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POTENTAIL USE OF BACTERIAL COMMUNITIES AS INDICATORS OF SOIL QUALITY IN SELECTIVE ECOSYSTEMS OF SRI LANKA

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ABSTRACT

Bacteria are the most populated and diverse organisms in soil microbial communities. Therefore, they are used as indicators of C substrate and soil pollution. The diversity of bacterial populations differed among soils collected from Siharaja forest and cultivated lands under rice and *mung* bean grown in Bathalagoda due to differences in quantity and the quality of C substrate. In agricultural soils, application of pesticides over a long period lead to development of pesticide tolerant bacteria. Elevated levels of heavy metals resulted by impurities in agrochemicals enhance the development of metal tolerance in bacteria. Therefore, investigations provide evidence that culturable fraction and total population of soil bacteria could be used as soil quality parameters. particularly in agricultural soils.

INTRODUCTION

The soil environment is highly populated and, as a result, organisms live interacting with each other, often developing into an assemblage called 'community' (Atlas and Bertha, 1993). As revealed by the information gathered using the latest technology, only about 1-5% of bacteria in soil is culturable (Watson and Heywood, 1995). Therefore, it has been predicted that the number of distinct populations of microorganisms in soil are several hundred folds higher than the estimations made previously based on the culturable fraction (Torsvik *et al.*, 1998; Amann, and Ludwig, 2000).

In general, microbial diversity varies over the space and the time responding to the differences existing in the growth regulating factors (Buckley and Schmidt, 2003). Availability of the substrate, moisture and oxygen, acidity and alkalinity, and salt and toxic substances control the growth of soil microorganisms. Since these factors varied between soil types and land use, diversity of microbial communities differed in soils at different scales. Variability in the diversity of microbial communities has been demonstrated at the highest scale between different geographical locations across the world (Cho and Tiedje, 2004). At the micro-scale level *i.e.* over a distance of few micro meters, differences have been documented between, on and around root, which is known as rhizosphere, and between root environment and the bulk soil (Joergensen, 2000). The rhizosphere soils are generally rich in substrates for microorganisms and consequently, inhabited by several folds higher populations than the bulk soil (Lu *et al.*, 2002).

In Sri Lanka, habitat diversity is tremendous due to presence of large number of soil types and differences in land use patterns. As a result, remarkable diversity is expected in soil microbial communities. The assessments carried out using biomarkers such as phospholipid fatty acids and DNA reflects the diversity of total communities (Torsvik *et al.*, 1998). On the other hand, adaptations of microorganisms to environmental changes could be assessed in detail using the culturable fraction. For example, the fraction of communities that could grow on recalcitrant substances provides evidence on the substrate availability, whereas resistance to heavy metals and pollutants such as pesticides provide an indication on the degree of exposure to contaminants.

This paper compiled information reported on the diversity of bacteria with respect to their response to carbon substrate, heavy metals and pesticides, associated with selective ecosystems of Sri Lanka.

1. Soil bacteria as an indicator of substrate availability.

Substrate versatility is demonstrated by soil bacteria for the source of C and the energy. In general, there is a positive relationship between population of soil microorganisms and its biomass with the amount of available C in soil. However, addition of fresh plant residues to soils enhances growth of cellulose decomposing bacteria and fungi. Therefore, the total population and cellulose decomposing populations are good indicators of available C.

Table 1: Organic C contents, composition of PLFAs and populations of cellulolytic and Azotobacter bacteria of soils collected from a disturbed area of Sinharaja forest. (Source: Rajapaksha, 2009/2010)

Landscape position	Organic C (%)	PLFAs (%) designated for			Cellulolytic	*Azotobacter*
		Fungi	Bacteria	Fungi / bacteria	(cfu x 10^5 g^{-1} soil)	
Shoulder	$3.5^b \pm 0.18$	7.9	48	0.16^b	18.9^{ab}	5.72^c
Mid slope	$3.8^{ab} \pm 0.11$	7.5	43	0.17^b	16.8^{ab}	18.04^a
Footslope	$4.4^a \pm 0.24$	10.8	45	0.24^a	69.1^a	9.29^b
Fernland	$2.4^c \pm 0.11$	10.3	48	0.21^{ab}	1.13^b	1.43^d

