Making of indicators biocatalytic Of the pesticides Imidazols in natural water

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Abstract- This work aims at the installation of an indirect technique of evaluation of the pesticides in surface water. This technique calls upon a proportioning of the peroxidase activity in water suspected of being contaminated by the pesticides. Aqueous artificial systems containing ranges of the pesticide, the Imazehapyr, were used as a model to work out a technique of qualitative and quantitative analysis. The results obtained present a linear correlation (0,99) between the amounts and the evaluated peroxidases activities. This technique was validated later on surface water contaminated by the pesticides. The producing germ of the enzyme peroxidase (*Aspergillus Niger*) showed a metabolic modulation which is expressed by a repression by Glucose (0,5%) and an induction by the Phenol (0,5%).
The results of this technique open prospects on the evaluation for the pesticides for less expensive methods.

Key words- Surface water, Imazethapyr, peroxidase, induction and repression and linear correlation.

Résumé- Ce travail a pour objectif la mise en place d'une technique indirecte d'évaluation des pesticides dans les eaux de surface. Cette technique fait appel à un dosage de l'activité peroxydase dans les eaux soupçonnées d'être contaminées par les pesticides. Des systèmes artificiels aqueux contenant des gammes du pesticide l'Imazéthapyr ont été utilisées comme modèles pour élaborer une technique d'analyse qualitative et quantitative. Les résultats obtenus présentent une corrélation linéaire (0,99) entre les doses et les activités peroxydases évaluées. Cette technique a été validée ultérieurement sur les eaux de surface contaminées par les pesticides. Le germe producteur de l'enzyme peroxydase (*Aspergillus Niger*) a montré une modulation métabolique qui s'exprime par une répression par le Glucose (0,5%) et une induction par le phénol (0,5%).
Les résultats de cette technique ouvrent des perspectives sur l'évaluation des pesticides par des méthodes moins coûteuses.

Mots clés- Eaux de surface, Imazéthapyr, peroxydase, induction et répression et corrélation linéaire.

1. INTRODUCTION

The increased use of the pesticides has resulted in an increase in the vegetable production, and reduced the maintenance costs and the control of the risks for the public health. On the other hand, however, the concerns involving the potential negative effects of the pesticides on the environment and human health have also increased. In many regards, the greatest potential of undesirables adverse effects of the pesticides is the contamination of the hydrological system, which supports the watery life and the related food chains and is used for leisure purposes, the drinking water and many other uses. Water is one of the principal mechanisms of transport of the pesticides of the zones of application towards other parts of the environment, which can involve movements in and through all the components of the hydrological cycle.

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The surface water is particularly vulnerable to the contamination by the pesticides, because the majority of the agricultural and urban zones flow in surface water. Once the pesticides are in the water supply network of surface moving (river and rivers), they can be largely transported downstream and dispersed in other rivers, lakes, tanks and, in the final analysis, in the oceans. The presence of pesticides in surface water has been recognized since the years 1940 (Butler, 1966). With the discovery of the harmful ecological effects of the pesticide DDT and the awakening increase of the environmental problems in the years 1960, the problem of the pesticides in surface water has been the subject of an increasing attention for the last decades.
Thus, a number of studies have shown the presence of residues of pesticides in food as well as the contamination of subsoil waters and surface; hence, the need for studying the outcome of these components in water in order to better measure the consequences and in particular their environmental impact.
The pesticides in general, and the weed-killers in particular, are regarded so dangerous micro-pollutants even with the state of traces. Adsorption and photo-degradation are among the principal ways of their dissipation in the environment. Library searches have proved that the toxicity of the Imazethapyr, (weed-killer used for the weeding of railways and the protection of certain cultures), is relatively

low and its adsorption by the grounds is generally weak, but part of this product can remain in the ground. Despite this, this weed-killer is likely to pollute water.

Today, these pesticides are omnipresent in the rivers in the agricultural medium. Two principal phenomena are responsible for this situation; the streaming (runoff) and scrubbing (leaching). The streaming is the result of the water run-off of surface following the rains or the irrigation. Scrubbing (sometimes called leaching) is caused by the drive of water through the ground. The weed-killers can be found in the drains, the wells, and the sheets of surface or underground water.

