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Autor: Celardin, Fredi / Chatenoux, Luc
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CORRELATIONS BETWEEN SOIL MICROBIOLOGICAL PARAMETERS (CO_2 RESPIRATION, ATP-BIOMASS) AND HEAVY METALS IN FIELD SOILS

BY

Fredi CELARDIN & Luc CHATENOUX

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ABSTRACT

Correlations between Soil Microbiological Parameters (CO_2 Respiration, ATP-Biomass) and Heavy Metals in Field Soils. - The impact of agriculture on soil microbiology in field situation was investigated by chemical and biological analysis of topsoils from a survey network covering cultivated and natural areas in Geneva canton. Microbial respiratory activity was measured by CO_2 production, microbial biomass was determined by Adenosine triphosphate (ATP), total heavy metals were determined by extraction with HNO_3 (2M) and the bio-available heavy metals soluble in $NaNO_3$ (0.1 M) were calculated using empirical relations. Spearman rank order correlations between these parameters were calculated. The inhibitory effect of Cu on the initial respiratory activity (A_{pot}) could be significantly detected in vineyard soils. The apparently stimulating effect of small concentrations of soluble Cd, Pb, Zn on A_{pot} could not be clearly established due to interdependence with pH.

INTRODUCTION

Sustainable agriculture promotes soil management which does not impair microbiological activity. In this respect, the assessment of the impact of heavy metals on soil micro-organisms in realistic environmental conditions is an important question. The toxicity of heavy metals on soil microbial activity has been reviewed by various authors (BABICH and STOTZKY, 1985) (BAATH, 1989). Low microbial biomass and significant reductions of enzyme activities were reported for heavily contaminated sites (KUPERMAN & CARREIRO, 1997). Soil variability and pollution levels are a source of confounding effects (INSAM *et al.*, 1996). Since soil microbiological analytical techniques are often lengthy and elaborate, efforts to estimate soil microbial activity in routine diagnosis have focused on relatively easily measured global parameters. Basic respiration and microbial biomass are currently applied to field studies despite the multiple causes affecting these parameters (PAUL & VORONEY, 1994). In a long-term field trial, basal respiration determined by Isermeyer's technique (JÄGGI, 1976) and microbial biomass evaluated by Adenosine triphosphate (ATP) extracted by sulfuric acid (MAIRE, 1987) proved to be suitable in differentiating the influence of mineral and organic fertilizer application on soil microbiology (MAIRE *et al.*, 1990). As a means to assess the effect of agriculture on

Ecole d'ingénieurs HES de Lullier, Filière Agronomie, 1254 Jussy (Switzerland)

microbiology in actual field conditions, we looked for eventual relations between measurements using these routine diagnosis methods and heavy metal concentrations in non cultivated and cultivated soils of Geneva canton.

MATERIALS AND METHODS

Topsoils from 99 sites in various environments (crop fields 61, horticulture 2, arboriculture 1, vineyards 9, meadows 16, woodlands 9, nature reserve 1) of a long term observation network in Geneva canton, were sampled in autumn 1995, at the end of harvest before tillage. These sites are situated in a temperate zone and they spread over 110 km^2 of agricultural land and 30 km^2 of deciduous forests. In majority, soils are loamy clay, calcic cambisols on moraine with A(B)C type profiles. On agricultural land Ap horizons are 20 to 30 cm deep; for the same depth, woodland profiles are differentiated into Ao E Bg decalcified horizons with slight gleyization (GRATIER & DE PURY, 1994).

Prior to sampling, the litter at the surface of the soil was removed, and a core at a depth of 20 cm was taken with an Edelman auger.

The samples were prepared according to the usual routine soil analysis operations of drying at 40°C, grinding and sieving (2mm). These treatments alter the soil microbial status. Drying causes a notable decrease in ATP. However a stable level of ATP, which is still lower but significantly related to ATP in lyophilised soil, is reached within 15 days after the moistening of the samples (MAIRE, 1987). Grinding increases the initial respiration rate by increasing the exposure of organic matter to micro-organisms (POWLSON, 1980) and sieving does not have a determining effect on biomass (JENKINSON, 1980). The overall image of biotic parameters remains rather well related to results from wet-sieved, frozen, thawed and incubated soils as recommended by other routine practices (FRANZLUEBBERS, 1996).

