

**Trees protection measures during decontamination
process of mercury polluted soil**

First Interim Report

To

**Hindustan Unilever Limited
Kodaikanal**

By

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Assessment of risk potential and recommendations for the protection of trees & plants during excavation of surface soil up to 30 cm depth

Removal of top soil up to 30cm depth from the mercury polluted site of HUL, Kodaikanal particularly from the steep slope is quite dangerous for the growing trees as well as other associated vegetation. This needs to be looked carefully for the sustenance of many old trees (≈ 300) in the area of 2.3 acre of the contaminated site. Some of our preliminary investigations made so far reveal possible damage to the Rhizosphere ecology in terms of following losses:

Forest floor litter:

Organic matter of the forest floor litter serves a buffer stock for release of nutrients to the plants slowly, conserves soil moisture and checks soil runoff during rainwater flow or windstorms. It is decomposed and mineralized gradually forming ectoorganic layers (LFH) on the mineral soil in different state of decay (Table1). It has been estimated that about 5.9Mg/ha (Area A) to 12.4Mg/ha (Area B) organic carbon will be washed away during decontamination process (Fig1). It is, therefore, recommended to replenish the same from the nearby forest areas within a safe limit of $<25\%$ removal at one time.

Table 1: Litter mass on the ground floor

Sampling Site	Ground floor litter (Mg/ha)			Mean \pm LSD 01 0.42
	L layer	F Layer	H layer	
Area- A	0.66	2.11	3.12	1.96
Area- B	1.19	5.33	5.91	4.14
Area- C2	1.41	2.29	4.26	2.65
Area- C1	0.80	3.10	6.01	3.30
control	1.55	1.08	2.79	1.80
Mean \pm LSD 01 0.54	1.12	2.78	4.42	

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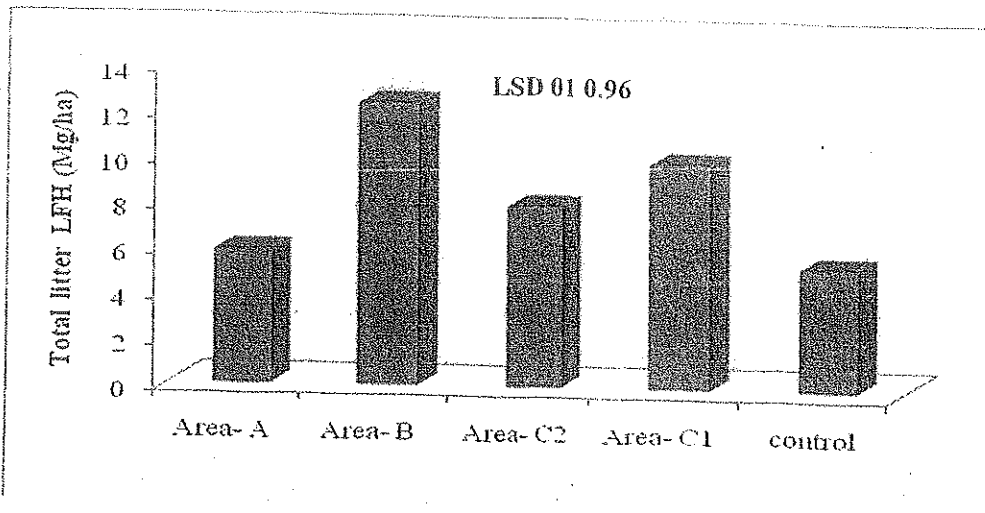


Fig 1 : Total ground floor litter (LFH) on different sites

Tree status and fine roots:

A tentative assessment of the biomass status of the standing trees and fine roots indicates the highest index for area C1 (Table 2). There is a great risk of falling of the trees during soil excavation process and hence, precautions are required to save the maximum intact roots on the site. Even then it could not be possible to safeguard the fine roots. Fine roots absorb water and minerals from the soil solution required to perform the various metabolic processes/physiological functions for the sustenance of plants. It has been estimated that about 7Mg/ha (Area A) to 24Mg/ha (Area C1) fine root mass will be cut out and removed from different contaminated area. However, fine roots are ephemeral (short lived) and it will be generated in due course of time between 3-6 months. It is estimated that nearly 60-70% of the fine roots remain confined to upper soil layer up to 30cm depth. So the trees will be under nourished till the formation of their fine root network and sustain their physiological function in a great stress. Some of them may become dry at wilting coefficient when the water absorption from the soil through fine roots fails to compensate the transpirational losses. It is, therefore, suggested to fill-up the dug- out soil from the uncontaminated site near by as soon as possible.

