

Urban Gardening and Open Crown Trees: Insiduous Lethal Implications on Human and Animal Health

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Abstract

Urban agriculture is gradually gaining acceptability in most parts of the world. The act of urban gardening is food and vegetable production in urban setting. The environmental and social positive effects of open crown trees as well as vegetables of various varieties to urban dwellers cannot be trivialized. This is more importantly so considering the current threat of climate change to man and animals through microclimatic element reverses. The use to which these agricultural products so produced in urban areas are put is diverse. From their use as vegetables to their use as herbal products as well as to their use as chewing sticks. Urban arable crops are generally consumed whole while parts of open crown trees serve as chewing sticks and medicinal concoctions. Combined, vegetables and open crown trees regulate the amount of sunlight intensity that reaches man and animals residing in urban areas thereby regulating heat energy.In urbanization however, serious inherent danger is unconsciously being exposed to. Lethal bye –products are emitted through the use of both degradable and non-degradable materials. Since the ecosystem is a complex self-sustaining system, much of the lethal bye-products eventually find their ways back into living tissues via urban plants which are finally consumed by man and animals. In heavy traffic roads which are highly correlated with urban areas, the concentration of lead (Pb) for example which is a heavy metal and very lethal to man and animals ranges between 0.18 mg/kg and 0.38 mg/kg while the concentration of leaf acidity in polluted *Azadirachta indica* compared to unpolluted plant stands at a ratio of 1:10. Similarly, heavy metal concentration in soil and the population of microorganisms are generally inversely related. Whereas some microorganisms are needed for the crops well-being and for seed production, the alteration of environmental stability by heavy metals are rife.Conclusively, there is more work to be done in environmental microclimatic cleansing if urban gardening and open crown trees will serve the plus purpose for which they are preferred.

1. Introduction

Trees that grow in open spaces devoid of crown closure with other trees are referred to as Open Crown trees. The crown of a tree is defined as the upper part of a tree which includes the branches and leaves (Mc Graw- Hill 2003). Open crown trees are useful for shade production in places like Mechanic workshops, farmlands, front and sides of houses, front of companies, along road sides et cetra.

These trees and some other vegetation including crops are common on either living or abandoned dumpsites in towns and villages, even along roadsides. These plant forms are always with contaminants depending on the standard of living of the populace residing around the dumpsites which determines the composition of the refuse. Soil organisms, from microbes to macroorganisms suh as earthworms are also victims of the effects of the toxicants. Effluents from such toxic soils find their ways into surface and/or underground water thereby contaminating urban and village water sources. Still further, the major available refuse treatment predominant in Nigeria is burning *in situ* in which the ambient air get polluted with toxic gases emanating from such burning. In addition, edible fruits and vegetables either to domesticated animals or humans are considered of value by inhabitants. In essence, there is always a serious food chain contamination in the cities and villages. The immediate and long term effects of these are seen either as acute toxicity or chronic toxicity manifesting in the form of terminal diseases.

Oily effluents (are fluids mostly in liquid forms) flow out or forth, for instance an outflow from a sewage system, a discharge of liquid waste as from a factory or nuclear plant (Alamu and Samsongrace 2012). Spent engine oil is the hydrocarbon product of crude oil with $C_{12} – C_{20}$ in molecular nature. The damage to soil of black oil is basically caused by two components, the nature of the oil which can lead to physical contamination or smothering and the chemical makeup of the oil which can have toxic effects. Accumulation of such chemicals can cause certain abnormalities to the soil organisms. The effect on plants and animals are separated to external and internal. The external effects are the most noticeable and are most immediate to destruction.

2.1 Urbanization

Urbanization is a sign of development but fraught with environmental problems because of the increased population. Environmental pollution is always on the increase due to human activities such as agricultural operations, sewage discharge, energy production, and smelting, refining, disposal of waste, industrial and vehicular emission. Emission of pollutants into the air amounted to the greatest source of heavy metals pollution which in the soil, enhance plant uptake causing accumulation in plant tissues and eventual phyto toxicity and change in plant community (Ernst 1996; Zayed *et al*. 1998; Gimmler *et al*. 2002). It is a common feature to find huge refuse dump sites within residential areas and along major and minor roads in Nigeria.

