BIOASSAY OF BIOSOLIDS IN AN OPERATIONAL SCALE FIELD TRIAL

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Abstract

Soil microbial biomass was used in the last two years of a five-year, randomized replicated field trial as a bioassay for potentially harmful substances in biosolids-amended soil. The purpose of using a bioassay was that it was not necessary to know the identity of harmful substances *a priori*. It answered the question "what about all the chemicals you do not monitor?".

Anaerobically digested biosolids were spread at operational rates (about 8 to 10 tDS/ha) as lagooned liquid and as dewatered cake. The trial was farmed as an arable rotation. The impact on soil biomass was studied by collecting soil samples at 2 or 3 monthly intervals. The size of the biomass and its metabolic quotient were measured.

Soil microbial respiration quotient proved very sensitive. As expected it showed the stress of seasonal climatic variation. One microbial stress event was detected across all the plots, irrespective of whether biosolids had been applied or not. The field activity diary revealed the stressor was an application of selective herbicide that had been applied two days before sampling. The metabolic quotient had recovered by the next sampling. The size of the biomass increased after biosolids were applied and then decreased again as the organic matter and nutrients were used. The metabolic quotient was unaffected by biosolids showing that no constituent or cocktail of constituents in the biosolids stressed the microbial biomass. Crop yield data enabled the construction of a reliable model for predicting fertiliser replacement value.

Key words

Agronomy, bioassay, biosolids, cocktail, fertiliser value, potential pollutant, soil microbial respiration quotient, substance of concern

Introduction

It is possible to find in sewage sludge and biosolids measurable amounts of most of the chemicals used in society. However, mere presence is not the question; the question should be whether there is unacceptable risk. As Paracelsus said 500 years ago "the dose makes the poison". Sewage sludge might be a source but is the concentration sufficient and is there a pathway to deliver a harmful dose to a receptor? In the case of sewage sludge produced in developed countries and used or disposed in accordance with today's rules, the consensus of informed scientific opinion is that the answer to this question is "no". Through a combination of hazardous substances regulations, which have eliminated some chemicals, changes in industrial practices and restricting discharges from factories, the concentrations of hazardous substances in sewage are dramatically

less then they were a few decades ago. Despite this the "urban myth" of "heavy metals" in sewage sludge persists.

A report that a "substance of concern" has been detected in biosolids has on occasions led to devastating consequences such as the collapse in confidence by farmers' organisations in Sweden following a report by the Environmental Protection Agency (de Wit, 2000) that brominated flame retardants had been found in biosolids. However they did not take the trouble or intellectual rigour to estimate risk. When risk was estimated subsequently, biosolids were found to be of no concern, but the harm to land application had been done.

In addition to the question of single substances or families there is the question of the cocktail effect, i.e. might a mixture be more potent than the sum of its components? There can be many motives for fanning such controversies, such as not liking the smell when biosolids are spread, a fear of science, seeking research funding, etc.

A bioassay has the potential to sense whether hazardous substances are present at concentrations that pose risk. It is not necessary to know the identity of the substance in advance. Like a food taster in ancient times, a king did not need to know the identity of the poison; the bioassay gave a dose related response. If a taster died or became ill, the identity of the poison and the poisoner could be investigated. Soil microbial respiration quotient (SMRQ) is a sensitive bioassay for chemical challenges. The SMRQ (the respiration rate per unit mass of biomass) increases when the biomass is stressed.

Biochemical processes in soil are mediated by the soil microbial biomass (see for example Powlson *et al*., 2011). The size of the microbial biomass normally increases after available carbon substrates have been added, e.g. bulky organic manures, or crop residues. Soil microbial activity can also be affected by soil temperature, moisture, pH, agrochemicals and heavy metals or organic contaminants, which could accumulate in soil following the long-term application of enriched organic manures including biosolids and livestock wastes.

Materials and Methods

The field experiment was established in 1993 at Model Farm, Watlington in Oxfordshire. Day to day farming was undertaken by the farmer, which ensured that operations were timely and appropriate. The soil was free draining with a sandy clay loam texture overlying Greensand. The P, K, Mg and lime status were good (Table 1).