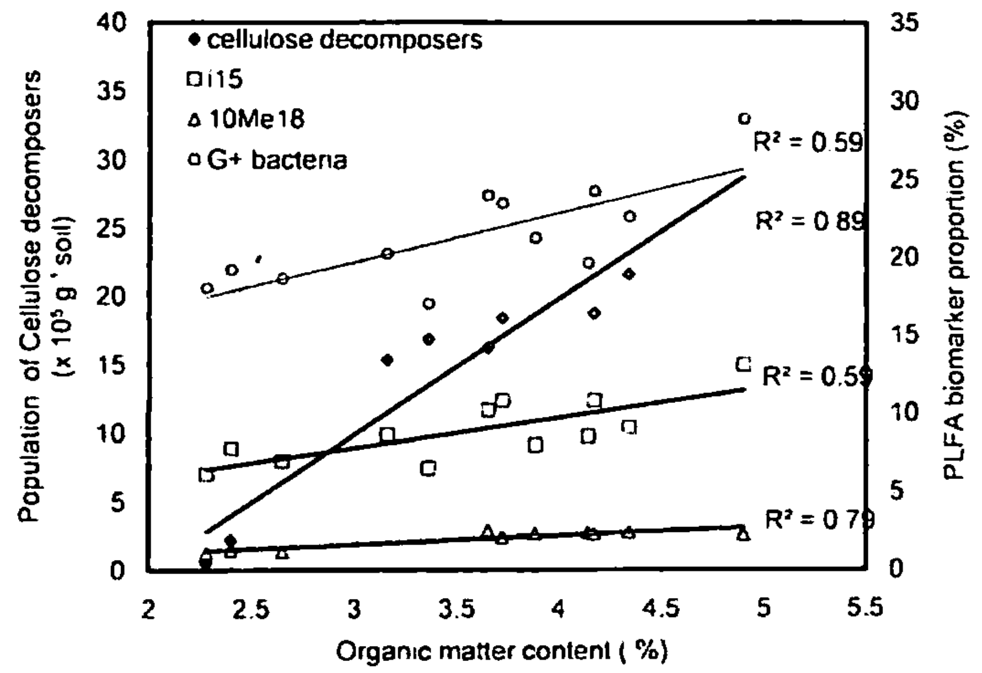

Fig. 1. Relationship between Organic C and microbial populations and selective PLFA biomarks on Sinharaja Forest.

A study conducted in Sinharaja forest revealed that the total population of bacteria and fungi and their biomarker PLFAs did not vary across the landscape significantly irrespective of the differences observed in the organic C contents (Table 1) (Rajapaksha, 2009/2010). However, in the culturable fraction, populations of *Azotobacter* and cellulolytic bacteria differed between landscape positions significantly partly due to differences in the substrate availability (Table 1). Positive correlations were observed for cellulolytic bacteria population, PLFA biomnarker i15, 10Me18 alone and total PLFA biomarkers that represent G+ bacteria and actinomycetes showed a positive correlation with organic matter content (Fig. 1) suggesting that their growth is governed mainly by the available C. However, the growth of fungi was not limited by the C. Positive correlations were observed for agricultural soil under intensive vegetable cultivation in Nuwara Eliya (Rajapaksha and Raufa, 1999).

Table 2. Phospholipid fatty acid biomarker contents (mol %) of microbial communities in rhizosphere soils of crops (n = 3) (Source: Rajapaksha and Ranasinghe, 2007)

Crop	Fungal	Actinomycetes	Bacterial	Fungi / bacteria
	(mol %)			
Beetroot	9.1^a	9.3	49	0.19
Carrots	5.9^b	10.7	49	0.12
Leeks	5.7^b	11.2	48	0.12
Lettuce	5.4^b	9.1	51	0.10

Rhizosphere characteristics varied with the plant species and crop varieties mainly due to the differences in the carbon substances secreted and leaked out from the plant roots (Bai *et al.*, 2000; Reichardt *et al.*, 2001). As a result, diversity of the microorganisms that colonize the rhizosphere also varied. There is evidence that microbial communities and culturable fractions of rhizosphere soils collected from vegetables grown in the Nuwara Eliya area differed significantly (Rajapaksha and Ranasinghe, 2007). A higher proportion of fungi, as indicated by designated phospholipids fatty acids (PLFA) was evident in the rhizosphere of beetroot in comparison to carrots, leeks and lettuce (Table 2). As a result, ratio between fungi to bacteria remained higher for the beetroot rhizosphere. Although mol % of bacterial PLFA indicate more or less similar values, the composition of PLFA representing G+ and G- bacteria differed between four crops.

