Enregistrement scientifique n° : 2525
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Présentation : poster

Impact of heavy metal on phosphatase activities in the industrial fallow soil (Nord-Pas de Calais, France)
Impact des métaux lourds sur l'activité de phosphatases dans les friches industrielles (Nord-Pas de Calais, France)

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As part of the Concerted Research Programme about the environmental impact of pollutants in the industrial fallow soil of Mortagne du Nord (Nord-Pas de Calais), the researches conducted aim at identifying the bioindicators of the soil quality in order to determine the effect of pollution by heavy metals. The research on pollution bioindicators was conducted by associating the effect of pollution on the enzymatic activities which contribute to the degradation of plant residues and soil organic materials. The phosphatase activities involved in the energy metabolism of the soil microflora of this industrial fallow have been measured.

This study was conducted on five alluvial sites with a pollution gradient ranging from 26 363 to 2 812 µg of Zn/soil g in 0-15 cm surface horizon; some controls on alluvial soils covered with grass or forest have been selected.

The toxicity of heavy metals can result in a dysfunction of the enzymatic systems of the microflora, but our study did not allow to reveal a close relation between the phosphatase activities of surface and deep horizons and Zn content.

Other parameters are currently measured along this pollution gradient of the site : composition, density and biomass of the populations of soil macroinvertebrates, nitrogen and carbon mineralization by microflora.

The researches conducted should allow to better understand the effect of pollution by heavy metals on the soil quality and to develop an integrated system of bioindication including the parameters which influence the biological activity of these soils.

Keywords : bioindicator, heavy metals, pollutants, soil enzyme, enzymatic activity, polyphenoloxidases and phosphatases
Mots clés : bioindicateur, métaux lourds, polluants, enzyme, activité enzygmatique, polyphénoloxidases, phosphatases

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As part of the Concerted Research Programme about the environmental effect of pollutants in the industrial fallow of Mortagne du Nord (Nord-Pas de Calais), the researches conducted aim at characterizing pollution, evaluating its impact on soils, fauna, flora and health, and at analysing the mechanisms governing the action of heavy metals in the environment. Pollutants have four main effects on the soil biological activity: they accumulate in organisms, in various organs and at various concentrations (Diercksens et al., 1985; Yeates et al., 1994, 1995; Hopkin and Martin, 1983; Yeates et al.; Migula et al., 1993), they have a direct toxic effect, thus decreasing the abundance and the diversity of populations and leading sometimes to extinction. They affect some general biological functions of the soil such as the decay rate of organic residues and of the nutrient recycling (Rühling and Tyler, 1973; Aoyama et al., 1993; Edwards and Bohlen, 1995) and various enzymatic activities (Burns, 1978; Dzantor and Felsot, 1991; Obbard et al., 1994; Yeatles et al., 1995; Doelman and Haanstra, 1989, 1994), they show some dynamics of space dispersal and of time disappearance and fixation which are influenced by biological activities.

A deterioration of such biological systems due to the toxic effects of heavy metals is likely to be associated with some modifications in the space and time dynamics of the microbial activity (Doelman and Haanstra, 1994), and particularly some modifications in the enzymatic systems. Currently, numerous researches deal with the identification of bioindicators of the soil quality (Blandin, 1986; Dick, 1994; Tortenson, 1997). Our study aims at determining whether the soil enzymatic activities, and mainly phosphatases could be bioindicators. Phosphomonoesterases called “phosphatases” catalyze the hydrolysis of orthophosphoric monoesters and of H_3PO_4 anhydrides: they are acid and alkaline phosphatases (Dick and Tabatabai, 1984) whose activities are optimal in the acid (pH 6.5) and alkaline (pH 11) environments (Eivazi and Tabatabai, 1977; Tabatabai, 1982; Rojo et al, 1990). Phosphatases are involved in the organic phase of the phosphorus cycle (Mc Gill and Cole, 1986; Stevenson, 1985), and they lead to the release of inorganic phosphorus which can be assimilated by living organisms. Acid and alkaline phosphatases are involved in the energy metabolism of the microflora. Furthermore, these activities decrease with increasing concentrations of heavy metals (Marzadori and al., 1996).

