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LACTUCA SATIVA AS BIOINDICATOR OF ALUMINUM CONTAMINATION IN WATER TREATMENT PLANT SLUDGE

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ABSTRACT

Highlights: Water Treatment Plant sludge is a residue consisting of metals, such as aluminum, from the coagulants used in the process. Aluminum can be responsible for environmental impacts that cause symptoms of toxicity in vegetables, such as greens, and are also used as indicators of the presence of this element in the environment. **Aim:** Thus, this study sought to analyze the possibilities of using lettuce, *Lactuca sativa L.*, as a bioindicator of environmental contamination by aluminum from the sludge generated in Water Treatment Plants. **Design/Methodology/Approach:** For this, a weighted analysis was performed that considered qualitative and quantitative parameters for the determination of the ideal bioindication, in which weights and grades were assigned to the aspects related to lettuce characteristics, comparing the sum to values presented in studies that used the same methodology. **Results:** In the weighting performed, it was observed that the sum of the scores presented in relation to the parameters of ideal bioindication for lettuce was quite satisfactory, with values close to and higher than those observed in other studies. **Practical Implications:** From this, it was possible to analyze that lettuce presents a potential use in bioindication of environmental quality, mainly for environmental impacts by metals, such as aluminum, present in Water Treatment Plant sludge. **Originality/value:** Therefore, this study contributed to the understanding regarding the good performance of lettuce in the identification studies of the ideal bioindication and the characterization of the species as a good bioindicator of environmental quality, as well as the fact that bioindicators are essential elements for the evaluation of tolerance levels related to possible effects from environmental pollution. **Research limitations:** It is assumed that field studies and laboratory analyses can prove the aspects evidenced here, especially the ability of the vegetable to indicate environmental impacts by metals present in the Water Treatment Plants (WTP) sludge, in this case, aluminum.

Keywords: Lettuce; Bioaccumulation; Bioindication; WTP waste; Phytotoxicity.

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1. INTRODUCTION

In the process of removing impurities from the natural water collected for human consumption, a process carried out in Water Treatment Plants (WTP), it is necessary to add products known as coagulants that help and facilitate this process (Kamiwada; Andrade; Reis, 2020; Lopes *et al.*, 2020). The most commonly used coagulant products, especially in Brazil, are made of aluminum and iron, which contributes to the formation of a residue rich in these metals (Bartiko; Julio, 2015; Tavares, 2016; Russo *et al.*, 2020).

WTP sludge, known as the residue generated in water treatment, is characterized by the properties of a dense and viscous mass discarded, most often in water bodies and on land near the stations, without undergoing any type of treatment. Besides being composed of metals such as aluminum and iron, the sludge presents biodegradable organic compounds and other inorganic compounds (Shahin; *et al.*, 2019; Liu *et al.*, 2020; Mañosa *et al.*, 2020). In this context, the great discussions on the issues related to the final waste disposal arise from the possible impacts that the sludge discharging into the environment can cause. Among these impacts, one can highlight the unavailability of phosphorus in the soil, characterized as an essential nutrient, mainly for agricultural crops (Tavares, 2016).

For some years, it has become increasingly common, and at the same time indispensable, to use creatures as biological indicators to assess the state and dynamics of an ecosystem. Some studies have shown the importance of using different species that can prove and indicate, in a clear way, possible negative effects related to pollution, be it water, air, or soil pollution (Dube *et al.*, 2018; Ng *et al.*, 2020; Terneus-Jácome *et al.*, 2020).

In this sense, this paper will present some discussions on the use of bioassays in studies with waste generated in the water treatment process, as well as some impacts of aluminum bioaccumulation for vegetables and the properties that can make lettuce a potential bioindicator of environmental contamination by metals, mainly the aluminum present in the WTP waste.

In a study, Barceló *et al.* (2020) analyzed the use of bioassays in monitoring the decrease in toxicity associated with pollutants in treated water. According to the authors, bioassays are based on measuring the response of organisms or cell lines exposed to different types of contaminant classes and have been used to establish the toxicity levels of target contaminants, individually or as mixtures. According to the above, the test organisms generally used are microorganisms, pseudomonads, plants, algae, invertebrates, fish, and cell lines. Among some species used to assess toxicity removal efficiency in water treatment systems, the authors highlighted *Cyprinus carpio*; *Vibrio fischeri*; and *Daphnia magna* as species with potential for bioindication or biomonitoring.