Table 1: **Properties of soil at the experimental trial site** (means of samples from control plots sampled on 26 October 1994)

Parameter		Value	Parameter		Value
pH		6.6	Extractable P	(mg l^{-1})	88.6
Conductivity	(dS m^{-1})	2.1	Extractable K	(mg l^{-1})	228
Cation exchange capacity	(me $100g^{-1}$)	15.0	Extractable Mg	(mg l^{-1})	129
Organic carbon	(%)	1.2	Total Zn	(mg l^{-1})	41.3
			Total Cu	(mg l^{-1})	15.1
Particle Size Distribution:			Total Ni	(mg l^{-1})	18.7
Sand	(%)	52.6	Total Cd	(mg l^{-1})	0.54
Silt	(%)	27.9	Total Pb	(mg l^{-1})	20.0
Clay	(%)	19.5	Total Cr	(mg l^{-1})	28.1

The biosolids used for the trial were lagoon-thickened liquid mesophilic anaerobic digested (MAD) biosolids (TL) from Perry Oaks biosolids treatment works, and dewatered MAD biosolids (TC) also from Perry Oaks or from Maple Lodge wastewater treatment works (Table 1). Biosolids were applied at rates that were operationally typical at the time, i.e. TL was applied at 100 m^3 ha^{-1}, and TC at 45 t ha^{-1} (fresh weight); approximately 8 and 10 tDS ha^{-1} respectively. Application dates were February 1993, September 1993, September 1994, August 1995 and September 1996 (five cropping cycles) using calibrated field application equipment (Figure 1).

Figure 1: **Biosolids application equipment**

Table 2 shows some of the chemical properties of the biosolids used in the trial. Both Perry Oaks (1.8 million p.e.) and Maple Lodge (0.8 million p.e.) treat wastewater from domestic and industrial customers. Each has primary settlement and diffused air activated sludge; the feed to the digesters is approximately 60% primary to 40% waste activated sludge. The wastewater that generated the sludge that became the lagooned biosolids was approximately 6 years older than that which became the cake biosolids. This was reflected in the higher metals concentrations.

Note, the metals are considerably less now because of continued source control and phosphate is greater because of phosphate removal at the WwTWs.

The trail area was cropped in blocks with an arable rotation consisting of winter wheat, spring barley, and a break crop, which was either field beans, oilseed rape or winter linseed depending on the year (Figure 2).

Figure 2: Aerial view of the trial site: the arable plots are right of centre

The design within each block was a factorial combination with ammonium nitrate mineral nitrogen fertiliser. Although operational and practical constraints limited the amount of randomisation that was possible for the biosolids treatments, the treatments were replicated three times and the inorganic nitrogen applications were randomised within the blocks. The winter wheat block was selected for microbial sampling; the treatments selected were:

T0/N0, T0/N2, TL/N0, TL/N2, TC/N0 and TC/N2
(T0 = control, no biosolids; TL = liquid lagoon thickened biosolids; TC = dewatered biosolids
N0 = control, no inorganic nitrogen; N2 = 100 kg N ha^{-1})

Table 2: Some chemical properties of the biosolids used in the trial

Parameter		Lagooned Liquid TL				Dewatered Cake TC			
		1993	1993	1994	1995	1993*	1993*	1994	1995
Dry solids	(%)	7.4	6.9	7.6	7.9	22.7	21.7	22.6	24.3
Volatile solids	(%)	68.8	64.4	65.2	64.7	68.1	67.4	69.7	69.9
pH		7.4	7.3	7.0	7.3	8.0	7.9	8.0	8.3
NH_4-N	(%)	1.4	1.6	1.5	1.5	0.85	0.8	1.1	0.9
Total N	(%)	4.1	3.8	4.9	5.3	5.4	4.0	4.4	5.4
Total P	(%)	2.7	2.5	2.9	2.9	1.7	2.7	2.0	2.5
Zn	(mg kg^{-1})	1141	1067	963	913	700	540	805	710
Cu	(mg kg^{-1})	830	790	792	764	734	723	762	683
Ni	(mg kg^{-1})	203	179	157	111	65	40	82	49.5
Cd	(mg kg^{-1})	25.2	20.2	12.0	10.0	5.1	1.8	9.5	6.6
Pb	(mg kg^{-1})	431	460	514	390	228	183	380	360
Cr	(mg kg^{-1})	466	413	286	221	150	93	164	108
Hg	(mg kg^{-1})	5.0	4.6	4.3	4.4	3.6	3.8	4.1	2.8
Mo	(mg kg^{-1})				7.9				7.7
As	(mg kg^{-1})				4.7				3.8
Se	(mg kg^{-1})				3.7				2.4
F	(mg kg^{-1})				176				182

