University of Eswatini

Department of Chemistry

November 2018 Re-Sit Examination

TITLE OF PAPER : **Research Methods**

COURSE NUMBER : **CHE 303**

TIME : **2 Hours**

Important Information : Answer question 1 and two (2) other questions

You are not supposed to open this paper until permission has been granted by the Chief Invigilator

Question 1: Compulsory [40 Marks]

a) Define simple random sampling and give an example where it would work [5]

b) Compare and contrast between induction and deduction, as used in research [7]

c) Discuss 3 methods of your choice for data validation in scientific research [13]

d) Write short notes on a good researcher [10]

Question 2 [30 Marks]

a) Write short notes on the following;

i. Literature review, [5]

ii. Hypothesis [5]

iii. Research design [5]

b) The research process can be summarized in a sequence of steps which defines a systematic procedure for the objectives of a research to be met. Describe the sequence of steps involved. [10]

c) List qualities of a bad research [5]

Question 3 [30 Marks]

Given the article attached, formulate and write the abstract [30]

Question 4 [30 Marks]

a) Critique and praise (where necessary) the research as presented in article provided in Question 3 above [20]

b) Identify a topic of your choice that could be the focus of a research study in Water treatment and water resource management, and write out a possible hypothesis on the topic [10].

The end

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SHORT COMMUNICATION

HOT-WATER-SOLUBLE C AS A SIMPLE MEASURE OF LABILE SOIL ORGANIC MATTER: THE RELATIONSHIP WITH MICROBIAL BIOMASS C

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Many monitoring programs advocate that soil organic matter contents should be measured to assess effects of land use on soil quality. Rates of change in the total organic pool are relatively slow and other more labile soil C pools, such as soil microbial C, have been suggested as more sensitive indices to monitor longer-term trends in organic matter (Powlson *et al.*, 1987; Doran and Parkin, 1994; Larson and Pierce, 1994; Gregorich *et al.*, 1994). The fumigation-extraction methods to determine microbial C (Vance *et al.*, 1987) are relatively complex and time-consuming, which could limit their inclusion in routine monitoring programs. In contrast, water extracts of moist and air-dried soils are simple to obtain and can provide a labile C fraction (McGill *et al.*, 1986; Davidson *et al.*, 1987; Haynes and Swift, 1990; Zsolnay and Gorlitz, 1994; DeLuca and Keeney, 1994; Harris and Safford, 1996). Cold-water extracts from moist soil contain very little C derived from microbial cells (van Ginkel *et al.*, 1994), and there is poor agreement between the microbial biomass C and amounts of soluble C extracted from moist soils (DeLuca and Keeney, 1994). However, extracts from dried, fumigated or partially-sterilized soil contain much greater amounts of soluble C, some of which is derived from killed microbial cells (Powlson and Jenkinson, 1976; Vance *et al.*, 1987; West *et al.*, 1989; Badalucco *et al.*, 1992). Blagodatskyi *et al.* (1987) used soil desiccation as an alternative to fumigation to release microbial C and, on a very limited range of soils, Sikora *et al.* (1994) found good agreement between microbial biomass measured by the desiccation method, by fumigation-extraction or the substrate-induced respiration methods.

The proportion of soil organisms surviving air-drying can be variable, clays and organic matter protect against desiccation and there may have been selection for drought tolerant organisms (Powlson and Jenkinson, 1976; Sparling *et al.*, 1986). To maximize the amount of microbial material in a water extract of dry soils it will be necessary to solubilize the surviving cells. Boiling water at 100°C kills vegetative microbial cells (Stanier *et al.*, 1968) but has the disadvantage of extracting appreciable amounts of non-microbial organic C (Haynes and Swift, 1990). A temperature of 70°C is sufficient to kill vegetative microbial cells (Stanier *et al.*, 1968) and still makes microbial biomass components extractable (Speir *et al.*, 1986). Our preliminary studies on conditions to extract microbial C suggested a suitable combination was to air-dry soil at 20–25°C followed by a water extraction at 70°C for 18 h. We report results obtained using this approach compared against those obtained by fumigation-extraction.

