59226

Soil Biol. Biochem. Vol. 11, pp. 475 to 479
© Pergamon Press Ltd 1979. Printed in Great Britain

LANL Ref

0038-0717/79/1001-0475$02.00/0

EFFECT OF LEAD ON SOIL RESPIRATION AND DEHYDROGENASE ACTIVITY

P. DOELMAN and L. HAANSTRA

Research Institute for Nature Management, Kemperbergerweg 67, 6816 RM Arnhem, The Netherlands

(*Accepted* 30 *May* 1979)

Summary—The effect of added Pb on the respiration and dehydrogenase activity of two sandy soils, a clay soil and a peat soil, (all with different physico-chemical properties), was studied.

A concentration of 375 $\mu g\ Pb \cdot g^{-1}$ inhibited the respiration of the sandy soil by *ca.* 15%, 1500 $\mu g\ Pb \cdot g^{-1}$ *ca.* 50%. In the clay soil 1500 $\mu g\ Pb \cdot g^{-1}$ caused a 15% reduction in respiration. The inhibition of respiration in the sandy soil was still *ca.* 30% 40 months after the addition of Pb. Respiration of the peat soil was not affected by even 7500 $\mu g\ Pb \cdot g^{-1}$.

Dehydrogenase activity was affected by Pb in a similar way to soil respiration. In the sandy soil a considerable reduction occurred, while in the clay and peat soils dehydrogenase activity was not reduced.

It was concluded, that a relationship exists between the inhibitory effects of Pb and the buffering capacity of the soil as expressed by its cation-exchange capacity. Because of these different effects of the same Pb concentration on the various soil types, no single value for the permitted concentration of lead pollution in soil could be established.

INTRODUCTION

In industrialized countries, the contamination of soil with a variety of heavy metals has become very common. However, the effects of this pollution, which may be of a lasting nature, are largely unknown.

Lead dispersion in the environment is mainly caused by activities such as mining and smelting, combustion of coal, production of pesticides and fertilizers and the use of Pb compounds in gasoline. In Pb pollution, the distance between discharge and ultimate destination is determined by local climate, geography and particle size. In this way, Pb concentrations along busy roads and around other sources of pollution have reached about 400 $\mu g\ Pb \cdot g^{-1}$ in The Netherlands (Rameau, 1968), 2000 $\mu g\ Pb \cdot g^{-1}$ in the USA (Page and Ganje, 1970) and even 8000 $\mu g\ Pb \cdot g^{-1}$ (Khan *et al.*, 1973), while the natural Pb content of soils has an average value of 15 $\mu g\ Pb \cdot g^{-1}$ (Swaine, 1955, Fishbein, 1972). In agricultural research, some attention has been given to the consequences of Pb accumulation in the soil but a reduction in crop production has not been found (Zuber *et al.*, 1970; Smilde, 1976).

Little attention has so far been paid to the effects of Pb on the activity of the soil microflora. Some studies have dealt with aspects of the N cycle such as ammonification and nitrification and of the C cycle (Lipman and Burgess, 1914; Bhuiya and Cornfield, 1974; Morissey *et al.*, 1974; Tyler *et al.*, 1974) and with soil respiration (Fujihara *et al.*, 1973; Ruhling and Tyler, 1973; Mikkelson, 1974; Ebregt and Boldewijn, 1977; Jensen, 1977). These studies have yielded conflicting results as to the concentrations at which serious disturbance of these processes may be expected and the length of time that inhibitory effects would continue after serious pollution.

The above investigations were mostly made from an agricultural point of view, i.e. the effects of Pb on crop production. No research has been done from an ecological point of view, for example in many places nowadays soil microorganisms have to cope with a continuous accumulation of Pb, which is proceeding at an ever increasing rate. Vital soil functions such as cycling of elements and its contribution to the flow of energy, may be seriously affected which could impair the functioning of the whole ecosystem.

Our aim was to find out to what degree soils can be charged with Pb without seriously disturbing general microbial processes, and to investigate the possibilities of developing general criteria for this pollution which can be used in practice. The effects of Pb on soil respiration were therefore investigated. It has been shown by Heal and McLean (1975) that about 90% of the primary production on land is consumed by the decomposers in the soil. The influence of Pb on the dehydrogenase activity was also studied, as it was shown by Stevenson (1959) that a significant correlation exists between the rate of respiration and the dehydrogenase activity in soil.

