# Temperature effect on seed germination of four plants in sand from coastal sand dunes in Greece

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***Abstract*—** *In high temperatures of coastal sand dunes, salt may limit seed germination and thus strogly limit plant survival. The relationship between germination and sand dunes soil temperatures has never been evaluated. The effect of coastal dunes sands on plant germination at 20°C and 28°C was studied. Sand samples of coastal dunes from coastline regions in Greece were analyzed and used as substrates. The Si content (89-97%) is the representative one in all soils. The Si, Al and Mg contents in the Aegean Sea sand dunes were higher than the Ionian Sea. Pigweed germination at 28°C in sand from mainland Greece was higher than the corresponding one from insular Greece. Purslane germination at 28°C in sand from the Ionian Sea (52%-57%) was higher than the corresponding one from the Aegean Sea (49%-50%) and at 20°C it remained unaffected by the soil. Chervil and coriander germination at 20°C was very low, while no germination occurred in pigweed at 20°C and chervil and coriander at 28°C in all sand soils. The study has shown that the coastal dune soils favoured germination of pigweed and purslane at 28°C. These results indicate that those plants have adapted to the coastal sand dunes environment.*

***Keywords*— sand elements, pigweed (Amaranthus retroflexus), purslane (Portulaca oleracea), chervil (Anthriscus cerefolium), coriander (Coriandrum sativum).**

## I. INTRODUCTION

The sand dunes occurred in rows along the coast. Coastal dunes have an extensive global distribution (Martinez et al. 2004). A variety of abiotic and biotic factors including sand instability, salinity, extreme high or low temperatures, drought, and herbivore predation may limit seed germination and seedling emergence in coastal sand dunes (Sykes & Wilson 1988; Maun 1994; Maun 2009). Major problems faced by seeds are sand accretion and soil salinity.

Seed germination is a critical stage for the establishment of a species. Salt inhibits seed germination in saline soil, and high salinity is injurious to most glycophytes. Ungar (1978) suggested that there is a complex interaction between salinity and temperature, especially for coastal halophytes. Salinity and temperature interact in their control of seed germination (Khan & Ungar 1999), with the greatest inhibition due to salinity usually found at the minimum or maximum limits of tolerance to temperature (Badger & Ungar 1989). In sand dune environments, seed germination is strongly related to available moisture and seedling survival is strongly limited by temporal changes in water availability (Maun 2009). Very small seeds showed a high germination rate because of the small amount of moisture required for inbibition (Stairs 1986; Zheng et al. 2005).

Purslane (Portulaca oleracea) is a spontaneous wild forb, herbaceous annual plant, from the Portulacaceae family. It is one of the world's most aggressive and worst weeds, a unique plant that has the ability to adapt to many diverse environments (Rahdari et al. 2012). It is more tolerant to salinity and drought conditions (Hamidov et al. 2007). The plant produces numerous minute seeds, many of which have primary dormancy and require warm temperatures and light for germination (Egley 1984). The plant grows in the wild and is cultivated around almost the whole world. Purslane is a heat- and drought-tolerant plant, and is an important vegetable crop (Anastácio & Carvalho 2012; Dadkhah 2013). Moreover, purslane is an edible halophyte (Grieve & Suarez 1997) and a promising crop for saline agriculture (Kiliç et al. 2008), which has been studied for its relatively high salinity tolerance (Teixeira & Carvalho 2009). The optimal seed germination occurs at temperatures > 30°C, while poor germination occurs at temperatures < 24°C (Miyanishi & Cavers 1980; Zimmerman 1976). Germination was 96% when seeds were exposed to 35/25°C day/night alternating temperatures, but 25/10°C day/night resulted in 15% germination (Miyanishi & Cavers 1980). In the light/dark regime, the germination was lower at 25/15°C than at 30/20°C (70%, and 81% germination, respectively) (Chauhan & Johnson 2009).

Pigweed (*Amaranthus retroflexus*) is a common annual worldwide weed of 60 crops in 70 countries. It can be found in a wide range of habitats and causes substantial yield reduction in many different agricultural crops through competition (Ghorbani et al. 2000; Holm et al. 1997). Pigweed is a thermophyte to sub-thermophyte, often highly stress-tolerant, growing in sandy and saline habitats (Costea et al. 2004; Robertson & Clemants 2003). It reproduces by seeds which can only remain

viable for at least 6–10 years (Costea et al. 2004). Seed germination of pigweed differed in its response to temperature. Pigweed had higher germination rates at 35/30°C than at 25/20°C or 45/40°C, and at 15/10°C no seed germination was observed (Guo & Al-Khatib 2003). Ghorbani et al. (1999) found that the minimum temperature for pigweed germination was greater than 5°C, whereas maximum germination occurred between 35 and 40°C. Pigweed was sensitive to Al, Mn, NaCl and Na2SO4.

