



Activated charcoal from green coconut as an alternative to remove 2,4-D from water and reduce toxicity in *Lactuca sativa* L.

*Carvão ativado de coco verde como alternativa para remover 2,4-D da água e reduzir toxicidade em *Lactuca sativa* L.*

Joice Lazarin Romão¹, Luis Fernando Cusioli¹, Rafaela Lanças Gomes², Ana Luiza de Brito Portela Castro¹, Rosangela Bergamasco¹, Raquel Guttierres Gomes¹

¹ Universidade Estadual de Maringá, Maringá, Paraná, Brasil

² Universidade Estadual Paulista, Botucatu, São Paulo, Brasil

Contato: rggomes@uem.br

Keywords

mitotic index
water pollution
cell cycle
biological indicator

ABSTRACT

Water quality is essential for the maintenance of all forms of life on the planet since the consumption of contaminated water can pose health risks. In this study, green coconut-activated charcoal was used in the treatment of contaminated water at concentrations of 2, 5, 10, and 20 mg/L of the herbicide dichloro phenoxy acetic acid (2,4-D). To verify the efficiency of the adsorption process, germination bioassays, and cytogenetic analyses were performed with seeds of *Lactuca sativa* L. as bioindicator. The germination bioassays were carried out with a germination paper roll in triplicate, with 300 seeds per treatment. As for the cytogenetic analysis, 3000 cells were analyzed per treatment. The results showed that the green coconut activated charcoal has adsorptive potential to remove 2,4-D from water, with germination results of 89.6% for treated water, 92% for pure water, and 0% for contaminated water. In the cytogenetic analysis, the Mitotic Index (MI) values were high and did not differ statistically for the pure and treated water sample, since the average between the four concentrations was 11.41 for the pure water sample, 10.64 for the treated and 7.15 for the contaminated water samples. As for chromosomal abnormalities (CA), there was a gradual increase from 0.47 to 1.10 according to exposure to 2,4-D concentrations. We thus conclude that 2,4-D has a toxic action for the development of lettuce seeds, and activated carbon from green coconut was efficient in adsorption.

Palavras-Chave

índice mitótico
poluição hídrica
ciclo celular
indicador biológico

RESUMO

A qualidade da água é indispensável para a manutenção de todas as formas de vida do planeta, uma vez que o consumo de água contaminada pode oferecer riscos à saúde. Neste trabalho foi utilizado o carvão ativado de coco verde no tratamento da água contaminada nas concentrações de 2, 5, 10 e 20 mg/L do herbicida ácidodichlorofenoxiacético (2,4-D). Para verificar a eficiência do processo de adsorção foram realizados bioensaios de germinação e análise citogenética com sementes de *Lactuca sativa* L. como bioindicador. Os bioensaios de germinação foram feitos em rolo de papel de germinação em triplicata, totalizando 300 sementes por tratamento, e para a análise citogenética foram analisadas 3.000 células por tratamento. Os resultados obtidos mostraram que o carvão ativado de coco verde possui potencial adsorptivo para remover o 2,4-D da água, com resultados de germinação de 89,6% para água tratada, 92% para água pura e 0% para água contaminada. Na análise citogenética os valores de Índice Mitótico (IM) foram altos e não diferiram estatisticamente para as amostras de água pura e tratada, uma vez que a média entre as quatro concentrações foi 11,41 para amostras de água pura, 10,64 para amostras tratadas e de 7,15 para as amostras de água contaminada. Quanto as anormalidades cromossômicas (AC) houve um aumento gradual de 0,47 para 1,10 conforme exposição às concentrações de 2,4-D. Concluímos assim que o 2,4-D possui ação tóxica para o desenvolvimento das sementes de alface, e o carvão ativado de coco verde se mostrou eficiente na adsorção.

Informações do artigo

Recebido: 26 de abril, 2023

Aceito: 19 de agosto, 2023

Publicado: 23 de agosto, 2023

Introduction

Water is essential when it comes to the maintenance of all forms of life on Earth. Its use must be sustainable and rational because it is a resource of great social and economic value. When poorly managed, the characteristics of water can be compromised (WHO; DODDS, PERKIN, GERKEN, 2013; BEHMEL et al., 2016).

One of the major causes of water contamination is the presence of herbicides, including 2,4-D (dichloro phenoxyacetic acid), which is an organic, systemic selective herbicide for the control of weeds, including broadleaf ones (AQUINO et al., 2007), and has been widely used since the 1940s (ISLAM et al., 2018). Since 2,4-D is a selective herbicide, it only affects dicots, not monocots (GROSSMANN, 2003). Therefore, it is used to combat weeds in soybean, corn, wheat, rice, and sugarcane crops, as well as pastures (ZAFRA-LEMOS et al., 2021). There are about 1,500 herbicides/pesticides that contain 2,4-D as their main ingredient (AYLWARD; HAYS, 2008), and which are widely used all around the world (CHEN et al., 2018). When applied to target plants, it acts as an auxin signaling agent, which leads to uncontrolled plant growth, epinasty, and death (GOGGIN; CAWTHRAY; POWLES, 2016; GROSSMANN, 2010).

The effect of 2,4-D on non-target organisms is alarming. In animals, studies report interference of 2,4-D with the metabolism of fish, amphibians, insects, rodents, and small ruminants (STEBBINS-BOAZ et al., 2004; LACHAPPELLE et al., 2007; CATTANEO et al., 2008; FONSECA et al., 2008; MICHAUD; PARK; KWAK, 2010; VARGAS, 2010; IKECHUKWU et al., 2012; MENEZES et al., 2015; LAJMANOVICH et al., 2015; FREYDIER; LUNDGREN, 2016; DAKHAKHNI; RAOUF, QUSTI, 2016; AMEL et al., 2016; ZAFRA-LEMOS et al., 2021). When it comes to humans, some studies relate infertility in men to exposure to 2,4-D in cases in which spermatozooids, when exposed to this contaminant, had their total/progressive mobility and ability to penetrate a viscous medium compromised. This indicates that exposure to 2,4-D and its accumulation in seminal plasma may increase infertility risks, as stated by TAN et al., 2016).

Therefore, it is necessary to remove 2,4-D and other contaminants from water to ensure quality standards. There are several methodologies used to purify water contaminated with emerging contaminants, such as photocatalytic oxidation (GIRI et al., 2010; LEE et al., 2015); electrocoagulation (KAMARAJ et al., 2014; KAMARAJ et al., 2015); Fenton degradation (CHEN et al., 2015); biodegradation (FERREIRA-GUEDES; MENDES; LEITÃO, 2012) and the adsorption process in which activated charcoal can be used (COELHO et al., 2019; WANG et al., 2020).

The use of activated charcoal in the water purification process is widespread worldwide due to its surface area and porous structure, functional groups, chemical properties, surface texture, and other characteristics important for processes such as adsorption.

In addition, it is important to emphasize that the activated charcoal production process can improve these characteristics even more (ALVES et al., 2019).

For the production of activated charcoal, the raw material must be rich in carbonaceous content, such as wood and agro-industrial waste (CHOI et al., 2009). Activated charcoal produced from biological raw material is called a biosorbent (CUSÍOLI et al., 2019) which can be a good alternative due to its low costs of application. Besides, rice husk and cereal residues can be used for that purpose, since they turn into activated charcoal after processing (BOONAMNUAYVIRAYA et al., 2004; SATARI; KARIMI, 2018).

