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Morphological response of a cactus to cement dust pollution

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ABSTRACT

Cement dust from cement plants around the world has multiple negative effects on organisms and their environment. Cement's effects come from its strongly alkaline nature and high content of heavy metals. Previous studies on plants have documented that cement dust deposition can influence plant vegetative growth, the lipid and ionic composition of tissues, and foliar temperature. Here we evaluate the effects of cement dust coming from a plant in western Argentina on the morphology of the cactus *Tephrocactus aoracanthus*. In sites located at 0.15 km, 2 km and 6 km from the cement plant, we recorded five morphological attributes of the cactus: length and number of spines, cladode (stem) diameter, and fresh and dry weight. We also transplanted plants in situ to evaluate the effect of distance from the cement plant. In addition, we set an experiment spreading cement dust weekly on the aerial and ground parts of the cactus. Results of our field observational and experimental studies indicate that cement dust deposition on aerial parts of the plant leads to increased spine length, number of spines, and wet and dry weights of cladodes.

1. Introduction

Atmospheric dust is a major source of pollution, especially in dry climates. Mineral dust from industrial activities is a source of heavy metals, many of which are known to be toxic (Gbadebo and Bankole, 2007). Industrial production of cement is prominent among industrial activities as a major emitter of noise, gas and particulate matter (Farmer, 1993).

Portland cement is the most widely used cement in civil constructions worldwide, particularly for its being an essential ingredient of concrete. It is primarily composed of oxides of calcium, silicon, aluminum, iron, magnesium, sulfates and other substances in small percentages. During the production process, the mixture of minerals is sent through a rotating tube furnace set almost horizontally, a so-called "burning" process. Different pollutants, such as heavy metals, dioxins, particulate matter, iron, aluminum, silicon, copper, sulphur dioxide and nitrogen dioxide are emitted during this process (Tajudeen and Okpuzor, 2011).

Numerous previous studies conducted in different regions of the world have addressed the effect of cement dust on wild and cultivated plants (Dziri and Hosni, 2012; Prajapati, 2012; Iqbal and Shafig, 2001; Pärn, 2006; Liblik et al., 2003). All of them show its negative effects on plant growth and productivity. Dust deposition on the plant results in decreased light available for photosynthesis and increased leaf temperature due to the change in leaf surface optical properties (Prajapati, 2012). Iqbal and Shafig (2001) observed a significant reduction in size, number of leaves and foliar area in herbaceous plants exposed to cement dust in cultivation experiments. Pärn (2006) found that pollution from cement dust negatively affected the radial growth of pine tree trunks in forests of Estonia. Also in conifers, Dziri and Hosni (2012) noticed that cement dust negatively affected lipid peroxidation, essential oil composition and the activity of antioxidant enzymes on Aleppo pine needles (leaves). Overall, a negative effect is observed on the physiology of plants exposed to cement dust (Prajapati, 2012; Iqbal and Shafig, 2001; Pärn, 2006; Liblik et al., 2003).

With respect to the impact of cement dust pollution at the plant community level, in Estonia's boreal forests, Paal et al. (2012) observed that calcium and pH levels increased as the distance from Kunda cement plant decreased. Said change was accompanied by an increase in species richness and a change in plant community composition toward a greater presence of alkali-tolerant calcareous species. Similarly, in a forest of Lithuania, Stravinskienė (2011) noticed that the number of tree, shrub and bryophyte species increased with decreasing distance from Akmenės Cementas plant, while the number of herbs decreased.

The gradual deposition of cement dust also has a strong impact on the physical and chemical properties of the soil. Paal et al. (2012) found high levels of calcium, potassium and magnesium as distance decreased from Kunda cement plant in the north of Estonia, which has been

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operating and emitting dust for over 140 years. Calcium levels increased up to tenfold from the unpolluted to the most polluted sites. A reduction was also recorded in the levels of nitrogen at smaller distances from the plant. With regard to heavy metals, Abimbola et al. (2007) found high concentrations of cadmium, lead, copper, zinc and nickel in the soil, coming from cement dust deposition in the vicinity of a cement plant in the southeast of Nigeria. They associated these high concentrations of heavy metals with a greater preponderance of diseases occurring in the human population living in the surrounding areas. In the peat that covers the ground of a Nordic forest, Stravinskienė (2011) recorded an increased concentration of strontium, barium, titanium, manganese, nickel, chromium, copper and boron microelements as the distance from Akmenės Cementas plant in Lithuania decreased. Besides the high concentration of metals, many studies have documented a strong increase in pH in the soil surrounding cement plants due to deposition of the strong basic oxides that cement dust is composed of (CaO, SiO, Al_2O_3 and Fe_2O_3) (Liblik et al., 2003; Paal et al., 2012; Rahman and Ibrahim, 2012; Iqbal and Shafig, 2001).

