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Are *Myriophyllum alterniflorum* biomarker responses to arsenic stress differentially affected by hydrodynamic conditions?

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20 **Abstract**

21 Arsenic (As) is a significant contaminant in the environment and its detection through
22 macrophytes can provide a powerful tool. *Myriophyllum alterniflorum* constitutes a good
23 candidate by virtue of its ability to accumulate contaminants, and moreover its biomarkers can
24 respond to the presence of trace metals and metalloids. The objective of this study therefore is to
25 evaluate the watermilfoil response to As exposure under several hydrodynamic conditions since
26 it is well known that hydrodynamics affect plant functioning. For this purpose, fresh watermilfoil
27 plants are subjected to three hydrodynamic conditions, namely laminar, turbulent and calm, in a
28 synthetic medium either enriched or not by 100 $\mu\text{g}\cdot\text{L}^{-1}$ arsenic for 21 days. Growth, pigment
29 content (chlorophyll a, b and carotenoids), respiratory and photosynthetic activities, osmotic
30 potential and hydrogen peroxide concentration are all monitored. Arsenic accumulation is
31 measured separately in the roots and shoots of *Myriophyllum alterniflorum*. On the one hand, it
32 should be noted that arsenic induces: (i) a significant increase in H_2O_2 content; (ii) a decrease in
33 osmotic potential, pigment content, photosynthesis and respiration rates, shoot and root growth;
34 and (iii) an inhibition of shoot branching. Moreover, a higher accumulation of this metalloid in
35 roots than in shoots, regardless of the hydrodynamic condition, is witnessed. While on the other
36 hand, hydrodynamic conditions only affect watermilfoil morphology and arsenic accumulation.
37 Also, the younger and older parts have experienced differential toxic effects. Overall, our results
38 suggest the effective use of *M. alterniflorum* in both water quality biomonitoring and
39 phytoremediation studies.

40 **Keywords:** Alternate watermilfoil, biological and physiological endpoints, arsenic,
41 hydrodynamic conditions, oxidative stress.

43 **Abbreviations:**

44 LQ: limit of quantification

45 MTES: France's Ministry for the Ecological and Solidarity Transition

46 FW: fresh weight

47 DW: dry weight

48 SL: mean total main shoot length

49 RamifL: mean total length of shoot ramifications

50 RL: mean total root length.

51

52 **1- Introduction**

53 Arsenic (As), a metalloid with atomic number 33 and a molar mass of $74.92 \text{ g}\cdot\text{mol}^{-1}$, constitutes
54 nearly 0.00021% of the natural elements of the earth's crust. Its critical concentration in streams
55 is $4.2 \mu\text{g}\cdot\text{L}^{-1}$ (MTES and French Water Agencies, 2003). Arsenic has natural sources like
56 volcanism, wildfires, and the weathering of bedrock regions that contributes to dispersing
57 primary arsenic minerals like arsenopyrite (FeAsS), realgar (AsS) and orpiment (As_2S_3), known
58 to form a geochemical background. The main sources of arsenic are industrial and agricultural
59 uses, including (gold) mining and smelting activities, the combustion of municipal solid wastes
60 and coal fly ash, pharmaceutical wastes, and the use of arsenic pesticides (Laperche *et al.*, 2003;
61 Punshon *et al.*, 2017). Four degrees of oxidation have been identified for As, i.e.: -III, 0, +III,
62 and +V. The toxicity of As compounds decreases as follows: $\text{AsH}_3 \text{ gas} > \text{As} (-\text{III}) > \text{As} (\text{III}) >$
63 $\text{As} (\text{V}) >$ methylated compounds (Sharma and Sohn, 2009). In addition, arsenic can be

64 distinguished in: (i) organic forms, which are mainly arsenobetaine, arsenocholine and mono-
65 and di-methyl derivatives; and (ii) inorganic compounds constituted primarily of arsenite (AsIII)
66 and arsenate (AsV) (Farooq *et al.*, 2016; Singh *et al.*, 2015; Tangahu *et al.*, 2011). The speciation
67 and mobility of arsenic in water are related to both pH and oxido-reduction potential. In natural
68 waters, the main forms are arsenite under reducing conditions in groundwater and arsenate in
69 surface waters (Cullen and Reimer, 1989; Villaescusa and Bollinger, 2008).

70 Arsenic as a non-essential element for plant growth has no specific and selective transporters (Li
71 *et al.*, 2016). Due to the arsenate/phosphate analogy, arsenate uptake in plant cells occurs via the
72 phosphate transport system (Bakhat *et al.*, 2017; Farooq *et al.*, 2016). In aqueous solutions,
73 arsenate, an oxyanion, actually exists in three forms, with corresponding pKa values (2.2, 7.0 and
74 11.5) similar to phosphate pKa (2.1, 7.2 and 12.7) (Elias *et al.*, 2012; Tawfik and Viola, 2011).
75 In plants, arsenic is preferentially accumulated in roots (Mazej and Germ, 2009; Vromman *et al.*,
76 2011; Yabanli *et al.*, 2014). It is well known that As reduces photosynthesis and growth, destroys
77 the chloroplast membrane, reduces chlorophyll synthesis, produces reactive oxygen species
78 (ROS) in leading to deleterious effects in cells (membranes, DNA, photosynthetic pigments) and
79 lastly decreases osmotic potential (Cao *et al.*, 2004; da-Silva *et al.*, 2017; Li *et al.*, 2011; Meharg
80 and Macnair, 1992; Mkandawire and Dudel, 2005; Patra *et al.*, 2004; Rahman *et al.*, 2007b;
81 Robinson *et al.*, 2006; Stoeva *et al.*, 2005; Stoeva and Bineva, 2003; Krayem *et al.*, 2017).

82 The bioindication of water pollution has been using aquatic macrophytes for several decades
83 (Lagadic *et al.*, 1998), and this process became incorporated into the European Water
84 Framework Directive (Aguiar *et al.*, 2014; Delmail, 2014). Aquatic macrophytes may be
85 impacted not only by the toxic effects of xenobiotics (Pollard *et al.*, 2014) but also by other
86 biotic and abiotic factors, such as hydrodynamic conditions (Atapaththu *et al.*, 2015; Atapaththu

87 and Asaeda, 2015) or nutrient enrichment (Krayem *et al.*, 2016a, 2016b). It has been
88 demonstrated that water movement plays a key role since it regulates freshwater macrophyte
89 metabolism and growth (Madsen *et al.*, 2001). Furthermore, mechanical stresses leading to
90 hormonal imbalance, ROS generation or lipoperoxidation can be generated by water movement
91 (Ellawala *et al.*, 2013, 2011). To reinforce the use of macrophyte biomarkers in environmental
92 conditions, the interactions between aquatic plants and these environmental factors must be
93 elucidated.

94 *Myriophyllum alterniflorum*, also known as alternate watermilfoil, is a submerged aquatic
95 macrophyte found in European inland oligotrophic freshwaters. As demonstrated previously,
96 watermilfoil can accumulate metal or metalloid like copper, cadmium and arsenic (Decou *et al.*,
97 2019, 2018; Delmail *et al.*, 2011a, 2011b; Krayem *et al.*, 2016b, 2018); until now however, the
98 effect of hydrodynamics on the physiology of submerged macrophytes has rarely been evaluated
99 (Atapaththu *et al.*, 2015; Atapaththu and Asaeda, 2015). In watermilfoil, arsenic could induce a
100 stress leading to a change in biochemical, physiological and morphological biomarkers. In
101 addition, arsenic stress may be modulated by hydrodynamic conditions affecting this macrophyte
102 in the stream.

103 The aim of this study therefore is to evaluate the combined effect of arsenic stress and
104 hydrodynamics on the response of watermilfoil biomarkers. Moreover, the influence of
105 hydrodynamic conditions on arsenic accumulation is determined and the effects of this
106 accumulation in *Myriophyllum alterniflorum* roots and shoots are investigated.

107 **2. Materials and methods**

108 **2.1 Plant material, growth conditions and acclimation**

109 The *M. alterniflorum* strain was previously established by Delmail *et al.* (2011a). After 30 days
110 of proliferation in Murashige and Skoog medium (Murashige and Skoog, 1962), the plant clones
111 were acclimatized for 21 days in Vienne oligotrophic synthetic medium (Krayem *et al.*, 2016a,
112 2016b, 2018) (the Vienne oligotrophic medium composition is given in the Supplementary
113 Data). Nitrate, ammonium and phosphate concentrations were fixed according to the system of
114 surface water quality evaluation (MTES and French Water Agencies, 2003) at 2 mg.L^{-1} ($32 \text{ }\mu\text{M}$),
115 0.1 mg.L^{-1} ($5.5 \text{ }\mu\text{M}$) and 0.1 mg.L^{-1} ($1.05 \text{ }\mu\text{M}$), respectively. Plant acclimation was performed in
116 a culture box (Steri Vent High Model $80 \times 100 \times 100 \text{ mm}$, KALYS SA) containing 400 mL of
117 sterilized medium (20 min, 121°C in an autoclave (DB-90, Systec)) and maintained in a growth
118 cabinet set at $26^\circ \pm 2^\circ\text{C}$, with a 16-h photoperiod and a light intensity of $13.1 \pm 0.6 \text{ W.cm}^{-2}$
119 (Supra'Lux, Actizoo, 30 W).

120 **2.2 Experimental set-up**

121 In order to simulate environmental conditions, an aquarium system was created with water
122 recirculation (see Fig. 1 in the Supplementary Data). Each aquarium (L=120 cm; W=40 cm,
123 H=49 cm) was filled with 150 L of Vienne oligotrophic synthetic medium (see composition in
124 the Supplementary Data). The treated aquarium was supplemented with $100 \text{ }\mu\text{g.L}^{-1}$ of As (V)
125 $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$ (Normapur, Prolabo 99.9%), while another aquarium with no added arsenic
126 served as the control. A channel (pipe half-sectioned in the lengthwise direction, L = 135 cm; \emptyset
127 = 16.5 cm) was installed over the aquarium. Inside the aquarium, a pump (Laguna power jet,
128 7600 L/h, 75 W) transferred water from the aquarium to the top of the channel, allowing water to
129 cascade into the aquarium at the bottom of the channel. Three distinct hydrodynamic zones were
130 thus created in this system, namely: calm (in the aquarium), turbulent (at the waterfall from the
131 channel into the aquarium), and laminar (in the channel with a water velocity of approx. 3 cm.s^{-1}

132 ¹). In each zone, 35 grams of fresh watermilfoil were exposed. The younger and older parts of
133 watermilfoils shoots were distinguished. For a morphological biomarker analysis, six node
134 explants (six replicates) were affixed onto a Plexiglas square (10 cm x 10 cm) in two
135 hydrodynamic zones, an intermediate zone and a laminar zone, for 19 days. Three independent
136 experiments were conducted and for each experiment, at least three replicates were analyzed.
137 (For more details about the experimental set-up, see Supplementary Data.)