The number of toxic residues of agricultural, industrial, or domestic origin used in the environment without proper treatment has been increasing exponentially. As a response, the use of bioassays to monitor toxicological effects on living organisms has been explored (BADERNA et al., 2011). To carry out bioassays, organisms that are bioindicators of environmental pollution are used. They can be species or communities, such as animals, plants, and microorganisms, which can detect the presence of toxic substances in the environment (HOLT; MILLER, 2011).

Plants are excellent indicators of genetic damage when exposed to chemical products, and tests that make use of them are simple and inexpensive (GRANT, 1999; MONTEIRO et al., 2007). For instance, *Lactuca sativa* L., popularly known as lettuce, has several advantages. It is used in studies for toxicity analysis, due to rapid germination, uniformity, and sensitivity (TIGRE et al., 2012), in addition to stable and well-defined cytogenetic characteristics, such as large chromosomes in reduced number with karyotypic characteristics that facilitate a microscopic view of the chromosomes allowing a clear assessment of their alterations (SOUZA et al., 2009; HOU et al., 2014; PALMIERI et al., 2014; ARAGÃO et al., 2015; WANG et al., 2016; CARVALHO et al., 2019; VIEIRA et al., 2022).

There are studies on the removal of 2,4-D, but none of them verified the toxicity of treated water. This work, in turn, aimed to study the use of green coconut activated charcoal as an adsorbent for removing 2,4-D from water; verify the efficiency of *Lactuca sativa* L. as a bioindicator; and perform genetic analysis of the aforementioned contaminant.

Materials and methods

Preparation of solutions for the germination test

First, 2,4-D herbicide solutions (Sigma –Aldrich PA > 98%) were prepared at 2, 5, 10, and 20 mg/L concentrations. The solutions used for the germination test were: a) pure water (PW), b) water contaminated with 2,4-D (CW) and c) treated water (TW). To obtain the treated water sample, water was experimentally contaminated with different concentrations. Then, it was passed through a gravitational filter with green coconut-activated charcoal.

The solutions were analyzed with aspectro photometer (HACHDR5000) at a wavelength of 230 nm, before and after filtration.

Preparation of the gravitational filter

The gravitational filter was prepared with the aid of hermetic support as a container, provided by Carbontec®, a company based in Maringá, state of Paraná, Brazil (Purific - Brazil). Approximately 140 g of activated charcoal purchased from the company Bahia Carbon was added to the hermetic support, according to the size and maximum capacity. At first, about 20 L of deionized water was passed through the filter, so that the color would not interfere with the germination analysis. Right after that, the contaminated water (CW) was passed through the filter in continuous flow, and, after filtration, treated water (TW) was obtained. These steps were performed separately for all concentrations of the contaminant (SHIMABUKU et al., 2016).

Preparation of the germination test

The germination test was performed to verify the effect of solutions (a), (b), and (c) as described in item 2.1. Seeds of *Lactuca sativa* L. (TopSeed®) were used as bioindicators. For each solution, 100 seeds were used. All tests were performed in triplicate. We used germination paper (J. Prolab®), which was submerged in solutions (a, b, and c) for 24 hours. The seeds were sown on moistened paper and kept in an oven for 7 days at a temperature of 20°C ± 1°C. After this period, normal, abnormal, and dead seedlings were counted, and the proportions of normal seedlings per treatment were calculated. Regarding the classification of the seedlings, normal ones are understood as those that present all their essential structures, whereas the abnormal ones have defects in some parts and have no potential to develop (BRASIL, 2009).

Cytogenetic analysis

Seeds of *Lactuca sativa* L. were sown on Petri dishes and germination paper moistened with 5 mL of the respective solutions and remained in an oven for 48 hours at 20°C ± 1°C. The methodology used for preparing the slides was that by Freitas et al. (2016) with modifications. The roots used for the cytogenetic analysis were collected and fixed into a solution of ethanol and acetic acid (3:1) for 24 hours. To prepare the slide, the meristematic region was cut and boiled in a 2% acetic orcein solution (Dinâmica®) transferred to the slide, covered with a cover slip, and care fully crushed over a drop of 2% acetic orcein solution. The slides were analyzed under an optical light microscope. There were 1,000 cells per slide and 3 slides per sample, with a total of 3,000 cells per sample.

The analyzed parameters were calculated by Çildirand Liman (2020). The Mitotic Index (MI) was calculated as the number of dividing cells divided by the total number of observed cells x 100. Chromosomal

aberrations (CA) were replaced as the total number of cells with AC divided by the total number of observed cells x 100. After viewing under an optical microscope, the slides were viewed under a photographic microscope to capture images of the cell cycle and the chromosomal aberrations found.

Statistical analysis

The normality and homogeneity of variances were verified by using Shapiro-Wilk and Bartlett tests respectively. As the germination data did not show any of these characteristics, they were transformed using the natural log of the proportion of normal seedlings, divided by the subtraction of the proportion of normal seedlings from one unit (*logito*) (Eq.1).

$$\text{Logito} = \ln\left(\frac{p}{1-p}\right) \quad (\text{Eq.1})$$

Where *p* is the proportion of normal seedlings. Initially, normality and homogeneity of variances were verified by using Shapiro-Wilk and Bartlett tests respectively. As the germination data did not show any of these characteristics, they were transformed using the natural log of the proportion of normal seedlings, divided by the subtraction of the proportion of normal seedlings from one unit

For the genetic data, normality and homogeneity of variances were verified using Shapiro-Wilk and Bartlett tests, respectively. As the MI and CW data did not show normality or homogeneity of variances, generalized linear models with gamma distribution and logarithmic link function were fitted for each variable, considering water and herbicide as factors. Fit quality was initially addressed by analyzing the deviations by degrees of free demand, later, by the standardized Pearson residuals graphs (NELDER; WEDDERBURN, 1972).

Results and discussion

Table 1 shows the results of the germination test for samples of pure, contaminated, and treated water.

Table 1. Mean and standard deviation of the proportions of germinated seeds according to the water treatments

Water sample	Mean and standard deviation
Pure	0.9216 ± 0.027A
Treated	0.8966 ± 0.031B
Contaminated	0 ± 0C

Means followed by the same letter do not differ from Tukey's test (p<0.05). Source: Own authorship (2023)

According to Table 1, regarding the germination test, there was no interaction between the two factors, namely water treatment and herbicide concentration (p=0.1333). There was a main effect of water (p=0.0443), and no main effect of herbicide (p=0.8544). All water samples differed from each other according to Tukey's test

($p < 0.05$). Pure water had the highest average proportion of seedlings with normal structures, whereas contaminated water did not show any normal seedlings, as root growth was inhibited at all concentrations, making them normal. The percentage of germination of the sample with treated water (89.6%) using green coconut activated charcoal differed statistically from the percentage of germination of the sample with pure water (92%).

This suggests that there may have been a factor that influenced the process of water treatment, causing some residual contaminants to remain even after the adsorption process. Dabrowski et al. (2005) listed some factors that can influence the process of water treatment by activated charcoal, such as the type of activated charcoal precursor (wood, petroleum residues, bituminous coal, lignite, between others), aqueous solubility of the compound and availability of oxygen in the solution.

Akzo and Kabasakal (2004) analyzed the influence of temperature on adsorption, and the results showed that higher adsorption of 2,4-D occurs at higher temperatures. Thus, the water treatment process can be affected, and its results can be altered due to these parameters. This possibly explains the statistical difference in germination percentage between treated water and pure water samples.

Table 2. Mean and standard deviation of the mitotic index (MI) for all samples of *Lactuca sativa* L.

