

## Peroxidase Effect of the Root of *Brassica napus var. rapifera* (turnip) Phenol Concentration in Water Samples of Lake Chapala, Jalisco Mexico

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### ABSTRACT

**Objective** To determine the effect of peroxidase enzyme root *Brassica napus var. rapifera* (turnip) in the concentration of chlorophenol in water samples from Lake Chapala.

**Material and methods** An observational study was carried out, such study consisted on collecting ecological water samples from Lake Chapala Jalisco, Mexico and comparing those to the Industrial and Technical Education Center's (CETI) drinking water by using a phenol solution at 2% and root extract of *Brassica Napus var. rapifera* (turnip) which contains a high percentage of peroxidase enzyme.

**Results** Water samples collected from Lake Chapala Jalisco reacted to peroxidase in a period of 5-10 minutes, which denoted that the enzyme is able to act on this substrate.

**Conclusions** The oxidizing action of the chlorophenol in wastewater treatment removes carcinogenic and mutagenic actions and therefore reduces public health risks to human population.

**Keywords:** Peroxidase, Chlorophenol, Enzyme activity, *Brassica NapusVar*, Reduction. Risk in the Health

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### INTRODUCTION

"The phenol was obtained by Runge in 1834, which he called carbolic acid" In 1867, the British surgeon Joseph Lister first used phenol as a disinfectant to sterilize wounds, bandages and surgical instruments. Dilute solutions are very as antiseptics, but the concentrated solutions are caustic and leave scar tissue"<sup>1,2,3b</sup>

Currently, the phenol has been replaced by germicides less irritating and more effective. It is used on the manufacturing of resins, plastics, pesticides, explosives, dyes and detergents, and as feedstock for the production of some drugs, such as aspirin. Until the First World War, the phenol or carbolic acid had only been obtained by removing the asphalt, until the process was established to be based on the sulfonation of benzene hydrolyzed with sodium hydroxide (phenol synthetic). In 1930, the process becomes monochlorobenzene hydrolysis to yield the phenol in the steam phase to monochlorobenzene hydrolyzing with water in what is known as the process of Rashig.

In 1950, B. P. Internacional. Ltd. and Hercules Chemical, Inc., institute a new process for obtaining phenol oxidizing cumene to cumenehydroperoxide and catalyzing the reaction thereof to obtain phenol and a second commercial product acetone. This process arose due to overproduction of cumene was the reaction product in obtaining synthetic rubber GR - S , in addition to the need for obtaining acetone was used as an additive for aviation gasoline .

In 1961 the Dow Chemical of Canada, Ltd., obtained by the oxidation of toluene to benzoic acid, and reoxidation of the latter to obtain fenol<sup>3</sup>.



Lake Chapala Jalisco is one of the largest natural lakes in Mexico. America and the world, is the main source of water to supply the same to the Guadalajara metropolitan area, unfortunately receives contaminated water such as laden stools pig and anthropogenic materials that threaten endemic species of fauna and promote an increase in pest lily, and among other important works on issues related to human health risks.

It represents a major touristic attraction and constitutes one of the sources who collaborate in the ecological balance and is involved in maintaining climate stability of the environment.