A plant exposed to cement dust pollution might perceive the damage produced in its tissues by pollutants as damage caused by herbivores, which might trigger responses similar to those triggered against herbivores. When a plant is attacked by herbivores, a series of “defense” responses are activated to deter the attack and reduce the likelihood of a new attack; also activated are a series of “civil” responses that enable the plant to recover lost tissues and reorganize those tissues that had remained safe, in order to optimize its growth rate. These physiological responses are part of an integral response of the plant that affects its distribution patterns and its use of resources. Civil-type responses include a decreased root growth, mobilization of reserves from roots to stems, increased resource assimilation by roots and stems, increased photosynthesis rate in undamaged leaves, activation of new meristems and alteration of leaf senescence patterns (Karban and Baldwin, 1997). Defense responses are based on the development of structures of physical or chemical nature. Chemical defenses include secondary metabolites, higher fiber content and low nutrient levels in tissues. Physical defenses are based on increasing the number and size of thorns (Brooks and Owen-Smith, 1994) and trichomes, and production of defensive compounds which are released to the surface, such as lignin, resins, silica or waxes which cover the epidermis and alter the texture of plant tissue, making it slippery or sticky.

In this work, we studied the effects of cement plant emissions on the cactus *Tephrocactus aoracanthus*. In a field trip we observed that plants of this cactus species occurring in the vicinity of Holcim plant have much longer spines than those growing far from it (J.M.E. Drack and D.P. Vázquez, personal observation). We also observed an important deposition of whitish dust, presumably from quarries and plant chimneys, on the plants and rocks of the area. In areas closest to the cement plant, dust accumulation forms hard greyish crusts that cover the ground, evidencing an important presence of dust resulting from reduced products such as Portland cement dust and cement kiln dust, and which grow hard in contact with environmental moisture. Based on these observations, we hypothesized that pollution generated by the plant is the cause for cacti, perceiving the impact of pollutants on their tissues as a herbivory action, possibly due to the damages produced by the strongly alkaline nature of dust and its physical effects on their tissues (for example, an increase in temperature of their photosynthetic surface area), to activate their civil and defense response mechanisms, promoting growth of the plant and multiplication and growth of its spines, as has been documented to occur in plants under attack by herbivores (Karban and Baldwin, 1997). If so, the damage caused by the alkalinity of cement dust could occur when dust is deposited on the photosynthetic aerial portion of the plant or when it modifies soil properties and acts through contact with cactus roots.

In particular, we pose two hypotheses: First, that aerial deposition of cement dust on the photosynthetic surface area of *T. aoracanthus* induces an increase in its biomass (biomass of cladodes and spines). Second, that cement dust, by modifying the physical, chemical and nutritional properties of soil, induces an increase in biomass (biomass of cladodes and spines) in *T. aoracanthus*. For both hypotheses we expect that (1) cactus biomass will increase as distance from the cement plant decreases, and that (2) cactus biomass will increase if cacti are transplanted from sites far from the cement plant to sites near it. These two predictions are compatible with both hypotheses and do not allow us to rule out one or the other, but help us understand the nature of the effects of proximity to the cement plant on the cactus population. Predictions that allow us to discriminate between one or the other hypothesis are the following. For the first hypothesis we expect that (3) cacti that are experimentally sprayed with cement dust on their photosynthetic surface area will develop a greater biomass than non-sprayed cacti growing in the same conditions. For the second hypothesis we expect that (4) cactus biomass will increase if the substrate they live in is experimentally treated with cement dust.

2. Materials and methods

To evaluate our hypotheses, we conducted observational and experimental studies to assess the morphological response of the cactus to cement dust. These studies included in situ measurements and transplantation experiments, and experimental cultivation of cladodes in the experimental field in the campus of CCT CONICET Mendoza.

