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PHYTOREMEDIATOR EFFECT OF AZOLLA SP., IN WATERS ARTIFICIALLY CONTAMINATED WITH LEAD AND SOWN WITH RICE

EFECTO FITOREMEDIADOR DE *AZOLLA* SP., EN AGUAS CONTAMINADAS ARTIFICIALMENTE CON PLOMO Y SEMBRADAS CON ARROZ

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ABSTRACT

The objective of this work was to demonstrate the phytoremediation effect of *Azolla* sp. in water artificially contaminated with lead and planted with rice seeds. A completely randomized 4x3 design was applied. The percentage of germination of the rice seeds, the length of the radicle and the epicotyl were determined. Chemical analyzes were performed using the inductively coupled plasma optical emission spectroscopy (ICP-OES) method. The results showed that 81.03% of the rice seeds germinated in contact with lead-contaminated water, which when combined with Azolla in the lead-contaminated medium only germinated 66.60%, while when they were both rice seeds like Azolla without the contaminant germinated 66.1%. Regarding the length of the epicotyl, it could be observed in the treatment where the rice seeds were in contact with the contaminant. Regarding the phytoremediation effect of Azolla, it was found that Azolla has the ability to capture lead, however, when rice seeds are in an aquatic environment not associated with Azolla, they can bioaccumulate this heavy metal.

Keywords:

Water fern, heavy metal, environmental pollution, rice seed germination.

RESUMEN

El presente trabajo tuvo como objetivo demostrar el efecto fitorremediador de *Azolla* sp. en aguas contaminadas artificialmente con plomo y sembradas con semillas de arroz. Se aplicó un diseño completamente al azar 4x3. Se determinó el porcentaje de germinación de las semillas de arroz, el largo de la radícula y del epicotilo. Los análisis químicos fueron realizados por el método de espectroscopia de emisión óptica con plasma acoplado inductivamente (ICP–OES). Los resultados demostraron que el 81,03% de las semillas de arroz germinaron en contacto con el agua contaminada con plomo, que cuando estuvo combinada con la Azolla en el medio contaminado con plomo solo germinó el 66,60 %, mientras que cuando estuvieron tanto las semillas de arroz como de Azolla sin el contaminante germinó el 66,1%. En lo que se refiere al largo del epicótilo se pudo observar en el tratamiento donde las semillas de arroz estaban en contacto con el contaminante. En relación al efecto fitorremediador de Azolla, se comprobó que la Azolla tiene capacidad de captar plomo, sin embargo, cuando las semillas de arroz se encuentran en un ambiente acuático no asociadas con Azolla, pueden realizar una bioacumulación de este metal pesado.

Palabras clave:

Helecho acuático, metal pesado, contaminación ambiental, germinación de semillas de arroz.

INTRODUCTION

Agriculture is an activity that has been carried out worldwide since the Neolithic period, it has allowed the development of great cultures and evolved with the improvement of new technological and scientific tools, among them, the use of agrochemicals, genetic improvements, contributing to the needs diets of more than seven billion inhabitants (Leiva, 2014) and where cereals have played an important role in obtaining high productions.

Rice (***Oryza sativa*** L.) is one of the three food grains that predominates in area and production together with wheat and corn; worldwide, it ranks second after wheat with respect to harvested area; began to be used as a cropapproximately 10,000 years ago, in humid regions of tropical and subtropical Asia; it constitutes the basic food for more than half of the world's population; Yit contributes to the agro-industrial and economic development of several countries (Mendoza et al., 2019).

The Food and Agriculture Organization of the United Nations (FAO) has established that worldwide the main producing countries of paddy rice are mainland China, India, Indonesia, Bangladesh, Vietnam, Thailand, Thailand, the Philippines, Japan and Brazil. In 2020, the world annual production of paddy rice was 756.7 million tons, of which 90.5% was produced in Asia, in a cultivated area of 164.2 million ha, for an agricultural yield of 4.6 t ha-1 in this same year, the cultivated area of rice in Ecuador was 315,023 ha, with a production of 1,336,502 t (FAO, 2022).