Several factors influence the displacement of the weed-killers. Here are some: 1) Volatility Property of the product to change into gas and to escape in the atmosphere. 2) Adsorption Attraction of a weed-killer on particles (organic matter, clay, etc). The more the weed-killer is absorbed, the more the risks to find it in water are decreased. The phenomenon is, however, reversible. 3) Solubility capacity of a weed-killer to be dissolved in water; the more soluble the product is, the larger the risk is to find it in water (for example the Imazethapyr). 4) Type of ground 5) Pluviometry 6) Remanence.

The properties of bio-accumulation of the pesticides facilitate their reaching the human being and their accumulation in the body, and generate health problems: poisoning, skin diseases, respiratory problems, sterility, cancers, asthma, effects on the nervous system and on the reproductive system.

According to what precedes, the pesticides present a potential threat for the water resources.

This pollution, therefore, affects in priority surface water, where one observes a contamination of all the rivers. In order to protect the watery environment, the natural water treatment charged in Imazethapyr is essential. The treatment can be carried out using various processes which are currently well controlled on a laboratory scale and are applied on a large scale including the physical treatments while using fluids (water or gas), present in the ground or are injected, like vector to transport pollution towards points of extraction or to immobilize it, the chemical treatment which uses the chemical properties of the pollutants, using adapted reactions, for the inerter (precipitation, etc), to destroy them (oxidation, etc) or to separate them from the polluted medium (surfactant, etc.) and the biological treatment which consists of using micro-organisms, generally bacteria (but also of mushrooms and the plants), to support total or partial degradation of the pollutants. Some bioprocesses also make it possible to fix or solubilize certain pollutants.

The analysis of the pesticides is a long-term job, it requires the knowledge to make and a very important infrastructure, which makes their analyses not accessible to everyone.

Several analytical attempts were targeted to facilitate the evaluation of the presence of the pesticides in biological sites, thereby positioning our work to find analytical methods of biological nature, which gives quantitative and qualitative indications pesticides or their residues in a watery system.

2. MATERIAL AND METHODS

2.1 Presentation of the Imazethapyr as a model

The Imazethapyr or acid 5-éthyl-2- (4-isopropyl-4-methyl-5-oxo-2-imidazoline-2-yl) nicotinic, is an organic compound of formula C15H19N3O3 pertaining to the chemical family of the Imidazolines. This substance is used as active matter of selective weed-killers marketed under various marks. This weed-killer is classified in the group B (inhibitors of enzyme acétolactate synthase or ALS) of the HRAC classification of weed-killers. It is used to fight against various types of bad grasses, as well as with broad sheets (dicotyledones) such as graminaceous (monocotylédones). One uses it in particular in the cultures of beans, peas, soya, groundnut, alfalfa and corn. It is manufactured by several industrialists: BASF (Germany, Canada), American Cyanamid (the United States), Agsin (Singapore) 1 and sold under marks such as Contour, Hammer, Odyssey (Imazamox + Imazethapyr), Overtop, Passport, Patriot (Atrazine + Imazethapyr), Pivot, Pursuit (Ammonium salt), Pursuit More, Solves, Valor (Imazethapyr + Pendiméthaline).

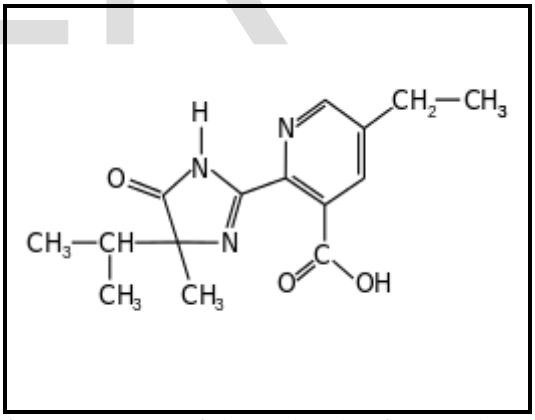

Fig. 1: Structure of the molecule of Imazethapyr

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2.2 Culture media

The various reagents employed during this study were used without preliminary purification. The solutions of the

organic compounds were prepared by dissolution in aqueous medium. The pH of the solutions was adjusted by hydrochloric addition of acid HCl.