* cake from Maple Lodge; all other biosolids were from Perry Oaks

The soil samples for microbial biomass analysis were taken on 15 September 1994 (before biosolids application and ploughing), 26 October (after biosolids and soil cultivation) and 13 December 1994, 20 February 1995, 24 April, 13 June and finally on 31 August 1995. Soils were sampled to a depth of 15 cm and 10 soil cores were collected from the centre of each replicate plot in the pattern of a 'W' and combined. Soil samples were stored in loosely tied polyethylene bags to allow gaseous exchange, but avoiding desiccation. They were taken to the laboratory in cool boxes with minimum delay. At the laboratory they were maintained at 4 °C in the dark until further treatment.

For the 1994/95 season, biosolids were applied on 17 September 1994 at 100 t ha^{-1} as liquid or 45 t ha^{-1} as cake. The plots were ploughed to 20 cm on 27 September, power harrowed on 3 October and again the next day, Dutch harrowed twice on 5 October and drilled the same day with Consort winter wheat at 375 seeds m^{-2} with methiocarb at 220 g active ha^{-1}. Agrochemicals comprised:

- herbicides isoproturon (2.5 l ha^{-1}) and Trifluralin (960 g ha^{-1}) on 24 October ‘94;
- fungicides Hispro (0.5 kg ha^{-1}) on 4 April ’95; Tilt (0.35 l ha^{-1}) and Tebuconazole (0.35 l ha^{-1}) on 23 May ’95; Plover (0.15 l ha^{-1}) and MBC (0.5 l ha^{-1}) on 21 June ‘95
- insecticide Ambush (0.25 l ha^{-1}) on 2 November ‘94
- growth regulators Holdup and Permeate were applied at 2.8 l ha^{-1} on 3 April ‘94.

The first nitrogen was applied on 30 March '95 at 50 kgN ha^{-1} and a second application was made on 19 April '95 at 50 or 100 kgN ha^{-1} for the N100 and N150 treatments respectively. The trial was harvested on 2 August '95 with a plot combine harvester and the straw removed from the experimental plots.

For the 1995/96 season, mustard was drilled on 18 August 1995 to take up residual mineral nitrogen and act as a green manure. Biosolids were applied two days later at 100 t ha^{-1} as liquid or 45 t ha^{-1} as cake. This is earlier than would have been normal for a spring sown cereal but was necessary to coincide with the winter wheat application in the same experimental area; nitrogen loss from the biosolids was reduced to some extent by the mustard. The plots were ploughed to 20 cm on 4 January '96, Dutch harrowed three times on 19 March '96 and drilled the next day with Chariot spring barley at 400 seeds m^{-2} with methiocarb at 220 g active ha^{-1} and rolled 2 days later. Agrochemicals comprised:

- herbicides Ally (metsulfuron-methyl 20 g ha^{-1}) and MCPA (1 l ha^{-1}) on 4 June '96
- fungicides Colstar (fenpropimorph + flusilazole 1 l ha^{-1}) on 1 June '96, and Glint (fenpropimorph + propiconazole 1 l ha^{-1}) on 24 June '96.

Nitrogen was applied on 29 March '96. A helium kite was used to scare birds. The trial was harvested on 8 August '96 with a plot combine harvester and the straw removed from the experimental plots.