In Ecuador until 2014, 53.2 kilograms per inhabitant were consumed annually, which defines the level of importance compared to neighboring countries such as Colombia and Peru that consume 40.0 and 47.4 kilograms per inhabitant annually respectively (Gavilánez et al., 2016).

According to Zambrano et al. (2019) in Ecuador arefive cantons that stand out for the area planted with rice, an activity that is carried out on land owned by them using a wide variety of seeds, standing out the SFL11 and INIAP 14 seeds. Among the cantons that stand out in rice production are in Babahoyo is in first place, followed by Baba, Montalvo, Ventanas and Vinces.

The sowing of the rice crop is carried out in the traditional way by continuous flooding during most of its growth cycle, for this reason, it is called a semi-aquatic plant. The lack of water causes a reduction in the yield potential of the crop, influencing the phenological processes, which cause physiological, physicochemical and microbiological changes in the soil-plant-water interaction (Winkel et al., 2013).

The use of water in agriculture continues to depend on the constant growth in demand for agricultural products to meet the food needs of a constantly growing population. According to the Food and Agriculture Organization of the United Nations (FAO, 2022), agriculture consumes 70% of the total water extracted, in Ecuador around 82%, and in some developing countries up to 95%.

Rice is the crop that needs the most water for irrigation compared to other crops. According to Bouman et al., (2006) irrigated rice cultivation represents between 34 and 43% of water used for irrigation, with respect to the total used in agriculture. This same author states that in the Asian countries that are the main rice producers in the world, the use of water for rice flood irrigation is around 1300 and 1500 mm (13000 and 15000 m^3/ha). In Ecuador, rice cultivation uses between 800 and 1240 mm (8000 and 12400 m^3/ha) of water. Due to the importance of water use in rice cultivation, it is imperative to seek strategies to improve its use and conservation.

Considering that the functions of the ecosystems depend on the Water Resource and therefore all the social and economic activities of the human being, it is important to consider that its quality affects, since poor water quality influences the degradation of the ecosystems, impact on health, agriculture, tourism, industrial production, which ultimately represents economic costs for its recovery. Heavy metals are pollutants that affect the soil, water and people's health (Rivera et al., 2021).

Heavy metals can reach groundwater and surface water through uncontrolled wastewater seepage. Rodriguez (2018), mentions that heavy metals bioaccumulate in plant tissuesby capillary action, which is associated with surface tension; principle based on cohesion and that allows the entry of water through the roots and stems of the plants, to later ascend and accumulate said metals within the microphyte.

Studies carried out by Rodríguez et al., (2002) in the lower basin of the Bogotá river, mention that since this is the only source of irrigation, it has been detected that pollutants such as Mercury (Hg), Pb, Nickel (Ni), Cd, Arsenic (As) deteriorate the quality of water and contaminate both plants, animals and soils affecting the agricultural exploitation of the sector.

Lead is an element that accumulates over time, it is difficult for it to be eliminated by the body when it has been ingested. Within the human health problems caused by lead, neurological alterations, nephrotoxicity, anemia, and kidney cancer are cited. Animals can also be affected by lead through inhalation or ingestion. From the environmental point of view, lead represents a permanent problem since it can remain in the soil for thousands of years, therefore, it is necessary to decontaminate and remedy soils that are contaminated by heavy metals.(Shock, 2010).

The environment has been affected by various pollutants that come from industrial, mining, agricultural, artisanal and domestic activities, which represent a risk to human health, therefore, alternatives have been sought that can amend the impact caused, being phytoremediation, an economical and sustainable alternative; according to Jara et al. (2014) consists of using plants to remediate in situ soils, sediments, water and air contaminated by organic waste, nutrients or heavy metals, eliminating contaminants from the environment making them harmless. This technique consists of a rhizofiltration process, through which the metals are absorbed by the roots of the plant in a hydroponic medium.

The phytoremediation technique is more effective through genetic manipulation, since it improves the remediation capacity in plants by absorbing Cd, Hg and PCBs.

