

## **Heavy Metals Accumulation in Soil and Mango Leaf and Their Effects on Soil Microbial Population along Road Sides in Southwest, Nigeria**

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**Abstract:** This study examined microbial population (MP) and heavy metal load in the soil and mango leaf, along Ogbomoso-Oyo road (high traffic density) and Ogbomoso-Ifeodan (low traffic density). Mango leaves (about 3m from the ground) were sampled at three spots (10km intervals) and three distances of 0-10, 10-20 and 20-30m from the edge of the road. Soil was sampled at 0-15 and 15-30cm depth under mango trees. The samples were analyzed for Pb, Cd, Cr, Zn with atomic absorption spectrophotometer and total MP using nutrient media preparations. On the High Traffic Density (HTD) road, 20-30m distance, the soil contained higher amount of Lead ( $7.71 \pm 0.76$ ), Cadmium ( $8.36 \pm 0.59$ ), Chromium ( $0.48 \pm 0.02$ ) and Zinc ( $5.58 \pm 0.37$  mg/kg). MP was higher at 0-10m with Total microbial count of  $10.5 \pm 1.46 \times 10^5$  Cfu $g^{-1}$  and Total fungi count of  $0.83 \pm 0.23 \times 10^5$  Cfu $g^{-1}$ . Microbial population was higher along LTD than HTD road. Mango leaf contained significantly higher metal content along HTD road Pb (4.31), Cd (4.63), Cr (0.03) and Zn (5.04mg/kg) than LTD road. Microbial population was higher at LTD than HTD, and Mango accumulated higher heavy metals at HTD than LTD. Implications of heavy metals and microbial populations on food chain were discussed.

**Keywords:** Heavy metal, microbial population, Mango, Nutrient media, traffic densities,

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### **I. Introduction**

Pollution of the biosphere by heavy metals due to industrial, agriculture, domestic and social activities has created a serious problem for the safe and rational utilization of soils [1,2,3,4,5]. Motor vehicle emission is one of the leading causes of air pollution. Exhaust emission and burning of fossil fuel were identified as primary sources of atmospheric metallic burden [6]. Man, animals, vegetation and soil act as ‘Sinks’ for atmospheric pollutants [7,8].

In general, an increase in metal concentration adversely affects soil microbial properties such as respiration rate, enzyme activities, which appears to be very useful indicators of soil pollution [9,10]. Heavy metals exert toxic effects on soil microorganisms [11], hence, results in the change of diversity, population size and overall activities of the soil microbial communities [12]. There is increasing public concern about health effects resulting from ingestion of food containing toxic metals such as cadmium and lead. A wide range of metabolic disorders and neuropsychological deficits in children have been noted, and chronic exposure to cadmium has been linked to kidney failure and bone disease.

Plants have a higher capacity to take up metals from soils or atmosphere than their physiological needs. While this may not have any adverse effect on plant itself, it may expose the consumer to higher intake of concerned metals. Since plants are the major source of human food, a study of metal-content in plants and crops indicates the extent of heavy metal contamination in human beings by consuming them [13]. It has been reported that nearly half of the mean ingestion of lead, cadmium and mercury through food is due to plant origin (fruit, vegetables and cereals). Several studies revealed that 60-80% of heavy metal toxins found in urban areas were the results of consuming contaminated foods rather than air pollution [14]. Nigerian roads are characterized by farm sites with all types of crops on the edge of the roads. This study examined the effects of traffic densities on lead (Pb), Cadmium (Cd), Chromium (Cr) and Zinc (Zn) concentrations in soil and mango leaf, and the affects of these metals on microbial population along two roads in Ogbomoso.

### **II. Materials and methods**

The study was conducted in Ogbomoso (Longitude  $4^{\circ} 10'E$ , Latitude  $8^{\circ} 10' N$  and altitude 213 m asl) Oyo state, Nigeria in July, 2010. The main socio-economic activities along the roads are farming and trading farm produce. One major and minor road were chosen for the study, Ogbomoso-Oyo as high traffic density (HTD) and Ogbomoso-Ife Odan road as low traffic density (LTD) in Oyo state.

