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Engaging the Senses: The Association of Urban Green Space with General Health and Well-Being in Urban Residents

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Abstract: This study evaluated the short-term responses of physiological and psychological indices and examined the human senses that are mostly engaged during a green space and urban exposure in residents of Athens, Greece. The forest had beneficial effects for human physiology, anxiety and mood states and was also associated with all five senses and positive reactions, while the opposite was observed in the urban center. The difference of pre- and post-green space exposure salivary cortisol was correlated with the participants' environmental profile and body mass index. Green spaces can alleviate stress and improve overall mood, while helping individuals experience their surroundings with all five senses.

Keywords: greenspaces; mental health; blood pressure; salivary cortisol; Profile of Mood States



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1. Introduction

We live in a time of extreme urbanization. Fifty-five percent of the world's population now live in urban areas, a proportion expected to rise to 68% by 2050 [1,2]. At the same time, the conditions prevailing in urban environments are responsible for psychological distresses. Research has shown that air pollution, noise and daily stressful routines negatively affect both body and mind [3–5] and have contributed to the growing number of individuals around the world suffering from intense anxiety [6]. Stress has been dubbed as the “Health Epidemic of the 21st Century” by WHO, with devastating emotional and physical effects. Stress manifestation includes three phases: the alarm stage, during which individuals are faced with a “fight or flee” reaction against the stressful stimulus [7], the resistance stage during which the human body repairs itself after combating the stressful event and the exhaustion stage. In the latter phase, the organism shows signs of adaptation failures, as well as negative effects on mental health, the circulatory, digestive, immune and other systems [8]. Stress manifestation is linked to fluctuations of cortisol, a glucocorticoid hormone released at the alarm stage. Cortisol secretion and blood levels follow a diurnal pattern, being at its highest values early in the morning and gradually decreasing throughout the day.

Acknowledging anxiety as a global health challenge, some countries shifted to alternative forms of therapy using natural means. The therapeutic effects of natural environments have been known since ancient times [9]. Today, two main theories explain their beneficial effects: Ulrich's (1983) Stress Reduction Theory (SRT), and Kaplan and Kaplan's (1989) Attention Restoration Theory (ART). SRT is a psycho-evolutionary health theory that considers non-threatening natural settings as restorative environments leading to a more positive emotional state and an optimal level of physiological arousal [10]. On the other hand, ART describes a set of properties in natural environments that can be effective in

restoring a person's ability to focus and concentrate [11]. During the last decade, research interest has shifted to green spaces (GS), particularly in urban areas, and their impact on human health. GSs include natural surfaces, natural settings or urban greenery [12]. Based on the European Urban Atlas [13], important elements of the definition of green urban areas are open accessibility and being used for recreational purposes, such as public parks, gardens, children's play areas and forests. The latter is linked to "shinrin-yoku" (forest bathing), a nature therapy that originated in Japan and uses the forest atmosphere and the human body's five senses in order to trigger pleasant emotions and other physiological functions [14].

Numerous studies have shown that GSs are strongly related to psychological recovery [15–19]. More specifically, they contribute to combating stress-related diseases [11,20–30], burnout [18,31] and negative mood states, such as depression and aggression [32–36], enhance mental health and well-being [21,37–40], and improve restrictiveness, concentration and performance [41,42]. Additionally, GSs are positively related to general health and better physiological conditions [24,43–49]. Such conditions refer to the improvement of immune functions [50–52], the diversification of the human microbiome [52–55] and the reduction of cardiovascular risks [56–59], pulmonary diseases [60,61] and blood pressure [62–64]. Moreover, parasympathetic nerve activity is induced [65], while sympathetic nerve activity is suppressed [66].

According to the Gallup 2019 Global Emotions Study [67], Greek citizens are among the most stressed people in the world, particularly those who live in densely populated cities like its capital, Athens. The recent economic crisis [68,69], intense urbanization and poor environmental conditions, such as noise and air pollution [70], have put a burden on the inhabitants' well-being and mental health. Even though GSs and forest bathing focus on nature connectedness through our body's senses, only a few studies have tested which sense(s) is/are mainly used during nature exposure, or the correlation between their functionality and the restorative effects of GSs for human body and mind. In this study, we examined the potential beneficial effects of GSs for human health and well-being in a group of Greek city-dwelling subjects by measuring physiological and psychological indices of well-being, including changes in the 5 basic human senses, in response to exposure to the natural environment. Thus, we evaluated short-term responses to forest and urban environments in Athens, Greece, to assess immediate changes in physiological and psychological indices of well-being. These included testing of the senses mostly prevailing during a nature exposure.

