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Biomonitoring of ozone: A tool to initiate the young people into the scientific method and environmental issues. A case study in Central Italy

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ABSTRACT

The wide diffusion of tropospheric ozone is a major environmental problem, in urban area as well as in rural and remote localities. Its increasing man-related levels are connected to severe impacts on human life and welfare, in terms of adverse health effects, damage to manufacts and injury to plants. Biological monitoring is a powerful tool for filling the gap between the causes and the effects of environmental toxins, as bioindication agents assess in an easy-to-detect fashion the effects of pollution on (selected) biota. A project was launched in 2012 to involve some 190 students (ages 11–16) from three schools in Central Italy in biodetection of ozone effects with the hypersensitive plant *Nicotiana tabacum* Bel-W3. The project also involved teachers and families of the pupils. Results implied the reading of 12,000 biological data (ozone injury on cotyledons) and were fortified by data captured by four automatic analyzers (1300 raw data of hourly means). Biological and chemical data compared favorably and were treated with geostatistical methods; results are exposed in the form of cartographic restitutions. Under the guidance of their teachers, the pupils had several opportunities to practice with many basic and applied study areas and disciplines and were initiated into the scientific method in a simple and absorbing manner. Though primarily an educational exercise, the survey provided sound research elements and the picture of pollution that emerged has increased the knowledge of air quality in the area. Biomonitoring is confirmed to be a powerful tool to involve young people in environmental topics.

Introduction

Air quality is likely to be the environmental issue of most concern in the future (Beaumont et al., 1999). In industrialized countries of northern hemisphere, ground-level (tropospheric) ozone (O_3) concentration has risen by 1–2% per year, so that nearly one-quarter of the earth's surface is currently at risk from this pollutant in excess of 60 part per billion (ppb) (Ashmore, 2005) (for O_3, 1 ppb is 1.96 $\mu g\,m^{-3}$ at 20 °C and 101.325 kPa). Studies have shown that ambient O_3 not only induces airway inflammation and decreases lung function, but also is associated with elevated mortality from morbidity of respiratory disorders; it is accepted that impacts on human health occur above 50 ppb (daily 8 h average concentration, WHO, 2006), even if negative effects are thought to occur at lower concentrations (such as those around 35 ppb), some individuals being more susceptible than others. Environmental impact especially on natural ecosystems and crop productivity (quantity and quality) is relevant as well (The Royal Society, 2008). O_3 is also an important greenhouse gas with a relevant radiative forcing. So, O_3 must be regarded as a key environmental and sanitary problem, affecting well-being of citizen, plants, ecosystems and even non-living materials.

Unlike many other air pollutants, O_3 is not directly emitted: it is a secondary pollutant formed by sunlight driven reactions involving volatile organic compounds and nitrogen oxides. These precursors may arise from natural biogenic sources, but are mainly related to vehicular traffic. In Italy, as well as in the Mediterranean countries, ground-level concentrations of ozone are relevant due to the abundance of precursors and the favorable ambient conditions (high temperature and solar radiation) (González-Fernández et al., 2013). Climatic models predict that record-breaking temperature anomalies (heat waves) will become more intense, longer lasting and/or more frequent; as a consequence exposure of sensitive targets to high levels of O_3 is to be expected (Pellegrini et al., 2007). Several projections indicate O_3 concentrations reaching 75 ppb (daily 8 h of summer seasonal average concentration) over much of Europe

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by 2100 (Mills et al., 2013). The issue is a priority not only in urban areas (where the greatest production of anthropogenic precursors takes place, mainly as a consequence of road traffic), but also in rural and remote areas (Sicard et al., 2013).

The only practical management strategy is to control the emissions from human activities that lead to O_3 formation. As combustion processes due to vehicular traffic represent the prominent source of O_3 precursors, the only realistic way to minimize the presence of this pollutant in the troposphere is to reduce drastically traffic burden. This requires viable solutions that have at their core measures that mean deep modifications in the mobility habits of many people and a shift away, by the general public, from complete reliance on the car for all their transport needs. This is a deep cultural change and relevant popular consensus should be reached by decision makers to overcome traditional resistance against such initiatives.

Environmental education provides high potential to provide information to young people to reflect upon environmental questions. Environmental education programs enhance the environmental literacy of participants, which is generally comprised of knowledge, awareness, skills, attitudes, dispositions, and competencies believed to equip people with what they need to effectively analyze and address important environmental problems (Stern et al., 2013).

