Soil Microbial Biomass-C as a Possible Indicator of Soil Pollution

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Introduction

Soil quality is defined in several ways (PARR et al., 1992; SMITH et al., 1993; DORAN & PARKIN, 1994; YAKOVCHENKO et al., 1996). The traditional means of characterizing soil quality by crop yield as a bioassay is simple but incomplete and focuses only on productivity. In terms of soil quality other aspects, like buffering, filtering and transformation capacities of the soil and sustainability of the food production system are also considered. Besides physical and chemical properties biological properties should also be investigated if we want to get information about the soil system as a whole (SZABOLCS, 1994).

Soil quality can be deteriorated by various anthropogenic factors. Especially the accumulation of heavy metals and xenobiotics may endanger the soil biota, soil transformation processes and the quality of plant products. Air deposition of pollutants from industrial sources may seem negligible but persistent materials can accumulate in significant amounts in the topsoil. It is necessary, for this reason, not only to measure the concentration of harmful materials but also to estimate the level of significant changes which can affect the ecological functions of soil (FILIP, 1995). However, there are a number of criteria that a microbiological property should fulfill as an indicator in monitoring soil pollution (BROOKES, 1995; FILIP, 1995): sensitivity, low variability of data, wide range of applicability, appropriate control and background measurements, how easy it is to standardize, general scientific validity based on reliable and contemporary scientific knowledge and ecological importance of the results. No single microbial parameter is known to satisfy the criteria mentioned and can be used universally.

Microbial biomass is one of the most important parameters of the soil microbial community and, this point of view may gain an indicative importance (BROOKES, 1995). However, the interpretation of results can be difficult because of natural fluctuations caused by changes in environmental factors (soil

moisture, temperature, nutrients etc.). There is a great variety of methods for the measurement of microbial biomass but, the most frequently used techniques are the chloroform fumigation incubation, FCI (JENKINSON & POWLSON, 1976), chloroform fumigation extraction, CFE (VANCE et al., 1987) and substrate induced respiration methods, SIR (ANDERSON & DOMSCH, 1978). The possibility of using microbial biomass-C as an indicator of soil pollution was demonstrated in the Woburn Market Garden Experiment at Rothamsted (BROOKES & MCGRATH, 1984). Microbial biomass-C considerably decreased at current EU limits of Cu, Ni and Zn, and Cd increased up to three times the limit. KNIGHT et al. (1997) also reported the decrease in microbial biomass-C with Cu contamination. Many studies showed that increased concentration of heavy metals in soil decreased microbial biomass-C (BROOKES et al., 1986; DUMONTET & MATHUR, 1989; CHANDER & BROOKES, 1991, 1993; BARDGETT et al., 1994; FLIESSBACH et al., 1994; LEITA et al., 1995; KANDELER et al., 1996; AOYAMA & NAGUMO, 1996; KNIGHT et al., 1997.) On the other hand, OHYA et al. (1988) – investigating 30 urban soil samples contaminated with Zn and Pb in the range of 49-4680 and 3-1510 ppm, respectively – did not find correlation between the amount of heavy metals and microbial biomass (using the ATP method). Other studies indicate that biomass-C per total organic-C (DUMONTET & MATHUR, 1989; BARDGETT et al., 1994; FLIESSBACH et al., 1994; AOYAMA & NAGUMO, 1996), specific respiration activity (BARDGETT et al., 1994; FLIESSBACH et al., 1994; LEITA et al., 1995) and the formation of biomass-C from added C-source (CHANDER & BROOKES, 1991; DUSEK & TESAROVA, 1996) can also be used as an indicator of soil pollution.

VALSECCHI et al. (1995) found a strong positive correlation between organic-C and heavy metal content in 16 investigated soils, while a negative correlation was established between heavy metal content and specific respiration activity of microbial biomass. It means that microbial biomass was less effective in the mineralization of soil organic matter when soils were contaminated with heavy metals.