2. Materials and Methods

2.1. Participants

A total of 24 volunteers participated in the two-day field experiment (10 males, 14 females). Inclusion criteria were age (participants' age should be between 20 and 60 years old), and no history of any psychological disorder. The participants should be able to walk at a self-directed pace for 60 min. The individuals who participated in the study had a mean age of 34.9 ± 11.0 and a Body Mass Index (BMI) of 23.8 ± 2.92 . All subjects were asked to refrain from eating, smoking, caffeine, and alcohol consumption for one hour before field exposures.

2.2. Experimental Design

The field experiment was performed in November 2019 according to the Declaration of Helsinki and its later amendments. A crossover design was used, according to which participants were exposed to two environments: an urban space on the first day and a green space on the second one. In both settings, environmental conditions such as temperature, humidity, air pollution and noise were measured. It is also important to note that measurements of salivary cortisol took place at exactly the same time on both days, in order to avoid a diurnal effect.

Upon the subjects' arrival in the morning (10:00 a.m.), instructions were provided. The experimental protocol was distributed along with an application form. All participants had to sign a written informed consent and turn over their mobile phones and/or tablets. Following that, participants were asked to fill out a questionnaire with demographic, lifestyle and environmental attitude questions. They then remained in a seated position for 30 min. During that period, the Profile of Mood States (POMS) was completed, saliva samples were taken, and lastly, pulse rate and blood pressure (diastolic and systolic) were measured. Following the pre-measurements, the intervention was performed. Subjects walked a guided, equally distanced (3 km), pre-determined course (Supplementary Materials Figures S1 and S2) at a comfortable and relaxing pace for 60 min. The time of intervention represents the average time of forest walking based on previous studies in the literature. Participants were able to interact with other people and behave as their usual selves.

After the intervention, participants had to sit for another 30 min. They filled out a new POMS questionnaire and a descriptive question oriented to the senses mostly engaged during the walk, and their negative or positive influence. Post-saliva samples, pulse rate and blood pressure measurements were taken. Each exposure lasted approximately 2.5 h. Figure 1 shows the experimental protocol of the study.

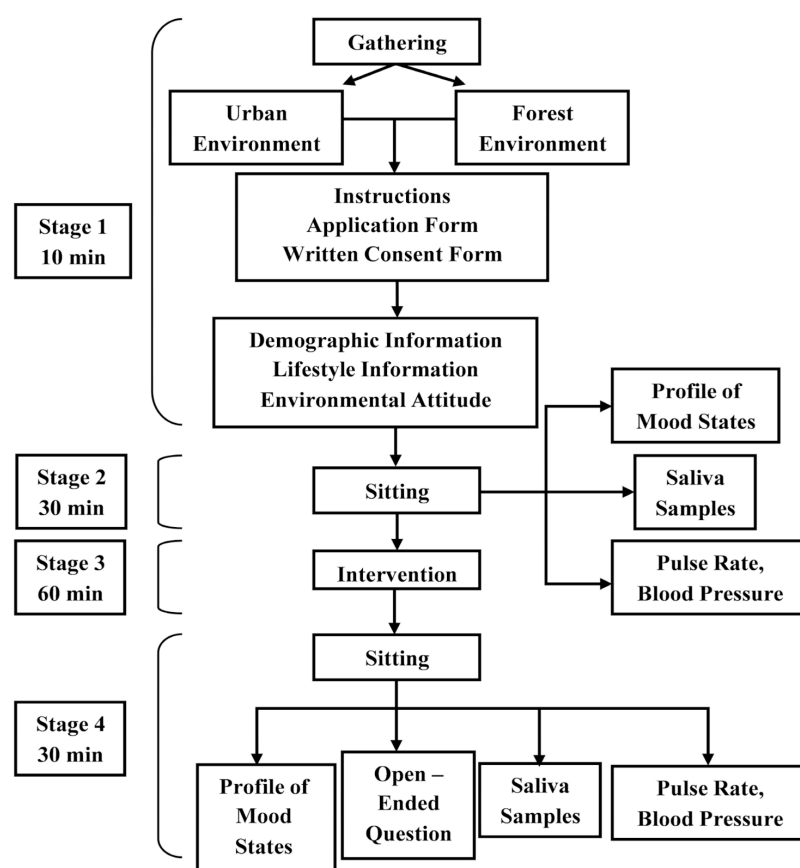


Figure 1. Environmental Protocol.