The present paper describes an holistic experience (*sensu* Stern et al., 2013) based on a biomonitoring campaign devoted to mapping O_3 distribution in a large area of Central Italy by means of sensitive indicator plants. The entire process included a pre-experience preparation and a post-experience follow up: teachers, young people and their families have been involved in theoretical and practical open-air activities aimed at evidencing severe biological damage induced by 'normally' polluted air.

Materials and methods

The actual O_3 levels in the study area during the campaign were measured by four photometric analyzers operated by local environmental authorities (ARPAT) and located in Pisa (86,000 inhabitants), Pomarance (6000 inhabitants), Pontedera (28,000 inhabitants) and Santa Croce sull'Arno (14,000 inhabitants). Raw data (in terms of some 1300 hourly averages) were processed to describe the typical daily profile, to individuate the maximum daily hourly mean (M1) and compute the AOT40s (accumulated exposure over a threshold of 40 ppb, 08.00 a.m.–08.00 p.m. – *sensu* de Leeuw and van Zantwoort, 1997).

Meteorological data were measured at Pontedera; the data were compared to the historical series (2000–2011) provided by Centro Funzionale Regionale di Monitoraggio Meteo-Idrologico of Regione Toscana.

The methodology of biological monitoring described in Lorenzini and Nali (2004) and Nali and Lorenzini (2007) was accurately followed. The ready-to-use miniaturized kit (which is $13 \times 9 \times 2$ cm high and contains 24 mini-wells) developed and patented by Lorenzini (1994) and validated by the Italian Environmental Protection Agency was adopted together with tobacco germlings (*Nicotiana tabacum*) cv. Bel-W3 (O_3-hypersensitive) raised in charcoal-filtered air before ambient air exposure. In brief, realistic O_3 mixing ratios (above 40 ppb for at least 4 h) induce on leaves and cotyledons minute bifacial grayish necrotic spots; such symptoms are pathognomonic, easily detectable (an hand lens may be useful) and evaluated in quantitative terms with the help of proper iconography. The percentage of the area of each cotyledon and leaf covered by the characteristic lesions produced by O_3 was assessed visually by comparison with spot standards and recorded on standard score sheets. Five classes of injury were distinguished, covering the following ranges of necrotic leaf and cotyledon adaxial surface: 1, no lesions; 2, l–10%; 3, ll–25%; 4, 26–50%; 5, >50% area covered by lesions. This scale was adopted to quantify the extension of cotyledonar damage after a week of exposure to ambient air. All the pupils were asked to assess visual symptoms. The readings were also verified independently by an experienced operator, whose scores were regarded as 'reference value'.

Three schools (8 classrooms, 188 students) have been involved; they were attended by pupils living in 14 municipalities of Valdera District (Province of Pisa, Tuscany, Central Italy), covering an area of some 600 km^2 (elevation 5–270 m a.s.l.). One hundred and twenty four pupils were attending secondary schools of the first level (age 11–12) and 64 secondary schools of the second level (age 15–16). Monitoring points were 132, mainly represented by private gardens owing to students' families or their neighbors.

The first step consisted of brief but intensive educational training sessions devoted to the teachers ('teacher training') (spring 2012): topics covered included the basic issues connected with O_3 pollution, its genesis and spatial/temporal diffusion, its biological impact, with special regard to human health. Basic concepts of biological monitoring were also discussed and international specialist literature shared. The results of previous similar experiences, collected in a CD, were critically analyzed. Communication was based upon simple but effective concepts, often treated in popular terms; special care was devoted to the difference between 'good' (stratospheric) and 'bad' (tropospheric) O_3 (the friend-or-foe approach was particularly appreciated). Photographic material (symptoms induced by O_3 on sensitive plants) was distributed and discussed.

Some 12,000 cotyledons were read and Cotyledonar Injury Index (CII) data processed. Spatial distributions of CIIs were obtained through interpolation, transforming discrete data into a continuous distributional model, using kriging as an interpolation algorithm (using Surfer 5.02 software, Lorenzini and Nali, 2004). The entire campaign lasted two weeks (18–31 May 2012) and consisted of two successive exposures of fresh kits of indicator plants.