Chervil (*Anthriscus cerefolium*) is a fragrant, delicate annual herb, belonging to the Apiacae family. Its seeds need light and wet porous soil to germinate. Seeds of chervil have germinated at a 13°C temperature (Bubel 1988). The seed germination percentage of chervil at 22°C in H2O was 44%. In the 80mM NaCl concentration there was an increase in the germination (64%), while in higher NaCl concentrations the germination reduced (Liopa-Tsakalidi & Barouchas 2011).

Coriander (Coriandrum sativum) is an annual herb of the Apiaceae family, grown primarily for its seed and seed oil all over the world (Verma & Sen 2008). It is known as a species moderately tolerant to salinity. Zidan & Elewa (1995) mentioned that during germination, coriander tolerated salinity up to 200mm NaCl. Liopa-Tsakalidi et al. (2011) reported that no seeds germinated in high salt concentrations (0.5 and 1.5 mol/l NaCl). In addition to this, the seed germination of coriander showed a progressive decrease as salinity levels increased (Ewase et al. 2013; Fredj et al. 2013). The salinity effect appears mainly during germination and plant growth (Aymen & Cherif 2013).

Little research has been conducted about the application of seed germination on coastal sand dunes and the studies on their relationship with seedlings are still inadequate. For example, the growth characteristics of seedlings in Greek coastal sand dunes have not been investigated with respect with to initial seed germination.

The focus of the current study was to provide knowledge on the germination behavior of pigweed (*Amaranthus retroflexus*), purslane (*Portulaca oleracea*), chervil (*Anthriscus cerefolium*), and coriander (*Coriandrum sativum*) in two temperatures and different sand soils of coastal sand dunes in Greece, and to ascertain the effects of different characteristics of sand soils on germination.

## II. MATERIAL AND METHOD

### 2.1 Analysis of sand dune soils

In the present paper, the study area was located in the mainland (Kastro Kyllinis and +Vartholomio Ileias) and the insular (Zakynthos) coastline of the Ionian Sea, and the insular (Milos and Tinos) coastline of the Aegean Sea, in Greece (Fig. 1).

**FIG. 1: STUDY AREA IN THE MAINLAND AND THE INSULAR COASTLINE OF THE IONIAN SEA, AND THE INSULAR COASTLINE OF THE AEGEAN SEA, IN GREECE.**

For comparison purposes, sand samples that represent the sand soils of various coastal sand dunes of Greece, i.e. representing areas of the Ionian and Aegean Seas, were randomly collected from 0 to 10 cm below the surface of the coastal sand dunes, using metal spatula. At each study area, 3 samples (approximately 1-2 kg) were collected randomly from different sand dunes within the radius of 200m. The samples were transferred to the Laboratory of Soils and Irrigations of the Technological Educational Institute of Western Greece for further studies. Sand samples were collected from coastal sand dunes from five regions of Greece: the Alykanas area in Zakynthos, the Kastro Kyllinis area and the Vartholomio area in Ileias (Ionian sea), and the Milos and Tinos islands (Aegean sea).

The total content of silicon (Si), calcium (Ca) chromium (Cr), aluminium (Al), magnesium (Mg), potassium (K), titanium (Ti), manganese (Mn), iron (Fe), sodium (Na), and zirconium (Zr) elements were determined using microwave digestion and the Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES). ICP-AES is the preferred method of elemental analysis for chemical stratigraphy. Methods: Approximately 1g ± 0,1mg of sample was digested with 2.35 mL of HNO3 65% and 7 mL of HCl 37% (aqua regia) in microwave digestion system (Berghof speedwave MWS-3+). The temperature program was as follows: 5 min for temperature 145°C, 10 min for temperature 170°C and 15 min for temperature 180°C. The resulting solutions were cooled for 30 minutes and diluted to 10 mL with distilled water. The clear solutions were analyzed by ICP-OES (Thermo Scientific iCAP 6000). The operating conditions were: nebulizer gas flow rates: 0.5 l/min; auxiliary gas flow: 0.5 L min−1; plasma gas flow: 15 L min−1; Pump rate: 45 rpm; ICP RF power: 1100 W. Aliquots of an ICP multielement standard solution (100 mg/L Merck) containing the analyzed elements, was used in the preparation of calibration solutions. Working standard solutions were prepared by dilution of the stock standard solutions to desired concentration in 1% HNO3. The ranges of the calibration curves (6 points) were selected to match the expected concentrations for all the elements of the sample studied by ICP-OES. The correlation coefficient r2 obtained for all cases was 0.9999. The detection limits (LOD) were calculated as the concentrations of an element that gave the standard deviation of a series of ten consecutive measurements of blank solutions. Soil samples from BIPEA's proficiency testing scheme A15 were used to ascertain the accuracy of the measurements. All above-mentioned sand testings were performed in the Laboratory of Soils and Irrigations of the Technological Educational Institute of Western Greece.

