USE OF ENZYME ACTIVITIES TO MONITOR POLLUTION OF AGRICULTURAL LAND

Carmen Trasar-Cepeda[(1)*], Fernando Gil-Sotres[(2)], Diana Bello[(1)]

[(1)] Departamento de Bioquímica del Suelo, IIAG-CSIC, Santiago de Compostela, Spain
[(2)] Departamento de Edafología y Química Agrícola, Santiago de Compostela, Spain

* Corresponding author E-mail: ctrasar@iiag.csic.es

Abstract

Concern about environmental pollution has grown in the last few decades, amongst both specialists in the field and society at large. This concern is reflected in the numerous studies that have been published on this topic in recent years. Soil pollution has been somewhat neglected as a topic of study, relative to air and water pollution, which are considered harmful to human life. However, soil pollution is a particularly serious problem because of the impact on soil functioning and on the ecosystem as a whole. Although natural processes such as volcanic activity and weathering of the parent material contribute to soil pollution, anthropogenic activities represent the main cause of soil pollution. Apart from some accidental events, most pollution is generated by human activities such as industrial processes, transportation, construction, uncontrolled discharges, waste generation and agriculture. Agricultural land is particularly sensitive to pollution, partly because certain agricultural practices (soil preparation and tillage, soil fertilization, grazing, etc.) may affect basic soil properties, and partly because the soils usually display poor resilience. However, pollutants often reach already degraded agricultural soils and their impact will therefore be added to existing effects. The pollutants most frequently encountered in the agricultural sector include heavy metals, petroleum derived products, persistent organic pollutants, pesticides and fertilizers. Soil enzymes such as oxidoreductases and hydrolases have been widely used to investigate the impact of different pollutants on agricultural soils. However, the study findings are often inconclusive, because the impact of a given pollutant on the activity of different soil enzymes is influenced by various factors. In this report, we analyze the findings of different studies concerning pollution of agricultural soils.

Keywords: *Agricultural soils, soil pollution, soil degradation, soil quality index,*

Soil pollution

Concern about environmental contamination has increased in recent years at both scientific and community levels. Contamination has increased as a direct consequence of the worldwide increase in human population and industrial development. Soil is a non-renewable resource on a human timescale (Fitzpatrick,

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1980) and preservation of soil quality is therefore of high priority for environmental conservation (Cihacek et al., 1996). Soil pollution is of particular concern because of the strong impact it may have on the whole ecosystem, as it negatively affects soil functioning.

Soil pollutants may comprise compounds and elements derived from natural sources or substances of anthropogenic origin. Natural processes such as volcanic eruptions and weathering of the parent material can release amounts of toxic substances to soil, while desertification, forest wildfires and marine aerosols can cause changes in the structure or physicochemical characteristics of soil. However, soil pollution is mainly caused by human activities, such as industrial processes, transportation, construction, uncontrolled discharge, waste generation and agriculture. Although agriculture is not considered a main cause of pollution, the agricultural use of soils is not a negligible cause of soil pollution, especially in intensive agriculture.

Problems associated with pollution of agricultural soils

Agricultural land is continuously exploited to produce crops used both as food for humans and fodder for animals. Agricultural land is particularly sensitive to the presence of pollutants because the soils are often already degraded. Agricultural management practices can affect many basic soil properties, leading to degradation of the soil. Agricultural management practices and the increasingly frequent use of fertilizers, pesticides, etc. are generating large amounts of toxic organic products in the edaphic environment and disrupting soil functioning. For example, the preparation and tillage of crop soil may cause either a short-term increase in biochemical activity due to exposure of new surfaces (Dick, 1984; McGill et al., 1986; Latif et al., 1992; Khan, 1996) or a decrease in biochemical activity via reduction of the organic matter content (although this could also be a long-term effect) (Carter, 1986; Dick, 1994; Jensen et al., 1996). Other agricultural practices such as the application of organic fertilization may increase the biochemical activity through the addition of organic matter and microorganisms (Dick et al., 1988; Jenkinson, 1980; Kandeler et al., 1999), although the biochemical activity may also decrease in response to the addition of poor quality manure or other types of fertilizer scarce in nutrients or with a high heavy metal content. Moreover, inorganic fertilization may also either increase the biochemical activity of agricultural soils, by stimulating plant growth and secretion of enzymes by plant roots (Lynch and Panting, 1980), or decrease the activity, via inhibition of enzyme synthesis (Dick, 1992; Olander and Vitousek, 2000).