Ng *et al.* (2020) studied the potential effect of water treatment plant sludge as a soil substrate on the greening process of a grass species. To this end, the authors conducted a bioassay with bermudagrass *Cynodon dactylon (L.) Pers*. In a research conducted in order to evaluate the use of WTP waste (sludge) in the alteration of a soil affected by mining activities, Alvarenga *et al.* (2018) conducted bioassays, seeking to verify the effects of the alteration caused by the sludge on the ecotoxicological properties of the soil. According to the study, four different types of bioassays were used, each with a bioindicator organism of different trophic level. The following bioassays were structured: *Vibrio fischeri* luminescence inhibition; *Daphnia magna* acute immobilization test; 24-hour mortality test with *Thamnocephalus platyurus*; and 72-hour growth inhibition of the green microalgae *Pseudokirchneriella subcapitata*.

Howells *et al.* (2018) conducted a study on the effects of wastes generated from the water treatment process as soil corrective media. This research sought to understand the behavior and effects of WTP waste when applied to soil. For this, some specific analyses took place, where one consisted in determining the influence of the incorporation of waste to the soil on the survival, growth, and reproduction of a earthworm species (*Eisenia fetida*).

In a study developed by Dube *et al.* (2018), the effects of irrigation with different dilutions of WTP sludge water were evaluated. With this, the analyses were carried out with two vegetation species (*Brachiaria decumbens* and *Medicago sativa*), seeking to identify how the irrigation process developed in the study could impact the production, development, and uptake of these species, as well as the properties of the crop soils.

Bioaccumulation and biomagnification are criteria for assessing the risks that contaminants from ecosystems may pose to organisms (PENG *et al.*, 2017). Bioaccumulation is associated with the absorption and accumulation of chemical substances or compounds from the environment, while biomagnification occurs from the progressive increase of the concentrations of these contaminants at each trophic level of the food web (Romero-Romero *et al.*, 2017).

Direct absorption and accumulation of substances from the environment, such as metals, can lead to bioaccumulation of contaminants in organisms. This can be

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seen from the action of abiotic agents that start to cause a disturbance in organisms, causing toxicity to be identified at different environmental levels (Romero-Romero *et al.*, 2017; Cunha Neto *et al.*, 2020).

In addition, metal bioaccumulation can cause changes in the plasma membrane, as well as compromise photosynthesis and cause detrimental effects on the electron transport chain, in the inactivation of Calvin Cycle enzymes and in the reduction of stomatal conductance (Cunha Neto *et al.*, 2020). According to Santos (2009), metal toxicity in plants comes from a series of processes that start at the molecular level, causing cellular alterations, until the moment when a modification occurs in the plant tissue.

Among the metals present in the soil, aluminum is characterized for being one of the most abundant. This is mainly due to the fact that most rocks formed through weathering have minerals classified as aluminosilicates that can release exchangeable aluminum (Al^{3+}). Aspects associated with aluminum solubility can lead to conditions of ionic toxicity in the soil, nutrient limitation, and low phosphorus availability, and processes such as leaching and percolation can intensify soil acidity (Miguel *et al.*, 2010).

In the soil, excessive acidification contributes to the release of some metals, mainly phytotoxic aluminum, which can pose a threat to the development of agricultural crops (Niu *et al.*, 2020; Shetty *et al.*, 2020). When bioavailable aluminum accumulates in soils, it can interfere with plant growth and development (Silambarasan *et al.*, 2019). High concentrations of aluminum can inhibit root growth, damage root tissues, and cause calcium, magnesium, and phosphorus deficiencies in plants. In addition to limiting nutrient supply and development, aluminum can compromise the plant's ability to inhibit pathology. This is mainly because the plant depends on the interaction between the root and the soil microbiota, and aluminum toxicity can affect the microorganisms in the region where this relationship occurs (Niu *et al.*, 2020).

Miguel *et al.* (2010) presented a discussion of several aspects associated with the effects of aluminum toxicity on plant species. According to the authors, in roots, for example, the tips may become thick and yellowish, crooked, and corrupted, and the epidermal tissues may disintegrate, and, at the top of the roots, the cells become wrinkled and collapsed. In turn, there may be a reduction in the root cap size and a misalignment in the meristematic tissue. Also, according to Miguel *et al.* (2010), the leaves may turn yellow and there may be a purplish aspect to the sheaths and edges of the blade, as well as atrophy.