## **2.1 Collection and preparation of samples**

Soil (0-15 and 15-30cm depth under mango trees) and mango leaf (about 3m from the ground) samples were collected from the road edge along three transects inward within 0-10, 10-20 and 20-30m away from the road sides. The samplings were replicated thrice along the roads at intervals of 10km. Ten core soil samples randomly distributed round the observation points were taken with soil auger. The soil and mango leaf samples were analyzed for lead (Pb), Cadmium (Cd), Chromium (Cr), Zinc (Zn) and soil pH and Total microbial population. Sample analyses were carried out by spectrophotometer MILTON ROY 2ID.

The soil samples were air dried and sieved with 2mm sieve. The air dried soil sample was weighed for wet digestion. The samples were oven-dried at 70° C to constant weight, ground inside a hammer mill incorporated with 2mm sieve. 2g of the ground samples was then put in crucible and ashed inside furnace at 580°C. The ash was washed into 100ml volumetric flask and wet digested with a mixture of 1:1 perchloric acid and nitric acid. The digested samples were then read from a MILTON ROY 2ID Atomic Absorption Spectrophotometer (AAS) using their respective lamp and wavelengths. Calculation was done using: Meter Reading x slope x Dilution Factor [15].

Standard methods were used to prepare nutrient agar (NA) and potato dextrose agar (PDA) for estimation of microbial population. One gramme each of the soil samples were measured into the test tube containing 9ml sterile distilled water and serially diluted to dilution factor ( $10^{-5}$ ) and 1ml of the last dilution was put into sterile plate which were incubated at 37°C for NA and PDA incubated at 28°C-30°C. All plates were incubated inverted wise. The plates were counted at 48hrs for NA and 72 hrs for PDA.

## **III. Results**

The pH of the soil samples from the sites ranges from 6.40 to 6.70 for low traffic density road and 6.60 to 6.90 for high traffic density road and are said to be slightly acidic.

The results of heavy metals extracted from the soil under mango trees sampled were presented in the Table 1 below. Only zinc was significantly higher at high traffic density (HTD) (5.33mg/kg) than low traffic density (LTD) (4.06mg/kg) road. The total microbial count and the total fungi count were not significantly different at both traffic situations ( $P=0.05$ ) (Table 1).

**Table 1** Heavy metal concentrations in soil and microbial population along roadsides with different traffic densities

Traffic	METALS (mg/kg)				Microbial counts (Cfug <sup>-1</sup> (x10 <sup>5</sup> ))	
	Pb	Cd	Cr	Zn	Tmc	Tfc
High	6.71a	7.87a	0.46a	5.33a	9.48a	0.74a
Low	6.95a	7.84a	0.39a	4.06b	9.67a	0.64a

Numbers in column followed by the same letters are not significantly different.

**Tmc** = Total microbial count

**Tfc** = Total fungi count      **Cfug<sup>-1</sup>** = Colony forming unit per gramme

Generally, all the metals (Pb, Cd, Cr, and Zn) were significantly higher at 20-30m distance from the road edge than 10-20m and 0-10m (Table 2). The total microbial count and total fungal count were higher near the road (0-10m) than far away (20-30m) from the road. ( $P=5\%$ ) (Table 2).

**Table 2** Heavy metal concentrations in soil and microbial population under mango trees along roadsides with different distances from the road

Distance	METALS (mg/kg)				Microbial counts		
	Pb	Cd	Cr	Zn	Tmc	Cfug <sup>-1</sup> (x10 <sup>5</sup> )	TfcCfug <sup>-1</sup> (x10 <sup>5</sup> )
0-10m	5.98c	7.25c	0.43ab	4.22b		10.78a	0.88a
10-20m	6.73b	7.89b	0.41ab	4.90a		10.52a	0.68b
20-30m	7.78a	8.42a	0.44b	4.97a		7.42b	0.50c

Numbers in column followed by the same letters are not significantly different.

