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Phytotoxicity test of effluent and sludge from a rice parboiling industry

Teste de fitotoxicidade de efluente e lodo de uma indústria de parboilização de arroz

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ABSTRACT

The rice represents a great sector inside of Brazil's economy, witch around all the rice consumed in the country 25% is the parabolized type. It is known that the beneficiation process of this product involves a high generation of effluent with the high organic loads and elevated levels of nitrogen and phosphor. The effluent treatment stations of these industries rely on anaerobic digestion to reduce the organic matter content, accordingly they generate anaerobic sludge. The possibility of the use of anaerobic sludge, treated or not, in agricultural soil awakens the need for studies that assure its practice in a sustainable way to the environment. The phytotoxicity is characterized as a highly important analysis, since it presents a general idea of the complexity of the interactions of the different compounds present in the residue or effluent. Germination tests have been the most used to evaluate phytotoxicity. This study aimed to evaluate the phytotoxicity of crude effluent and untreated sludge from a rice parboiling industry using three types of seeds, watercress, lettuce and cucumber. Germination rates varied from 1.432±0.370% to 78.176±15.340% indicating biomass phytotoxicity.

Keywords: Bioindicators; Germination index; Inhibition

RESUMO

O arroz representa um grande setor dentro da economia brasileira, sendo que de todo o arroz consumido no país 25% é do tipo parabolizado. Sabe-se que o processo de beneficiamento deste produto envolve uma alta geração de efluentes com altas cargas orgânicas e níveis elevados de nitrogênio e fósforo. As estações de tratamento de efluentes dessas indústrias dependem da digestão anaeróbica para reduzir o conteúdo de matéria orgânica e, consequentemente, geram lodo anaeróbico. A possibilidade do uso de lodo anaeróbico, tratado ou não, em solo agrícola desperta a necessidade de estudos que garantam sua prática de forma sustentável ao meio ambiente. A fitotoxicidade é caracterizada como uma análise altamente importante para tal, pois apresenta uma ideia geral da complexidade das interações dos diferentes compostos presentes no resíduo ou efluente. Os testes de germinação têm sido os mais utilizados para avaliar a fitotoxicidade. Este estudo teve como objetivo avaliar a fitotoxicidade do efluente bruto e do lodo não tratado de uma indústria de parboilização de arroz utilizando três tipos de sementes: agrião, alface e pepino. As taxas de germinação variaram de 1,432 ± 0,370% a 78,176 ± 15,340%, indicando fitotoxicidade da biomassa.

Palavras-chave: Bioindicadores; Índice de germinação; Inibição

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1 INTRODUCTION

The rice represents a large sector within the Brazilian economy, since the country is a major producer and consumer of the grain, with per capita consumption around 25 $kg.year^{-1}$ (QUEIROZ et al., 2007, SPINOSA et al., 2016), and of all rice consumed, about 25% is the parboiled type (PARAGINSKI et al., 2014).

The processing of this type of rice involves the drenching of the grain in tanks with water at 60 °C (AGEITEC, 2016). This stage results in a high volume of effluent generated with grain properties acquired during the wetting period (AMATO, 2017; BASTOS et al., 2010, QUEIROZ et al., 2007). The process generates about 4 liters of effluent per kilogram of rice beneficiated, these effluents have high loads of organic substances, nitrogen, and phosphorus, from the fertilization of the grain, generating an effluent characterized by high levels of Biochemical Oxygen Demand (BOD) , Chemical Oxygen Demand (COD), solids and acid pH (ABIAP, 2006).

Because of the influence of the grain culture, as seasonal aspects, there are great variability of the parameters in the characterization of these effluents (BASTOS et al., 2010). It is possible to find in the literature variations between 398.70 and 6447.50 $mg.L^{-1}$ of COD (GERBER et al., 2018; NADALETI et al., 2018), 23.10 and 120.90 mg.L-1 of Nitrogen Total Kjeldahl (NTK) (GERBER et al., 2018; ISODIL et al., 2003) and ph varyng from 3.47 to 6.30 (GERBER et al., 2018; NADALETI et al., 2019). However, according to Bastos et al. (2010), which shows the greatest variation is the ratio between the carbon load for the nitrogen load (C/N), ranging from 21.00 to 72.73 according to data obtained in the literature (ISODIL et al, 2003; QUEIROZ et al., 2007).