Samples	Herbicide Solution			
	2mg.L ⁻¹	5mg.L ⁻¹	10mg.L ⁻¹	20mg.L ⁻¹
P	11.20±0.36Aa	11.47±0.42Aa	11.47±0.40Aa	11.50±0.20Aa
T	10.9±0.09Aa	10.83±0.06Aa	10.87±0.12Aa	9.98±1.01Aa
C	7.79±0.38Ab	7.06±0.02Bb	6.96±0.07Bb	6.81±0.04Bb

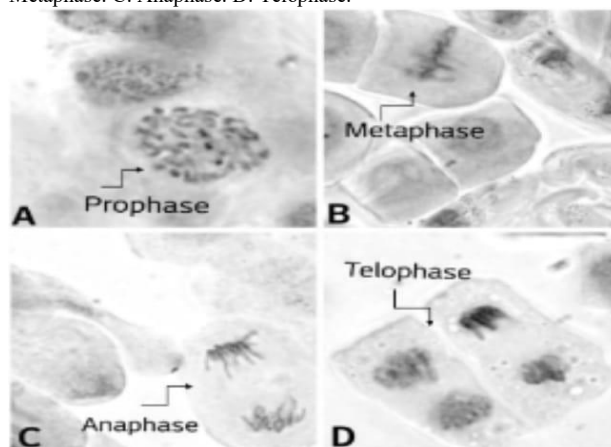
The uppercase letters in the rows and lowercase letters in the columns do not differ statistically by Tukey's test ($p < 0.05$). (P = pure; T = treated; C = contaminated). Source: own authorship (2023).

For the Mitotic Index (MI), which corresponds to the total number of dividing cells during the cell cycle (Figure 1), there was an interaction between the factors of water treatment and herbicide concentration ($p = 0.001$). There was no statistical difference between the 2,4-D concentrations for the pure and treated water samples since the average between these two parameters was 11.41 and 10.64 respectively. As for contaminated water at a concentration of 2 mg/L of 2,4-D, presented a statistically higher mean (7.79) than the other concentrations (7.06, 6.96, and 6.81) according to Tukey's test ($p < 0.05$) (Table 2). For all 2,4-D concentrations tested, treated and pure water has statistically higher MI means (11.41 and 10.64) than contaminated water (7.15).

The 2,4-D showed a toxic effect for *L. sativa* L. when in higher concentrations (10 and 20 mg/L). Thus, the sample with water contaminated with different concentrations of 2,4-D showed lower MI, respectively 7.79, 7.06, 6.96, and 6.81, about the indices in the samples with pure water, where the results were: 11.20, 11.47, 11.47 and 11.50, and treated (10.90, 10.83, 10.87, 9.98). For samples with treated water whose average between the four concentrations was 10.64, the MI values did not differ statistically about the pure water sample (average of

11.41), thus evidencing the efficiency in the treatment process, and samples with contaminated water were superior (mean MI of 7.15), regardless of concentration (Figure 1).

Figure 1. Phases of Mitosis in *L. sativa* L in pure water. A: Prophase. B: Metaphase. C: Anaphase. D: Telophase.



Source: Own authorship (2023)

As discussed above, even if contaminant residues remained in the water (Table 2), they were not enough to affect the mitotic index of the seeds. The mitotic index can assess the cytotoxicity of several agents (FERNANDES; MAZZEO; MARIN-MORALES, 2007) such as 2,4-D, and is indicative of environmental toxicity. Compounds that can interfere with the metabolism of plants represent a danger to human health, and when present in water, even in small amounts, they are mutagenic and can cause birth defects (PATEL et al., 2019). Many toxic compounds can affect DNA and cause mutations (KLAUNIG; KAMENDULIS; HOCEVAR, 2010). The mixture of toxic compounds found in industrial effluents may be related to carcinogenicity (OHE; WATANABE; WAKABAYASHI, 2004; RICE et al., 2018). Table 3 shows the values obtained for Chromosomal Abnormalities (CA) in samples treated with 2,4-D.

Table 3. Means and Standard Deviation of Chromosomal Abnormalities (CA).

Samples	Herbicide Solution			
	2mg.L ⁻¹	5mg.L ⁻¹	10mg.L ⁻¹	20mg.L ⁻¹
P	0.10±0.10Aa	0.10±0.17Aa	0.10±0.001Ab	0.10±0.10Ab
T	0.30±0.20Aa	0.23±0.15Aa	0.20±0.10Ab	0.30±0.10Ab
C	0.47±0.15Ca	0.60±0.10Ba	1.00±0.10Aba	1.10±0.20Aa

The uppercase letters in the rows and lowercase letters in the columns do not differ statistically by Tukey's test ($p < 0.05$). (P = pure; T = treated; C = contaminated). Source: Own authorship (2023)

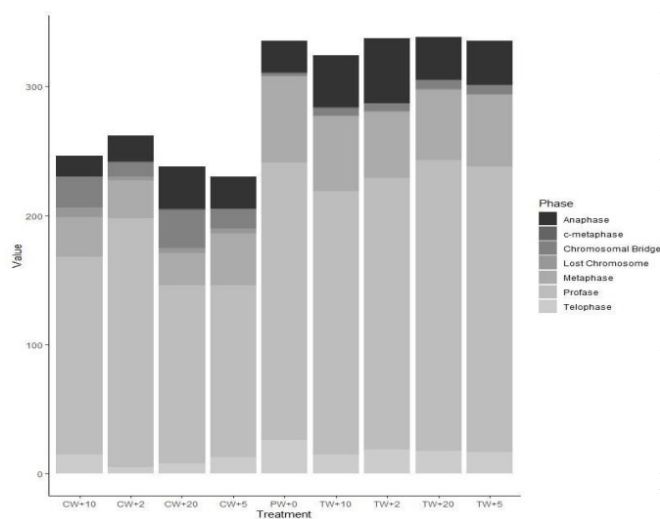
For Chromosomal Aberrations (CA) there was an interaction between water treatment and herbicide concentration ($p = 0.0062$). CA gradually increased according to exposure to 2,4-D concentrations in contaminated water, values ranged from 0.47 to 1.10 according to the increase in herbicide concentration (table). The CA, as well as the MI, varied according to the concentrations. At higher concentrations of 2,4-D (10 and 20 mg/L), CA rates also increased in treated (0.20 and 0.30) and contaminated (1.00 and 1.10) water samples,

demonstrating a concentration-dependent positive effect, while at lower concentrations (2 and 5 mg/L), there was no significant difference between CA rates.

Figures 2 and 3 list the cell cycle phases and chromosomal aberrations found for each sample and 2,4-D concentrations. We can observe that the prophase phases stood out, being found more frequently, followed by metaphase, anaphase, and, finally, telophase. Regarding the analysis of chromosomal aberrations, chromosome bridges in anaphase were more frequent, followed by chromosomes lost in metaphase and, finally, c-metaphases.

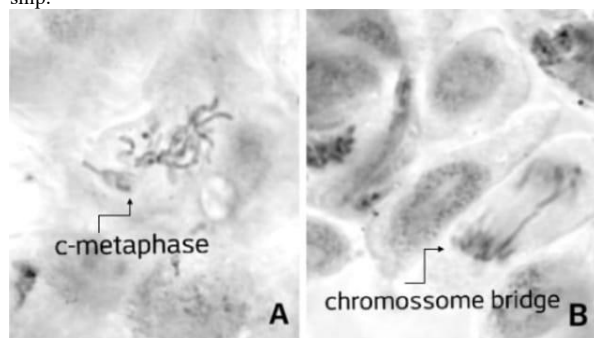
Chromosomal aberrations (Figure 3) correspond to abnormalities found during the cell cycle, such as c-metaphases, chromosomal bridges, chromosome loss, and others. In this study, we detected 3 of them, namely: metaphase and chromosome loss, which indicate aneugenic damage, and chromosomal bridges, due to clastogenic effects. The genotoxic potential of 2,4-D has been identified in different plant species, whose alterations induced by this herbicide involve chromosomal fragmentation, bridges, chromosomal adhesion, lagging chromosomes, micronucleus, and in addition, strand breaks in DNA (ENAN, 2009).