**Tmc** = Total microbial count      **Tfc** = Total fungi count

**Cfug<sup>-1</sup>** = Colony forming unit per gramme

The microbial populations were higher at 0-15cm than 15-30cm depth (Table 3). Soils from 15-30cm depth under mango trees contained significantly higher Pb, Cd, and Zn than 0-15cm (Table 3).

**Table 3** Heavy metal concentrations soil and microbial population under mango tree along roadsides with different soil depths

Soil depth	METAL (mg/kg)			Microbial counts			
	Pb	Cd	Cr	Zn	Tmc (x10 <sup>5</sup> ) Cfug <sup>-1</sup>	Tfc (x10 <sup>5</sup> ) Cfug <sup>-1</sup>	
0-15cm	6.55b	7.66b	0.43a	4.61b	10.89a	0.82a	
15-30cm	7.11a	8.05a	0.43b	4.78a	8.26b	0.56b	

Numbers in column followed by the same letters are not significantly different.

**Tmc** = Total microbial count

**Tfc** = Total fungi count

**Cfug<sup>-1</sup>** = Colony forming unit per gramme

Mango leaves at HTD contained significantly more lead, cadmium, and zinc while chromium was higher at LTD (Table 4).

**Table 4** Heavy metal concentrations in mango leaf along roadsides with different Traffic densities.

Traffic	Metal (mg/kg)			
	Pb	Cd	Cr	Zn
High	4.31a	4.63a	0.03b	5.04a
Low	3.24b	3.76b	0.07a	3.91b

Values in the column followed by the same letters are not significantly different.

Generally, there were no significant differences in heavy metal content of mango leaves considering the distance from the edge of the road (Table 5).

**Table 5** Heavy metal concentrations in mango leaf along roadsides with different distances from the edge of the road

Distances	Metal (mg/kg)			
	Pb	Cd	Cr	Zn
0-10m	4.01a	4.40a	0.06a	4.53a
10-20m	3.58a	4.00a	0.05ab	4.63a
20-30m	3.74a	4.19a	0.04b	4.29a

Numbers in column followed by the same letters are not significantly different.

The average metal concentration of mango leaf (mg/kg) at different distances from the road is shown in Table 6. There is inconsistency in the concentration of metals in mango leaves in relation with distance from the edge of the roads.

**Table 6** Average metal concentration in mango leaf along roadsides with different distances from the road

Level of traffic And distance	Metal ( $\pm SD$ ) (mg/kg)			
	Pb	Cd	Cr	Zn
Ha	4.59 $\pm$ 0.02	4.51 $\pm$ 0.20	0.03 $\pm$ 0.00	5.15 $\pm$ 0.55
Hb	4.00 $\pm$ 0.70	4.30 $\pm$ 0.60	0.03 $\pm$ 0.00	5.17 $\pm$ 1.07
Hc	4.32 $\pm$ 0.42	4.69 $\pm$ 0.34	0.02 $\pm$ 0.00	4.80 $\pm$ 0.51
La	3.42 $\pm$ 0.52	3.00 $\pm$ 0.50	0.10 $\pm$ 0.03	3.90 $\pm$ 0.20
Lb	3.15 $\pm$ 0.45	3.70 $\pm$ 0.20	0.07 $\pm$ 0.01	4.00 $\pm$ 0.55
Lc	3.16 $\pm$ 0.51	3.69 $\pm$ 0.27	0.05 $\pm$ 0.00	3.78 $\pm$ 0.22

Key: **H** = high traffic

**L** = low traffic      **a** = 0-10m

**b** = 10-20m **c** = 20-30m

At HTD road, 20-30m from the edge of the road, the soil contained higher amount of lead (7.71 $\pm$  0.76mg/kg), Cd (8.36 $\pm$ 0.59), Cr (0.48 $\pm$ 0.02) and Zn (5.58 $\pm$ 0.37mg/kg) than the region closer (Table 7). Table 7 showed the microbial content along the two roads with the distances from the edge of the road. The microbial population showed the reverse order with higher population recorded near the road (0-10m) with total microbial count of 10.50 $\pm$ 1.46 $\times$ 10<sup>5</sup> Cfug<sup>-1</sup> and total fungal count of 0.83 $\pm$ 0.23 $\times$ 10<sup>5</sup> Cfug<sup>-1</sup>. The same trend was observed at LTD. However, microbial population was slightly higher along the low traffic than the high traffic road.