Most soil biological data are based on the laboratory incubation of soil enriched with single or complex pollutants. In field trials or observations the effects of sewage sludge or heavy metal enriched sludge on microbial biomass have been mainly investigated. Only few investigations have dealt with soil contamination originating from air pollution. DUMONTET & MATHUR (1989) investigated soil microbial biomass at different distances from a copper smelter. They found significant decrease in the ratio of microbial biomass-C per soil organic matter due to the accumulation of Cu, Zn, Cd and Pb elements. DUSEK & TESAROVA, (1996) studied the effect of PCB's in grassland soils. The control plot contained 4.4 ng PCB/g soil and the contaminated 14.0 ng PCB/g soil. Microbial biomass-C and specific respiration rate were significantly lower in PCB-contaminated soils. The biosynthesis of new microbial C from glucose in model experiments was also lower in contaminated soil (DUSEK & TESAROVA, 1996).

Microbial biomass-C as an indicator was studied in the present paper. Two anthropogenically polluted soils contaminated by air depositions from power plants were used and were compared with control sites. The seasonal variability in biomass was also investigated over the vegetation season.

Materials and Methods

Five sampling sites (arable land) were selected near two power stations (Table 1). Two of them are situated close to the „Mátraalja" Power Plant (Visonta) which is in North-East Hungary about 80 km from Budapest. This power plant uses lignite from the local open-cut mines and brown coal from other sources for energy generation. The contaminated site is near the power station (2 km) at a village named DETK. The control site is situated at KOM-POLT, a village at a 15 km distance. Chernozem brown forest soil (acidic) is typical of both sites. Only the cadmium concentration was found significantly higher in the DETK soil in comparison to that of KOMPOLT (Table 2).

Table 1
Chemical characteristics of the investigated soils

Sampling site	pH (H$_2$O)	pH (KCl)	CaCO$_3$ (%)	C	N	NH$_4^+$-N	NO$_3^-$-N	CEC meq/100 g
						mg/kg		
DETK	5.64	4.95	0.21	1.93	1994	17.16	20.59	53.913
KOMPOLT	6.25	5.75	0.17	1.66	1681	13.72	6.86	35.217
SZBATTA-1	6.30	6.25	0.34	1.53	1694	3.43	17.16	21.739
BESNYO	7.25	7.15	1.19	2.30	2517	0.00	10.29	24.783
SZBATTA-2	7.35	7.19	5.10	1.64	1788	0.00	10.29	22.826

Table 2
Total amount of heavy metals in the investigated soils (ppm)

Element	DETK	KOMPOLT	SZBATTA-1	BESNYO	SZBATTA-2	National limit*
As	6.0	7.9	8.3	9.1	8.3	15
Hg	0.083	0.11	0.095	0.076	0.062	1
Cr	50.0	45.0	34.0	37.0	34.0	100
Zn	76.0	72.0	56.0	65.0	64.0	300
Cd	0.57	0.19	0.16	0.20	0.33	3
Pb	21.0	21.0	20.0	18.0	20.0	100
Co	14.0	13.0	9.2	9.7	8.8	50
Ni	27.0	33.0	32.0	33.0	30.0	50
Cu	12.0	15.0	13.0	18.0	18.0	100

* in case of soils with CEC between 25-35 meq/100 g soil

The other three sampling sites are located near the "Dunamenti" Power Plant (Százhalombatta), south from Budapest. Sites in the contaminated area were located nearby the Százhalombatta Power Plant (SZBATTA-1) in which gas and oil are burnt. It causes a significant air pollution in the surroundings and around the oil refinery (SZBATTA-2). However, no accumulation in heavy metals or hydrogencarbons were detected in soil samples from these sites. The control site (BESNYO) is located 20 km from the power plants. Soils in these sites belong to the calcareous chernozem type.