### 2.2 Seed germination

Four plant species were utilized for this study; two weeds, pigweed (Amaranthus retroflexus) and purslane (Portulaca oleracea), and two herbs, chervil (Anthriscus cerefolium) and coriander (Coriandrum sativum) (seed supplied by SAIS Sementi, Cesena-Forlì, Italy). Forty seeds were placed on Petri dishes (10cm diameter) containing 15g of river sand and 15ml of distilled water (control) or an equal quantity of the respective test sands, i.e. coastal dune sand from Alykanas area of Zakynthos island (Ionian sea), coastal dune sand from Kastro area of Kyllinis (Ionian sea), coastal dune sand from Vartholomio area of Ileias (Ionian sea), coastal dune sand from Milos area island (Aegean sea), coastal dune sand from Tinos area island (Aegean sea). The petri dishes were arranged in a completely randomized block design with three replicates of each treatment, and were transferred in a controlled plant growth chamber under 20±1°C or 28±1°C temperature regimes, a 16h photoperiod and 80±2% relative humidity (RH). The number of the germinated seeds was recorded every day, starting from day 2 after the seeds were initially placed on Petri dishes. The experiment consisted of three replications.

### 2.3 Data and Statistical analysis

The germination percentage is an estimate of the seed viability. The equation to calculate the final germination percentage (GP) is:

$$GP = \frac{\text{number of germinated seeds}}{\text{number of total seeds}} \times 100$$

The germination rate was estimated by using a modified

$\text{Timsons index of germination velocity} = \sum G/t$ , where G is the percentage of seeds which germinated after 2-day intervals and t is the total germination period.

All data was tested by the analysis of variance (ANOVA), using the SPSS 21 software. Duncan's multiple range test was performed at p = 0.05 for each of the significant variables measured. Duncan's multiple range test was performed at p = 0.05 for each of the significant variables measured.

## III. RESULTS

### 3.1 Chemical characterization of dune sand

The chemical characteristics of the sand tested are presented in Figures 2 and 3 respectively. They can provide useful information about plant survival.

The results show that Si (89-96%) is the most represented mineral. The mean values of total Si contents for the Milos (97%) and Tinos (96%) islands (Aegean Sea) sand dunes were significantly higher than the rest of the sand dunes. Alykanas Zakynthos and Kastro Kyllinis (14%) were significantly lower than the rest of the dunes (Fig. 2).

When compared to the other elements, calcium contents present an important concentration. The sand chemical analysis contains a non-negligible part of Ca, Cr, Al and Mg. The Ca and Cr contents for the Milos and Tinos islands (Aegean Sea) sand dunes were significantly lower than the rest of them, namely Alykanas Zakynthos and Kastro Kyllinis, Vartholomio Ileias (Ionian Sea). Similarly to Si contents, the results show that aluminium (Al) and magnesium (Mg) contents in the sand dunes of the Aegean Sea are significantly higher than those of the Ionian Sea (Fig. 1). The K, Ti, Mn, Fe, Na, and Zr contents do not show clear group separations like that shown by the Al contents (Fig. 2, 3).