Soils from a wide range of locations in New Zealand were sampled to 0–10 cm depth. Also included were a small number of peats and subsoils sampled to greater depths (Table 1). The soils were sieved (<4 mm) while field moist, adjusted to −5 kPa moisture content, and conditioned at 25°C for 7 d before measuring microbial biomass (triplicates) of the moist soil by the fumigation-extraction technique (Vance *et al.*, 1987). Exceptions were the peats and the sawdust amended soil for which the microbial biomass was measured without conditioning and peats were cubed (1 cm) rather than sieved. A *k*-factor of 0.41 was used to calculate microbial C from the C-flush. A small (*ca.* 100 g) subsample of the soil was spread thinly on plastic sheeting and air-dried at 20–25°C for up to 96 h. The air-dried soil was stored in sealed plastic jars at ambient laboratory temperature (18–22°C) until analyzed (triplicates) for water-soluble components up to 6 months later. Hot-water soluble C was obtained by incubating 2 g air-dry soil or 0.4 g peat with

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Table 1. Soil type, classification, land use and C pools of the soil used in the study

Soil	NZ classification*	USDA soil taxonomy suborder	No	Land use	Depth (cm)	Total C (%)	Microbial C ($\mu g\ g^{-1}$)	Hot-water§ extractable C ($\mu g\ g^{-1}$)
Blackstone sandy loam	pallic soil	ochrept	6	hill pasture	0–10	3.27–3.69	548–805	208–553
Teviot silt loam	brown soil	ochrept	13	hill pasture	0–10	3.52–6.19	965–1736	439–1068
Teviot/Blackstone	recent soil	orthent	4	hill pasture	0–10	3.05–3.34	552–1115	272–358
Te Awa silt loam	recent soil	fluvent	3	arable	0–90	0.11–3.25	1–288	1–95
Twyford sandy loam	recent soil	fluvent	3	arable	0–70	0.28–1.90	22–232	1–184
Horotiu sandy loam	allophanic soil	udand	3	pasture	0–150	0.13–9.28	44–1378	41–738
Tirau loamy silt	allophanic soil	udand	4	pasture and forestry	0–10	6.88–8.52	786–1374	802–891
Templeton silt loam	pallic soil	ochrept	2	arable	0–10	2.42–2.58	356–457	192–211
Temuka deep clay loam	gley soil	aquept	2	pasture	0–10	4.76–6.18	2090–2332	912–970
Patamahoe clay loam	granular soil	humult	2	arable and pasture	0–10	2.11–6.05	166–2152	136–867
Timaru silt loam	pallic soil	ochrept	2	arable and pasture	0–10	2.31–2.75	536–964	256–387
Waitohi silt loam	pallic soil	udalf	2	arable and native bush	0–10	2.79–2.52	778–900	352–574
Warkworth clay	ultic soil	udult	1	pasture	0–10	4.98	1032	336
Ruakaka loamy peat	organic soil	hemist	2	pasture	0–10	11.57–13.24	1640–1657	436–657
Pumice sand and silt	anthropic soil	arent	6	sawdust amended soil	50–150	0.07–5.48	42–754	1–787
Peat (unnamed)	organic soil	fibrist	5	restiad peat dome	0–10	41.8–47.6	3257–10 639	6249–12 216
Rangitaiki pumice sand	pumice soil	vitrand	5	*Dracophyllum* heathland	0–10	6.16–7.43	901–1063	424–600

*After Hewitt (1993).
§18 h extraction at 70°C.

10 ml water in a capped test-tube at 70°C for 18 h. The tubes were shaken by hand to resuspend the soil at the end of the incubation and then filtered through Toyo 5C paper. Soluble C was measured on a Shimadzu TOC-5000 analyzer (Wu *et al.*, 1990) and total soil C was measured on ground air-dried soil by Leco combustion furnace (Blakemore *et al.*, 1987). All results are expressed on an oven-dry soil basis.