Soils were sampled (core, 5 cm diameter) from the top 0-20 cm layer at 20 points and then were mixed. Samples were transported to the laboratory in polyethylene bags in cooling box. The thoroughly mixed soil samples were passed through a 5-mm sieve the next day. Each original mixed soil sample was divided into three subsamples. One was used for moisture determination, another was air-dried for physical and chemical analyses and the remaining part was placed into a refrigerator for microbiological analyses. Samples were pre-incubated for 7 days at room temperature (22 °C) before microbiological analyses. Soil samples were taken seven times: in 1995 on 2 March, 18 April, 6 June, 28 September and 7 November, and in 1996 on 26 March and 22 April.

Soil pH was determined by combined glass electrode (RADELKIS) in distilled water and 1 M KCl extract. $CaCO_3$ was determined by Scheibler's method, organic-C by the wet oxidation with potassium dichromate, total-N by the Kjeldahl method. Extractable NH_4^+ and NO_3^- from 1 N KCl extract were measured by steam distillation. Cation exchange capacity (CEC) was determined according to the USDA Riverside laboratory method.

75 g of soil (in dry weight) was placed into a 100 cm^3 flask in three replicates. A flask was placed into a jar (800 cm^3) containing 25 cm^3 0.1 N NaOH solution, then tightly closed. Entrapped CO_2 was measured by potentiometric titration of the alkali solution after 24 hours of incubation.

Microbial biomass-C was determined by the chloroform fumigation extraction method (VANCE et al., 1987) using ethanol free chloroform, prepared according to WILLIAMSON et al. (1995). Four replications were used (30 g of each). After fumigation (24 hours) chloroform was removed from the desiccator. Fumigated and non-fumigated samples were extracted in 0.5 M potassium sulphate for 30 minutes, then filtered. Carbon content in the extract was determined using dichromate wet combustion. Biomass-C was estimated according to VANCE et al. (1987): $[(C_{fum.}-C_{nonfum.})/k_C]$, ($k_C = 0.38$).

In addition to field measurements, a model experiment was run. Fresh soil samples were sieved (5 mm mesh-size) and enriched with lucerne meal in an amount of 0.5% (dry weight). Samples with no addition of plant materials were used as a control. Soil samples were incubated at 25 °C for 30 days. Microbial biomass-C was measured at the beginning and at the end of the experiment. Soil CO_2 evolution was also monitored during the incubation. Soil moisture of the investigated samples was kept at the starting level by regular moistening. Lucerne meal used in this experiment contained 39.2% C and 2.48% N.

Results and Discussion

Microbial biomass-C

Microbial biomass-C data showed the following tendency during the sampling period (Figure 1). The minimum value was obtained in June, then it increased in October and November. The highest biomass-C was found in March. However, there was no sampling from mid-June to the end of September and from mid-November to March. A decrease in microbial biomass-C during the winter season usually occurs (KAISER & HEINEMEYER, 1993).

In the Visonta area the results showed that microbial biomass-C was higher at the slightly polluted DETK site as compared to the unpolluted KOMPOLT site at each sampling time. This is probably due to the higher organic matter content of the DETK soil (Table 1) and is not to be assigned to heavy metal pollution. In addition, this soil contains a higher amount of clay with high cation exchange capacity. Thus, heavy metals can be adsorbed in significant concentrations and organic matter is protected for a longer time.

