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Quality of lettuce *Lactuca sativa* (var. *Tropicana* M1) grown with two low-salinity shrimp effluents

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ABSTRACT

In a previous study, we investigated the use of shrimp effluents from well water (WW) and diluted seawater (DSW) (both with $2.7\,\mathrm{dS\,m^{-1}}$ electrical conductivity (EC)), and a hydroponic solution (HS) as the control treatment in greenhouse lettuce production. This new paper completes the previous one by focusing on the quality of lettuce harvested. Compared to the lettuce from the other treatments, WW-lettuce exhibited higher levels of phenolic compounds and a higher antioxidant capacity, mainly in the soluble fraction. The lettuce cultivated with DSW showed no significant difference in total phenolics and flavonoids with respect to the HS lettuce. These results reveal that the functional properties (antioxidant properties, polyphenols and flavonoid content) are even better in the lettuce produced with WW and DSW shrimp effluents. In contrast, agronomical properties (weight, number of leaves and yield) were found to be better in the case of lettuce grown with the hydroponic solution (control).

1. Introduction

Shrimp farming has been criticized for its use of large water exchanges to adequately sustain the water quality for shrimp growth and for the discharge of effluents enriched with nutrients, which contribute to water quality deterioration in the ecosystems in which effluents are released. One way to mitigate these environmental effects is by integrating shrimp farming with agriculture through the irrigation of plants in hydroponic systems, which could be achieved by growing halophyte plants in marine shrimp cultures or growing vegetables when low-salinity or fresh water is used.

In a recent paper, León-Cañedo et al. (2019) investigated the concentrations of heavy metals in the lettuce *Lactuca sativa* grown in a deep flow technique (DFT) hydroponic system with shrimp culture effluent. In the present study, we include results obtained during the same experiments, on the quality of harvested lettuce in terms of phenolic compounds and their antioxidant capacity. It has been shown that when plants are exposed to some types of abiotic stress during their cultivation, their defense mechanism that involves an increase in compounds

with antioxidant properties is activated, which increases their nutraceutical properties (Oh, Carey, & Rajashekar, 2009). However, salt stress could also affect the nutrient content and growth of plants. Therefore, this study evaluated the performance of a hydroponic lettuce culture of the *Tropicana* M1 variety grown in a DFT system with two low-salinity shrimp effluents (well and diluted seawater at 2.7 dS m⁻¹); this could demonstrate the hypothesis that the lettuce grown with low-salinity shrimp effluents exhibits a quality comparable to that grown with a traditional hydroponic solution.

2. Materials and methods

2.1. Experimental

Two types of shrimp culture effluent were used: (i) from shrimp tanks filled with well water (treatment WW; EC $2.7\,dS\,m^{-1}$) and (ii) from shrimp tanks filled with diluted sea water (treatment DSW; CE $2.7\,dS\,m^{-1}$) (Fig. 1). Additionally, a control treatment consisting of one hydroponic DFT system in triplicate was constructed to grow lettuce

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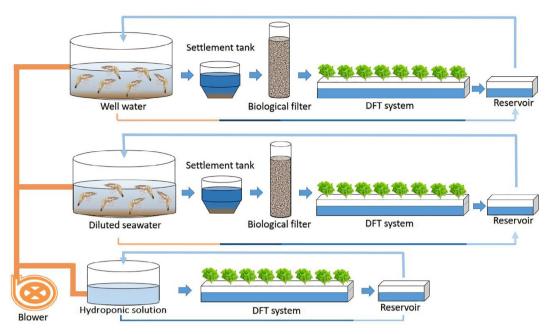


Fig. 1. Scheme of the experimental system used in this study.

using a nutritive solution (HS), and was used to compare the growth of the lettuce plants and their quality. The lettuce and the shrimp tanks were coupled after the shrimp were harvested (50 days of growth). After harvest, both the root and foliage tissues of the lettuces were individually weighed, and the production variables were analyzed. The shrimp effluents from both treatments, WW and DSW, were characterized for physicochemical parameters, major components, trace metals and nutrients. This system, and the operating conditions, are described in detail in our previous paper (León-Cañedo et al., 2019) and in the supplemental section.