The soil samples were sieved to pass 5.6 mm and examined for plant roots and soil animals, which were removed. The water holding capacity (WHC) was determined following the procedure of Harding and Ross (1964) using a composite sample of soil representative of each experimental treatment. Soil moisture status was adjusted to 40% of the WHC by the addition of appropriate volumes of water to dry soil or, in the case of wetter soil, carefully drying soil samples in open trays on the laboratory bench avoiding soil surface desiccation by regular turning. Moisture adjusted soils were incubated at 25 °C in the dark for a period of 7-10 days to allow equilibration prior to the determination of soil microbial biomass content and soil respiration rate. The moisture content of each soil was measured again at the end of the equilibration period to check for water loss during the incubation phase.

A sub-sample of each soil was air dried at 30 °C and ground to pass 2 mm. This was used for measuring the pH value (MAFF 1986), the organic carbon content (based on the loss on ignition at 430 °C) (Davies 1974) and the electrical conductivity (MAFF 1986). Soil samples collected on the 26 October were also analysed for their aqua regia extractable concentrations of Zn, Cu, Ni, Cd, Pb, Cr and Hg by the Thames Water laboratory using inductively-coupled plasma emission spectroscopy.

Soil microbial biomass was measured on each soil in duplicate. Sub-samples of soil weighing 25 g (fresh weight) were transferred into 100 cm^3 glass bottles with screw caps in quadruplicate. Two bottles were sealed and stored at 4 °C in the dark. The remaining bottles were exposed to

chloroform fumigation for 24 h following the method of Jenkinson and Powlson (1976), but using chloroform stabilised with 25 ppm amylene. After evacuating the fumigated soils, both the fumigated and the unfumigated samples were extracted with 50 cm^3 of 0.5 M K_2SO_4 solution for 30 min (Tate *et al*. 1988) in the glass bottles to minimise possible errors. The soil extracts were filtered through GF/C filter paper and the total organic carbon content (TOC) of the filtrate was determined using a Sartec 190 TOC analyser. The mean value of the non-fumigated samples was subtracted from the mean value of the fumigated samples giving the amount of organic carbon released by fumigation. This figure was converted to biomass carbon (C_{mic}) by multiplying by 2.22 (Wu *et al*. 1990).

Soil respiration rate was measured from the efflux of CO_2 using an infrared gas analyser (IRGA)(Smith and Hadley, 1990). Ambient air entered the gas manifold through an intake mounted externally to the laboratory at a height of approximately 6 m above the ground. The air was passed through a large equilibration chamber (125 l) with a laboratory pump and distributed to 24 independent gas lines regulated by in-line needle valves and flowmeters. Flow rates through each air line were maintained at 200 cm^3 min^{-1}. A rotary switch mechanism, actuated by an electronic programmable timer, controlled two-way solenoid valves located in each sample line. The position of the valves determined which air line was sampled by the IRGA. The timer was set so that each sample line was monitored for 6 minutes in sequence. Ambient air carried to the IRGA in the reference line was conducted through the same flow path as the sample gas, but was independent of the switching manifold.

Duplicate portions of each preincubated and moisture adjusted soil, weighing approximately 150 g (fresh weight), were transferred into specially constructed glass microcosms (140 mm high, 50 mm diameter). The soil was placed on an integral coarse sintered glass platform positioned above the air inlet at the base of the microcosm. Filled microcosms were sealed with neoprene stoppers fitted with an air outlet, and were placed upright in a temperature controlled incubator maintained at 25 °C in the dark. The rate of moisture loss from the soil samples was minimised by air spargers containing 200 cm^3 of sterile deionised water positioned within each air line. Condensation traps were also installed to avoid fouling of the gas switching system or IRGA.

Carbon dioxide evolution was monitored for a minimum of 12 h. The mean concentration of CO_2 in air due to soil respiration was measured from when a steady state had been reached until the end of the monitoring period. The IRGA was calibrated regularly with prepared gas mixtures of CO_2. The data were analysed statistically by analysis of variance using the Genstat computer programme (Genstat 5 Committee, 1987).

Results and Discussion

Crop yield

Figures 3 and 4 show that biosolids increased the yields of winter wheat and of spring barley. In this trial, 100 kgN ha^{-1} was the optimum complimentary mineral nitrogen fertiliser for winter wheat; spring barley reached its optimum yield with biosolids alone.