138

139 **2.3 Arsenic monitoring**

140 The As concentrations in plants (roots and shoots) were determined by means of ICP-MS
141 (Agilent Technology, ICP-MS 7700). SLRS-5 (river water reference for trace metals, density =
142 1.0007 g.mL⁻¹) was used as the certified reference for purposes of analysis, with a limit of
143 quantification (LQ) = 0.05 µg As.L⁻¹. 500 mg of fresh watermilfoil were therefore dried at 60°C
144 for 48 h and then digested in 4 mL of a mixture of HNO₃ (Xilab for trace element analysis, 69%)
145 and H₂O₂ (Prolabo, 30%) (50/50 v/v) at 150°C for 2 h, adjusted afterwards to 10 mL with
146 MilliQ-Water and filtered with a 0.2-µm syringe filter (0.2 µm, Whatman). The Bioconcentration
147 Factor (BCF) was calculated by applying Equation 1:

$$148 \quad \text{BCF (L.kg}^{-1}\text{)} = [\text{M}] \text{ plants} / [\text{M}] \text{ water (Eq. 1)}$$

149 where [M] plants is defined as the metal concentration in plants (shoots or roots), in µg.kg⁻¹ FW,
150 and [M] water the metal concentration in water, in µg.L⁻¹. All these calculations were performed
151 on three replicates of plants and water samples in each of the three independent experiments.

152

153 **2.4 Biomarker analysis**

154 **2.4.1 Biochemical biomarkers**

155 **2.4.1.1 Hydrogen peroxide content (H₂O₂)**

156 The H₂O₂ content was measured according to Zhou *et al.* (2006); details are provided in the
157 Supplementary Data.

158 **2.4.1.2 Chlorophyll a, b and carotenoid contents**

159 Pigment quantification was based on the methods implemented by Delmail *et al.* (2011a), with
160 these experimental conditions also being detailed in the Supplementary Data.

161 **2.4.2 Physiological biomarkers**

162 **2.4.2.1 Osmotic potential (Ψ_{scor})**

163 The osmotic potential (Ψ_s) was measured using a vapor pressure osmometer (Micro Osmometer
164 Manual, Löser Messtechnik) and in accordance with the method implemented by Krayem *et al.*
165 (2018). Further details are provided in the Supplementary Data.

166

167 **2.4.2.2 Photosynthetic and respiration rates**

168 For each independent experiment, both photosynthetic and respiration rates were measured on
169 three replicates at 1 and 21 days in an aquarium system using a Clark-type oxygen electrode
170 (DW 1 Oxygen Electrode Chamber, Hansatech Instruments, Ltd.) and by following the method
171 detailed in the Supplementary Data.

172 **2.4.3 Morphological biomarkers**

173 For each independent experiment, six watermilfoil shoots affixed onto Plexiglas squares were
174 monitored in an aquarium for 19 days. Two squares per aquarium were laid out in the
175 intermediate and laminar zones, as previously described in Section 2.2. For each square, the
176 mean total main shoot length (SL), mean total length of shoot ramifications (RamifL) and mean

177 total root length (RL) were measured on plant pictures taken by a Sony camera (14 M pixels)
178 every third day, then analyzed with the Visilog Viewer 6.8 software (NOESIS).

179 **2.5 Statistical analysis**

180 For each independent experiment and for each hydrodynamic zone, our results relied on three
181 replicates (N=3), except for the growth parameters, which were based on six replicates (N=6).
182 Data normality was verified by a kernel density estimation, along with typical Lilliefors and
183 Shapiro tests. The effects of As on pigment content, hydrogen peroxide, osmotic potential and
184 morphology at 21 days were assessed by the mixed ANOVA method, followed by a Sidak test to
185 compare differences between control plants and arsenic-treated plants, as well as between
186 younger and older plant parts. Arsenic accumulation in the various plant parts and the
187 bioconcentration factor (BCF) were both tested by means of the Bonferonni test. All tests were
188 performed at a 5% significance level using the Systat 11 software package for Windows.

189 **3. Results and discussion**

190 **3.1 As internalization in watermilfoil**

191 Arsenic concentrations ranged between 200 and 500 $\mu\text{g}\cdot\text{g}^{-1}$ plant dry weight (DW) in this
192 experiment, while they only reached 150 $\mu\text{g}\cdot\text{g}^{-1}$ DW in a previous box experiment (Krayem *et*
193 *al.*, 2016a) conducted at 21 days of exposure for each system (Table 1). This higher arsenic
194 accumulation in watermilfoil from the aquarium system was mainly due to the higher level of
195 exposure to arsenic. If the arsenic concentration was indeed identical ($100 \mu\text{g}\cdot\text{L}^{-1}$) in the two
196 systems (150-L aquarium and 400-mL boxes), then the biomass/As ratio in the aquarium was 89
197 times higher than in the boxes.

198 In addition, hydrodynamics affected the differential accumulation of arsenic by roots and shoots.
199 A 57%, 57% and 44% higher As accumulation in roots than in shoots was recorded (p-value =

200 0.001) under calm, turbulent and laminar conditions, respectively. These results were also
201 assessed by calculating the BCFs in roots and shoots within each of the three hydrodynamic
202 zones. A 58% and 51% higher BCF (see Table 1) in roots than in shoots was recorded in the
203 calm and turbulent zones, respectively. Similarly, in the laminar zone, a 45% increase in the BCF
204 in roots, compared to shoots, was found. It appears that in roots, As accumulation was highest in
205 the calm zone. An increase in the arsenic content of roots located in the calm zone, by about 8%
206 and 23%, was noted compared to the turbulent and laminar zones, respectively. It could be
207 hypothesized that these differences in As accumulation are correlated with water flow velocity. It
208 could indeed be assumed that changes in water velocity affect plant metabolism and growth, as
209 has been reported by many authors (for a comprehensive review, see Bornette and Puijalon,
210 2011; Franklin *et al.*, 2008). For example, Strand and Weisner (2001) indicated that *M. spicatum*
211 at sheltered sites increase (or at least do not decrease) their below-ground biomass. This greater
212 allocation to below-ground organs could influence absorption and explain the increase in arsenic
213 content observed in our experiment. Moreover, as reported by Franklin *et al.* (2008),
214 photosynthetic and nutrient uptake rates are positively correlated with low water velocity (0 - 0.1
215 m.s⁻¹). Accordingly, our results on *M. alterniflorum* should be explored by further experiments
216 since the influence of flow velocity on aquatic macrophytes is a very complex process. It is well
217 documented that at moderate water flow rates as velocity increases, the diffusive boundary layer
218 around the plant surface erodes more substantially, thus leading to enhanced nutrient and gas
219 fluxes and hence photosynthesis and growth. In contrast, Franklin *et al.* (2008) indicated that *M.*
220 *spicatum* belong to the species most susceptible to increased velocity due to high hydraulic
221 resistance and low anchoring strength. Increasing the velocity actually leads to physical and

222 mechanical stresses due to greater drag forces, which in turn can lead to stem breakage and
223 uprooting.

224

225 In conclusion, a higher flow velocity induces the lowest As accumulation in roots, while in
226 shoots, the As level was practically similar across the three hydrodynamic zones. The BCF was
227 higher in roots than in shoots for all three conditions and higher in the calm zone, whereas the
228 shoot/root ratio was lower in the laminar zone than in the two other zones (1.77 vs. 2.29).
229 Arsenic is a non-essential element for plants, and it is well known that the root endodermis
230 constitutes a barrier to the root-to-shoot translocation of toxic contaminants (Mazhoudi *et al.*,
231 1997). In addition, several studies have pointed to roots as the preferential site of arsenic
232 accumulation in plants (Mazej and Germ, 2009; Vromman *et al.*, 2011; Yabanli *et al.*, 2014). A
233 BCF of 4,000 L.kg⁻¹ (based on dry matter) was observed in the roots of *Hydrilla verticillata* after
234 exposure to 0.15 µg.L⁻¹ of arsenic for 4 days (Xue and Yan, 2011). Mazej and Germ (2009)
235 found a BCF of 1.5 L.kg⁻¹ in the roots, compared with a BCF of 0.045 L.kg⁻¹ in the shoots, of
236 *Najas marina* after a treatment with 10.6 mg/kg of arsenic in the sediment. This reduced root-to-
237 shoot transfer of arsenic may be due to phytochelatin-mediated As vacuolar sequestration in root
238 cells (Gupta *et al.*, 2004; Hasanuzzaman *et al.*, 2015; Zhao *et al.*, 2009).

239 **3.2 Biomarker analysis**

240 **3.2.1 Biochemical biomarkers**

241 **3.2.1.1 H₂O₂ content**

242 H₂O₂ content was not affected by the hydrodynamic conditions in control plants (Fig. 1). When
243 comparing young and old parts, as opposed to As-treated plants, the control plants did not
244 display any significant difference (p-value = 0.154). At 21 days, in the As-treated plants, a

245 significant increase in H₂O₂ content of 96%, 106% and 100% (p-value = 0.001) in the young
246 parts and 85%, 140% and 83% (p-value = 0.004) in the old parts of As-exposed plants, compared
247 to the control specimens, were observed for the calm, turbulent and laminar zones, respectively
248 (Fig. 1). Arsenic toxicity appears on the tissues of both young and old parts, generating an
249 oxidative stress characterized by the formation of hydrogen peroxide (Hasanuzzaman *et al.*,
250 2015; Mallick *et al.*, 2011).