After removal of organic matter by oxidation with H_2O_2, particle size distribution was analysed by sedimentation according to the pipet method with sodium hexametaphosphate as dispersing agent (GEE & BAUDER, 1990).

Organic carbon (C_{org}) was determined by dichromate oxidation of organic matter according to Walkley - Black procedure and photometric measurement of Cr (III) at 610 nm (Cary 3E, Varian) (NELSON & SOMMERS, 1990). Organic carbon multiplied by 1.732 yields organic matter (OM).

Soil pH was measured with a glass electrode (Metrohm) in a suspension of 10 g of soil in 25 ml of deionized water, after standing 18 hours at room temperature.

Total heavy metal concentrations in soils are used to assess the degree of contamination; the fractions soluble in neutral salt solutions are related to their bio-availability (GUPTA *et al.*, 1984) (HÄNI & GUPTA, 1985).

Here, total heavy metals were extracted with HNO_3 (2M) at a soil to solution ratio of (1:10 weight/volume) on a boiling water bath, during 120 minutes, according to the method described in the Swiss Ordinance on Soil Contaminants (ANONYMOUS, 1986). Heavy metal concentrations (Cd, Cr, Cu, Ni, Pb, and Zn) in the filtrate were determined by atomic absorption spectroscopy (Perkin Elmer 2100 spectrophotometer).

Since soluble heavy metal concentrations (m) in $NaNO_3$ (0.1 M), representing the bio-available fractions, were below the practical detection limits, they were estimated using their empirical relations with the total concentration (M) and soil pH (CELARDIN, 1999):

$\log m = \log M - A\,pH - B$ for Cd, Pb and Zn (1)

$\log m = A \log M + B$ for Cu (2)

A, B are linear regression parameters listed in Table 1.

TABLE 1. Linear regression coefficients between soluble heavy metal concentrations (m), total concentrations (M) in mg kg^{-1} and soil pH

$\log m = \log M - A\,pH - B$ for Cd, Pb and Zn
$\log m = A \log M + B$ for Cu

	A	B	r^2
Cd	1.196	-5.666	0.893
Cu	0.687	-1.812	0.927
Pb	1.029	-2.516	0.756
Zn	1.295	-6.091	0.880

Microbial parameters (CO_2-respiration and ATP-biomass) were determined in 10 g moistened soil samples, during their incubation for 15 days at 25°C. In this period, the initially variable soil microbial activity and biomass reach stable levels which are lower but related to the soil in place (MAIRE, 1987).

During the 15-days incubation, momentary soil respiration was estimated in closed bottles by trapping in 20 ml NaOH solution (0.05 M) the CO_2 produced in 24 hours (JÄGGI, 1976) (ALEF, 1995) (ÖHLINGER, 1996). The resulting carbonate was precipitated by addition of 2 ml $BaCl_2$ (0.05 M) and the excess NaOH titrated to pH 9.30 with HCl (0.1 M) by means of a Metrohm Titrino 719 S automatic titrator. Respiratory activity is expressed as (CO_2 $\mu g\ g^{-1}\ h^{-1}$).

The evolution of respiratory activity was estimated by three CO_2 measurements on days 4, 9 and 15. The initial activity (defined here as potential activity A_{pot}) was calculated by extrapolating the activities on the days 4 and 9 back to the day 0. This initial activity is related to the metabolism of the stress resistant biomass after drying at 40°C. The available substrate is the easily decomposable fraction of soil organic matter as well as the remnants of the stress vulnerable biomass having not resisted to the drying process.

Carbon mineralisation rate (C_{min} $\mu g\ g^{-1}\ h^{-1}$) was estimated by dividing the total area under the activity response curve by the total incubation time (MAIRE, 1987).