#### **WHAT IS PHENOL AND CHLOROPHENOLS ?**

Phenol is both a manufactured substance and natural. Pure phenol is colorless or white, the commercial product is a liquid with characteristic smell sickeningly sweet and tarry, is used to manufacture drugs, adhesives, plastics and rubber, 6,7, 8 is part of the list of dangerous substances (Azardaus substance List) regulated by OSHA; legal limit air exposure in workplace 5 ppm / 8 hours, NIOSH of 5 ppm for 8 hours and 15.6 ppm with less than 15 minutes of exposure. ACGIH : 5 ppm for 8 hours / average workplace and EPA cited among others , is mutagenic and may cause risk for cancer<sup>4,5</sup>. May cause effects on the human health , it is corrosive and causes burns when being in contact with it.<sup>6,9</sup> Chlorophenols are a group of chemicals that are formed by Cl - ion binding to phenol group . Is derived from phenol and benzene is an aromatic compound used in the synthesis of pesticides and antiseptic use, among others. Chlorophenols are a group of chemicals that are formed by adding chlorine (between one and five) to phenol. Five basic types of chlorophenols are mentioned as follows: monochlorophenols, dichlorophenolstrichlorophenols, tetrachlorophenols, and pentachlorophenols. In total , we have identified so far 19 different chlorophenols , the most common are : 2-chlorophenol , 4 - chlorophenol, 2,4- dichlorophenol , 2,4,5 - trichlorophenol , 2,4,6 - trichlorophenol , 2, 3,4,5 - tetrachlorophenol , 2,3,4,6- tetrachlorophenol and 2,3,5,6-tetrachlorophenol. 2-chlorophenol, is a liquid at room temperature, all the solids are chlorophenols. Chlorophenols have strong medicinal taste and smell and the taste can be seen in the water even at low concentrations of parts per million (ppm). Chlorophenols at least two chlorines have been used either directly as pesticides or pesticides have been converted. Also, chlorophenols, especially 4-chlorophenol, have been used as antiseptics. Besides commercial production can be formed small amounts of chlorophenols, especially mono dichlorophenols to chlorinated waste water or drinking water for the presence of phenol and also produced during the chlorination of wood pulpin the processes for papermaking<sup>10</sup>. Chlorophenols entering the environment in a general way when applying pesticides or by ion exchange reactions, we generated a chain reaction when chlorophenols reach the environment (air, water, soil) the light and the high volatility of this group because this group becomes steam or gas and adhere to the floor of lakes, rivers, its long elimination

process lasting from days to months maybe. Most chlorophenols released into the environment is water, and in very small amounts in the air. Compounds that are more likely to enter the air are the mono - and dichlorophenol, as they are the most volatile (ie, have the greatest tendency to become a vapor or gas). Once in the air, sunlight helps destroy these compounds and the rain out of the air. Chlorophenols stick to soil and sediment at the bottom of lakes, rivers and streams. However, some low levels of chlorophenols in water, soil or sediment are broken down by the action of microorganisms and environmental removed within days or weeks<sup>11</sup>.

### **CHLOROPHENOL IMPACT ON THE ENVIRONMENT**

The chlorophenol by contact with water forms highly toxic solutions. It also contributes to air pollution because chlorophenol vapors are heavier than air, so these particles tend to fall and because of their density and people breath them in. However, the problem can be diminished due to both aerobic and anaerobic microorganisms which disable them to succeed in the sedimentation process. On the Water: Phenol is heavier than water and sinks. It slowly dissolves and forms, including dilution, toxic solutions. Air: Vapors are heavier than air and exposed to heat, form explosive mixtures. Phenol oxidation in the air is accelerated by the effect of light or impurities which act as catalysts and soil: Decomposition in the surface water is accomplished in about 7 days to 90% (stagnant) and soil reaches the same proportion in about 1 day according to the micro flora and concentration<sup>18,19</sup>.

Metabolites of phenols can also be extremely toxic:"the incomplete combustion 2,4,5 - trichlorophenol can lead to the formation of TCDD (dioxin) . Biodegradation produces acetic acid and CO<sub>2</sub> via catechol, o- chinona and dicarboxylic acids. Phenol is eliminated from the body via urine, after oxidation or conjugation with sulfuric or glucónico<sup>18</sup>.

### **CHLOROPHENOL ADVERSE EFFECTS IN HUMANS**

Prolonged exposure paralyzes the central nervous system and causes kidney and lung injuries, which could later result in death. The symptoms that accompany the condition are headaches , buzzing ears, dizziness, gastrointestinal upset, drowsiness , collapse, poisoning , loss of consciousness , irregular breathing , respiratory arrest (apnea ) , heart failure and, in some cases , seizures . "According HORN (1989), the phenol exerts teratogenic and carcinogenic. According to the Ames test, phenol not without mutagenic. Usually, the smell and taste alarming avoid injuries from ingestion "(Horn, 2001). 9 chlorophenol exposure can occur through contact , inhalation and ingestion , in producing people , to use pesticides from drinking water contaminated with chlorinated phenols have been measured concentrations of chlorophenols in chlorinated drinking water at levels of chlorophenols ( weight) parts per billion (volume) of water. In lakes, rivers and streams chlorophenols were found in amounts less than 1 percent of the water tested. In urban air concentrations were recorded chlorophenols under one part per billion (the amount of chlorophenols per billion parts of air) and this impacts on the environment<sup>4,6,19</sup>.

People who work in the production of chlorophenols or uses those during the application of pesticides are the most likely to have a high exposure to these chemicals. E.i. tetrachlorophenols mixtures are used at sawmills and wood preservatives. The most likely source of exposure is when the skin comes in contact with treated wood tetrachlorophenols. Another possible route of exposure is contaminated air and dichlorophenols monkey.