251 In contrast, at 21 days in As-treated plants, a significant increase in H₂O₂ content of 28%, 49%
252 and 29% (p-value = 0.049) is observed in the older parts for the calm, turbulent and laminar
253 zones, respectively (Fig. 1), as compared to the younger parts. Similarly, a higher As
254 accumulation in older than younger fronds of the terrestrial plant *Pteris vittata* could be observed
255 (7.575 µgAs.g⁻¹ DW / 4.893 µgAs.g⁻¹ DW) after 12 weeks of cultivation in 97 µgAs.g⁻¹ soil. This
256 differential accumulation appears to produce an As detoxification mechanism as older senescent
257 leaves fall (Zhang *et al.*, 2002). Bouazizi *et al.* (2010) discovered that, unlike in older leaves,
258 hydrogen peroxide content remained stable in the young leaves of *Phaseolus vulgaris* watered
259 with 3.2 mg.L⁻¹ Cu, thus suggesting a greater stimulation of peroxidase activity in older rather
260 than younger tissues. Along the same lines, our results have suggested a higher oxidative stress
261 in older shoots. This differential effect of As on older parts could be explained by the increased
262 activity of the enzymatic antioxidant system (superoxide dismutase, ascorbate peroxidase,
263 peroxidase) in younger parts, contributing to ROS limitation in order to protect young tissues
264 from ROS degradation, i.e. a phenomenon previously observed in several species in response to
265 various trace elements (da-Silva *et al.*, 2017; Hasanuzzaman *et al.*, 2015; Delmail and Labrousse,
266 2012). Another aspect could be the preferential As sequestration in old leaves to protect the
267 younger ones.

268

3.2.1.2 Pigment content

269 Pigment levels were not affected by the hydrodynamic conditions in control plants (Fig. 2). In
270 As-treated plants, a significant decrease in pigment content, equal to 22%, 29% and 22% for Chl
271 a (p-value = 0.026) (Fig. 2) and 31%, 23%, and 10% for Chl b (p-value = 0.048), was observed
272 (Fig. 2) in the younger parts for the calm, turbulent and laminar zones, respectively. This
273 reduction reached 22%, 24% and 18% for Chl a (p-value = 0.026) (Fig. 2) in the older parts
274 compared to the control for the calm, turbulent and laminar zones, respectively. On the other
275 hand, chlorophyll b in the older parts of the As-treated plants did not change significantly (p-
276 value = 0.091) over time compared to the controls for the three hydrodynamic zones,
277 respectively (Fig. 2). In contrast, the carotenoid concentration (Fig. 2) did not differ significantly
278 in the young shoots vs. the control (p-value = 0.387), whereas in old shoots a significant increase
279 of approximately 25% (p-value = 0.014) was observed for the calm, turbulent and laminar zones.
280 Arsenic therefore caused a decrease of Chl a and b, while the carotenoids only increased in the
281 older parts. Duman *et al.* (2010) found in *Lemna minor* a decrease in chlorophyll pigment
282 content during arsenic stress (0.079-5.056 mg.L⁻¹ As (V) in 400-mL Hoagland medium over 6
283 days). Similarly, Ozturk *et al.* (2010) demonstrated this same feature in *Nasturtium officinale*
284 (after exposing 4 g of plants to doses of 0.79 mg.L⁻¹ to 3.9 mg.L⁻¹ of As(III) in 400 mL of
285 Hoagland medium for 7 days). Srivastava *et al.* (2013) indicated that in *Hydrilla verticillata*,
286 exposure to 37.5 mg.L⁻¹ As for 96 hours induced a 24% decrease in chlorophyll content.
287 The increase in carotenoid content observed in this study may however reflect one of the non-
288 enzymatic defense mechanisms adopted by the plant to protect its chlorophyll pigments from
289 oxidative degradation (Ferrat *et al.*, 2003). In contrast, a 2-mg.L⁻¹ arsenate exposure on two
290 aquatic species, *Vallisneria gigantea* and *Azolla filliculoides*, induced an increase of fluorescence

291 in the range of 400 to 500 nm; this finding was correlated with an increase in the biosynthesis of
292 carotenoids and flavonoids, i.e. two compounds occurring in the arsenic defense system (Iriel *et*
293 *al.*, 2015).

294 When comparing older and younger parts of watermilfoil under each hydrodynamic condition, a
295 decrease of 26%, 38% and 38% (p-value = 0.002) for Chl a and 45%, 39%, and 46% (p-value =
296 0.009) for Chl b were observed in the older parts relative to the younger parts of the control
297 plants in the calm, turbulent and laminar zones, respectively (Fig. 2). Similarly, in As-treated
298 plants, a reduction of 26%, 33% and 35% for Chl a (p-value = 0.005) (Fig. 2) and 37%, 47% and
299 46% (p-value = 0.003) for Chl b (Fig. 2) had also been recorded. Alternatively, the carotenoid
300 contents remained stable in both the control specimens (p-value = 0.959) and As-treated plants
301 (p-value = 0.086) regardless of the hydrodynamic condition. Therefore, the As effect on older
302 parts was greater than on younger ones, especially for chlorophylls a and b. The decrease in
303 photosynthetic pigment contents could be due to: (i) lipid peroxidation by ROS and chloroplast
304 membrane degradation (Upadhyay and Panda, 2009), (ii) chlorophyll degradation by
305 chlorophyllase (Sharma and Dubey, 2005), or (iii) acceleration of a tissue aging process called
306 "senescence" resulting in a decrease in pigment content due to resource remobilization (Delmail
307 and Labrousse, 2012). The stability of the carotenoid contents in the younger parts and their
308 increase in the older parts is reflected by the major role these contents play in defending and
309 protecting the other pigments from oxidative degradation (Ferrat *et al.*, 2003).

310 **3.2.2 Physiological biomarkers**

311 **3.2.2.1 Osmotic potential**

312 The hydrodynamic conditions did not affect the osmotic potential of control plants (p-value =
313 0.316). In addition, no significant difference was observed between younger and older parts in
314 the control specimens (p-value = 0.416) (Fig. 3).

315 However, in As-treated plants (Fig. 3), significant decreases in osmotic potential of 37%, 19%
316 and 22% in the younger parts (p-value = 0.002) and 26%, 24% and 26% in the older parts (p-
317 value = 0.002) were observed for the calm, turbulent and laminar zones, respectively (Fig. 3).

318 The exposure of plants to arsenic can in fact cause an increase in the protein, soluble sugar,
319 proline and glycine-betaine contents in vacuoles (Hasanuzzaman *et al.*, 2015). This accumulation
320 leads to an increase in solute concentrations and, consequently, contributes to decreased osmotic
321 potential (Garg and Singla, 2011; Hasanuzzaman *et al.*, 2015; Stoeva *et al.*, 2005; Yabanli *et al.*,
322 2014). Tripathi *et al.* (2014) studied the effect of increasing concentrations of As (V) from 0 to
323 18.5 mg.L⁻¹ on the macrophyte *Najas indica* by measuring the amino acid concentration. They
324 found that the total concentration of amino acids and proteins increased by 54% and 42%
325 respectively after exposure to 18.5 mg.L⁻¹ of As over 2 days. This increase in the concentration
326 of amino acids and proteins in plant cells could induce a decrease in osmotic potential and
327 moreover explains the result obtained in this study. In addition, Srivastava *et al.* (2013) showed
328 that *Hydrilla verticillata* reduced its water use and increased its transpiration during As exposure
329 (37.5 mg.L⁻¹ and after 96 hours). This reduction led to an increased solute concentration and
330 hence a decrease in osmotic potential. Also, this result is similar to the observation by Delmail *et*
331 *al.* (2011a) highlighting a 27% and 65% decrease in the osmotic potential of younger and older
332 leaves of *Myriophyllum alterniflorum* during a 30-day exposure to 100 µg Cu.L⁻¹. Moreover,
333 Krayem *et al.* (2018) reported a decrease in the osmotic potential of 22%, 16% and 20% in
334 younger parts (p-value = 0.008) and of 22%, 18%, and 17% in older parts (p-value = 0.008)

335 compared to the control specimens in the calm, turbulent and laminar zones, respectively, in
336 *Myriophyllum alterniflorum* plants after exposure to 100 $\mu\text{g}\cdot\text{Cu}\cdot\text{L}^{-1}$.

337 No significant difference between young and old parts was found for either the control group (p-
338 value = 0.416) or the As-treated plants (p-value = 0.420). In addition, there was no significant
339 difference in osmotic potential across the three zones and between the control and As-treated
340 plants (p-value = 0.388). Under our experimental conditions therefore, the effect of arsenic did
341 not vary between the older and younger parts of watermilfoil. This result differs from the 38%
342 decrease in osmotic potential observed in older leaves of *Myriophyllum alterniflorum*, compared
343 to younger leaves, at 30 days of exposure to 100 $\mu\text{g}\cdot\text{Cu}\cdot\text{L}^{-1}$, as reported by Delmail *et al.* (2011a),
344 whose medium differed from ours, particularly in nitrates and phosphates, which could explain
345 this discrepancy.

346 **3.2.2.2 Photosynthetic and respiratory activities**

347 No significant effect of hydrodynamic conditions on respiration and net photosynthesis (NP) in
348 the control plants was observed (p-values = 0.138 and 0.855, respectively) (Fig. 4). In the As-
349 treated plants however, a significant decrease in respiration, ranging from 30% to 50% (p-value
350 = 0.001), and in photosynthesis, ranging from 30% to 40% (p-value = 0.001), was recorded on
351 Day 1 and Day 21, respectively, within the three zones (Fig. 4).

352 The As toxicity clearly induced a response of watermilfoil biochemical and physiological
353 biomarkers. Exposure to 143 $\mu\text{g}\cdot\text{As}\cdot\text{g}^{-1}\cdot\text{FW}$ led to a decrease in respiration and NP. The oxidative
354 stress generated by arsenic can indeed destroy thylakoid membranes, thus reducing pigment
355 contents and affecting the photosystem II (Prasad *et al.*, 2001). Stoeva and Bineva (2003)
356 showed that arsenic can induce chloroplast membrane destruction, leading to decreased
357 photosynthesis. In *Hydrilla verticillata*, a 32 $\text{mg}\cdot\text{L}^{-1}$ As exposure for 96 h induced a decrease in

358 net photosynthesis and in Chl a, Chl b and Car contents (Srivastava *et al.*, 2013). This reduction
359 could be due to: (i) chlorophyll degradation by ROS, (ii) a reduction in photosystem II
360 efficiency, and/or (iii) a dysfunction of the thylakoid electron transport chain (Duman *et al.*,
361 2010; Hasanuzzaman *et al.*, 2015; Rahman *et al.*, 2007a). Furthermore, arsenic and especially As
362 (V) can form a complex with adenosine diphosphate by means of phosphate substitution. ADP-
363 As (V) inhibits the synthesis of the adenosine triphosphate (ATP) necessary for respiration in the
364 mitochondrial electron transport chain as well as for photosynthesis in the thylakoid electron
365 transport chain. Such a mechanism leads to a reduction of respiratory and photosynthetic
366 activities in plant cells (Elias *et al.*, 2012; Finnegan and Chen, 2012).