Plants are important in the preparation of local medicine, air pollution control, noise level reduction, microclimatic amelioration, water and soil conservation etc. (Rao 1981). Monitoring of pollutants with higher plants presents several advantages as compared to other systems based on animals or microorganisms mainly because of the wider range of situations which can be monitored with these organisms. Plants have a higher capacity to take up metals from the soil or atmosphere than their physiological needs. In heavy-metal-polluted soil, 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 (Oliver 1997). Since plants are the major sources 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 (Athar & Vohora 1995). It has been reported that nearly half of the mean injection of lead, cadmium, and mercury through food is due to plant origin (fruits, vegetables and cereals) some of which are grown in the urban centers either along the roads or on living and abandoned dumpsites. 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 (Quijano 2001).

Contamination of the environment by heavy metals is of major concern because of their toxicity and threat to human life and environment (Purves 1985). 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. Jaya (2009) reported that the concentration of heavy metals, lead and cadmium in raw mangoes (fruits) were beyond the permissible levels given by WHO (1983).

Urban agriculture is being practiced mostly either on living or abandoned waste dump sites because of the fertility usually associated with the sites. These sites may also be located along road sides thus exposing the plants to vehicular emission as well as pollutants from the soil. Plants growing on municipal dump site soils accumulate higher concentrations of metals than those on rural waste dump sites (Amusan *et al.* 2005).The plants take up heavy metals either as mobile ions present in the soils solution through the roots (Davies 1983) or through foliar absorption. The uptake of the metals by crops results in the accumulation of these metals in plant tissues. This is known to be influenced by the metal species, plant species and plant age as well as plant parts (Juste & Mench 1992; Amusan *et al*. 2005).

3. The method of operation

3.1 Some waste dump sites in Ogbomoso city were surveyed for sample collection of leaves, stems and roots of pawpaw, tomato and cocoyam. Soil samples were also collected in polythene bags with core sampler *in situ* at 15 cm depth. The plant materials and the soil were taken to the laboratory for heavy metal analysis using standard methods.

3.2 Twelve waste dumpsites in Ogbomoso were selected for sampling of some plant species to assess them for heavy metal content in their tissues. Shoots of the plants were collected for the purpose of the research and taken to the laboratory for heavy metal analysis using standard procedures.

3.2a. One major and one minor roads were selected for the study, Ogbomoso-Oyo as high traffic density (HTD) and Ogbomoso-Ife Odan road as low traffic density (LTD) in Oyo state. Soil (0-15 cm and 15-30 cm depth under mango trees) and mango leaves (about 3 m from the ground) samples were collected from the road edge along three transects inward within 0-10, 10-20 and 20-30 m away from the road sides. The samples were replicated thrice along the roads at intervals of 10 km. Both soil and the mango leaves were taken to the laboratory for microbial population and heavy metal analysis - lead, cadmium, chromium and zinc using standard methods.

3.2b. In line with the above method, vegetation along the roads was randomly sampled using systematic random sampling methods with the aid of a1 m by 1 m quadrat at the indicated distances. The vegetation and the corresponding soils were taken to the laboratory for the same analyses.

4. Leaf samples of *Azerdracta indica* and *Gliricidia sepium* were collected from;

* three mechanic workshops for each plant (polluted)
* three farmlands for each plant (unpolluted)

Parameters studied were:

(i) stomata quantity determined by the use of microscope. The stomata count was taken from the uppersurfaces of the five leaf samples tagged for each plant.

(ii) leaf acidity was determined by dissolving 5g of granulated leaves in 50 ml of distilled water and homogenised appropriately. The mixture obtained was filtered through a whatman number 42 filter paper into a 100 ml conical flask. The filterate was titerated against 0.1M NaOH. Acidity rate was obtained using the formular;

Titeratevalue x 0.1 x 0.0089/ volume of extract obtained x 100.

(iii) leaf colour differences was carried out by determining the chlorophil content which is the green colour pigment in the leaf. One gram of fresh leaf was crushed in an HPLC grade acetone 80% with pestle and morter. This was mixed up to 100 ml volume and the absorbence read at the desired wavelength of chlorophyl "a", "b" and total chlorophyl.The difference between chlorophyl a and b gives the leaf colour difference in µg/g.

Table 1 indicated the heavy metal content of the edible part of the crops. All the crops contained heavy metals no matter how small except chromium that was not detected in tomato from control site.