Effluent Treatment Plants (ETP) of the rice parboiling industries rely on the anaerobic digestion process to reduce the organic matter content (CREMONEZ et al., 2013). Thus, ETP generate anaerobic sludge in their biological treatments (BARCELOS, 2009; STEIL, 2001). Several studies point to the use of anaerobic sludge, treated or not, in agricultural soil as a destination and may also contribute positively to agricultural production (BORBA et al., 2017; PEREIRA, GARCIA, 2017). For many authors, physicochemical parameters are not enough for assessing the effects of the compounds on the environment, and a biological response is required (HANANEN et al., 2012; CHOWDHURY et al., 2013; KAPANEN et al., 2013). Furthermore, phytotoxicity is characterized as an extremely important analysis, and germination tests are the most commonly adopted (WU; MA, 2001), since they are simple, fast, reliable and reproducible methods (TIQUIA et al., 1996).

The present work had as objective to evaluate the phytotoxicity of the raw effluent and the untreated sludge from Upflow Anaerobic Sludge Blanket Reactor (UASB) from ETP of a rice parboiling industry located in the city of Pelotas-RS through the biological response of three types of seeds.

2 MATERIALS AND METHODS

Several species of plants are currently used for this type of study, however, the most commonly used are watercress, cabbage, lettuce, carrot, tomato and cucumber. The sensitivity of the seeds to the toxicity depends on the amount of reserve they have, accordingly the seeds of tubers, grains and vegetables have low sensitivity to toxicity (OLESZCZUK et al., 2011). Thus, the experiment was carried out in a 2x3 factorial arrangement with a control, blank (B) for each seed, necessary to calculate the Relative Germination and the Radical Length of each plot. One of the factors used in the study was the biomass employed, with effluent (E) and sludge (S) as the other factors, and the other factors were the seeds used, with levels of watercress (*Rorippa nasturtium-aquaticum*) (WA), lettuce (*Lactuca sativa L.*) (L) and cucumber (*Cucumis sativus L.*) (C), generating nine different treatments performed in triplicates (Table 1):

Table 1 – Treatments of the factorial arrangement 2x3 and a reference element in triplicates

	Watercress seed			
Effluent	EWA1	EWA2	EWA3	
Sludge	SWA1	SWA2	SWA3	
Blank	BWA1	BWA2	BWA3	

The phytotoxicity test was performed using metrology adapted from Tiquia and Tam (1998) and Zucconi et al. (1988). As far as sample preparation is concerned, slurry, as a solid residue, had to be diluted in distilled water, 10g.100mL-1, needed to be stirred for one hour on a magnetic stirrer and filtered on filter paper.

The seeds were placed in petri dishes with a bottom covered with filter paper, each plate received ten seeds. Finally, with the aid of a graduated pipette, the plates received 5 mL of the biomass. Blanks received 5 mL of distilled water. The plates were closed and sealed with cling film and allocated in B.O.D., at 25 °C by the germination period of each seed.

After the incubation period, the number of seeds germinated per plate was quantified and with a pachymeter, the root length of the germinated seeds was determined. The formulas used to determine the relative germination and root length of each triplicate, as well as the germination index were adapted from Zucconi et al. (1988):

Relative Germination (RG):

$$RG\ (\%) = \frac{\text{number of seeds germinated in the extract of the compound)}}{\text{number of seeds germinated in the blank}} * 100 \qquad (1)$$

Radicle Length (RL):

$$RL\ (\%) = \frac{\text{Sum of the elongation of the radicles in the compound}}{\text{Summation of the elongation of the radicles in the Blank}} * 100 \qquad (2)$$

Germination Index (GI):

$$GI\ (\%) = \frac{[\text{RG}(\%) * RL(\%)]}{100} \qquad (3)$$

The parameters used to determine the phytoxicity classification of the biomasses are shown in Table 2. In addition, the results were analyzed statistically by Tukey's Variance and Test Analysis.