Therefore, the phosphatase activities in the soil of the industrial fallow of Mortagne-du-Nord have been measured in order to determine the impact of heavy metals on the biological quality of these soils.

Material and methods

Five contaminated sites have been selected according to a heavy metal pollution gradient. Sites I, II and III are characterized by an herbaceous zone covered with a very abundant litter; sites IV and V are situated in a poplar plantation covered with a less abundant litter. The phosphatase activities of soil samples from control sites under grassland (G) or poplar plantation (P) have been compared with those from five contaminated sites (I to V). The soil layers which are interesting are those which are likely to show a root system, therefore the enzymatic activities have been measured in the horizons between 0 and 15 cm and between 15 and 25 cm in depth; they will be called reference 1 surface horizon and reference 2 deep horizon.

1.- pH measurement in soil samples

Phosphatase activities depend in particular on pH; it has been measured in water (pH H_2O) and in potassium chloride (pH KCl).

2.-Measurement of the total heavy metals content in soil samples

The total zinc and copper contents have been measured by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) and the total lead and cadmium contents have been measured by Furnace Atomic Absorption Spectrometry (FAAS). These contents are expressed in µg/soil g.

3.- Measurement of phosphatase activities in soil samples

Phosphatase activities have been measured according to Tabatabai’s method (1969, 1982); the phosphatase activity is expressed in µg of para-nitrophenol/g of dry soil at 105 °C/hour of incubation (µg of pN/g/h). All the analyses result from three assays.

Results

1.- pH of soil samples

Figure 1 shows soils with different pH: soils with pH close to neutral (sites I, II and III), alkaline soils (sites IV and V) and acid soils (control sites under grassland (G.1 and G.2) and control sites under poplar plantation (F.1 and F.2)). Whatever the site may be, pH does not vary between the surface horizon and the deep horizon.

2.- Total heavy metal content

The total heavy metal content of contaminated and control sites under study are shown in table 1. It seems that the Zn content is the highest of the whole contaminated sites, therefore, only this content is considered in the study. In contaminated sites, this zinc content ranges from 26 363 µg/g of soil (II-1) to 671 µg/g of soil (IV-2); it is always higher in surface horizons and in particular in II.1 (26 363 µg) and in I.1 (15 576 µg). In control sites, this content is clearly lower than that of contaminated sites and it is higher in surface horizons.

3.- Phosphatase activities

Alkaline and acid phosphatase activities have been revealed in all horizons studied, whatever the soil pH may be. They are prevailing in surface horizons as compared to deep horizons (figures 2 to 5).

Alkaline phosphatases (figures 2 and 3) show an optimal activity in surface horizons IV.1 and V.1 and in deep horizons IV.2 and V.2 from alkaline contaminated soils. Alkaline phosphatase activities have also been revealed in surface horizons of contaminated soils with a pH close to neutral (I.1, II.1 and III.1) and control soils (F.1 and G.1); on the contrary, these soils show very low activities in deep horizons (I.2, II.2, III.2 and F.2, G.2).

Acid phosphatases (figures 4 and 5) show an optimal activity in surface horizons F.1 and G.1 from control soils, while these activities are clearly lower in contaminated soils. In deep horizons, contaminated soils with alkaline pH and control soils show alkaline phosphatase activities which are clearly higher than those of soils with a pH close to neutral.

4.- Relations between the different parameters: influence of soil pH and of the zinc content on phosphatase activities

The values of the correlation coefficients between alkaline and acid phosphatase activities and the Zn content or pH are expressed in table 2.

Only the surface and deep acid phosphatase activities are linked to soil pH ($R^2 = 0.54$, $p = 0.0596$ and $R^2 = 0.5$, $p = 0.0764$); only the deep alkaline activities are linked to the zinc content ($R^2 = 0.5$, $p = 0.0752$).

