EFFECTS OF PETROLEUM HYDROCARBONS ON *SALICORNIA PERENNANS* GERMINATION AND GROWTH UNDER SALINE CONDITIONS

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Abstract – The research aimed at studying the effects of petroleum hydrocarbons (PHs) on *Salicornia perennans* Willd. growth at different saline concentrations. The study investigated 1) germination and seedling growth and 2) plant growth and photosynthetic performance in hydroponics conditions. Results show that PHs, together with salinity, strongly affected the germination. In freshwater, final germination did not differ between control and PHs treated plants. Under saline conditions, PHs generally stimulated germination, especially at the highest concentration (6000 ppm). Germination did not occur at 50 and 70 NaCl g/l. PHs and salinity strongly affected radicle growth: at 0 up to 15 NaCl g/l germination was reduced by both PHs treatments, while at higher salinity, no statistically significant differences were observed. Plant growth was significantly affected at 0 g/l of salt and with 6000 ppm PHs addition. The photosynthetic performance, evaluated through the maximum quantum yield of photosystem II (F_V/F_M), was slightly affected by the treatments, showing only a transient decrease in the plants treated with PHs in the absence of NaCl. On the contrary, plants grown in saline conditions maintained optimal values of F_V/F_M throughout the treatment period, highlighting a good tolerance of photosynthetic apparatus towards PHs.

Introduction

Petroleum hydrocarbons (PHs) are among the most concerning pollutants, especially for wetlands. Besides entailing the modification of some physical and chemical properties of water and soils, PHs are toxic for the environment: plants, animals and microbes (1). Coastal wetlands are unique and fragile environments that have relatively limited distribution and are inhabited by several specialized species. Moreover, they often encompass habitats of conservation interest, such as those of the Natura 2000 network. Halophytes are specialized plants adapted to thrive in salty habitats and represent interesting candidates to restore contaminated sites where salt content deeply impacts the environment. To test the possible use of halophytes as plants to employ in the remediation of PHs contaminated areas, *Salicornia perennans* Willd. (Chenopodiaceae), an annual succulent halophyte inhabiting brackish and saline environments, was chosen. The annual species belonging to the genus *Salicornia*, commonly known as glasswort, are reported to tolerate a broad range of saline conditions. They can produce a very high number of seeds

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which can easily germinate in vitro. Moreover, their cultivation is easy both in-soil and off-soil, possibly yielding relatively high biomass in a short time. Thus, this species can be considered a good candidate to test their capacity to tolerate and remediate petroleum hydrocarbons-contaminated saline waters.

In order to evaluate *S. perennans* resistance to salinity and to petroleum hydrocarbon stress, we investigated the germination capability and plant growth under different combination of salt and PHs concentrations.

Materials and Methods

Germinations trials

Fifty fruits of *S. perennans* (collected at Calambrone on the coastal area of Tuscany, Italy) were placed in each Petri dish, and germination was monitored daily. Salt treatments were: 0; 7,5 g/l (0,13 M); 15 g/l (0,26 M); 30 g/l (0,51 M); 50 g/l (0,86 M); 70 g/l NaCl (1,20 M). PHs treatments: A) 600 ppm B) 6000 ppm. When germination did not occur anymore, non-germinated seeds were washed and transferred in Petri dishes containing distilled water for the recovery experiment and then germination was monitored. In order to evaluate the effects of PHs on seedling growth, 30 seedlings were grown in the same conditions of salinity and PHs, after germination in distilled water. After 10 days, radicle, hypocotyl and cotyledons were measured using a stereomicroscope. Finally, the dry weight ratio was calculated on the same seedlings Germination was monitored daily until no germinating seeds were observed. The final germination rate has been statistically analysed through two-way ANOVA tests with the PAST software. Differences among means and the interaction among factors have been evaluated using Tukey's post hoc test.

Plant growth trials

After 30 days from germination plants were transferred in 500 ml flask in hydroponic solution and salt was gradually added with different concentration of NaCl: 0 g/l; 7,5 g/l (0,13 M); 15 g/l (0,26 M); 30 g/l (0,51 M); 50 g/l (0,86 M); 70 g/l (1,20 M). PHs addition was performed after 30 days from plants adaptation to salt treatment at two increasing concentrations: 600 ppm and 6000 ppm.

