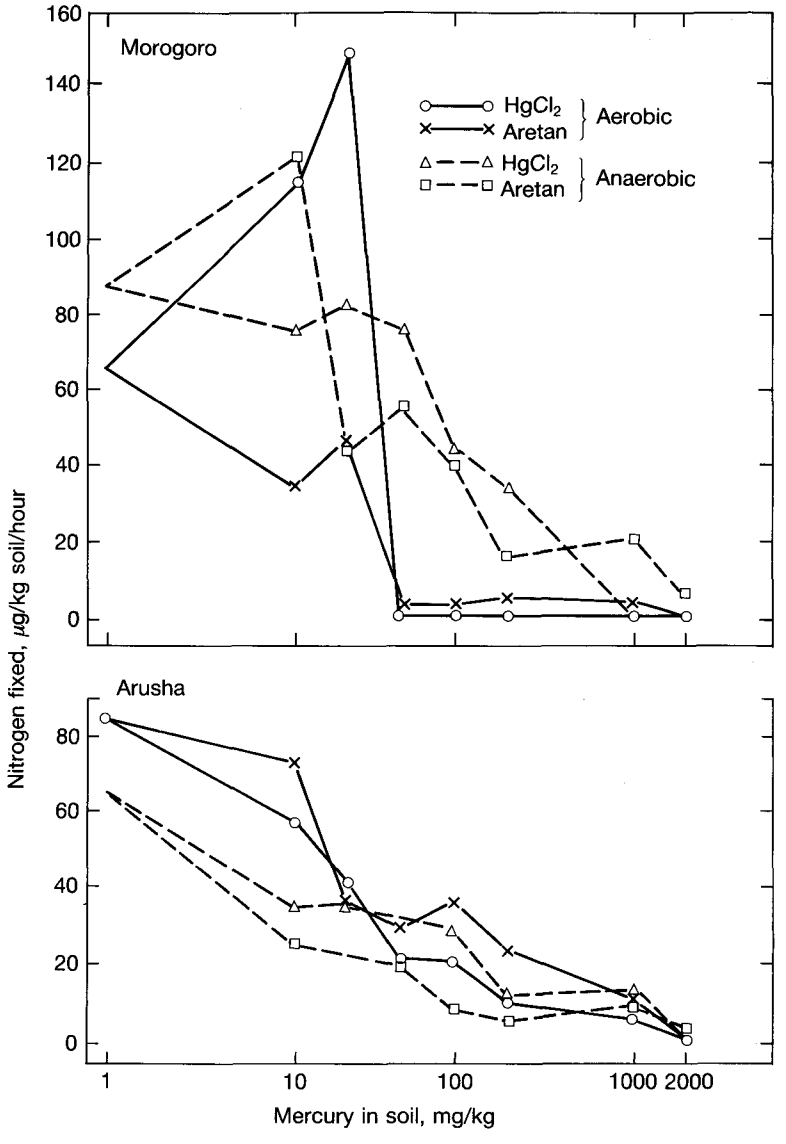

Fig. 2 Effect of mercury on non-symbiotic nitrogenase activity in Morogoro and Arusha soils.

mercuric chloride on nitrogenase activity was the opposite of that observed for Aretan. The activity increased up to 24 mg Hg/kg, showing a peak at this level, dropped sharply at 50 mg Hg/kg and remained very low thereafter. In the Arusha soil, nitrogenase activity in aerobically incubated soil was reduced gradually by increasing concentrations of both Aretan and mercuric chloride, with relatively sharper decreases at 24 and 50 mg Hg/kg, respectively. These trends of response were rather similar to those observed for the general population.

In the Morogoro soil, the response of nitrogenase activity under anaerobic incubation was the opposite of that under aerobic incubation in that Aretan increased the activity at lower Hg levels while mercuric chloride had little effect. Sharp decreases in activity occurred at 24 and 100 mg Hg/kg soil due to Aretan and mercuric chloride, respectively. The anaerobic activity in the Arusha soil decreased gradually over the entire range of added Hg.

The different responses in nitrogenase activity between the two soils, under aerobic incubation, may suggest that different microbial types may have been present in the two soils since the soils were of different pH values. The Morogoro soil was acidic while the Arusha soil was nearly neutral. The generally higher rates of nitrogenase activity during anaerobic incubation in the Morogoro compared with the Arusha soil may indicate that anaerobiosis was achieved better in the Morogoro soil. This is probable because with 43% clay (compared with only 5% in the Arusha soil) (see Table 1), the Morogoro soil also had greater water-holding capacity of 56% (compared with 32% for the Arusha soil). This may have enhanced the anaerobiosis conducive to proliferation of the anaerobic nitrogen fixers.

The decrease in nitrogenase activity with increasing Hg concentration in the soils of the present study may not be due to an effect of Hg on the nitrogenase enzyme alone. Cellular respiration, a process vital in the provision of energy and metabolites necessary for nitrogen fixation, is inhibited by Hg (van Faassen 1973; Cornfield 1977; Sinha *et al.* 1979; Spalding 1979) and this may also contribute to decreased nitrogen fixation.

