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Chapter 1

Agrochemicals and Bacterial Diversity in Cultivated Tropical Soils

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Additional information is available at the end of the chapter

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1. Introduction

1.1. General

Agrochemicals provide yield protection, prevent and cure crop disease, provide insulation to reduce energy use and provide countless other benefits that increase the standard of living for civilization as a whole. While the chemicals industry has made good progress reducing its overall environmental footprint, chemicals can also create a negative impact on human health and the environment when their production and use are not managed responsibly (Edwards, 1975; Tu, 1990; Zulalian, 1990).

1.2. Agriculture in Ghana

Urban food needs is increasing in developing countries, including Ghana (Figure 1), with growing populations. Increasingly vegetables are grown in urban and peri-urban areas to meet this demand. Agriculture has been Ghana's most important economic sector over the years, employing more than 60% of the national work force, mainly in the small landholders on formal and informal basis (Gerken *et al*, 2001) and accounting for about 40% of the total GDP and export earnings. Ghana has climatic zones that range from dry savanna to wet forest and run in east-west bands across the country.

Agriculture crops including yams, grains, cocoa, oil palms, kola nuts and timber, form the base of Ghana's economy. Compared to this, the contribution of the more traditional vegetables such as okra, pepper, tomato, onion and egg plants to the agricultural GDP is low. However, considering their contribution to agricultural GDP and the land area devoted to the cultivation of all crops, the traditional vegetables produce more value per area. For example, the share in total area cultivated and value for vegetables (tomato, okra, pepper and

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eggplant) is 3.0 and 7.0%, respectively, while for cereals (maize, millet, sorghum and rice), these values are 35.0 and 8.0%, respectively (Table 1).

Figure 1. Map of Ghana, its boundaries and Lake Volta. Also shown are some major rivers.

Crops	Share in value (%)	Share in area (%)	Rate of growth (%)	
			Area	Yield
Maize (*Zea mays*)	4.80	17.0	2.00	3.00
Rice (*Oryza glaberrina*)	1.00	2.23	8.00	2.00
Millet (*Pennisetum glaucum*)	1.30	7.27	2.00	-
Sorghum (*Sorghum bicolor*)	1.24	8.41	2.00	1.50
Cassava (*Manihot esculenta*)	19.2	12.1	2.00	3.00
Cocoyam (*Xanthosoma roseum*)	10.2	6.08	2.00	-
Yam (*Dioscorea* spp.)	16.7	6.32	2.36	0.75
Plantain (*Musa* spp.)	13.0	5.32	2.00	-
Groundnut (*Arachis hypogaea*)	3.83	4.66	2.36	3.00
Tomato (*Solanum lycopersicum*)	2.79	0.61	1.00	2.00
Okra (*Hibiscus esculentus*)	3.16	0.85	3.00	2.00
Pepper (*Capsicum annuum*)	0.14	1.71	3.00	2.00
Eggplant (*Solanum melongena*)	0.56	0.07	3.00	2.00
Beans (*Phaseolus vulgaris*)	15.3	5.12	1.00	2.00
Cocoa (*Theobroma cacao*)	0.92	18.8	5.00	2.00
Oil Palm (*Elaeis guineensis*)	0.07	1.58	2.00	2.00
Rubber (*Ficus elastic*)	0.06	0.06	4.00	2.00
Coffee (*Coffea Arabica*)	0.16	0.02	4.00	2.00
Cotton (*Gossypium hirsutum*)	0.12	0.32	8.00	2.00
Tobacco (*Nicotiana tabacum*)	3.47	0.06	8.00	2.00
Orange (*Citrus sinensis*)	0.09	0.55	3.00	2.00
Pineapple (*Ananas comusus*)	0.09	0.06	3.00	2.00
Total crops/growth rates	98.2%	99.2%	3.31%	1.58%