In the Százhalombatta region there were no significant differences in microbial biomass-C among the investigated sites. The seasonal changes were similar
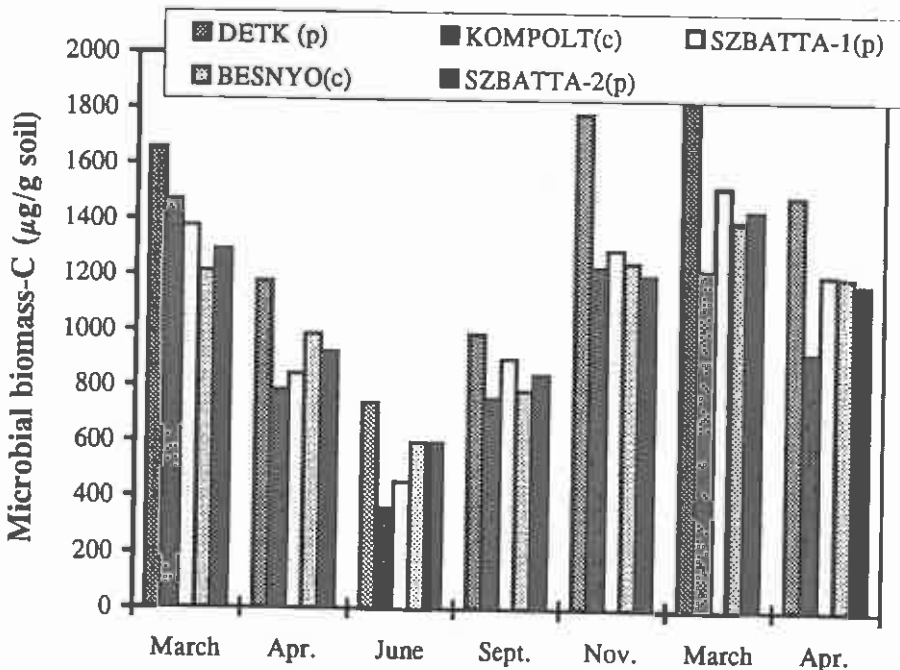

Figure 1

Microbial biomass-C changes during the sampling season (March 1995 to April 1996). Polluted (p) and unpolluted control (c) sites

to the Visonta region. There is not enough data to determine the factors affect-
ing the fluctuations of microbial biomass during the year but, probably tem-
perature, vegetation and the amount of plant residue after harvest can play an
important role. The effect of soil moisture could be excluded as water content
was similar at all sampling dates; rainy days mostly preceded soil sampling
(Figure 2).

Biomass specific respiration rate of soils

Biomass specific respiration (BSR) values fluctuated in the range of 7-40 μg
CO_2-C/day/mg biomass-C. In general, BSR was lower in the DETK and KOM-
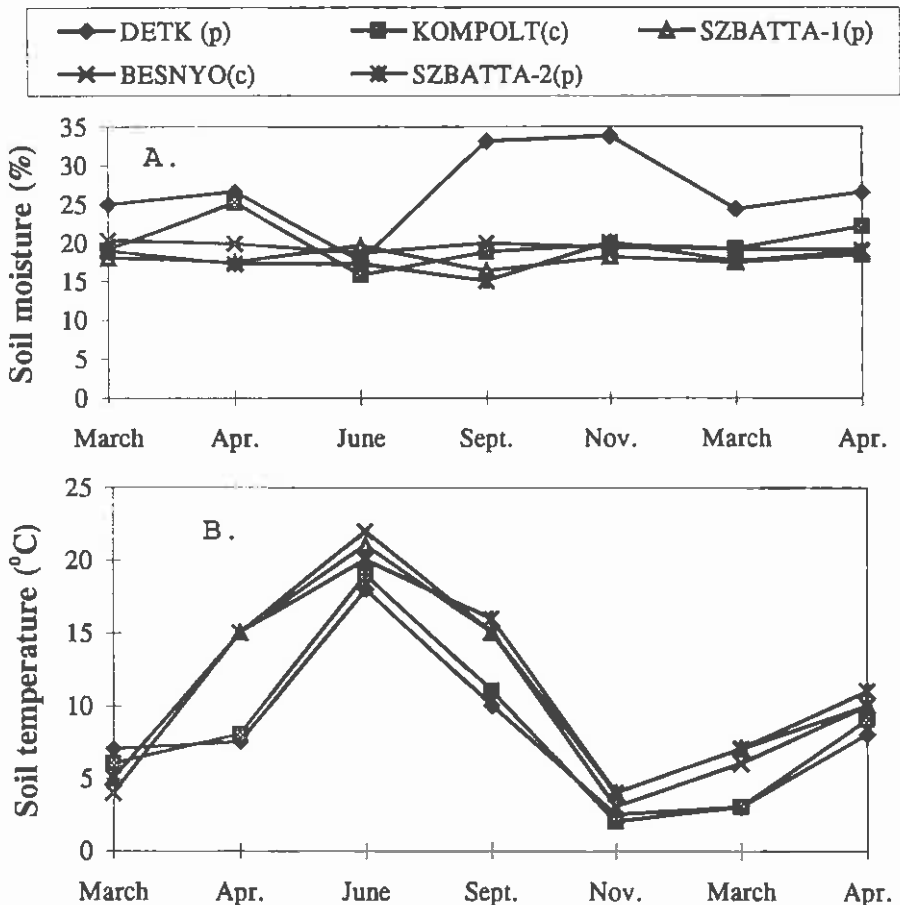
Figure 2
Soil moisture (A) and temperature (B) on the sampling dates.
Polluted (p) and unpolluted (c) sites