**Table 7.** Average metal concentration of soil and microbial population under mango tree at different distances from the road

Level of Traffic And distance	Metals ( $\pm SD$ ) (mg/kg)				Tmc	Tfc
	Pb	Cd	Cr	Zn	Cfug <sup>-1</sup> (x10 <sup>5</sup> )	Cfug <sup>-1</sup> (x10 <sup>5</sup> )
Ha	5.58 $\pm$ 0.48	6.95 $\pm$ 0.54	0.45 $\pm$ 0.12	4.73 $\pm$ 0.32	10.50 $\pm$ 1.46	0.83 $\pm$ 0.23
Hb	6.91 $\pm$ 0.40	8.29 $\pm$ 0.67	0.44 $\pm$ 0.05	5.69 $\pm$ 0.51	10.29 $\pm$ 2.02	0.85 $\pm$ 0.26
Hc	7.71 $\pm$ 0.76	8.36 $\pm$ 0.59	0.48 $\pm$ 0.02	5.58 $\pm$ 0.37	7.64 $\pm$ 0.97	0.53 $\pm$ 0.10
La <sub>6</sub>	6.46 $\pm$ 0.82	7.55 $\pm$ 0.71	0.40 $\pm$ 0.10	3.71 $\pm$ 0.43	11.07 $\pm$ 3.07	0.93 $\pm$ 0.52
Lb	6.55 $\pm$ 1.06	7.48 $\pm$ 1.09	0.39 $\pm$ 0.08	4.10 $\pm$ 0.47	10.75 $\pm$ 2.59	0.52 $\pm$ 0.08
Lc	7.85 $\pm$ 0.47	8.47 $\pm$ 0.47	0.40 $\pm$ 0.04	4.37 $\pm$ 0.36	7.21 $\pm$ 1.01	0.47 $\pm$ 0.08

Key: **H** = high traffic      **Tmc** = Total viable count

**L** = low traffic

**Tfc** = Total fungi count

**a** = 0-10m

**Cfug<sup>-1</sup>** = Colony forming unit per gramme

**b** = 10-20m

**SD** = Standard Deviation.

At 0-15cm depth along the HTD road, all the metals were lower in quantity than at 15-30cm depth. The only exception was the chromium recorded at 10-20m along the road where higher Cr (0.46 $\pm$ 0.07mg/kg) was recorded, and 20-30m with higher Zn (5.84 $\pm$ 0.05mg/kg). The same trend was observed at LTD at 10-20m distance from the road edge for Pb, Cd, and Zn. Chromium recorded higher amount, 0.42 $\pm$ 0.11 and 0.39 $\pm$ 0.05mg/kg than 0.38 $\pm$ 0.11 and 0.38 $\pm$ 0.02mg/kg respectively for 0-15cm depth and 15-30cm at 0-10 and 10-20m distance from the road. At 20-30m distance from the edge of the LTD road, 0-15cm depth recorded higher metal content than 15-30cm for Pb, Cd, Cr and Zn (Table 8). Table 8 showed the microbial population in the soil according to depth of sampling along the two roads. Total microbial count and total fungi count of the microbial population were higher at 0-15cm (7.47 $\pm$ 1.21 $\times$ 10<sup>5</sup> Cfug<sup>-1</sup>) than 15-30cm (6.94 $\pm$ 0.95 $\times$ 10<sup>5</sup> Cfug<sup>-1</sup>) depth throughout the experimental sites and the depth of soil sampling. Microbial populations were a little higher at low traffic density than high traffic density road.