Exposure occurs by ingestion and inhalation and through the skin, monochlorophenols do not stay long in the body are converted into less harmful and most leave through the urine within 24 hours. The other chlorophenols (dichlorophenol, trichlorophenol,tetrachlorophenols), which also leave the urine, can remain in the body for several days, even months<sup>18</sup>.

This health problem affects society and the environment due to sewage and chlorinated water have this group of chemicals , the impact on public health, the environment , and the effects that pollution has water bodies conducting necessary research projects which seek chemical - biological mechanisms to inhibit the carcinogenic action of chlorophenol using as peroxidase enzymes found in turnip root and so reduce and / or inhibit mutagenic characteristics , which would contribute to public health by reducing exposure to substances such as this andthus reduce risks highlighted as obvious to cancer in people who have contact with this chemical group<sup>11, 12, 13</sup>.

**ENZYME ACTIVITY**

Factors that may interfere with enzyme activity include pH, temperature, substrate concentration, the presence of enzyme inhibitors, light, enzyme inhibitors which are enzymes that prevent receptor interact with its center, or can be enzyme activators which tend to increase catalyzed reactions. Some RNA molecules are capable of catalyzing reactions (such as the ribosome 16S subunit resides in peptidyltransferase activity). Including synthetic molecules called artificial enzymes capable of catalysing chemical reactions such as the classical enzymes. Furthermore, many enzymes require cofactors for activity. Many drugs is inhibitory molecules. Likewise, the activity is affected by temperature, pH, concentration of the enzyme itself and the substrate, and other physical factors - chemical<sup>20, 21, 22</sup>.

This project focused on answering the following research question:

Is there chlorophenol in water samples taken from Lake Chapala and what is the effect of root peroxidase Brassica Napus var. rapifera (turnip) in the concentration of chlorophenol in water samples of Lake Chapala?

The aim of this investigation was to determine the effect of peroxidase enzyme root Brassica Napus var. rapifera (turnip) in the concentration of chlorophenol in water samples from Lake Chapala. For this it was necessary to:

- Isolate peroxidase enzyme root Brassica napus var. rapifera (turnip).
- Determine the presence of chlorophenol in water samples from Lake Chapala.
- Measure chlorophenol concentrations in drinking water samples.
- Quantify the concentration levels of phenol in water samples from Lake Chapala and chlorophenol in water, before and after the action of peroxidase.

**The assumptions that guided to guide this research project were as follows**

HT = Working Hypothesis

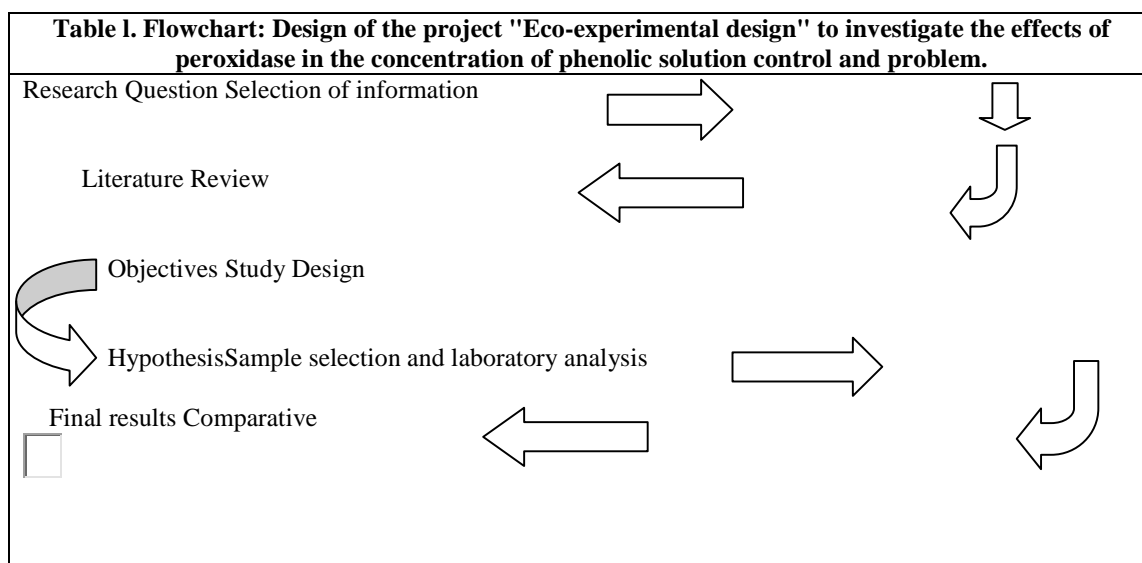
Water samples from Lake Chapala contain chlorophenol and this can be reduced by the action of the root extract of Brassica napus var. Rapifera (turnip) in the concentration of a phenolic solution.