Table 3: Predominant bacterial species associated with the rhizosphere of rice varieties and their ability to solubilize $Ca_3(PO_4)_2$ and production of indole acetic acid (IAA) (Unpublished data)

Sample ID	Rhizosphere	Taxonomy	$Ca_3(PO_4)_2$ Solubilization	IAA production
pdnKhBG30	*Kaluheenati*	*Paenibacillus apiarius*	+	
pdnKhBG31	*Kaluheenati*	*Pseudomonas chlororaphis*	+	
pdnShBGg32	*Suduheenati*	*Pseudomonas vesicularis*	+	+
pdnShBG33	*Suduheenati*	*Staphylococcus sciuri*	+	
pdnShBG34	*Suduheenati*	*Staphylococcus arlettae*	+	+
pdnShBG35	*Suduheenati*	*Staphylococcus cohini*	+	+
pdnSsBG36	*Suduru samba*	*Rhodococcus rhodochrous*	+	+
pdnSsBG37	*Suduru samba*	*Staphylococcus xylosus*	+	
pdnRaBG38	*Rathal*	*Staphylococcus gallinarum*	+	+
pdnRaBG39	*Rathal*	*Acidovorax facilis*	+	

Due to the differences in the composition of soluble C, predominant species associated with the rhizosphere would be different. In general, bacteria associated with the roots are known to enhance the plant growth and called 'plant growth promoting rhizo-bacteria' (PGPR). They are known to enhance plant growth through performing various functions such as di-nitrogen fixation, solubilizing of phosphate minerals, increasing nutrient acquisition, producing growth hormones, and controlling pathogens while living in the rhizosphere of a wide range of crops (Bacilio-Jimenez *et al.*, 2003; Sorense and Sessitch, 2007). Comparisons made for the rhizosphere of different rice varieties grown at Bathalagoda revealed significant differences among the predominant bacterial species in the root zone irrespective of the fact that they are grown in the same field (Table 3).

The composition of PLFA of total microbial communities of Sinharaja forest, rhizosphere soils of an improved rice variety grown under wetland conditions and maize and *mung* bean grown under upland conditions in Bathalagoda, rhizosphere soils of leeks, carrot, lettuce and beet root from Nuwara Eliya were subjected to principal component analysis. The variability in the composition of PLFAs of these soils is indicated in the Fig. 2. There is a clear difference between microbial communities of uplands and wetlands. This separation could be attributed to unique cultural practices imposed on wetland rice that lead to development of distinct microbial populations, particularly facultative and strict anaerobes. Among upland crops, soils of vegetable rhizosphere are separated from the rhizosphere of maize (OPM) and *mung* bean (OB, EM) of Bathalagoda. Soils from the forested tree species and fern of Sinharaja forest have been separated clearly from all agricultural soils indicating the greater impact of plant type and soil disturbances on microbial communities.