Figure 2. Grouped graph of all cell cycle phases and chromosomal aberrations found.



Source: Own author ship (2023)

Figure 3. Chromosomal abnormalities found. A: C-metaphase, B: anaphase— an arrow indicates a chromosomal bridge. Source: own author ship.



Source: Own authorship (2023)

The effects of 2,4-D on seeds of *L. sativa L.* are still unknown, but its seeds treated with different concentrations of the herbicide glyphosate demonstrated chromosomal anomalies such as chromosomal ossand stickychromo some, anaphase and telophase with bridges, multipolar anaphase, and C-metaphase, in addition to the formation of micronuclei (VIEIRA et al., 2022).

Plants have been used as bioindicators of environmental pollution for a long time, as they of ferthe assessment of toxicity and mutagenicity present in the environment (SANDALIO et al., 2001). Chromosomal aberrations determine the genotoxicity of compounds or substances, where as the mitotic index is used to determine cytotoxicity. The mechanisms of action of the substances can be clastogenic when they involve breaking chromosomes and aneugenic when there are chromosomally and spindle alterations (LEME; MARIN-MORALES, 2009, VIEIRA SILVEIRA, 2018; VIEIRA et al., 2022).

Activated charcoal is widely used to remove pollutants not only from waste water streams but also from drinking water sources, such as ground water, rivers, and lakes (Crini et al., 2019). Brito et al. (2020) used coconut shell and babassu endocarp-activated bio charcoal, and achieved a 2,4-D removal index of 97% and 99%, respectively. These results are similar to that of our study, in which, through the use of bioassays with *L. sativa L.*, the water sample treated with green coconut activated charcoal showed results very similar to those of the pure water samples.

In the same way that the 2,4-D herbicide caused root growth inhibition in *L. sativa L.* seedlings, the study by Brito et al., (2017) tested two bases of the glyphosate herbicide (Roundup® and Glyphosate AKB 480 ®) to evaluate the effects on lettuce seed germination. The authors detected are duction of the root system at all concentrations tested. Reduced root growth affects the growth of an entire seedling by restricting water and nutrient uptake. The phytotoxic effects of 2,4-D are directly related to these restrictions imposed on seedling growth, and lead to inhibition of enzyme activity and membrane instability with detrimental changes in the physiology of lettuce sedgings (LAMHAMDI et al., 2011). For contaminated water, there was no normal seedling in any of the repetitions, as there was no proportional growth and development of the root system.

The 2,4-D is harmful to the environment, for has phytotoxic, cytotoxic, and genotoxic effects on several plants. The results obtained in our research corroborate studies by Okzul et al., (2016) where *Allium cepa L.* bulb roots were exposed to different concentrations of 2,4-D at a higher concentration, which was 4.02 mg/L. The phytotoxic and cytotoxic effects of 2,4-D led to root growth inhibition and a decreased mitotic index in addition to chromosomal aberrations which was the case in this study. Both are closely related since plant growth demands cell proliferation (HARASHIMA; SCHINITTGER, 2010).

The results of our study are in linewith other published studies that addressing these of vegetables as bioindicators of environmental pollution, both for pollutants in general and for pesticides, oils, drugs, and dyes. In the study by Pawlowski et al., (2013) whose dan essential oil from *Schinus Lentisci folius March.*, in

bioassays with *L. sativa* L. and *Allium cepa* L., both species had a decrease in their MI by 25.14% and 19.35%, respectively. There was also induction of aneugenic and clastogenic effects in both of them. Alves *et al.*, (2018) studied the effect of two phenolic compounds (Timol and Carvacrol) on *L. sativa* L. seeds, and both showed a toxic effect. Carvacrol showed a genotoxic effect on *L. sativa* L., with chromosomal aberrations, effects that are similar to those caused by 2,4-D.

About humans, the presence of the 2,4-D poses health risks. Some studies have suggested that exposure to 2,4-D is related to the risk of developing Parkinson's (Tanner *et al.*, 2009), as well as soft sarcoma, non-Hodgkin's, bladder, and skin cancers in farmers and workers exposed to 2,4-D during manufacturing and handling processes (GOODMAN; LOFTUS; ZIL, 2017; BOERS *et al.*, 2010; KOUTROS *et al.*, 2016; COGGON *et al.*, 2015; AYLWARD, HAYS, 2015). In light of the foregoing, removing 2,4-D and other pollutants from water is necessary to maintain its quality standards for human, animal, and vegetable consumption.

With all this, the contribution of the study was to point out important results, addressing the removal of 2,4-D from water, which is an environmental and public health concern and which has been of great concern worldwide. Contamination of water by herbicides can have adverse effects on human health and aquatic ecosystems. The adsorption process with green coconut activated carbon together with the test of *L. sativa* L. seeds was effective, this can contribute to the improvement of water quality and the reduction of the negative impacts of this contaminant and also give a better destination to the waste agroindustrial. Drinking water is a fundamental human right, and ensuring the supply of clean and safe water is essential for the health and well-being of the population.

Conclusion

The use of green coconut activated charcoal proved to be efficient in the treatment of water contaminated with different concentrations of 2,4-D, since the results of the germination bioassays and cytogenetically were very similar. Seeds of *Lactuca sativa* L. showed to be sensitive in the detection of 2,4-D in water, which makes them a good indicator for this compound in an aqueous medium. Herbicide 2,4-D caused phytotoxic damage to *Lactuca sativa* L. to seedlings, inhibiting the growth and development of their root system at all concentrations (2, 5, 10 e 20 mg/L). Regarding the cytogenetic analysis, the reduction of the MI (11,41 of pure water, 10,65 treated water, to 7,16 of contaminated water) and CA rates increased in treated (0.20 and 0.30) and contaminated (1.00 and 1.10) water samples indicated cytotoxicity and genotoxicity of 2,4-D, by the concentrations used, which did not occur significantly in treated water. Due to the toxic potential of 2,4-D, it is crucial to remove it from water, for it interferes with the metabolism of several organisms. Therefore, the use of green coconut-activated charcoal is an excellent alternative to address this use.