2.2. Biochemical parameters in lettuce

Proximate and physicochemical analysis (crude protein, fat, pH, total soluble solids (TSS), titration acidity (TA) and chlorophyll) were performed according to the AOAC (1997). Colour was measured by reflectance in leaves and free proline according to the method of Bates, Waldren, and Teare (1973). To extract the free phenolic compounds and phenolic compounds from the insoluble fraction, samples were processed according to Nguyen and Niemeyer (2008) and Mora-Rochin et al. (2010), respectively. The total phenolic content (TPC) of the soluble and insoluble fraction extracts was determined by colorimeter (Singleton, Orthofer, & Lamuela-Raventós, 1999). The total flavonoid content (TFC) of the free and bound methanolic extracts was determined by a colorimetric assay (Kim, Chun, Kim, Moon, & Lee, 2003).

Antioxidant capacity assays. For the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay, the procedure described by Arnao, Cano, and Acosta (2001) was followed. For the 2,2-diphenyl1-picrylhydrazyl (DPPH) assay, the procedure in Brand-Williams, Cuvelier, and Berset (1995) was followed. More details on the analyses are given in the supplementary section.

2.3. Statistical analysis

The data were subjected to the Shapiro-Wilk test, to check the normality. The obtained data were analyzed using the software JMP ver. 10.0.0 (Copyright © 2012 SAS Institute Inc.) by non-parametric one-way Kruskal-Wallis test, and comparisons for each pair using the Wilcoxon method performed at a significance level of P < 0.05 at a 95% confidence limit to establish the significant differences between

the mean parameters of the three treatments (WW, DSW, and control). To determine differences between the water quality parameters of the shrimp effluents a one-way ANOVA was used.

3. Results and discussion

Both shrimp effluents (WW and DSW) exhibited comparable physicochemical values and elemental composition (Table 1). Concentrations of both ${\rm Cl^-}$ and ${\rm Na^+}$ in the WW effluents from the shrimp culture were higher than the reported characteristic concentrations for this type of water or those specified as restriction limits (${\rm Cl^-}$, 355 mg ${\rm l^{-1}}$; León-Cañedo et al., 2019). The two shrimp effluents evaluated exhibited concentrations below those from the guidelines for trace metals in irrigation water so that the plants do not exhibit toxic effects. The production variables of lettuce showed a production trend of HS > WW > DSW (Table 2) for the individual average weight and yield variables, with the exception of the number of leaves for which the DSW treatment was higher than WW. Supplementary information on the growth and the salt stress effect was discussed elsewhere (León-Cañedo et al., 2019).

There were no significant differences in pH between the lettuces from the two treatments and the control (P>0.05) (Table 2). A significant difference in TSS was observed between the lettuce from the WW treatment and the control HS; WW-cultivated lettuce showed a high concentration of TSS. The titration acidity was similar in the lettuce obtained from the three treatments, and only slight differences were observed, in which the lowest value was found for the WW treatment. Zushi, Matsuzoe, and Kitano (2009) reported that seawater and NaCl irrigation may increase the quality of the crop, though it may lead to a reduction in the size and yield of the fruit.

The leaf colour results indicated that this characteristic was affected by the WW and DSW treatments (Table 2). Both parameters L and C showed significant differences on the CIELAB scale, with the chroma value decreasing in both the WW and DSW treatments in comparison to that in HS. The colour of the leaves changed progressively from vivid green to dull green in the plants exposed to both types of effluents, which may be due to salt stress (Bartha, Fodorpataki, del Martinez-Ballesta, Popescu, & Carvajal, 2015). WW-cultivated lettuce had the lowest chroma value compared to that of the control. It was observed that the chlorophyll a content was significantly lower in the WW-

Table 1
Chemical characterization of the shrimp culture effluents used for lettuce (mean ± SD) during the crop cycle. WW, DSW and HS refer to the shrimp tanks filled with well water, diluted sea water and the nutritive solution (control).