The chemicals and the standards used during the experiments and the analyses are of following analytical quality:

Medium PDA (Potato Dextrose Agar) 2% (pre-culture of the stock): for the conservation of the long-term stock.

Liquid minimum medium composed of: Source of carbon (Glucose 0,5g/l or the Phenol 0,5g/l); 1.36 g/l of Dibasic potassium phosphates K2HPO4; 0.1 Epsom salt g/l MgSO4; 0,6 Sulphate g/l of Ammonia SO4 (NH4) 2; 0,02 Calcium Chloride g/l CaCl2; 0,5 Sodium chloride g/l NaCl; 1,1 mg/l Sulphate MnSO4 Manganese; 0.2 mg/l Sulphate ZnSO4 Zinc; 0,2 Copper sulfate mg/l CuSO4; 0,14 Ferrous Sulphate mg/l FeSO4; (Adjusted pH with 7 with a solution 1M HCl)]. **The mediums are sterilized before their use during 20 min with 121°C.**

The culture of the stock *Aspergillus Niger* was carried out under the optimum conditions for mushroom: pH= 7; (Glucose 0,5 g/l or Phenol 0,5 g/l); T=37°C; Agitation (100 tr/min); Pesticide 20 mg/l.

2.3 Source of carbon

In order to follow the kinetics of degradation, to determine the breakdown products and the compositions of the solution, during the biological treatment, several aqueous solutions were employed under the conditions fixed above and which are as follows:

Minimum medium + Glucose (0,5%)

Minimum Medium + Phenol (0,5%)

Minimum medium + Glucose (0,5%) + Imazethapyr (0,002%)

Minimum Medium + Phenol (0,5%) + Imazethapyr (0,002%)

The induction of the catalysis was evaluated during the growth.

2.4 Search for peroxidase activity

Peroxidases are oxydoreductases which catalyze the oxidation of a substrate (donor of protons) in the presence of hydrogen peroxide (H_2O_2). They use hydrogen peroxide as acceptor of electrons coming from various molecules. Moreover, they can catalyze the oxidation of some molecules in the presence of oxygen. The catalysis started by H_2O_2 allows the formation of transitory enzymes. While governing with a donor of proton (AH_2), they give rise to free radicals (AH) which can form polymers while reacting with one another. The total reaction is of the type:

$$AH_2 + H_2O_2 \rightarrow A + 2\,H_2O$$

2.5 Preparation of the samples

Two aqueous artificial systems containing ranges of the Imazethapyr pesticide were used as a model to work out a technique of qualitative and quantitative analysis:

Minimum medium (distilled water);

Formed minimum medium (water contaminated by the pesticide).

2.6 Test of peroxidase activity

Peroxidases are enzymes which oxidize a substrate defined in the presence of hydrogen peroxide (H_2O_2). They are regarded as enzymatic antioxydants.

These oxidoreductases recognize natural and synthetic substrates. One of the reactions used to test their activity is that using the guaiacol like substrate, generating the tetraguaiacol absorbing the light with 470 nm.

3. Results and Discussions

The results of the technique of qualitative and quantitative analysis, obtained by using the aqueous artificial systems containing ranges of the Imazethapyr pesticide present a linear correlation (0,99) between the amounts and the evaluated peroxidases activities. The producing germ of the peroxidase enzyme (*Aspergillus Niger*) showed a metabolic modulation which is expressed by a repression by Glucose (0,5%) and an induction by the Phenol (0,5%).

3.1. Effect of the source of carbon on the peroxidase activity and the abatement of the pesticide

Table 1: Effect of the source of carbon on the peroxidase activity and the abatement of the pesticide

Source of carbon	Glucose 0,5%	Phenol 0,5%	Glucose (0,5%) + Imazéthapyr (0,002%)	Phenol (0,5%) + Imazéthapyr (0,002%)
Activity peroxidase (UE)	5	9	8	24
Percentage of abatement	34	54	51	83

Table 1 shows that the Phenol (0,5%) and the Imazethapyr (0,002%) give Enzymatic values of 24 Units (EU) and 83% for the peroxidase activity and the percentage of abatement respectively. However, one notices an effect slightly less marked for Glucose (0,5%), Phenol (0,5%) and the combination Glucose + Imazethapyr.