Source: Nurah, 1999

Table 1. Growth rates and contributions of different crops to agricultural GDP

1.3. Application of agrochemicals in agriculture

Vegetable production in Ghana typically occurs in intensely managed, irrigated smallholder farms with relatively high pesticide inputs. Surveyed vegetable crops (tomato, pepper,

okra, eggplants (or garden eggs) and onion) cover approximately 0.4% of the cultured land of Ghana, equating to 58,270 ha in 1998 (Gerken *et al*., 2001). An estimated average pesticide rate of 0.08 liters ai/ha is applied to these vegetables. Relatively lower quantities are applied to cereals (0.03 liters ai/ha1) and higher quantities to cocoa (0.5 liters ai/ha). The compounds applied in vegetable production include organochlorine and organophosphate insecticides. In contrast to the traditional organochlorines, organophosphates are not highly persistent, but some can be highly toxic to some aquatic organisms (Castillo *et al*., 2006). Intensively managed vegetable farms are also characterized by an extensive network of drainage systems where surplus water may flow into local streams and rivers. Consequently, the aquatic ecosystems located downstream of vegetable farmlands might be vulnerable due to intensive pesticide use, drainage systems, and high precipitation rates typical for tropical areas where vegetable production occurs.

1.4. Non-point source agricultural pollution

Non-point source agricultural pollution is regarded as the greatest threat to the quality of surface waters in rural areas. One of the most important routes leading to non-point source agricultural pollution of surface waters in rural areas is runoff. Runoff from agricultural fields introduces pesticides, soil, organic matter, manure and fertilizer into small streams, increasing the volume of stream discharge and changing water quality (Neumann and Dudgeon, 2002). The impacts of such runoff are well documented (Cooper, 1993; Castillo *et al*., 1997; DeLorenzo *et al*., 2001).

Traditional vegetable farming systems (i.e. without any chemical input) are incapable of meeting demand. For instance, pests and diseases, which pose big problems in vegetable production, require intensive pest management to control them. Chemical pesticide use is a common practice to control pests and diseases in vegetable cultivation. However, besides their beneficial effects, pesticides are accepted as having potential environmental and public health impacts as well. If improperly used, pesticides can cause direct human poisoning, accumulate as residues in food and the environment or lead to the development of resistant strains of pests. These problems can arise from misuse of the pesticides or over-reliance on them, particularly if the users are not aware of these potential problems.

A total of 43 pesticides are in use in vegetable farming in Ghana (Table 2). This figure was obtained as a direct summation of pesticides applied on farms, but it could be lower than the actual number of pesticides in use. The pesticides comprised insecticides, fungicides and herbicides. Herbicides (44%) were the class of pesticides most used in vegetable farming in the areas surveyed, followed by insecticides (33%) and fungicides (23%).

In Table 2 the classification of these pesticides by the type of pests they control, active ingredient, chemical group and World Health Organization (WHO) Hazard Category is presented. The herbicides and fungicides used are mostly under WHO Hazard Category III, with a few under Hazard Category II. All the insecticides used are under Hazard Category II, which WHO classifies as moderately hazardous. This category includes organochlorines (OCs), organophosphates (OPs) and pyrethroids. Endosulfan was the only OC mentioned in use in the survey.

Pesticide type (% of total number in use)	Active Ingredient	Chemical Group	Hazard Category (WHO)	Registered for use on
Herbicide (44%)	Pendimethalin	Dinitroaniline	III	Tomatoes, onions
	2, 4-D	Aryloxyalkanoic acide	II	Rice, sugarcane
	Propanil	Anilide	III	Rice
	MCPA-Thioethy1	Aryloxyalkanoic acide	III	Not registered
	Oxadiazon	Oxadiazole	III	Not registered
	Oxyfluorfen	Diphenyl ether	III	Not registered
	Bensulfuron-methy1	Sulfonylurea	III	Rice
	Glyphosate	Glycine derivative	III	Various crops
	Paraquat dichloride	Bipyridylium	II	Various crops
	Acifluorfen	Diphenyl ether	III	Not registered
	Metolachlor	Chloroacetamide	III	Not registered
	Phenmedipham	Carbamate	III	Not registered
Fungicide (23%)	Mancozeb	Carbamate	III	Mangoes, vegetables
	Matalaxyl-M	Acylalanine	II	Not registered
	Thiophanate-methyl	Benzimidazole	III	Various crops
	Carbendazim	Benzimidazole	III	Not registered
	Benomyl	Benzimidazole	III	Not registered
Insecticide (33%)	Lambda-cyhalothrin	Pyrethroid	II	Vegetables
	Chlorpyrifos	Organophosphorus	II	Citrus, public health
	Endosulan	Organochlorine	II	Cotton
	Dimethoate	Organophosphorus	II	Not registered
	Cypermethrin	Pyrethroid	II	Not registered
	Deltamethrin	Pyrethroid	II	Various crops