Total C ranged from 0.07% to 13.24% for the mineral soils and up to 47.6% for the peats. Microbial biomass C ranged from 1 $\mu g\ g^{-1}$ in the mineral subsoils to 2332 $\mu g\ g^{-1}$ in the mineral top-

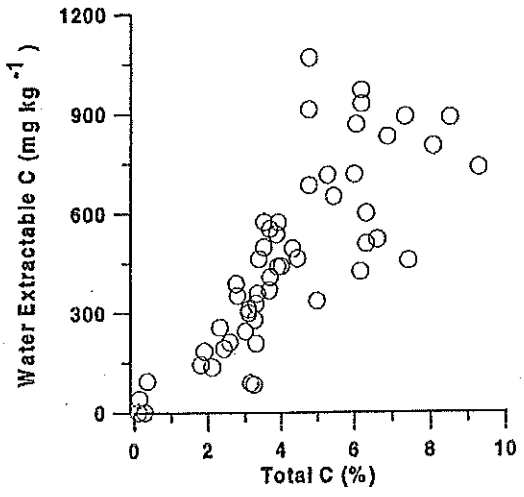

Fig. 1. Relationship between hot-water extractable C (WEC, 18 h at 70°C) and total organic C (%), excluding organic and sawdust-amended soils.

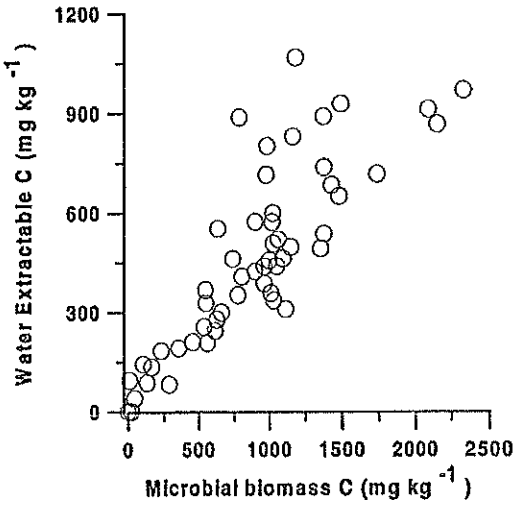

Fig. 2. Relationship between hot-water extractable C (18 h at 70°C) and the microbial biomass C determined by fumigation extraction, excluding organic and sawdust-amended soils.

soils, and up to 10.6 mg g^{-1} in the peats. Hot-water-extractable C ranged from 1 $\mu g\ g^{-1}$ in the subsoils up to 1068 $\mu g\ g^{-1}$ in topsoils and 12.2 mg g^{-1} in peats. Replicates generally differed by < 5%, and mean values for each soil were used for regression analyses.

Overall, there was a linear relationship (Fig. 1) between total C and hot-water-extractable C ($R^2 = 0.86$, $n = 66$, $P < 0.001$). This correlation was biased by two groups of soils forming clear outliers: the sawdust-amended (Anthropogenic) soil, and peaty soils with a total C content > 10%. Excluding these soils from the regression because of their high leverage gave the regression (± standard errors of parameters): water-extractable C = 105 ± 11 (Total C) − 25 ± 52 ($R^2 = 0.63$; $n = 53$; $P < 0.001$).

Generally, there was a linear relationship and reasonable correlation between hot-water extractable C and microbial biomass C ($R^2 = 0.79$, $n = 66$, $P < 0.001$). The sawdust-amended soil and peaty soils again formed outlier points. Regression analyses excluding these soils (Fig. 2) gave the relationship: water-extractable C = 0.43 ± 0.39 (microbial biomass C) + 77.3 ± 40.5 ($R^2 = 0.71$; $n = 53$, $P < 0.001$), demonstrating a closer agreement than between water-extractable C and total C. Extending this relationship to the microbial quotient (microbial C/Total C percentage) and the analogous water-extractable C/total C percentage, showed a general agreement between the two ratios, but revealed subsoils with very low biomass as a distinct group of outliers. These subsoils were also excluded from analyses (Fig. 3). Regression analyses gave the relationship $y = 0.30\,x + 0.44$ ($R^2 = 0.47$, $n = 47$, $P < 0.001$).