All the plants from the dump sites contained high but variable amount of heavy metals. Metal ion concentrations in plants also vary from one plant to another. *Tithonia diversifolia, Cassia obtusifolia, and Ageratum conyzoides* accumulated high amount of lead. *Eupatorium odoratum* contained the lowest percentage of lead (36.82%) (Adelasoye *et al.* 2012). *A. conyzoides, Triumfetta rhomboidea,* and *E. odoratum* absorbed highest amount of cadmium. *Sida corymbosa* (96.96%) and *T. rhomboidea* (72.72%) were best assimilator of chromium. Cupper was absorbed to a highest extent by *C. obtusifolia* (59.52%), *T. diversifolia* (52.58%) and *T. rhomboidea* (50.6%). *T. rhomboidea* (24.71%), *C. obtusifolia* (24.71%), *A. conyzoides* (26.06%) and *Amarathus spinosus* (23.61%) (Adelasoye *et al.*, 2012) absorbed the high manganese which was similar to the report of Roechan *et al*. (2000). Heavy metals are generally abundant in Ogbomoso metropolis. The absolute total metal concentration from the polluted soil ranged from the lowest Cr (990µgg^{-1}) to (24, 200µgg^{-1}).

Mango leaves at HTD contained significantly more lead (4.31 mgkg^{-1}) cadmium (4.63 mgkg^{-1}) and zinc (5.04 mgkg^{-1}) while chromium (0.07mgkg^{-1}) was higher at LTD (Adelasoye & Oyeyiola, 2014).

At HTD road, 20-30 m from the edge of the road, the soil contained higher amount of lead (7.71±0.76), Cd (8.36±0.59), Cr (0.48±0.22) and Zn (5.58±0.37 mgkg^{-1}) than the region closer (Adelasoye and Oyeyiola, 2014). Table 1 also showed the microbial content of the soil 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 (1-10 m) with total microbial count of 10.50±1.46x10^{5} Cfug^{-1} and total fungal count of 0.83±0.23x10^{5} Cfug^{-1}. The same trend was observed at LTD. However, microbial population was slightly higher along the low traffic than the high traffic density road.

The following plant species were among the vegetation samples taken along the roads: *Tectona grandis* (Linn.), *Daniella oliveri* (Rolfe), *Gmelina arborea* (Roxb), *Sida acuta* (Burm), *Gliricidia sepium* (Jacq), *Chromlena odorata* (Linn.), *Tithonia diversifolia* and *Panicum maximum*. Only

Tectona grandis (Linn.) and *Gmelina arborea* (Roxb) were not found at the sampling point along low traffic density road.

A comparison of the average heavy metal content in vegetation revealed that HTD recorded higher metal concentration than LTD. At HTD road, Pb (4.00±1.53 mgkg^{-1}) and Cd (4.34±1.67 mgkg^{-1}) were higher at 10-20 m than 0-10 m and 20-30 m from the road. Chromium (0.15±0.01 mgkg^{-1}) was higher at 20-30 m than the other distances closer to the road. Zinc was highest (4.10±0.01 mgkg^{-1}) at 0-10 m from the road followed by 20-30 m and lowest (3.90±0.01 mgkg^{-1}) at 10-20 m from the road (Adelasoye & Alamu 2016).

In Table 2 the soil concentration of heavy metals along high density traffic road was averagely higher than that of LTD. The table further indicated that concentration of all the metals in the soil decreased with increasing distances from the edge at the HTD road. Lead and chromium did not follow the same trend at LTD road. Total microbial population was higher at HTD than LTD while the reverse trend was observed with Total fungi count in which LTD road had higher Total fungi count than HTD (Table 2).

table 3, the leaf of *Azerdraita indica* on polluted trees had a higher average weight of 1.84 mm^{2} compared to that of unpolluted tree with 1.75 mm^{2}. Litterfalls are likely to be more due to a higner weight. The leaf stomata (upper surface) of polluted trees had a higher average value of 38 mm^{2}while the leaf acidity indicated that trees in unpolluted sites had a greater acidity value in average (0.142 ug/g) compared to the average acidity value of 1.423 ug/g for polluted trees. *Azerdraita indica* on unpolluted site had the highest colour difference of 0.156 ug/g.