Table 2 - Classification of IG based phytotoxicity

Classification	GI (%)
Stimulate soil properties	GI>100
Does not inhibit plant growth	80>GI ≤100
Moderate inhibition	60>GI ≤80
Strong inhibition	40>GI≤60
Severe inhibition	≤40

Source: authors

The analysis concerning the characterization of the sludge were carried out according to Tedesco et al (1995), already the characterization of the effluent was carried out according to APHA (2002). The statistical analysis of the results was performed in the IBM SPSS Statistics software through the F Test and the Tukey Test, both at 5% significance.

3 RESULTS AND DISCUSSION

From the equations concerning the relative germination, which considers the number of germinated seeds in each plate and the length of the radicle of each treatment and of the blanks (Equations 1 and 2), analyzed in the laboratory, it was possible to obtain the results presented in Table 3:

Table 3 - Relative germination and radicular length in each treatment

	Repetition	EWA	SWA	EL	SL	EC
RG (%)	1	52.000	92.308	33.333	79.000	88.889
	2	62.000	105.000	33.333	88.889	100.000
	3	61.538	92.308	22.222	79.000	100.000
RL (%)	1	66.745	46.769	4.818	93.000	49.765
	2	72.635	67.000	5.216	70.000	44.581
	3	66.000	39.977	4.636	84.036	54.349

Source: authors

Table 4- Germination index and repetitions obtained in each treatment

GI (%)			
	WA	L	C
E	34.707	1.577	44.235
	45.034	1.707	44.581
	37.714	1.011	54.349
S	40.088	73.470	61.934
	70.350	61.091	80.174
	34.266	66.388	92.419

Source: authors

From the results presented in Table 4, the analysis of the variance was performed, where Test F indicated that at least one of the averages presents a significant difference at 5% significance (Table 5). Due to the result of Test F, the initial hypothesis was rejected, in which there would be no significant difference between the averages obtained in the study, and was followed up in the statistical analysis, where the Tukey averages test was applied. The Tukey test (q 5% (6.12) = 4.750, Δ = 28.406) and the phytotoxicity classification of the compound used in the treatments allowed us to determine that the only treatment that presented severe inhibition germination index was the EL:

Table 5 - Treatment GI Result

GI (%)	
SC	78.76 ± 15.340^{a}
SL	66.983 ± 6.211^{ab}
SWA	48.235 ± 19.373^{b}
EC	47.722 ± 5.742^{b}
EWA	39.152 ± 5.311^{b}
EL	1.432 ± 0.370^{c}

Source: authors

Although the phytotoxicity classification indicates that only the SWA, EC and EWA treatments showed strong inhibition, the means test indicates that the LA treatment, classified with moderate inhibition, does not differ significantly from the others mentioned. Accordingly, statistically, only SC treatment shows moderate inhibition. It is important to emphasize that Brazil does not have specific legislation for phyto-toxicological tests as a biological response to environmental impacts, accordingly that the studies of the area (TAMADA et al., 2012, TIQUIA, TAM, 1998), as well as the State's Environmental Protection Agency (EPA, 2016), consider that values below 30% are considered highly phytotoxic. The California Compound Quality Council (2001) indicates that when the GI does not reach 80%. The compound is considered as phytotoxic and may indicate the presence of soluble salts, organic compounds, inorganic elements or heavy metals.

A study by Spessat et al. (2016) to evaluate the phytotoxicity of residues from a rice oil extraction industry (activated sludge, physic-chemical sludge, distillation residue, defatted rice bran and rice hull ash) using cucumber seeds and cabbage determined that the activated sludge residues and physic-chemical sludge for the cabbage seed presented a potential growth promoter, while the defatted rice bran presented high phytotoxicity, requiring treatment for its use in soil. However, the cucumber seed did not show a significant difference between the residues analyzed and accordingly this study it did not show efficacy as a bioindicator of phytotoxicity.

Gerber et al. (2018) investigated the crude and treated effluent phytotoxicity of a parboiled rice industry using lettuce seeds (*Lactuca sativa L.*) and cucumber (*Cucumis sativus L.*) as bioindicators. The study determined that crude effluent presented phytotoxicity, GI of 46.6±16% for lettuce seeds and 77.1±7.0% for cucumber seeds. The authors pointed out that iron (Fe) and manganese (Mn) concentrations were the main cause for phytotoxicity, according to statistical correlation. The characterization of the effluent also determined the presence of essential nutrients such as nitrogen and phosphorus, for agricultural use already treated, its use for this purpose would be impracticable due to its phytotoxicity potential for cucumber seeds, GI of 77.3±5.8 %.