Table 2. Types of Pesticides applied in vegetable production in Ghana

Technical endosulfan, a mixture of two stereoisomers, that is, α- and β-endosulfan in the approximate ratio of 7:3 (Shetty *et al*., 2000; Kennedy *et al*., 2001), is a chlorinated pesticide for control of a large spectrum of insect pests on a wide range of crops (Aguilera-del Real *et al*., 1997). It is used in many countries throughout the world for the control of pests on fruits, vegetables, tea, tobacco, and cotton (Antonious and Byers, 1997; Sethunathan *et al*., 2002). Because of such abundant usage, and the potential for accumulation in the environment (endosulfan is not readily detoxified by soil microorganisms), residues are detectable in soils, sediments, and crops at harvest time (Goebel *et al*., 1982; U.S. Department of Health and Human Services, 1990). Although the metabolites of endosulfan, that is., sulfate, diol, ether,

hydroxy ether, and lactone, have been shown to occur (Maier-Bode, 1968; Schuphan *et al.*, 1968), only the sulfate metabolite is significant as a residue (Antonious and Byers, 1997).

Although the impacts are complex and often unknown or sometimes open to debate, some negative effects are well documented, such as chemicals found in the environment that are persistent, bioaccumulative and or toxic (e.g. PCBs, dioxins) (Waiwright, 1978; Moorman, 1989). Concern has been raised about chemicals which interfere with the normal function of hormonal systems of human and animals (i.e. endocrine disrupters), and substances which impact on children's health (De Reuck, *et al*, 1979).

1.5. Activity of selected agrochemicals

Glyphosate, the main component of Ceresate, is a non-selective, non-residual herbicide used against annual or biennial herbaceous monocotyledons, herbaceous dicotyledonous and perennial weeds. It is absorbed by foliage and transported through plant and is very effective on many deep-roots perennial species. It is metabolized or broken down by some plants, while other plants do not break it down. It is not usually absorbed from the soil by plants (Rueppel *et* al, 1977; McEwen & Stephenson, 1979; Eberbach & Douglas, 1983). Glyphosate remains unchanged in the soil for varying lengths of time, depending on soil texture and organic matter content. The half-life of glyphosate can range from 3 to 130 days.

Dimethoate (Cerox) is an organophosphorus insecticide with a contact and systemic action. Dimethoxon, an oxygen analogue metabolite of dimethoate, appears to play a dominant role in its toxicity for insects and mammals (Bohn, 1964; Koppel etal, 1986). Hydrolytic degradation is the main inactivating pathway of dimethoate in the environment. The half-life of dimethoate in different plants is between 2 and 5 days. Degradation in soil is dependent on the type of soil, temperature, moisture, and pH level.

Paraquat is a selective herbicide used to control most annual grasses and certain broadleaf weeds in field corn, potatoes, rice, cotton, soybeans, tobacco, peanuts and sunflowers. It is used in both pre-emergence and early post-emergence weed control (McErtenson, 1992). Paraquat is not subject to microbial degradation. Slight losses of paraquat can result from photodecomposition and volatilization. Its soil half-life is 90 days.

1.6. Microorganisms in soil

Microorganisms present in soil include Actinomycetes, Fungi, Algae, Bacteria and Protozoa. Most organisms are found in the top layers of soil, usually the top 2-3 centimeters, since this is typically where most of the organic matter is located (Alexander, 1979). The organisms are usually concentrated close to root surfaces in the rhizosphere, within living and dead roots, on soil particles, or among aggregates of soil particles. The rhizosphere is the region of the soil that is immediately adjacent to and affected by plant roots. It is a dynamic region where interaction takes place between plants, soil, microorganisms, nutrients and water.

Microorganisms play a major role in the breakdown of pesticides in the soil. Many microbes are capable of utilizing pesticides as sources of carbon and most pesticides studied are attacked

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at one or more sites by microorganism e.g. the bacteria *Hydrogenomones* can degrade DDT completely to carbon dioxide (McEwen & Stephenson, 1979).