The yield data from the four cropping cycles and from the grass-clover plots enabled the construction of a model for advising farmers the fertiliser replacement value that their biosolids treatments would provide. It took account of the timing and method of application, the soil type (in terms of leaching loss potential) and the chemical composition of the biosolids. The model built on the national fertiliser recommendations, with an allowance for the climate in the region where biosolids were being used.

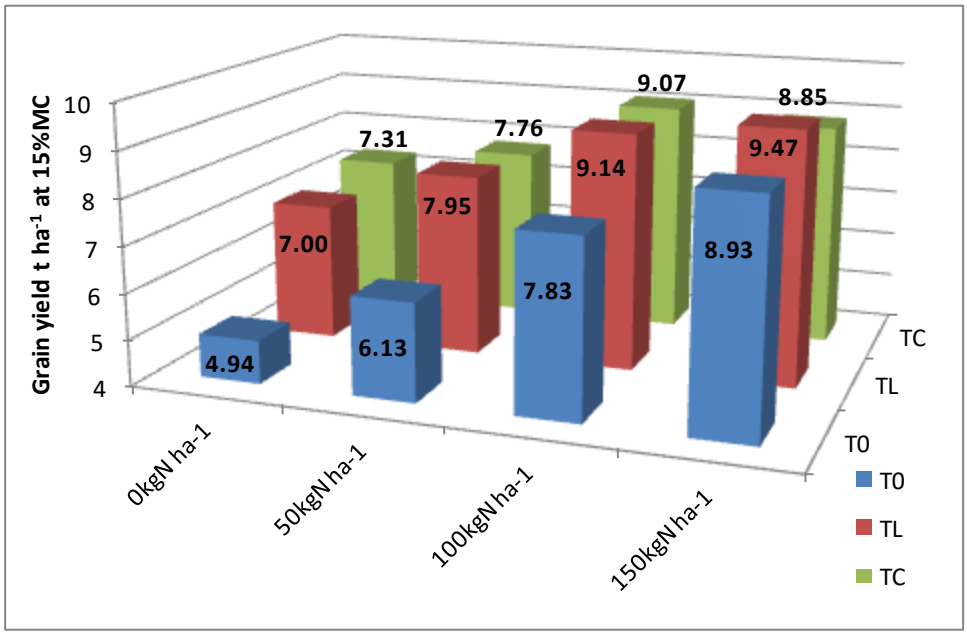

Figure 3: Effect of biosolids and/or ammonium nitrate fertiliser on winter wheat yield

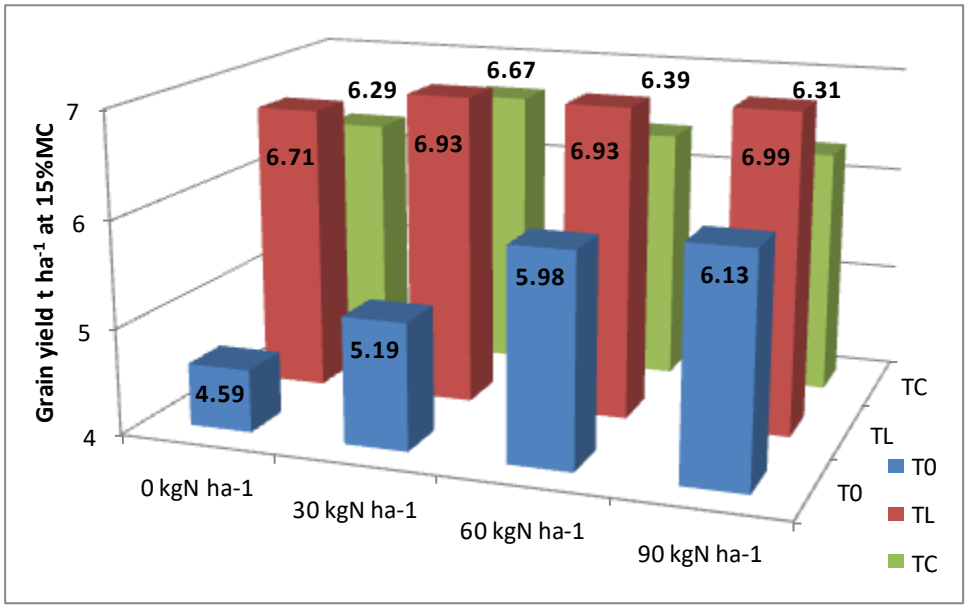

Figure 4: Effect of biosolids and/or ammonium nitrate fertiliser on spring barley yield