367 **3.2.3 Morphological biomarkers**

368 In As-treated plants, a significant decrease in shoot length (SL) (Fig. 5a) by 15% and 16% (p-
369 value = 0.0260) at 19 days was observed for the intermediate and laminar zones, respectively,
370 compared to the control plants. Similarly, RL (Fig. 5b) decreased by 35% and 52% (p-value =
371 0.005) at T = 19 days of As exposure for the intermediate and laminar zones, respectively,
372 relative to the control plants. This significant reduction in stem and root lengths reflects As
373 toxicity (Hasanuzzaman *et al.*, 2015). Gusman *et al.* (2013) observed a 38% decrease in *Lactuca*
374 *sativa* leaf length and a 50% decrease in root length after exposure to 3.4 mg.L⁻¹ As. Liu *et al.*
375 (2005) found a reduction in the length of the main stem and root of 3.9% and 8.6%, respectively,
376 after treating *Triticum aestivum* with 0.5 mg.L⁻¹ As. In *Pisum sativum* after 24 h of exposure to
377 0.2 mg.L⁻¹ As (V), Dho *et al.* (2010) observed that the root growth and mitotic index decreased
378 by 42% and 20%, respectively. This reduction in meristematic activity was correlated with ROS
379 production affecting root cells mitosis. In addition, in *Oryza sativa* exposed to 7.8 and 15.6
380 mg.L⁻¹ As for 24 h, Singh *et al.* (2009) recorded a 23% and 37% decrease in root length; these

381 authors also noted a 81% and 30% increase in peroxy radical $O_2^{\cdot-}$ and hydrogen peroxide at
382 15.6 mg.L^{-1} As. As previously observed in the literature, oxidative stress due to As could thus
383 occur in roots, decreasing root length after an exposure to $100 \mu\text{g As (V).L}^{-1}$.

384 No shoot branching was observed in control and As-treated plants in both hydrodynamic zones.

385 In addition, a significant decrease in SL (p-value = 0.05) and RL (p-value = 0.001) was noted in
386 plants from the intermediate zone as compared to the laminar zone (Figs. 5a, b). As previously
387 pointed out, flow velocity exerts a significant effect on macrophyte growth, from a stimulating
388 effect at low and moderate velocities to an inhibiting effect at higher velocities; moreover, water
389 movement could generate physical and mechanical stresses, in inducing morphological changes
390 like dwarfed growth form (Bornette and Puijalon, 2011; Franklin *et al.*, 2008; Madsen *et al.*,
391 2001). It should be noted that water turbulence can affect growth more drastically than laminar
392 flow, as demonstrated in *Elodea nuttallii* by Atapaththu and Asaeda (2015). Water turbulence
393 can induce chlorosis, plasmolysis in leaf cells, and the accumulation of organic acids (like
394 citrate, oxalate and ascorbate) and amino acids, which could interfere with cell metabolism and
395 inhibit plant growth (Atapaththu *et al.*, 2015; Atapaththu and Asaeda, 2015). Lastly, these
396 authors also reported ultrastructural changes, such as starch granule accumulation in chloroplasts
397 and cell wall thickening linked to cellulose and lignin accumulation.

398

399 **Conclusion**

400 In our study, hydrodynamics did not influence the response of the watermilfoil biomarkers
401 tested, except for the growth parameters; this response of several aquatic macrophytes species to
402 water movement is well known since water velocities and turbulence induce mechanical stress

403 and lead to a decline in plant growth. Hydrodynamics did however affect arsenic accumulation
404 and acted differentially on the roots and shoots. The arsenic concentration observed in roots was
405 higher than that in shoots, most likely resulting from a mixed response of water velocity inducing
406 a below-ground biomass accumulation along with stress-inducing As sequestration in the
407 vacuoles of root cells. In this manner, the watermilfoil protected its shoots from As toxicity, and
408 particularly the younger parts since As accumulation and toxicity biomarker responses are higher
409 in the older parts.

410 Further studies are needed to determine the exact contribution of flow velocity, water turbulence
411 and trace element stress on aquatic macrophyte biomarkers.

412

413

414

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423

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425 **References**

- 426 Aguiar, F.C., Segurado, P., Urbanič, G., Cambra, J., Chauvin, C., Ciadamidaro, S., Dörflinger,
427 G., Ferreira, J., Germ, M., Manolaki, P., Minciardi, M.R., Munné, A., Papastergiadou, E.,
428 Ferreira, M.T., 2014. Comparability of river quality assessment using macrophytes: A
429 multi-step procedure to overcome biogeographical differences. *Sci. Total Environ.* 476–
430 477, 757–767. <https://doi.org/10.1016/j.scitotenv.2013.10.021>
- 431 Atapaththu, K.S.S., Asaeda, T., 2015. Growth and stress responses of Nuttall's waterweed
432 *Elodea nuttallii* (Planch) St. John to water movements. *Hydrobiologia* 747, 217–233.
433 <https://doi.org/10.1007/s10750-014-2141-9>
- 434 Atapaththu, K.S.S., Miyagi, A., Atsuzawa, K., Kaneko, Y., Kawai-Yamada, M., Asaeda, T.,
435 2015. Effects of water turbulence on variations in cell ultrastructure and metabolism of
436 amino acids in the submersed macrophyte, *Elodea nuttallii* (Planch.) H. St. John. *Plant*
437 *Biol.* 17, 997–1004. <https://doi.org/10.1111/plb.12346>
- 438 Bakhat, H.F., Zia, Z., Fahad, S., Abbas, S., Hammad, H.M., Shahzad, A.N., Abbas, F., Alharby,
439 H., Shahid, M., 2017. Arsenic uptake, accumulation and toxicity in rice plants: Possible
440 remedies for its detoxification: A review. *Environ. Sci. Pollut. Res.* 24, 9142–9158.
441 <https://doi.org/10.1007/s11356-017-8462-2>
- 442 Bornette, G., Puijalón, S., 2011. Response of aquatic plants to abiotic factors: a review. *Aquat.*
443 *Sci.* 73, 1–14. <https://doi.org/10.1007/s00027-010-0162-7>
- 444 Bouazizi, H., Jouili, H., Geitmann, A., El Ferjani, E., 2010. Copper toxicity in expanding leaves
445 of *Phaseolus vulgaris* L.: antioxidant enzyme response and nutrient element uptake.
446 *Ecotoxicol. Environ. Saf.* 73, 1304–1308. <https://doi.org/10.1016/j.ecoenv.2010.05.014>

447 Cao, X., Ma, L.Q., Tu, C., 2004. Antioxidative responses to arsenic in the arsenic-
448 hyperaccumulator Chinese brake fern (*Pteris vittata* L.). Environ. Pollut. 128, 317–325.
449 <https://doi.org/10.1016/j.envpol.2003.09.018>

450 Cullen, W.R., Reimer, K.J., 1989. Arsenic speciation in the environment. Chem. Rev. 89, 713–
451 764. <https://doi.org/10.1021/cr00094a002>

452 da-Silva, C.J., Canatto, R.A., Cardoso, A.A., Ribeiro, C., Oliveira, J.A., 2017. Arsenic-
453 hyperaccumulation and antioxidant system in the aquatic macrophyte *Spirodela*
454 *intermedia* W. Koch (Lemnaceae). Theor. Exp. Plant Physiol. 29, 203–213.
455 <https://doi.org/10.1007/s40626-017-0096-8>

456 Decou, R., Bigot, S., Hourdin, P., Delmail, D., Labrousse, P., 2019. Comparative *in vitro/in situ*
457 approaches to three biomarker responses of *Myriophyllum alterniflorum* exposed to metal
458 stress. Chemosphere 222:29-37. <https://doi.org/10.1016/j.chemosphere.2019.01.105>

459 Decou, R., Laloi, G., Zouari, M., Labrousse, P., Delmail, D., 2018. Evaluation of the Relevance
460 of *Myriophyllum alterniflorum* (Haloragaceae) Cadmium-Sensitive Biomarkers for
461 Ecotoxicological Surveys. Bull Environ Contam Toxicol. 101 (4):458-466
462 <https://doi.org/10.1007/s00128-018-2433-2>

463 Delmail, D., 2014. Risk management of European inland waters using macrophyte
464 biomonitoring. Front. Environ. Sci. 2. <https://doi.org/10.3389/fenvs.2014.00031>

465 Delmail, D., Labrousse, P., 2012. Plant ageing, a counteracting agent to xenobiotic stress, in:
466 Nagata, T. (Ed.), Senescence. InTech. <https://doi.org/10.5772/31944>

467 Delmail, D., Labrousse, P., Hourdin, P., Larcher, L., Moesch, C., Botineau, M., 2011a.
468 Differential responses of *Myriophyllum alterniflorum* DC (Haloragaceae) organs to

469 copper: physiological and developmental approaches. *Hydrobiologia* 664, 95–105.
470 <https://doi.org/10.1007/s10750-010-0589-9>

471 Delmail, D., Labrousse, P., Hourdin, P., Larcher, L., Moesch, C., Botineau, M., 2011b.
472 Physiological, anatomical and phenotypical effects of a cadmium stress in different-aged
473 chlorophyllian organs of *Myriophyllum alterniflorum* DC (Haloragaceae). *Environ. Exp.*
474 *Bot.* 72, 174–181. <https://doi.org/10.1016/j.envexpbot.2011.03.004>

475 Delmail, D., Labrousse, P., Hourdin, P., Larcher, L., Moesch, C., Botineau, M., 2011c.
476 Differential responses of *Myriophyllum alterniflorum* DC (Haloragaceae) organs to
477 copper: physiological and developmental approaches. *Hydrobiologia* 664, 95–105.
478 <https://doi.org/10.1007/s10750-010-0589-9>