MATERIAL AND METHODS

Respiration experiments were done on a number of different soil types common in The Netherlands. All but one were agricultural soils. "Eng" and "Es" were sandy soils, "Nude" was a clay soil and "Trio" was a peat soil fortified with carbonate and fertilizers. The sandy soil "Eng" and 'Es" and the clay soil "Nude" are arable soils from the region around Wageningen (The Netherlands). Some physico-chemical properties of these soils are given in Table 1, which also gives the "number" of microorganisms.

The soils were sampled to a depth of 10 cm. For each sample 15 sub-samples were taken. The soils were sieved (<2 mm) and mixed thoroughly with different quantities of $PbCl_2$, being one of the main Pb

9278

Table 1. Some physico-chemical properties of the various soil types

	pH (H_2O)	Organic matter (%)	CEC (m-equiv/100 g)	Mineral fraction (%) Clay	Silt	Sand	Number of microorganisms ($10^6 \cdot g^{-1}$ dry soil)
Sandy soil "Eng"	5.7	2.8	5	12	81	7	22
Sandy soil "Es"	5.4	6.7	10	28	66	6	38
Clay soil "Nude"	7.5	3.2	30	99	0	1	105
Peat soil "Trio"	5.7	46	80	6	22	72	480

CEC = cation-exchange capacity.

salts in gasoline exhaust (Brunner, 1966). After mixing, the soils were re-moistened to about field capacity, which is 10, 12, 20 and 70%, respectively. Four or five grams of the soils were put in a Gilson respirometer vessel and the O_2 consumption was measured at 25°C (Umbreit *et al.*, 1964). Measurements were made between 24 and 42 h after the start of the experiment because of the irregular results during the first hours due to handling of the samples. The experiments were performed in triplicate and repeated once or twice. The standard error of the mean value of a set of respirometer experiments never exceeded 5% (σ) (Table 2, Fig. 1). In this study a difference (δ) of 15% was assumed to be important. It was also assumed that the significant level (α) could be 5% and the power (β) should be 80%, so 3 replicates were enough to make a detected difference (δ) of 15% significant (Cochran and Cox, 1968).

The total number of microorganisms in the various fresh soils was determined by the most probable number method (Pochon and Tardieux, 1962) on enriched soil extract medium, which contained yeast extract, 1 g per litre of soil extract, pH 6.6–6.8. Stock solutions of soil extract were prepared by adding 1 kg clay soil to 1 l. demineralized water, followed by autoclaving for 1 h at 120°C. Filtering and autoclaving was done twice.

To investigate long-term effects of lead, the respiration of "Eng" soil samples was measured, to which 0, 375, 750 and 1500 $\mu g\ Pb \cdot g^{-1}$ (as $PbCl_2$), respectively, was added. The samples were then taken back to the field. After 1, 2, 3, 5 and 40 months subsamples were taken and the O_2 consumption measured using a Gilson respirometer.

The level of dehydrogenase activity was measured using a method described by Naumann (1970). The enzyme activity was expressed as optical density units at 465 nm, using a Beckmann spectrophotometer.

RESULTS AND DISCUSSION

Influence of lead on soil respiration

Respiration was seriously inhibited in both sandy soils, at the highest concentration of $PbCl_2$ (7500 $\mu g\ Pb \cdot g^{-1}$) (Fig. 1). At intermediate concentrations the respiration was also affected. In the clay soil, respiration was also retarded at high concentration of $PbCl_2$, but at intermediate concentrations the effects were only small and hardly significant. In peat soil, however, no effects on soil respiration could be observed, even at the highest concentration of $PbCl_2$.

A significant correlation exists between the inhibition of respiration by Pb and the cation-exchange capacity (CEC) of these soils (Tables 1–2). Thus, in peat soils, which have a high CEC, no inhibition took place. The results indicate that "adsorbed" Pb has no inhibitory effect on soil respiration and that the inhibition may be caused by more "soluble" forms of Pb, which may be hard to define since after addition $PbCl_2$ is immediately bound (adsorbed and chelated) or converted to compounds such as Pb phosphate, Pb carbonate or Pb oxide (Ter Haar, 1971).