Discussion and conclusion

This study did not reveal any significant relation between the phosphatase activities (alkaline and acid) and the Zn content in the industrial fallow of Mortagne du Nord, except the alkaline phosphatase activities of deep horizons; however this relation is not very close.

Optimal alkaline phosphatase activities have been revealed in the alkaline soils of surface horizons IV.1 and V.1 (2 100 and 1 862 µg of pN/g/h) and deep horizons IV.2 and V.2 (997 and 947 µg of pN/g/h), while a phosphatase inhibition due to the Zn content could be expected. In these contaminated soils with alkaline pH, the Zn action is compensated by this pH for metal cations Zn^{++} are chelated by hydroxide ions (OH-) in the form of metal oxides (Morel, 1977); in the absence of the soluble form of Zn, the alkaline phosphatase activities reach normal values and even values higher than those of the control sites. It is interesting to point out that in contaminated soils with neutral pH, phosphatase activities are always lower than those of alkaline contaminated soils and control soils; it could be explained by the Zn action which is in the soluble form at this pH and leads to an enzymatic inhibition. This study revealed that the phosphatase activities of surface horizons are higher than those of deep horizons. Our results are consistent with those obtained by Juma and al. (1977) who showed that the phosphatase activity in different types of soil is always higher on the surface than in depth.

As a conclusion, it seems that the phosphatase activities can be measured only in acid or neutral soils in order to evaluate the impact of heavy metals on the soil biological quality.

Currently, researches are conducted about the effects of heavy metals on other soil enzymatic systems such as polysaccharidases and polyphenoloxidases in contaminated sites of Mortagne du Nord. Other parameters are measured along this pollution gradient: composition, density and biomass of soil macroinvertebrates, nitrogen and carbon mineralization by microflora.

Researches should allow to understand the effect of pollution by heavy metals on the soil biological quality and to develop a bioindication system including the different parameters which influence the soil biological activity.

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Table captions

Figure 1: pH measurement in soil samples (pH H_2O and pH KCl).

Figure 2: Alkaline phosphatase activity (µg pN/soil g/h) in surface horizons (1) of contaminated sites (I to V) and in control sites under forest (F) and grassland (G). Mean of three independent assays ± standard error.

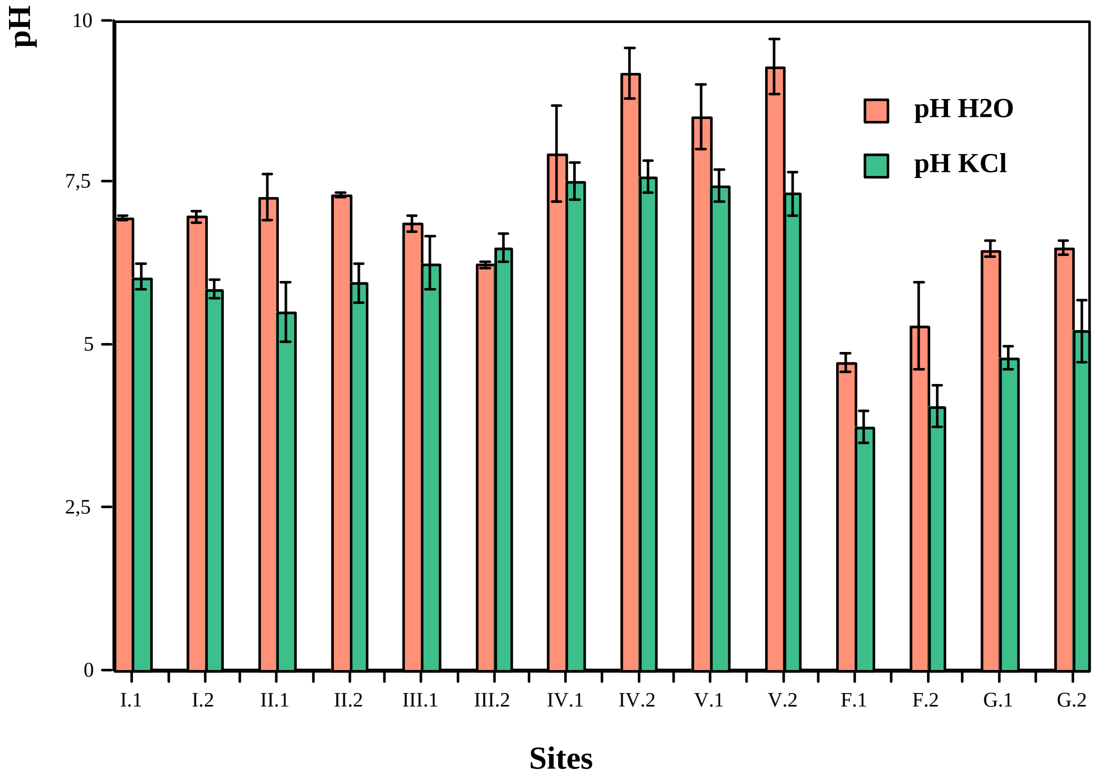
Figure 3: Alkaline phosphatase activity (µg pN/soil g/h) in deep horizons (2) of contaminated sites (I to V) and in control sites under forest (F) and grassland (G). Mean of three independent assays ± standard error.

