TOOL WORKSHEET NO.4

# Enzymatic activities

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## DESCRIPTION OF THE INDICATOR

**Name of the indicator:** For several decades, enzymatic activities have been considered good indicators of the biochemical functioning of soils, whether they are natural or anthropised. Often associated with microorganisms' activities, they ensure the distribution through the soil of many ecosystem services such as the development of cultivated plants, animal productivity, quality of the environment, human health…

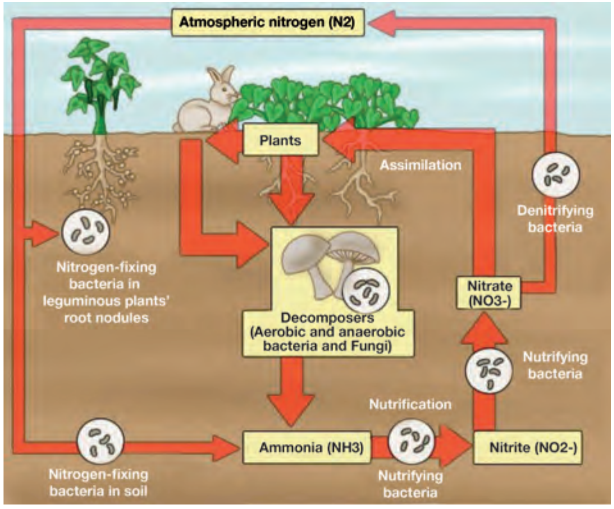



*Figure 1:* Roles played by microorganisms and their enzymes in nitrogen cycle

**Type of indicator:** Associated with major geochemical cycles such as nitrogen cycle (Figure 1), sensitive to a great amount of anthropic pressure, soil enzymatic activities show a great potential of bioindication. They are biomarkers of the functioning of soils.

## DESCRIPTION OF THE METHOD

Despite great controversy regarding interpretation of results from soil enzymatic activities measurements, many techniques have been developed for a large range of enzymes, including hydrolases and oxidoreductases. They differ in respect of the used substrate, operational conditions (especially pH), incubation time and detection methods (colourimetry, fluorometry or radiolabelling).

Different steps are (Fig. 2):

- Collection on 0-20 cm of a soil sample representative of the plot (3-5 spots per plot)
- Homogenisation, sieving and weighing of soils
- Preparation of a soil solution, and distribution in microplates
- Addition of a specific substrate and incubation
- Stopping the reaction and readings on the spectrophotometer
- Retrieval and analysis of results

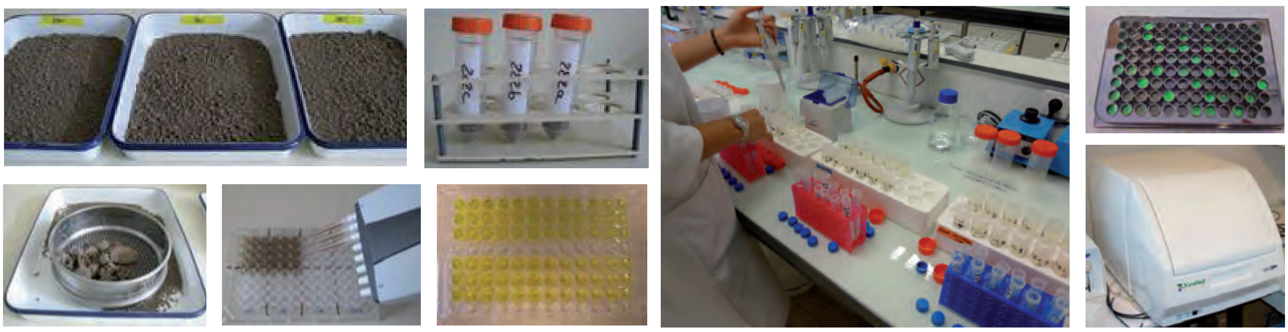


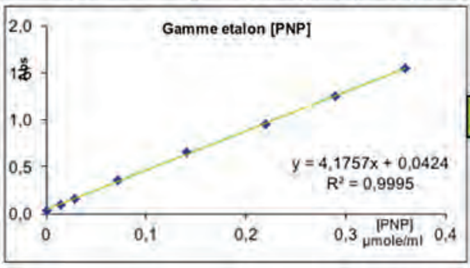
*Figure 2:* Description of the different steps of enzymatic activities measurement

Activities are automatically calculated in standard files (Figure 3), and expressed as unit (U) or milliunit (mU) per gram of dry soil, directly in a file. Data can therefore be quickly generated and analysed.