479 Dho, S., Camusso, W., Mucciarelli, M., Fusconi, A., 2010. Arsenate toxicity on the apices of
480 *Pisum sativum* L. seedling roots: Effects on mitotic activity, chromatin integrity and
481 microtubules. *Environ. Exp. Bot.* 69, 17–23.
482 <https://doi.org/10.1016/j.envexpbot.2010.02.010>

483 Duman, F., Ozturk, F., Aydin, Z., 2010. Biological responses of duckweed (*Lemna minor* L.)
484 exposed to the inorganic arsenic species As(III) and As(V): effects of concentration and
485 duration of exposure. *Ecotoxicology* 19, 983–993. [https://doi.org/10.1007/s10646-010-](https://doi.org/10.1007/s10646-010-0480-5)
486 [0480-5](https://doi.org/10.1007/s10646-010-0480-5)

487 Elias, M., Wellner, A., Goldin-Azulay, K., Chabriere, E., Vorholt, J.A., Erb, T.J., Tawfik, D.S.,
488 2012. The molecular basis of phosphate discrimination in arsenate-rich environments.
489 *Nature* 491, 134–137. <https://doi.org/10.1038/nature11517>

- 490 Ellawala, C., Asaeda, T., Kawamura, K., 2013. Water movement induced variations in growth
491 regulation and metabolism of freshwater macrophyte *Vallisneria spiralis* L. in early
492 growth stages. *Hydrobiologia* 709, 173–182. <https://doi.org/10.1007/s10750-013-1447-3>
- 493 Ellawala, C., Asaeda, T., Kawamura, K., 2011. Influence of flow turbulence on growth and
494 indole acetic acid and H₂O₂ metabolism of three aquatic macrophyte species. *Aquat.*
495 *Ecol.* 45, 417–426. <https://doi.org/10.1007/s10452-011-9364-x>
- 496 Farooq, M.A., Islam, F., Ali, B., Najeeb, U., Mao, B., Gill, R.A., Yan, G., Siddique, K.H.M.,
497 Zhou, W., 2016. Arsenic toxicity in plants: Cellular and molecular mechanisms of its
498 transport and metabolism. *Environ. Exp. Bot.* 132, 42–52.
499 <https://doi.org/10.1016/j.envexpbot.2016.08.004>
- 500 Ferrat, L., Pergent-Martini, C., Roméo, M., 2003. Assessment of the use of biomarkers in aquatic
501 plants for the evaluation of environmental quality: application to seagrasses. *Aquat.*
502 *Toxicol.* 65, 187–204. [https://doi.org/10.1016/S0166-445X\(03\)00133-4](https://doi.org/10.1016/S0166-445X(03)00133-4)
- 503 Finnegan, P.M., Chen, W., 2012. Arsenic Toxicity: The Effects on Plant Metabolism. *Front.*
504 *Physiol.* 3. <https://doi.org/10.3389/fphys.2012.00182>
- 505 Franklin, P., Dunbar, M., Whitehead, P., 2008. Flow controls on lowland river macrophytes: A
506 review. *Sci. Total Environ.* 400, 369–378. <https://doi.org/10.1016/j.scitotenv.2008.06.018>
- 507 Garg, N., Singla, P., 2011. Arsenic toxicity in crop plants: physiological effects and tolerance
508 mechanisms. *Environ. Chem. Lett.* 9, 303–321. [https://doi.org/10.1007/s10311-011-0313-](https://doi.org/10.1007/s10311-011-0313-7)
509 7
- 510 Gupta, D.K., Tohoyama, H., Joho, M., Inouhe, M., 2004. Changes in the levels of phytochelatin
511 and related metal-binding peptides in chickpea seedlings exposed to arsenic and different
512 heavy metal ions. *J. Plant Res.* 117, 253–256. <https://doi.org/10.1007/s10265-004-0152-8>

513 Gusman, G.S., Oliveira, J.A., Farnese, F.S., Cambraia, J., 2013. Arsenate and arsenite: the toxic
514 effects on photosynthesis and growth of lettuce plants. *Acta Physiol. Plant.* 35, 1201–
515 1209. <https://doi.org/10.1007/s11738-012-1159-8>

516 Hasanuzzaman, M., Nahar, K., Hakeem, K.R., Öztürk, M., Fujita, M., 2015. Arsenic toxicity in
517 plants and possible remediation, in: *soil remediation and plants*. Elsevier, pp. 433–501.

518 Iriel, A., Dundas, G., Fernández Cirelli, A., Lagorio, M.G., 2015. Effect of arsenic on reflectance
519 spectra and chlorophyll fluorescence of aquatic plants. *Chemosphere* 119, 697–703.
520 <https://doi.org/10.1016/j.chemosphere.2014.07.066>

521 Krayem, Baydoun, M., Deluchat, V., Lenain, J.-F., Kazpard, V., Labrousse, P., 2016a.
522 Absorption and translocation of copper and arsenic in an aquatic macrophyte
523 *Myriophyllum alterniflorum* DC. in oligotrophic and eutrophic conditions. *Environ. Sci.*
524 *Pollut. Res.* 1–8. <https://doi.org/10.1007/s11356-016-6289-x>

525 Krayem, Deluchat, V., Rabiet, M., Cleries, K., Lenain, J.F., Saad, Z., Kazpard, V., Labrousse, P.,
526 2016b. Effect of arsenate As (V) on the biomarkers of *Myriophyllum alterniflorum* in
527 oligotrophic and eutrophic conditions. *Chemosphere* 147, 131–137.
528 <https://doi.org/10.1016/j.chemosphere.2015.12.093>

529 Krayem M., Deluchat V., Decou R., and Labrousse P, 2017. The arsenic in aquatic
530 environments: the plant's point of view. Chapter 1 in *Arsenic: risks of exposure, behavior*
531 *in the environment, and toxicology*. Knežević R, editor. Hauppauge, New York: Nova
532 Science Publishers, Inc; 2017. (Chemistry research and applications).

533 Krayem, M., Deluchat, V., Hourdin, P., Fondanèche, P., Lecavelier Des Etangs, F., Kazpard, V.,
534 Moesch, C., Labrousse, P., 2018. Combined effect of copper and hydrodynamic

535 conditions on *Myriophyllum alterniflorum* biomarkers. *Chemosphere* 199, 427–434.
536 <https://doi.org/10.1016/j.chemosphere.2018.02.050>

537 Lagadic, L., Caquet, Th, Amiard, JC, Ramade, F, 1998. Utilisation de biomarqueurs pour la
538 surveillance de la qualité de l'environnement [Use of biomarkers for monitoring the
539 quality of the environment.]. Tec & Doc Lavoisier.

540 Laperche, V., Bodéan, F., Dictor, M.C., Baranger, P., 2003. Guide méthodologique de l'arsenic,
541 appliqué à la gestion des sites et sols pollués. BRGM/RP-52066-FR, 90 p., 5 fig., 10 tabl.,
542 3 ann [Methodological guide to arsenic, applied to the management of polluted sites and
543 soils. BRGM / RP-52066-EN, 90 p., 5 fig., 10 tabl., 3 ann] [WWW Document]. URL
544 <http://infoterre.brgm.fr/rapports/RP-52066-FR.pdf> (accessed 12.23.16).

545 Li, H., Ye, Z.H., Wei, Z.J., Wong, M.H., 2011. Root porosity and radial oxygen loss related to
546 arsenic tolerance and uptake in wetland plants. *Environ. Pollut.* 159, 30–37.
547 <https://doi.org/10.1016/j.envpol.2010.09.031>

548 Li, N., Wang, J., Song, W.-Y., 2016. Arsenic Uptake and Translocation in Plants. *Plant Cell*
549 *Physiol.* 57, 4–13. <https://doi.org/10.1093/pcp/pcv143>

550 Liu, X., Zhang, S., Shan, X., Zhu, Y.-G., 2005. Toxicity of arsenate and arsenite on germination,
551 seedling growth and amylolytic activity of wheat. *Chemosphere* 61, 293–301.
552 <https://doi.org/10.1016/j.chemosphere.2005.01.088>

553 Madsen, J.D., Chambers, P.A., James, W.F., Koch, E.W., Westlake, D.F., 2001. The interaction
554 between water movement, sediment dynamics and submersed macrophytes.
555 *Hydrobiologia* 444, 71–84. <https://doi.org/10.1023/A:1017520800568>

556 Mallick, S., Sinam, G., Sinha, S., 2011. Study on arsenate tolerant and sensitive cultivars of *Zea*
557 *mays* L.: Differential detoxification mechanism and effect on nutrients status. *Ecotoxicol.*
558 *Environ. Saf.* 74, 1316–1324. <https://doi.org/10.1016/j.ecoenv.2011.02.012>

559 Mazej, Z., Germ, M., 2009. Trace element accumulation and distribution in four aquatic
560 macrophytes. *Chemosphere* 74, 642–647.
561 <https://doi.org/10.1016/j.chemosphere.2008.10.019>

562 Mazhoudi, S., Chaoui, A., Ghorbal, M.H., El Ferjani, E., 1997. Response of antioxidant enzymes
563 to excess copper in tomato (*Lycopersicon esculentum*, Mill.). *Plant Sci.* 127, 129–137.

564 Meharg, A.A., Macnair, M.R., 1992. Suppression of the high affinity phosphate uptake system: a
565 mechanism of arsenate tolerance in *Holcus lanatus* L. *J. Exp. Bot.* 43, 519–524.

566 Mkandawire, M., Dudel, E.G., 2005. Accumulation of arsenic in *Lemna gibba* L. (duckweed) in
567 tailing waters of two abandoned uranium mining sites in Saxony, Germany. *Sci. Total*
568 *Environ.* 336, 81–89. <https://doi.org/10.1016/j.scitotenv.2004.06.002>

569 MTES et Agences de l'eau, 2003. Système d'évaluation de la qualité de l'eau des cours d'eau
570 SEQ-Eau, Grilles d'évaluation version 2,21 mars 2003 [Water quality assessment system
571 for watercourses SEQ-Water, Evaluation grids version 2, 21 March 2003] [WWW
572 Document]. URL [http://www.observatoire-eau-bretagne.fr/Ressources-et-](http://www.observatoire-eau-bretagne.fr/Ressources-et-documentation/Documents-de-planification/Systeme-d-evaluation-de-la-qualite-de-l-eau-des-cours-d-eau-SEQ-Eau)
573 [documentation/Documents-de-planification/Systeme-d-evaluation-de-la-qualite-de-l-eau-](http://www.observatoire-eau-bretagne.fr/Ressources-et-documentation/Documents-de-planification/Systeme-d-evaluation-de-la-qualite-de-l-eau-des-cours-d-eau-SEQ-Eau)
574 [des-cours-d-eau-SEQ-Eau](http://www.observatoire-eau-bretagne.fr/Ressources-et-documentation/Documents-de-planification/Systeme-d-evaluation-de-la-qualite-de-l-eau-des-cours-d-eau-SEQ-Eau) (accessed 12.23.16).