These results prove that the presence of Phenol with the Imazethapyr posts the best rate of abatement as well as a high peroxidase activity. This explains why the Phenol induces the synthesis of peroxidase.

3.2. Kinetics of enzymatic activity

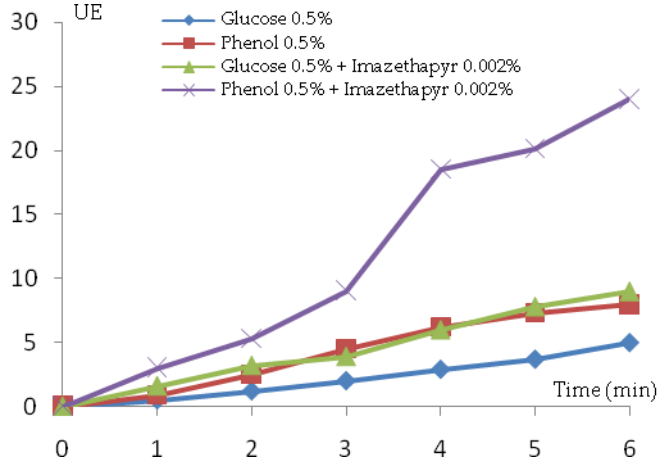

Fig. 2 : Kinetics of enzymatic activity

The figure studies the evaluation of the activity peroxidase (EU) according to time (min) while varying the sources of carbon (Glucose, Phenol, Glucose + Imazethapyr and Phenol + Imazethapyr).

The kinetics of enzymatic activity of Phenol + Imazethapyr increases with the variation of time. It is also noticed that the presence of Phenol as a source of carbon gives better enzymatic activity than that Glucose. This shows that the Phenol is an inductor of the metabolisation of the pesticide Imazethapyr and Glucose is a repressor of the metabolisation of the pesticide Imazethapyr.

3.3. Study of the correlation between the concentration of the comparable source of carbon and the peroxidase activity

The variety of the reactions which peroxidases catalyze led in their exploitation to the biotechnological applications. Recently, peroxidases receive a more detailed attention like biocatalysts for the applications of biosynthesis, of bio-transformations and others.

Their industrial application was the subject of several studies, like the elimination of the industrial wastes, discoloration and water treatment and other reactions of bio-transformations. Thus, their application was shown in biochemistry.

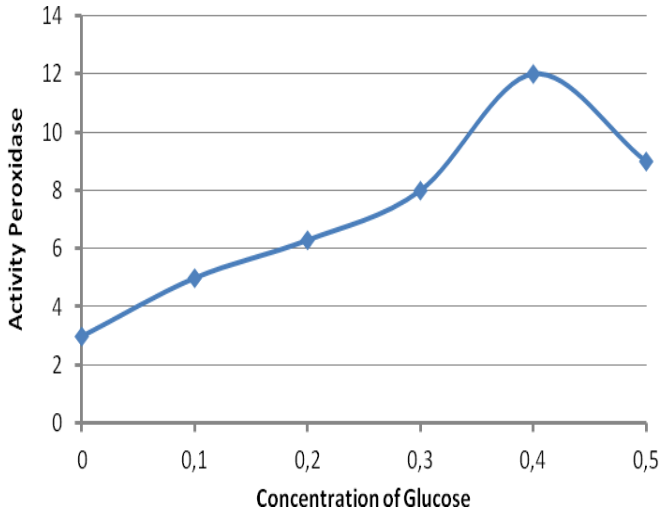

Fig. 3 : Peroxidase Activity in the presence of Glucose (AS)

Fig. 3 studies the variation of the peroxidase activity in the presence of Glucose, which gives a factor of correlation of 0,9545 showing that there is a catabolic repression. Catabolic repression is the fact that in the presence of Glucose (glucids quickly metabolizable) the synthesis of the enzymatic systems is more or less strongly inhibited. It is necessary to stress that the significant systems to this end (indicated sometimes under the name of Glucose effect) are implied in the first stages of the degradation of the organic substances metabolizable by the bacterium.