Reproducibility (i.e. the concordance between estimates of pupils and expert scorer) was evaluated using k statistics (Francini et al., 2009), which is an estimate of the agreement between qualitative responses of two independent observers: $k = (p_o - p_c)/(1 - p_c)$, where p_o is the total proportion observed and p_c is the expected one. When p_o equals p_c, $k = 0$; maximum k value is 1 and this occurs when there is total agreement between scorers. To test for significance of an obtained k, a z statistic is used $\left[z = k/\sqrt{var(k)}\right]$. In order to maintain consistent nomenclature when describing the relative strength of agreement associated with k statistics the following labels were assigned to the corresponding ranges of k: $k > 0.75$ = excellent; $0.40 \leq k \geq 0.75$ = moderate/substantial; $k < 0.40$ = fair (Fleiss et al., 1979). The self-directed learning coefficient (L) has been estimated for a set of students (one classroom of the first level and another of the second level) who have been called to evaluated the very same experimental material in two successive readings: L = (number of exact responses at the 2nd reading − number of exact responses at the 1st reading)/(total number of cotyledons read − number of exact responses at the 1st reading) (Lorenzini et al., 2000).

Results and discussion

Ozone pollution (instrumental monitoring)

As expected, O_3 was a relevant presence in the troposphere of the investigated area. Even if the true 'photochemical season' usually starts from the beginning of June, the concentrations observed during the 3rd and 4th week of May were significant under the ecological and sanitary points of view. Fig. 1 describes the daily

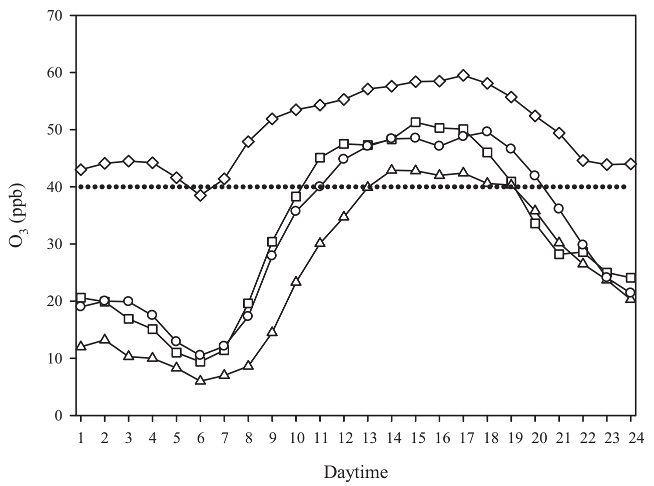

Fig. 1. Daily mean profiles ('typical days') of the ground level ozone concentrations as detected by automatic analyzers in the four monitoring locations [Pisa (○), Pomarance (◊), Pontedera (□) and Santa Croce sull'Arno (△)] from 18 to 31 May 2012. Dashed line parallel to the *x*-axis shows the threshold of 40 ppb. Day time is expressed in terms of 'local solar time' (GMT+1).

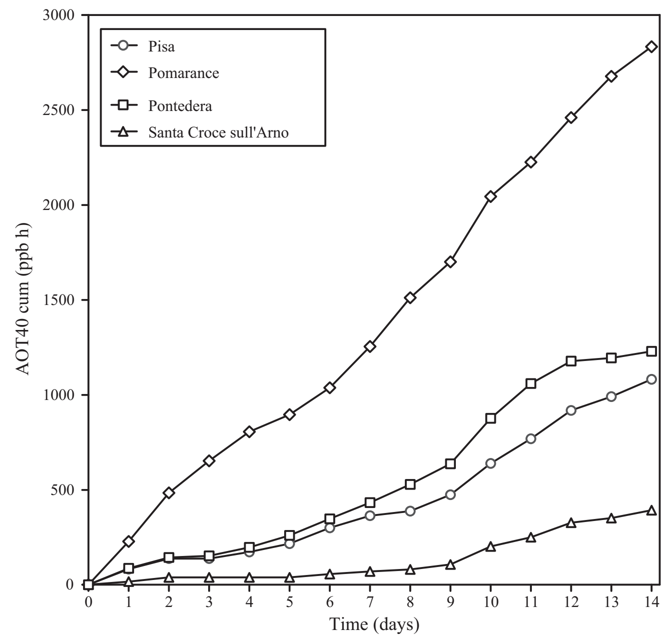

Fig. 2. Cumulated course of ozone AOT40s for the period 18–31 May 2012 as observed in the four instrumental monitoring stations (Pisa, Pomarance, Pontedera and Santa Croce sull'Arno.