At the end of the incubation period, the soil reaches a level related to its field microbial status. At this stage, ATP is used as a parameter to estimate microbial biomass and is determined by extraction in H_2SO_4. The original method (MAIRE, 1987; MARGESIN, 1996) was modified to simplify routine analytical work: 2 to 4g of moistened sample is extracted with 50 ml of H_2SO_4 (0.3 M) + 3-[N-Morpholino]propanesulfonic acid

(MOPS) (0.053 M), by horizontal shaking (17 mm amplitude, 400 r.p.m) during 3 minutes at room temperature. A 0.5 ml aliquot of the filtrate is diluted to 10 ml with a MOPS solution (0.053 M, pH 9.2) in order to buffer the final pH to 7.4, where ATP is relatively stable. The concentration of ATP in solution is determined by luciferin-luciferase bioluminescence (Hamilton-Lumicon luminometer). In order to correct for ATP losses during the extraction, each sample is extracted in parallel with a second sample to which 2 ml ATP (10^4 nM) is added as an internal standard. ATP loss calculations are developed by MAIRE (1984).

Because of uncontrolled true field conditions and data failing normality tests, Spearman rank order analysis was applied to calculate the correlations (Sigma Stat version 2.0 software by Jandel).

RESULTS AND DISCUSSION

The descriptive statistics of all the parameters are presented in Table 2. The maximum heavy metal concentrations are due to a few sites which, in the past, have received contaminated sewage sludge. The largest biotic values were observed on high altitude (1300 m) meadows.

TABLE 2. Descriptive statistics of soil parameters (n=99). OM: organic matter; A_{pot}: initial CO_2 production (potential activity); C_{min}: average carbon mineralisation rate (C-CO_2) during 15 days incubation; ATP: adenosine triphosphate.

	Mean	Std Dev	Max	Min	Median	25%	75%
Clay %	20.5	7.2	43.6	3.8	19.6	16.6	24.3
Silt%	45.8	9.7	81.1	17.4	44.8	40.5	51.6
OM %	3.2	2.3	21	1.5	2.6	2.3	3.5
pH	7.1	0.9	8.1	4.8	7.5	6.5	7.8
A_{pot} ($\mu g\ g^{-1}\ h^{-1}$)	6.4	6.6	42.2	0.1	4.6	2.9	7.8
C_{min} ($\mu g\ g^{-1}\ h^{-1}$)	1.4	1.3	10	0.4	1.1	0.9	1.5
ATP (ng g^{-1})	1168	1099	6492	47	823	610	1281
CO_2/ATP (h^{-1})	4.6	2.8	19.5	0.1	4.2	2.8	6.1
Cd (mg kg^{-1})	0.214	0.212	1.45	0.02	0.18	0.12	0.22
Cr	44.4	20.3	160	16.6	37.7	32.6	49.5
Cu	44.8	75.1	497	6.4	20.9	16.2	31.8
Ni	42.5	1 9.7	122	12.5	37.4	31.7	48.6
Pb	31.2	46.3	363	11.6	19.2	16.6	25.3
Zn	52.4	47.9	440	20.0	42	34	55.3

The results of Spearman rank order analysis are listed in Tables 3 and 4. Each correlation is characterised by three terms: the correlation coefficient ($0<r<1$), the significance of the correlation (P) and the sample size. The following discussion will be based first on the existence of a significant correlation, that is for $P<0.02$ and then the sign (positive or negative) of the correlation coefficient.

Biological parameters and organic matter are positively correlated. Potential activity A_{pot}, which is negatively correlated to pH, is the only pH dependent biological parameter.