**Table 8.** Average metal concentrations in soil and microbial population at different depths under mango tree along roadsides with different distances from the road.

Metals ( $\pm SD$ ) (mg/kg) Microbial count

Distance and soil depth	Pb	Cd	Cr	Zn	Tmc Cfug <sup>-1</sup> (x10 <sup>5</sup> )	Tfc Cfug <sup>-1</sup> (x10 <sup>5</sup> )
Had <sub>1</sub>	5.10 $\pm$ 0.26	6.60 $\pm$ 0.10	0.44 $\pm$ 0.14	4.55 $\pm$ 0.35	11.63 $\pm$ 1.20	1.00 $\pm$ 0.20
Had <sub>2</sub>	5.90 $\pm$ 0.18	7.30 $\pm$ 0.60	0.48 $\pm$ 0.13	4.90 $\pm$ 0.20	9.37 $\pm$ 0.25	0.67 $\pm$ 0.06
Hbd <sub>1</sub>	6.56 $\pm$ 0.15	7.93 $\pm$ 0.65	0.46 $\pm$ 0.07	5.35 $\pm$ 0.35	11.53 $\pm$ 2.10	1.00 $\pm$ 0.30
Hbd <sub>2</sub>	7.27 $\pm$ 0.05	8.65 $\pm$ 0.55	0.43 $\pm$ 0.02	6.03 $\pm$ 0.40	9.04 $\pm$ 1.05	0.70 $\pm$ 0.10
Hcd <sub>1</sub>	7.25 $\pm$ 0.85	8.20 $\pm$ 0.80	0.46 $\pm$ 0.01	5.84 $\pm$ 0.05	8.14 $\pm$ 1.25	0.60 $\pm$ 0.10
Hcd <sub>2</sub>	8.17 $\pm$ 0.31	8.51 $\pm$ 0.40	0.50 $\pm$ 0.01	5.32 $\pm$ 0.30	7.13 $\pm$ 0.12	0.47 $\pm$ 0.06
Lad <sub>1</sub>	5.90 $\pm$ 0.80	7.05 $\pm$ 0.55	0.42 $\pm$ 0.11	3.57 $\pm$ 0.46	13.8 $\pm$ 0.80	1.39 $\pm$ 0.02
Lad <sub>2</sub>	7 $\pm$ 0.36	8.05 $\pm$ 0.45				

			0.38±0.11	3.85±0.45	8.37±1.04	0.47±0.15
Lbd <sub>1</sub>	6.45±1.65	7.50±1.70	0.39±0.05	4±0.51	12.80±1.51	0.47±0.06
Lbd <sub>2</sub>	6.65±0.25	7.46±0.23	0.38±0.02	4.20±0.50	8.70±1.35	0.57±0.06
Lcd <sub>1</sub>	8.05±0.65	8.65±0.65	0.41±0.06	4.36±0.44	7.47±1.21	0.47±0.12
Lcd <sub>2</sub>	7.05±0.05	.30±0.20	0.38±0.02	4.37±0.37	6.94±0.95	0.47±0.06

Key: **H** = high traffic      **Tmc** = Total viable count

**L** = low traffic      **Tfc** = Total fungi count

**a** = 0-10m    **Cfug<sup>-1</sup>** = Colony forming unit per gramme

**b** = 10-20m    **d<sub>1</sub>** = 0-15cm    **d<sub>2</sub>** = 15-30cm

**c** = 20-30cm SD = Standard Deviation

#### IV. Discussion

The results indicated that mango leaves contained the investigated heavy metals Pb, Cd, Cr and Zn. Jaya [16] reported that the concentration of heavy metals, lead and cadmium in raw mangoes (fruits) were beyond the permissible levels given by WHO [17] for human consumption. The soil content of the metals was higher than that in the mango leaves along both roads. Microbial populations were also affected adversely as indicated by the lower microbial population recorded where higher metal concentrations abound. Nwuche [18] reported additive or synergistic effects of metals on soil microbes in that the effect of Zn alone was not significant on the activities of soil microbes but in combination with copper, it engenders a more toxicological effect than even copper itself.