For *Gliricidia sepium*, leaf weight of polluted trees had a higher average weight of 2.81 mm^{2} compared to that of unpolluted trees of 1.42 mm^{2}. This is the same trend with was is obtainable in *Azerdracta indica.* The leaf stomata (upper surface) of polluted trees had a higher average value of 58 mm^{2}.

For leaf acidity, unpolluted trees had a greater value of 0.142 ug/g compared to the average acidity value of 1.423 ug/g for polluted trees. The colur difference showed that, Gliricidia sepium on unpolluted site had the highest colour difference of 0.156 ug/g.

5. Discussion with Policy Recommendation

Cadmium concentrations was high in most plant organ assessed in the study, even sometimes higher than lead. It reaches variable levels in different organs of different species of such plants as oats, soybean, corn and tomato which accumulate more cadmium in roots than in aerial parts of the plant. Conversely, lettuce, carrot and potato accumulate more in leaves. Soybean accumulates more in the seeds than in the leaves (Sauebeck 1991; Kabata-Pendias & Pendia 1992). In the study, cocoyam corm and tomato fruits from the waste dump sites accumulated highest amount of lead, cadmium and chromium. The health implication of this fact can be devastating in that most backyard farms are on abandoned dumpsites. There is an urgent need to start educating the general public about metal toxicity and their media of exposure so that the number of seemingly incurable or otherwise terminal diseases which are products of acute and chronic metal toxicity can be drastically reduced.

Most of the plant encountered in the study may be useful for one herbaceous medicine preparation or the other particularly *T. diversifolia* and *A. corymbosa* which are used for malaria and gastric ulcer treatment respectively. *Tridax procumbens* is a well-known rabbit food in many places. These plants will introduce heavy metals in to the food chain if the utilization of the contaminated ones continues unabated.

The European Union ranged Cd in the soil of 0-1 $mgkg^{-1}$ indicated non-contaminated soil, 1-3 $mgkg^{-1}$ indicated slight contamination and 3-10 $mgkg^{-1}$ indicated a contaminated soil. Thus, both the high and low traffic density roads assessed in the study were contaminated in terms of cadmium. FAO/WHO recommended maximum tolerable intake of Cd of 0.4 -0.5 $mgkg^{-1}$ per week or equal to 0.07 mg per day. Korean women were estimated in 2007 to take 14.82 mg Cd day^{-1} of which 9.74µg Cd day^{-1} was from plant foods and 5.09µg Cd day^{-1} was from animal source. The daily cadmium intake of plant origin accounted for 65.7% (Chan-Seok Moon *et al*. 2012).

The results when compared with the WHO/FAO standard lower limits of Pb (0.3 $mgkg^{-1}$), Cd (0.2 $mgkg^{-1}$), and Cr (0.05 $mgkg^{-1}$) 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 the general public about toxicity of heavy metals. Agricultural activities should be discouraged within 30 meters from road edge, so also is animal grazing if heavy metal exposure routes are to be reduced to the barest minimum.