In our study, the hot-water-extractable C content of the mineral soils after air drying was about 43% of the microbial C, although there was considerable variability around this value (C.V. = 9%). The proportion of microbial C becoming extractable was similar to the 40–45% of the microbial C, obtained by fumigation-extraction (Wu *et al.*, 1990; Joergensen, 1996). This similarity may be coincidental, because in some cases non-microbial pools definitely contributed to the hot water-extractable C. This was especially so with soils of organic C contents > 10%, where, in some instances, more C was extracted in hot water than was originally present in the microbial biomass. We regard hot water extractable C as a measure of labile soil C, not necessarily a substitute for microbial C. However, for topsoils with < 10% organic C, water-extractable C was more closely related to the microbial biomass C than total C. The ratio of water extractable C-to-total C could be used in an analogous way to the microbial quotient which has been proposed for soil quality monitoring (Doran and Parkin, 1994). The method requires further validation on other soils but is worthy of attention for wide scale soil quality monitoring in that it is simple, rapid, does not require toxic fumigants, and the soils may conveniently be stored in an air-dry state at room temperature until analyzed.

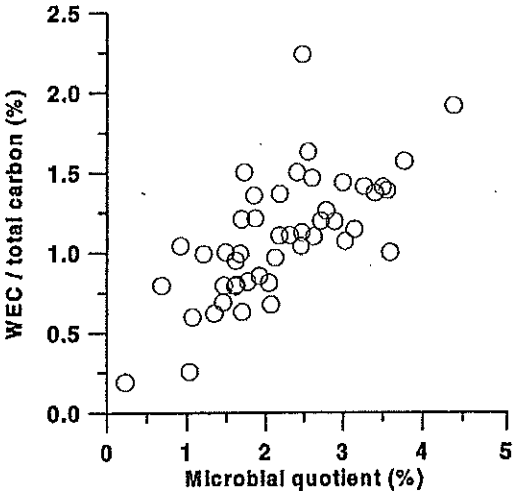

Fig. 3. Relationship between the microbial quotient (microbial C/total C) and the ratio of water-extractable C-to-total C.

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REFERENCES

Badalucco L., Gelsomino A., Dell'Orco S., Grego P. and Nannipieri P. (1992) Biochemical characterization of soil organic compounds extracted by 0.5 M K_2SO_4 before and after chloroform fumigation. *Soil Biology & Biochemistry* 24, 569–578.

Blagodatskyi S. A., Blagodatskaya E. V., Gorbenko A. Y. and Panikov N. S. (1987) Rehydration technique for microbial biomass determination in soil. *Pochvovedenie* 4, 64–71.

Blakemore L. C., Searle P. L. and Daly B. K. (1987) Methods for chemical analysis of soils. New Zealand Soil Bureau Scientific Report No. 80, Lower Hutt.

Davidson E. A., Galloway L. F. and Strand M. K. (1987) Assessing available carbon: Comparison of techniques across selected forest soils. *Communications in Soil Science and Plant Analysis* 18, 45–64.

DeLuca T. H. and Keeney D. R. (1994) Soluble carbon and nitrogen pools of prairie and cultivated soils: Seasonal variation. *Soil Science Society of America Journal* 58, 835–840.

Doran J. W. and Parkin T. B. (1994) Defining and assessing soil quality. In *Defining Soil Quality for a Sustainable Environment*, eds., J. W. Doran, D. C. Coleman, D. F. Bezdicek and B. A. Stewart, pp. 3–21. Soil Science Society of America, Madison.