Edwards (1975) lists possible effects on living organisms in soil contaminated with insecticides to include (i) direct toxic effect to microbial life in the soil, (ii) affecting organisms genetically to produce populations resistant to pesticides, (iii) sub-lethal effects resulting in alterations in behavior or changes in metabolic or reproductive activities, and, (iv) absorption into the bodies of soil fauna and passing on to other organisms.

The study determined the effects of some selected agrochemicals on bacterial population in the soil, and investigated the effect of agrochemicals on plant growth.

2. Materials and methods

2.1. The agrochemicals used

The agrochemicals used were obtained from the open market. Each agrochemical was new and sealed in bottles of one litre volume. The products included (i) Cerox, an insecticide containing dimethoate 400g / L; (ii) Ceresate, a herbicide containing glyphosate IPA 4% w/w SL; and, (iii) Paraquat, a herbicide with composition, paraquat DCL 24% w/w SL.

2.2. Selection of viable seeds for planting

Undamaged bambara groundnut (*Vigna subterranea*) seeds of similar sizes were surface-sterilized by immersing for 5 minutes in 0.1% mercuric chloride (HgCl) solution and washed in six changes of sterile distilled water. The seeds were next washed in 70% ethanol for 3 minutes, and rinsed twice with sterile distilled water. The sterile seeds were placed on water agar (0.1% agar) in large Petri dishes and incubated at room temperature for 5 days. The vigorously germinating seeds were selected for planting.

2.3. Planting of seeds in experimental soil

Five seeds were sowed in each pot, and the seedlings were thinned to one after they had survived. There were four replicates for each soil treated type. The plants received full sunlight up to mid-day each day and were protected from rains. They were watered daily with 20ml tap water per pot. Once a week, each pot received, in addition, 10ml Sachs' solution to augment the nutrient content in the soil.

2.4. Application of agrochemicals

On the second week of planting, the agrochemicals were applied to the soil with the seedlings. Pots labelled A served as control, no agrochemical was applied. Pots labelled B were sprayed with Cerox. Pots labelled C were sprayed with Ceresate, and Pots labeled D were sprayed with Paraquat. Spraying was done using a spray bottle. The agrochemicals were diluted with sterile distilled water according to the manufacturer's recommendation as follows:

Ceresate:	5 ml:300 ml of distilled water
Cerox:	1.8 ml:300 ml of distilled water
Paraquat:	1.2 ml :300 ml of distilled water

2.5. Assessment of extent of growth of experimental plants

The following records were made of the bambara groundnut plants: number of leaves, leaf length and leaf broadness. These measurements were taken once a week, for four weeks with the use of a ruler.

2.6. Assessment of nodulation

After six weeks, the plants were harvested and the roots thoroughly washed. The nodules were detached and counted.

2.7. Enumeration of total heterotrophic bacterial populations in soils

Soil samples were taken one day after planting of the seedlings and another set of samples on the third week after planting, from each of the pots to determine the bacterial population present in each soil treatment type. The soil samples were taken from between 2 - 8cm away from the stem of each seedling. One gram of each soil sample was dissolved in 9ml of sterile saline water and thoroughly mixed. Serial dilutions were made of each solution and 1ml plated on Nutrient Agar supplemented with yeast extract. All plates were incubated at 37^0C for a maximum of 48hrs.

2.8. Enumeration of *Rhizobium* sp. populations in each soil

Congo red Yeast-extract Mannitol Agar (YMA) (Hann, 1966) was inoculated with 1g of each soil treatment type. Incubation was at 30^0C for 5 days.

2.9. Determination of bacterial diversity in the soils

The Phene Plate (PhP) System which deals with Finger Printing of Bacteria in Microplates (Kuhn *et* al, 1991; Kuhn & Mollby, 1993) was used in the determination.

The bacteria to be tested were first pre-cultivated on appropriate agar media such as Blood agar, Brain Heart Infusion agar, Brilliant Green agar, Cereus selective agar, Deoxycholate Citrate agar, Eosin Methylene Blue agar, KF Streptococcus agar, MacConkey agar, Nutrient agar, Standard Plate Count agar, SS agar, Staphylococcus Medium, Triple Sugar Iron. The same pre-cultivation conditions were used for all strains in the test series.