| | | | | |
|---|---|---|---|---|
| ACTIVITE IU = | ([PNP]$_{essai}$ - [PNP]$_{témoin}$) x Volume Réactionnel | | | |
| µmole[PNP] synt/min/g sol sec | Tps (min) X Masse de sol sec | | | |
| [PNP]$_{essa}$ | Conc PNP essai (mmol/l) | | durée (MIN) | 30 |
| [PNP]$_{témo}$ | Conc PNP temoin (mmol/l) | $M_{NH4+}$ | Volume réactio | 25 |

Solution 1 de p-nitrophénol à 0,05g/L (0,36 mmole/l)

| CONC | 0 | 0,014 | 0,029 | 0,072 | 0,14 | 0,22 | 0,29 | 0,36 |
|---|---|---|---|---|---|---|---|---|
| volume PNP(µL) | 0 | 5 | 10 | 25 | 50 | 75 | 100 | 125 |
| masse PNP(µg) | 0 | 3E-07 | 5E-07 | 1E-06 | 3E-06 | 4E-06 | 5E-06 | 6E-06 |
| eau (ml) | 150 | 145 | 140 | 125 | 100 | 75 | 50 | 25 |
| DO1 | 0,033 | 0,097 | 0,156 | 0,358 | 0,662 | 0,947 | 1,239 | 1,546 |
| DO2 | 0,037 | 0,095 | 0,155 | 0,353 | 0,641 | 0,955 | 1,252 | 1,548 |
| MOYENNE | 0,035 | 0,096 | 0,156 | 0,356 | 0,652 | 0,951 | 1,246 | 1,547 |
| ECARTYPE | 0,003 | 0,001 | 0,001 | 0,004 | 0,015 | 0,006 | 0,009 | 0,001 |




| | |
|---|---|
| pente dte de regression | 4,176 |
| Ordonnée à l'origine | 0,042 |

| ECH | pesee | abs 1 | abs 2 | abs 3 | T | ABS | [PNP]mM | mU (activité galactosidase) = nmole [PNP] synth/min/g sol sec | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | g | | | | | DO | Calcul | MOY | VALEURS | MOYENNE | ECARTYPE |
| B09Me98FA1 | 4,3959 | 0,273 | 0,241 | 0,244 | 0,067 | 0,253 | 0,050 | | 11,85 | | |
| | 4,3113 | 0,24 | 0,308 | 0,214 | 0,067 | 0,254 | 0,051 | | 12,16 | 11,82 | 0,29 |
| HUMIDITE | 4,3442 | 0,307 | 0,219 | 0,207 | 0,067 | 0,244 | 0,048 | | 11,45 | | |
| 0,7115569 | | | | | | 0,067 | 0,006 | 5,89E-03 | | | |
| B09Me98Fa2 | 4,3423 | 0,329 | 0,303 | 0,311 | 0,067 | 0,314 | 0,065 | | 17,83 | | |
| | 4,1282 | 0,303 | 0,296 | 0,324 | 0,067 | 0,308 | 0,064 | | 18,25 | 16,93 | 1,57 |
| HUMIDITE | 4,3384 | 0,295 | 0,269 | 0,249 | 0,066 | 0,271 | 0,055 | | 14,72 | | |
| 0,6384436 | | | | | | 0,067 | 0,006 | 5,81E-03 | | | |
| B09Me98Fa3 | 4,4244 | 0,314 | 0,295 | 0,316 | 0,071 | 0,308 | 0,064 | | 14,60 | | |
| | 4,218 | 0,259 | 0,24 | 0,275 | 0,073 | 0,258 | 0,052 | | 12,06 | 13,30 | 1,04 |
| HUMIDITE | 4,4134 | 0,312 | 0,27 | 0,275 | 0,071 | 0,286 | 0,058 | | 13,23 | | |
| 0,7312607 | | | | | | 0,072 | 0,007 | 7,01E-03 | | | |
| | 4,0095 | 0,244 | 0,215 | 0,235 | 0,069 | 0,231 | 0,045 | | 10,84 | | |
| B09Me98Fa4 | 4,2871 | 0,241 | 0,258 | 0,284 | 0,08 | 0,261 | 0,052 | | 12,02 | 11,36 | 0,49 |
| HUMIDITE | 4,1078 | 0,227 | 0,253 | 0,244 | 0,067 | 0,241 | 0,048 | | 11,24 | | |
| 0,7319364 | | | | | | 0,072 | 0,007 | 7,09E-03 | | | |
| B09Me117F1 | 4,1717 | 0,173 | 0,173 | 0,157 | 0,066 | 0,168 | 0,030 | | 6,45 | | |
| | 4,0465 | 0,179 | 0,187 | 0,164 | 0,065 | 0,177 | 0,032 | | 7,23 | 6,29 | 0,84 |
| HUMIDITE | 4,1187 | 0,146 | 0,138 | 0,155 | 0,062 | 0,146 | 0,025 | | 5,19 | | |
| 0,7661989 | | | | | | 0,064 | 0,005 | 5,25E-03 | | | |
| B09Me117F2 | 4,2363 | 0,183 | 0,194 | 0,206 | 0,065 | 0,194 | 0,036 | | 7,80 | | |
| | 4,2079 | 0,181 | 0,189 | 0,178 | 0,065 | 0,183 | 0,034 | | 7,14 | 7,43 | 0,28 |
| HUMIDITE | 4,3669 | 0,208 | 0,17 | 0,194 | 0,068 | 0,191 | 0,036 | | 7,35 | | |
| 0,7749794 | | | | | | 0,066 | 0,006 | 5,65E-03 | | | |