Ho =Null hypothesis

Water samples from Lake Chapala contain no chlorophenol and it can not be reduced by the action of the root extract of Brassica napus var. Rapifera (turnip) in the concentration of a phenolic solution.

**MATERIAL AND METHODS**

We conducted an ecological experimental study, measuring the effect of turnip root extracts in water samples from Lake Chapala, Jalisco. Mexico (Table 1).



Source: Conceptual Construction Ruvalcaba Cobian Jesus Carlos.

## GETTING PEROXIDASE

Roots are ground to extract from them the vascular fluid in a solution of phosphate buffer to extract enzymes such as pH stable as are globular proteins are sensitive to extreme pH. This solution is homogenized so that it can remove all liquid from the roots may be removed by centrifugation and waste to 12,000 rpm for 10 minutes, the supernatant is obtained where the enzymes are dissolved by centrifugation. Different concentrations between the extract and the hydrogen peroxide also modifies the reaction rate. In the same volume of solution variations between aggregates volumes also modifies the amount of substrate to react and result in product. 4 tubes of which are used in checking of essentiality of the components have small changes in pairs between each other reagents and allowing the reaction and comparing the effect of different concentrations of substrates.

The speed of an enzyme catalysed reaction is determined by the enzyme and substrate concentrations. Absorbance at 470 nm is an indicator of the reaction and its speed measured in 3 minutes to every 30 seconds. After modifying the three dimensional structure of an enzyme, it cannot perform its physiological function nor catalyze reactions. It is noteworthy that the pH of a solution can be changed by changing the protein structure interactions to protonate or deprotonate groups R of the side chains of amino acids. The effect on the structure of enzymes trichloroacetic acid and ammonium sulfate will be compared as though both are weak acids, ammonium is a much weaker acid. Precipitating protein exists and must determine whether the peroxidase enzyme on the surface of the solution or in the precipitate.<sup>23-25</sup>

## METHOD FOR DETERMINING TURNIP PEROXIDASE ENZYME ACTIVITY

Peroxidase is an oxidoreductase that catalyzes the oxidation of phenolic compounds with H<sub>2</sub>O<sub>2</sub> as a final electron acceptor. This enzyme efficiently removes wastewater phenolic compounds by oxidative polymerization. The method is based on the oxidation of guaiacol ( ) to tetraguaiacol (brick red). Guaiacol substrate is hydrogen peroxide and the reaction is catalyzed by peroxidase enzyme. Attaching the method to a 2% solution of phenol crystals, further preparing a guaiacol control, as specified above.

## PREPARATION OF CRUDE EXTRACT

Homogenising the sample (60 to 150 g) in an omni -mixer at maximum speed for 3 minutes, keeping the temperature at 0 ° C in an ice bath. The extract obtained in 0.6 M citrate buffer at pH 4.5 (3 ml per g of tissue) and about 1 g of calcium carbonate. Centrifugation was done in a centrifuge type Sorvall 0 ° C and a speed of 12,000 rpm for 10 minutes. Some authors saturate the sample, using a glass tube current. The extract is filtered through cotton, discarding the first 10 to 20 ml of the filtrate.

Dissolve 126 g of citric acid in about 800 ml of distilled water, add sufficient NaOH solution to bring the pH to 4.5 (7.5 N NaOH solution). Make up to 1000 ml , the concentration of citrate is 0.6 M. Add a few ml of toluene as a preservative , stir and using dilute with equal part of distilled water to refrigerator to slow the growth of fungi.

## PROCEDURE

Diagnose the existence of peroxidase in the samples (extracts of Brassica Napus var. Rapifera), their enzymatic activity and their ability or response time on the samples specified above. Four test tubes were taken and in which 20 ml of distilled water plus 2 ml of enzyme extract were added, one tube was added 1 ml of guaiacol phenol solution 2% lake water samples Chapala, and municipal network Tonalá, respectively and letting them run through the tube walls. 1 ml of 0.085 % hydrogen peroxide was added to each tube, the tube was capped and mixed well, and then it was left for ten minutes until visibly precipitated or some type of enzymatic activity as bubbles existed or precipitated and turbidity diminished.