A multichannel pipette with sterile tips was used to fill all wells in the PhP plate with suspending substrate. Aliquots of 0.320 - 0.375ml of the substrate were dispensed into all eight wells of 'Column 1' in the plate, and 0.150ml into all the other wells. All wells in 'Column 1' were inoculated with eight different types of bacteria colonies. The plates were left for at least one hour, after which the bacterial suspensions in the first column were homogenized with

the aid of the multichannel pipette. Quantities of 25μl of the bacterial suspensions in the first column were then transferred to all the other wells in each row with the multichannel pipette. Colonies suspected to be anaerobic were covered with sterile paraffin oil. Each plate was covered by a sterile lid and put in a wet chamber to avoid drying. The plates were incubated at 37^0C. The colour of each well was assessed after 16, 40 and 64 hours of incubation. An optical microplate reader connected to a computer with the PhP software was used. Three readings were made, after 16, 40 and 64 hours, respectively. The absorbance was measured at 620nm.

2.10. Statistical analysis

The Statgraphics Plus for Windows version 4.0 was employed to test for significant differences between the various means of parameters of the differently treated soils and those of the untreated soil.

3. Results and discussion

3.1. Enumeration of population of rhizobia in the differently treated soil types

Total viable count studies using Congo red YMA produced the *Rhizobium* sp. population numbers indicated in Table 3. There were high population numbers per gram of soil in the cerox-treated soil and the non-treated soil, respectively. Ceresate- and paraquat-treated soils had very low population numbers per gram of soil. There was no statistically significant difference between the means of the population numbers in the ceresate-treated and paraquat-treated soils. There was, however, statistically significant difference in the means of the population numbers in the non-treated soil, cerox-treated soils and those of the ceresate-treated and paraquat-treated soils.

Treatment Type Soil	Mean number of *Rhizobium*spp. Population (g^{-1}soil) x 10^4
Cerox-treated soil	138 (± 12.11)
Ceresate-treated soil	20 (± 4.11)
Paraquat-treated soil	12 (± 3.55)
Non-treated soil	180 (± 9.99)

Table 3. Rhizobium spp. population numbers in soils with the different treatments.

3.2. Assessment of extent of growth of experimental plants

Plants were assessed four weeks after germination. On the basis of plant growth and the extent of nodulation recorded in Table 4, the plants could be described as follows:

- Non-treated soil:- plants grew well with deep green foliage, had highest mean leaf number and mean leaf length. They also formed the highest number of nodules and were the largest.

- Cerox-treated soil:- plants showed moderate growth and nodulation.
- Ceresate- and paraquat-treated soils:- plants showed stunted growth and yellowish-green foliage and formed the smallest number of mean number of nodules per plant, 3 – 10 nodules, as compared to 30 – 44 mean nodules per plant of the non-treated soil and the cerox-treated soil.

There was no statistically significant difference between the means of leaf length of plants cultured in cerox-treated soil and those of plants in paraquat-treated soil. There was also no significant difference between means of leaves of plants in cerox-treated soil and those in the non-treated soil.

There was no statistically significant difference between the mean leaf numbers of plants cultured in cerox- and ceresate-treated soils. There was no significant difference between the mean leaf sizes of plants cultured in cerox-treated soil, paraquat-treated soil and the non-treated soil. There was also no significant difference between the mean leaf sizes of plants cultured in the cerox-treated soil and the ceresate-treated soil.

There was no significant difference between the mean number of nodules of plants cultured in ceresate- and paraquat-treated soils.

Treatment Type	Mean leaf length (cm)	Mean leaf Number	Mean leaf size (cm)	Mean number of nodules
Cerox-treated	6.13 (±0.79)	11 (±2.94)	2.29 (±0.55)	30 (±5.16)
Ceresate-treated	3.36 (±0.86)	10 (±1.15)	1.57 (±0.28)	3 (±2.83)
Paraquat-treated	5.45 (±0.55)	6 (±2.16)	2.06 (±0.39)	8 (±5.45)
Non-treated soil	6.36 (0.40)	15 (±2.50)	2.37 (±0.24)	44 (±5.72)

Table 4. Growth and nodulation of the bambara groundnuts raised in the differently treated soils.