After 30 days from PHs addition, plants were collected and separated into aerial and root parts. Plant organs were gently washed, oven-dried at 60 °C and weighted.

During the experiment, the photosystem II (PSII) functionality was evaluated by chlorophyll fluorescence measurements conducted on leaves of the *S. perennans* grown under different NaCl treatments (0 and 30 g/l NaCl) and PHs (600 and 6000 ppm).

Chlorophyll fluorescence was measured using a portable miniaturized pulse-amplitude- modulated fluorimeter (Mini-PAM; Heinz WalzGmbH, Effeltrich, Germany). Measurements were conducted in vivo on the green leaves after 1 week and 3 weeks from the beginning of the treatments. Plants were pre-darkened for 30 min, and the maximum quantum yield of photosystem II was evaluated as $F_V/F_M=(F_M - F_O)/F_M$, where F_O and F_M are the minimum and the maximum fluorescence yield emitted by the leaves in the dark-adapted state, respectively. The F_V/F_M values have been statistically analysed through three-way ANOVA to test main and interactive effects of NaCl concentration (0 and 30 g/l NaCl),

PHs concentration (600 and 6000 ppm) and treatment time (1 and 3 weeks). Differences among means and the interaction among factors have been evaluated using Tukey's post hoc test.

Results

Salt and PHs effects on germination

Salicornia perennans showed a great germination capability when growing without salt (100 % seeds germinated). Salinity remarkably affected germination of *S. perennans* as just 7,5 g/l of NaCl were enough to lower the maximum germination to 68 %, while under 15 g/l germination was reduced to 27,3 % and with 30 g/l, it was barely detectable (just one seed germinated in the three replicates). With 50 and 70 g/l of NaCl, no germination has been observed (data not shown).

PHs, in general, accelerated and increased germination percentages (Fig.1). As regards in the case of no salt added, conditions of 6000 ppm PHs allowed *S. perennans* seeds to germinate faster than in control conditions and with 600 ppm of hydrocarbons. Similarly, plants grown with 7,5 g/l or 15 g/l of NaCl and 600 ppm of hydrocarbons reached final germination rates almost equal to those observed without salinity. Finally, for the highest NaCl treatment that allowed a certain number of seeds to germinate, only in the case with 6000 ppm of hydrocarbons germination occurred not at a barely detectable level (mean final germination 22 %).

The three tested salinity concentrations exerted statistically significant effects on germination. On the other hand, only the highest PHs treatment exerted statistically significant effects on germination.

PHs exerted important effects on the morphology of the germinated seedlings since, under the highest treatment, roots did not develop (Fig. 2 A, B).

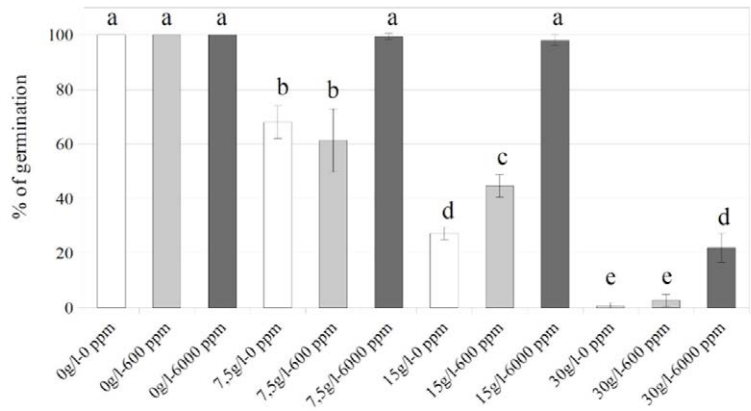

Figure 1 – Final germination rates of *S. perennans* under salinity and PHs treatments. White bars, no PHs; grey bars, 600 ppm PHs; black bars, 6000 ppm PHs. Different letters show statistically different means among treatments.