It may be inferred from the results of the present study that relatively low amounts of Hg in soil, as could be introduced via fungicide use (Gowen *et al.* 1976), may not drastically reduce non-symbiotic nitrogen fixation in these soils. The contribution of this process to the overall nitrogen status of these soils is not known. Amounts of nitrogen of up to nearly 100 kg/ha.yr fixed non-symbiotically have been cited for various soils (Alexander 1977). No attempt is made to similarly translate the values obtained in the present study, and to compare them with literature values, because the present soils were amended with glucose prior to incubation.

It is clear (Fig. 2) that, generally, severe reductions in nitrogenase activity occurred at about 50 mg Hg/kg soil or lower. This is in contrast with the response of the general microbial population (Fig. 1) whereby severe effects of Hg were not observed below 100 mg Hg/kg soil. The ability of the general population to withstand relatively higher levels of Hg may be ascribed to its large diversity of species, which may differ in their susceptibility or tolerance to Hg (van Faassen 1973; Sinha *et al.* 1979). The non-symbiotic nitrogen fixing bacteria, on the other hand, comprise a relatively more restricted group of genera, bound to lack the wide variability of the general population. This low variability may make them relatively less resistant to high levels of Hg in soil.

Nitrification

The ability to oxidize ammonium-N to nitrate-N was nearly the same in the unamended soils, resulting in 21 and 24 mg nitrate-N/kg in the Morogoro and Arusha soils, respectively. In the Morogoro soil, nitrate production decreased with increasing levels of mercuric chloride, remaining very low at Hg rates above 10 mg/kg soil (Fig. 3). In the Arusha soil, the decrease was dramatic at 2 mg Hg/kg soil, and dropped further at levels above 10 mg Hg/kg soil. In both soils, as nitrification became inhibited, there was an accumulation of ammonium-N with increasing soil Hg, over and above the 50 mg N/kg previously added to the soils.

The almost similar capacities for nitrification in the controls of these soils suggest that either their populations of efficiencies of their nitrifiers were nearly the same.

The inhibition of nitrification observed after adding 1000 mg Hg/kg soil in the present investigation was comparable to that reported by Liang & Tabatabai (1977, 1978), using the same concentration of Hg in the soil. The results of the present study indicate, moreover, that inhibition of nitrification takes place even at substantially lower levels of Hg in soil, as was also observed by van Faassen (1973). It is probable that the impaired nitrification under the lower soil Hg levels in the present study (e.g. 2 to 10 mg Hg/kg soil) could recover upon prolonged incubation. Sinha *et al.* (1979) reported recovery of nitrification after 45 d of incubation with Aretan at 2.5 mg Hg/kg soil. Van Faassen (1973) observed increased nitrification in a clay soil treated with 10 mg Hg/kg as mercuric chloride or phenylmercury acetate relative to controls. This

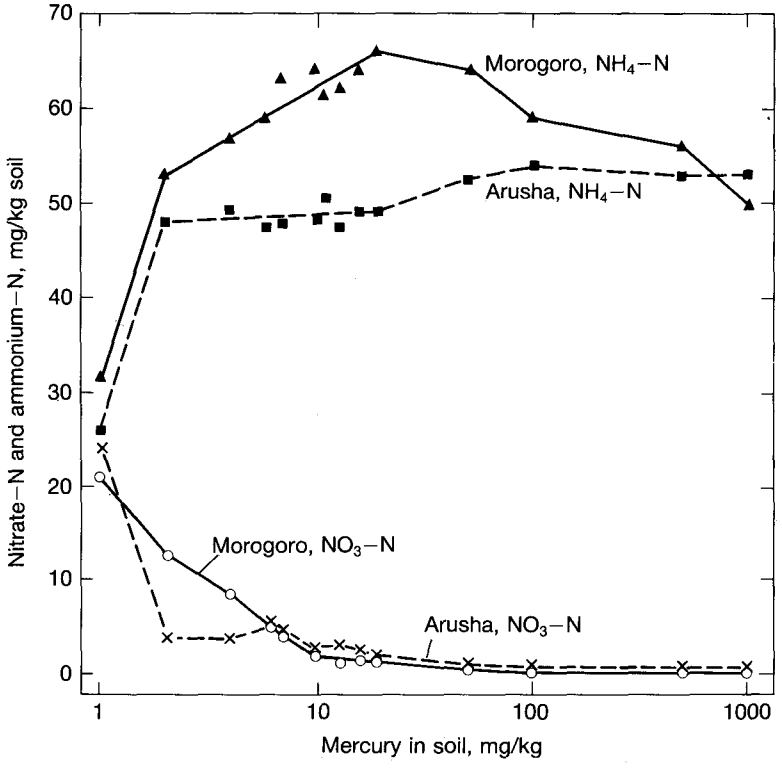

Fig. 3 Effect of mercuric chloride on nitrification in Morogoro and Arusha soils.

may indicate that the physico-chemical properties of a soil may influence the response of the nitrifying population to Hg.