2.1. Study area and species

The study area stretches between 760 m and 830 m elevation in the Monte ecoregion, in the eastern foothills of the Precordillera, in Las Heras department, Mendoza province, Argentina. The flora of the area is composed of shrubs such as *Larrea divaricata*, *L. cuneifolia*, *L. nitida*; grasses like *Trichloris crinita*, *Stipa* sp., and *Papophorum* sp.; cacti such as *Trichocereus candicans*, *Teprocactus articulatus*, *Opuntia sulphurea* and *Tephrocactus aoracanthus* (Dalmasso et al., 1999).

T. aoracanthus is an opuntioid cactus plant inhabiting the provinces of La Rioja, San Juan, west of Cordoba, and north of Mendoza in Argentina (Fig. S1). It occurs in sandy or clayey plains, sometimes somewhat saline, or on stony slopes, between 400 m and 850 m elevation in the driest area of Argentina. This species reaches a height of about 35 cm, has globose cladodes, smooth spines, dehiscent dry fruit, and is dark green in color (Kiesling, 1984). It has great capacity for clonal reproduction by detachment of its cladodes and their subsequent rooting in the soil (Almirón and Martínez Carretero, 2013). This property, along with the low mortality of cladodes after being detached from their mother plant, facilitated their transport and establishment of experiments.

Holcim cement plant is ca. 6 km south from the south-eastern edge of Villavicencio Nature Reserve, in Las Heras department, Mendoza province, in Argentina's west (Fig. S2). This plant is operating since 1936, originally and up to a few years ago by the name of Minetti. It produces mainly Portland cement and provides it to the regional market in Mendoza and neighboring provinces.

2.2. Observational field studies

To assess prediction 1 (cactus biomass increases as distance from the Holcim plant decreases), six study sites were selected, located at growing distances to the north and south of the cement plant. Northwards, a site was selected at 0.15 km from the plant (“0.15-km north” site), another at 2 km (“2-km north” site), and a third site at 6 km (“6-km north” site). Similarly, southwards, a site was selected at 0.15 km, another one at 2 km, and the last one at 6 km (“0.15-km south”, “2-km south” and “6-km south” respectively). These six sites represent an approximately straight transect in north-south direction, where there is a significant reduction in elevation with respect to sea level (830 m elevation for the 6 km north site; 740 m elevation for the 6 km south site) (Fig. S3, Table S1). Measurements towards the east and west were hindered by the presence of mountains and a private property of Holcim on the west, and clayey soils on the east where *T. aoracanthus* does not grow.

At each site, we selected 10 cactus plants, located approximately every 20 m along a transect. Diameter of cladodes and length and number of spines in all terminal cladodes of each "branch" were determined for each plant. Each cladode has many helicoidally arranged brachiblasts from which spines sprout (Fig. S4). Each brachiblast has several spines emerging from the same point, but one of them is always longer than the others. Therefore, within the same cladode, we determined the total number of spines and the length of spines longer than the five brachiblasts closer to the apex of each cladode.

In addition, we collected the largest terminal cladode from 12 plants selected about every 20 m along a transect and, once in the laboratory, their wet and dry weights were measured using a precision scale. Dry weight was obtained after chopping the cladodes and oven-drying them for five days at 70 °C.

2.3. Transplanting experiment in the field

To evaluate prediction 2 (the length of cactus spines will increase if cacti are transplanted from sites far from the cement plant to sites near it, and it will decrease if they are transplanted from sites near the plant to sites far from it), 12 terminal cladodes (those at the end of each branch in the plant) were transplanted, all containing a newly forming cladode (a process that starts at the beginning of spring) from the "0.15-km north" site to a site four kilometers south of the cement plant ("4-km south" site), both sites being at 735 m above sea level. The same was done from the site four kilometers south of the plant to the "0.15-km north" site. At both sites, another 12 "control" cladodes were also collected, which were planted in an unshaded spot in their same place of origin, so as to monitor the stress caused by their separation from the mother plant.

Spine length was measured twice; the first measurement was made six months after transplanting, and the second one eighteen months after transplanting. At the beginning of the project, spine length was the only morphological attribute of plants used for assessing the predictions. The idea of also considering number of spines, cladode diameter and wet and dry weight (cactus biomass) came up later on. It is for this reason that only spine length was measured during the experiment, so prediction 4 was assessed considering only this variable.