mean profiles ('typical day') on the basis of data captured by the automatic analyzers located in four stations related to the study area. The peculiar, bell-shaped circadian profile is similar in Pisa, Pontedera and Santa Croce sull'Arno; this is a classic behavior, with low levels during the night (10–20 ppb), a negative peak around 6–7 a.m. (beginning of traffic, production of nitrogen monoxide and 'titration'/depletion of O_3); afterwards we observe rapidly increasing concentrations following of a couple of hours the profile of solar radiation; peak values are reached early in the afternoon (45–50 ppb, hourly average concentration). Slightly different is the profile of Pomarance (which is the only rural/remote location amongst those investigated). Here values are constantly higher than the other stations and night time concentrations do not fall below the threshold of 40 ppb; maximum daily values are around 60 ppb. The low levels of NO may be related to this behavior. All these findings are in full agreement with general trends of ground level O_3 in Europe (Vingarzan, 2004). Maximum M1 (76 ppb) has been observed on May 27, between 04.00 and 05.00 p.m. in Pontedera. Data of the cumulative descriptor AOT40 are synthetized in Fig. 2. As expected, the highest values (2833 ppb h, which means a daily average of 202 ppb h) are recorded at Pomarance, the station which shows the highest number of daily means above the threshold of 40 ppb; on the other side, at Santa Croce sull'Arno the lowest values (393 ppb h) were recorded. It should be stressed that the Directive 2008/50/EC of the European Parliament and of the Council of 21 May 2008 on ambient air quality and cleaner air for Europe reports as target value for the protection of vegetation an AOT40 of 9000 ppb h (averaged over 5 years) in the averaging period May–July. Taking into account the mean AOT40 observed at Pomarance (202 ppb h) we must stress that in three months the total bulk would be an AOT40 of some 18,000 ppb h. The 'critical level' for protecting agricultural crop species from 5% yield losses was indicated as an AOT40 threshold of 3000 ppb h, not to be exceeded over a 3-month period (WHO, 2006); in our case this limit value has been approached after a mere couple of weeks. In all cases O_3 concentrations were well above background levels and potentially able to induce visible symptoms on the leaves of sensitive biological targets.

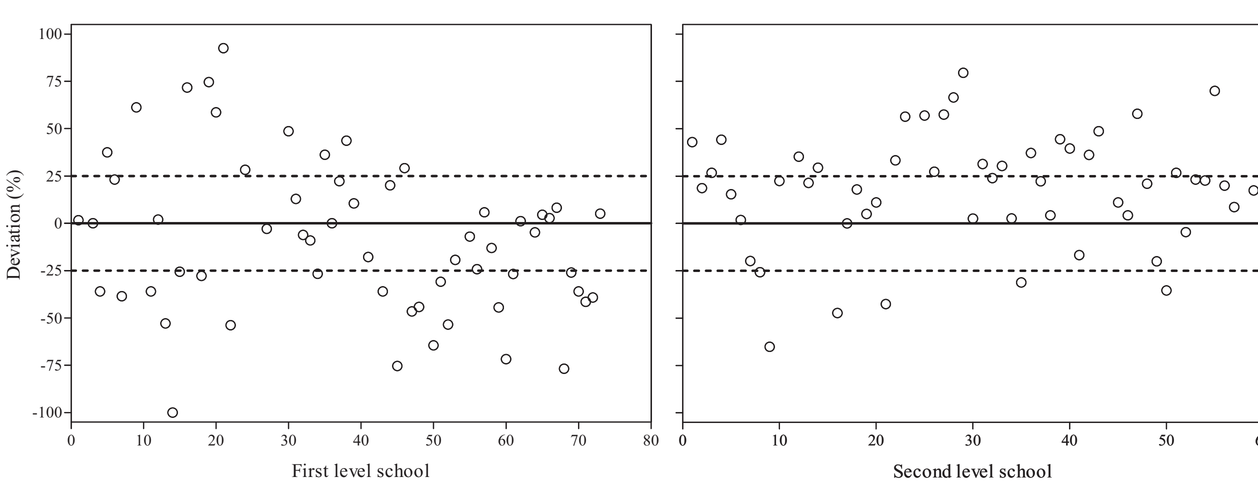

Fig. 3. Relative scattered distribution of the average Cotyledonar Injury Index reported by each pupil from the 'actual' figure as detected by the expert scorer (full line); dotted lines show the interval between plus and minus 25%. Values above or below 100% are not shown; they are 14 over-estimators and none under-estimator in the first level schools and 5 over-estimators and none under-estimator in the secondary school.