Soil chemical properties

A number of soil chemical properties were significantly different from the untreated control as a result of the biosolids applications although the differences were relatively small (Table 3). For example, biosolids increased the organic carbon content of soil by approximately 17 % compared to the control and the pH value of sludge-treated soil was reduced by 0.2 - 0.3 pH units. No main effect of biosolids application on mean soil moisture content was observed although ANOVA indicated a significant (P=0.01) interaction with sampling date. The soil moisture content of biosolids treated soils was greater than control plots, except for the June sampling date when the opposite trend occurred because of the greater transpiration of the heavier yielding crops (Figure 5). There was no significant effect (P>0.05) of the experimental treatments on soil electrical conductivity.

Biosolids increased the concentrations of Zn (P=0.001) and Cu (P=0.035) significantly compared with the untreated control although no significant effects on the amounts of the other elements examined were detected by ANOVA (Table 3). On average, soil Zn content increased by approximately 12 % and Cu content was raised by 24 % relative to the control. Nitrogen fertiliser treatment did not change any of the soil analysis data significantly. There was no difference in the rate of organic matter degradation in biosolids-treated soil compared to the unamended control.

Soil pH and electrical conductivity varied significantly (P<0.001) with sampling time. Soil pH was significantly greater in March compared with the pH values measured at the other sampling times which were not significantly different from each other. The electrical conductivity of soil was significantly less during the spring and summer period compared with soil sampled during autumn and winter. These trends are typical of farmed soils.

Table 3: Effect of biosolids on mean(1) soil chemical properties

Parameter		T0	TL	TC	cv (%)	$LSD_{0.05}$
pH value		6.8a	6.6b	6.5b	3.6	0.12
Electrical conductivity	(dS m^{-1})	2.1a	2.1a	2.1a	5.4	ns
Organic carbon	(%)	1.20b	1.42a	1.35a	17.9	0.08
Moisture content	(%)	12.8a	13.4a	13.3a	28.7	ns
Total Zn	(mg kg^{-1})	43.8b	50.5a	47.5ab	7.9	4.7
Total Cu	(mg kg^{-1})	13.2b	16.6a	16.2ab	15.2	3.3
Total Ni	(mg kg^{-1})	19.7a	20.2a	19.6a	7.1	ns
Total Cd	(mg kg^{-1})	0.33a	0.42a	0.58a	45.8	ns
Total Pb	(mg kg^{-1})	23.6a	29.8a	27.2a	23.1	ns
Total Cr	(mg kg^{-1})	27.8a	28.4a	28.1a	9.7	ns
Total Hg	(mg kg^{-1})	0.05a	0.08a	0.7a	30.2	ns
Total As	(mg kg^{-1})	11.9a	12.8a	12.3a	7.2	ns
Total Se	(mg kg^{-1})	0.2a	0.2a	0.2a	2.7	ns
Total F	(mg kg^{-1})	46.8a	39.5a	46.5a	28.0	Ns

(1) n = 24 for pH, EC, organic carbon and moisture content; n = 6 for the potentially toxic elements.

Soil microbial biomass and activity

Neither biosolids nor nitrogen fertiliser affected the soil microbial biomass carbon content (C_{mic}) (P=0.34 and P=0.938, respectively) nor (as Table 4 shows)the C_{mic} : C_{org} ratio (P=0.096 and 0.954, respectively) significantly (ANOVA). C_{mic} was influenced more by the changes in soil properties that occurred with time than by the direct effects of biosolids application on soil conditions. Thus, the C_{mic} increased significantly (P<0.001) with increased C_{org} and soil moisture content, which were in turn correlated (r = 0.36**). There was some indication in the data that C_{org} might be the principal factor controlling the biomass content of soil but it was not possible to discriminate this statistically.