Although a strong relationship exists between inhibition of respiration of CEC, the nature of this relationship is still rather complex. It can be calculated, that when the "adsorption sites" of the soils are completely saturated with Pb, "Eng", "Es", "Nude" and "Trio" soils will bind 5000, 10,000, 30,000 and

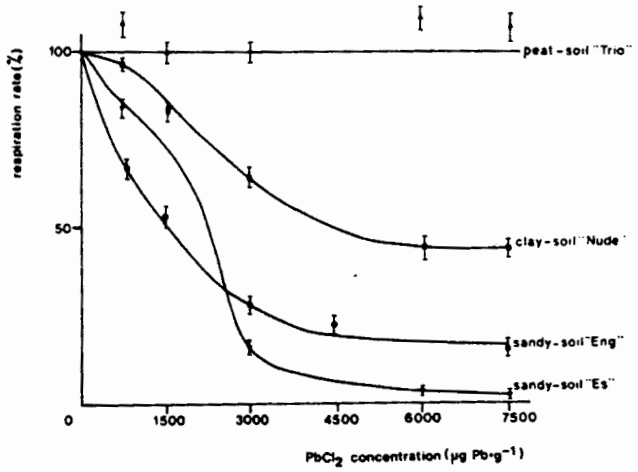

Fig. 1. The influence of various amounts of Pb on the respiration rate of "Eng", "Es", "Nude" and "Trio" soil, expressed as percentage of untreated soil.

Table 2. The effect of the addition of various concentrations of Pb (added as $PbCl_2$) on the soil-respiration rate, measured over 18 h after stabilization of the respiration rate. The standard error of the mean value of a set of experiments never exceeded 5%

$PbCl_2$ $\mu g\ Pb \cdot g^{-1}$	Sandy soil "Eng"		Sandy soil "Es"		Clay soil "Nude"		Peat soil "Trio"	
	A*	%**	A	%	A	%	A	%
0	1.7	100	6.5	100	11.5	100	1.1	100
375	1.42	83	5.5	84	11.7	101	1.1	100
750	1.11	65	5.8	89	10.7	93	1.2	109
1500	0.94	55	5.5	85	10.0	87	1.1	100
3000	0.56	33	1.0	16	7.4	65	1.25	113
4500	0.36	21	ND	ND	ND	ND	ND	ND
6000	ND	ND	0.25	4	5.1	44	1.2	110
7500	0.32	19	0.25	4	5.0	44	1.2	108

* A = respiration rate, $\mu l\ O_2 \cdot g^{-1}$ dry soil $\cdot h^{-1}$ at 25°C.
** = respiration rate as percentage of untreated soil.
ND = not determined.

80,000 $\mu g\ Pb \cdot g^{-1}$, respectively. From Harmsen's (1977) experiments done on two clay soils with a CEC of 200 and 150 m-equiv/100 g, respectively, it may be concluded that the general term CEC could be used for both metal ions, such as Ca^{2+}, and for heavy metal ions, such as Pb^{2+}. In the first three soils, inhibition of respiration was already higher than 50% when about one third of this calculated amount was added. Also, a comparison of both sandy soils shows that at 1500 $\mu g\ Pb \cdot g^{-1}$ the inhibition of the "Es" soil was only about 15% but of the "Eng" soil about 50%. At 7500 $\mu g\ Pb \cdot g^{-1}$, however, the opposite occurred and inhibition of the "Es" soil was higher, with a CEC twice as high. Apparently, other factors such as concentration of anions, pH, properties of chelation sites, etc. determine the availability of Pb to the microorganisms.