3.3. Total heterotrophic bacteria counts in soils after chemical application

The results in Table 5 show that all the differently treated soil types had total viable bacteria present. The mean number of viable heterotrophic bacteria recorded for the soil samples varied from 40 x 10^4 cfu g^{-1} to 61 x 10^5 cfu g^{-1}. Paraquat-treated soil recorded the least number of heterotrophic bacteria followed by Ceresate and the Cresox. The non-treated soil recorded the highest mean viable heterotrophic bacteria count. There was statistically significant difference between the means of the 4 variables at the 95.0% confidence level with the treatment types. There was, however, no significant difference between the various means before treatment, treatment after day 1, and treatment after 3 weeks at the 95.0% confidence level.

Treatment Type	Value Before Treatment	Value after Treatment 1 Day	Value After Treatment 3 weeks
Cerox	60×10^5 (±90.65)	18×10^5 (±258.20)	10×10^5 (±182.57)
Ceresate	40×10^5 (±75.28)	73×10^4 (±29.44)	70×10^4 (±52.28)
Paraquat	56×10^5 (±45.09)	41×10^4 (±54.77)	40×10^4 (±65.83)
Non-treated	52×10^5 (±19.90)	54×10^5 (±496.66)	61×10^5 (±258.20)

Table 5. Mean values of total heterotrophic bacteria count surviving after treatment with Agrochemicals.

3.4. Analysis of the diversity indices of the bacterial flora in the soils

The diversity indices of the bacterial flora were high (more than 0.90) for both the non-treated soil and the cerox-treated soil (Tables 6a and 6b). However, the diversity indices of the bacterial flora for the ceresate- and paraquat-treated soils had values of less than 0.90 (Table 6c and 6d). A high Di (maximum value is +1) means that the assayed isolates were evenly distributed into different types, whereas low Di (minimum value is 0) means that one or few types of bacteria dominated the studied population (Kuhn *et al,* 1991; Kuhn & Mollby, 1993; Ampofo & Clerk, 2003).

3.5. Similarities between the bacterial populations in the differently treated soil

The PhP software used (Kuhn & Mollby, 1993) also calculated the population similarity coefficients (Sp) between the different treatments. Sp coefficients were performed according to the unweighted-pair group method using the average linkages method. High Sp coefficients (<0.5) means that the two compared samples shared many identical genera. Low Sp coefficients (>0.5) means different bacterial populations (Sneat & Sokal, 1973). The mean similarities are presented in Table 7. Comparison between the differently treated soil types, i.e. cerox-treated soil, ceresate-treated soil, and paraquat-treated soil showed Sp values all below 0.50, an indication of related populations with high diversity indices of bacteria. Comparison between the populations of bacteria from the non-treated soil and the agrochemically treated soils, however, showed Sp values greater than 0.5, an indication that the related populations were of low diversity indices Kuhn *et* al, 1991; Kuhn & Mollby 1993; Ampofo & Clerk, 2003; Gabrielson *et* al, 2003).

This study has confirmed detrimental effect of insecticide on bacterial populations in the soil. Total heterotrophic counts, rhizobial counts as well as the number of nodules of all samples taken from the chemically treated soils were all low as compared to values obtained for the untreated soil. However the effect of the insecticide was minimal in all cases as compared to the effects of the herbicides on the soil fauna.

Sample Name and No.		No. of isolates	Di value
Non-treated soil	1	24	0.992
	2	24	0.908
	3	24	0.974
	4	24	0.962
Mean Diversity			**0.959**
		a)	
Sample Name and No.		**No. of isolates**	**Di value**
Non-treated soil	1	24	0.989
	2	24	0.978
	3	24	0.987
	4	24	0.962
Mean Diversity			**0.980**
		b)	
Sample Name and No.		**No. of isolates**	**Di value**
Non-treated soil	1	24	0.898
	2	24	0.862
	3	24	0.855
	4	24	0.915
Mean Diversity			**0.880**
		c)	
Sample Name and No.		**No. of isolates**	**Di value**
Non-treated soil	1	24	0.842
	2	24	0.814
	3	24	0.882
	4	24	0.880
Mean Diversity			**0.850**
		d)	

Table 6. a) Diversity among bacterial flora in the non-treated soil,b) Diversity among bacterial flora in the cerox-treated soil, c) Diversity among bacterial flora in the ceresate-treated soil, d) Diversity among bacterial flora in the paraquat-treated soil.