2.4. Experiments in experimental field

In many experimental studies assessing the effects of pollution by cement plants, the treatment used was dust emitted by rotating furnaces during the burning of the raw materials of Portland cement and during the cooling and spraying of the clinker (cement kiln dust, CKD; Raajasubramanian et al., 2011b; Nanos and Ilias, 2007; Darley, 1966). CKD is the main particulate pollutant emitted by cement plants due to its small particle size and the high temperatures of the processes emitting this dust. CKD has a similar composition to that of Portland cement, sharing with the latter most of its main chemical compounds, but slightly differing in their proportions (Baghdadi et al., 1995). Hence, we consider the use of Portland cement appropriate as treatment for our experiment, as other authors have done (Raajasubramanian et al., 2011a; Ade-Ademilua and Obalola, 2008).

To evaluate predictions 3 and 4 (cacti that are experimentally sprayed with cement dust on their photosynthetic surface area will develop a greater biomass than non-sprayed cacti growing in the same conditions, and cactus biomass will be greater if the substrate they live in is experimentally treated with cement dust), 88 terminal cladodes were moved to the experimental field of CCT CONICET Mendoza. These cladodes, all of them in branching stage (with a newly forming cladode), were obtained from a site six kilometers south of Holcim plant (760 m elevation). Each cladode was randomly assigned to one of four treatments: aerial cement, a treatment that consists in weekly spraying 500 mg of "Holcim Fuerte" Portland cement dust only on the aerial part of cacti to assess the effect of dust only on their photosynthetic areas; cement on the soil, consisting in weekly spraying 500 mg of "Holcim Fuerte" Portland cement on the pot's soil, not on the plant's aerial part, so as to assess a possible fertilizing effect of dust; aerial cement and cement on the soil, weekly spraying 250 mg on the soil and another 250 mg on the plant's aerial part; and control, which was not applied any cement. Individual two-liter plastic pots were used, filled with soil prepared for gardening. A 0.5 cm-thick layer of sand was placed upon the soil to avoid excessive moisture on the pot's soil surface and thus prevent fungal infections in the cacti. All pots were deposited on the grounds of CCT CONICET Mendoza, outdoors, with no irrigation and directly exposed to sun and rainfall. The experiment was performed between November 2015 and March 2016.

Spraying 500 mg of cement dust per plant also mean a 2.3 g m^{-2} day^{-1} application rate. Mandre and Klosieko (1997) set an experimental sample plot for taking a 6 year's data from a very polluted place situated close to Kunda cement factory, north of Estonia. They found a 1.64–6.6 g m^{-2} day^{-1} dust deposition rate depending on velocity and direction of the winds. So, we think that a 2.3 g m^{-2} day^{-1} application rate is a good option to simulate the conditions of a highly polluted environment.

At the beginning of the project, this experiment was done using the dust obtained by grinding the hard grayish crusts that cover the soil of the 0.15-km north site as treatment. We thought that the dust removed from these crusts could have the same properties as the dust recently emitted by the cement plant. Thereafter, the experiment was developed and six months later no significant differences were recorded in the length of spines among groups under the soil treatment and the control. However, we realized that the dust from the crust, because of being exposed for a long time to rainfall, sunlight and air oxygen, loses its chemical and physical properties, so it presumably resembles more an inert dust. For this reason, we decided to perform another experiment, but this time using packaged "Holcim Fuerte" Portland cement dust, as described in the first paragraph of Experiments in experimental field section.

2.5. Statistical analyses

To evaluate the predictions of our hypotheses, we used linear models, assessed by using the summary function of R statistical environment (R Core Team, 2016). In all linear models, the response variable used was one of the biomass variables recorded in the study (average length of spines, number of spines, cladode diameter and wet or dry weight of cladodes). For predictions 1 and 2 (experimental application of cement in the experimental field), the predictor variable was treatment (aerial spraying of cement, soil spraying, aerial + soil spraying, and no spraying [control]). For prediction 3 on the change in cactus biomass in function of distance from the Holcim plant, the predictor variables were distance from the plant (0.15 km, 2 km or 6 km) and direction (north or south). For prediction 4 (transplanting experiments in the field), the predictor variables were treatment (transplanting or control) and distance from the cement plant (0.15 km and 4 km).