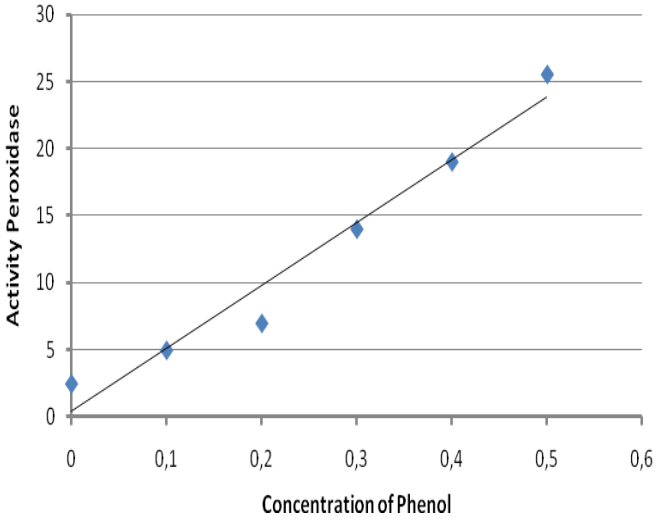

Fig. 4 : Peroxidase Activity in the presence of Phenol (AS)

The extent of catabolic repression depends on the nature of the source of carbon. The most degree of repression is obtained under conditions where the source of carbon is quickly metabolized, therefore under conditions where the

accumulation of the metabolites of the source of carbon is favoured. These facts result in supposing that some of the metabolites formed during the decomposition of glucids are the agents of this regulation, perhaps acting as corepressors. This assumption is not completed in the case of Phenol like source of carbon.

Figure 4 analyzes the variation of the peroxidase activity in the presence of Phenol, which interprets that the peroxidase activity changes relatively with the increase in the percentage of the concentration of Phenol with a factor of correlation of 0,9539, which leads to the absence of catabolic repression, which is considered in a very new light and which shows that the Phenol seems to play an important part in this phenomenon.

3.4. Validation of the concept

To choose a method of analysis, many criteria have to be taken into account, such as, the number and the rate of the analyses which one carries out, but the essential goal will always be to produce at a lower cost data that nobody can dispute: a result of validated analysis, called analytical data. To achieve this goal requires, on the one hand, to be ensured of the exactitude of the method chosen, on the other hand, to reduce the number of repetitions of the analyses intended to produce the same data. In this regard, it is necessary to prepare a reference sample of the laboratory which will be used first of all to study and validate the method then, as a standard, to set up an internal system of audit of the quality of the analyses.

This sample must represent an "average" of the samples which will be received later on by the laboratory for an analysis, average with regard to the content of the analysis or of the required analysts. To measure the polluted waters contaminated by the pesticides, one has to prepare the reference sample of the laboratory starting from a great number of polluted and non polluted samples; those which are polluted will be crushed and closely mixed with one another to obtain a homogeneous sample. One has to be ensured then of the conditions of conservation of this sample so that it does not undergo modifications in space (homogeneity) and time (various contaminations, reactions, etc).

Table 2: Biocatalytic validation of the concept of indicator of the pesticides Imidazols

Sample	AS Peroxidase (Distilled Water)	AS Peroxidase (Distilled Water + Pesticide)
1	15,3	22
2	12,4	25
3	10,7	24,5
4	11,3	28,6
5	14,4	25,5
6	15	23,3
7	13,4	21
8	14,6	26
9	12,3	22,5
10	14	25
Average	13,34	24,34
Standard deviation	1,5	2,1

As shown in table 2, the fourth stage of an analysis is the treatment of the samples. The treatments are carried out on solutions of several distilled water and water samples distilled only initially associated with the pesticide in a second place. Afterwards, one proceeds directly at the stage of measurement of the peroxidase activity. The value of this peroxidase activity varies from a sample to another in the two cases recorded below (distilled water/distilled water + pesticide). Hence, the importance of the calculation of the average and the standard deviation. The importance of the average value of all the data also depends on the importance of the standard deviation. For the first type of samples (distilled water) value 1,5 of the standard deviation is low, which means that the values are dispersed little around the average of the values of the peroxidases activities which is 13,34. The second type of samples (distilled water + pesticide) value 2,1 of the standard deviation is raised a little, which means that the values are dispersed around the average of the values of the peroxidases activities which is 24,34.

In conclusion, it is interesting to stress that the peroxidase activity as bioindicator of the contamination of the pesticides of the Imidazols family is accomplished.