Increasing soil heavy metal pollutants with increasing traffic density, and its decreased concentration with increasing distance away from the edge of the road inward is similar to the report of Haygarth and Jones (1992) that metal concentrations decreased with increasing distance from the road. It was suggested that this phenomenon might be due to heavy metals emitted from vehicle exhausts in particulate forms which are forced to settle under gravity closer to the edge of the road. The emission could be from petrol, diesel and alternative fuel engine including benzene, lead, organic compounds, carbon monoxide, oxides of nitrogen, un-burnt hydrocarbons, Sulphur dioxide and other suspended fine particulate matter like smoke, metals (Cd, Co, Cu, Zn, etc.) as well as inert dust (Gaighate & Hasan 1999). Vegetation along high traffic road contained higher concentration of heavy metals than low traffic density. However, irregular distribution of the metals was observed along the gradient from the road edge inwards these results agreed with that of Voegborlo and Chirgawl (2007) who reported clearly defined gradients of lead contamination beside road ways, and less-defined gradients of other metals including cadmium, chromium, copper, nickel, vanadium and zinc. Phyto toxicity of heavy metals and other particles emitted from auto-exhaust have been shown to accumulate and may cause damage and death of plant species growing along roadside (Alfani *et al*. 1996). Total fungi count was higher at low traffic density where heavy metals were low than high traffic density where total microbial population were lower. 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 (Marschner & Kalbitz, 2003; Utgikar *et al*. 2003). Rabia & Tansneem (2007) reported that lead and silver were found to be toxic for the growth of microorganisms. Toxic effects of heavy metals on microorganisms manifest in numerous ways such as decrease in litter decomposition and nitrogen fixation, less efficient nutrient cycling (Baath 1989). As the metals are immobile in the soil, they accumulate in top soil thereby endangering crops, plants and microbial population and composition. Food chain may be contaminated as organisms interact majorly in terms of energy transfer from producers to consumers (Athar & Vohora 1995). Continuous deposition of these heavy metals indicates potential health risk for humans through the food chain. Exposure to metals is normally chronic due to transfer to the food chain (USDA 2000). Children and developing fetuses appear to be particularly vulnerable to the neurotoxic effect of lead. It has been demonstrated that low-level lead exposure in children less than five years of age with blood lead levels in the 5 - 25µdL^{-1} range results in deficits in intellectual development as manifested by lost intelligent quotient points (Banks *et al*. 1997). The loss of calcium caused by cadmium's effects on kidney can be severe enough to lead to weakening of bones, "Itaitai" disease, an epidemic of bone fracture in Japan from gross cadmium contamination of rice stocks, has been shown to happen in more subtle fashion among a general community living in area of relatively modest cadmium concentration (Staessen *et al*. 1999). Controlling air pollution from motor vehicle is essential if adverse effects will be curtailed (EPA 2012). Proper maintenance of vehicle and regulation of truck emission control systems will not only limit harmful emission but will also improve fuel use efficiency and extend the life of vehicle. Zhang *et al*. (2012) findings indicated that trees growing linearly along roadways can effectively reduce the heavy metals' concentrations in the road side farmland.

5.1 Leaf stomata

The main function of leaf stomata is to allow gases such as CO_2, water vapour to move rapidly in and out of the leaf. They are formed at the initial stages of the development of the various plant organs and therefore reflect the environmental conditions under which they grow. Trees that have been grown on good soil and conducive environmental condition but later on exposed to soil pollution may become growth distressed. Crude or used Engine oil makes unsatisfactory condition for effective plant growth (Alamu & Samsongrace 2012). This is due to insufficient aeration of the soil because of the displacement of air from the spaces between the soil particles by oily effluents. In bid to adapt to the present status and maintain constant growth, it may increase the number of stomata. This is another form of stress that may affect other physiological functions of the plants.

5.2 Leaf acidity

In general terms, the leaf percentage acidity of samples collected from the polluted sites is lower than that for samples collected from unpolluted sites. Acidity may be accounted for by the deposition of heavy metals on trees leaves. This process is a result of mineral transportation up the tree for photosynthetic activities and eventual translocation to plant parts as photosynthates. Acidic and medium fertility soils support the growth of most tropical trees (Stewart *et al*. 1992). A sudden increase in acidity therefore is inimical to tree health. The end-users of such trees' parts transfer lethal materials through the ingestion of heavy metals.

Leaf colour

In general terms, leaf colour different is more in polluted tree leaf samples. Udo and Fayemi 1975) opined that growths of plants in oil polluted soils are generally retarded and chlorosis of the leaves results coupled with dehydration of the plants indicating water deficiency. The greenish colour is rare in such cases making other physiological activities difficult for the trees. A policy statement mandating articians using the trees for their operations to always be mindful of the trees under which they work is imperative. The policy should make it mandatory for them to dispose off the oily effluents properly and not to spill them directly under the tree which they operate. There is also the need for proper education of the general public on the need to be careful with the use of parts of such trees as sources of chewing stick, herbal prepation and other sundry uses for human consumption.

6. Conclusion

Population increase in any human settlement brings about enormous competition on material resources. The saying, necessity is the mother of invention is rife as a result of competition for material resources for human comfort.Urban farming is a bid to beat dearth of food as a result of population increase that brings about competition for food. Noble as urban farming may be in the eyes of policy makers and practitioners, environmental concerns should be expressed by all. However, environmental concerns can only be expressed if the lethal effects of effluent contaminants of urban crops are researched into and the level of danger known. This research looked at the lethal implication of contamination of products from urban farming on human and animals.