Mentaberry (2010) mentions that bioremediation has several advantages, among them that theplants can be used as low-cost extractor pumps to purify contaminated soil and water; plants use solar energy, some degradation processes occur faster than using microorganisms; less secondary waste is generated and it is a technique that is accepted by the community.

For a species to be considered as a bioremediation, it must have a high population growth rate and a high capacity to extract, accumulate, transform, degrade or volatilize contaminants. Research has been conducted to identify several plant species that have phytoremediation capacity, including duckweed (***Lemna*** spp.), Azolla (***Azolla*** spp.), Water Hyacinth (***Eichhornia crassipes***) (García, 2012).

The Azolla is an aquatic fern that floats freely in the water, it belongs to the Azollaceae family, it spreads easily in humid environments with warm temperatures, it develops optimally in environments with an organic substrate with a water column and without a roof.

In Ecuador, through research carried out by Montaño (2008), of the four Azolla species that have been identified, it has been possible to establish their geographical distribution: Micropylla Kaulf in the Galapagos and Guayas; Caroliniana wild in the Guayas, Filiculoides Lam in Cotopaxi in Imbabura and in the Napo; and Mexicana, Caroliniana in the Cotopaxi Province, of which Azolla Caroliana is considered native.

The Azolla is an aquatic species constitutes an adequate alternative that can contribute to the improvement of water quality, especially those that come from treated urban wastewater.

The objective of the research was to demonstrate the phytoremedial capacity of Azolla sp. in samples of water contaminated with lead and planted with rice seeds.

MATERIALS AND METHODS

The experimental tests were carried out in the Biomaterials laboratory of the Faculty of Chemical and Health Sciences, Technical University of Machala, Province of El Oro-Ecuador, located at coordinates 3.2914037 South latitude and -79.9137593 West longitude. Prior to the start of the study, 750 grams of the aquatic fern Azolla were obtained in its natural environment, at the El Palenque site, in the city of Pasaje-El Oro Province (Figure 1). The rice seeds used in the trial were obtained at the La Cuca experimental farm, Arenillas canton, El Oro Province, Ecuador.

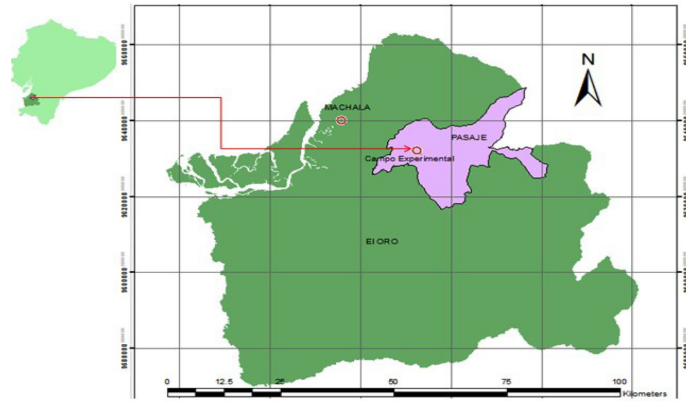

Figure 1. Georeferenced location of the area where the Azolla samples used in the experiment were collected

Experimental design

A completely randomized 4x3 balanced design was used, generating 12 experimental units (plastic trays). Each experimental unit of each treatment was randomly located at the same distance, temperature, position, with direct lighting, so that all are in the same conditions.

The experiment was carried out during 72 hours. The treatments under study are described in Table 1.

Table 1. Description of the treatments under study

Treatments	Treatment Description
T_1	*Azolla* sp. + water with lead solution (50 ppm).
T_2	Rice seeds + water with lead solution (50 ppm).
T_3	*Azolla* sp. + rice seeds + lead solution (50 ppm).
T_4	*Azolla* sp. + rice seeds + distilled water (control).

Prior to the beginning of the experiment, the water of each experimental unit was contaminated with lead, at a concentration of 50 ppm in 1 liter; and the pH was adjusted to 5 to prevent lead from precipitating.