Diverse agricultural practices may contribute to modifying soil biochemical activity in cropland and pasture land. Thus, increasing the frequency of mowing may enhance the biochemical activity as a result of the presence of larger amounts of dead roots (Holland, 1995; Mawdsley and Bardgett, 1997), although the biochemical activity may not be affected by this practice. By contrast, grazing can increase soil biochemical activity as a result of the input of nutrients from livestock excreta and the associated fertilization effect; however, grazing can also reduce the

biochemical activity as a result of loss of soil structure due to trampling by livestock (Haynes and Williams, 1999; Zacheis et al., 2002).
The application of various different management practices during reforestation with fast growing species may affect soil biochemical activity. The presence of fast growing tree species may increase the soil carbon content, due to the lower metabolic activity (at least at the surface layer), and decrease biochemical activity (especially enzyme activity), due to the toxic effects of allelopathic compounds released from the leaves of some species (Badiane et al., 2001; Nsabinama et al., 2004; Rutigliano 2004). However, such changes are almost imperceptible, as the vegetation-induced effects take place very slowly. Thus, the alterations caused by agricultural activities and land use lead to intense, often irreversible, modification of the filtering and degradation processes in agricultural soils. Agricultural land is generally degraded and displays poor resilience. Agricultural soils are therefore incapable of recovering from any type of aggression, and exposure to any type of pollutant is likely to lead to further degradation. It is difficult to differentiate between the two causes of soil degradation (Table 1).

Table 1. *Modification of biochemical activity of agricultural soils caused by management practices.*

Soil Use	Management practice	Effect on biochemical activity	Cause
CROP	Tillage and soil preparation	Increase	Exposure of new surfaces
		Decrease	Reduction of organic matter content
	Organic fertilization	Increase	Addition of organic matter and microorganisms
		Decrease	Low quality manure or high heavy metal content
	Inorganic fertilization	Increase	Plant growth and secretion of enzymes by plant roots
		Decrease	Inhibition of soil enzyme synthesis
PASTURE	Frequency of grass cutting	Increase	Increase in amounts of dead roots
		Decrease	Decrease in amount of root exudates
	Grazing	Increase	Fertilizing effect and increased input of nutrients from livestock excreta
		Decrease	Loss of soil structure by trampling
FOREST	Soil preparation before reforestation	Increase	Closer contact between organic matter and soil microorganisms
		Decrease	Negative effect of heavy machinery on soil structure
	Pruning and Thinning	Decrease	Loss of plant cover and modification on the amounts of available substrates
	Fertilization	Increase	Increased amounts of available nutrients
		Decrease	Inhibition of enzyme activity due to presence of the enzyme reaction product

<u>Types of pollutants affecting agricultural soils</u>

The pollutants that most frequently affect agricultural soils are heavy metals, petroleum derived products, persistent organic pollutants (POPs), pesticides and fertilizers. The effects of pesticides have been widely investigated and several review articles have considered this type of contamination. Pesticides comprise a

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very wide group of substances with very different chemical structures that are targeted at different pest species and act very differently on soils (Fernandes et al., 2003; Arias et al., 2008; Gamble 2013). We therefore we did not consider them in this report. The same applies in the case of fertilizers, and some review articles have already considered how fertilizers affect soil enzymes. However, the effects of fertilizers, especially organic fertilizers, are very complex, as it is difficult to distinguish between positive and any possible negative effects of a given fertilizer on soil biochemical activity (Piotrowska-Długosz, 2014). We therefore did not consider the role of fertilizers as soil pollutants in this report.

Type of studies investigating pollution of agricultural soils

This report considers studies published between 1995 and 2014 involving the effects on soil of pollutants such as heavy metals, hydrocarbons and persistent organic pollutants. The effects of different heavy metals on soil functioning were investigated in 65% of the studies carried out in the last few decades, whereas hydrocarbons and persistent organic pollutants were considered in respectively 26% and 9% of studies (Fig. 1)..

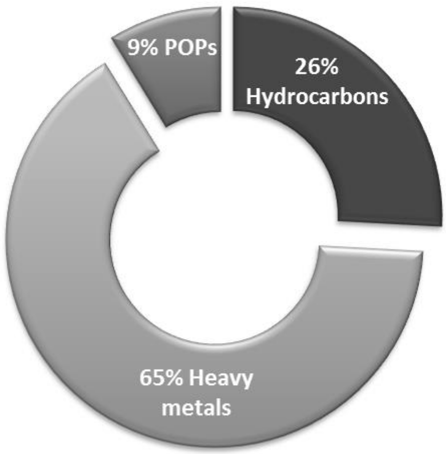

Figure 1
Percentage distribution of articles (published between 1995 and 2014) reporting data on agricultural soils polluted with heavy metals, persistent organic pollutants (POPs) and hydrocarbons.