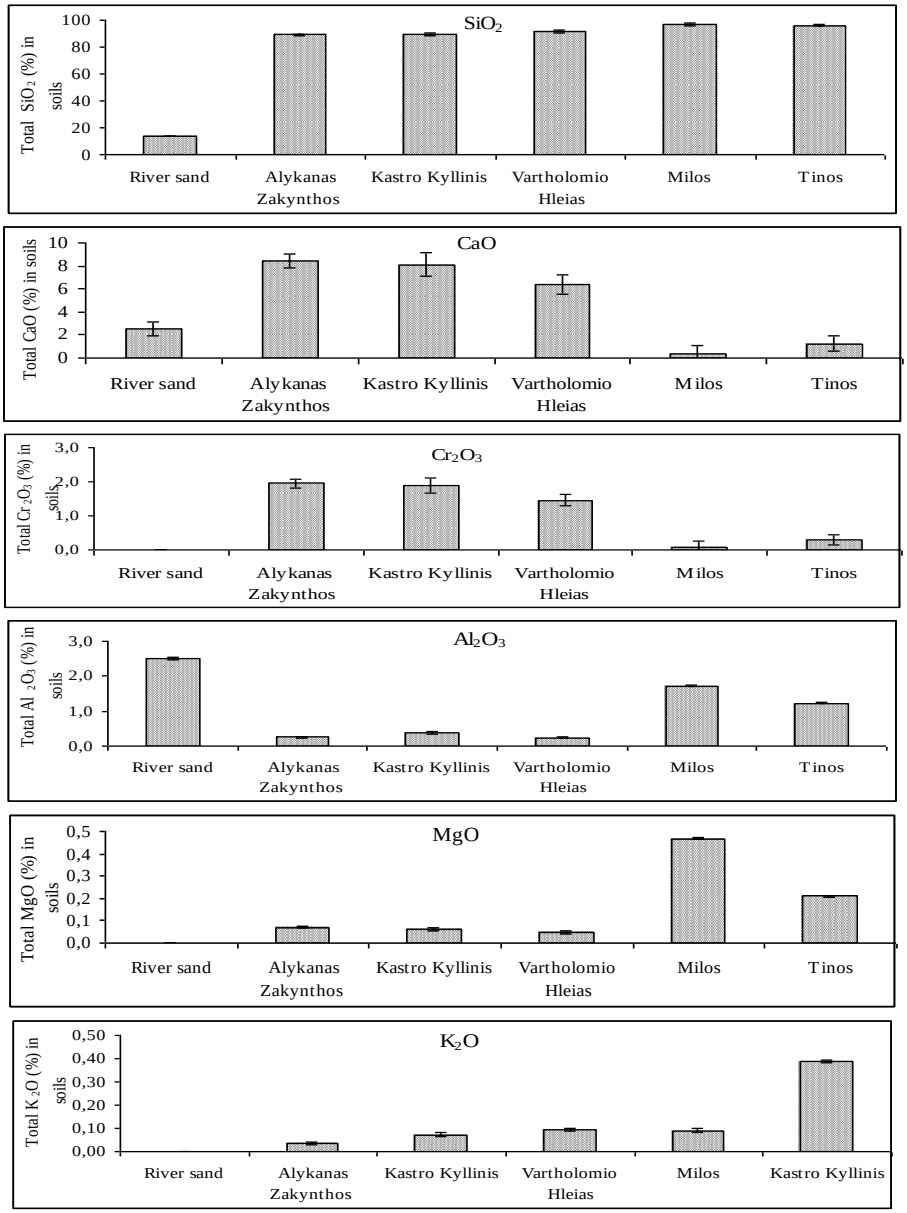


**FIG. 2: CHEMICAL CHARACTERIZATION (%) OF THE SAND DUNES OF FIVE AREAS OF GREECE; SILICON (SI), CALCIUM (CA) CHROMIUM (CR), ALUMINIUM (AL), MAGNESIUM (MG), POTASSIUM (K) CONTENTS.**