Data collection

In rice seeds, the measurements made were seed germination (%), epicotyl length (mm) and radicle length (mm). Both variables were collected 72 hours after starting the experiment.

Analytical determinations

From the chemical point of viewAzolla and seed samples weredried at 80 degrees Celsius for a period of 48 hours and then the samples were digested (Cohen-Shoel et al., 2002). The process consisted of an open acid digestion carried out on heating plates inside a fume hood, for which 300 mg of Azolla and rice seeds were weighed, which were boiled in 10 ml of 70% HNO_3 until they were evaporated and the sediment was dissolved in 50 ml of distilled water, which was subsequently filtered and stored in 50 ml plastic tubes.

Chemical analysis

The chemical analysis of the samples was carried out by the method of optical emission spectroscopy with inductively coupled plasma (ICP-OES) in the laboratories of the National Institute of Agricultural Research (INIAP) in the city of Quevedo-Ecuador.

Statistical procedure

To determine the presence or not of statistical differences between the treatments based on the percentage of germination of rice seeds, epicotyl and radicle length; as well as the amount of Cd absorbed (phytoremediation capacity) by Azolla, the Analysis of Variance (ANOVA) test of an intergroup factor was used. To define between which treatments there are differences or similarities, Duncan's test of ranges and multiple comparisons was applied. Compliance with the assumptions of data normality and variance homogeneity were previously statistically verified. The data processing was carried out with the statistical package SPSS version 22 for Windows, and a reliability in the estimation of 95% (α=0.05) was used.

RESULTS AND DISCUSSION

Rice germination percentage

The ANOVA of an intergroup factor carried out shows that there are significant statistical differences between the treatments under study based on the percentage of rice germination, evidencing that the aquatic fern when associated with rice plants in lead-contaminated environments has an effect on the amount of rice seeds that germinate (Table 2).

Table 2. Result of the ANOVA of an intergroup factor for the comparison between the different Azolla-rice associations in relation togermination percentage of rice seeds

Sources of variation	Sum of squares	Degrees of freedom	Mean squares	F calculated	p-value
Treatments	433,642	2	216,821	5,276	0.048
Mistakes	246,553	6	41,092		
Total	680,196	8			

When rice was not associated with Azolla in an environment contaminated with Pb, it presented the highest percentage of seed germination (81.03%), a higher value and statistically different from the treatments where Azolla and rice were associated in contaminated environments (66.6%) and not contaminated with lead (66.03%), evidencing that the contamination with the heavy metal does not influence the hydrolysis and oxidation processes that take place inside the seed, however, the aquatic fern that is already a plant constituted can affect the decrease in the amount of rice seeds that germinate (Figure 2).

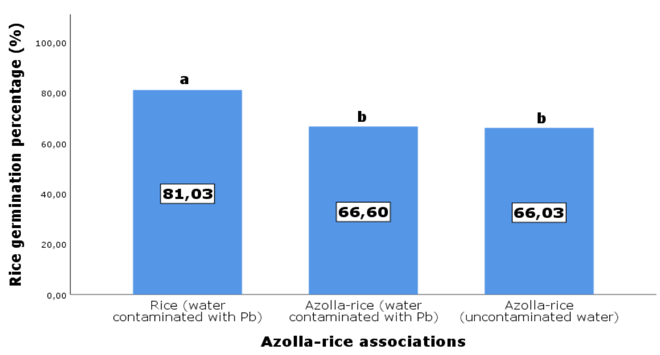

Figure 2. Effect of the Azolla-rice association on the germination of cereal seeds in lead-contaminated environments

*Different letters indicate statistical differences between Azolla-rice associations for a p-value≤0.05 (Duncan's test)

According to work carried out with the aquatic fern Salvinia minima to determine its phytoremediation capacity for lead, they established that its capacity is a function of the concentration of the metal (3.22 mgPb/l) and that physical factors also influence such as temperature and light, noting that high levels its phytoremediation capacity is more efficient (Vidal, 2009).