From this, it can be seen that metals such as aluminum are potentially toxic to organisms. Plants, for example, are sensitive to these contaminants, showing symptoms of toxicity, which may indicate the presence of these elements in the environment (Cunha Neto *et al.*, 2020). Thus, plants are potential bioindicators of environmental contamination.

In this context, this paper aims to analyze the possibilities of using lettuce (*Lactuca sativa L.*) as a bioindicator of environmental contamination by aluminum from the sludge generated in water treatment plants, from qualitative and quantitative parameters. The use of lettuce is motivated by the fact that it is characterized as a species among vegetables with moderate tolerance to salts, besides its application in some bioindication studies (Guerber *et al.*, 2017; Santos *et al.*, 2017). Lettuce is the most sold and consumed leafy vegetable in Brazil, mainly for its ease of production and acquisition, thus being responsible for the movement of a high volume of financial resources (Souza *et al.*, 2019; Aires *et al.*, 2020; Carini *et al.*, 2020).

2. METHOD

This study is characterized as a descriptive/exploratory research. Initially, a bibliographical survey was carried out, as from the compilation of data from specialized literature, aiming to identify, understand, and define the main aspects related to the theme under study.

Searching for methods that would allow the definition of parameters and criteria to analyze lettuce (*Lactuca sativa L.*) as a bioindicator, we used the considerations presented by Neumann-Leitão and El-Deir (2009), ratified by Guimarães and El-Deir (2019). According to the aforementioned, an ideal bioindicator should be characterized from aspects such as these: present a well-defined taxonomy; be easily recognized by non-specialists; present a wide geographic distribution; be abundant; have low genetic and ecological variability; be preferably large in size; have well-defined ecological characteristics; have a long lifecycle; present low mobility; and have the possibility of use in laboratory studies. In relation to the aspects previously presented, we consider the definitions and characteristics (Chart 1) presented by Paz *et al.* (2013).

Chart 1. Aspects associated with ideal bioindicators and related characteristics

Aspect	Characteristics
Taxonomy	Study the taxonomy of the species and verify whether it is well defined and analyze other species of the same genus and verify their morphological and functional characteristics, as well as their responses to specific contaminants.
Easily recognizable by non-specialists	This aspect is of great relevance, because collection is facilitated when the species has well-defined physical characteristics.
Wide geographic distribution	It allows the work to be replicable and not restricted to local research.
Abundant	It is reflected in the ease of collection of the species to be analyzed.
Genetic and ecological variability	It is related to evolution, mutation, and adaptability. The chosen species should have low variability and well-defined characteristics.
Size	Macroscopic organisms are visualized with the naked eye and are thus better identified.
Lifecycle	Longer cycles facilitate temporal analysis.
Mobility	Species with low mobility are indicated, because they suffer changes in the same environment in which they provide the answers, contributing to the cause-and-effect analysis.
Ecological characteristics	Niche, habitat, and the interactions of biotic and abiotic factors with the species are some of the characteristics that need to be collected from the bioindicator species.
Laboratory studies	Some responses provided by the species can only be diagnosed through laboratory analysis.

Source: Elaborated from Paz *et al.* (2013)

The analysis consisted in using the aspects previously presented as parameters that could characterize a species and the potential for bioindication. Thus, a weighting was performed for the aspects, defining weights that varied according to the relevance of the parameter to the reality analyzed, according to the scale used in other studies (Guimarães *et al.*, 2014; Pinheiro *et al.*, 2015; Santos *et al.*, 2016; Guimarães; El-Deir, 2019): 1 - low relevance; 2 - medium relevance; 3 - high relevance. In this context, the entire analysis process was carried out considering the weighting premises (Table 1).

Table 1. Weighting assigned to the parameters that characterize a bioindicator

Parameters	Weight
Well-defined taxonomy	3
Having well-known ecological characteristics	3
Easily recognized by non-specialists	2
Having a wide geographic distribution	2
Having low genetic and ecological variability	2
Preferably large in size	2
Having a long lifecycle	2
Presenting low mobility	2
Having the possibility of being used in laboratory studies	2
Being abundant	1

Source: The authors themselves

Finally, scores were assigned for each parameter in order to obtain a score that would allow verifying the potential of lettuce as a bioindicator, based on score analyses already performed in some studies (Guimarães *et al.*, 2014; Pinheiro *et al.*, 2015; Santos *et al.*, 2016; Guimarães; El-Deir, 2019). For this, a criterion of values ranging on a scale of 1 to 5 was used, according to the relevance of the characteristic for the species under study, where: 1 - minimal representativeness; 2 - low representativeness; 3 - medium representativeness; 4 - good representativeness; and 5 - excellent representativeness.