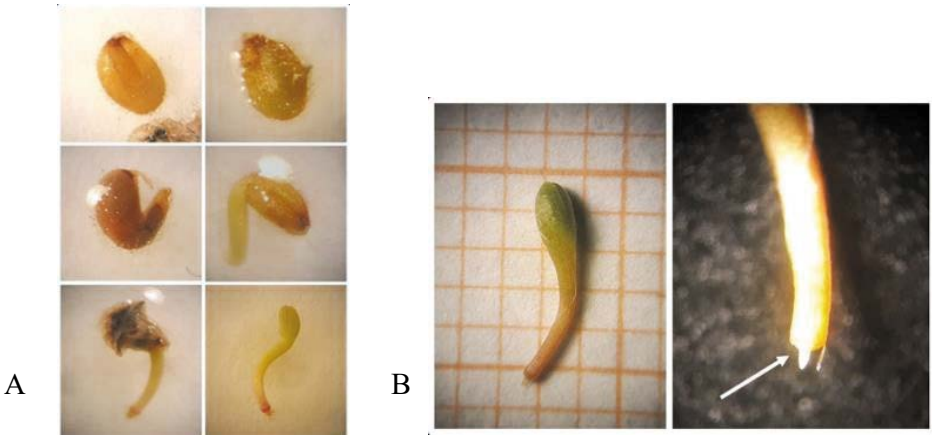

Figure 2 – A) Different germination stages of *S. perennans* grown in control conditions (no salt, no PHs); B) Morphological Effects on roots of 6000 ppm hydrocarbons-treated *S. perennans* seedlings.

Salt and PHs effects on plants

Salicornia perennans showed to tolerate the highest doses of salt (50-70g/l) even if anticipating the blooming phase. Hence, the PHs addictions were performed only at the salt rate of 0 and 30 g/l. In figure 3, biomass data registered at the end of the trials are shown. The aerial biomass was reduced under the treatment of 0 g/l NaCl combined with the highest rate of PHs (6000 ppm); in the root biomass, a reduction was observed at 30 g/l compared to 0 g/l, while the PHs addition seemed to not alter the root biomass (data not shown).

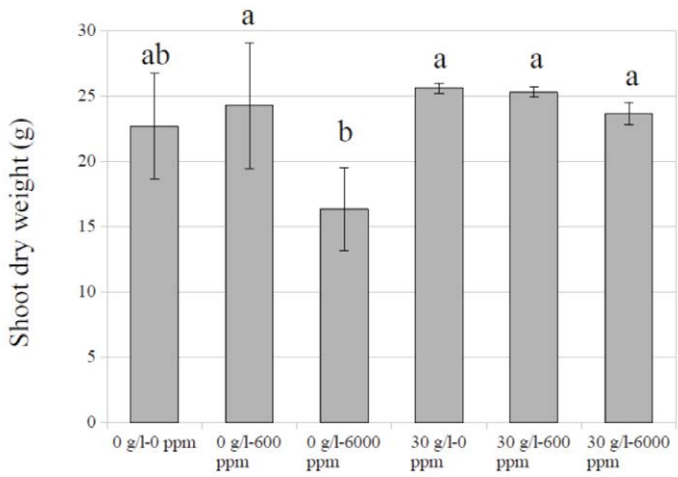

Figure 3 – Shoot dry weight of *S. perennans* plants treated with and without PHs at 0 and 30 g/l of NaCl. Different letters show statistically different means among treatments.

The effects of salt and PHs treatments on the physiological performance of plants were evaluated through the analysis of F_V/F_m on leaves (Fig. 4). Indeed, the presence of a stress factor generally results in a lowering of F_V/F_m, indicating an inactivation or damage of PSII functionality (2). Our results indicate that at 0 g/l NaCl, plants showed a slight reduction of Fv/Fm at increasing PHs concentration after one week of treatment, although they recovered the optimal values after three weeks. On the contrary, plants treated with 30 g/l NaCl maintained optimal values of F_V/F_m (close to 0.8) throughout the treatment period, highlighting their higher stress tolerance (Fig. 4).

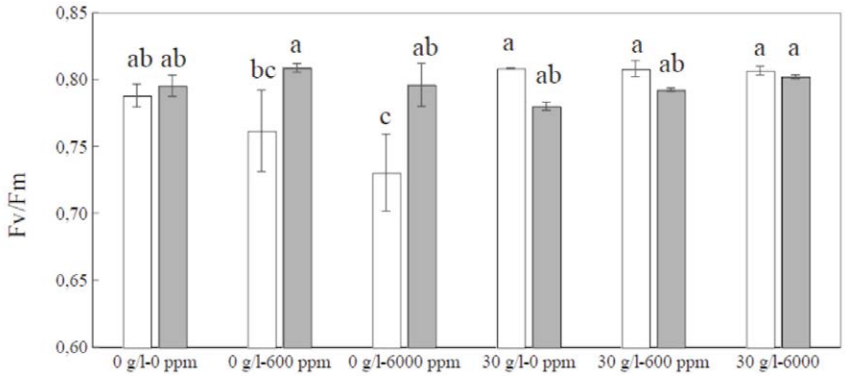

Figure 4 – Effects of salinity and PHs on Fv/Fm value after one week (white) and after three weeks (grey). Different letters show statistically different means among treatments.