References

- AKZU, Z.; KABASAKAL, E. Batch adsorption of 2,4-dichlorophenoxyacetic acid (2,4-D) from aqueous solution by granular activated carbon. **Separation and Purification Technology**, v. 35, p. 223-240, 2004. Doi: [http://10.1016/S1383-5866\(03\)00144-8](http://10.1016/S1383-5866(03)00144-8).
- ALVES, A.C.F.; ANTERO, R.V.P.; OLIVEIRA, S.B.; OJALA, S.A.; SCALIZE, P.S. Activated carbon produced from waste of fee grounds for an effective removal of bisphenol-A in aqueous medium. **Environmental Science and Pollution Research**, v. 24, p. 24850-24862, 2019. Doi: <https://doi.org/10.1007/s11356-019-05717-7>.
- ALVES, T.A.; PINHEIRO, F.P.; PRAÇA-FONTES, M.M.; ANDRADE-VIEIRA, L.F.; CORRÊA, K.B.; ALVES, T.A.; CRUZ, F.A.; JÚNIOR, L.V. FERREIRA, A.; SOARES, T.C.B. Toxicity of thymol, carvacrol and their respective phenoxyacetic acids in *Lactuca sativa* and *Sorghum bicolor*. **Industrial Crops Products**, v. 114, p. 59-67, 2018. Doi: <https://doi.org/10.1016/j.indcrop.2018.01.071>.
- AMEL, N.; Wafa, T.; SAMIA, D.; YOUSRA, B.; ISSAM, C.; CHERAIF, I.; ATTIA, N.; MOHAMED, H. Extra virgin olive oil modulates brain docosahexaenoic acid level and oxidative damage caused by 2,4-Dichlorophenoxy acetic acid in rats. **Journal of Food Science and Technology**, v. 53, n. 3, p. 1454-1464, 2016. Doi: <https://doi.org/10.1007/s13197-015-2150-3>
- AQUINO, J.A.; TUNEGA, D.; HARBEHAUER, G.; GERZABEK, M.H.; LISCHKA, H. Interaction of the 2,4-dichlorophenoxy acetic acid herbicide with oil organic matter moieties: A theoretical study. **European Journal of Soil Science**, v. 5, n. 4, p. 889-899, 2007. Doi: <https://doi.org/10.1111/j.1365-2389.2007.00928.x>.
- ARAGÃO, F.B.; PALMIERI, M.J.; FERREIRA, A.; COSTA, A.V.; QUEIROZ, V.T.; PINHEIRO, P.F.; NADRADE-VIEIRA, L.F. Phytotoxic and cytotoxic effects of Eucalyptus essential oil on *Lactuca sativa* L. **Allelopathy Journal**. Haryana, v. 35, n. 2, p. 259-272, 2015.
- AYLWARD L.L.; HAYS, S.M. Interpreting bio monitoring data for 2,4-dichlorophenoxy acetic acid: Update to Biomonitoring Equivalents and population biomonitoring data. **Regulatory Toxicology and Pharmacology**, v. 73, n. 3, p. 765-769, 2015. Doi: <https://doi.org/10.1016/j.vrtph.2015.11.001>.
- AYLWARD L.L.; HAYS, S.M. Biomonitoring Equivalents (BE) dossier for 2,4-dichlorophenoxy acetic acid (2,4-D) (CAS No. 94-75-7). **Regulatory Toxicology and Pharmacology**, v. 51 (3SUPPL.), p. 37-43, 2008. Doi: <https://doi.org/10.1016/j.vrtph.2008.05.006>.
- BADERNA, D.; MAGGIONI, S.; BORIANI, E.; GEMMA, S.; MOLTENI, M.; LOMBARDO, A.; COLOMBO, A.; BORDONALI, S.; ROTELLA, G.; LODI, M.; BENFENATI, E. A combined approach to investigate the toxicity of an industrial landfill leachate: Chemical analyses, risk assessment and in vitro assays. **Environmental Research**, v. 111, n. 4, p. 603-613, 2011. Doi: <https://doi.org/10.1016/j.envres.2011.01.015>.
- BEHME S.; DAMOUR, M.; LUDWIG, R.; RODRIGUEZ, M.J. Water quality monitoring strategies— Are viewing future perspectives. **Science of the Total Environment**, v. 571, p. 1312-1329, 2016. Doi: <https://doi.org/10.1016/j.scitotenv.2016.06.235>.
- BOERS, D.; PORTENGEN, S.; BAS BUENO DE MESQUITA, H.; HEEDERIK, D.; VERMEULEN, R. Cause-specific mortality of Dutch chlorophenoxy herbicide manufacturing workers. **Occupational and Environmental Medicine**, v. 67, n. 1, p. 24-31, 2010. Doi: <https://doi.org/10.1136/oem.2008.044222>.