3. RESULTS

Lactuca sativa has a high socio-economic importance due to its contributions in several aspects, such as food and human health, mainly for being a source of several vitamins, minerals, and dietary fiber, besides being one of the most commercialized vegetables in Brazil (Moraes *et al.*, 2020; Maione *et al.*, 2022). Moreover, lettuce is characterized by successive crops throughout the year, due to its features mainly related to wide adaptation to different climatic conditions, short cycle, low production cost, low susceptibility to several pests and diseases, and post-marketing safety (Pereira *et al.*, 2019).

Taxonomically, lettuce (*Lactuca sativa L.*) is of the genus *Lactuca L.*, tribe *Lactuceae*, subfamily *Cichorioideae*, family *Asteraceae*, order *Asterales*, subclass *Asteridae*, class *Magnoliopsida*, division *Magmoliophyta*, superdivision *Spermatophyta*, subkingdom *Tracheobionta*, kingdom *Plantae* (Neves, 2011).

Regarding the morphological aspects, *Lactuca sativa* presents some characteristics such as herbaceous

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growth with alternate leaves attached to a short stem. The leaves can be smooth or curly, loose, or forming a head that, according to the cultivar, can vary in color from yellowish green to dark green to different shades of purple (Azevedo Filho, 2017).

The system performed in open field is still the predominant lettuce cultivation, and temperatures between 18 and 25 ºC are the most indicated for the cultivation of this vegetable. In relation to the conditions of the cultivation soil, it is necessary to pay attention to some characteristics, such as these: organic matter content of, at least, 2.5%; Cation Exchange Capacity (CEC) consisting of 50% calcium, 10% magnesium and 3.5% potassium; phosphorus content with values of 20ppm; good drainage; water availability; and good exposure to the sun (Azevedo Filho, 2017).

There are a few different types of lettuce crops that vary mainly according to the type of leaf. The following groups can be mentioned: American, which presents a head with thick leaves; smooth, which presents a head with smooth leaves; romaine, characterized by an elongated head with smooth, elongated, hard, and thick leaves; curly, which does not present a head formation and the leaves are curly; and mimosa, which also does not present a head formation, but the leaves are characterized by having replicated edges (Azevedo Filho, 2017).

There are some aspects that justify the use of lettuce in toxicity tests in effluents, soils, or sediments. Among these, we highlight the rapid growth (rapid germination), the need for little energy for seed germination, sensitivity to chemical agents, linear growth in a wide range of pH variation, and low sensitivity to osmotic potentials, as well as the low cost, easy cultivation, availability throughout the year, and the possibility of use in toxicity tests in the laboratory and in the field. There is also the fact that the vegetable cypsela allows the inspection of phytotoxicity effects according to different variants, such as germination, plant biomass, and root elongation (Simões *et al.*, 2013; Bolonhesi; Lopes, 2018). For Silva *et al.* (2020), *Lactuca sativa* is a reliable bioindicator, particularly because it is simple, inexpensive, and requires a relatively small amount of sample.

Regarding the aspects analyzed on the ideal bioindication, the values assigned to the defined parameters (Table 2) were defined based on the study for lettuce (*Lactuca sativa L.*). Through the results analyzed, it can be seen that the sum of the scores presented in relation to the parameters of ideal bioindication for lettuce proved to be quite satisfactory, reaching a total value of 88 points. This score is close to the values presented in studies that investigated and sought to define ideal bioindicators using the same methodology presented here (Guimarães *et al.*, 2014; Pinheiro *et al.*, 2015; Santos *et al.*, 2016; Guimarães; El-Deir, 2019).

Table 2. Weighted analysis of optimal bioindication parameters for lettuce

Parameters	Weight	Score	Total
Have well known ecological characteristics	3	5	15
Well-defined taxonomy	3	4	12
Easily recognized by non-specialists	2	5	10
Have a wide geographic distribution	2	5	10
Preferably large in size	2	5	10
Have low mobility	2	5	10
Possibility of use in laboratory studies	2	5	10
Abundant	1	5	5
Low variability	2	2	4
Have a long lifecycle	2	1	2
Sum			88

Source: The authors themselves

In this context, it is important to highlight that some characteristics of the lettuce were preponderant for the defined value, such as the fact that the species has a wide geographic distribution (the most consumed leafy vegetable in Brazil), presents well-defined characteristics that can be visualized in a macroscopic aspect, and can be used in laboratory analysis. In order to complement the analysis performed, some aspects related to lettuce use in further bioindication studies were verified (Lombi *et al.*, 2010; Santos *et al.*, 2017; Gerber *et al.*, 2017).