Table 2: Tree and fine root biomass at various sites

Sampling Site	Aboveground tree biomass (Mg/ha)	Fine root biomass (Mg/ha) <2mm diameter
Area- A	124	7
Area- B	269	14
Area- C2	99	10
Area- C1	321	24
Control	276	6
±LSD 0.01	10.09	6.19

Soil Nature:

Soil appears to be slightly acidic with low concentrations of soluble salts (Table 3,4). Efforts have to be made to ensure almost same level of pH and EC concentration in the refilled soil as it affects the microbial activity and nutrient availability in the soil.

Table 3: Soil pH

Mercury levels (µg/g)	pH		
	Depth 0-15 (cm)	Depth 15-30 (cm)	Mean± LSD 0.01
50-100	6.69	6.57	6.63
100-500	5.40	5.68	5.54
> 500	6.06	6.34	6.20
Mean± LSD 0.01 NS	6.05	6.20	

Table 4: Soil Electrical conductivity

Mercury levels (µg/g)	EC values (µS/cm)		
	Depth 0-15 (cm)	Depth 15-30 (cm)	Mean LSD 0.01 ± 17.93
50-100	87.2	60	73.6
100-500	100	72.2	86.1
> 500	171	140	155.5
Mean± LSD 0.01 21.95	119.40	90.73	

Soil organic matter:

Organic carbon in the soil is considered as one of the major parameters for the determination of soil fertility for the growth and development of plants. The soil appears to be very rich in % carbon between 100-500µg/g Hg level in lower depth (Table5). Since organic carbon will be removed to the extent of 60-120Mg/ha from the sites having > 500 to 100-500µg/g of mercury levels (Fig 2), it is recommended that the excavated sites should be refilled next day from the surface soil of nearby forest area by scraping up to 10cm depth only.

Table 5: Organic carbon

Mercury levels in ppm	Organic Carbon (%)		
	Depth 0-15cm	Depth 15-30cm	Mean ±LSD 0.01 0.305
50-100	2.24	3.23	2.74
100-500	3.69	4.42	4.06
> 500	1.53	2.50	2.02
Mean± LSD 0.01 0.43	2.49	3.39	

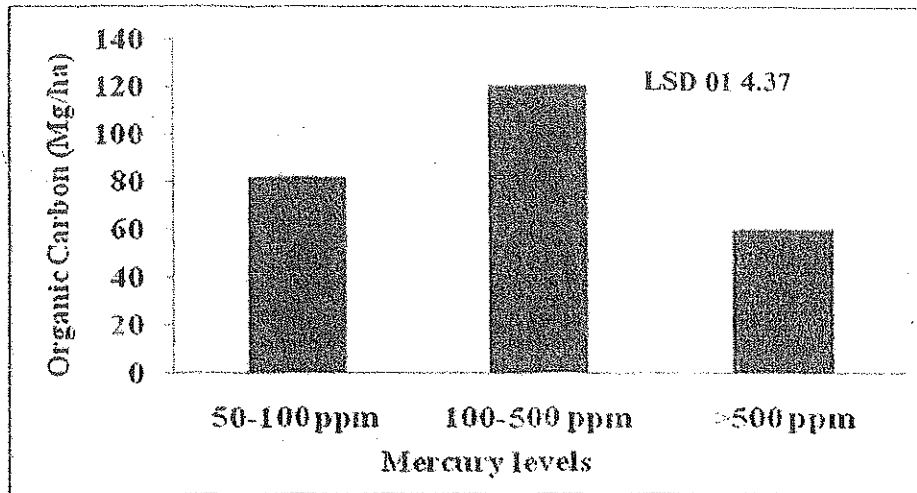


Fig 2: Loss of organic carbon per ha

Nitrogen availability:

Nitrogen is required in high amount during active growth period which is released by the mineralization of the soil organic matter. At a given time, available nitrogen fraction is directly proportional to organic carbon (Table 6). The entire fraction of the available nitrogen will be washed away during decontamination process. This loss is measured as 319 -578kg/ha from the sites of different Hg levels (Fig 3.) It is to be compensated in the same way as suggested for carbon.