4. Conclusion

The intensive use of the pesticides in agriculture generates an unprecedented contamination of surface water as well as that of the ground water. The traditional treatments applied to water containing polluting organic materials are based on the biological breakdown or physical methods of mass transfer (decantation, filtration, adsorption of the pollutants on activated carbon) or chemical processes such as

oxidation to ozone or chlorine. However, these processes remain ineffective in the case of water treatment contaminated by the organic pollutants.

This work lies within the scope of the natural water treatment charged in pesticides by an experimental protocol which can destroy the pesticides effectively, chemicals largely used in agriculture in Morocco, such as the Imazethapyr by using the biological process of biocatalytic indicator. The results confirm the effectiveness of the process of peroxidase activity in the water treatment polluted by the Imazethapyr. This weedkiller was selected due to its strong use on a worldwide scale and its environmental impact. The main result obtained during this work is that the peroxidase activity can be used as bioindicator of the contamination of the pesticides of the Imidazols family.

In prospect, it would be interesting to make tests on the other pesticides of the same family and to create kits of water treatment contaminated by the pesticides.

REFERENCES

[1] Observatory of the Residues of Pesticides in PACA, Full report of the results 2013 of ORP PACA. Publication of the 12/2014, 94-102;

[2] Technical elements on the compilation of the plant health indices Acta Index ACTA; Heap, 2014; Weedkiller Resistance Committee Action 3-8-11;

[3] Butler (P.A.), 1966. - *The problem off pesticides in estuaries*-Spec. Publ. Amndt. Fish. Ploughshare 3: 110-115. -1966. - *Pesticides in the marine environment*- The newspaper off applical ecology, 3 (Suppl.) 253;

[4] Meyer A., Chrisman J., Moreira J.C., Koifman S., 2003. Agricultural *cancer mortality among workers from Serrana Area*, state of Rio de Janeiro, Brazil. Approximately Res., 93, 264-271;

[5] HRAC (Weedkiller Resistance Committee Action) "*Classification off Weedkillers According to Site of Action*" [files], (consulted on March 8, 2015);

[6] Stanley A. Greene, *Sittig's Handbook of Pesticides and Agricultural Chemicals Personal care and cosmetic technology*, William Andrew, 2013, 1213 p. (ISBN 9780815519034), p. 526-527 ;

[7] BONTOUX J. (1993). *Introduction to the study of fresh waters, natural water used water, drink water*. Cebedoc ED., Liege, p169;

[8] Duda A.M. and Al, 1996. Copper (II) *complex of Imidazolinone Imazapyr* weedkiller. J. Agric. Food Chem., 44, 3698-3702;

[9] "Imazethapyr - *Pesticide information profiles*" [file], on Extoxnet - Extension Toxicology Network (consulted on April 7, 2016);

[10] Mechrafi E., 2002. *Adsorption, desorption and mobility of the weedkillers in contact with the organic and mineral adsorbents*. Thesis of doctorate: University Mohammed V, Faculty of Science, Folds back (Morocco);

[11] Verneaux J. Reflexions on the appreciation of the quality of running waters using biological method. Year. Sci. Univ. Besancon, 1982: 3-9 ;

[12] Beaudoin, S. (*2011, Analyze transverse of three generations of pesticides (conventional, OGM-pesticides and nano pesticides): strategies, regulation, evaluation, impacts environmental, medical and economic*. Environmental review article, ISE-UQAM, June;

[13] Rodier J. *Analysis of water: Natural water, waste water, sea water*. ED. Dunod Paris 1984;

[14] Devillers J., Farret R., P G., River J-L. and Soulas G., Eds. (2005). *Indicators to evaluate the risks related to the use of the pesticides*, ED. Tce & Doc.;

[15] - Simeonov V., Stratis J., Samara C., Zachariadis G., Voutsa, D., Anthemidis A., Sofoniou Mr., & Kouimtzis T. *Assessment off the surface toilets quality in Northern Greece.Water* Research37, 4119-4124 (2003);

[16] - Viel J.F., Challier B., Pitard A., Pobel D., 1998. *Brain cancer mortality among French farmers: the vineyard pesticide hypothesis*. Arch. Environ. Health., 53, 65-70 ;