- BOONAMNUAYVITAYA V.; CHAYA, C.; TANTHAPANICHAKOON, W.; JARUDILOKKUL, S. Removal of heavy metals by adsorbent prepared from pyrolyzed of free residues and clay. **Separation and Purification of Technology**, v. 35, p.11-22, 2004. Doi: [https://doi.org/10.1016/S1383-5866\(03\)00110-2](https://doi.org/10.1016/S1383-5866(03)00110-2).
- BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Regras para Análise de Sementes**. Ministério da Agricultura, Pecuária e Abastecimento. Brasília: MAPA/ACS, 2009. 399p. Secretaria de Defesa Agropecuária. Brasília, DF :MAPA/ACS, 2009, 399p., ISBN 978-85-99851-70-8.
- BRITO, G.M.; ROLDI, L.L.; SCHEITINO, L.A.; FREITAS, J.C.C.; COELHO, E.R.C. High-performance of activated biocarb on based on agricultural biomass waste applied for 2,4-D herbicide moving from water: adsorption, kinetic and thermodynamic assessments. **Journal of Environmental Science and Health-Part B Pesticides, Food Contaminants, and Agricultural Wastes**, v.55, n.9, p.767-782, 2020. Doi: <https://doi.org/10.1080/03601234.2020.1783178>.
- BRITO, L.R.; OLIVEIRA, R.; ABE, F.R.; BRITO, L.B.; MOURA, D.S.; VALARES, M.C.; GRISOLIA, C.K. OLIVEIRA, D.P.; OLIVEIRA, G.A.L. Ecotoxicological assessment of glyphosate-based herbicides: Effects on different organisms. **Environmental Toxicology and Chemistry**, v.36, n.7, p.1755-1763, 2017. Doi: <https://doi.org/10.1002/etc.3580>.
- CATTANEO, R.; LORO, V.L.; SPAVENELLO, R.; SILVEIRA, F.A.; LUZ, L.; MIRON, D.S.; FONSECA, M.B.; MORAES, B.S.; CLASEN, B. Metabolic and histological parameters of silver cat fish (*Rhamdia quelen*) exposed to commercial formulation of 2,4-D dichlorophenoxyacetic acid (2,4-D) herbicide. **Pesticide Biochemistry and Physiology**, v.92, n.3, p.133-137, 2019. Doi: <https://doi.org/10.1016/j.pestbp.2008.07.004>.
- CARVALHO, M. S. S.; ANDRADE-VIEIRA, L. F.; DOS SANTOS, F. E.; CORREA, F. F.; DAS GRAÇAS CARDOSO, M.; VILELA, L. R. Allelopathic potential and phytochemical screening of ethanolic extracts from Five species of *Amaranthus* spp. in the plant model *Lactuca sativa*. **Scientia Horticulturae**, v.245, p.90-98, 2019. Doi: <https://doi.org/10.1016/j.scienta.2018.10.001>.
- COGGON, D.; NTANI, G.; HARRIS, E. C.; JAYAKODY, N.; PALMER, K. T. Soft tissue sarcoma, non-Hodgkin's lymphoma and chronic lymphocytic leukaemia in workers exposed to phenoxy herbicides: Extended follow-up of a UK cohort. **Occupational and Environmental Medicine**, v. 72, n.6, p.435-441, 2015. Doi: <https://doi.org/10.1136/oemed-2014-102654>.
- CUSIOLI, L. F.; BEZERRA, C. D. O.; QUESADA, H. B.; ALVES BAPTISTA, A. T.; NISHI, L.; VIEIRA, M. F.; BERGAMASCO, R. Modified *Moringa oleifera* Lam. Seed husks as low-cost biosorbent for atrazine removal. **Environmental Technology**, p. 1-12, 2019. Doi: <https://doi.org/10.1080/09593330.2019.1653381>.
- CHEN, X.; ZHANG, H.; WAN, Y.; CHEN, X.; LI, Y. Determination of 2,4-Dichlorophenoxyacetic acid (2,4-D) in rat serum for pharmacokinetic studies with a simple HPLC method. **PLoS ONE**, v. 13, n.1, p. 1-10, 2018. Doi: <https://doi.org/10.1371/journal.pone.0191149>.
- CHEN, H.; ZHANG, Z.; YANG, Z.; YANG, Q.; LI, B.; BAI, Z. Heterogeneous Fenton-like Catalytic Degradation of 2,4-Dichlorophenoxyacetic Acid in Water with FeS. **Chemical Engineering Journal**, v. 273, p. 481-489, 2015. Doi: <http://doi.org/10.1016/j.cej.2015.03.079>.
- CHOI, H. D.; CHO, J. M.; BAEK, K.; YANG, J. S.; LEE, J. Y. Influence of cationic surfactant on adsorption of Cr(VI) onto activated carbon. **Journal of Hazardous Materials**, v.161, n. 2-3, p.1565-1568, 2009. Doi: <https://doi.org/10.1016/j.jhazmat.2008.04.067>.
- ÇILDIR, D.S.; LIMAN, R. Cytogenetic and genotoxic assessment in *Allium cepa* exposed to imazalil fungicide. **Environmental Science and Pollution Research**, v. 27, n.16, p. 20335-20343, 2020. Doi <https://doi.org/10.1007/s11356-020-08553-2>.
- COELHO, E. R. C.; BRITO, G. M. D.; FRASSON LOUREIRO, L.; SCHEITINO JR, M. A.; FREITAS, J. C. D. 2,4-dichlorophenoxyacetic Acid (2,4-D) Micropollutant Herbicide Removing from Water Using Granular and Powdered Activated Carbons: A Comparison Applied for Water Treatment and Health Safety. **Journal of Environmental Science and Health. Part. B, Pesticides, Food Contaminants, and agricultural wastes**, v. 55, p.361-375, 2019. Doi: <http://doi:10.1080/03601234.2019.1705113>.
- CRINI, G.; LIGHTHOUSE, E.; WILSON, L.; MORIN-CRINI, N. Conventional and non-conventional adsorbents for wastewater treatment. **Environmental Chemistry Letters**, v. 17, n.1, p.195-213, 2019. Doi: <https://doi.org/10.1007/s10311-018-0786-8>.
- DA BROWSKIA.; PODKOŚCIELNY, P.; HUBICKI, Z.; BARCZAK, M. Adsorption of phenolic compounds by activated carbon: A critical review. **Chemosphere**, v.58, n.8, p. 1049-1070, 2005. Doi: <https://doi.org/10.1016/j.chemosphere.2004.09.067>.
- DAKHAKHNI, T. H.; RAOUF, G.A.; QUSTI, S.Y. Evaluation of the toxic effect to the herbicide 2, 4-D on rat hepatocytes: an FT-IR spectroscopic study. **European Biophysics Journal**, v.45, n.4, p. 311-320, 2016. Doi: <https://doi.org/10.1007/s00249-015-1097-7>.
- DODDS, W.K.; PERKIN, J.S.; GERKEN, J.E. Human impact on fresh water ecosystem services: A global perspective. **Environmental Science and Technology**, v.47, n.16, p. 9061-9068, 2013. Doi: <https://doi.org/10.1021/es4021052>.
- ENAN, M.R. Genotoxicity of the herbicide 2, 4-dichloro phenoxy acetic acid (2, 4-D): Higher plants as monitoring systems. **American-Eurasian Journal of Sustainable Agriculture**, v.3, n.3, p. 452-459, 2009.
- FERNANDES, T.C.C.; MAZZEO, D.E.C.; MARIN-MORALES, M.A. Mechanism of micronuclei formation in polyploidized cells of *Allium cepa* exposed to trifluralin herbicide. **Pesticide Biochemistry and Physiology**, v.88, n.3, p. 252-259, 2007. Doi: <https://doi.org/10.1016/j.pestbp.2006.12.003>.
- FERREIRA-GUEDES, S.; MENDES, B.; LEITÃO, A.L. Degradation of 2,4-Dichlorophenoxyacetic Acid by a Halotolerant Strain of *Penicillium chrysogenum*: Antibiotic Production. **Environmental Technology**, v.33, p. 677-686, 2012. Doi: <http://doi.org/10.1080/09593330.2011.588251>.
- FONSECA, M. B.; GLUSZACK, L.; MORAES, B.S.; MENEZES, C.C.; PRETTO, A.; TIerno, M.A.; ZANELLA, R.; GONÇALVES, F.F.; LORO, V.L. The 2,4-D herbicide effects on acetylcholinesterase activity and metabolic parameters of piava fresh water fish (*Leporinus obtusidens*). **Ecotoxicology and Environmental Safety**, v. 69, n.3, p.416-420, 2008. Doi: <https://doi.org/10.1016/j.ecoenv.2007.08.006>.
- FREITAS, A.S.; CUNHA, I.M.F.; ANDRADE-VIEIRA, L.F.; TECHNO, V.H. Effect of SPL (Spent Pot Liner) and its main components on root growth, mitotic activity and phosphorylation of Histone H3 in *Lactuca sativa* L. **Ecotoxicology and Environmental Safety**, v.124, p. 426-434, 2016. Doi: <https://doi.org/10.1016/j.ecoenv.2015.11.017>.
- FREYDIER L.; LUNDGREN, J.G. Unintended effects of the herbicides 2,4-D and dicamba on lady beetles. **Ecotoxicology**, v. 25, n. 6, p.1270-1277, 2016. Doi: <https://doi.org/10.1007/s10646-016-1680-4>.
- GIRI, R.R.; OZAKI, H.; OTA, S.; TANIGUCHI, S.; TAKANAMI, R. Influence on Inorganic Solids on Photocatalytic Oxidation of 2,4-Dichlorophenoxyacetic Acid with UV and TiO₂ Fiber in Aqueous Solution. **Desalination**, v. 255, p. 9-14, 2010. Doi: <http://doi.org/10.1016/j.desal.2010.01.025>.