Gregorich E. G., Carter M. R., Angers D. A., Monreal C. M. and Ellert B. H. (1994) Towards a minimum data set to assess soil organic matter quality in agricultural soils. *Canadian Journal of Soil Science* 74, 367–385.

Harris M. M. and Safford L. O. (1996) Effects of season and four tree species on soluble carbon content in fresh

and decomposing litter of temperate forests. *Soil Science* 161, 130–135.

Haynes R. J. and Swift R. S. (1990) Stability of soil aggregates in relation to organic constituents and soil water content. *Journal of Soil Science* 41, 73–83.

Hewitt A. E. (1993) *New Zealand Soil Classification.* Landcare Research, Lincoln.

Joergensen R. G. (1996) The fumigation-extraction method to estimate soil microbial biomass: Calibration of the k_{EC} value. *Soil Biology & Biochemistry* 28, 25–31.

Larson W. E. and Pierce F. J. (1994) The dynamics of soil quality as a measure of sustainable management. In *Defining Soil Quality for a Sustainable Environment*, eds., J. W. Doran, D. C. Coleman, D. F. Bezdicek and B. A. Stewart, pp. 37–51. Soil Science Society of America, Madison.

Powlson D. S. and Jenkinson D. S. (1976) The effects of biocidal treatments on metabolism in soil – II. Gamma irradiation, autoclaving, air-drying and fumigation. *Soil Biology&Biochemistry* 8, 179–188.

Powlson D. S., Brookes P. C. and Christensen B. T. (1987) Measurement of soil microbial biomass provides an early indication of changes in total soil organic matter due to straw incorporation. *Soil Biology & Biochemistry* 19, 159–164.

McGill W. B., Cannon K. R., Robertson J. A. and Cook F. D. (1986) Dynamics of soil microbial biomass and water-soluble organic C in Breton L after 50 years of cropping to two rotations. *Canadian Journal of Soil Science* 66, 1–19.

Sikora L. J., Yakovchenko V. and Kaufman D. D. (1994) Comparison of the rehydration method for biomass determination to fumigation-incubation and substrate-induced respiration method. *Soil Biology & Biochemistry* 26, 1443–1445.

Sparling G. P., Speir T. W. and Whale K. N. (1986) Changes in microbial biomass C, ATP content, soil phosphomonoesterase and phosphodiesterase activity following air drying of soils. *Soil Biology & Biochemistry* 18, 363–370.

Speir T. W., Cowling J. C., Sparling G. P., West A. W. and Corderoy D. M. (1986) Effects of microwave radiation on the microbial biomass, phosphatase activity and the levels of extractable N and P in a low fertility soil under pasture. *Soil Biology & Biochemistry* 18, 377–382.

Stanier R. Y., Doudoroff M., and Adelberg E. A. (1968) *General Microbiology.* MacMillan, London.

van Ginkel J. H., Merckx R. and van Veen J. A. (1994) Microbial biomass method based on soluble carbon in the soil solution. *Soil Biology & Biochemistry* 26, 417–419.

Vance E. D., Brookes P. C. and Jenkinson D. S. (1987) An extraction method for measuring soil microbial biomass C. *Soil Biology & Biochemistry* 19, 703–707.

West A. W., Sparling G. P. and Speir T. W. (1989) Microbial activity in gradually dried or rewetted soils as governed by water and substrate availability. *Australian Journal of Soil Research* 27, 747–757.

Wu J., Joergensen R. G., Pommeraning B., Chaussod R. and Brookes P. C. (1990) Measurements of soil microbial biomass C by fumigation-extraction – an automated procedure. *Soil Biology & Biochemistry* 22, 1167–1169.

Zsolnay A. and Gorlitz H. (1994) Water extractable organic matter in arable soils: Effects of drought and long-term fertilization. *Soil Biology & Biochemistry* 26, 1257–1261.