## READ IN THE SPECTRUM AT 420 nm USING DISTILLED WATER AS A BLANK

In a test tube containing 20 ml of distilled water, add 2 ml of enzyme extract. Add 1 ml of reagent guaiacol drain leaving the walls of the tube to form an intermediate layer. Added one ml of hydrogen peroxide solution 0.085 %, the same as the above reagent , the tube is capped , inverted quickly 2 or 3 times to mix and place in the quickest way into the tube Klett- Summerson colorimeter reading at 420 nm. Previously the unit must be set to 0 with distilled water at the same wavelength. Periodically measuring the intensity of color development is done in triplicate. The reaction is linear in time up to 15 minutes. Considered the best time 5 minutes.

**QUANTIFICATION OF ENZYME ACTIVITY**

Peroxidase activity is expressed as Klett units / mg protein for 5 minutes of reaction. For quantitation 12 tubes were prepared divided into 3 sections of 4 tubes to read at 5 , 10 and 15 minutes at a wavelength of 420 nm using the same method as that for the qualification in this method is added guaiacol to all tubes to also act with the standard and be more efficient and to better quantify.

**RESULTS**

Baseline readings at 420 nm. Denote that drinking water has 0.507, the water of Lake Chapala 0.370, 0.438 guaiacol and phenol solution 2% to 0.791 (Table 1).

**Table 1.** Qualification of enzyme activity.

Type of sample analyzed	Absorbance at 420 nm
Phenol solution sample 2%	0.791
Guaiacol sample	0.438
Lake water sample and Chapala	0.370
Drinking Water Sample CETI	0.507

**Source:** Direct, basal test readings for Lake Chapala water and drinking water CETI. CETI Laboratory, 2010

Samples and previous results have only one tube with guaiacol as standard, to verify the peroxidase enzyme activity for samples of phenol solution 2% Chapala lake water and drinking water CETI; qualification using the same method with the same processes using a standard guaiacol (Table 1).

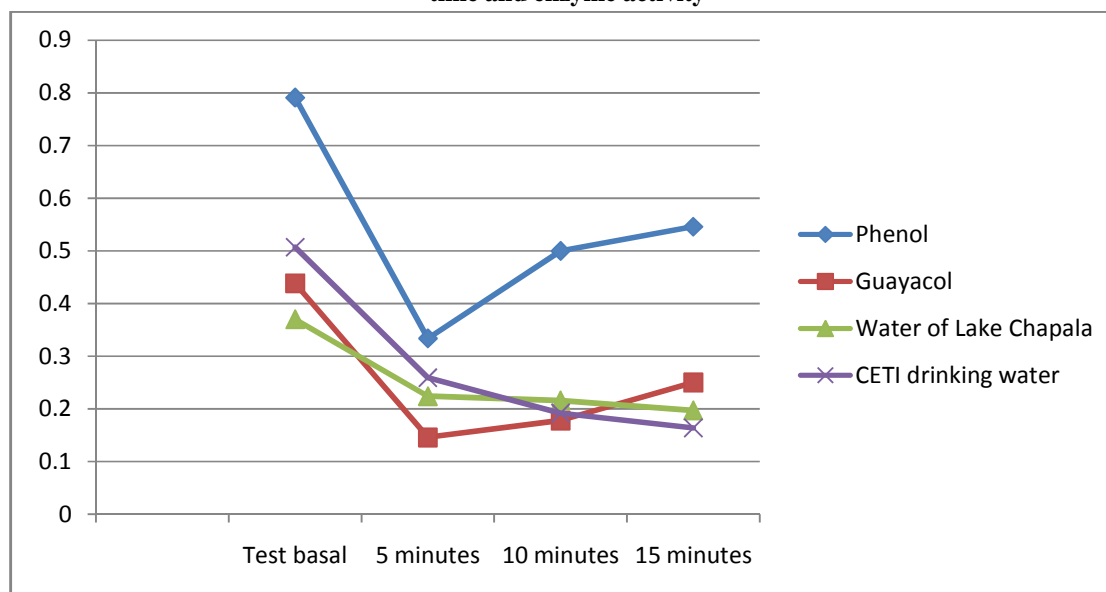
Water samples ( Chapala , CETI ) decreased absorbances , its highest or highest reading was at 5 minutes after that rank the following samples of water, decreased at 10 and 15 minutes , indicating that enzyme activity vs phenol or chlorophenol was effective and decreased the % phenol in the samples , and in the case of guaiacol standards for 5, 10, 15 minutes their absorbances rose because the guaiac is a standard and the sample containing phenol solution was prepared at 2% which was a high concentration for the enzymatic activity of the peroxidase, and thus be able to metabolise or unfold the phenol to other compounds, that is why along predominated readings guaiacol and phenol on the activity of the enzyme extract, which is correct for the quantification of the enzymatic method with phenol and its derivatives. (Table 2).