The change in the metal concentrations with distance from the edge of the road was not consistent as reported by different authors. These results agreed with that of Voegborlo [19] who reported clearly defined gradients of lead contamination beside roadways, and less-defined gradients of other metals including cadmium, chromium, copper, nickel, vanadium, and zinc. Variations in the concentration of heavy metals in soil profiles are normally not consistent and readily interpreted because a number of factors can influence the level of metals in a soil profile and these include nature of parent material, the organic matter content environmental factors and chemical properties of the soil as well as the metal solubility. Soil pH greatly influences the solubility, availability and toxicity of metal elements in the soil [20]. It has been reported that a neutral soil may contain high levels of Mn, Al or Pb without any sign of toxicity to microorganisms whereas toxicity may develop with certain organisms at much lower metal concentrations in acid soils [21]. Thus, if appropriate pH for maximum dissolution of a metal is not in place, the toxicity of such metal would be seriously hindered, if not entirely stopped. In heavy-metal-polluted soils, plant growth can be inhibited by metal absorption. However, some plant species are able to accumulate fairly large amounts of heavy metals without showing stress which represents a potential health risk for animals and humans [23].

Toxic effects of heavy metals on micro-organisms manifest in numerous ways such as decrease in litter decomposition and nitrogen fixation, less efficient nutrient cycling [24], impaired enzyme synthesis and activity in soil, sediments and water. It has been reported that the rate of mineralization of acid forest soils decreased inversely with the log of the heavy metal concentration [20, 21]. Synergism was reported by [25,26] as responsible for the inhibition of soil-litter respiration by a combination of Se and Cd. Chander [27]) also reported that Cu and Zn in combination caused larger decreased in soil microbial biomass compared to the sum of either metals alone. These may eventually result in yield depression. Heavy metal uptake by crops growing in contaminated soil such as road side soil is a potential hazard to human health because of transmission in the food chain [28, 29, 30].

It has been reported that prolonged consumption of sub-lethal concentrations of heavy metals through food may lead to their chronic accumulation which hinder proper functioning of the kidney and liver of humans thereby causing disruption of numerous biochemical processes, leading to cardiovascular, nervous, kidney and bone diseases [31, 32, 33, 34, ]. Zhang [35] findings indicate that trees growing linearly along roadways can effectively reduce the heavy metals' concentrations

Continuous deposition of these heavy metals indicates potential health risk for human through the food chain.

Chronic exposure to metals at high enough level to cause chronic toxicity effects (such as hypertension in individuals exposed to lead, and renal toxicity in individuals exposed to cadmium) can occur without symptoms. Several studies have revealed that 60-80% of heavy metal toxins found in human bodies in urban areas were due to consumption of contaminated foods rather than air pollution [14]. The European Union ranged Cd in soil of 0-1mg/kg indicated non-contaminated soil, 1-3mg/kg indicated slight contamination and 3-10mg/kg indicated a contaminated soil. Thus, both the high and low traffic density roads assessed in this study were contaminated in terms of cadmium. FAO/WHO recommended maximum tolerable intake of Cd of 0.4-0.5mg/kg per week or equal to 0.07mg per day. The Korean women were estimated in 2007 to take 14.82mg Cd/day of which 9.74 $\mu$ g Cd/day was from plant foods and 5.09 $\mu$ g Cd/day was from animal source. The daily cadmium intake of plant origin accounted for 65.7% [36].

The results of this study when compared with the WHO/FAO standard lower limits of Pb (0.3mg/kg), Cd (0.2mg/kg) and Cr (0.05mg/kg) indicated that both the soil and mango trees along both roads were contaminated with heavy metals. The health implications of this are enormous and there is the need to educate policy makers, scientists and general public about toxicity of heavy metals. Agricultural activities should be discouraged within 30meter from road edge so also is animal grazing if heavy metal exposure routes are to be reduced to the barest minimum.

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