3. Results

3.1. Change in cactus biomass in function of distance from Holcim cement plant (prediction 1)

There was a significant effect of distance from the cement plant, direction (north or south) and interaction between distance and direction on the variables spine length and cladode diameter (Figs. 1a and

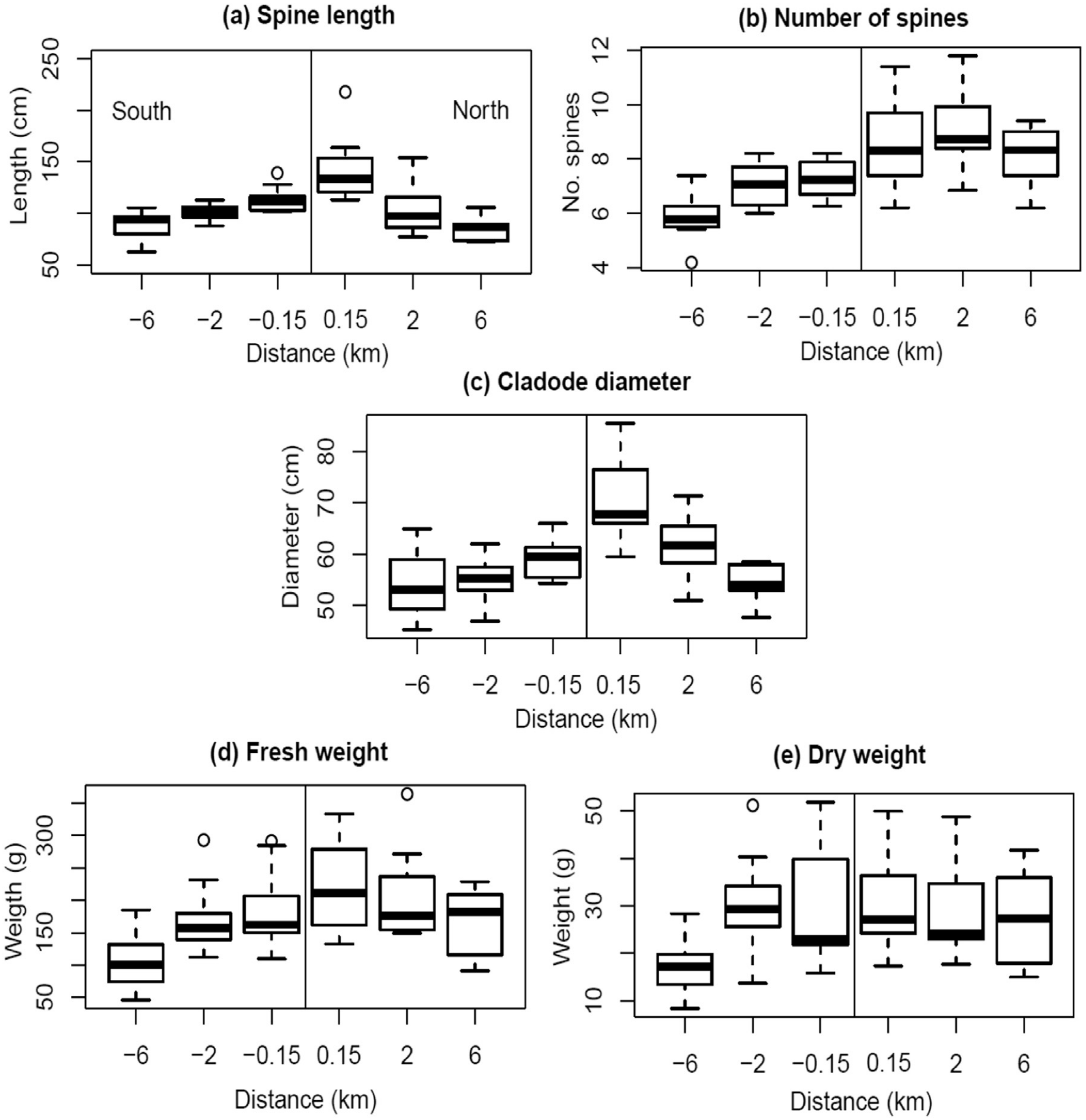

Fig. 1. Results of observational studies of morphological attributes of *Tephrocactus aoracanthus* as a function of distance to Holcim cement plant (prediction 1). In each box, the middle line represents the median, the lower and upper box limits represent the first and third quartiles of the data distribution, respectively, the error bars indicate the points within 1.5 times the interquartile distance, and the circles represent the extreme values beyond the error bars.