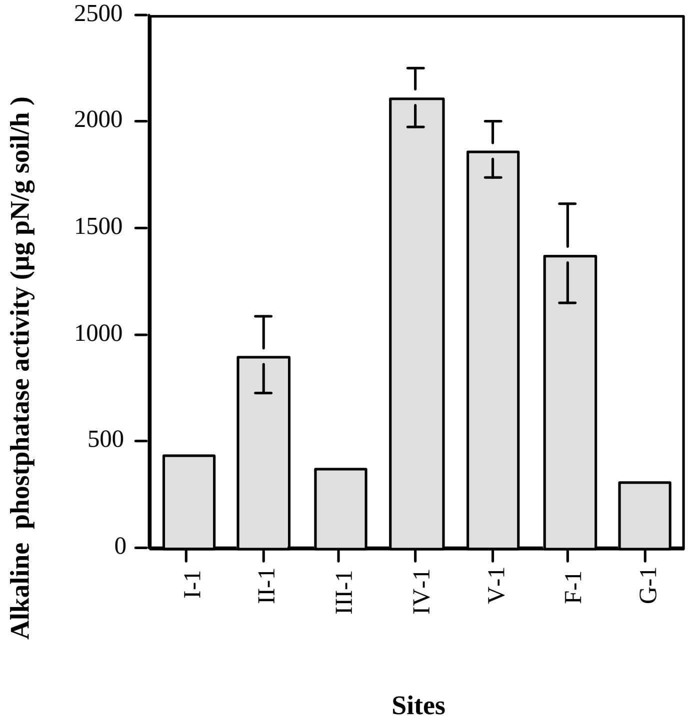
Figure 4: Acid phosphatase activity (µg pN/soil g/h) in surface horizons (1) of contaminated sites (I to V) and in control sites under forest (F) and grassland (G). Mean of three independent assays ± standard error.