Table 6: Concentration of available nitrogen

Mercury (µg/g)	Available Nitrogen (mg/kg)		Mean LSD 0.01= ± 16.68
	Depth 0-15	Depth 15-30	
50-100	142.68	165.76	154.22
100-500	176.96	208.32	192.64
> 500	122.72	90.04	106.38
Mean, LSD 0.01= NS	147.45	154.71	

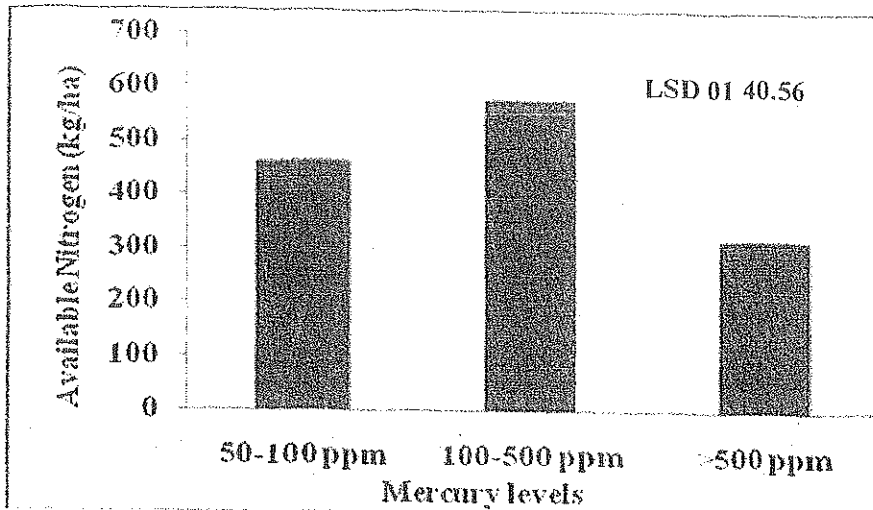


Fig 3: Loss of available nitrogen per ha

Other nutrients:

It is estimated that different magnitudes of Ca, Mg, K and P will be lost when the contaminated soil is treated for Hg removal and their replenishment in the soil may not be possible quickly to sustain the trees. So we must replace it by fertile soil as soon as possible in order to sustain the growing trees. Till now, we have estimated the levels phosphorus and potassium (Table 7, 8 and Fig 4,5)

Table 7: Concentration of available Potassium

Mercury ($\mu\text{g/g}$)	Available Potassium (mg/kg)		Mean LSD 0.01= ± 15.16
	Depth 0-15	Depth 15-30	
50-100	156.00	123.00	154.22
100-500	120.00	80.00	192.64
> 500	136.00	80.00	106.38
Mean, LSD 0.01= \pm 18.57	137.33	94.33	

Table 8: Concentration of available Phosphorus

Mercury (µg/g)	Available Phosphorus (mg/kg)		Mean LSD 0.01= ± 10.21
	Depth 0-15	Depth 15-30	
50-100	55	30	42.65
100-500	45	30	37.52
> 500	9	7	8.25
Mean, LSD 0.01= ± 12.51	36.54	22.41	