Different types of study have been conducted to investigate the effect of diverse pollutants on agricultural soils, usually depending on the type of pollutant considered (Bello et al., 2014). Thus, 52% of studies on heavy metals were performed under laboratory conditions, while 57% were carried out under field conditions, and 39% of the latter involved accidental contamination. In the case of hydrocarbons, 43% of the studies were performed under laboratory conditions, 39% involved accidental contamination in the field and only 12% were conducted under experimental field conditions

In the case of POPs, no experimental field studies have been carried out, and only 28% of studies involved accidental contamination in the field and the remaining 72% of studies were carried out under experimental laboratory conditions (Table 2;

Bello et al., 2014). Of the recently (1995-2014) published studies investigating heavy metal pollution in agricultural soils, 66% were devoted to Cd (18%), Zn (17%), Cu (16%) and Pb (15%), while 15% involved Cr and Ni (considering both together) and very few were dedicated to other metals (Hg, As, etc.). Most studies regarding hydrocarbons involved the effect of crude oil (73%), while only 19% were dedicated to diesel contamination and the remaining 8% investigated contamination of soil by gasoline, kerosene and motor oil (Bello et al., 2015). Studies of the effects of numerous POPs as soil pollutants have also been published, although most of these concerned pollution by different chlorophenols, naphthalene, anthracene, pyrene, benzanthrazene and phenentrene (Bello et al., 2014).

Table 2. *Percentage distribution of the type of studies carried out to investigate the effect of some contaminants, i.e. hydrocarbons and persistent organic pollutants (POPs), on agricultural soils*

	Field studies		**Laboratory studies**
	Accidental	**Experimental**	**Experimental**
Hydrocarbons	**18**	**39**	**43**
Heavy metals	**12**	**36**	**52**
POPs	**0**	**28**	**72**

Problems regarding the use of soil enzymes as indicators of soil contamination

Soil enzymes have been widely used as indicators of soil pollution by different heavy metals, hydrocarbons and POPs (Bello et al., 2014). The enzymes used include oxidoreductases, mainly dehydrogenase, and different hydrolases involved in carbon, nitrogen, phosphorus and sulphur cycling. Different enzymes have been used as indicators of soil pollution, depending on the pollutant and the study. Nevertheless, the enzymes most commonly used in the different studies considered (irrespective of the type of pollutant) were dehydrogenase (19-22% of the studies), urease (14-16% of the studies) and phosphatase (13-25%), although enzymes such as arylsulphatase and glucosidases were also quite often used, especially in soils polluted by heavy metals (Table 3); other enzymes, such as laccase and peroxidase, have rarely been used (Table 3, Bello et al., 2014). Despite the large number of different studies carried out to investigate the effects of different doses of heavy metals, POPs and hydrocarbons on different soils, by means of different types of experiments and using different enzymes, no clear conclusions have been reached about the effects of any of these contaminants on soil enzymes, as variable responses have been observed. Thus, contamination of soil by a particular compound may cause the activity of a given enzyme to increase, decrease or remain unaltered (Zimenka and Kartyzhova, 1986; Kandeler et al., 1996; Margesin et al., 2000; Scelza et al., 2008; Moreno et al., 2009).

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Table 3. *Enzyme activities most commonly studied to estimate effect on soil biochemical activity in agricultural soils contaminated by hydrocarbons, heavy metal and persistent organic pollutants (POPs), reported in articles published between 1995 and 2014.*

	Dehydrogenase	Urease	Phosphatase	Arylsulphatase	Glucosidase	Others
Hydrocarbons	**22**	**16**	**13**	**1**	**3**	**45**
Heavy metals	**19**	**14**	**25**	**14**	**8**	**20**
POPs	**20**	**17**	**16**	**6**	**8**	**33**

The lack of clear, consistent responses can be explained by diverse factors, of which the following are some of the most frequently indicated: i) the lack of standardized protocols for determining enzyme activities; ii) the small number of soils usually analyzed in each study (large numbers of samples should be used to overcome the statistical uncertainty due to the usual variability of soil enzymes); iii) the small number of enzyme activities usually analyzed, as often only one enzyme activity is measured in each study (Margesin et al., 1999; Shen et al., 2006; Guo et al., 2012). It has been demonstrated that the behavior of a single enzyme does not necessarily reflect the responses of other enzymes, as enzymes associated with different cycles and participating in different stages of degradation will not necessarily respond in the same way.