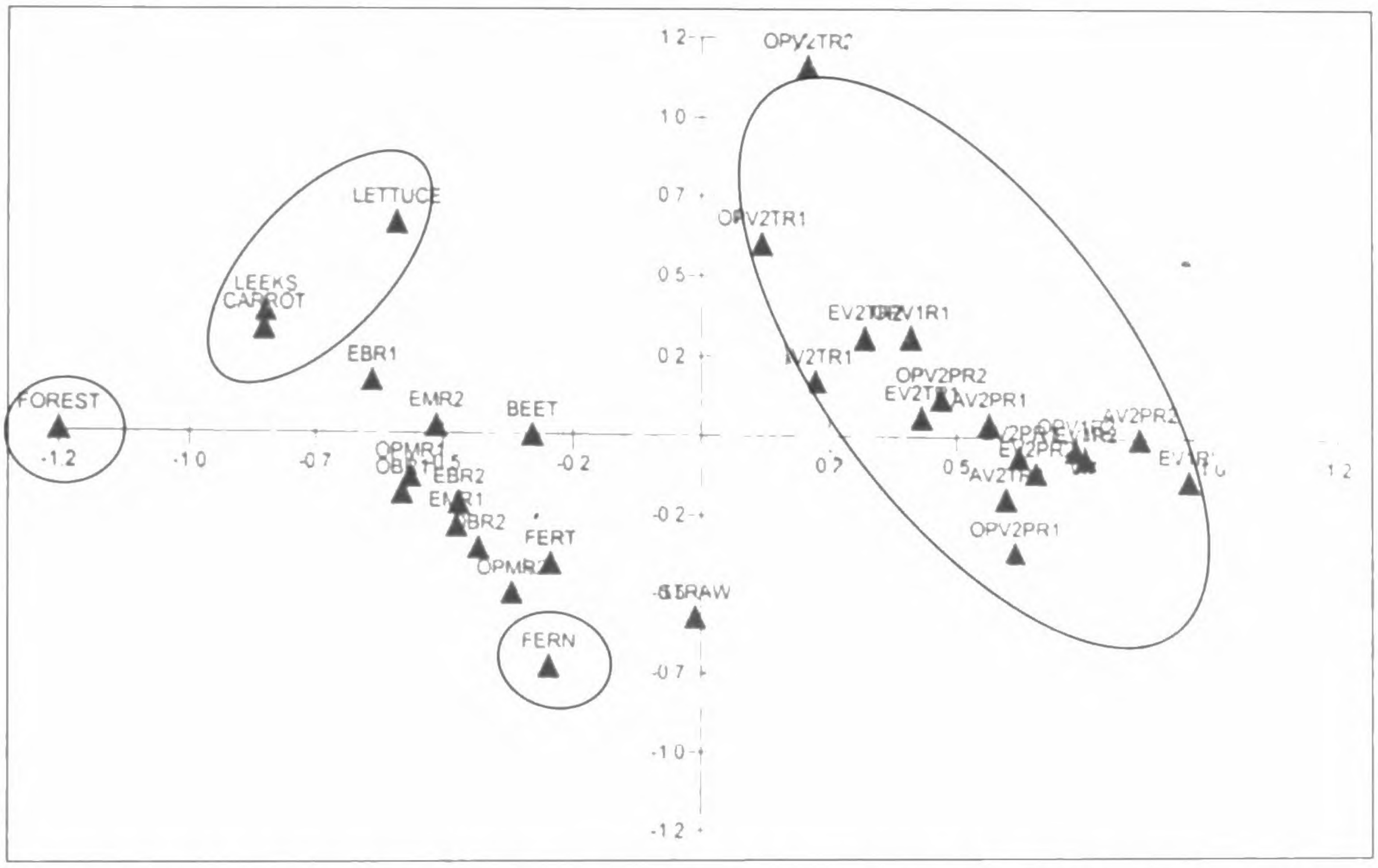

Fig. 2. Score plots generated form the mol percentage of phospipholipids futty acids extracted for Sinharaja forst up plan and wetlend rice in Bathalagoda and vegetable plots of Nuwara Eliya.

2. Soil bacteria tolerant to pesticides

Pesticides are toxic on non-target organisms and shift in microbial communities are well known over a short- or long-run (Ratcliff *et al.*, 2006). Pesticides, particularly herbicides, are often used in rice cultivation. Many wetland rice fields in the island are having a cultivation history of growing rice for more than a half century with high inputs. Differences in the culturable fraction of microorganism were reported between two fields that received fertilizer and pesticide inputs for 19 years and that did not receive those in Bathalagoda (Table 4) (Rajapaksha *et al.*, 2009). Results also revealed that a drastic reduction in the algae population by 96% has taken place due to cultivation of rice with chemical fertilizers. In addition to soil acidity, extensive use of herbicides may be a main reason for this as documented previously (Wegener *et al.*, 1985).

Table 4. Population of fungi and selective groups of soil bacteria in a fertilized and unfertilized rice grown soils in Bathalagoda (n=10)

Microbiological population (g^{-1} soil)	Agronomic practice		't' value
	Fertilized	Unfertilized	
Fungi (cfu x 10^5)	33 ± 8	10 ± 1.4	21 (***)
Aerobic bacteria (cfu x 10^8)	4.7 ± 0.9	3.7 ± 0.29	4.9 (**)
Actinomycetes (cfu x 10^4)	4.2 ± 1.6	8.7 ± 1.5	-22 (***)
Autotrophic nitrifiers (cells x 10^4)	3 ± 0.6	7 ± 1.1	-12 (***)
Azotobacter (cfu x 10^4)	1.6 ± 0.2	1.7 ± 0.17	-0.41
Azozpirillum (cfu x 10^4)	0.13 ± 0.2	1.8 ± 0.92	-28 (***)
Blue green algae (cells x10)	2 ± 0.57	52 ±12.4	-24 (***)