As with microorganisms, the availability of Pb to other biological systems, such as plant roots, is subject to confusion. Various methods have been suggested to characterize the available Pb fraction, e.g. extraction with 0.1 N NH_4NO_3 (Keaton, 1937), with 2% acetic acid (Rameau, 1968), with 2.5% acetic acid in 45 min. (Alloway and Davies, 1971) or with $CaCl_2$ and $BaCl_2$ (Harmsen, 1977). However, it was shown by John (1972) that the availability of Pb should be related to the plant species, because of the different uptake power of their root systems. He, therefore, suggested using 1 N HNO_3 for lettuce and 0.01 N HNO_3 for wheat. It should be noted that the use of these extraction techniques is recommended irrespective of soil types. It is, therefore, not surprising that conflicting results were often obtained. Therefore more recent investigations have been aimed at determining the concentrations of free Pb ions in soil. Extremely low values were usually found. The pK values of Pb^{2+} range from 12.8 for $PbCO_3$ to 44.6 for $Pb_3(PO_4)_2$, PbO and $Pb(OH)_2$ having intermediate values (Santillan and Jurinate, 1975). These values suggest that phosphates are indeed an important sink for Pb, which is in accordance with the results already mentioned. The pK values are influenced by the pH of the soil, e.g. at low values the solubility of heavy metal compounds increases. The presence of Cl^- ions also contributes to their solubility (Hahne and Kroontje, 1973). However, the concentration of free Pb ions is considerably decreased by clay minerals such as montmorillonite, illite and kaolinite. The adsorption of Pb to these minerals is 2–3 times as strong as that of other divalent cations such as Ca and Cd (Bittell and Miller, 1974). The organic matter fraction of the soil also has a strong binding capacity for Pb ions which is influenced by the pH 8, 7, 6, 5, 4 and 3, respectively, 112, 105, 85, 65, 41 and 24 m-equiv Pb were bound per 100 g of humic acids (Hildebrand and Blum, 1974). These binding mechanisms explain why hardly any free Pb ions can be detected in soil. Therefore, it may be concluded that the chemical nature of the Pb fraction available to plants and microorganisms is obscure and cannot be satisfactorily established with any of the techniques that have been used so far.

Kloke (1974), suggested that an accumulation of 2000 $\mu g\ Pb \cdot g^{-1}$ in soil can be considered as a concentration below which no damage is to be expected. According to our study, such a generalization is not justified. In Pb-sensitive (sandy) soils, 375 $\mu g\ Pb \cdot g^{-1}$ causes a significant decrease in respiration. For the peat soil "Trio", 1500 $\mu g\ Pb \cdot g^{-1}$ did not affect the respiration at all, but in the sandy soil "Eng" there was an inhibition of about 50%. Furthermore, Kloke (1974) suggested that Zn in soil is four times as toxic as Pb, and Cd is assumed to be 40 times as toxic. According to Kloke, total Pb + 4 times total Zn + 40 times total Cd should not be higher than 2000 μg M (heavy metals) $\cdot g^{-1}$. As there may be synergistic effects between these cations, as shown by Gray and Ventilla (1973) for some aquatic microorganisms, it is our opinion that the suggestion of Kloke, as to general values for pollution by heavy metals, should be viewed with suspicion.

Influence of lead on dehydrogenase activity

Dehydrogenase inhibition was similar in both the sandy soils (Table 3). In the peat and clay soils, however, there was no inhibition. The results obtained with clay soil in which the respiration rate was seriously retarded at concentrations at which no inhibition of the dehydrogenase was found, strongly suggest that respiration rate is more sensitive in characteriz-

Table 3. The influence of Pb (added as $PbCl_2$) on the dehydrogenase activity in various soil types (values are optical density units at 465 nm)

$PbCl_2$ $\mu g\ Pb \cdot g^{-1}$	Sandy soil "Eng" (air-dried)	Sandy soil "Eng" (fresh)	Sandy soil "Es" (air-dried)	Clay soil "Nude" (air-dried)	Peat soil "Trio" (air-dried)
0	0.07	0.31	0.18	0.45	0.11
375	0.07	0.31	0.14	0.32	ND
750	0.06	0.25	0.15	0.30	ND
1500	0.04	0.20	0.04	0.32	0.09
3000	0.03	0.11	0.08	0.41	0.10
6000	0.01	0.06	0.01	0.32	ND
7500	0.01	0.07	0.01	0.40	0.11

ND = not determined.

ing inhibitory effects of heavy metals on the soil microflora.

Length of inhibition

After more than 3 yr, respiration in Pb-amended soils was still considerably retarded (Table 4). It should be noted that this result contradicts that of Mikkelson (1974), who found that Pb only retarded the respiration of a sandy soil for the first 10–14 days. However, when the present experiments with sandy soil was repeated under controlled conditions in the laboratory, respiration was still retarded after 200 days.