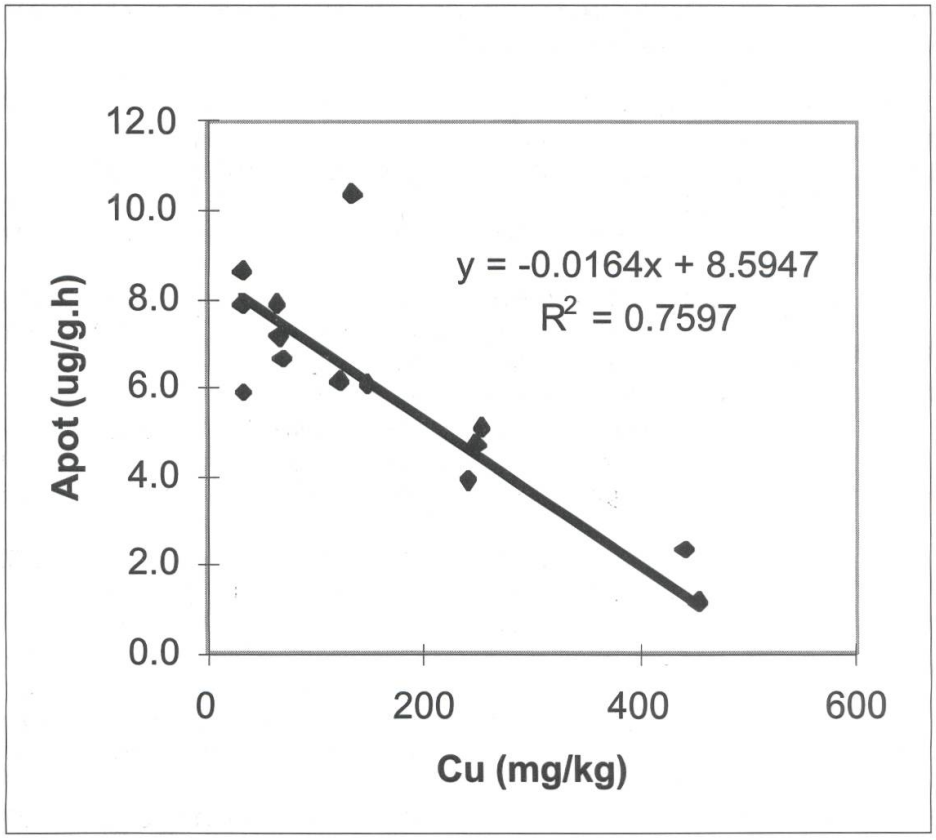
TABLE 3. Spearman rank order correlations (significant for P<0.02). For each pair of variables the correlation coefficient, the P value and the size of the sample are listed

	OM	ATP	C_{min}	CO_2/ATP	A_{pot}	Cd	Cr	Cu	Ni	Pb	Zn
pH	-0.365	-0.068	-0.164	-0.043	-0.571	0.409	-0.058	0.476	0.152	-0.07	0.232
	0	0.503	0.104	0.669	**0**	**0**	0.57	**0**	0.134	0.489	**0.021**
	99	99	99	99	97	99	99	99	99	99	99
OM		0.566	0.664	-0.0796	0.594	0.195	0.241	-0.118	0.141	0.301	0.364
		0	**0**	0.433	**0**	0.053	**0.017**	0.243	0.163	**0.003**	**0**
		99	99	99	97	99	99	99	99	99	99
ATP			0.517	0.486	0.416	0.236	0.008	-0.198	0.023	0.069	0.16
			0	**0**	**0**	**0.019**	0.939	0.049	0.823	0.499	0.114
			99	99	97	99	99	99	99	99	99
C_{min}				0.15	0.618	0.118	0.007	-0.091	0.035	0.182	0.235
				0.139	**0**	0.246	0.944	0.37	728	0.071	**0.019**
				99	97	99	99	99	99	99	99
CO2/ATP					0.155	-0.16	0.138	0.081	0.063	0.037	0.081
					0.129	0.114	0.172	0.427	0.535	0.719	0.423
					97	99	99	99	99	99	99
A_{pot}						-0.086	0.089	-0.242	-0.021	0.274	0.196
						0.4	0.382	**0.0173**	0.837	**0.007**	0.055
						97	97	97	97	97	97

TABLE 4. Spearman rank order correlations (significant for P<0.02) between soluble heavy metal concentrations and biological parameters. For each pair of variables the correlation coefficient, the P value and the sample size are listed

	Cd soluble	Cu soluble	Pb soluble	Zn soluble
A_{pot}	0.574	-0.242	0.592	0.595
	0	**0.0171**	**0**	**0**
	97	97	97	97
C_{min}	0.194	-0.0916	0.163	0.176
	0.0546	0.366	0.107	0.0813
	99	99	99	99
ATP	0.0834	-0.199	-0.0102	0.026
	0.411	0.0482	0.92	0.798
	99	99	99	99
CO_2/ATP	0.0199	0.0805	0.105	0.0863
	0.845	0.428	0.302	0.395
	99	99	99	99

There are few correlations between microbiology and total heavy metals due to the low contamination levels of the majority of the samples and also because total concentrations alone do not represent bio-availability.