Table 6 presents the characterization of effluent and sludge analyzed and used in the study:

Effluent		**Sludge**	
COD (mg O_2L^{-1})	8595.04	Humidity %	70.79
P (mg.L^{-1})	8.11	P (g.kg^{-1})	16.11
Mn (mg.L^{-1})	0.13	Mn (mg.kg^{-1})	704.23
N (mg.L^{-1})	89.44	N (g.kg^{-1})	29.45
Zn (mg.L^{-1})	0.09	Zn (mg.kg^{-1})	182.88
Fe (mg.L^{-1})	0.70	Fe (mg.kg^{-1})	1636.25
C (mg/L)	2303.00	%C	15.51
C/N	25.75	C/N	5.27
pH	4.48	pH	7.78

Source: authors

Kader et al (2015) report the possibility that the presence of acid cations, such as Fe and Mn, in the soil may influence phytotoxicity, because although they are considered micronutrients, in certain concentrations and pH ranges can lead to toxicity and interfere in RL (SEDIBE ET AL. 2013, SHAIBUR, KAWAI 2010).

The phytotoxicity can reveal the interference of the effluents in the environment, being a bioindicator that considers not only the individual effect of each substance present in the effluent, but also the synergism of these and its effects on the seed germination index. Thus, effluents and residues that fit physicochemical parameters may reveal high environmental risk, while the synergism of the parameters can generate toxic effects that overcome their individual properties (BADERNA et al., 2010).

4 CONCLUSIONS

The crude effluent and sludge from the UASB of the ETP of the rice parboiling industry presents phytotoxicity to watercress, lettuce and cucumber seeds, according to this study, thus results of the study do not corroborate the use of the sludge and effluent in agricultural soil, being necessary the treatment of the same accordingly that its phytotoxic effects are reduced or annulled. The phytotoxicity of biomasses ranged from moderate to severe, and most of them presented a strong inhibition.

Furthermore, the results indicate high phytotoxicity of the effluent, being possible that its presence in the untreated sludge caused the phytotoxicity of the sludge, being recommended the test of phytotoxicity of the sludge after removal of the effluent through different treatments in order to know those able to promote the viability of sludge application in agricultural soil.

Thus, the presented findings direct future studies to enable the anaerobic sludge disposal of rice parboiling in agricultural soil. The application of a pre-treatment in sludge for dispose of it in agricultural soil can be represent a decrease in the expenses of industries of the sector that today need hired licensed companies for the correct disposal of the waste.

REFERENCES

SPESSATO AG, PAZ MF, FONSECA CB, CORRÊA LB, CORRÊA EK. **Alongamento relativo de sementes de pepino (*C. sativus*) e repolho (*B. oleracea*) expostos a diferentes resíduos oriundos da índustria de extração de óleo de arroz.** In: Proceedings of XVIII Encontro de Pós-Graduação; 2016; Pelotas, Brasil.

AGEITEC. EIFERT EC, ELIAS MCE, FRANCO DF, FONSECA JR. **Árvore do Conhecimento.** Arroz. Beneficiamento; 2018. [cited 2019 jan 12]. Available from: https://www.embrapa.br/busca-de-noticias/-/noticia/43177238/embrapa-disponibiliza-atualizacao-da-arvore-do-conhecimento-do-arroz

AMATO, G. W. **Arroz no programa mundial de alimentação das Nações Unidas.** 2nd ed. Porto Alegre: Instituto Rio Grandense do Arroz; 2017.

BADERNA D, LOMAZZI E, POGLIAGHI A, CIACCIA G, LODI M, BENFENATI E. **Acute phytotoxicity of seven metals alone and in the mixture: are Italian soil threshold concentrations suitable for plant protection?** Environ Res 2015;140:102-11.

BARCELOS BR. **Avaliação de Diferentes Inóculos na Digestão Anaeróbia da Fração Orgânica de Resíduos Sólidos Domésticos [dissertation].** Brasília: Departamento de Engenharia Civil e Ambiental, Universidade de Brasília; 2009. 89 p.