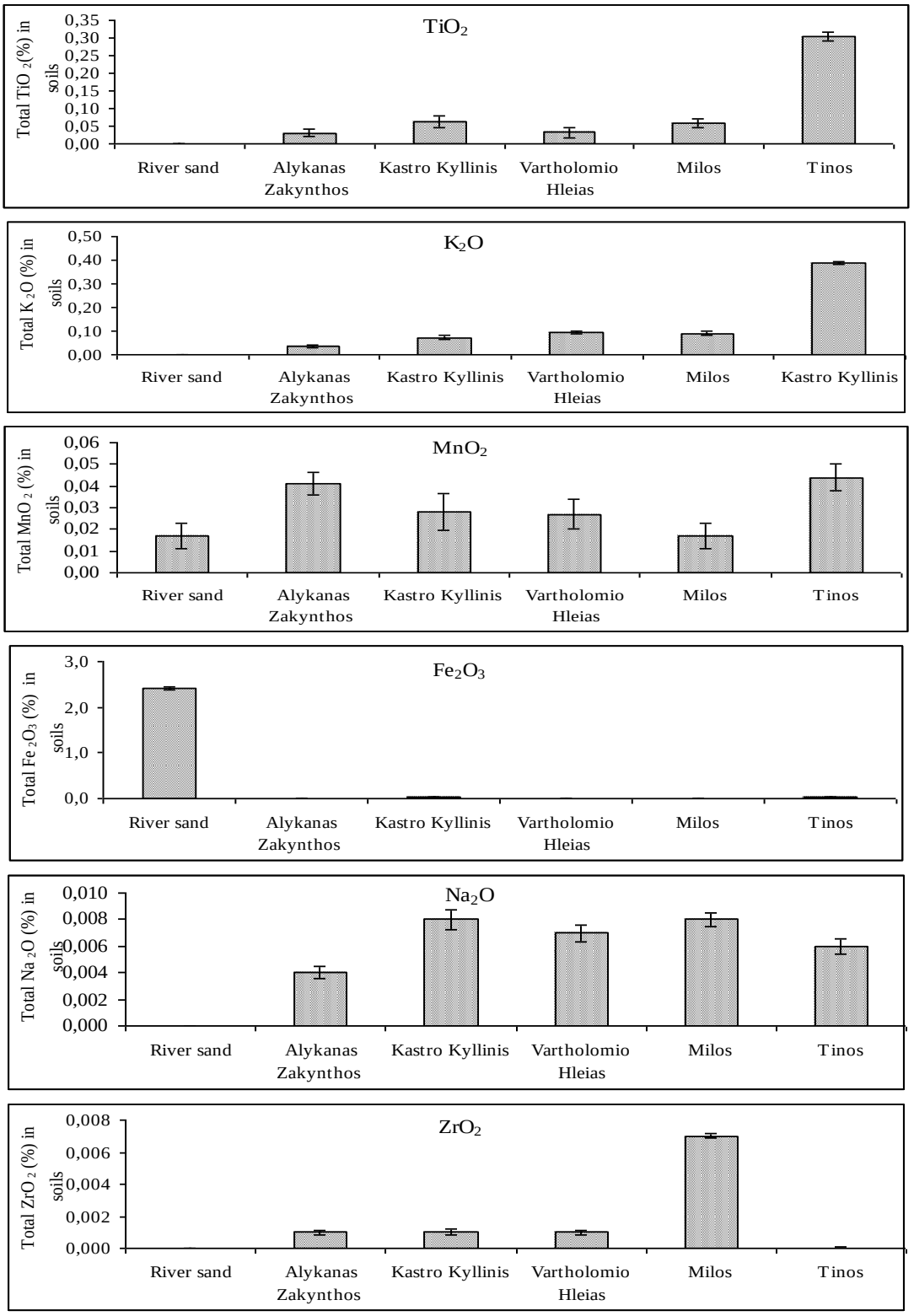


**FIG. 3: CHEMICAL CHARACTERIZATION (%) OF THE SAND DUNES OF FIVE AREAS OF GREECE; TITANIUM (TI), MANGANESE (MN), IRON (FE). SODIUM (NA), ZIRCONIUM (ZR) CONTENTS.**

### 3.2 Coastal dunes effects on seed germination

The seed germination observation of pigweed, purslane, chervil and coriander plants lasted 10 days at a temperature of 20°C and 28°C in a controlled plant growth chamber (Fig. 3). Temperature had a significant effect on the germination percentage. No seed germination was observed at the lowest temperature (20°C) in all sand soils.