Monitoring the loss of biochemical quality caused by pollution of agricultural soils

We previously demonstrated that it is not possible to use a single enzyme to estimate the loss of biochemical quality caused by soil pollution, in a study in which we examined three situations involving different degrees of pollution (Trasar-Cepeda et al., 2000). We investigated three different pasture soils affected by effluent from a tannery, effluent from an urban waste storage depot and fuel oil spills. In all cases we analyzed samples from three areas at different distances from the source of pollution, so that in each case three different degrees of contamination were considered. We also analyzed samples from a nearby uncontaminated area. As these were cases of accidental contamination, the soils were affected by different, unknown quantities of contaminants. We determined the activity of four enzymes (phosphomonoesterase, urease, ß-glucosidase and dehydrogenase) in contaminated and uncontaminated samples. The activities were generally affected by all of the contaminants. However, the activity of the diverse enzymes expressed as a percentage of the activity in uncontaminated samples was not very conclusive, as none of the activities provided a clear indication of the level of contamination, even when the specific activities (expressed either by unit of total carbon or total nitrogen content) rather than absolute values were considered.

However, in an earlier study (Trasar-Cepeda et al., 1998), we showed that there is an ecological equilibrium paralleled by a biochemical equilibrium between organic matter content and biochemical activity in correctly functioning soils, i.e. undisturbed native soils under climax vegetation (Dick, 1994; Doran & Parkin, 1994). For climax soils in Galicia (NW Spain), this equilibrium can be expressed by an equation in which total N content (Nk, measured by the Kjeldahl method) is predicted by a function (Nc) of some biochemical parameters. The function identified by the aforementioned is expressed as follows:

$$\text{Total N} = 0.38\text{x}10^{-3}\ \text{Microbial biomass C} + 1.40\text{x}10^{-3}\ \text{Mineralized N} + 13.60\text{x}10^{-3}\ \text{Phosphomonoesterase} + 8.90\text{x}10^{-3}\ \text{ß-glucosidase} + 1.60\text{x}10^{-3}\ \text{Urease}$$

Application of this equation to native soils under climax vegetation yielded an Nc/Nk ratio of 1 (100% when expressed as a percentage). In other words, the Nc/Nk ratio is a good indicator of equilibrium between organic matter content and biochemical activity (Gil-Sotres et al., 1998; Trasar-Cepeda et al., 1998). When a soil becomes contaminated, this balance should be disrupted (Leirós et al., 1999; Gil-Sotres et al., 2005), and the Nc/Nk ratio may therefore be a good indicator of soil contamination, as the disturbance of soil biochemical quality caused by contamination should be reflected by changes in this ratio. Moreover, as the extent of the disturbance increases, the difference between the value for good quality soil, i.e. 100% and the value obtained for contaminated soil will also increase and thus indicate the degree of soil disturbance in response to contamination. However, as already mentioned, agricultural soils may already be degraded before accidental contamination. The ratio should also enable estimation of the degradation of the agricultural soils prior to contamination, thus distinguishing between the disturbance caused by contamination and the previous degradation of the soil caused by management and agricultural practices. In the contamination situations considered, the three pasture soils were already degraded, although each to a different extent (Table 4). Moreover, we were able to estimate the degree of contamination at each site and to establish the level of degradation caused by soil contamination in each of the three situations (Table 4).

Table 4. *Values of the 100 Nc/Nk ratio in soils contaminated by landfill effluents, tannery effluents and hydrocarbon spills, as well as in pasture land in a nearby uncontaminated area (control soils)*

	Tanning effluent	**Landfill effluent**	**Fuel oil spill**
Contaminated sample 1	**28**	**35**	**74**
Contaminated sample 2	**25**	**19**	**55**
Contaminated sample 3	**15**	**31**	**52**
Pasture soil	**75**	**79**	**56**

Finally, when the values of the ratio for the three control soils were normalized to 100%, we were able to establish the degree of contamination caused by the tannery and the urban waste storage effluents and by the fuel oil spill.

Moreover, we were also able to differentiate situations in which the biochemical and microbial activity were reduced by the presence of the contaminant from other

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situations in which there was a transient state of high biochemical and microbiological activity (Table 4). This state was attributed to utilization of the hydrocarbons as degradable substrate and the subsequent stimulation of the proliferation of part of the soil microflora (Joergensen et al., 1995; Braddock and McCarthy, 1996; Leirós et al., 1999)).

Conclusions

In summary, we conclude that the impact of any contaminant on the enzymatic activity in an agricultural soil depends on the previous level of degradation of the soil caused by management and agricultural practices. Dehydrogenase, urease, phosphatase and, to a lesser extent, arylsulphatase are the enzymes most commonly used as indicators of contamination in agricultural soils. For the diagnosis and quantification of contamination of agricultural soils, we recommend using different enzymes and, when possible, to combine analysis of enzyme activities with analysis of other soil biochemical properties

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