BORBA RP, CAMARGO OA, COSCIONE AR, MARIA IS. **Dessorção de P de sedimentos provenientes de latossolo tratado com lodo de esgoto.** Revista de Geociências. 2017;36(2).

CHOWDHURY AKMMB, AKRATOS SC, VAYENAS DV, PAVLOU S. **Olive mill waste composting: A review.** International Biodeterioration & Biodegradation. 2013;85:108-19.

CREMONEZ PA, FEIDEN A, ZENATTI DC, CAMARGO MP, NADALETI WC, ROSSI E, ANTONELLI J. **Biodigestão Anaeróbia no Tratamento de Resíduos Lignocelulósicos.** Revista Brasileira de Energias Renováveis. 2013;2:21-35.

GERBER MD, ARSAND DR, LUCIA T, CORREA ÉK. **Phytotoxicity Evaluation of Wastewater from Rice Parboiling.** Bulletin of Environmental Contamination and Toxicology. 2018; 2455-9.

HIMANEN M.,PROCHAZKA P, HANNINEN K, OIKAR, A. **Phytoxicity of lowweight carboxylic acids.** Chemosphere. 2012;88:426-31.

KADER M, LAMB DT, CORRELL R, MEGHARAJ M, NAIDU R. **Porewater chemistry explains zinc phytotoxicity in the soil.** Ecotox Environ Safety. 2015;122:252-9

KAPANEN A, VIKMAN M, RAJASARKKA J, VIRTA M, ITAVAARA M. **Biotests for environmental quality assessment of composted sewage sludge.** Waste Management. 2013;33:1451-60.

OLESZCZUK P, HOLLERT H. 2011. **Comparison of sewage sludge toxicity to plants and invertebrates in three different soils.** Chemosphere. 2011;83(4):502-9.

PARAGINSKI RT, ZIEGLERM V, TALHAMENTO A, ELIAS MC. **Propriedades tecnológicas e de cocção em grãos de arroz condicionados em diferentes temperaturas antes da parboilização.** Brazilian Journal of Food Technology. 2014;17(2):146-153.

PEREIRA ACA, GARCIA ML. **Efeitos da disposição de lodo de estações de tratamento de efluentes (ETE) de indústria alimentícia no solo: estudo de caso.** Eng. Sanit. Ambient. 2017;22(3).

QUEIROZ MI, LOPES EJ, ZEPKA LQ, BASTOS RG, GOLDBECK R. **The kinetics of the removal of nitrogen and organic matter from parboiled rice effluent by cyanobacteria in a stirred batch reactor.** Bioresource Technology. 2007;98:2163-9.

SEDIBE M, MGCOYI W, COMBRINK N. **Quality assessment of agricultural water used for fertigation in the Boland region of South Africa.** Life Sci J. 2013;10:19-1327.

SHAIBUR M, KAWAI S. **Effect of arsenic on nutritional composition of japanese mustard spinach: an Ill effect of arsenic on nutritional quality of a green leafy vegetable.** Nature Sci. 2010;8:186-94.

SPINOSA WA, JÚNIOR VS, GALVAN D, FIORIO JL, GOMEZ RJHC. **Syrup production via enzymatic conversion of a byproduct (broken rice) from rice industry.** Acta Scientiarum Technology. 2016;38(1):13-22.

STEIL L. **Avaliação do uso de inóculos na biodigestão anaeróbia de resíduos de aves de postura, frangos de corte e suínos [dissertation].** Araraquara: Biotecnologia/UNESP; 2001. 127p.

TIQUIA SM, TAM NFY. **Elimination of Phytotoxicity during cocomposting of spent pig-manure sawdust litter and pig sludge.** Bioresource Technology. 1998;65:43-49.

WAN Y, BAO Y, ZHOU Q. **Simultaneous adsorption and desorption of cadmium and tetracycline on the cinnamon soil.** Chemosphere. 2010;80:807-12.

WU L, MA LQ. **Effects of Sample Storage on Biosolids Compost Stability and Maturity Evaluation.** Journal of Environmental Quality. 2001;30(1):222-8.

ZUCCONI F et al. **Evaluating toxicity in immature compost.** Biocycle, Emmaus. 1988;22:54-57.