1c, Table S2). There was also an impact of distance on the wet weight of cladodes, but no effect of direction (Fig. 1d, Table S2). Instead, there was no significant impact of distance on number of spines or dry weight of cladodes (Figs. 1b and 1e, Table S2). Therefore, the plants growing near the cement plant tend to have higher values for some of the morphological variables studied than plants growing in more faraway sites but, in some cases, the magnitude of this effect depends on the direction in which distance increases.

3.2. Change in cactus biomass in transplanting experiments in the field (prediction 2)

According to the hypotheses put forward in the introduction, cactus biomass was expected to increase if cacti were transplanted from sites far from the cement plant to sites near it, and to decrease if they were transplanted from sites near the plant to sites far from it (prediction 2). In the first measurement, made in 2015 (Fig. 2a, Table S3), this prediction was confirmed when transplanting cacti growing near the plant to a faraway site (0.15 → 4 vs. 0.15 → 0.15 in Fig. 2a). Nevertheless, the prediction was not confirmed for cacti transplanted from a faraway to a nearby site (4 → 0.15 vs. 4 → 4 in Fig. 2a). In the measurement of 2016 (Fig. 2b, Table S3) there was also a significant difference in comparing 0.15 → 0.15 vs. 0.15 → 4 and a non-significant difference for the groups 4 → 0.15 vs. 4 → 4, but there was a tendency to longer spines in the group 4 → 0.15, consistent with prediction 2.

3.3. Change in biomass of cement dust-treated cacti in experimental cultivation (predictions 3 and 4)

There was no significant impact of cement dust treatments on spine length or number of spines in cladodes (Fig. 3, Table S4). For cladode diameter, only the aerial treatment had a marginally significant effect (Fig. 3, Table S4). The aerial and aerial-soil treatments had a significant effect on the wet weight of cladodes (Fig. 3, Table S4), and only the aerial-soil treatment had a significant effect on the dry weight of cacti (Fig. 3, Table S4). Thus, over these six months of treatment, we did not

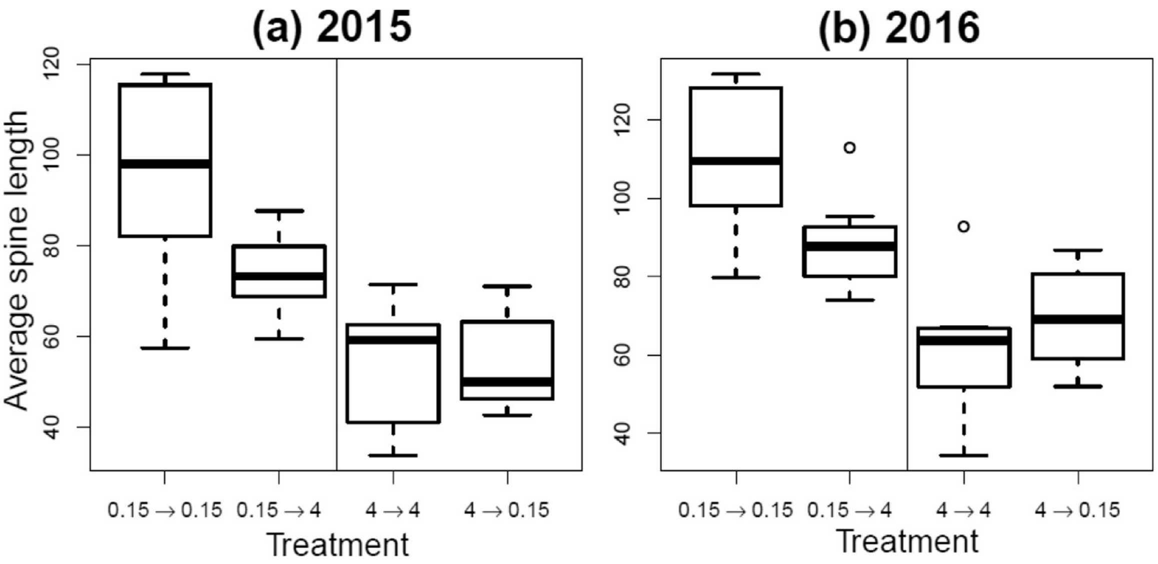

Fig. 2. Results of in situ transplant experiments in 2015 and 2016 to evaluate prediction 2. In the horizontal axis, arrows indicate the direction of cactus transplant; for example, "0.15 → 4" represents cacti originally growing at 0.15 km from the Holcim cement plant that were transplanted to 4 km, whereas "0.15 → 0.15" represents cacti originally growing at 0.15 km from Holcim that were transplanted in their site of origin (control). Other conventions as in Fig. 1.

detect any effect of treatments on spine length or number, but we did find an impact on the variables related to biomass and cladode diameter.