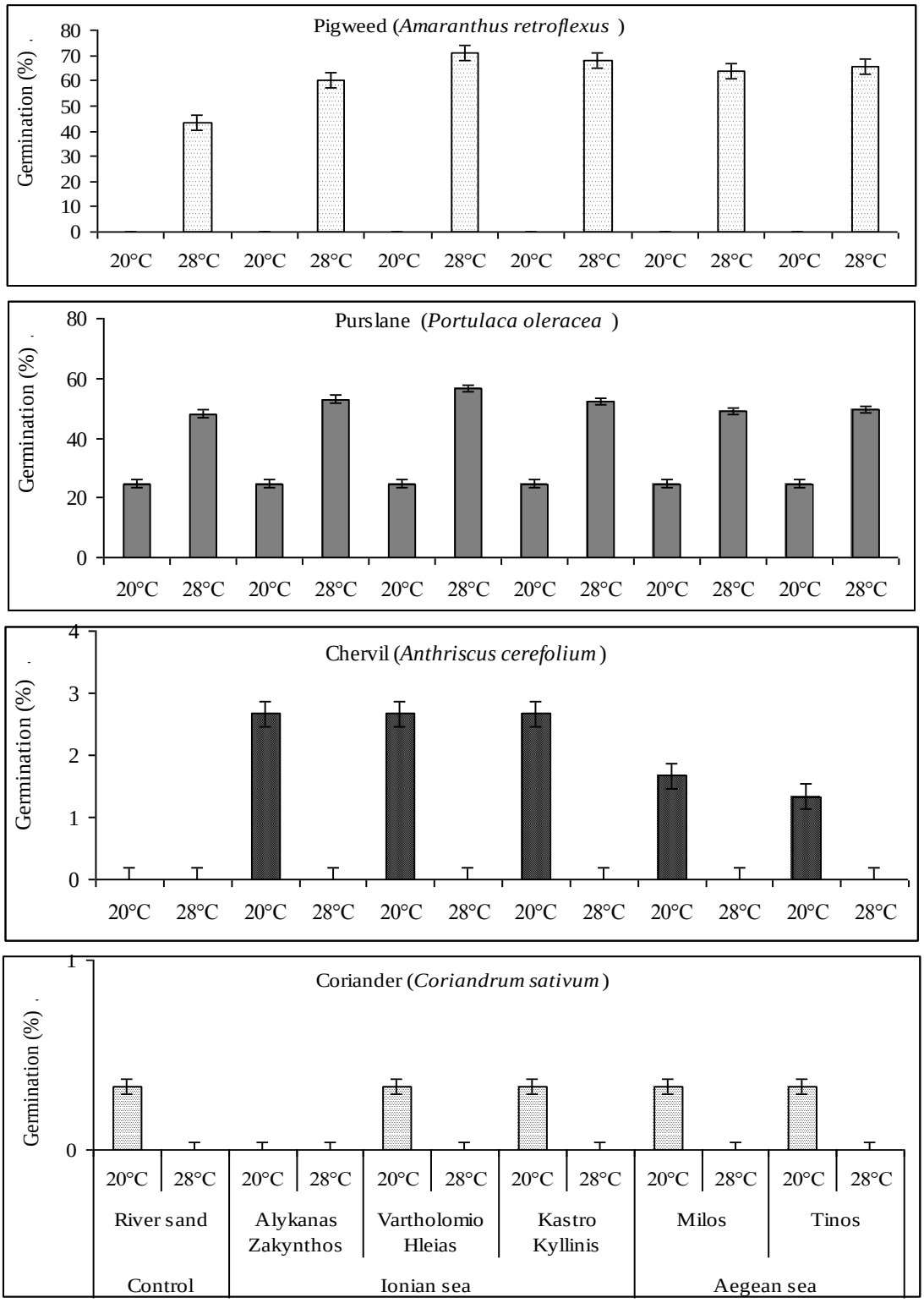


**FIG. 4: EFFECT OF SAND DUNES ON GERMINATION (EXPRESSED AS %) OF PIGWEED (*AMARANTHUS RETROFLEXUS*), PURSLANE (*PORTULACA OLERACEA*), CHERVIL (*ANTHRISCUS CEREFOLIUM*), AND CORIANDER (*CORIANDRUM SATIVUM*).**

The seed germination percentage of pigweed at 28°C in the river sand (control) was 44%, significantly lower than the coastal dune percentages. Its seed germination percentage in sand of coastal dunes from mainland Greece (Kastro Kyllinis (68%) and Vartholomio Ileias (71%) was significantly higher than the corresponding one from insular Greece (Zakynthos (60%), Milos (64%) and Tinos (66%) islands) (Fig. 4, 5).