The period 18–31 May 2012 in the investigated area was characterized by a daily mean temperature of 17.5 °C (absolute minimum 12.3 °C; absolute maximum 23.1 °C) and 4 rainy days (for 2.74 mm of rain). Compared to the historical set of data (the same temporal window in the period 2000–2011), this span should be regarded as almost 'typical', as differences were negligible (historical data were: absolute minimum 13.3 °C; absolute maximum 25.4 °C, rainy days 2.5, for 1.30 mm of rain).

Biological responses (indicator plants)

Most of the kits delivered to pupils (263 out of 294, i.e. 89%) were safely recovered. All the germlings exposed in all the stations showed typical bifacial necrotic symptoms after a few days of exposure to ambient air. The response was fast and spectacular and surprised the pupils. They, however, were well aware of the phenomenon. Fig. 3 describes the results referred to the differences between CIIs given by pupils and those given by the expert scorer. After the first week of exposure, 33% of the secondary schools of the first level had expressed scores within a range of ±25% in comparison with the reference scorer. There was a balance between under- and over-estimations. Results of the second week are alike and are not reported. Forty-four of the scores of the students of the secondary school of the second level fall in the range of ±25% in comparison with the results given by the reference scorer.

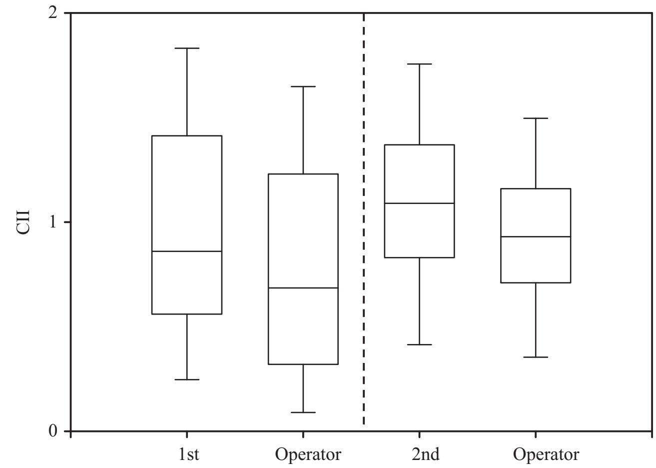

Fig. 4. Box and whisker representation of CIIs as scored by the pupils of the schools of the first (*left*) or second level (*right*) in comparison with those of the experienced scorer. Each box encloses the 25th, 50th (median) and 75th percentiles; whiskers above and below the box indicate the 95th and 5th percentiles, respectively.