CONCLUSIONS

Contamination of the soil by 375 $\mu g\ Pb \cdot g^{-1}$, which is locally very common in industrialized countries, may decrease the rate of respiration of the microflora. This is especially true of soils having a low cation-exchange capacity, such as sandy soils. In these soils, the influence of Pb can still be detected more than 3 yr after its addition.

It was concluded that the total number of microorganisms, the level of respiration or the dehydrogenase activity of a soil can only be considered as a measure of the toxic effects of Pb on soils when compared with an unpolluted sample of the same soil. When determining the effect of Pb experimentally, soil respiration is a more sensitive measure than dehydrogenase activity.

When some physico-chemical properties of the soil are known, such as cation-exchange capacity, organic matter content and pH, cautious predictions can be made on the potential inhibitory effects Pb may have. It must be emphasized that contamination of soils with Pb is mainly an irreversible and, therefore, a cumulative process, as hardly any leaching of the component occurs (Rolfe *et al.*, 1975). This means that for areas in which soils are continuously exposed to contamination, calculations can be made of the time needed to saturate the soil with Pb.

REFERENCES

ALLOWAY B. J. and DAVIES B. E. (1971) Heavy metal content of plants growing on soils contaminated by lead mining. *Journal of Agricultural Science*. **76**, 321–323.

BHUIYA M. R. H. and CORNFIELD A. H. (1974) Incubation study on effects of pH on nitrogen mineralization and nitrification in soils, treated with 1000 ppm lead and zinc, as oxides. *Environmental Pollution* **7**, 211–217.

BITTELL J. E. and MILLER R. J. (1974) Lead, cadmium and calcium selectivity coefficients on a montmorillonite, illite and kaolinite. *Journal of Environmental Quality* **3**, 250–252.

BRUNNER M. (1966) Die Zusammensetzung der Auspuffgase bei Benzinmotoren. *Zeitschrift für Präventivmedizin* **11**, 77–103.

COCHRAN W. G. and COX G. M. (1968) *Experimental Design*, 2nd edn, Wiley, London.

EBREGT A. and BOLDEWIJN J. M. A. M. (1977) Influence of heavy metals in spruce forest soil on amylase activity, CO_2 evolution from starch and soil respiration. *Plant and Soil* **47**, 137–148.

FISHBEIN L. (1972) Natural non-nutrient substances in the food chain. *Science of the Total Environment* **1**, 229–244.

FUJIHARA M. P., GORLAND T. R., WILDUNG R. E. and DRUCKER H. (1973) Response of microbiota to the presence of heavy metals in soils. *Annual Meeting of the American Society of Microbiology* **73**, 32.

GRAY J. S. and VENTILLA R. J. (1973) Growth rates of sediment-living marine protozoan as a toxicity indicator for heavy metals. *Ambio* **2**, 118–121.

Table 4. The influence of Pb (added as $PbCl_2$) on the respiration rate of samples of the sandy soil "Eng", 1, 2, 3, 5 and 40 months after the addition of $PbCl_2$ in March 1974, measured for 18 h after stabilization of the respiration rate. The standard error of the mean value of a set of experiments never exceeded 5%

$PbCl_2$ $\mu g\ Pb \cdot g^{-1}$	(1)		(2)		(3)		(5)		(40)	
	A*	%**	A	%	A	%	A	%	A	%
0	1.12	100	0.69	100	0.65	100	0.72	100	0.56	100
1500	ND	ND	0.38	54	0.45	69	0.42	59	0.39	70

* A = respiration rate, $\mu l\ O_2 \cdot g^{-1}$ dry soil $\cdot h^{-1}$ at 20°C.
** = respiration rate as percentage of untreated soil.
ND = not determined.

HAAR TER G. J. (1971) Composition of airborne lead particles. *Nature, Lond.* **232**, 553–554.

HAHNE H. C. H. and KROONTJE W. (1973) Significance of pH and chloride on behaviour of heavy metal pollutants: Mercury (2), Cadmium (2), Zinc (2) and Lead (2). *Journal of Environmental Quality* **2**, 444–450.

HARMSEN K. (1977) Behaviour of heavy metals in soils. (Thesis, Wageningen) Agricultural Research Report No. 866, pp. 171.