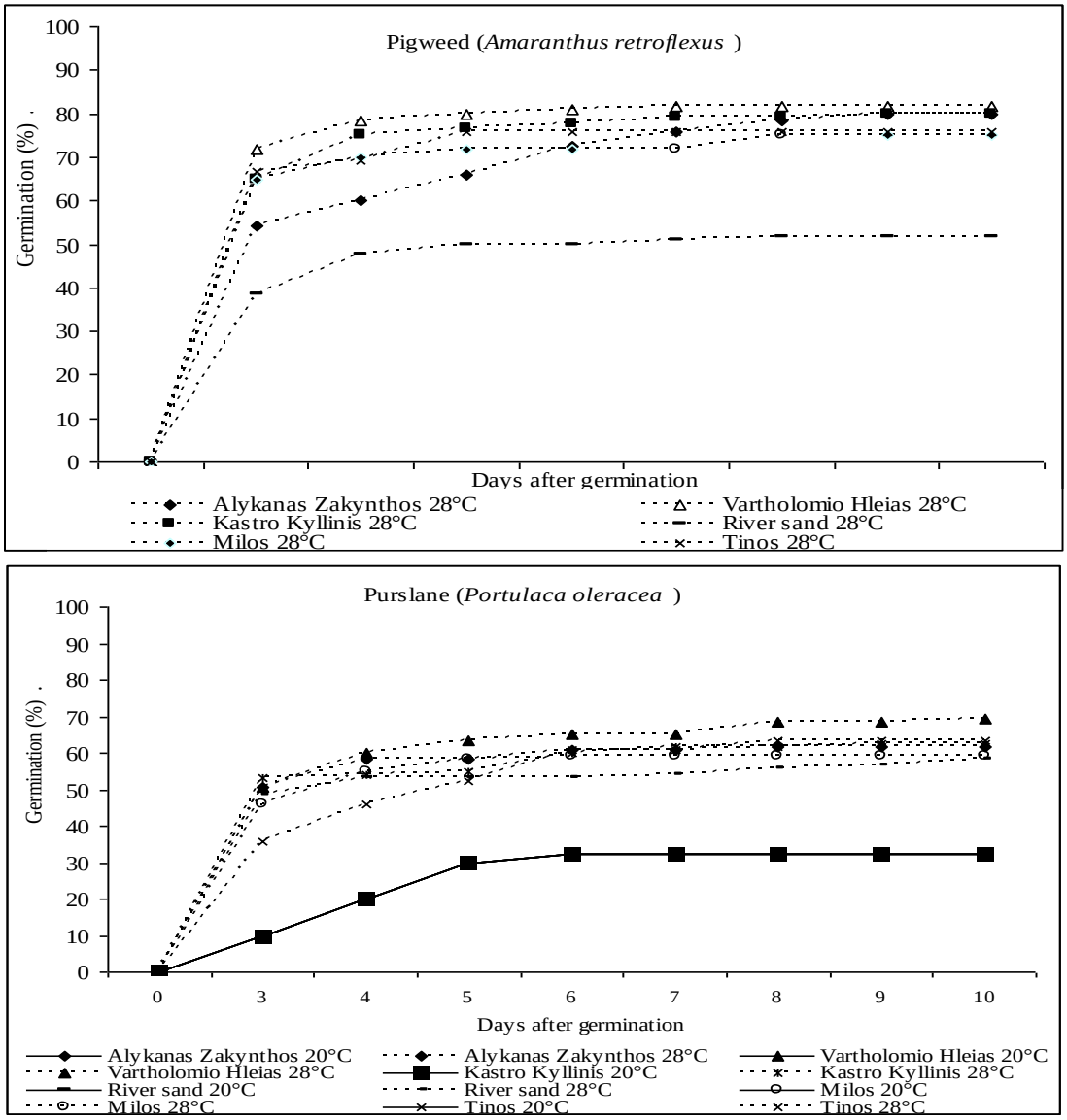


**FIG. 5: EFFECT OF SAND DUNES ON GERMINATION (EXPRESSED AS %) OF PIGWEED (*AMARANTHUS RETROFLEXUS*), AND PURSLANE (*PORTULACA OLERACEA*)**

The seed germination observation of purslane lasted 10 days at a temperature of 20°C and 28°C in a controlled plant growth chamber (Fig. 4, 5). The radicle emergence of the purslane seeds occurred in day 3 after the seeds were placed in Petri dishes.

Data presented in Figure 4 show that the seed germination percentage (25%) of purslane at 20°C did not affect any of the sand soils (Fig. 4, 5). Its seed germination percentage at 28°C in the river sand (control) was low (48%) and in sand of coastal dunes from the Ionian sea (Alykanas, Zakynthos island (53%), Kastro Kyllinis (52%) and Vartholomio Ileias (57%)) it was significantly higher than the corresponding one from the Aegean sea (Milos (49%) and Tinos (50%) islands) (Fig. 4, 5).

The germination percentages of chervil and coriander clearly showed different reactions to temperature. No germination occurred at the 28°C temperatures and germination at 20°C was also very low. Maximum germination percentage of chervil was recorded at 20°C in sand of coastal dunes from the Ionian sea (3%) (Alykanas Zakynthos island, Vartholomio Ileias and Kastro Kyllinis), whereas the minimum was recorded in sand of coastal dunes from the Aegean sea ((Milos area (2%), and Tinos area (1%)) islands (Fig. 4).

The results showed that the temperature and sands of coastal dunes significantly affected germination velocity. This was estimated by using a modified Timson index as a measure of pigweed seed germination Maximum germination velocity of pigweed seeds at 28°C was observed in sand of coastal dunes from Vartholomio Ileias (mainland Greece), whereas the

lowest rate was in the river sand (control). Moreover, the germination velocity of purslane seeds at 20°C in all sand soils was less than at 28°C. The germination velocity of chervil and coriander are null (Table 1).

**TABLE. 1: EFFECT OF SAND DUNES ON TIMSON INDEX GERMINATION VELOCITY OF PIGWEED (*AMARANTHUS RETROFLEXUS*), PURSLANE (*PORTULACA OLERACEA L.*), CHERVIL (*ANTHRISCUS CEREFOLIUM*) AND CORIANDER (*CORIANDRUM SATIVUM*).**