575 Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bio assays with tobacco
576 tissue cultures. *Physiol. Plant.* 15, 473–497. [https://doi.org/10.1111/j.1399-](https://doi.org/10.1111/j.1399-3054.1962.tb08052.x)
577 [3054.1962.tb08052.x](https://doi.org/10.1111/j.1399-3054.1962.tb08052.x)

578 Patra, M., Bhowmik, N., Bandopadhyay, B., Sharma, A., 2004. Comparison of mercury, lead and
579 arsenic with respect to genotoxic effects on plant systems and the development of genetic
580 tolerance. Environ. Exp. Bot. 52, 199–223.
581 <https://doi.org/10.1016/j.envexpbot.2004.02.009>

582 Pollard, A.J., Reeves, R.D., Baker, A.J.M., 2014. Facultative hyperaccumulation of heavy metals
583 and metalloids. Plant Sci. 217–218, 8–17. <https://doi.org/10.1016/j.plantsci.2013.11.011>

584 Prasad, M.N.V., Malec, P., Waloszek, A., Bojko, M., Strzałka, K., 2001. Physiological responses
585 of *Lemna trisulca* L. (duckweed) to cadmium and copper bioaccumulation. Plant Sci.
586 161, 881–889. [https://doi.org/10.1016/S0168-9452\(01\)00478-2](https://doi.org/10.1016/S0168-9452(01)00478-2)

587 Punshon, T., Jackson, B.P., Meharg, A.A., Warczak, T., Scheckel, K., Guerinot, M.L., 2017.
588 Understanding arsenic dynamics in agronomic systems to predict and prevent uptake by
589 crop plants. Sci. Total Environ. 581–582, 209–220.
590 <https://doi.org/10.1016/j.scitotenv.2016.12.111>

591 Rahman, M.A., Hasegawa, H., Mahfuzur Rahman, M., Nazrul Islam, M., Majid Miah, M.A.,
592 Tasmen, A., 2007a. Effect of arsenic on photosynthesis, growth and yield of five widely
593 cultivated rice (*Oryza sativa* L.) varieties in Bangladesh. Chemosphere 67, 1072–1079.
594 <https://doi.org/10.1016/j.chemosphere.2006.11.061>

595 Rahman, M.A., Hasegawa, H., Ueda, K., Maki, T., Okumura, C., Rahman, M.M., 2007b. Arsenic
596 accumulation in duckweed (*Spirodela polyrhiza* L.): A good option for phytoremediation.
597 Chemosphere 69, 493–499. <https://doi.org/10.1016/j.chemosphere.2007.04.019>

598 Robinson, B., Kim, N., Marchetti, M., Moni, C., Schroeter, L., van den Dijssel, C., Milne, G.,
599 Clothier, B., 2006. Arsenic hyperaccumulation by aquatic macrophytes in the Taupo

600 Volcanic Zone, New Zealand. Environ. Exp. Bot. 58, 206–215.
601 <https://doi.org/10.1016/j.envexpbot.2005.08.004>

602 Sharma, P., Dubey, R.S., 2005. Lead toxicity in plants. Braz J Plant Physiol 17, 35–52.

603 Sharma, Sohn, M., 2009. Aquatic arsenic: Toxicity, speciation, transformations, and remediation.
604 Environ. Int. 35, 743–759. <https://doi.org/10.1016/j.envint.2009.01.005>

605 Singh, H.P., Kaur, S., Batish, D.R., Sharma, V.P., Sharma, N., Kohli, R.K., 2009. Nitric oxide
606 alleviates arsenic toxicity by reducing oxidative damage in the roots of *Oryza sativa*
607 (rice). Nitric Oxide 20, 289–297. <https://doi.org/10.1016/j.niox.2009.02.004>

608 Singh, R., Singh, S., Parihar, P., Singh, V.P., Prasad, S.M., 2015. Arsenic contamination,
609 consequences and remediation techniques: A review. Ecotoxicol. Environ. Saf. 112, 247–
610 270. <https://doi.org/10.1016/j.ecoenv.2014.10.009>

611 Srivastava, S., Srivastava, A.K., Singh, B., Suprasanna, P., D'souza, S.F., 2013. The effect of
612 arsenic on pigment composition and photosynthesis in *Hydrilla verticillata*. Biol. Plant.
613 57, 385–389. <https://doi.org/10.1007/s10535-012-0288-7>

614 Stoeva, N., Berova, M., Zlatev, Z., 2005. Effect of arsenic on some physiological parameters in
615 bean plants. Biol. Plant. 49, 293–296.

616 Stoeva, N., Bineva, T., 2003. Oxidative changes and photosynthesis in oat plants grown in As-
617 contaminated soil. Bulg J Plant Physiol 29, 87–95.

618 Strand J.A., Weisner S.E.B. 2001. Morphological plastic responses to water depth and wave
619 exposure in an aquatic plant (*Myriophyllum spicatum*) J. Ecol. 89(2):166-175.
620 <https://doi.org/10.1046/j.1365-2745.2001.00530.x>

621 Tangahu, B.V., Sheikh Abdullah, S.R., Basri, H., Idris, M., Anuar, N., Mukhlisin, M., 2011. A
622 Review on Heavy Metals (As, Pb, and Hg) Uptake by plants through phytoremediation.
623 Int. J. Chem. Eng. 2011, e939161. <https://doi.org/10.1155/2011/939161>

624 Tawfik, D.S., Viola, R.E., 2011. Arsenate replacing phosphate: alternative life chemistries and
625 ion promiscuity. Biochemistry (Mosc.) 50, 1128–1134. <https://doi.org/10.1021/bi200002a>

626 Tripathi, R.D., Singh, R., Tripathi, P., Dwivedi, S., Chauhan, R., Adhikari, B., Trivedi, P.K.,
627 2014. Arsenic accumulation and tolerance in rootless macrophyte *Najas indica* are
628 mediated through antioxidants, amino acids and phytochelatins. Aquat. Toxicol. 157, 70–
629 80. <https://doi.org/10.1016/j.aquatox.2014.09.011>

630 Upadhyay, R.K., Panda, S.K., 2009. Copper-induced growth inhibition, oxidative stress and
631 ultrastructural alterations in freshly grown water lettuce (*Pistia stratiotes* L.). C. R. Biol.
632 332, 623–632. <https://doi.org/10.1016/j.crv.2009.03.001>

633 Villaescusa, I., Bollinger, J.-C., 2008. Arsenic in drinking water: sources, occurrence and health
634 effects (a review). Rev. Environ. Sci. Biotechnol. 7, 307–323.
635 <https://doi.org/10.1007/s11157-008-9138-7>

636 Vromman, D., Flores-Bavestrello, A., Šlejkovec, Z., Lapaille, S., Teixeira-Cardoso, C., Briceño,
637 M., Kumar, M., Martínez, J.-P., Lutts, S., 2011. Arsenic accumulation and distribution in
638 relation to young seedling growth in *Atriplex atacamensis* Phil. Sci. Total Environ. 412–
639 413, 286–295. <https://doi.org/10.1016/j.scitotenv.2011.09.085>

640 Xue, P., Yan, C., 2011. Arsenic accumulation and translocation in the submerged macrophyte
641 *Hydrilla verticillata* (L.f.) Royle. Chemosphere 85, 1176–1181.
642 <https://doi.org/10.1016/j.chemosphere.2011.09.051>

- 643 Yabanli, M., Yozukmaz, A., Sel, F., 2014. Heavy metal accumulation in the leaves, stem and
644 root of the invasive submerged macrophyte *Myriophyllum spicatum* L. (Haloragaceae):
645 An Example of Kadın Creek (Mugla, Turkey). *Braz. Arch. Biol. Technol.* 57.
646 <https://doi.org/10.1590/S1516-8913201401962>
- 647 Zhang, W., Cai, Y., Tu, C., Ma, L.Q., 2002. Arsenic speciation and distribution in an arsenic
648 hyperaccumulating plant. *Sci. Total Environ.* 300, 167–177.
649 [https://doi.org/10.1016/S0048-9697\(02\)00165-1](https://doi.org/10.1016/S0048-9697(02)00165-1)
- 650 Zhao, F.J., Ma, J.F., Meharg, A.A., McGrath, S.P., 2009. Arsenic uptake and metabolism in
651 plants: Tansley review. *New Phytol.* 181, 777–794. [https://doi.org/10.1111/j.1469-](https://doi.org/10.1111/j.1469-8137.2008.02716.x)
652 [8137.2008.02716.x](https://doi.org/10.1111/j.1469-8137.2008.02716.x)
- 653 Zhou, B., Wang, J., Guo, Z., Tan, H., Zhu, X., 2006. A simple colorimetric method for
654 determination of hydrogen peroxide in plant tissues. *Plant Growth Regul.* 49, 113–118.
655 <https://doi.org/10.1007/s10725-006-9000-2>
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659 **List of figures**

660 **Figure 1:** Effect of arsenic supply on hydrogen peroxide (H_2O_2) content (μM based on FW) in
661 shoots of watermilfoil plants - Controls and Treated with $100 \mu\text{g.L}^{-1}$ As in a recirculated system
662 at 21 days. The values shown are means \pm standard error of three independent experiments
663 (N=9). The uppercase letters compare the H_2O_2 concentrations at the same plant parts (old or
664 young) across the hydrodynamic zones, while lowercase letters compare the H_2O_2 concentrations
665 within the same hydrodynamic zone between the different plant parts (old vs. young) on the
666 Control and As-treated plants. The means followed by the same letter do not differ in the Sidak
667 test at a 5% significance level.