cfu : Colony forming units

*** and ** means are significant at P =0.001 and P =0.01, respectively

Investigation by Matiwalage and Rajapaksha, (2008) reported that percentages of bacteria resistant to paraquat and glyphosate in the soils treated with the respective herbicides remained significantly higher than the control even 45 days after applying herbicides (Fig. 3). Therefore, changes in the microbial communities would occur perhaps irreversibly due to repeated exposure to herbicides. The studies on soil bacteria further revealed that *Alcaligenes xylososxydans* and *Pseudomonas syringae* are predominant biodegrading agents of paraquat and *Aeromonas* and *Bacillus* as predominant glyphosate degraders in rice soils of Maha Illuppallama.

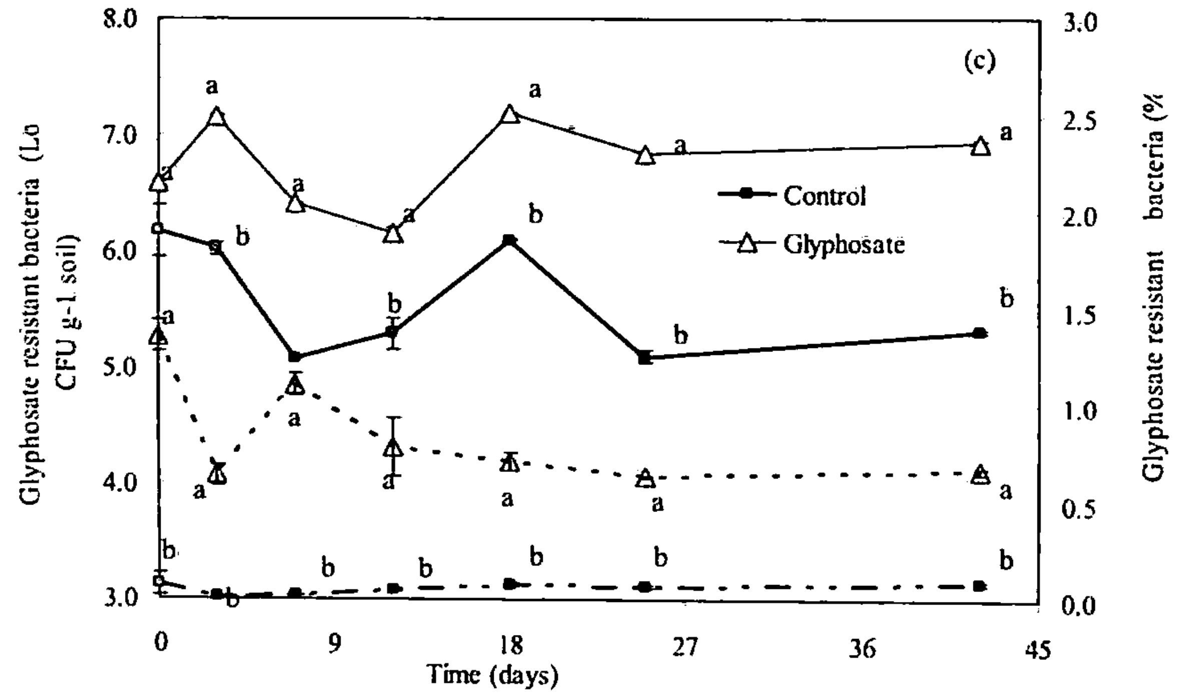

Fig. 3: Population dynamics in glyphosate resistant bacteria of control and herbicide treated soils (n =3). Solid lines indicate total bacteria population and broken lines indicate percentage of bacteria resistant to glyphosate

There is also evidence that bacteria of vegetable growing soils in Nuwara Eliya have developed resistance to pesticides due to repeated exposure (Scynthya and Rajapaksha, 2012). In this study, the highest percentages of bacteria tolerant to carbofuran, metribuzin and mancozeb were 45.7 %, 42.0 % and 0.88 %, respectively. Results further revealed that the bacterial tolerance to all three pesticides increased as the length of cultivation history increased. The lowest tolerance to mancozeb was exhibited by a soil collected from an organic farm (0%) and a virgin soil (0.01%). This suggests that the mancozeb was the most toxic pesticide and repeated exposure to it resulted in bacterial communities predominant with mancozeb tolerant bacterial populations.