Table 4: Effect of sewage sludge application on mean soil microbial properties (n = 24)

Microbial parameter	T0	TL	TC	cv (%)	$LSD_{0.05}$
Biomass (mg C_{mic} kg^{-1} dry soil)	195.5a	209.2a	213.2a	27.8	NS
Respiration (mg CO_2-C kg^{-1} dry soil h^{-1})	0.220b	0.247ab	0.274a	28.4	0.032
C_{mic} : C_{org} (%)	1.66a	1.49a	1.58a	25.1	NS
Metabolic quotient (mg CO_2-C h^{-1} $g^{-1}C_{mic}$)	1.14b	1.22ab	1.38a	30.7	0.19

As Table 4 shows, the mean C_{mic} and the mean C_{mic} : C_{org} ratio for T0, TL and TC were not significantly different (P>0.05 ANOVA). The CO_2 efflux (respiration) from T0 and TL did not differ significantly and neither did TL and TC but TC was significantly greater than T0 (P<0.001). This could not be explained by differences in soil moisture or organic matter content since these were not significantly correlated with respiration activity (Table 5). Nevertheless, soil respiration rate

was significantly related to C_{mic} (P<0.001). Thus the metabolic quotient of TC soil was significantly greater (P = 0.015) than T0 (Table 4), despite the greater C_{org} (Table 3) and moisture content of soil amended with biosolids. A difference in toxic effect of the different biosolids is very improbable because if anything the cakes were "cleaner" than the liquid. The most likely explanation is that TL was more stabilised than TC (6 year lagoon maturation compared with no maturation) and because of this the balance and composition of the soil microbial population were modified in the biosolids-treated soil. Thus the metabolic quotient was linked to microbial adaptation to the changes in substrate composition in the different biosolids and also to the increased prevalence of predator organisms such as bacteriophagic nematodes and that these factors caused a shift towards a younger, more active, microbial community (Insam and Haselwandter, 1989; Weiss and Larink, 1991). The seasonal trends suggested that moist soils and increased availability of carbon substrates reduced the extent of environmental stress experienced by soil microbial populations.

Table 5: Correlation coefficients (r) and significance (P) of relationships between soil microbial and chemical parameters

Parameter	C_{mic}		Respiration		$C_{mic} : C_{org}$		Metabolic quotient	
	r	P	r	P	r	P	r	P
pH	0.10	NS	-0.15	NS	0.11	NS	-0.26	0.027
Conductivity	0.46	<0.001	0.63	<0.001	0.55	<0.001	0.19	NS
C_{org}	0.49	<0.001	0.11	NS	-0.25	0.032	-0.50	<0.001
Moisture	0.47	<0.001	0.01	NS	0.21	NS	-0.45	<0.001

NS not significant at P = 0.05

The changes in soil microbial respiration quotient (SMRQ) with time and for the different treatments are shown in Figure 5 and for comparison the soil moisture contents are also shown. There was a spike in the SMRQ in the 26th October 1994 samples. Biosolids had been applied on 17th September 1994 but the spike was across all treatments so biosolids could not have been the cause. However, on 24th October, two days before the sampling, isoproturon herbicide (IPU) had been applied. The toxicity of IPU to the soil microbial biomass, as indicated by the increased rate of respiration of soil treated with the herbicide, was reported by Harden *et al*. (1993) and has been confirmed by others, e.g. Perin-Garnier *et al*. (2001). The SMRQ had returned by the next sampling (13th December) by which time the IPU would have biodegraded (Walker *et al*. (2001). IPU was withdrawn from use in the UK in 2009 because of leaching to water resources and what was considered the unacceptable risk to aquatic life and the cost of removing it for potable supply. SMRQ also spiked in the 31st August 1995 sampling, again this was consistent across all treatments irrespective of whether biosolids had been applied. Examination of the soil moisture data (Figure 6) shows that it was particularly low (<5%) at this time. By 23rd November, soil moisture and SMRQ had both recovered.

The data in Figure 6 demonstrate clearly that nothing in either the older lagooned MAD liquid biosolids or the newer dewatered MAD biosolids stressed the soil microbial biomass as evidenced by the SMRQ. However the sensitivity of SMRQ to both chemical additions to soil and to the physical conditions in the soil was demonstrated very clearly.