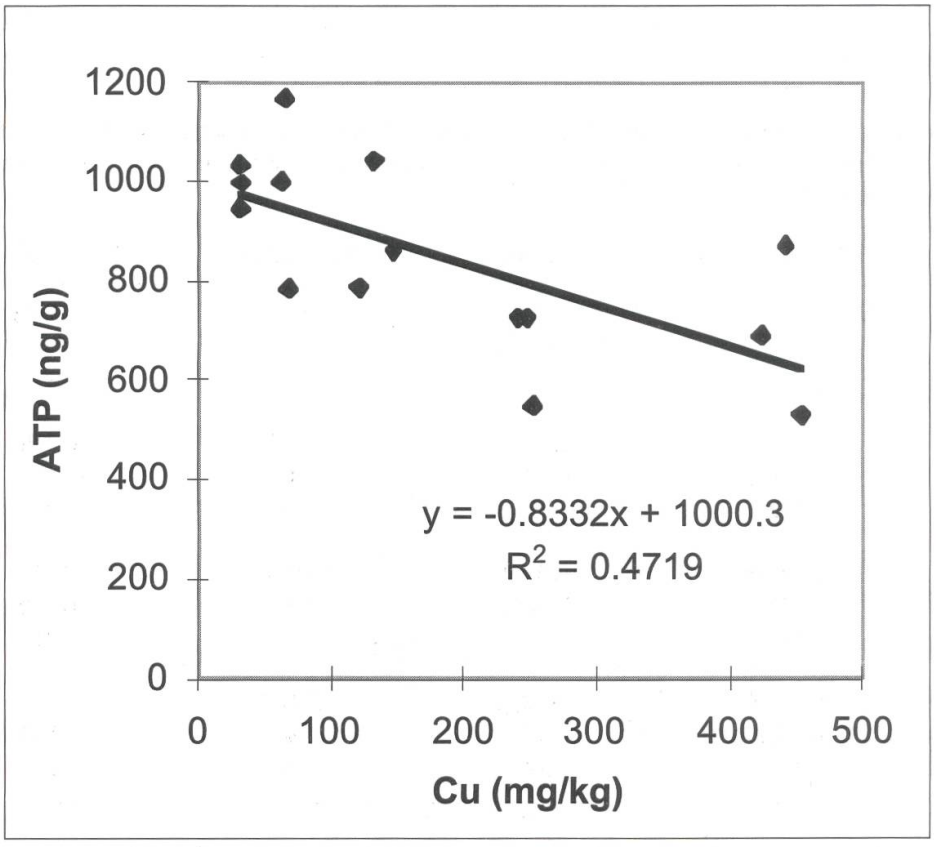


FIG. 1. Long term effects of Cu additions on the microbiology of a sandy loam acid soil (pH: 5.1). A_{pot}: potential respiratory activity; ATP: adenosine triphosphate; Cu: copper extracted with HNO_3 (2M).