POLT soils than in the SZBATTA-1, SZBATTA-2 and BESNYO soils (Figure 3). It can be explained by the higher clay content or lower pH of these soils. Higher clay content provides the storage of higher amounts of biomass, but the microbial activity can be limited due to the depressed oxygen diffusion.
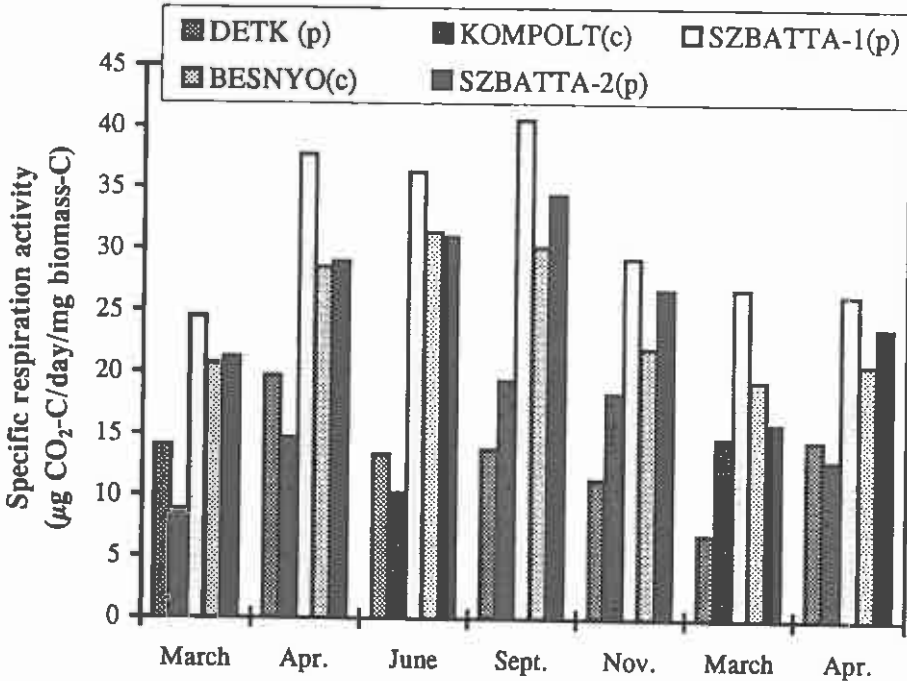

Figure 3
Biomass specific respiration (BSR) rates during the season.
Polluted (p) and unpolluted (c) sites

The BSR of the DETK soil was higher than that of the KOMPOLT soil at the first three sampling times, but later the BSR of the DETK soil was lower.