| Timson index, germination velocity | | | | | | |
|---|---|---|---|---|---|---|
| | **Sand dunes** | **Temperature °C** | **Pigweed (*Amaranthus retroflexus*)** | **Purslane (*Portulaca oleracea*)** | **Chervil (*Anthriscus cerefolium*)** | **Coriander (*Coriandrum sativum* )** |
| Control | River sand | 20°C | _ | 3,3 | _ | _ |
| | | 28°C | 5,2 | 5,8 | _ | _ |
| Ionian Sea | Alykanas Zakynthos | 20°C | _ | 3,3 | _ | _ |
| | | 28°C | 8,0 | 6,2 | _ | _ |
| | Vartholomio Hleias | 20°C | _ | 3,3 | _ | _ |
| | | 28°C | 8,2 | 6,9 | _ | _ |
| | Kastro Kyllinis | 20°C | _ | 3,3 | _ | _ |
| | | 28°C | 8,0 | 6,3 | _ | _ |
| Aegean Sea | Milos | 20°C | _ | 3,3 | _ | _ |
| | | 28°C | 7,5 | 5,9 | _ | _ |
| | Tinos | 20°C | | 3,3 | | |
| | | 28°C | 7,6 | 6,3 | _ | _ |

## IV. DISCUSSION

The distinctly different chemical composition of the coastal sand dunes in Kastro Kyllinis, Vartholomio Ileias, Zakynthos, Milos and Tinos islands, show different characteristics in comparison with the dunes in the mainland and insular coastline of the Ionian Sea and the insular coastline of the Aegean Sea of Greece (Fig. 3). Sand elements may differ along a given coastline. For instance, along the Florida coast, the beaches primarily consist of quartz and calcium carbonate sand in the north, and quartz sand in the south (Maun 2009). Variations in the chemical composition of frontal dune sediments along the west coast of Jutland, Denmark, have been investigated by Saye and Pye (2006). The silicon (Si) content (89-97%) is the one most represented in all soils of coastal sand dunes. Similar results have been reported by Maazouzi et al. (2013) for the $SiO_2$ content in Algeria sand dunes (97%) and by Muhs et al. (2013) for the Sinai–Negev dunes of Egypt and Israel (~76 to 98%).

It is known that the seed germination is an important phase of plant development, since it gives it a better start. The above differentiations of the coastal dunes' chemical composition of the Ionian and the Aegean Sea exhibit different effects on the seed germination of four plants. The higher Si contents in sand from the coastal dunes of the Aegean Sea seem to inhibit the seed germination of some pigweed, purslane and chervil species by causing a deficiency in organic matter. However, little information is available on the effects of silicon on seed germination under salinity.

Moreover, the high aluminium (Al) contents in sand soils from the Aegean Sea islands reduced the seed germination of pigweed, purslane and chervil. Alamgir and Akhter (2009) reported that aluminium (Al) affected seed germination of different wheat varieties and the inhibitory effect increased with the increase of $Al^{3+}$ concentration.

The high seed germination percentage of the small seeds of purslane (60-71%) and pigweed (49-57%) in all sand soils at 28°C could result in tolerance to coastal dunes sand. Many studies on sand dunes have reported that very small seeds showed a high germination rate because of the small amount of moisture required for inhibition (Stairs 1986; Zheng et al. 2005). In sand dunes, seed germination is strongly related to available moisture (García et al. 2002).

The results showed that the low seed germination percentage (25%) of purslane at 20°C did not affect any sand soil. Lower temperature low seed germination percentage of purslane was noted by Baskin and Baskin (1988) at 20/10°C and 15/6°C day/night temperatures and by Miyanishi and Cavers (1980) at 25/10°C. No seed germination was observed in pigweed at 20°C in all sand soils. These results are in agreement with earlier results, which showed that seed germination of pigweed is inhibited by low temperature **(**Ghorbani et al. 1999; Guo & Al-Khatib 2003; Steckel et al. 2004**)**.

In this paper, the absence of chervil seed germination at 28°C and the poor germination at 20°C in all sand soils showed that seeds are germinated at temperatures lower than 13°C (Bubel 1988).

The results of this study showed no seed germination at 28° and low germination at 20°C of coriander in all sand soils due to the fact that it is a plant sensitive to salinity (Liopa-Tsakalidi et al. 2011; Ewase et al. 2013; Fredj et al. 2013).

The study has shown that the soils of coastal sand dunes favoured seed germination of the weeds pigweed and purslane at 28°C, while inhibiting the seed germination of the herbs chervil and coriander under two different temperatures. These results indicate that weed seeds have adapted to the coastal sand dunes environment. In further laboratory and field experiments it would be interesting to understand the ecological role of the pigweed and purslane seeds during seed germination in coastal sand dunes environments.

## V. CONCLUSION

The study has shown differences between the percentage of various sand elements in the dunes of the mainland and insular coastline of the Ionian Sea and the insular coastline of the Aegean Sea of Greece. Moreover, the study has shown that there was no germination of chervil and coriander seeds in coastal dune soils and sand soils favoured germination of pigweed and purslane at 28°C. These results indicate that the two plants, pigweed and purslane, have adapted to the coastal sand dunes environment.

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