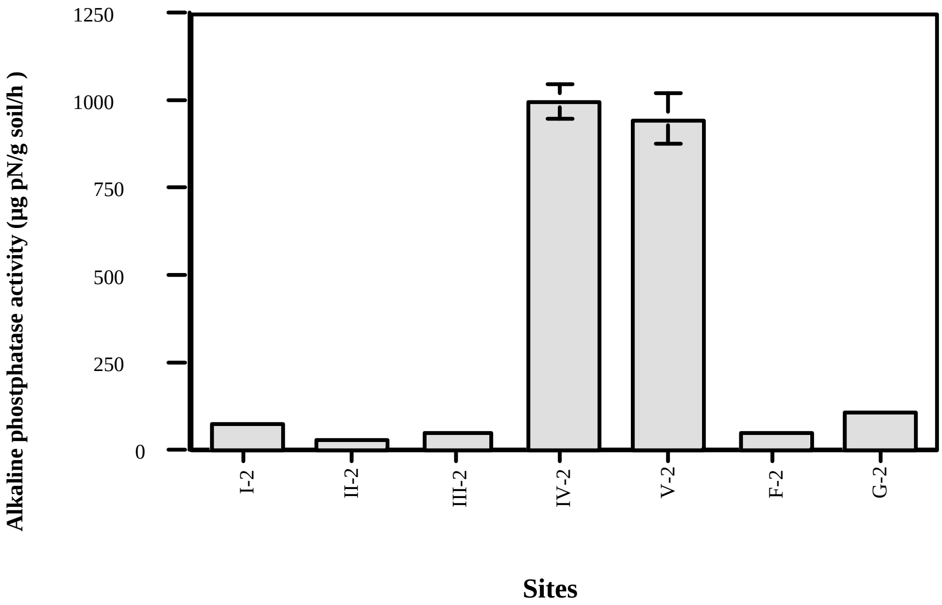
Figure 5: Acid phosphatase activity (µg pN/soil g/h) in deep horizons (2) of contaminated sites (I to V) and in control sites under forest (F) and grassland (G). Mean of three independent assays ± standard error.

Table 1: Total heavy metals content (µg/soil g) in surface (1) and deep (2) horizons of contaminated sites (I to V) and in control sites under forest (F) and grassland (G).

Table 2: Correlation between alkaline or acid phosphatase activity and Zn content or pH of contaminated sites.



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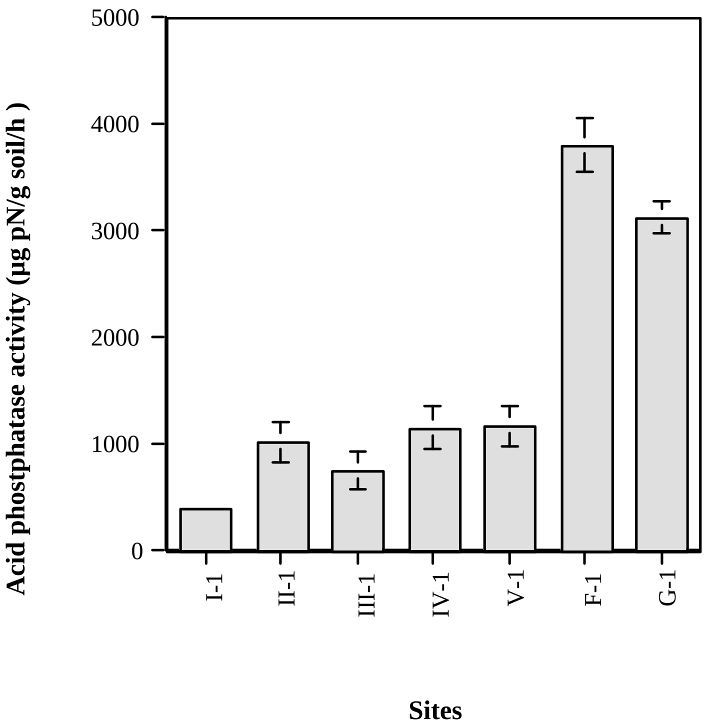
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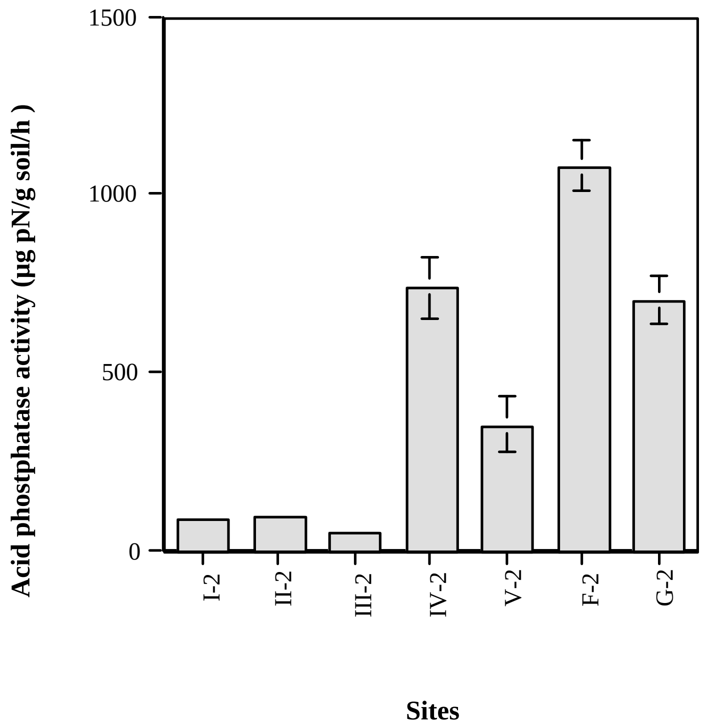
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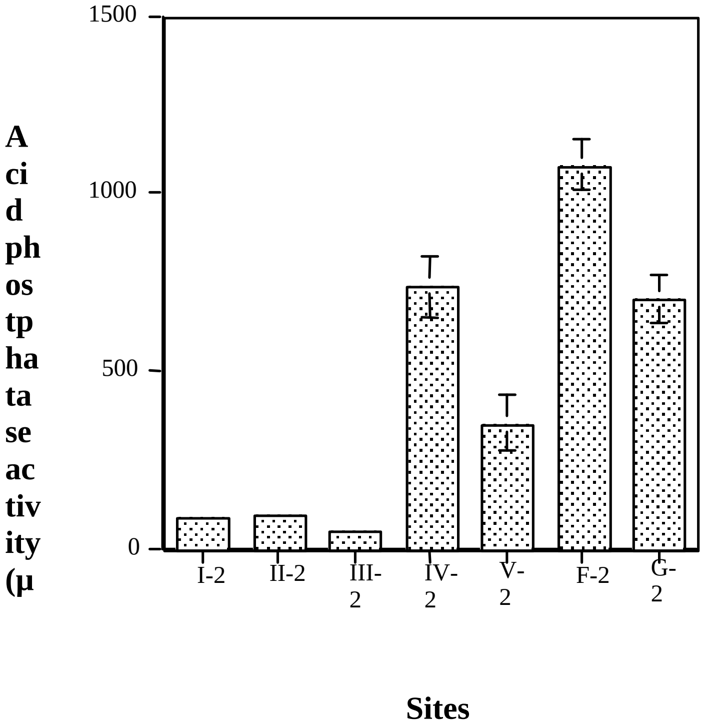
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Sites