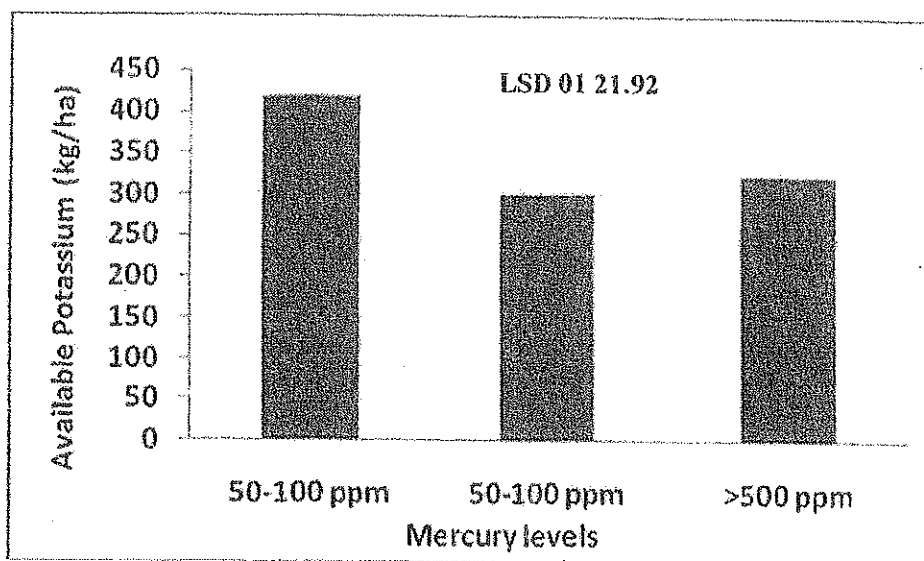


Fig 4: Loss of available potassium per ha

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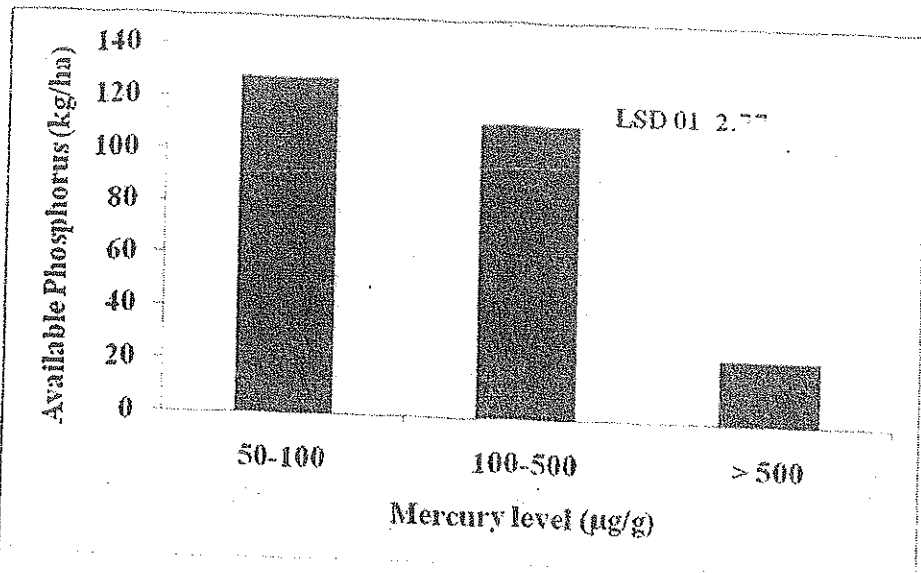


Fig 5: Loss of available Phosphorus per ha

Microbial Biomass Carbon:

It is an index to examine the biological health status of the soil, which varied from 113 to 534µg/g and closely relates the soil organic carbon with a greatest amount in the soils having 100-500 Hg level (Table 9). It has been estimated that microbial biomass carbon will be washed away to the extent of 738 to 1383kg/ha from the soils of different Hg levels of contamination and it will take much time to build up this level (Fig6). Therefore, suitable cultures, isolated from the nearby forest soils, need to be inoculated throughout the dug-out and refilled mineral soil.

Table 9: Microbial biomass carbon

Mercury (µg/g)	Microbial Carbon (µg/g)		Mean LSD 0.01= ± 28.39
	Depth 0-15	Depth 15-30	
50-100	211.00	413.40	154.22
100-500	265.00	533.60	192.64
> 500	113.20	253.20	106.38
Mean, LSD 0.01=± 34.77	196.40	400.07	

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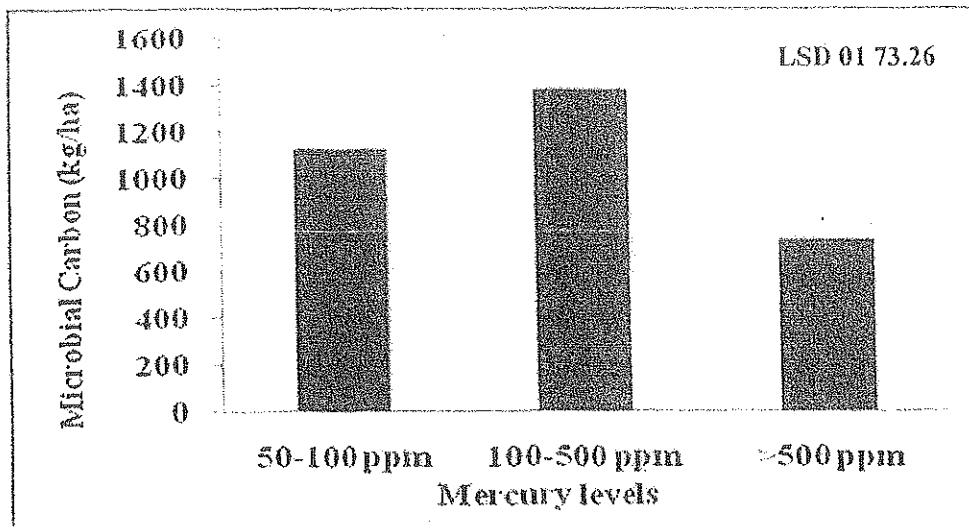