668

669 **Figure 2:** Effect of arsenic supply on pigment contents (in $\mu\text{g.g}^{-1}$ FW) in shoots of watermilfoil
670 plants - Controls and Treated with $100 \mu\text{g.L}^{-1}$ As in a recirculated system at 21 days. The values
671 shown are means \pm standard error of three independent experiments (N=9). The uppercase letters
672 compare the pigment concentrations at the same plant parts (old or young) across the
673 hydrodynamic zones, while lowercase letters compare the pigment concentrations within the
674 same hydrodynamic zone between the different plant parts (old vs. young) on the Control and
675 As-treated plants. The means followed by the same letter do not differ in the Sidak test at a 5%
676 significance level. (FW: Fresh Weight, Chl a: chlorophyll a, Chl b: chlorophyll b, car:
677 carotenoid)

678

679 **Figure 3:** Effect of arsenic supply on the osmotic potential (Ψ_{scor}) $\mu\text{osmol.kg}^{-1} \text{H}_2\text{O}$) in shoots
680 of watermilfoil plants - Controls and Treated with $100 \mu\text{g.L}^{-1}$ As in a recirculated system at 21
681 days. The values shown are means \pm standard error of three independent experiments (N=9). The
682 uppercase letters compare the osmotic potential at the same plant parts (old or young) across the

683 various hydrodynamic zones, while lowercase letters compare the osmotic potential within the
684 same hydrodynamic zone between the different plant parts (old vs. young) on the Control and
685 As-treated plants. The means followed by the same letter do not differ in the Sidak test at a 5%
686 significance level.

687

688 **Figure 4:** Effect of arsenic supply on the net photosynthesis and respiration in the watermilfoil
689 plants - Controls and Treated with $100 \mu\text{g.L}^{-1}$ As in a recirculated system. The values shown are
690 means \pm standard error of three independent experiments (N=9), expressed in $\text{mmolO}_2.\text{mL}^{-1}.\text{s}^{-1}.\text{g}^{-1}$
691 FW . Net photosynthesis (NP), Respiration (Res). The uppercase letters compare the respiratory
692 and photosynthetic activities in the control and As-treated plants separately across hydrodynamic
693 zones, while lowercase letters compare the respiratory and photosynthetic activities within the
694 same hydrodynamic zone between the Control and As-treated plants. The means followed by the
695 same letter do not differ in the Sidak test at a 5% significance level.

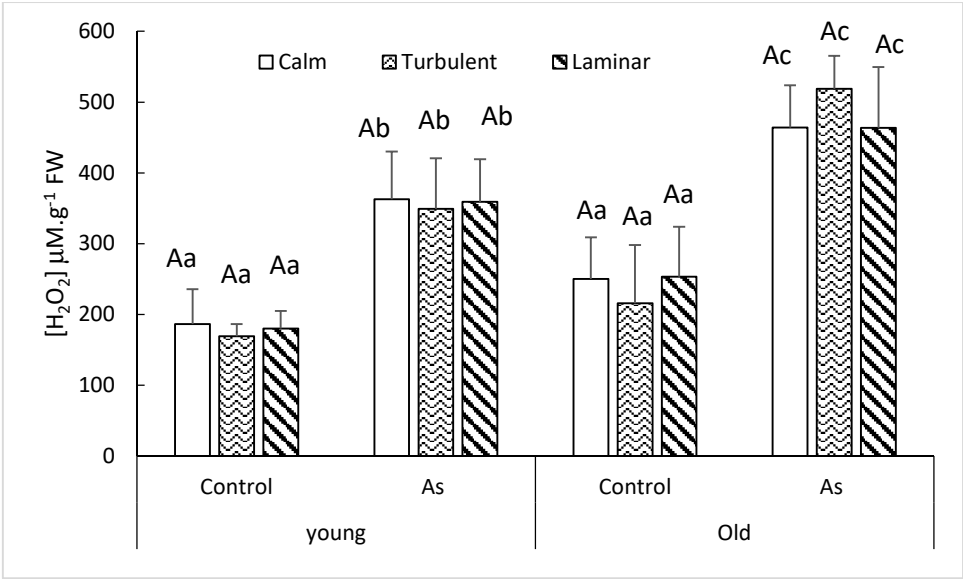
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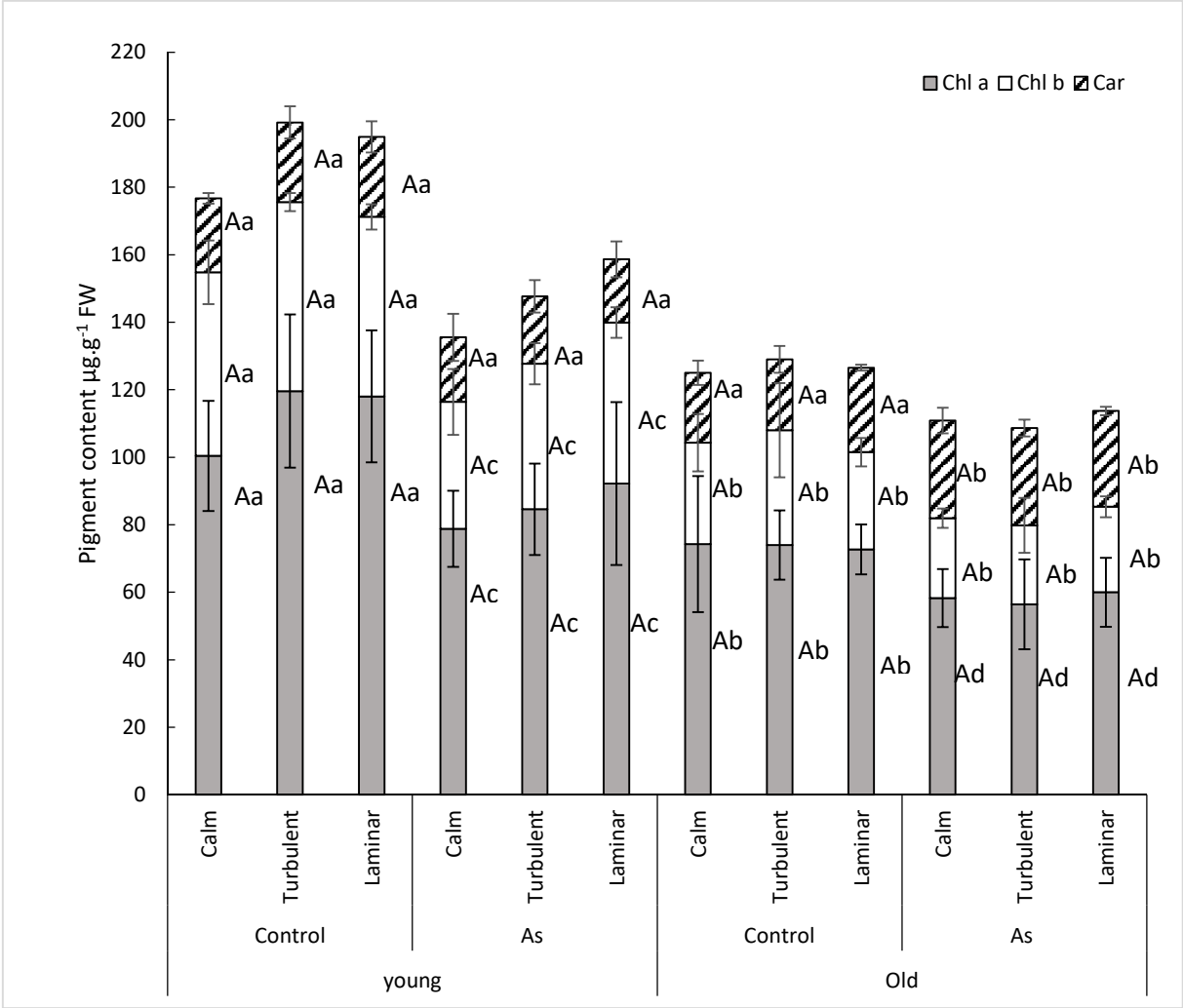
697 **Figure 5:** Effect of arsenic supply on the mean total main stem length (SL) (A) and mean total
698 root length (RL) (B) of watermilfoil plants - Controls and Treated with $100 \mu\text{g.L}^{-1}$ As in a
699 recirculated system for 19 days. The values shown are means \pm standard error of three
700 independent experiments (N=18), expressed in cm. The uppercase letters compare the SL and RL
701 in the control and As-treated plants separately across hydrodynamic zones, while lowercase
702 letters compare the SL and RL within the same hydrodynamic zone between the Control and As-
703 treated plants. The means followed by the same letter do not differ in the Sidak test at a 5%
704 significance level. Inter: intermediate, Lam: Laminar.