3. Soil bacteria tolerant to heavy metals

Elevated concentrations of heavy metals are known to affect microbial populations adversely. Some species of microorganisms are inherently tolerant to heavy metals whereas some develop tolerance when they are exposed repeatedly (Yamamato *et al.*, 1985; Diaz-Ravina and Baath, 1996). This phenomenon has been demonstrated for the microbial communities of sewage sludge amended soils (Oorts *et al.*, 2005) and manure amended soils (Huysman *et al.*, 1999).

In Sri Lanka, accumulation of heavy metals above the permissible levels in selective lands in Nuwara Eliya was verified based on soil and plant analysis (Premarathna *et al.*, 2005). The inherent tolerance of bacteria to Cu demonstrated for the culturable fraction of bacteria in a virgin montane forest in Nuwara Eliya (Fig. 4) (Dandeniya and Rajapaksha, 2008).

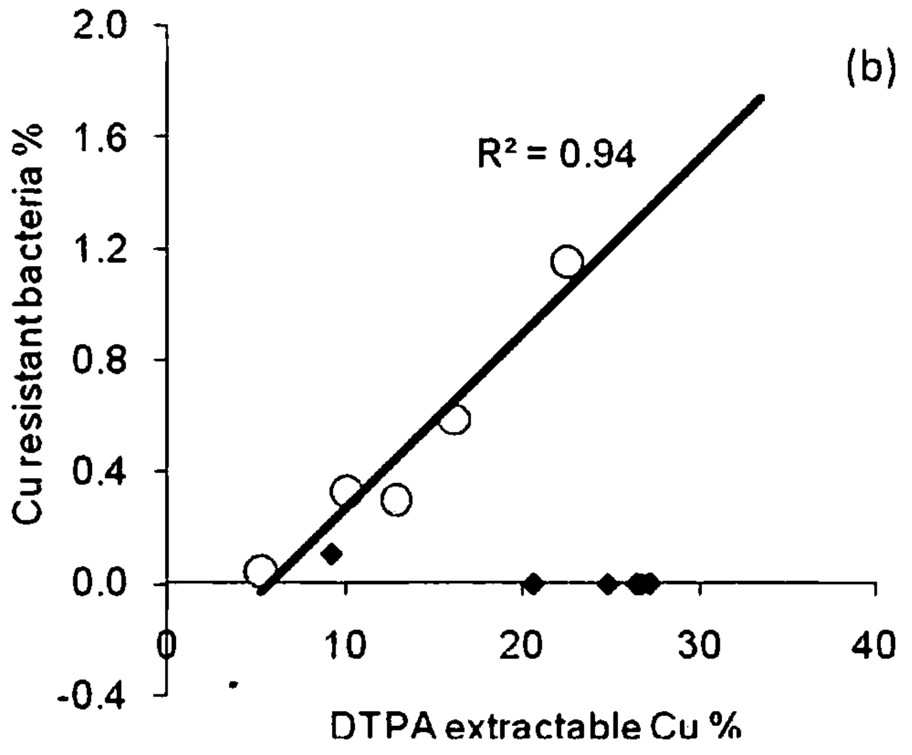

Fig. 4 Relationship between DTPA extractable Cu fraction and percentage of Cu resistant bacteria in an undisturbed montane forest soils

In vegetable growing soils in the same region, long term cultivation has resulted in irreversible changes in the microbial communities (Rajapaksha and Raufa, 1990) and shifting of bacterial communities towards a higher fraction of metal tolerant species (Rajapaksha, 2010; Rajapaksha and Raufa, 1999). These studies revealed that metal tolerance as expressed by IC_{50}(50% inhibition) levels and bacterial tolerance to metals increase with the increase in the metal concentration and duration of cultivation (Rajapaksha, 2010). The IC_{50} value of bacteria for Cd was -6.37 log Cd concentration in the virgin soil and it remained significantly lower than that of a 22 years cultivated soil (-4.03 log Cd concentration). This study further revealed that the bioavailable metal concentrations of the investigated soils are not high enough to induce metal tolerance in fungi. It has been previously documented that fungi are less affected by the elevated metal concentrations than the bacteria (Rajapaksha *et al.*, 2004).