Contaminated sites	Horizons	Depth (cm)	Cu (µg/g)	Zn (µg/g)	Pb (µg/g)	Cd (µg/g)
Site I	I.1	0-3	516	15576	4362	60
	I.2	5-15	68	2644	420	32
Site II	II.1	0-6	901	26363	244	149
	II.2	6-20	27	1561	32	14
Site III	III.1	0-4	126	6610	1100	34
	III.2	4-15	18	1227	130	12
Site IV	IV.1	0 -15	56	5397	580	33
	IV.2	15-25	21	671	102	6
Site V	V.1	0-5	63	2812	350	21
	V.2	5-20	22	1235	170	10
Control sites	**Samples**	**Depth (cm)**	**Cu (µg/g)**	**Zn (µg/g)**	**Pb (µg/g)**	**Cd (µg/g)**
Poplar plantation (F)	F.1	0-5	22	101	115	2
	F.2	5-25	20	39	31	<1
Grassland (G)	G.1	0-10	12	77	40	<1
	G.2	10-20	8	61	36	<1

	pH H_2O	**Zn content**	
Surface alkaline activities	R^2=0,16 p=0,3663	R^2=0,14 p=0,4022	
Deep alkaline activities	R^2=0,2 p=0,3174	R^2=0,5 p=0,0752	
Surface acid activities	R^2=0,54 p=0,0596	R^2=0,36 p=0,1566	
Deep acid activities	R^2=0,5 p=0,0764	R^2=0,01 p=0,8355	
Zn content	R^2=0,015 p=0,7252		