Rice radicle length

The hypothesis contrast executed with the ANOVA statistical test of an intergroup factor shows a p-value of 0.000; therefore, there are highly significant differences between the different Azolla-rice associations, evidencing that they influence the growth of the cereal radicle in lead-contaminated environments (Table 3).

Table 3. Result of the ANOVA of an intergroup factor for the comparison between the different Azolla-rice associations in relation to the length of the rice radicle

Sources of variation	Sum of squares	Degrees of freedom	Mean squares	F calculated	p-value
Treatments	39,142	2	19,571	30,807	0,000
Mistakes	66,705	105	0.635		
Total	105,848	107			

The presence of rice seeds without association with Azolla in Pb-contaminated waters presented the lowest average (0.00 cm), lower value and statistically different from the groups in which the Azolla and rice association was made in contaminated environments (1.36 cm) and not contaminated with lead (1.18 cm); and it is shown that heavy metal contamination had a marked influence on radicle growth, although when rice was associated with Azolla in an environment contaminated or not with lead, the values presented a minimal difference, evidencing the effect of the plant. of Azolla in root growth (Figure 3).

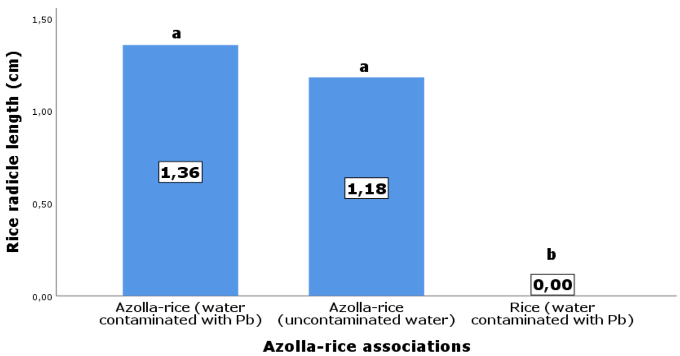

Figure 3. Effect of the Azolla-rice association on the growth of the cereal radicle in environments contaminated with Pb

*Different letters indicate statistical differences between Azolla-rice associations for a p-value≤0.05 (Duncan's test).

The root of the Azolla plant has negative charges in its cells due to the presence of carboxyl groups that interact with the positive charges of heavy metals, establishing a dynamic equilibrium, which facilitates the entry of the metal into the cell via apoplastic or symplastic (Torres et al., 2010).

Rice epicotyl length

The hypothesis contrast executed with the ANOVA statistical test of an intergroup factor shows a p-value of 0.000; therefore, there are highly significant differences between the different Azolla-rice associations, evidencing an incidence in the growth of the plant in the first stages of phenological development in an environment contaminated with lead (Table 4).

Table 4. Result of the ANOVA of an intergroup factor for the comparison between the different Azolla-rice associations in relation to the epicotyl length of rice (mm)

Sources of variation	Sum of squares	Degrees of freedom	Mean squares	F calculated	p-value
Treatments	111,154	2	55,577	18,255	0,000
Mistakes	319,663	105	3,044		
Total	430,817	107			

The length of the rice epicotyl, when the Azolla-rice association was presented in uncontaminated waters (anaerobic processes occur) with Pb, reached the lowest value of the median (0.00 mm) statistically different from the groups where it was carried out. Azolla and rice association in contaminated water (0.70 mm) and rice without association with Azolla in lead-contaminated water (2.90 mm); and it is evident that the development of the seedling (epicotyl) under waterlogged conditions in the presence of the aquatic fern is affected, in the presence or not of the contaminating metal, however, in the case where the rice was established without association with Azolla, the Lead contamination did not influence, which may be associated with the fact that the epicotyl develops with reserves found in the seed embryo (Figure 3).