4. Discussion

The results of observational studies in the field (prediction 1) indicate that proximity to the cement plant affects the cacti's morphology and that the magnitude of this effect tends to be greater as we get closer to the plant from the south. The two years of transplanting experiments (prediction 2) also show us that distance from the plant affects cactus morphology. In transplanting cactus plants far away from the plant, spines grow less than near it, whereas when we place the cacti near the plant their spines take two years to show a trend towards greater growth. The experiment with cement dust (predictions 3 and 4) show us that when dust is sprayed on the photosynthetic surface area of cacti, the size, wet and dry weights of cladodes increase.

On the whole, these results support the first hypothesis that aerial deposition of cement dust on the photosynthetic area of cacti induces an increase in their biomass (biomass of spines and cladodes) presumably because cacti activate their civil and defense response mechanisms when perceiving damage in their tissues caused by the strongly alkaline nature of dust. The results of the experiment of transplanting cladodes in the field show us that when we transplant cladodes living near the cement plant to a faraway site, they reduce their spine length but, in turn, these transplanted cladodes develop larger spines than those originating at the site far from the cement plant despite growing in the same conditions. This observation may be an indication that cacti living near the plant are genetically different from those living far from it, and develop longer spines without being in contact with cement dust. This hypothetical genetic change in the cactus population growing near the plant could result from the action of selective pressures caused by the pollutants which have been acting for over 80 years, the time over which the cement plant has been operating. Future genetic studies among close-to-factory-living cactus and far-to-factory-living cactus would be necessary to assess possible genetical differences related to evolutionary pressures.

Even though there are many field studies characterizing the effects of cement plant emissions on vegetation (Dziri and Hosni, 2012; Fakhry and Migahid, 2011; Iqbal and Shafig, 2001; Maletsika et al., 2015; Liblik et al., 2003; Pärn, 2006; Prajapati, 2012; Pärn, 2006; Prasad and Inamdar, 1990; Ots et al., 2011; Raajasubramanian et al., 2011a, 2011b; Salami and Farounbi, 2002), we could not find any specific study addressing these effects on succulent plants from arid environments. There are several studies with experiments where cement dust has been spread on plants (Iqbal and Shafig, 2001; Maletsika et al., 2015; Raajasubramanian et al., 2011a, 2011b), but always on the aerial part, mostly on leaves; no study was found to spray dust on both the aerial part of plants and the soil. Regarding results, most of the studies we found characterizing the effects of cement plant emissions on plants in situ showed a decrease in biomass and in the photosynthetic area (foliar area) of herbaceous, tree and shrub species (Pärn, 2006; Prasad and Inamdar, 1990; Ots et al., 2011; Raajasubramanian et al., 2011a, 2011b; Salami and Farounbi, 2002). The present study differs from others in that we found a larger photosynthetic area and a greater plant biomass associated with a larger size and diameter of the cladodes living near the cement plant. Only one study was found reporting an increase in the biomass of plants when exposed to cement dust (Fakhry and Migahid, 2011). All this makes the study reported here particularly original and novel.

In the future, it would be important to conduct studies that allow delving into the mechanisms involved in determining the morphological responses of cactus plants found in the present study. In particular, we suggest performing hormone analyses on cacti treated with cement dust, in order to examine the presence of hormones involved in systemic defense responses. Also interesting would be to analyze the soil pH and ion composition at different distances from the cement plant to assess the effects of cement deposition on the soil and nutrient availability for plants, and to use dust traps to quantify the magnitude of cement dust deposition at different distances from the plant.