Variable	n	WW Mean ± SD		DSW Mean ± SD		HS*
		Initial	Final	Initial	Final	
Temp. (°C)	6	$21.5 \pm 2.2^{a,1}$	19.3 ± 1.3 ^{a,1}	$21.2 \pm 1.0^{a,1}$	19.6 ± 2.1 ^{a,1}	21.6
$DO (mg l^{-1})$	6	$7.8 \pm 0.3^{a,1}$	$7.6 \pm 0.4^{a,1}$	$7.9 \pm 0.4^{a,1}$	$7.5 \pm 0.3^{a,1}$	7.1
pH	6	$6.9 \pm 0.4^{a,1}$	$7.0 \pm 0.3^{a,1}$	$7.0 \pm 0.3^{a,1}$	$6.7 \pm 0.2^{a,1}$	6.1
EC ($dS m^{-1}$)	6	$2.7 \pm 0.1^{a,1}$	$2.6 \pm 0.3^{a,1}$	$2.7 \pm 0.1^{a,1}$	$2.8 \pm 0.4^{a,1}$	2.3
TAN (μ g l ⁻¹)	3	$13.8 \pm 6.9^{a,1}$	$31.1 \pm 14.7^{a,1}$	$27.8 \pm 11.8^{a,1}$	$37.9 \pm 22.1^{a,1}$	41,200
NO_2^- -N (µg l^{-1})	3	$2.8 \pm 1.2^{a,1}$	$32.4 \pm 3.9^{b,1}$	$18.8 \pm 16.8^{a,1}$	$20.0 \pm 3.5^{a,1}$	2.5
NO_3^- -N (µg 1^{-1})	3	$489 \pm 156^{a,1}$	$668 \pm 171^{a,1}$	$640 \pm 139^{a,1}$	$587 \pm 168^{a,1}$	140,790
$TN (mg l^{-1})$	3	$3.4 \pm 1.3^{a,1}$	$2.5 \pm 0.1^{a,1}$	$2.4 \pm 0.4^{a,1}$	$3.1 \pm 0.5^{a,1}$	190.3
$PO_4^ P (\mu g 1^{-1})$	3	$82 \pm 76^{a,1}$	$262 \pm 21^{a,1}$	$133 \pm 62^{a,1}$	$211 \pm 103^{a,1}$	45,600
TP $(\mu g l^{-1})$	3	$171 \pm 42^{a,1}$	$318 \pm 25^{a,1}$	$164 \pm 81^{a,1}$	$303 \pm 125^{a,1}$	45,600
$K^+ (mg 1^{-1})$	3	$32.9 \pm 9.2^{a,1}$	$33.6 \pm 4.4^{a,1}$	$34.3 \pm 2.7^{a,1}$	$35.9 \pm 2.9^{a,1}$	211.6
$Mg^{2+} (mg l^{-1})$	3	$58.2 \pm 8.1^{a,1}$	$57.5 \pm 14.3^{a,1}$	$47.6 \pm 8.5^{a,1}$	$43.7 \pm 2.0^{a,1}$	49.6
$Na^{+} (mg l^{-1})$	3	$230 \pm 13^{a,1}$	$245 \pm 41^{a,1}$	$265 \pm 64^{a,1}$	$236 \pm 17^{a,1}$	41.6
$Cl^- (mg l^{-1})$	3	$729 \pm 38^{a,1}$	$669 \pm 45^{a,1}$	$690 \pm 132^{a,1}$	$620 \pm 34^{a,1}$	13.6
$Ca^{2+} (mg l^{-1})$	3	$59.8 \pm 6.1^{a,1}$	$61.4 \pm 8.9^{a,1}$	$52.1 \pm 3.3^{a,1}$	$53.7 \pm 2.7^{a,1}$	131.2
Fe (mg l ⁻¹)	3	ND	ND	ND	ND	2.9
Cu (μg l ⁻¹)	3	$19.6 \pm 1.2^{a,1}$	$22.0 \pm 0.7^{a,1}$	$22.0 \pm 5.3^{a,1}$	$20.9 \pm 4.9^{a,1}$	100
Mn ($\mu g l^{-1}$)	3	$1.0 \pm 0.4^{a,1}$	$1.9 \pm 0.7^{a,1}$	$1.2 \pm 0.6^{a,1}$	$1.7 \pm 0.4^{a,1}$	400
$\operatorname{Zn}(\mu g 1^{-1})$	3	$33.0 \pm 6.8^{a,1}$	$38.4 \pm 14.4^{a,1}$	$58.0 \pm 15.0^{a,1}$	$36.1 \pm 7.1^{a,1}$	100
Hg (μ g l ⁻¹)	3	$6.7 \pm 0.6^{b,1}$	$0.7 \pm 0.1^{a,1}$	$6.7 \pm 0.4^{b,1}$	$0.7 \pm 0.3^{a,1}$	< 0.2