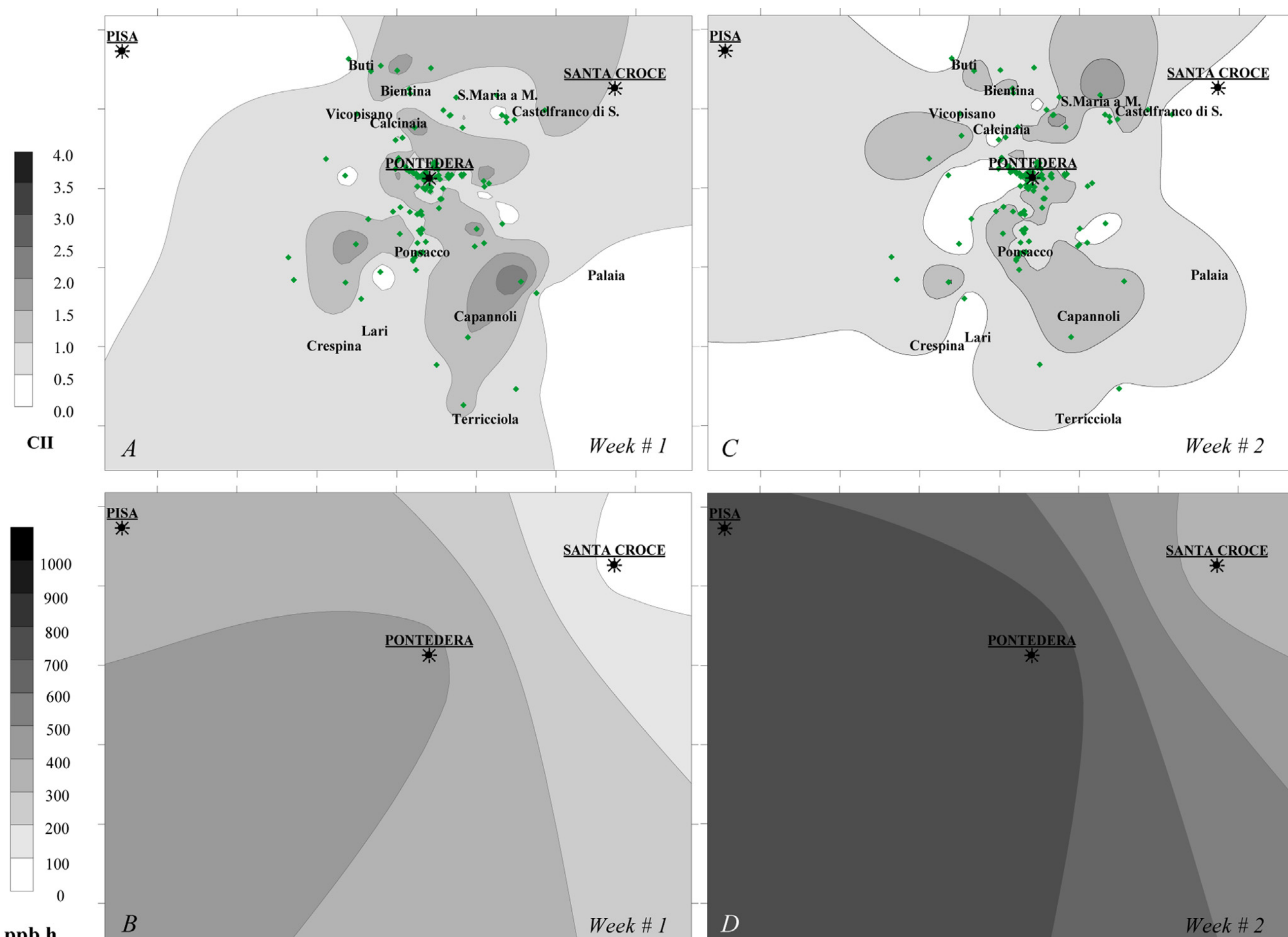

Fig. 5. Spatial distribution of the Cotyledonar Injury Index (CII, on a 0–4 scale) on tobacco Bel-W3 (ozone hypersensitive) seedlings (based on data recovered by the experienced scorer) in the study area during the first (A) and the second (C) week of exposure of the tobacco seedlings. Spatial distribution of ozone AOT40s, based on data captured by the automatic ozone analyzers in the study area during the first (B) and the second (D) week of exposure of the tobacco seedlings. Green dots and asterisks indicate locations of monitoring points and of automatic analyzers, respectively.

Table 1
Values of k as a measure of reproducibility of the readings of young students when compared with the scores of the expert scorer; z represents the statistical significance ($P \leq 0.05$) of k values.

Level of the secondary school	k	z	Reproducibility
First	0.29	53.4	Marginal
Second	0.45	46.6	Good

Over-estimation is much more relevant than under-estimation (46% vs 36%). Results of the second week (*data not shown*) are better, with a 55% of readings within the ±25% range. Over-estimation is still prevalent over under-estimation (36% vs 9%).