- GOGGIN, D.E.; CAWTHRAY, G. R.; POWLES, S. B. 2,4-D resistance in wild radish: Reduced herbicide translocation via inhibition of cellular transport. **Journal of Experimental Botany**, v.67, n.11, p. 3223-3235, 2016. Doi: <https://doi.org/10.1093/jxb/erw120>.
- GOODMAN, J.E. LOFTUS, C. T.; ZU, K. 2,4-D chlorophenoxyacetic acid and non-Hodgkin's lymphoma: results from the Agricultural Health Study and an updated meta-analysis. **Annals of Epidemiology**, v.27, n.4, p. 290-292.e5, 2017. Doi: <https://doi.org/10.1016/j.annepidem.2017.01.008>.
- GRANT, W.F. Higher plant assays for the detection of chromosomal aberrations and gene mutations- brief historical background and their use for screening and monitoring environmental chemicals. **Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis**, v. 426, n.2, p.107-112, 1999. Doi [https://doi.org/10.1016/S0027-5107\(99\)00050-0](https://doi.org/10.1016/S0027-5107(99)00050-0).
- GROSSMANN, K. Auxin herbicides: Current status of mechanism and mode of action. **Pest Management Science**, v. 66, n.2, p. 113-120, 2010. Doi: <https://doi.org/10.1002/ps.1860>.
- GROSSMANN, K. Mediation of herbicide effects by hormone interactions. **Journal of Plant Growth Regulation**, v. 22, p. 109-122, 2003. Doi: <https://doi.org/10.1007/s00344-003-0020-0>.
- HARASHIMA, H.; SCHNITTGER, A. The integration of cell division, growth and differentiation. **Current Opinion in Plant Biology**, v. 13, n.1, p. 66-74, 2010. Doi: <https://doi.org/10.1016/j.pbi.2009.11.001>.
- HOLT, E.A.; MILLER, S.W. Bioindicators: Using Organisms to Measure Environmental Impacts. **The Nature Education**, v.2, n.2, p.1-10, 2011.
- HOU, J.; LIU, G. N.; XUE, W.; FU, W. J.; LIANG, B. C.; LIU, X. H. Seed germination, root elongation, root-tip mitosis, and micronucleus induction of five crop plants exposed to chromium in flavor-aqua soil. **Environmental Toxicology and Chemistry**, v. 33, n.3, p. 671-676, 2014. Doi: <https://doi.org/10.1002/etc.2489>.
- IKECHUKWU, O. R.; MADUKA, A. S.; NKIRE, K. T.; SHOYINKA, O. S.; OKONKWO, A. L.; UNEOJO, O. V. Morphometric changes, biochemical values, and kidney morphology of male West African Dwarf goats exposed to 2,4-dichlorophenoxyacetic acid (2,4-D). **Comparative Clinical Pathology**, v.21, n.1, p.91-97, 2012. Doi: <https://doi.org/10.1007/s00580-010-1068-4>.
- ISLAM, F.; WANG, J.; FAROOQ, M. A.; KHAN, M. S.; XU, L.; ZHU, J.; ZHOU, W. Potential impact of the herbicide 2,4-dichlorophenoxy acetic acid on human decosystems. **Environment International**, October, v.111, p. 332-351., 2018. Doi <https://doi.org/10.1016/j.envint.2017.10.020>.
- KAMARAJ, R.; DAVIDSON, D. J.; SOZHAN, G.; VASUDEVAN, S. Adsorption of 2,4-Dichlorophenoxyacetic Acid (2,4-D) from Water by in Situ Generated Metal Hydroxides Using Sacrificial Anodes. **Journal Taiwan Institute Chemical Engineers**, v. 45, n.6, p. 2943-2949, 2014. Doi: <http://doi.org/10.1016/j.jtice.2014.08.006>.
- KAMARAJ, R.; DAVIDSON, D. J.; SOZHAN, G.; VASUDEVAN, S. Adsorption of Herbicide 2-(2,4-Dichlorophenoxy) Propanoic Acid by Electrochemically Generated Aluminum Hydroxides: An Alternative to Chemical Dosing. **RSC Advances**, v. 5, p. 39799-39809, 2015. Doi: <http://doi.org/10.1039/C5RA03339J>.
- KLAUNIG, J.E.; KAMENDULIS, L.M.; HOCEVAR, B.A. Oxidative stress and oxidative damage in carcinogenesis. **Toxicologic Pathology**, v.38, n.1, p.96-109, 2010. Doi: <https://doi.org/10.1177/0192623309356453>.
- KOUTROS, S.; SILVERMAN, D. T.; ALAVANJA, M. C.; ANDREOTTI, G.; LERRO, C. C.; HELTSHE, S.; BEANE-FREEMAN, L. E. Occupational exposure to pesticides and bladder cancer risk. **International Journal of Epidemiology**, v.45, n.3, p. 792-805, 2016. Doi: <https://doi.org/10.1093/ije/dyv195>.
- LACHAPPELLE, A. M.; RUYGROK, M. L.; TOOMER, M.; OOST, J. J.; MONNIE, M. L.; SWENSON, J. A.; STEBBINS-BOAZ, B. The hormonal herbicide, 2,4-dichlorophenoxyacetic acid, inhibits Xenopus oocyte maturation by targeting translational and post-translational mechanisms. **Reproductive Toxicology**, v. 23, n.1, p. 20-31, 2007. Doi: <https://doi.org/10.1016/j.reprotox.2006.08.013>.
- LAJMANOVICH, R. C.; ATTADEMO, A. M.; SIMONIELLO, M. F.; POLETTA, G. L.; JUNGES, C. M.; PELTZER, P. M.; CABAGNA-ZENKLUSEN, M. C. Harmful Effects of the Dermal Intake of Commercial Formulations Containing Chlorpyrifos, 2,4-D and Glyphosate on the Common Toad *Rhinella arenarum* (Anura: Bufonidae). **Water, Air, and Soil Pollution**, v. 226, n.12, p.1-12, 2015. Doi: <https://doi.org/10.1007/s11270-015-2695-9>.
- LAMHAMDI, M.; BAKRIM, A.; AARAB, A.; LAFONT, R.; SAYAH, F. Lead phytotoxicity on wheat (*Triticum aestivum* L.) seed germination and seedling growth. **Comptes Rendus Biologies**, v.33, p. 118-126, 2011. Doi: <https://doi.org/10.1016/j.crv.2010.12.006>.
- LEE, H.; PARK, S. H.; PARK, Y. K.; KIM, S. J.; SEO, S. G.; KI, S. J.; JUNG, S. C. Photocatalytic Reactions of 2,4-D chlorophenoxyacetic Acid Using a Microwave-Assisted Photocatalysis System. **Chemical Engineering Journal**, v.278, p. 259-264, 2015. Doi: <http://doi.org/10.1016/j.cej.2014.09.086>.
- LEME, D. M.; MARIN-MORALES, M.A. *Allium cepa* testis environmental monitoring: A review on its application. **Mutation Research**, v. 682, n.1, p. 71-81. Doi: <https://doi.org/10.1016/j.mrrev.2009.06.002>.
- MENEZES, C.; RUIZ-JARABO, I.; MARTOS-SITCHA, J. A.; TONI, C.; SALBEGO, J.; BECKER, A.; BALDISSEROTTO, B. The influence of stocking density and food deprivation in silver catfish (*Rhamdia quelen*): A metabolic and endocrine approach. **Aquaculture**, v. 435, p. 257-264, 2015. Doi: <https://doi.org/10.1016/j.aquaculture.2014.09.044>.
- MICHAUD, J.P.; VARGAS, G. Relative toxicity of three wheat herbicides to two species of Coccinellidae. **Insect Science**, v. 17, n.5, p.434-438, 2010. Doi: <https://doi.org/10.1111/j.1744-7917.2009.01308.x>.
- MONTEIRO, M.; SANTOS, C.; MANN, R. M.; SOARES, A. M.; LOPES, T. Evaluation of cadmium genotoxicity in *Lactuca sativa* L. using nuclear microsatellites. **Environmental and Experimental Botany**, v.60, n.3, p. 421-427, 2007. Doi: <https://doi.org/10.1016/j.envexpbot.2006.12.018>.
- NELDER, J.A.; WEDDERBURN R.W. Generalized linear models. **Journal of the Royal Statistical Society Series A**, v.135, n.3, p. 370-84, 1972. Doi: <https://doi.org/10.2307/2344614>.
- ÖZKUL, M.; ÖZEL, Ç. A.; YÜZBAŞIOĞLU, D.; ÜNAL, F. Does 2,4-dichlorophenoxy acetic acid (2,4-D) induce genotoxic effects in tissue cultured *Allium* roots? **Cytotechnology**, v.68, n.6, p.2395-2405, 2016. Doi: [10.1007/s10616-016-9956-3](https://doi.org/10.1007/s10616-016-9956-3).
- OHE, T.; WATANABE, T.; WAKABAYASHI, K. Mutagens in surface waters: A review. **Mutation Research - Reviews in Mutation Research**, v.567:(2-3 SPEC. ISS.), p. 109-149, 2004. Doi: <https://doi.org/10.1016/j.mrrev.2004.08.003>.