**Table 2.** Absorbances obtained at 420 nm in a certain period of time at 5 minutes, 10 minutes and 15 minutes.

Sample Type	Test basal	5 minutes	10 minutes	15 minutes
Phenol	0.791	0.334	0.500	0.546
Guaiacol	0.438	0.146	0.178	0.25
Lake Chapala water	0.370	0.224	0.216	0.197
CETI drinking water	0.507	0.259	0.192	0.164

**Source:** Direct laboratory analysis, which shows the absorbance values in laboratory CETI, 2010

**Figure 1. Comparative analysis on the effect of peroxidase from the baseline measurement with respect to time and enzyme activity**



**Source:** Direct, analysis of water samples from Lake Chapala and drinking water CETI. Laboratory of CETI.

Finally results obtained indicate that the presence of peroxidase enzymes exists in the root extracts of *B. napus* var "turnip", which is involved in the degradation of phenol and that the effect on the degradation of phenol group is effected with high enzymatic activity of approximately 98%. (Figure1).

**Figure A y B. Reactions samples peroxidase enzyme with 2% phenol, guaiacol, water samples of Lake Chapala and CETI drinking water**



## DISCUSSION

The existence of peroxidase enzymes in *Brassica Napus* var. Rapifera was demonstrated as well as its enzymatic activity, the ability or response time range was of 5 to 10 minutes, it was confirmed that it is suitable for inhibiting and disable the group phenol and chlorophenol. Enzymatic activity was evaluated approximately 98%, this result was based on the analysis previously conducted in other research. The methodology used for the development of this research was highly effective, in addition to that from the point of view of the social bio security level it's a biological alternative natural substance that does not cause adverse environmental effects, we expect that in the near future it is considered and used as an alternative to chemical control for phenols and chlorophenols in the processes of water treatment plants,

and other applications that are likely to be detected with the activity of the enzyme on this chemical, for example, could be used as a food turnip mixed in salads or even in fresh water to decrease the effect of chlorophenol on plants or even drinking water for human consumption.

The significance of this type of research is that it is possible to highlight public health risks and allows you to find new ways to address these findings. Deepen main ways of determining whether a chemical is harmful, evaluate how it is absorbed in the body, preventing exposure to its effects on the body, and removed, to set routes study to investigate how it is absorbed, how it is used and released by the body. In the case of certain chemicals, animal testing is required. Animal testing may also serve to identify adverse health effects such as cancer or birth defects. Without laboratory animals, scientists would lose a basic method to get information needed to make wise decisions to protect public health. The side effects caused by chlorophenol in some experiments and cases of people who by accident or lack of information came to be exposed to some derivative of chlorophenol, most cases of people accidentally exposed to chlorophenol had mild adverse effects such as burns, damage liver, acne and risk of cancer<sup>4-11</sup>.

**In the study conducted by Ila Bania and Rita Mahanta. Evaluation of plant peroxidases from various sources, (2012)** it can be perceived that the distribution of peroxidase activity and the kinetic parameters of three plant species [(*Nicotianatabacu*, *Brassica capitataroleraceae*. *Albal*. And *Raphanus sativus* (mooli in India, winter varieties)] were evaluated. To describe the rate of hydrogen peroxide decomposition for all the three plant peroxidases to first order kinetic expression with Respect to Both the substrate and the enzyme was used. The pH optima of the three peroxidases were determined to using acetate buffer (pH 4.0-5.0) and potassium phosphate buffer (pH 6.0-8.0) oleraceae *B. capitata* var. *albal* May prove peroxidase as a potential source for biotechnological application. Therefore, this can be advancement in purifying methods of peroxides plants due to the enzymatic potential source of this substance<sup>14</sup>.