Means with different letters (a,b) between the initial and final water for the same treatment indicate significant differences (P < 0.05); means with different numbers (1,2) between WW and DSW for the same stage (initial or final) indicate significant differences (P < 0.05); ND, not detected ($< 0.005 \, \text{mg l}^{-1}$); TAN, total ammonianitrogen; TN, total nitrogen; TP, total phosphorus.

Table 2 Colour L (lightness), C (chroma), chlorophyll a (Chl a) and chlorophyll b (Chl b) $(mg\,g^{-1}$ DW), total soluble solids (TSS), titration acidity (TA), crude protein, fat, ash, and fixed carbon (% of fresh weight) $(mean \pm SD)$ of lettuce cultured with different shrimp effluents from well water (WW), diluted seawater (DSW) and the control treatment (HS).

	ww	DSW	HS
pН	6.02 ± 0.03^{a}	6.00 ± 0.05^{a}	6.04 ± 0.04^{a}
TA	0.10 ± 0.01^{b}	0.12 ± 0.01^{a}	0.11 ± 0.02^{ab}
TSS	3.82 ± 0.65^{a}	2.63 ± 0.35^{b}	2.77 ± 0.27^{b}
L	44.5 ± 5.3^{c}	48.7 ± 5.4^{b}	53.5 ± 3.2^{a}
C	28.5 ± 2.9^{c}	32.2 ± 2.9^{b}	35.3 ± 2.8^{a}
Chl a	16.3 ± 1.3^{b}	18.1 ± 0.7^{a}	18.3 ± 0.6^{a}
Chl b	11.8 ± 0.2^{a}	11.6 ± 0.1^{b}	11.5 ± 0.1^{b}
Moisture	94.6 ± 0.5^{a}	93.2 ± 2.2^{a}	91.6 ± 1.6^{b}
Fat	0.47 ± 0.04^{a}	0.38 ± 0.02^{b}	0.49 ± 0.02^{a}
Ash	0.88 ± 0.08^{a}	0.75 ± 0.07^{b}	0.88 ± 0.05^{a}
Crude protein	0.72 ± 0.04^{b}	0.69 ± 0.05^{b}	1.07 ± 0.03^{a}
Fixed carbon	3.3 ± 0.5^{b}	4.9 ± 1.9^{a}	5.9 ± 1.3^{a}

For the same line, means with different letters between columns are significantly different (P < 0.05). Statistical analyses were performed by one-way Kruskal-Wallis tests

cultivated lettuce than in the lettuce from the other treatments. The decrease in the chlorophyll a content may be due to the inhibition of the synthesis and the degradation of chlorophyll (Ashraf, 2004). The results of the proximate analysis for the harvested lettuce revealed a general trend of HS > WW > DSW for the fat, ash and protein. The reduction in protein in the WW and DSW treatments compared to that in the control was significant (Table 2).

The results of this study showed that the lettuce grown with the WW exhibited higher TPC and TFC than the control lettuce cultivated under the hydroponic solution, while the lettuce grown with the DSW treatment was not different from that grown with HS (Fig. 2). The contribution of bound to total phenolics in the examined samples indicated that the bound phenolics were the minor phenolic fraction in lettuce, representing 19 to 23% of the total fraction. Contrary to the effect on

free phenolics, the bound phenolic compounds were found to be at a lower concentration (\sim 16%) in the lettuce cultivated under WW than under the HS treatment, which indicated that WW could mainly induce an increase in free phenolics, such as flavonoids and flavonois.

The growth conditions, along with the genetic background of the crop, is an important factor that determines crop quantity and quality because carbon partitioning to metabolism depends partially on the environmental conditions to which the crops are exposed (Ramakrishna & Ravishankar, 2011). In this study, both WW and DSW treatments were maintained at a same conductivity (2.7 dS m⁻¹), which causes salinity stress on plants. This effect has been shown to increase the synthesis of phenolic compounds in plants (Waśkiewicz, Muzolf-Panek, & Goliński, 2013). In this work, the lettuce grown with the DSW treatment did not exhibit differences in TFC compared to the control; therefore, these results suggest that there could be other factors that cause differences in phytochemicals.