Waste dumpsites, low density and high density roads as well as trees used for shade were visited and samples of leaves of trees and soil samples were taken to laboratory for analysis.

In heavy traffic roads,lead(pb) that is very lethal to man and animal ranged between 0.18mg/kg and 0.38mg/kg. Leaf acidity in polluted trees (*Azadrachta indica*) compared to unpolluted trees had a ratio of 1:10. A conscious effort is needed to educate settlers in urban centres and along major highways to be aware of the dangers inherrent in using plants and trees found at such arenas and desist from direct consumption of such products.

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APPENDIX

Table. 1: Metal concentration (mgkg^{-1}dry weight) in tomato and pawpaw fruits and cocoyam corms.

Metal	PA	PB	PC	Pco	TR	TA	TB	TC	Tco	TR	CA	CB	CC	Cco	TR
Pb	0.22	0.31	0.32	0.10	0.69	0.18	0.22	0.28	0.02	0.53	0.33	0.60	0.40	0.06	1.05
Cd	0.27	0.45	0.44	0.04	0.69	0.24	0.26	0.32	0.01	0.49	0.34	0.66	0.56	0.04	0.93
Cr	0.05	0.03	0.05	0.01	0.11	0.14	0.02	0.02	Nd	0.16	0.02	0.05	0.12	0.02	0.17
Mn	0.54	0.88	0.18	0.10	1.06	0.48	0.68	0.35	0.11	1.23	0.79	1.40	1.62	0.17	3.10
Cu	0.33	0.33	0.26	0.11	0.11	0.12	0.17	0.16	0.14	0.05	0.32	0.46	0.45	0.14	0.14

Note: PA= Pawpaw, Pco= control, TA= Tomato, Tco= control, CA= cocoyam, A, B, C= sites TR= Transfer ratio Nd= Not detected

Source: Adelasoye, 2012.

2: Soil heavy metal concentration (mgkg^{-1}) and microbial population along road sides with different distances from the road.

Level of traffic and distance		Metal(±SD)			Microbes	
	Pb	Cd	Cr	Zn	TMP Cfug^{-1}(x10^{5})	Tfc Cfug^{-1}(x10^{5})
Ha	6.93±1.00	7.89±1.29	0.23±0.31	4.07±0.26	2.12±1.03	0.62±0.17
Hb	6.18±0.84	7.18±1.12	0.10±0.03	4.03±1.05	11.10±0.93	0.05±0.11
Hc	5.34±0.72	6.15±0.62	0.08±0.05	3.96±1.13	10.20±0.79	0.48±0.16
La	4.47±1.19	5.42±1.27	0.06±0.01	2.74±0.31	11.1±0.88	0.83±0.29
Lb	4.51±0.83	5.21±0.90	0.06±0.02	2.62±0.36	9.72±1.00	0.62±0.40
Lc	4.11±0.61	4.97±0.66	0.06±0.02	5.50±0.34	9.10±0.81	0.40±0.22

Note: H = High traffic L = low traffic a = 0-10 m b = 10-20 m c = 20-30 mTMP = Total Microbial Population Tfc = Total fungi count Cfug^{-1}= Colony forming unit per gramme SD = Standard deviation

Source: Adelasoye & Alamu 2016.

Table 3: Effects of oily effluent on *Azerdraita indica and Gliricidia sepium*

Parameters	**Sample**	***Azerdraita indica***		***Gliricidia sepium***	
		Polluted	Unpolluted	Polluted	Unpolluted
Weight of leaf (g)	1	1.07	1.35	2.29	1.85
	2	2.56	1.84	2.08	1.49
	3	1.90	2.06	4.06	0.93
Stomata quantity/mm^{-2} (lower surface)	1	11	13	53	35
	2	61	36	48	16
	3	42	41	73	12
Stomata quantity/mm^{-2} (upper surface)	1	36	32	37	21
	2	23	45	32	34
	3	29	29	52	26
Percentage Acidity	1	0.086	0.097	0.243	0.118
	2	0.272	0.116	0.218	0.106
	3	0.121	0.214	0.396	0.078
Colour difference (a-b) ug/g	1	0.08	0.10	0.08	0.56
	2	0.15	0.16	0.23	0.18
	3	0.11	0.22	0.18	0.18