In another study, application of 25 mg Cd and 500 mg Zn kg^{-1} soil to an Entisol collected from Peradeniya resulted in 88% and 96% reduction in the bacteria population and mycorrhizal colonization, respectively (Fig. 5) (Rajapaksha and Amarakoon, 2011). This study further revealed that toxicity of heavy metals could be reduced to some extent if the pH of the soil is closer to neutral.

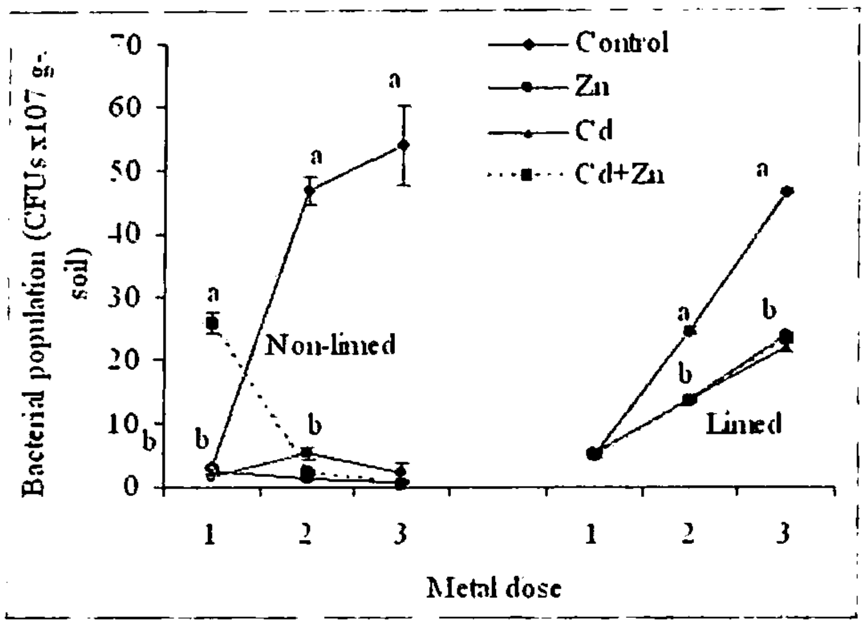

Fig. 5. Changes in bacterial population due to addition of Cd and Zn (Dose 1, 2 and 3 are 2.5 mg Cd and 50 mg Zn kg^{-1} soil a and their five and 10 folds, respectively).

CONCLUSIONS

The diversity of bacterial communities in forest and cultivated ecosystems and predominant bacteria in different rice varieties are governed by the availability of C substrate. The differences are partly due to the differences in the soil properties and carbon availability of the plant root systems. Long term cultivation with high inputs of agrochemicals has led to the development of bacterial communities predominant with resistance to pesticides and heavy metals.

REFERENCES

Amann, R. I., and W. Ludwig. 2000. Ribosomal RNA-targeted nucleic acid probes for studies in microbial ecology. FEMS Microbiol. Rev. 24: 555-565.

Atlas, R. M., and R.Bartha.1993. Microbial communities and ecosystems. Microbial ecology- Fundamentals and applications. The Benjamin / Cummings publishing company.130-162.

Bacilio-Jimenez, M., S. Aguilar-Flores, E. Ventura-Zapata, E. Perez-Campos, S. Bouquelet, and E. Zenteno. 2003. Chemical characteristics of root exudates from rice (*Oryza sativa*) and their effects on the chemotactic of endophytic bacteria. Plant Soil. 249:271-277.

Bai, Q., A. Gattinger, L. Zelles. 2000. Characterization of microbial consortia in paddy rice soil by phospholipids analysis Microbial ecology. 39: 273 – 281.

Buckley, D. H., and T.M. Schmidt. 2003. Diversity and dynamics of microbial communities in soils from agro-ecosystem. Environ. Microbiol. 5:441-452.

Cho, J., and J.M. Tiedje. 2004. Biogeography and degree of endemicity of fluorescent Pseudomonas strains in soil. Appl. Environ. Microbiol. 66:448-456.

Dandeniya, W. S., and R. M. C. P. Rajapaksha. 2008. Copper fractions and selective microbiological properties of intensively cultivated Ultisols of Nuwara Eliya. Journal of the National Science Foundation. 36: 307-313.