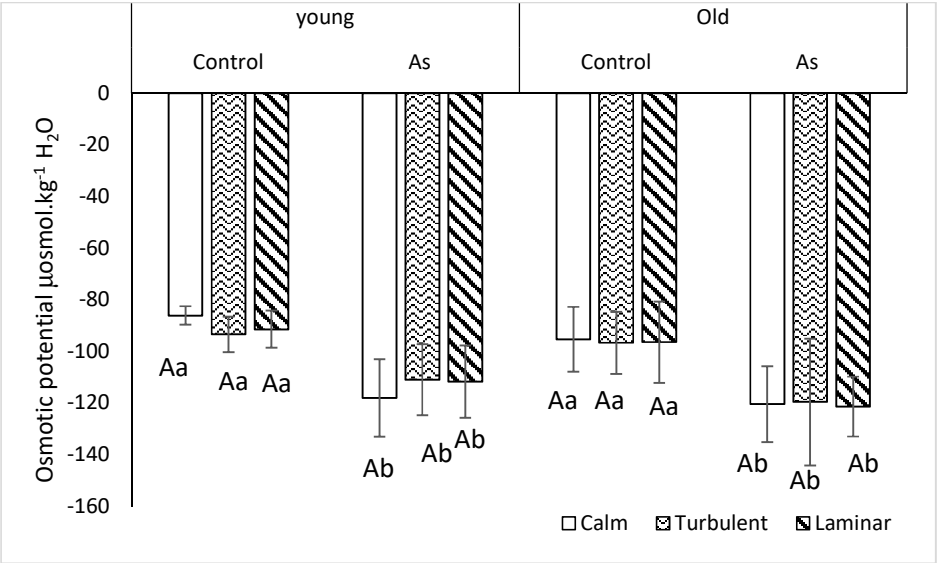
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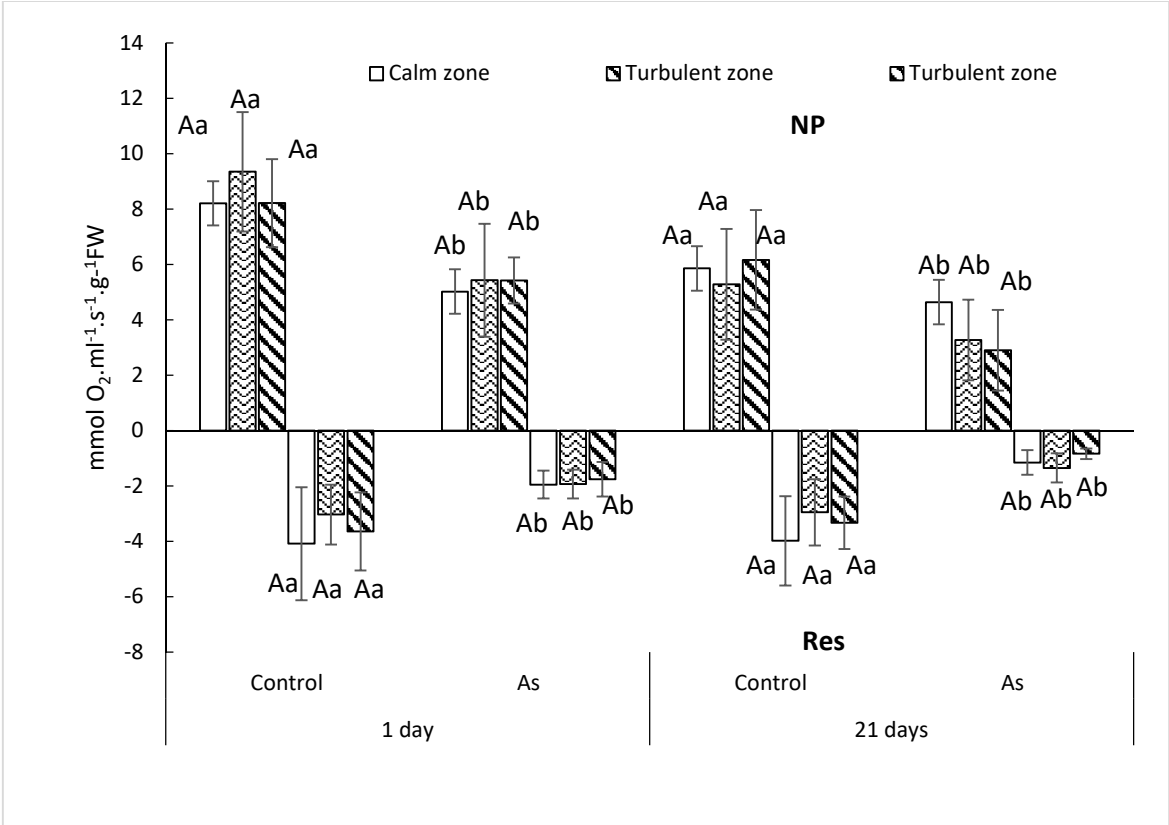
706 **List of tables:**

707 **Table 1:** Arsenic accumulation (in $\mu\text{g}\cdot\text{g}^{-1}$ DW) and bioconcentration factor (BCF, in $\text{L}\cdot\text{kg}^{-1}$) in
708 the shoots and roots of watermilfoil plants - Controls and Treated with $100 \mu\text{g}\cdot\text{L}^{-1}$ As in a
709 recirculated system by considering three hydrodynamic conditions (calm, turbulent and laminar)
710 at 21 days. The values shown are means \pm standard error of three independent experiments
711 (N=6). The uppercase letters compare the arsenic concentration for the same plant parts across
712 the various hydrodynamic zones, while lowercase letters compare the arsenic concentration
713 within the same hydrodynamic zone across the various plant parts. The means followed by the
714 same letter do not differ in the Bonferroni test at a 5% significance level.

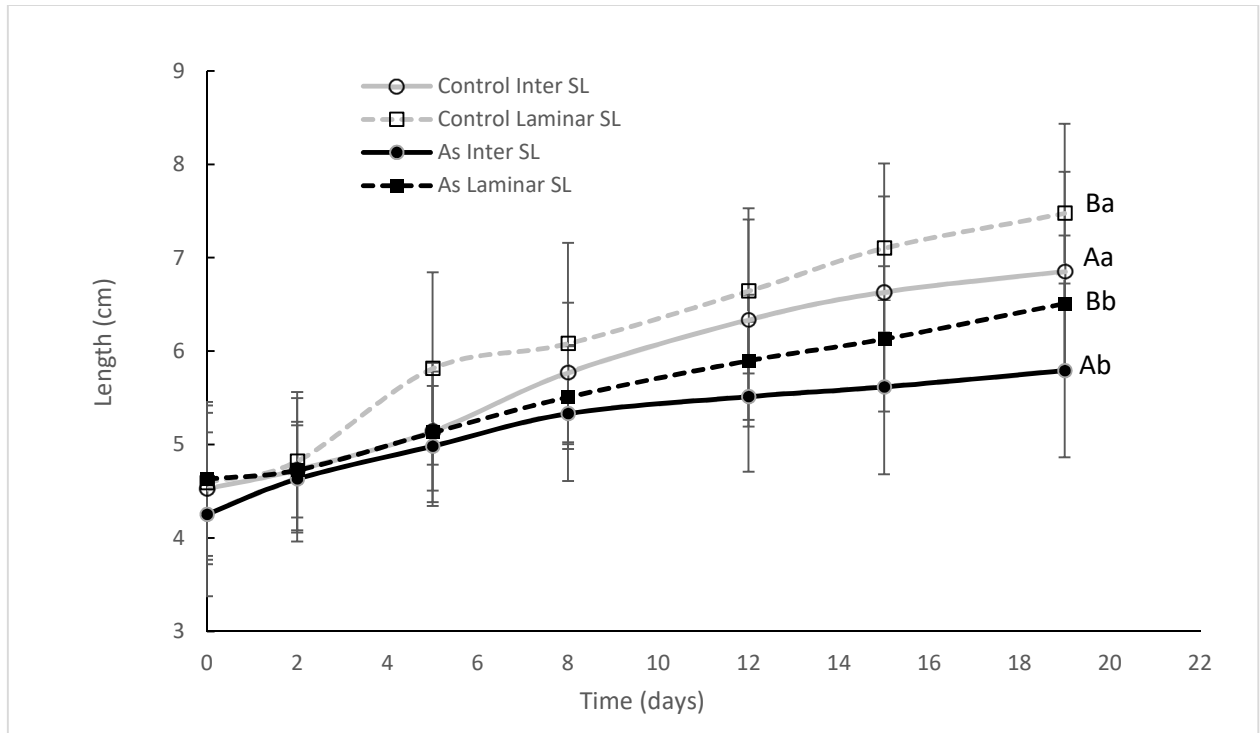




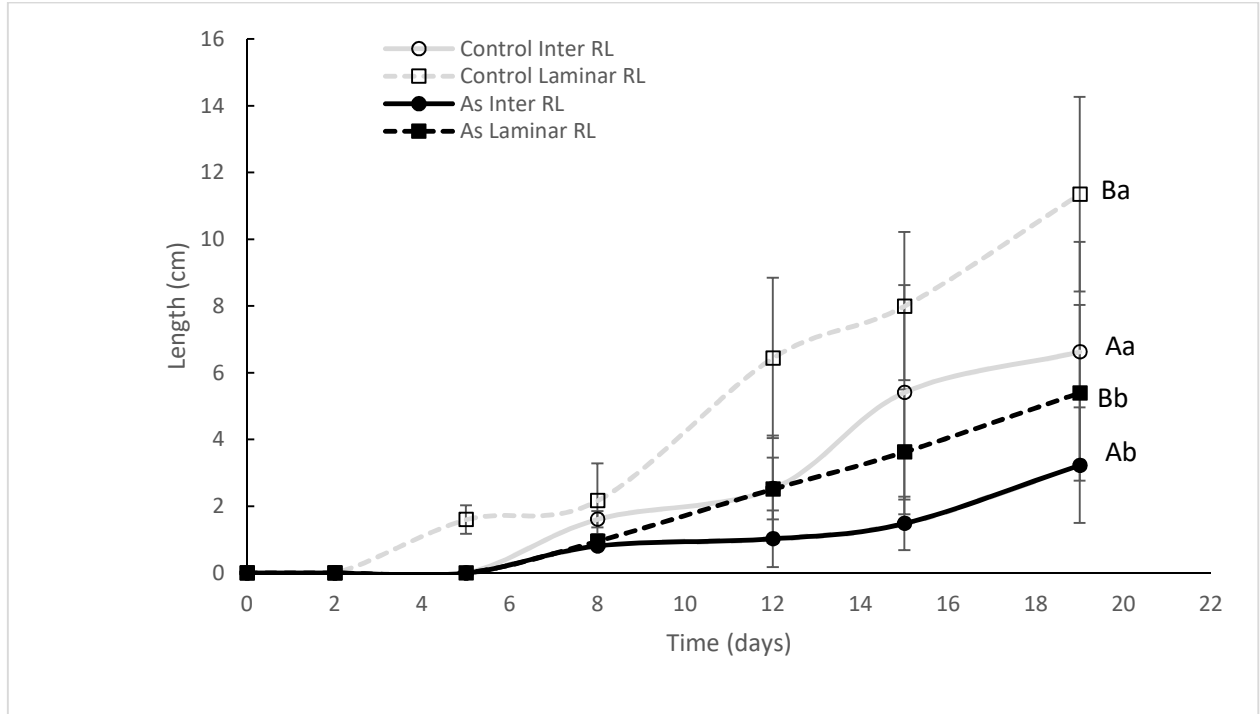




A



B



	Plant parts	Hydrodynamic conditions		
		Calm zone	Turbulent zone	Laminar zone
[As]	Stem+ leaves	218±1 ^{Aa}	200±1 ^{Bc}	218±2 ^{Ae}
μg.g ⁻¹ DW	Root	500±2 ^{Ab}	459±1 ^{Bd}	387±1 ^{Cf}
BCF (L.kg ⁻¹)	Stem+ leaves	176±40 ^{Aa}	172±60 ^{Aa}	169±70 ^{Aa}
	Root	414±80 ^{Ab}	346±50 ^{Bb}	308±44 ^{Cb}