*Figure 3 :* Example of enzymatic activities calculation table

## INTERESTS AND LIMITS OF THE INDICATOR

The interests of such indicators are to get a **global vision of all degradation cycles** of organic matter, to be **very selective** and **very sensitive**, thus usable at low levels of activity. Besides, soil enzymatic activities measurement methods are **easy** to implement and **cheap**.

However, monitoring these activities has its limits. Currently, the operation of activities measurements in soils leaves us with very few quick and automatable methods for the monitoring of a great number of samples. Collected data are therefore often fragmented. A better interpretation of results would imply to **have reference situations available**, in order to evaluate natural spatio-temporal variations. It is also necessary **to access multiple disruption situations** including soil use, plant cover, chemical inputs. These results, achieved according to standardised protocols, should ultimately lead to the **construction of frames of reference** for their interpretation. Another strong constraint lies in the availability of sampling storage methods that are not harmful to activities. Finally, enzymatic activities measurements must be associated with measurements of other biological and physic-chemical indicators.

## EXAMPLE OF APPLICATION

The measurements of **numerous enzymatic activities** involved in the cycles of carbon, nitrogen, phosphorus and sulphur have been conducted on a large scale in the course of the ADEME's Bioindicateurs I & II programs. One of these programs' goals was to improve our knowledge on the biological functioning of soils in order to provide **indicators of soil quality**, therefore leading to wiser soil management procedures. The idea was in particular to evaluate the sensitivity and limits of use of such indicators, to **build a frame of reference for data interpretation** and to transfer results for **risk assessment**.

### *Example of application for agricultural practices*

The effects of agricultural practices on enzymatic activities have been highlighted in many case studies (Figure 4) and we were able to demonstrate that:

- **Crop rotations** significantly impact the **13 enzymatic activities** under study (with different thresholds of significance)
- **Tillage** significantly impacts **7 enzymatic activities**
- **Organic amendments** have a significant impact on **6 enzymatic activities**