Fig 6: Loss of Microbial Biomass Carbon per ha

Microorganisms:

The entire population and diversity of microorganisms will disappear during treatment and their restoration is not quite easy unless it is well inoculated and incubated with the VAM fungi, nitrifying and phosphate solubilising bacteria. The rhizosphere is the soil zone in the immediate vicinity of a root system. Arbuscular mycorrhizal symbiosis affects the community and diversity of other organisms in the soil. Mycorrhizae diversity has been reported to increase plant species diversity as the potential number of associations increases. Dominant arbuscular mycorrhizal fungi can prevent the invasion of non-mycorrhizal plants on land where they have established symbiosis and promote their mycorrhizal host.

Recent research has shown that AM fungi release an unidentified diffusional factor, known as the **myc factor**, which activates the nodulation factor's inducible gene *mtENOD11*. This is the same gene involved in establishing symbiosis with the nitrogen fixing, rhizobial bacteria. When *Rhizobium* bacteria are present in the soil, mycorrhizal colonization is increased due to an increase in the concentration of chemical signals involved in the establishment of symbiosis. Effective mycorrhizal colonization can also increase the nodulations and symbiotic nitrogen fixation in mycorrhizal legumes. The extent of arbuscular mycorrhizal colonization and species affects the bacterial population in the rhizosphere. Bacterial species differ in their abilities to compete for carbon compound root exudates. A change in the amount or composition of root exudates and fungal exudates due to the existing

AM mycorrhizal colonization determines the diversity and abundance of the bacterial community in the rhizosphere. The influence of AM fungi on plant root and shoot growth may also have indirect effect on the rhizosphere bacteria. AMF contributes a substantial amount of carbon to the rhizosphere through the growth and degeneration of the hyphal network. There is also evidence that AM fungi may play an important role on mediating the plant species' specific effect on the bacterial composition of the rhizosphere.

Arbuscular mycorrhizae (AMs) are characterized by the formation of unique structures such as arbuscules and vesicles by fungi of the phylum Glomeromycota (AM fungi). AM fungi (AMF) help plants to capture nutrients such as phosphorus and micronutrients from the soil. It is believed that the development of the arbuscular mycorrhizal symbiosis played a crucial role in the initial colonisation of land by plants and in the evolution of the vascular plants. Arbuscular mycorrhizal fungi are most frequent in plants growing on mineral soils. The populations of AM fungi is greatest in plant communities with high diversity such as tropical rainforests where they have many potential host plants and can take advantage of their ability to colonize a broad host range. Kodaicanal lies in the broad zone of tropical rainforest.

It has been said that it is quicker to list the plants that do not form mycorrhizae than those that do. This symbiosis is a highly evolved mutualistic relationship found between fungi and plants, the most prevalent plant symbiosis known and AM is found in 80% of vascular plant families of today. The tremendous advances in research on mycorrhizal physiology and ecology over the past 40 years have led to a greater understanding of the multiple roles of AMF in the ecosystem. This knowledge is applicable to human endeavours of ecosystem management, ecosystem restoration and agriculture. The use of arbuscular mycorrhizal fungi in ecological restoration projects has been shown to enable host plant establishment on degraded soil and improve soil quality and health (phytoremediation).

Disturbance of herbaceous plant communities and plant propagules by excavating the soil from the contaminated areas will lead to degradation of physical and biological soil properties, soil structure, nutrient availability and organic matter. While reclaiming the polluted land, it is essential to restore the ground layer vegetation which also works as soil binders and controls run off and help to rejuvenate the biological and physical soil properties.

It is, therefore, suggested to inoculate the soil with arbuscular mycorrhizal fungi with the reintroduction of ground vegetation. A long term study demonstrated that a significantly greater long term improvement in soils' quality parameters was attained when the soil was inoculated with a mixture of indigenous arbuscular mycorrhizal fungi species compared to the non inoculated soil and soil inoculated with a single exotic species of AM fungi. The benefits observed were an increased plant growth and soil nitrogen content, higher soil organic matter content and soil aggregation. The improvements were attributed to the higher legume nodulation in the presence of AMF, better water infiltration and soil aeration due to soil aggregation. Inoculation with native AM fungi increases plant uptake of phosphorus, improves plant growth and health and it is a biological tool in the restoration of biotypes to self-sustaining ecosystems.

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