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Parameter	Population of	Compared to	Sp value
Soil treatment type	Cerox	Ceresate	0.35
	Cerox	Paraquat	0.49
	Ceresate	Paraquat	0.13
No treatment	Non-treated	Cerox	0.51
	Non-treated	Ceresate	0.55
	Non-treated	Paraquat	0.52

Table 7. Similarities between the bacterial populations for the different soil treatment types.

Chemicals exert number of different toxic effects on a bacterial cell. It is difficult or even impossible to deduce the toxic mechanism of a specific chemical by just looking at its molecular structure, although chemicals with similar structures and/or physico-chemical properties are expected to have similar modes of action. Several studies have been done on the Quantitative Structure-Activity Relationships (QARs), but still knowledge is scarce. There are general rules though, such as lipophilic chemicals being more prone to disturb the bacterial membrane than hydrophilic chemicals, and electrophilic chemicals often forming irreversible covalent bonds to their target site at nucleophilc entities in biological molecules, such as proteins and DNA. A chemical may have multiple modes of toxic action and at low concentration it may even be used as a nutrient.

The effect of glyphosate on soil microbes has been studied by several authors because glyphosate, unlike most other herbicides, kills the plant by blocking a biochemical pathway which is also essential for most of the bacteria and fungi. It is known (Eberbach & Douglas, 1983) that glyphosate blocks certain biochemical pathways that are essential for growth of bacteria and the low number in bacteria population (10×10^5) as compared to the population in the non-treated soil (61×10^5) is evident enough to support this. In similar experiments conducted in Australia by the CSIRO Lands and Water the herbicides Ally®, Hoegrass® and Paraquat® were applied directly onto soil without any stubble cover at two and five times the recommended rate. In most situations this low level of functioning continued up to nine weeks. However, when the chemical was applied directly to the soil or to growing plants, the stress time for soil organisms was reduced. The research showed that it takes six weeks for the microbial activity to return to normal.

The Phene-Plate (PhP) system for biochemical finger printing of bacteria, which is based on measurements of the kinetics of biochemical tests, was suitable in using to type total of 384 isolates of bacteria in microplates. The system included mathematical models and had the

advantage of calculating the diversity index (Di) of the bacterial populations present in each of the treated soils, as well as calculating the similarity coefficient (Sp) (Kuhn *et* al, 1991; Kuhn & Mollby, 1993) between the populations of bacteria in the different treatments.

The current gaps in knowledge about the characteristics effects and exposure patterns of existing chemicals must be filled. Given the large knowledge gaps about chemicals on the market, it is important to generate and assess information regarding their potential risks by means of appropriate legal and regulatory instruments, voluntary agreements and economic incentives. A scientific, rules-based approach requires reliable information on effects and exposure as the basis for risk management decisions, where such information is not available, more and more countries may take precautionary approach. Workers and the public must take a more active role in monitoring and contributing to chemical safety management discussions. To facilitate this, good data from research institutions on health and environmental impacts must be more widely available.

Policies need to be established to ensure that this information is reliable, and presented in a way that is useful to all potential users for decision-making, including workers, the general public and the government. Further, governments and industry should work toward educating the public with respect to chemical safety and, where feasible, provide public interest groups with resources that allow them to play the equitable role in policy discussions.

The half-life for glyphosate is between 3 -130 days (Eberbach & Douglas, 1983), hence the effect of glyphosate on the soil bacteria was still evident. Dimethoate has a half-life of about 3 - 5 days (Bohn, 1964), but its effect on bacteria growth was still evident after three weeks. This means, dimethoate had still not been degraded or the recovery rate for the microorganisms was very slow. The half-life of paraquat is not known, but it is known that paraquat is not easily degraded and sticks to the surface soil for a longer period, because it is not leached easily. Pots treated with this agrochemical showed the effects after three weeks of application.

The following recommendations are made from this study

- Agricultural biotechnology may offer a possible alternative that may permit higher yield levels without intensive use of agrochemicals.
- If the herbs to be eliminated can easily be uprooted, then it is more advisable to do manual elimination, especially in small farming systems.

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