The BSR of the SZBATTA-1 soil always exceeded the BSR values of BESNYO and SZBATTA-2 soils. The soil of the site next to the oil refinery (SZBATTA-2) did not differ significantly from the BSR of the control (BESNYO) soil. Data available are not sufficient enough to estimate the effect of seasonal changes on BSR during sampling times. KILLHAM (1985) established a higher respiration rate of microbial biomass under stress situation by ^{14}C labelled substrate. A larger amount of substrate is needed for maintaining a certain amount of microbial biomass. Due to the higher respiration rate and lower efficiency of biomass formation in environmental stress there is a decrease in biomass within a short time and also in soil organic matter in a longer period (BROOKES, 1995).

Biomass-C/total organic-C

Fluctuation of microbial biomass-C:soil organic-C (C_{bio}/C_{org}) can have a different tendency than microbial biomass-C expressed on dry soil basis. This is not surprising if it is considered that soil organic-C is a slowly changing component as compared to biomass-C.
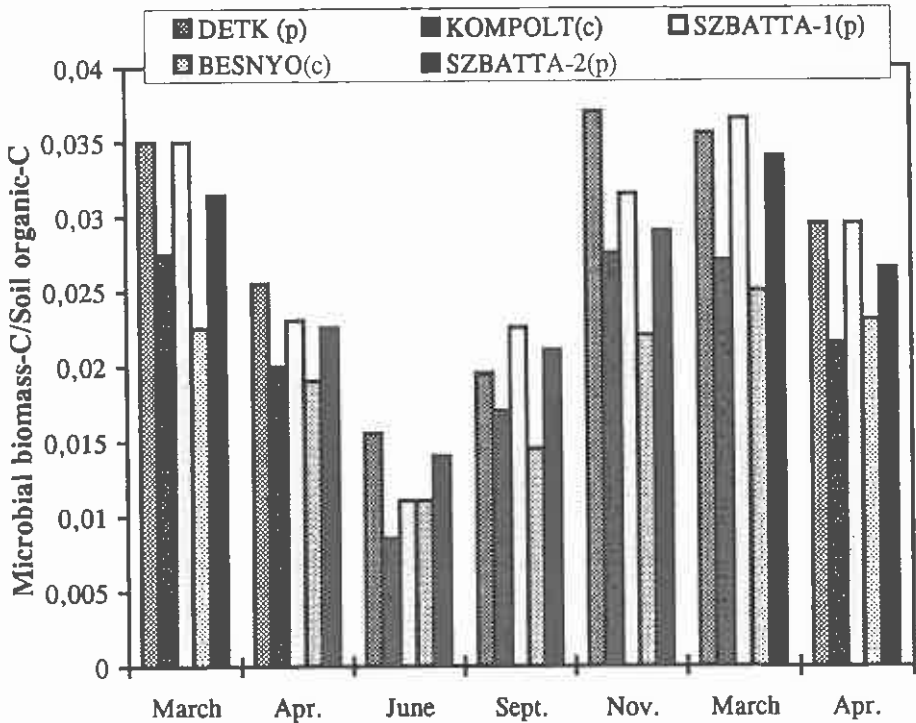

Figure 4
Changes in the ratio of microbial biomass-C per soil organic-C during the season.
Polluted (p) and unpolluted (c) sites

The C_{bio}/C_{org} ratio was higher in the DETK than in the KOMPOLT soil (Figure 4). This was probably related to the higher clay content of this soil rather than the level of pollution. The C_{bio}/C_{org} value is proposed as an environmental index of soils being under stress (e. g. heavy metal contamination) (CHANDER & BROOKES, 1991; DUSEK & TESAROVA, 1996).