In conclusion, the results of observations and field experiments show us that there are pollutants nearby the cement plant, which are either being emitted or were emitted and accumulated over time, and which manifest their effects on the morphology of the cacti growing in the area. Across the experiment with cement dust, we were able to observe that cacti are more sensitive to pollutants when these are acting in contact with their photosynthetic aerial parts, and the transplanting experiment suggests that pollutants are being emitted through the air near the cement plant, mainly dust. Deposition of great amounts of dust near the cement plant is much evident (Fig. S4), even forming thick hard cement crusts that cover the ground. Rocks, plants and earth have turned a grayish color due to deposition of this pollutant. There is an

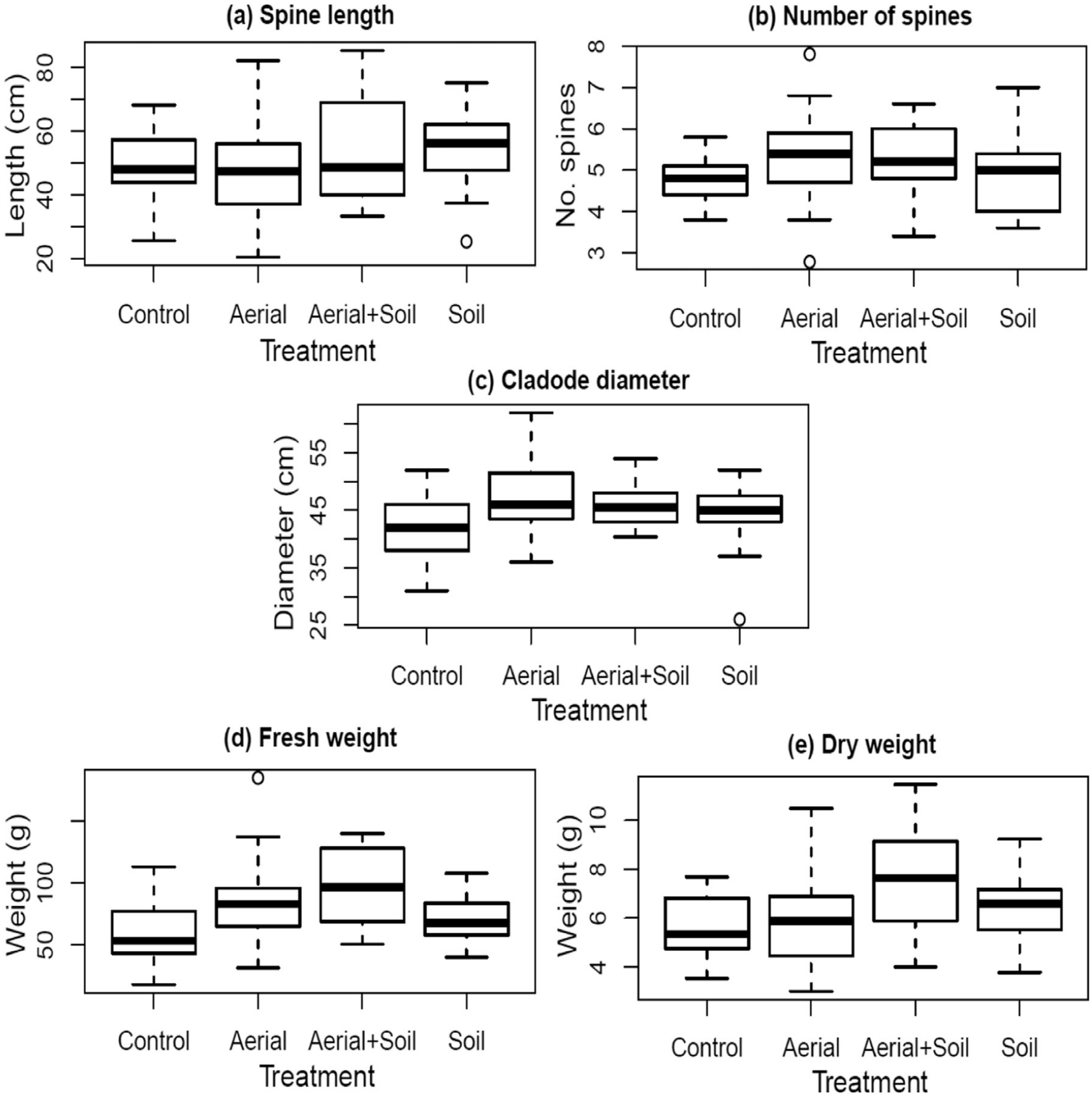

Fig. 3. Results of experimental application of cement dust to plants grown in pots in the in the campus of CCT CONICET Mendoza, to evaluate predictions 3 and 4. Other conventions as in Fig. 1.

urgent need to improve control of the emissions by Holcim cement plant and to clean the cement crusts covering the areas adjoining the plant. Unfortunately, there are no other studies addressing the impact of the pollution caused by this plant in particular, so a broad research area is open to further studies.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2017.10.046.

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