It has been demonstrated that a deficiency of Ca, particularly during cultivation of plants, could induce an increase in secondary metabolites in plants, mainly phenolic compounds, such as synaptic and p-coumaric acids (Chishaki & Horiguchi, 1997). However, the two low-salinity shrimp effluents, WW and DSW, showed similar reduced levels of Ca $(53.7 \pm 2.7 \text{ to } 61.4 \pm 8.9 \text{ mg l}^{-1})$ during the crop culture compared to the control (Ca, 131.2; Table 1), and this effect was not evidenced in the DSW treatment. In addition, the Mg content in WW was higher than that in HS and DSW (Table 1). Kobayashi, Masaoka, and Sato (2005) reported that the Ca content of plants was greatly decreased by excess Mg treatment in rice. Cationic antagonism between K-Mg-Ca, such as a high level of one or more of these nutrients, can result in the decreased uptake of the other elements. Exposure of plants to unfavourable conditions, such as nutrient deficiency, increases the production of reactive oxygen species (ROS). To protect themselves against this oxidative stress, plants employ antioxidant defense systems that activate pathways that lead to the production of phenolic compounds (Gill & Tuteja, 2010). Salinity has a pronounced effect on membrane and lipid peroxidation, resulting in changes to plant physiology, such as osmotic stress, nutrient deficiency and ion toxicity, which results in a high

^{*} HS was elaborated every 2-4 weeks; Statistical analyses were performed by one-way ANOVA.

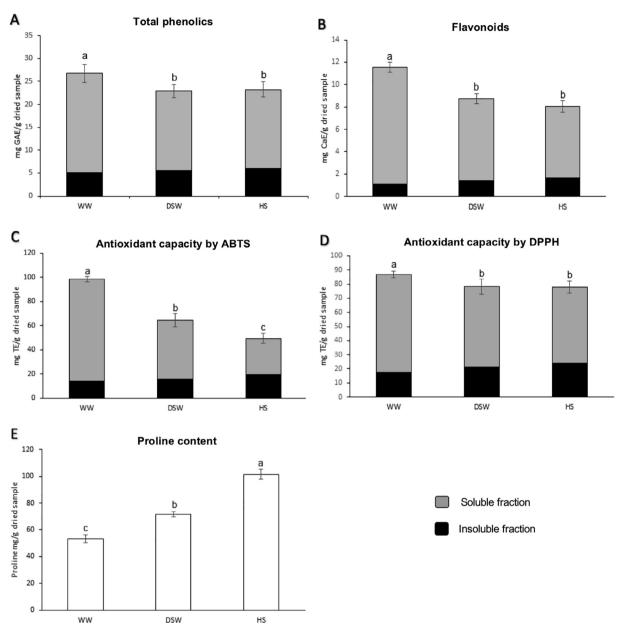


Fig. 2. Phenolic content (mean \pm SD) and antioxidant capacity of lettuce leaves grown with shrimp effluents from WW, DWW and HS. A, total phenolic (expressed as mg gallic acid (GAE)); B, flavonoid (as catechin equivalents (CaE)); C, antioxidant capacity by ABTS (as Trolox equivalents (TE)); D, antioxidant capacity by DPPH (as TE); E, proline. Different letters indicate significant differences (P < 0.05) between treatments. Statistical analyses were performed by one-way Kruskal-Wallis tests.

production of ROS (Munns, 2002). Although the lettuce in the WW treatment had a lower number of leaves and an intermediate weight per lettuce harvested, the WW treatment significantly increased (P < 0.05) the total phenolic compounds and total flavonoids by ~ 15 and $\sim 44\%$, respectively, in the lettuce. Similar results were reported by Garrido et al. (2014) who found a 36% increase in TPC in lettuce exposed to 50 mmol l^{-1} NaCl.