Discussion

There is little literature data concerning the effects of PHs on seed germination under saline conditions concerning the genus *Salicornia*; thus, studies are needed to clarify this important aspect. Salinity is well-known to deeply affect the germination of halophytes, and studies on *Salicornia* have shown that this factor, even at low concentrations, determines a significant reduction of final germination rate in the close congener *S. europaea* L. In this light, the tested *S. perenanns* appears to be more a halotolerant than a true halophyte. In fact, the natural process of germination occurs in the wet season, when the salt concentration lowers thanks to the rainfall.

Generally, the treatment with PHs on seed germination in soil has turned out to be detrimental to the final germination rate (1, 3). The low PHs treatment (600 ppm) generally slowed down the *S. perennans* germination but determined a lower final germination rate only in plants grown with 7,5 g/l of salt. On the other hand, the highest treatment with PHs (6000 ppm) caused unexpected effects. Indeed, the latter treatment accelerated the germination also for seeds grown without salinity, while in mild saline conditions (7,5-15 g/l), it succeeded in counterbalancing the inhibiting effect of salt on the final germination. Differently, the effects of the highest PHs treatment on seedling radicle are somehow

consistent with what was reported for other species in which a significantly reduced root growth has been observed.

Despite the positive action exerted by PHs on germination at certain saline concentrations, in consideration of the important effects on the development of the radicle in germinated seedlings, it is obvious that the overall action of PHs cannot be regarded as beneficial.

The obtained results show that PHs can enhance germination in *S. perennans* seedlings under saline conditions. This is in contrast with what was reported for a different species of the same genus: *Salicaria persica* grown in soil (3). The recovery experiment shows that PHs can exert their effects even after their removal, suggesting that hydrocarbons can possibly penetrate fruit teguments and directly affect the embryo. The measurements on germinated seedlings show that PHs, despite enhancing germination under saline conditions, at the same time exert important detrimental effects on radicle growth. This latter result can be only partially explained by the water stress, while the toxicity of PHs should be accounted for by the reduction of the radicle growth. Taken together, our results show that PHs are toxic for the seedlings of *S. perennans* and that this species reacts with an increase in germination. On the contrary, adult plants have turned out to be much more resistant and only slightly affected by PHs, depending on the saline conditions. In particular, PHs treatment exerted a transient reduction in photosynthetic potential and a slight decrease of aerial plant biomass in plants grown without salt, highlighting that saline conditions are necessary for this halophyte to enable the resistance to the PHs stress.

Conclusion

The outcomes of the present study represent the first data available in the evaluation of the salt and PHs stress on *S. perennans.*

Salicornia perennans showed to be more susceptible to the toxic effects of PHs under saline conditions at the stage of seedling than at the adult stage. In conclusion, petroleum pollution may be particularly critical at the early stages of the development of *S. perennans* seedlings, while the saline conditions are necessary for this halophyte to enable the resistance to the PHs stress. Further studies are necessary to elucidate the mechanism involved in germination and seedling radicle development and to investigate the *S. perennans* efficiency in degrading PHs in salty ponds at a field scale. Also, long-term observations are needed, and more information on other possible sources of impact should be gathered to obtain more reliable conclusions.

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References

[1] Khan MAI, Biswas B, Naidu ESR, Megharaj M (2018) - *Toxicity assessment of fresh and weathered petroleum hydrocarbons in contaminated soil- a review*. Chemosphere 212:755–767.

[2] Murchie EH, Lawson T (2013) - *Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications.* J Exp Bot 64(13): 3983-3998.

[3] Ebadi A, Sima NAK, Olamaee M, Hashemi M, Nasrabadi RG (2018) - *Remediation of saline soils contaminated with crude oil using the halophyte Salicornia persica in conjunction with hydrocarbon-degrading bacteria*. J Environ Econ Manag 219:260–268.