These results denote respect obtained similarity to B shows in activity *napusvar* important enzyme catalyzing and reducing the phenol, although it is a plant variety, in terms of species is likely to have similar mechanisms both species, but here we observed the highest enzyme activity in the first 5 minutes. Salah Israa Abdel Azim Hamad and Ali Ahmed in (2013) in their study, trials were made to use peroxidases from different weeds phenolic plants for removing pollutants from wastewaters. The used Plants (*Portulacaoleracea*, *Sonchusoleraceus*, *Xanthium strumarium*, *Cyperusrotandus* and *Trianthemaportulacastrum*). In conclusion The obtained results could possibly contribute to a better understanding of the enzymatic process involved in phytoremediation of aromatic compounds like phenol. Moreover, they supplied useful information to apply these plants, or their extracts, which contain these enzymes in industrial wastewater cleanup processes<sup>15</sup>.

Bazrafshan Edris *et al* (2012). In his studio entitled. Application of *Moringaperegrina* seed extract naturally as a coagulant for phenol removal from Aqueous solutions, found that the best pH was 5 and that of According To These results, it was defined That *M. peregrina* seed extract is not only an inexpensive coagulant, But Also quite an Effective factor in the removal of phenol from Aqueous environments, this represents an option to the current problems of water pollution from industrial wastes, this study is consistent with regard to pH worked best on the stability of the enzyme and used under the action of the root extract *B. napus* var. <sup>16</sup>. Montenegro (2010). Also observed that when bacterial growth is increased, the rate of degradation of phenol with the highest percentage increases when it is higher glucose concentration, the results matching the WHO pointing to phenol, its effects on the environment and can be biodegraded aerobically and anaerobically both in the water and on the ground<sup>17</sup>.

Currently determined that several chlorophenol derivatives may be carcinogenic to the human<sup>11,12,13</sup>.

The Department of Health and Human Services has determined that may reasonably be anticipated that the 2,4,6 - trichlorophenol is a carcinogen. The International Agency for Research on Cancer (IARC) has determined that the group of chlorophenols may be carcinogenic to humans. Protection Agency U.S. Environmental. UU. (EPA) has determined that the 2,4,6 -trichlorophenol is likely carcinógeno<sup>12,13</sup>.



Arellano et al. (2012) found peroxidase activity in synthetic waters loaded with phenol from the use of crude extracts of *Brassica napus*<sup>26</sup> and other researchers have found that enzyme activity, these studies strengthen the idea of considering the potential of *Brassica napus* var. "turnip" as an alternative to reduce the concentration of phenol and chlorophenol wastewater as those that have been chlorinated and used for consumption humano<sup>23-26</sup>.

### CONCLUSIONS

- Using the methodology was possible to demonstrate the presence of peroxidase enzyme activity derived from the plant known in Mexico as turnip (*Brassica Napus* var. Rapifera) in inhibiting and disabling chemicals such as phenol and chlorophenol group.
- The enzymatic activity of peroxidase was demonstrated, as well as its capacity of or time which ranged from 5 to 10 minutes, time in which its function to inhibit and off phenol and chlorophenol group was observed.
- It is interesting to consider the proposal that the enzyme activity of *Brassica napus* var. could form part of the strategies of Phyto- remediation of sewage and even those containing in its composition ancillary industrial waste. And even dabble in search of aquatic plants could be bio - remediating the aquatic ecosystem by decreasing concentrations of phenol.
- The results allow predicting the presence of the enzyme obtained using the root (*Brassica Napus* var. Rapifera ) in wastewater treatment and even as a food product added to help reduce the impact of exposure to phenol - chlorophenol from drinking water intake or farm products where pesticides have been used and also have been used in sewage to irrigate these crops , which has demonstrated the presence of these chemicals and it is unknown if after washing vegetables grown under the conditions described above , any residues or low concentrations remain, as the EPA noted that a substance that produces an effect at high concentrations also occur at low concentrations.

### Acknowledgements

The authors of the present research article would like to acknowledge and truly thank the collaboration of Yesenia Elizabeth Ruvalcaba Cobián who has a B.A in Teaching English as a Foreign Language, for her contributions on the revision and translation of the article; situation which allows the possibility to increase the transferring and modification of scientific knowledge.

The **authors** declare that no conflict of interests for the publication of this research paper.

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