[17] - Carole Bedos, Sophie Génermont, Edith the Framework, Lucas Garcia, Enrique Barriuso, Pierre Storeroom "*Modelling pesticide volatilization after soil application using the mechanistic model Volt' Air*" Atmospheric Environment, flight. 43, pp. 3630-3639, 2009 ;

[18] - Roberts EM, English PB, Grether JK, and Al .*Maternal residence near agricultural pesticide applications and autism spectrum disorders among children in the Central California Valley*. Approximately Health Perspect 2007; 115 : 1482-9 ;

[19] - Jollivet Mr., Mathieu NR., 1989. *The rural one with the environment: the question of nature today*. Paris: Harmattan. 351 p ;

[20] - Lyall K, Croen, Sjödin A, and Al. *Polychlorinated Biphenyl and organochlorine pesticide concentrations in maternal mid-pregnancy serum samples: association with autism spectrum disorder and intellectual disability*. Approximately Health Perspect 2016; [Epub ahead off print]

[21] - Tron I., Stake O., Cohuet S., 2001. *Chronic effects of the pesticides on health: current state of knowledge*. Rennes, Regional Observatory of Brittany Health. 88 p;

[22] - Gaspar FW, Harley KG, Kogut K, and Al. *Antenatal DDT and DDE exposure and child IQ in the CHAMACOS cohort*. Approximately Int 2015; 85 : 206-12 ;

[23] - Giroux, Isabelle, *Contamination of water by the pesticides in the areas of soya and corn culture in Quebec,* Results of the sampling campaigns 1999, 2000 and 2001, and temportelle evolution of 1992 to 2001 per Department of the Environment, Government of Quebec, December 2002, pdf, 78 pages;

[24] - Donauer S, Altaye M, Xu Y, and Al. *Year observational study to evaluate associations between low-level gestational exposure to organophosphate pesticides and cognition during early childhood*. Am J Epidemiol 2016; 184 : 410-8 ;

[25] - Mamy L., Barriuso E., Gabrielle B. (2008a). *To evaluate the environmental risks of the pesticides. Example of the weeding of the cultures resistant or not to the glyphosate*. Agronomic innovations 3: 121-143 ;

[26] - Engel SM, Bradman A, Wolf MA, and Al. *Antenatal organophosphorus pesticide exposure and child neurodevelopment At 24 months: year analysis off furnace birth cohorts*. Approximately Health Perspect 2016; 124 : 822-30 ;

[27] - Pingault NR. (2007). *To improve quality of water: An indicator to support a durable use of the plant health products*. Workshop OECD, 19 March 21, 2007, Washington, Ministry for Agriculture and Fishing: 10p;

[28] - Liu P, Wu C, Chang X, and Al. *Unfavourable associations off both antenatal and postnatal exposure to organophosphorous pesticides with infant neurodevelopment in agricultural year area off Jiangsu province, Clouded*. Approximately Health Perspect 2016; 124: 1637-43 ;

[29] - Zamy C., Mazellier P., Legube B. (2004). *Hydrolysis and photolysis off two organophosphorus pesticides in dilute aqueous solutions: Kinetics and by products*. European newspaper of Hydrology 35 (1): 47-58 ;

[30] - Stein LJ, Gunier RB, Harley K, and Al. *Early childhood adversity potentiates the unfavourable association between antenatal organophosphate pesticide exposure and child IQ: The CHAMACOS cohort*. Neurotoxicology 2016; 56 : 180-7 ;

[31] - Herrmann J.M., Guillard C., *Photocatalytic degradation off pesticides in agricultural used toilets* - C.R. Acad. Sci. Paris, Series LLC, Chemistry/Chemistry, 3, 23, (2000), 417-422 ;

[32] - Gunier RB, Bradman A, Harley KG, and Al. *Antenatal residential proximity to agricultural pesticide uses and IQ in 7-year-old children*. Approximately Health Perspect 2016 ;

[33] - Rowe C, Gunier R, Bradman A, and Al. *Residential proximity to organophosphate and carbamate pesticide uses during pregnancy, poverty*

during childhood, and cognitive functioning in 10-year-old children. Approximately LMBO 2016; 150 : 128-37 ;
[34] - Anses. *Professional exposures to the pesticides*. 2016 ; Volume 1.