Diaz-Ravina, M., and E. Baath. 1996. Development of metal tolerance in soil bacterial communities exposed to experimentally increased metal levels. Appl. Environ. Microbiol. 62:2970-2977.

Huysman, H., W. Verstraete, P.C. Brookes. 1994. Effect of manuring practices and increased copper concentrations on soil microbial populations. Soil Biol. Biochem. 26:103-110.

Joergensen, R. G. 2000. Ergosterol and microbial biomass in the rhizosphere of grasland soils. Soil Biol. Biochem. 32:647-652.

Lu, Y., A.Watanabe, M. Khumura. 2002. Contribution of plant-derived carbon to soil microbial biomass dynamics in a paddy rice microcosm. Soil Biol. Fertil. 36, 136-142.

Matiwalage, I. N. and R. M. C. P. Rajapaksha. 2008. Toxic effect of paraquat and glyphosate on bacteria of wetland rice soil. Journal of the Soil Science Society of Sri Lanka. 20: 9-16.

Oorts, H. Bronckaers, and E. Smolders 2005. Discrepancy of microbial response to elevated copper between freshly spiked and long term contaminated soils. Environ. Toxicol. Chem. 25, 845-853.

Premarathna, H. M. P.L., G. M. Hettiarachchi, S. P. Indraratne. 2005. Accumulation of cadmium in intensive vegetable growing soils in the up country. Trop. Agric. Res. 17:93-103.

Rajapaksha, R. M.C.P. and I. D. Amarakoon. Response of lettuce (*Lactuca sativa* L.) and rhizosphere biota to successive additions of Cd and Zn. Communications in Soil Science and Plant Analysis. In press

Rajapaksha, R. M. C. P. 2009/2010. Soil microbial diversity in a relatively disturbed area of Sinharaja forest. The Sri Lanka Forester. 32-33:27-39.

Rajapaksha, R. M. C. P. 2010. Heavy metal tolerance of culturable bacteria and fungi in a long-term cultivated tropical ultisol. European Journal of Soil Biology. 47:9-15.

Rajapaksha, R. M. C. P. and M. C. F. Raufa. 1999. Microbiological properties of an intensively cultivated land in up country wet zone of Sri Lanka. Journal of the Soil Science Society of Sri Lanka. 11:22-28.

Rajapaksha, R. M. C. P. and S. Ranasinghe. 2007. Arbuscular mycorrhizae associated with exotic vegetables grown in the up country wet zone of Sri Lanka. Journal of the Soil Science Society of Sri Lanka. 19:1-9.

Rajapaksha, R. M. C. P., M. A. Tobor-Kapoin, and E. A. Baath. 2004. Metal toxicity affects fungal and bacterial diversity differently. Appl. Environ. Microbiol. 70:2966-2973.

Rajapaksha, R. M. C. P., U. K. Wijekoon, and P. H. Yasanayake. 2009. Soil microbial populations as affected by intensive cultivation of exotic vegetables in Nuwaraeliya and rice in Bathalagoda. Sri Lankan Journal of Agricultural Science. 46:1-14.

Ratcliff, A. W., M.D. Busse, and C. J. Shestak. 2006. Changes in microbial community structure following herbicide (glyphosate) additions to forest soil. Appl. Soil Ecol. 34:114-124.

Reichardt, W., G. Mascarina, B. Padre, J. Doll. 1997. Microbial communities of continuously cropped irrigated rice fields. Appl. Environ. Microbiol. 63(1): 233-238.

Sorensen, J., and A. Sessitch. 2007. Plant associated bacteria-lifestyle and molecular interactions. *In* Modern Soil Microbiology (K. M.Van Elas, J. K. Jansson and J. T. Trevors . eds) CRC Press. 211-236.

Torsvik, V., F.L. Daae, R.A. Sandaa, L. Ovreas. 1998. Novel techniques for analyzing microbial diversity in natural and perturbed environments. J. bacteriol. 64:53-62.

Watson, R. T., and V. Heywood.1995.Global biodiversity assessment UNEP by Cambridge University Press. 1-140.

Wegener, K.E., R. Aldag, and B. Meyer.1985. Soil algae: Effects of herbicides on growth and C_2H_2 reduction (nitrogenase) activity. Soil Biol. Biochem. 17:641-644.

Yamamato, H., K. Tatsuyama, and T. Uchima. 1985. Fungal flora of soil polluted with copper. Soil Biol. Biochem. 17: 785 – 790.