- PALMIERI, M. J.; LUBER, J.; ANDRADE-VIEIRA, L. F.; DAVIDE, L. C. Cytotoxic and phytotoxic effects of the main chemical components of spentpot-liner: A comparative approach. **Mutation Research – Genetic Toxicology and Environmental Mutagenesis**, v.763, p.30-35, 2014. Doi: <https://doi.org/10.1016/j.mrgentox.2013.12.008>.
- PARK, K.; KWAK I.S. Molecular effects of endocrine-disrupting chemicals on the Chironomus riparius estrogen-related receptor gene. **Chemosphere**, v.79, n.9, p. 934-941, 2010. Doi: <https://doi.org/10.1016/j.chemosphere.2010.03.002>.
- PATEL, D. M.; JONES, R. R.; BOOTH, B. J.; OLSSON, A. C.; KROMHOUT, H.; STRAIF, K. Parental occupational exposure to pesticides, animals and organic dusts risk of childhood leukemia and central nervous system tumors: Findings from the International Childhood Cancer Cohort Consortium (I4C). **International Journal of Cancer**, v.146, n.4, p. 943-952, 2019. Doi: <http://doi.org/10.1002/ijc.3238>
- PAWLOWSKI, Â.; KALTCHUK-SANTOS, E.; BRASIL, M. C.; CARAMÃO, E. B.; ZINI, C. A.; SOARES, G. L. G. Chemical composition of Schinus lentiscifolius March. essential oil and its phytotoxic and cytotoxic effects on lettuce and onion. **South African Journal of Botany**, v.88, p. 198-203, 2013. Doi: <https://doi.org/10.1016/j.sajb.2013.07.026>.
- RICE, G. E.; EIDE, I.; FEDER, P. I.; GENNINGS, C. Assessing human health risks using in vitro formation on whole mixtures. **Chemical Mixtures and Combined Chemical and Nonchemical Stressors**, p.421-463, 2018. Doi https://doi.org/10.1007/978-3-319-56234-6_15.
- SATARI, B.; KARIMI, K. Citrus processing wastes: environmental impacts, recent advances, and future perspectives in total valorization, **Resources, Conservation and Recycling**, v.129, p.53-167, 2018. Doi: <https://doi.org/10.1016/j.resconrec.2017.10.032>.
- SANDALIO, L. M.; DALURZO, H. C.; GOMEZ, M.; ROMERO-PUERTAS, M. C.; DEL RIO, L. A. Cadmium-induced changes in the growth and oxidative metabolism of pea plants **Journal of Experimental Botany**, v.52, n.364, p. 2115-2126, 2001. Doi: <https://doi.org/10.1093/jexbot/52.364.2115>.
- SOUSA, M. S.; SILVA, P. S.; CAMPOS, J. M. S.; VICCINI, L. F. Cytotoxic and genotoxic effects of two medicinal species of verbenaceae. **Caryologia**, v. 62, n.4, p. 326-333, 2009. Doi: <https://doi.org/10.1080/00087114.2004.10589698>.
- SHIMABUKU, Q. L.; ARAKAWA, F. S.; FERNANDES SILVA, M.; FERRI COLDEBELLA, P.; UEDA-NAKAMURA, T.; FAGUNDES-KLEN, M. R.; BERGAMASCO, R. Water treatment with exceptional virus inactivation using activated carbon modified with silver (Ag) and copper oxide (CuO) nanoparticles. **Environmental Technology (United Kingdom)**, v.38, n.16, p. 2058-2069, 2016. Doi: <https://doi.org/10.1080/09593330.2016.1245361>.
- STEBBINS-BOAZ, B.; FORTNER, K.; FRAZIER, J.; PILUSO, S.; PULLEN, S.; RASAR, M.; WINGER, E. Oocyte Maturation in Xenopus laevis Is Blocked by the Hormonal Herbicide, 2,4-Dichlorophenoxy Acetic Acid. **Molecular Reproduction and Development**, v.67, n.2, p. 233-242. Doi: <https://doi.org/10.1002/mrd.10396>.
- TAN, Z.; ZHOU, J.; CHEN, H.; ZOU, Q.; WENG, S.; LUO, T.; TANG, Y. Toxic effects of 2,4-dichlorophenoxy acetic acid on human sperm function in vitro. **Journal of Toxicological Sciences**, v. 41, n.4, p.543-549, 2016. Doi: <https://doi.org/10.2131/jts.41.543>.
- TANNER, C. M.; ROSS, G. W.; JEWELL, S. A.; HAUSER, R. A.; JANKOVIC, J.; FACTOR, S. A.; LANGSTON, J. W. Occupation and Risk of Parkinsonism. **Archives of Neurology**, v. 66, n. 9, p. 1106-1113, 2009. Doi: <https://doi.org/10.1001/archneurol.2009.195>.
- TIGRE, R. C.; SILVA, N. H.; SANTOS, M. G.; HONDA, N. K.; FALCAO, E. P. S.; PEREIRA, E. C. Allelopathic and bioherbicide potential of Cladonia verticillaris on the germination and growth of Lactuca sativa. **Ecotoxicology and Environmental Safety**, v. 84, p. 125-132, 2012. Doi: <https://doi.org/10.1016/j.ecoenv.2012.06.026>.
- VIEIRA, L.F.A.; SILVEIRA, G.L. Cyto(geno)toxic endpoints assessed via cell cycle bioassays in plant models. In: T. A. ÇELIK, ed. **Cytotoxicity**. London: Intech Open, pp. 117-129, 2018
- VIEIRA, C.; MARCON, C.; DROSTE, A. Phytotoxic and cytogenotoxic assessment of glyphosate on *Lactuca sativa* L. **Brazilian Journal of Biology**, v.84, e257039, 2022. Doi: <https://doi.org/10.1590/1519-6984.257039>
- ZAFRA-LEMOS, L.; CUSIOLI, L. F.; BERGAMASCO, R.; BORIN-CARVALHO, L. A.; DE BRITO PORTELA-CASTRO, A. L. Evaluation of the genotoxic and cytotoxic effects of exposure to the herbicide 2,4-dichlorophenoxyacetic acid in *Astyanax lacustris* (Pisces, Characidae) and the potential for its removal from contaminated water using absorbent. **Mutation Research – Genetic Toxicology and Environmental Mutagenesis**, v.865, p.1-8, 2021. Doi: <https://doi.org/10.1016/j.mrgentox.2021.503335>
- WANG, C.; XIAO, H.; ZHAO, L.; LIU, J.; WANG, L. ZHANG, F.; SHI, Y.; DU D. The allelopathic effects of invasive plant *Solidago canadensis* on seed germination and growth of *Lactuca sativa* enhanced by different species of acid deposition. **Ecotoxicology**, v. 25, n.3, p. 555-562, 2016. Doi: <https://doi.org/10.1007/s10646-016-1614-1>.
- WANG H.; XU J.; LIU, X.; SHENG, L. Preparation of straw activated carbon and its application in waste water treatment: a review, **Journal of Cleaner Production**, v. 283,(124671), p.1-19, 2020. Doi: <https://doi.org/10.1016/j.jclepro.2020.124671>
- WHO. Water quality and health strategy 2013-2020 (2012) **World Health Organization**.