Soil incubation experiment

Microbial biomass-C increased during the 30-day incubation period in soils from all sampling sites and at all times, showing a significant incorporation of

lucerne carbon into the microbial biomass (Figure 5). The lucerne meal added to the soil contained 1.96 mg C/g soil and the carbon assimilation from this source was estimated to range from 15 to 40%, depending on the soil and the sampling date. The incorporation rates of C showed seasonal changes. The pre-
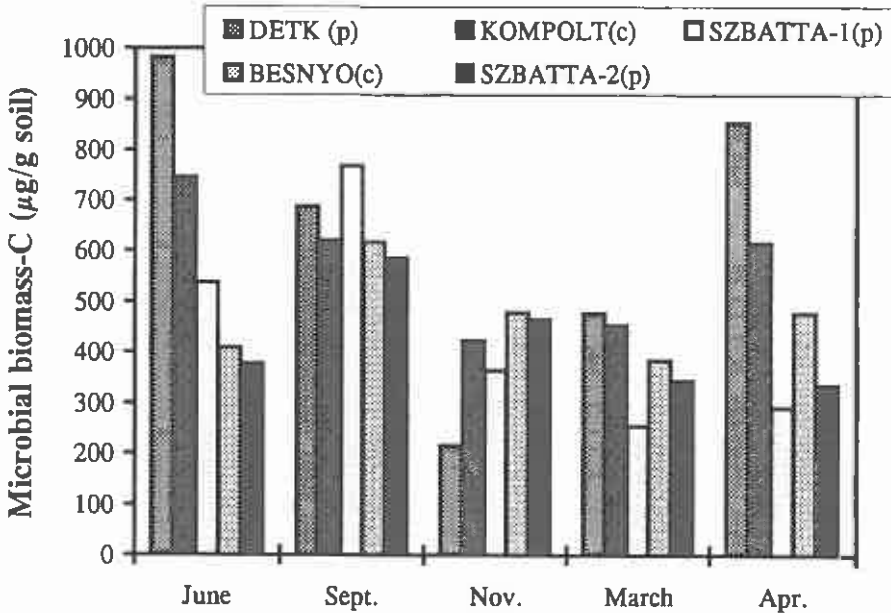

Figure 5
Net microbial biomass-C synthesized during 30 days after lucerne meal addition.
Polluted (p) and unpolluted (c) sites

sence of plant residues and perhaps root exudates in soil samples could be important in this respect. An interesting tendency was observed in the Visonta region where the seasonal change of carbon incorporation from lucerne to microbial biomass was higher in the DETK soil in comparison to the KOMPOLT soil. Similarly, but not to such a high extent, the carbon incorporation rate of the SZBATTA-1 soil fluctuated more than in BESNYO and SZBATTA-2 soils.

Conclusions

Soil microbial biomass and its formation in soil samples during a period of 30 days has not decreased as a result of soil contamination by atmospheric depositions from power plants. A rather slight increase could be observed during the one year sampling period. The biomass forming capacity also fluctuated during the sampling season in spite of the identical incubation conditions.

Seasonal changes of microbial biomass-C were higher than the difference among the polluted and control soils in our sampling data.

The chemical analysis of soils showed only a very little accumulation of heavy metals in the top soil layer. Microbial biomass as an indicator of soil biological functioning was not affected either.

For its general importance in soil fertility and ecological functions, microbial biomass-C undoubtedly represents an important indicator of soil quality. As the sampling season and soil characteristics other than pollution may strongly influence the analytical results, it is recommended to sample soils from control and polluted soils at the same time.

Summary

The indication value of microbial biomass-C was studied at two sites near power plants in the Visonta and Százhalombatta region (Hungary). The seasonal changes of microbial biomass-C, biomass specific respiration rate, relative proportion of biomass-C to organic-C, and the formation of biomass during 30 days' incubation with lucerne meal were studied. Soils were sampled at five locations seven times (from spring 1995 to spring 1996). Although the dust deposition rate was not high in the above regions, a small accumulation of inorganic pollutants could be observed, which remained below the official environmental limit values. The low level of pollution did not cause significant changes in microbial biomass-C. The results of our investigations show great differences in microbial biomass-C during the season, which can be related to the nutrient state of the soil rather than to soil moisture and temperature. Biomass specific respiration of soils differed significantly from site to site, but there is no evidence that it can be attributed to soil contamination. Carbon incorporation rates into biomass also changed during the year in spite of the identical incubation conditions.

Acknowledgement

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