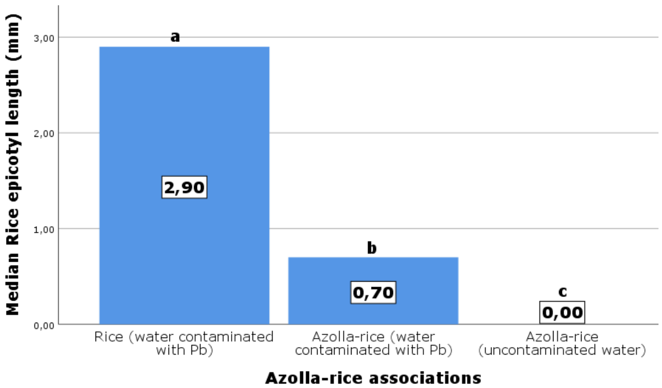

Figure 3. Effect of the Azolla-rice association on the epicotyl length of rice

*Different letters indicate statistical differences between Azolla-rice associations for a p-value≤0.05 (Duncan's test)

Research carried out on beans and corn by Isaza (2022), showed that lead affected the physiological processes of imbibition, germination and growth of these crops. Probably the effect of lead concentration in plants induces oxidative stress that occurs in roots and leaves. On the other hand, Lamhamdi (2011), mentions that the effects that can be produced in the plants, by lead, are related to the concentration of the heavy metal and type of soil, which is mainly evidenced in the germination process of the seeds.

Lead adsorption

The verification carried out using the ANOVA statistical test of an intergroup factor shows a p-value of 0.000; therefore, there are highly significant differences between the different Azolla-rice associations, demonstrating that the heavy metal present in an aquatic environment can be adsorbed in different amounts when Azolla and rice are associated or not associated (Table 5).

Table 5. Result of the ANOVA of an intergroup factor for the comparison between the different Azolla-rice associations in relation to the lead adsorption capacity in Azolla and rice

Sources of variation	Sum of squares	Degrees of freedom	Mean squares	F calculated	p-value
Treatments	13052306.23	3	4350768.74	23,026	0,000
Mistakes	1511583.43	8	188947.92		
Total	14563889.67	11			

Azolla in association with rice seeds in the presence of a lead-contaminated environment performs the highest uptake of the heavy metal (2156.5 mg/kg), statistically different from the value obtained in rice, when associated with the aquatic fern (176 0.4 mg/kg), although it does not differ from the treatments where Azolla (2708.9 mg/kg) and rice (2716.1 mg/kg) were present without association in environments contaminated with the heavy metal (Figure 4).

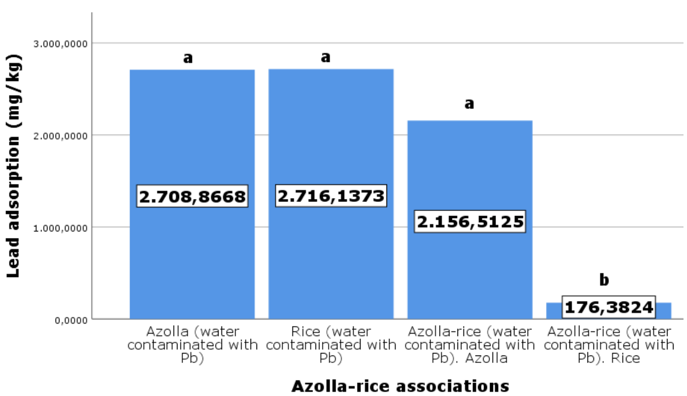

Figure 4. Effect of the Azolla-rice association on the absorbed lead content

*Different letters indicate statistical differences between Azolla-rice associations for a p-value≤0.05 (Duncan's test)

The Azolla aquatic fern has a hyperaccumulator capacity, that is, it can accumulate and also remove heavy metals from the water, including lead, which in high amounts affects plant growth. (Ramirez, 2017).

CONCLUSIONS

The Azolla is an aquatic fern that demonstrated phytoremediation capacity of the heavy metal lead in association with rice seeds in the germination process, evidencing; which, in an environment contaminated with 50 ppm of lead, adsorbs 92.4% of the lead present, proving to be a viable biological alternative to remedy lead-contaminated environments in rice cultivation.

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