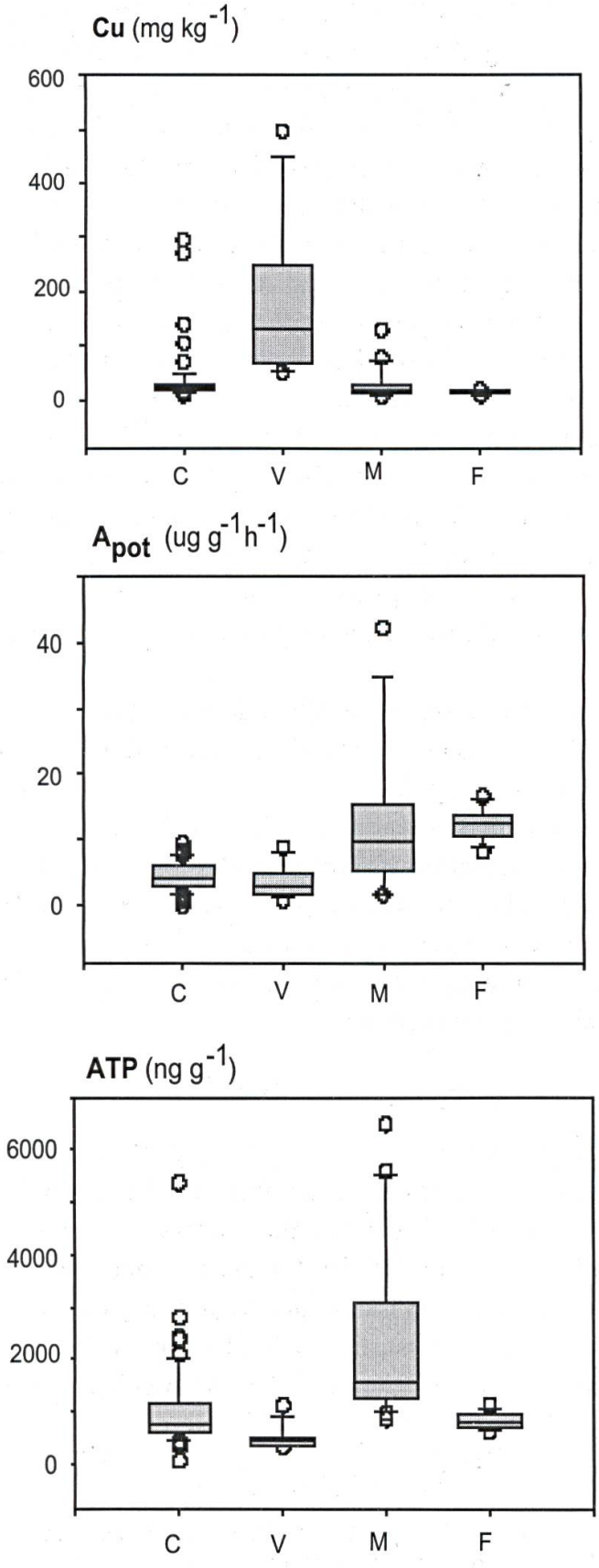


FIG. 2. Distribution of Cu, potential activity (A_{pot}) and ATP biomass in cropfields C (n=61), vineyards V (n=9), meadows M (n=16) and forests F (n=9).

Soluble concentrations of Cd, Pb and Zn, calculated with equation 1, are positively correlated with Apot indicating a stimulating effect at low concentrations. However, since with the present field data, A_{pot} and soluble Cd, Pb and Zn, are all negatively correlated with pH, this impact cannot be clearly differentiated. It is nevertheless interesting to mention that in pot experiments, bacterial growth stimulation by low Cd additions was observed (STADELMANN *et al.*, 1983) and also that up to 30 mg/kg additions of Ni to a soil at pH 4.9 showed an increase of carbon mineralisation measured by soil respiration (GUPTA, 1987). Stimulation of respiration and ammonification obtained immediately after addition of Pb, As, Se were reported by WILKE (1986). More recently, in a field study on contaminated soils, positive relations between heavy metals and soil respiration were also reported (VALSECCHI *et al.*, 1995).

Soluble Cu, which is apparently independent of pH (equation 2), has the same negative correlation with A_{pot} as total Cu. This negative correlation between A_{pot} and total Cu is also observed for activity and biomass measurements in sandy loam acid soil samples (pH: 5.1) having received, 13 years ago, progressively increasing Cu amounts during a field trial (Fig. 1). The comparison with the ATP regression shows A_{pot} to be a more sensitive parameter to Cu impact, with the practical advantage of being more easily determined.

The impact of Cu is also apparent in the relatively low average values of A_{pot} recorded in vineyard soils with heavy loads of this metal due to prolonged fungicidal treatments with copper sulphate (Fig. 2).

These observations show that potential activity may be used as an easily measured, sensitive index for routine evaluation of soil microbiology. Our data, obtained in uncontrolled field situations at relatively weak contamination levels shows the impact of Cu on the microbial activity in vineyard soils. In future work, the direct influence of the pH on microbial activity must be established first, in order to distinguish it from the pH dependent impact of soluble heavy metals.

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