HEAL O. W. and MCLEAN S. F. (1975) Comparative productivity in ecosystems-secondary productivity. In *Unifying Concepts in Ecology* (W. H. van Dobben and R. H. Lowe-McConnel, Eds). Pudoc, Wageningen. HILDEBRAND E. and BLUM W. E. (1974) Lead fixation by soil acids. *Naturwissenschaften* **61**, 128–129.

JENSEN V. (1977) Effects of lead on biodegradation of hydrocarbons in soil. *Oikos* **28**, 220–224.

JOHN M. K. (1972) Lead availability related to soil properties and extractable lead. *Journal of Environmental Quality* **1**, 295–298.

KEATON C. M. (1937) The influence of lead compounds on the growth of barley. *Soil Science* **43**, 401–408.

KHAN M. A. Q., COELLO W. F. and SALEEM Z. A. (1973) Lead content of soils along Chicago's Eisenhower and loop-terminal express ways. *Archives of Environmental Contamination and Toxicology* **1**, 209–223.

KLOKE A. (1974) Blei-Zink-Cadmium: Anreicherung in Boden und Pflanzen. *Staub* **34**, 18–21.

LIPMAN C. B. and BURGESS P. S. (1914) The effects of copper, zinc, iron and lead salts on ammonification and nitrification in soils. *University of California Publications in Agricultural Sciences* **1**, 127–139.

MIKKELSON J. P. (1974) Effects of lead on the microbiological activity in soil. *Tidsskrift for Plantea* vl **78**, 509–516.

MORISSEY R. F., DUGAN E. P. and KOTHS J. S. (1974) Inhibition of nitrification by incorporation of select heavy metals in soil. *Annual Meeting of the American Society of Microbiology* **74**, 2: E6.

NAUMANN K. (1970) Untersuchungen über die Wirkung von Parathion-methyl auf die Atmung und die Dehydrogenase-Aktivität des Bodens. *Zentralblatt für Bakteriologie Parasitenkunde* **125**, 119–133.

PAGE A. L. and GANJE T. J. (1970) Accumulation of lead in soils for regions of high and low vehicle density. *Environmental Science and Technology* **4**, 140–142.

POCHON J. and TARDIEUX P. (1962) *Microbiologie du Sol.* Editions de la Tourell, St. Mondé (Seine).

RAMEAU J. TH. L. B. (1968) Ernstige loodverontreiniging langs snelwegen. *TNO Nieuws* **23**, 54–57.

ROLFE G. L., HANEY A., GETZ L. L., HUDSON J. L. and LARIMORE R. W. (1975) An ecosystem analysis of environmental contamination by lead. Illinois University, at Urbana-Champaign. Research report on phase 1.

RUHLING A. and TYLER G. (1973) Heavy metal pollution and decomposition of spruce needle litter. *Oikos* **24**, 402–416.

SANTILLAN M. J. and JURINATE J. J. (1975) The chemistry of lead and cadmium in soil: Solid phase formation. *Soil Science Society of America Proceedings* **39**, 851–856.

SMILDE K. W. (1976) Toxische gehalten aan zware metalen in grond en gewas. Een literatuuroverzicht. Nota. Instituut voor Bodemvruchtbaarheid, nr. 25.

STEVENSON J. L. (1959) Dehydrogenase activity in soils. *Canadian Journal of Microbiology* **5**, 229–235.

SWAINE D. J. (1955) The trace element content of soils. Techn. Commun. Commonwealth Bureau of Soil Science **48**, pp. 157.

TYLER G., MORNSJO B. and NILSSON B. (1974) Effects of cadmium, lead and sodium salts on nitrification in a mull soil. *Plant and Soil* **40**, 237–242.

UMBREIT W. W., BURRIS R. H. and STAUFFER J. F. (1964) *Manometric Techniques*, 4th, edn, Burgess, Minnesota.

ZUBER R., BOVAY E., TSCHANNEN W. and QUINCHE J. P. (1970) Le plomb comme facteur de pollution atmosphérique et son accumulation sur les plantes croissant en bordure des artères à forte densité de circulation. *Schweizerische landwirtschaftliche Forschung* **9**, 83–96.