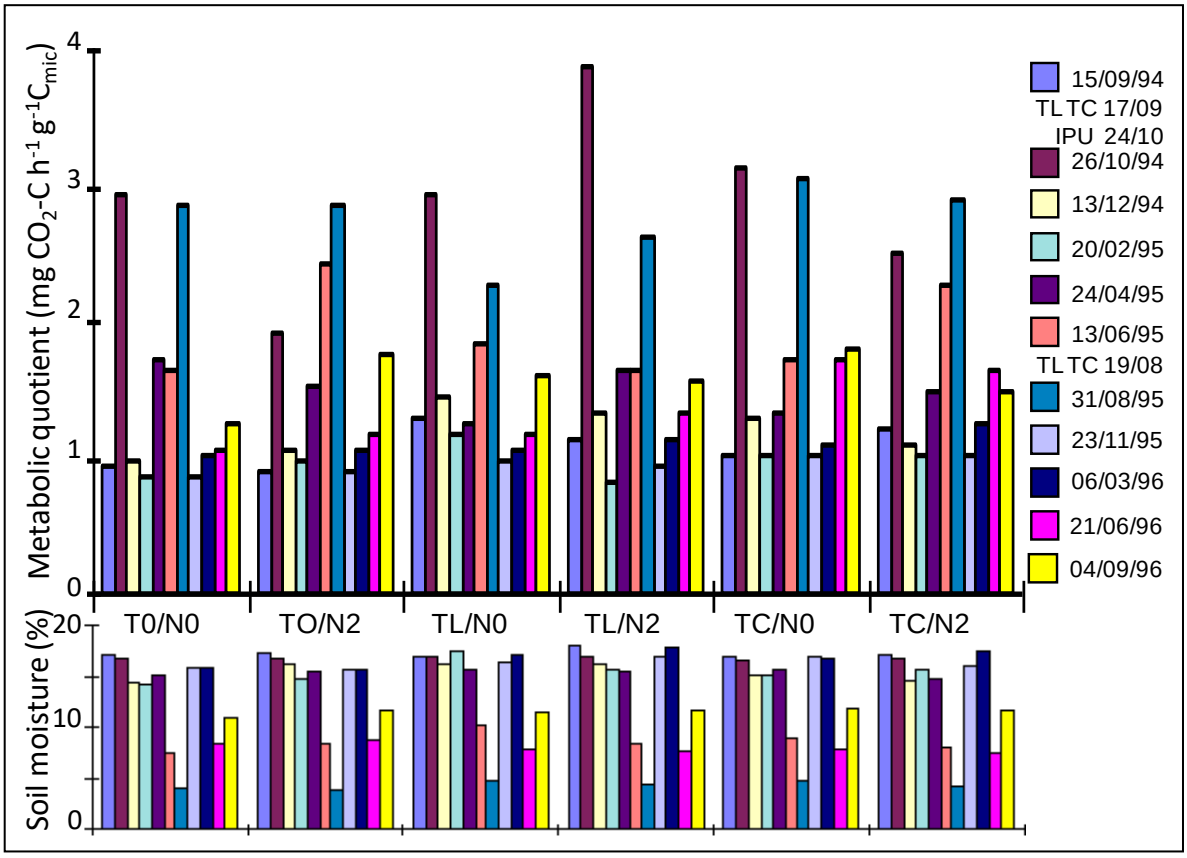

Figure 5: **Soil microbial respiration quotients (top) and soil moisture contents (bottom) September '94 to September '96**

Conclusions

Crop yield data proved that the fertiliser replacement value of biosolids could be predicted reliably from the chemical composition of the biosolids, the rate, method and timing of application, the soil type and the climate of the region where the biosolids were being used.

A comparison of the soil microbial biomass respiration quotient (SMRQ) for biosolids treated plots and control plots proved to be a very sensitive bioassay for chemical stressors. It was not necessary to pre-calibrate the assay, nor was it necessary to know the identity of the stressor in advance. The only chemical stress in the two years of the trial was a selective herbicide for grass weeds in cereals. There was no indication that any chemicals (either singly or as cocktails) in either of the domestic/industrial biosolids used in the trial stressed the bioassay.

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