The very low, but still detectable, concentrations of nitrate-N after incubation of soils with > 50 mg Hg/kg may suggest that the nitrifiers of these soils may have been largely eliminated by those high Hg levels. But heterotrophic nitrification (Quastel *et al.* 1950; Schmidt 1954) may probably account for such low rates of nitrate production, which may reflect background values to be expected in the absence of autotrophic nitrifiers.

The drastic effect of Hg on nitrification, at still lower soil Hg levels as compared with those which reduced the total population or nitrogenase activity, is consistent with the fact that nitrifiers are, comparatively, an even less diverse microbial group. This means that the nitrifiers have little variability, and hence will be even more susceptible to the toxicity effects of Hg.

The accumulation of ammonium-N with increasing soil Hg levels may suggest that ammonifiers exhibited some resistance to Hg. The comparatively lower ammonification rate in the Arusha soil may imply a small population of ammonifiers in this soil relative to the Morogoro soil, despite the fact that the two soils started with nearly equal total microbial populations of 10^7 c.f.u./g soil (Fig. 1) and contained similar quantities of organic carbon and total N (Table 1).

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Summary

The influence of mercury on microbial populations and activity of two soils from Tanzania was studied. Aretan (2-methoxyethylmercury chloride) slightly affected the microbial population of the Morogoro (Oxisol) soil, which was 10^7 c.f.u./g in control soil and 10^6 c.f.u./g in the presence of 2000 mg Hg/kg soil. Mercuric chloride at > 8 mg Hg/kg soil increased the population slightly, with a sharp decrease at > 100 mg Hg/kg soil, dropping ultimately to 10^3 c.f.u./g at 2000 mg Hg/kg soil. In the Arusha (Andept) soil, the microbial response to the two mercury compounds was the opposite of that for the Morogoro soil. Aretan sharply reduced the nitrogenase activity of aerobically incubated Morogoro soils at Hg levels > 24 mg/kg, resulting in very low activity at > 50 mg Hg/kg soil. Mercuric chloride increased the activity, which showed a peak at 24 mg Hg/kg soils, followed by a sharp drop at 30 mg Hg/kg and remained low thereafter. In the Arusha soil, the activity was reduced gradually by both Aretan and $HgCl_2$. The response of the activity under anaerobic incubation in the Morogoro soil was the opposite of that under aerobic incubation, in that it was Aretan which at first increased the activity. In the Arusha soil the activity under anaerobic incubation decreased gradually over the entire range of added Hg. Nitrification was decreased by $HgCl_2$ atlevels of < 2 and < 10 mg Hg/kg soil in the Arusha and Morogoro soils, respectively. The tolerance to Hg by microorganisms in this study was in the order: total population > nitrogen fixers > nitrifiers. This may be explained in terms of species diversity of the microorganisms, which may be expected to follow the same sequence.

Résumé

Population et activités microbiennes dans deux sols de Tanzanie sous l'influence du mercure

On étudie l'influence du mercure sur les populations et les activités microbiennes de deux sols en provenance de Tanzanie. L'Aretan (chlorure de 2-méthoxyéthylmercure) n'affecte que faiblement la population microbienne du sol de Morogoro (oxisol), qui compte 10^7 individus par g dans le sol témoin et 10^6 individus en présence de 2000 mg de mercure par kg de sol. Le chlorure mercurique, à une dose supérieure à 8 mg de mercure par kg de sol, augmente quelque peu la population. Celle-ci décroît brutalement au delà de 100 mg de mercure par kg de sol, pour tomber finalement à 10^3 individus par g à 2000 mg de mercure par kg de sol. Dans le sol d'Arusha (Andept), la réponse microbienne aux deux composés mercuriels est l'inverse de celle obtenue avec le sol de Morogoro. L'Aretan réduit fortement l'activité de la nitrogénase de sols de Morogoro incubés en aérobiose à des teneurs en mercure au delà de 24 mg par kg. L'activité devient très faible au delà de 50 mg de mercure par kg de sol. Le chlorure mercurique augmente cette activité, avec un pic de 24 mg de mercure par kg de sol, suivi d'une chute sévère à 30 mg de mercure par kg. L'activité demeure faible aux doses plus fortes. Dans le sol d'Arusha, l'activité est réduite progressivement tant par l'Aretan que par $HgCl_2$. La réponse de l'activité en

incubation anaérobie dans le sol de Morogoro est l'inverse de celle en incubation aérobie en ceci que c'est l'Aretan, cette fois-ci, qui augmente d'abord l'activité. Dans le sol d'Arusha, l'activité en incubation anaérobie décroît progressivement sur l'échelle entière des concentrations d'ajout de mercure. La nitrification est réduite par $HgCl_2$ à des seuils au dessous de 2 et 10 mg de mercure par kg de sol, respectivement pour les sols d'Arusha et de Morogoro. La tolérance des microorganismes au mercure dans cette étude est dans l'ordre: population totale > fixateurs d'azote > nitrificateurs. Ceci peut être expliqué en termes de diversité des espèces de microorganismes qui suit vraisemblablement la même séquence.