The most common antioxidant assays utilize DPPH and ABTS radicals; both determine the ability of the lettuce examined in this study to donate hydrogen atoms (Pisoschi & Negulescu, 2011). This study showed that the lettuces grown with the two shrimp effluents (WW and DSW) had the ability to inhibit the formation of these radicals (Fig. 2(c) and (d)). These results had a similar trend compared with that exhibited by total flavonoids. The lettuces grown with the WW and DSW shrimp effluent had an approximately ~ 80 and $\sim 60\%$ higher capacity in the soluble fraction to inhibit ABTS formation in comparison to the control plants (HS), whereas in the DPPH assay, the WW-cultivated lettuce

showed an approximately 25% higher antioxidant capacity than the control plants (HS). In contrast, the DSW-cultivated plants did not exhibit significant differences from the HS. Compared to the phenolic compound results, the insoluble fraction showed the opposite effect, showing a higher antioxidant capacity in the control plants than in the WW-cultivated plants (27-30%). Several studies correlate the use of agri-food waste for the production of lettuce with an increased content of phenolic compounds and flavonoids, as well as an increase in the antioxidant capacity by DPPH (e.g., Santos et al., 2016). The antioxidant properties of phenolic compounds are mainly due their ability to act as reducing agents, hydrogen donors, and free radical scavengers (Souza et al., 2011); our results only show such behaviour with respect to DPPH. Bondet, Brand-Williams, and Berset (1997) found that most phenolic compounds react slowly with DPPH, which is sensitive to an acidic pH, whereas ABTS has extra flexibility, and it can be used at different levels of pH, which is useful to explain the effect of pH on antioxidant activity. Furthermore, ABTS is soluble in aqueous and

organic solvents, and it has been shown that it can be inhibited by both hydrophobic and hydrophilic phenolic compounds, whereas DPPH is more easily inhibited by hydrophobic compounds (Pisoschi & Negulescu, 2011).

As mentioned above, the difference in the Mg content between the WW and DSW effluents could have caused Ca and K deficiencies due to ionic antagonism, causing an increase in the content of phenolic compounds that was reflected in a higher antioxidant capacity (Xu & Mou, 2015). An increased consumption of diets rich in phenolic antioxidants may contribute to reducing human diseases, which highlights the interest in the cultivation of foods under conditions that improve the antioxidant content of bioactive phytochemicals.

Proline accumulates in response to a wide range of biotic and abiotic stresses, such as those caused by salinity and heavy metals (Siripornadulsil, Traina, Verma, & Sayre, 2002). Bartha et al. (2015) observed a significant increase in the content of proline of lettuce, subjected to 50 and 100 mM NaCl, respectively. However, the results in our study showed the opposite effect; a decrease of 50 and 30% (Fig. 2) in the proline content for the lettuces from the WW and DSW treatments, respectively, compared that with HS. Shetty (2004) proposed a link between the accumulation of the stress metabolite proline and the energy transferred toward phenylpropanoid biosynthesis via the oxidative pentose phosphate pathway. While a number of proline syntheses are accompanied by the oxidation of NADPH, this enhances the oxidative pentose phosphate pathway, providing precursors for phenolic biosynthesis via the shikimic acid pathway (Sarkar, Bhowmik, Young-In-Kwon, & Shetty, 2011). This mechanism could explain why plants grown with effluents do not have a high content of proline.

4. Conclusions

These results confirm the hypothesis that the lettuce grown with low salinity shrimp effluents shows a quality comparable or better to that grown with a traditional hydroponic solution. Well water (WW) lettuce exhibited higher levels of phenolics and flavonoids, and a higher antioxidant capacity, mainly in the soluble fraction. The lettuce cultivated with diluted seawater (DSW) showed no significant difference in total phenolics and flavonoids with respect to the hydroponic solution (HS) lettuce. Results reveal that the functional properties (antioxidant properties, polyphenols and flavonoid content) are even better in the lettuce produced with WW and DSW shrimp effluents. In contrast, agronomical properties (individual weight, number of leaves, and yield) were found to be better in the case of lettuce grown with the hydroponic solution (control). In the future, it is worthy to conduct research on the performance of growth using other low-salinity shrimp effluents (2–5 g l^{-1}) to evaluate the quality and yield of harvested letfuce.

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Declaration of Competing Interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2019.100027.

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