In the study presented by Lombi *et al.* (2010), an analysis of the response of *Lactuca sativa* grown in soils containing the presence of WTP sludge was performed. The aforementioned authors then investigated the yield of lettuce in relation to concentrations of aluminum, phosphorus, and copper. In this context, it was possible to verify the possible effects of the application of sludge to the vegetable biomass of lettuce, as well as to understand how the concentration of metals present in the residue could compromise or not the quality of the cultivar. In view of the results analyzed in the study, it was possible to evidence the use of lettuce as a bioindicator species of impacts that may occur due to environmental contamination by metals, in this case, in the soil.

The study by Santos *et al.* (2017) took into consideration the use of lettuce (*Lactuca sativa*), specifically the seeds of the vegetable, to investigate the toxicity of water from two urban streams. According to the aforementioned authors, the use of lettuce in the study presented significant potential for research, since it was possible to identify the development of the radicle, which had its growth altered according to different dilutions of water from the streams used.

Gerber *et al.* (2017) evaluated the possible phytotoxic effects of raw and treated effluent from a swine slaughterhouse on lettuce seeds, mainly seeking to identify and determine correlations between effluent characteristics and seed germination. For this, a characterization of the effluent in both conditions (raw and treated) was made and, in relation to the seeds, the germination index, the root length, and the number of germinated seeds were defined. According to the analyses performed in the study, a phytotoxic potential of the effluent was found for the lettuce seeds, since germination was lower than in the control treatment, which used only distilled water. For the authors, it is important to analyze the characteristics of the effluent and phytotoxicity together, mainly because the reduction of some parameters may be related to lower phytotoxic effects on the bioindicator species used. Also, according to the above, a synergism between various contaminants present in the effluent may occur, not only increasing the overall phytotoxicity, but also possibly exceeding the individual toxic effects, making it difficult to accurately identify the causes of toxicity.

4. CONCLUSION

Through the aspects presented in this paper, it was possible to understand that bioindicators are essential elements for assessing the tolerance levels related to possible effects from environmental pollution. In this context, considering the potential contamination of metals present in the waste generated in water treatment plants, it was analyzed that bioassays are a relevant part for understanding the levels of intraspecific tolerance, seeking to establish the best bioindicators and/or biomonitors for environmental pollution arising from the inadequate disposal of aluminum-contaminated sludge.

Given this and the parameters analyzed in this study, it is believed that lettuce (*Lactuca sativa L.*) presents a use potential in the bioindication of environmental quality, not only by the characteristics of the species, but also by the validation already presented in further studies that used this vegetable as a biomonitor. It was found that the taxonomic and ecological characteristics of *Lactuca sativa*, wide geographical distribution, low mobility, size that favors macroscopic analysis, and the possibility of being easily recognized by non-specialists, as well as the properties of use in laboratory studies, were predominant factors for the value obtained in the weighted analysis performed. All these aspects enabled the understanding regarding the good performance of lettuce in the identification studies of ideal bioindication and the characterization of the species as a good bioindicator.

Considering that the metal aluminum is significantly present in the waste generated in water treatment plants and that it has mechanisms of action that can compromise the quality of vegetables, such as lettuce, it is assumed that field studies and laboratory analysis can prove the aspects evidenced here, especially the ability of the vegetable to indicate environmental impacts by metals present in the sludge of WTP, in this case, aluminum. In this context, it was analyzed that the metal accumulation processes in the environment, especially in the soil, can favor the bioaccumulation of these substances and, consequently, the impairment of plant development. Thus, it is understood that high concentrations of aluminum can interfere with essential processes in the constitution of plants, especially those that are sensitive to these contaminants.

Based on the results analyzed here, and seeking to contribute to the development of future research, it is recommended that further studies be carried out to: identify other species that have the potential to be used to bioindicate the effects of the aluminum present in the waste from the water treatment plant; determine aluminum absorption from the WTP sludge by lettuce; evaluate the effect of different concentrations of aluminum present in the WTP sludge, added to the soil, on the growth and development of lettuce; and investigate the possible effects of aluminum biomagnetic efficiency from the accumulation of the metal in lettuce.

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