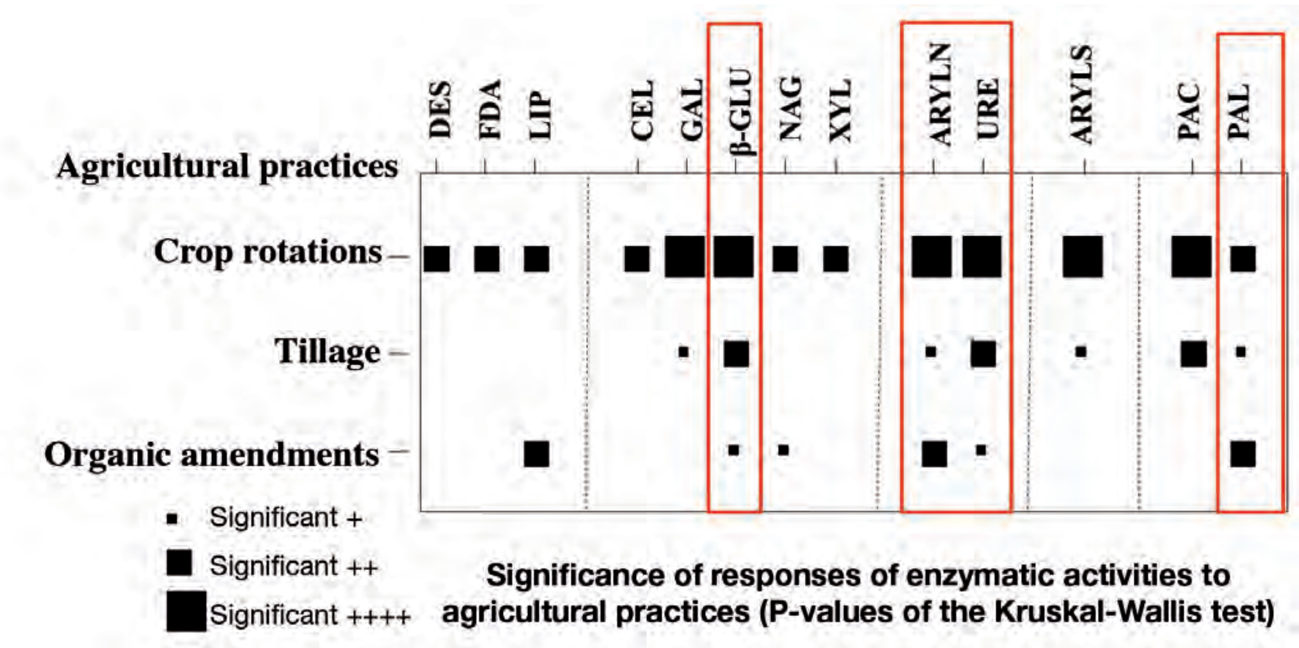



*Figure 4:* Responses of enzymatic activities to agricultural practices

**Four enzymatic activities** distinguish themselves and respond to **all 3 agricultural practices:**
**Alkaline phosphatase – Urease - β-glucosidase – Arylamidase**

### *Example of application for contaminated sites*

Results achieved on contaminated sites (Figure 5) show that:

- **Metal and arsenic contaminations** impact respectively **11 and 13 enzymatic activities**
- **Organic contamination** only impacts significantly **2 enzymatic activities**

Measurements conducted on the program's contaminated sites helped:

- Discriminate plots under cultivation from forested plots
- Discriminate different levels of forested contamination
- Rank the effects of contaminants on the biological functioning of studied plots
- Discriminate the effect of contamination levels in relation to plant cover by highlighting the leading role of specific enzymatic activities.
- Define 2 enzymatic activities that significantly respond, no matter what type of contamination they were exposed to: **Arylamidase (ARYL-N) and alkaline phosphatase (PAK)**

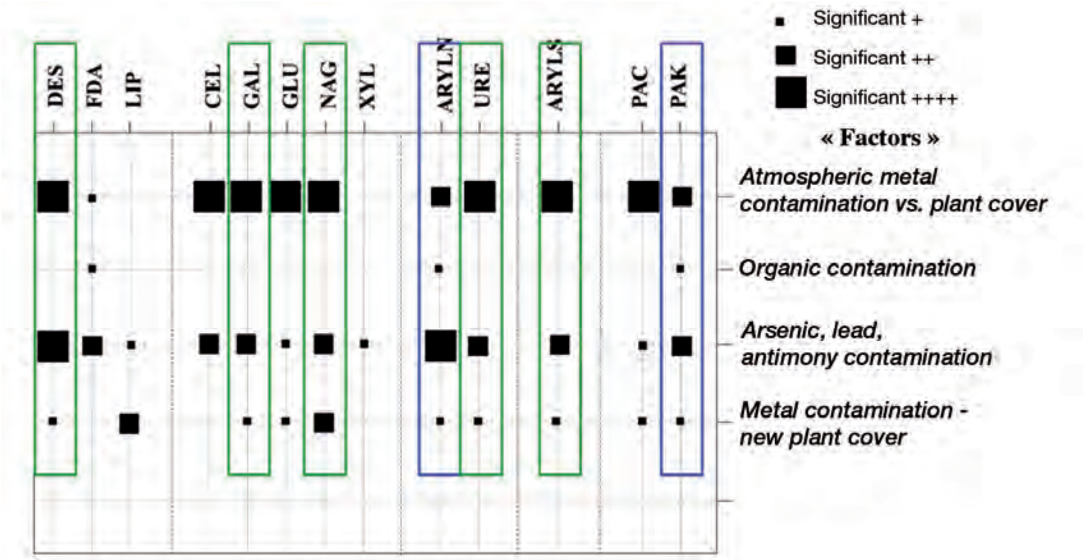



*Figure 5:* Responses of enzymatic activities to contaminations (blue frame: enzymes responding to all factors; green frame: enzymes only responding to metal contaminations)

## PERSPECTIVES

In order to facilitate the diagnosis of the biochemical state of soils based on enzymatic activities measurement, our goal is to be able to choose from these activities depending on the agronomic or environmental issue at stake, and to position them within a frame of reference. To achieve this, the construction of a multienzymatic index is essential. It will need to be based on enzymatic activities that are significantly impacted by a range of factors, agronomic data or ecosystem services, such as the Puglisi et al. index (2006).

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