Fig. 4 describes the variations of CIIs of the two groups in comparison with data captured by the reference scorer. Mean and median values from the students are higher than those of the reference scorer; this is true both for first level pupils (+19% and +25%, respectively) and for the second level students (+17% for both mean and median values). Boys and girls of the first level showed a low level of accuracy in scores and a marginal level of reproducibility, as put in evidence by k statistics; results from older scorers are better (Table 1). Pupils of the first level show a good repeatability ($88 \pm 17.5\%$). Time required for each single reading and attribution of CIIs ranged between 2 and 9 s. The self-directed learning coefficient (L) is null: there is no difference between the two successive readings, the first being the best for five students out of 10 but the worst for the remaining five. Repeatability is also good for the second level students ($76 \pm 32.7\%$), but their self-directed learning coefficient is scarce, the second reading being the best for 11 students but the worst for other 10 individuals. Time required for each single reading and attribution of CIIs ranged between 3 and 15 s.

The processing of all the data validated by the expert scorer allowed the creation of two thematic charts which describe the spatial distribution of CIIs in the 2 weeks of investigation (Fig. 5, left). CIIs values are grouped in classes with a width greater than standard deviation of mean values amongst all the stations. Fig. 5, right, describes the spatial distribution of AOT40 values in the same area. It appears how the capillary distribution of monitoring points of the biological networks allows a more detailed spatial distribution of experimental data, with respect to reduced number of the automatic analyzers. However, the correspondence between data from the photometric analyzers and those from the readings of bioindicators is fair, with some areas showing higher levels of pollution. Global wind regime, local conditions, orography, point like sources of pollutants may evoked to explain the geographical distribution of airborne chemical pollutants.

Conclusions

The project was a success on the educational plan and reached its aims. All the stakeholders (teachers, pupils and their families) had a first-hand experience of how polluted is their aerial environment, by using an extraordinary, reliable and cost-effective tool, such as biological monitoring. Curiosity and involvement were widespread and many participants were very excited. So, pupils (and their relatives) were the witnesses (and main actors playing a decisive role) of an inexperienced form of monitoring, on the basis of simple but scientifically sound procedures. By means of periodic press releases, local media underlined the importance of this innovative approach to evaluating the state of health of the environment. Some schools set up poster exhibitions describing their experiments. Several students proudly took pictures and videotapes with their cameras; some of them appeared to have developed a long lasting interest in environmental sciences. Though primarily an educational exercise, the survey provided sound research elements and the picture of pollution that emerged has increased the knowledge of air quality in the area. It must be stressed that the number of automatic O_3 analyzers displayed in the area by the regional environmental agency is insufficient to allow a detailed description of the distribution of the pollution on a local scale.

The use of the miniaturized kit described here is a solid example of good experimental method, reliable, specific, quick, very cost-efficient and easy to manage. The monitoring of environmental health with living organisms should be regarded as a highly politically correct issue, whose simplicity and readiness may be profitably used to trace formative pathways for students, so to improve their awareness and involvement in key topics of environmental quality, such as air pollution. Under the guidance of the teachers, tens of pupils had several opportunities to practice basic and applied study techniques in a multidisciplinary way, such as statistics (from simple arithmetic averages to data sheet management and standard deviation computing, according to their age), botany, chemistry, ecology, geography, word processing, computer graphics and navigation on the Internet. Moreover, they were introduced to the problem-solving world and the scientific method in a simple and absorbing manner. Intimacy with nature and direct caring for the biota were developed.

The 'Charter of European Cities and Towns Toward sustainability' adopted in 1994 in Aalborg, Denmark (http://ec.europa.eu/environment/urban/pdf/aalborg_charter.pdf), under the point I.13 ('Citizen as Key Actors and the Involvement of the Community') emphasizes the setting of mechanisms able to improve the awareness of problems and implies the active participation of citizens and interested groups in the assessment of several indicators of the state of the environment. Under this point of view, biomonitoring of airborne chemical pollutants may represent a key element and a new discipline ('environmental horticulture?') may be envisaged (Burchett, 1995). With these methodologies, air quality information can be provided to the public in a format which is understandable and relevant to their needs ('receiver-friendly').

As the degree of environmental concern has a strong and long-during impact on our behavior in specific environmental related issues such as mobility (Bamberg, 2003), developing in young people a precise scenario on the overall impact of present day way of life may be strategic. Pupils act as multipliers for information about environment-friendly behavior. They represent qualified sensitive targets in environmental issues: children are the future opinion formers and decision-makers.

A major limiting factor should be stressed in the present project: in Italy the (long) summer holiday period (usually from 10 June to mid-September) coincides with the 'true' photochemical season, so experiences with students must be carried out during periods (such as the second half of May) which are not totally representative of the worst environmental scenarios.

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