2.3. Study Locations

Exposures were conducted in the city center of Athens and Tatoi Forest, Attica, Greece (Figure 2). Weather conditions for each site are described as follows: mean temperature of 16 °C and 15 °C, and average humidity average at 70% and 99%, respectively. Noise pollution in the Athens city center fluctuated between 47 and 87 dB (mean: 70 dB), while at Tatoi forest the levels were 35–51 dB (mean: 41 dB). We also measured air pollution (by way of ultrafine particles, UFPs) using a miniature diffusion size classifier (miniDiSC). MiniDiSC is a nanoparticle detector, able to determine particle number concentration as well as the

average particle diameter. The measurements took place in both environments during the 60-min walk. An average of 54,885.6 pt/cm³/min was recorded in the urban area, whereas UFP concentration in the forest area was considerably lower at 2647.3 pt/cm³/min.



Figure 2. Study locations: city center of Athens (**left**) and Tatoi Forest (**right**), Greece.

Tatoi Forest Vegetation

The Tatoi area (60 km²) extends in an elevational range from 240 m to 1033 m, and is dominated mainly by an Aleppo pine forest (*Pinus halepensis* Mill.) [71]. Different vegetation types are also present, including sclerophyllous evergreen formations, phryganean ecosystems, deciduous broad-leaved forest stands as well as mixed coniferous forests at higher elevations [71–73].

2.4. Data Collection

At baseline, subjects completed their demographic information and questions regarding their lifestyle (smoking, exercise habits, medication). BMI was calculated based on their self-reported weight and height. Additional questions verifying the environmental attitude of each participant were also included. Pulse rate (PR), systolic (SBP) and diastolic blood pressure (DBP) were assessed as physiological indicators of the subjects cardiovascular system function [24]. The data were collected four times in total (twice pre- and twice post-intervention measurements) with a minute difference between measurements and using portable blood pressure monitors (M4; Omron, Kyoto, Japan) and the subjects' upper left arm [62]. Before the measurement, participants were sitting in an upright position so that any activity- or position-related effects were eliminated.

Cortisol is the hormone of stress. It has a diurnal rhythm; its levels increase during the early morning, with the highest value at about 8 a.m., and decrease slightly in the evening and during the early phase of sleep [74]. Sample collection can be done by measurements of serum, saliva, urine and hair. All choices have advantages and disadvantages, but recent research has proved salivary cortisol (SC) to be a useful tool [75]. In this study, saliva samples were taken for cortisol measurement using a salivette (Sarstedt, Numbrecht, Germany). Participants had to place cotton wads in their mouth and softly chew them for 1.5 min. Double measurements were made (before and after the intervention). The samples were stored at 4 °C and then transported to the laboratory for analysis. Salivary cortisol measurements were performed based on the electro-chemiluminescence immunoassay principle of Cobas e 411 analyzer (Roche Diagnostics, Mannheim, Germany) as previously described [76]. Further processing included saliva extraction via centrifugation at 1000 g for 8 min to separate off the saliva into the outer tube. In addition to baseline measures, cortisol total output was summarized using the area under the curve with respect to ground (AUC_G). AUC_G was calculated using the four serial salivary samples.

The Profile of Mood States (POMS) was first developed by McNair and Doppleman in 1971 [77]. It is composed of 65 items and its main purpose is to provide information on mood states. In 1983, a short form of 37 items was deducted by S. Shacham [78] as an alternative for rapidly measuring psychological distress. Ever since, it has become a widely acknowledged tool, mostly preferred for its rapid and accurate results. In this study, we used a modified version of the short form made up of 28 items and a 5-point scale (0 = not at all, 4 = extremely). The tool assesses 7 dimensions of mood: tension–anxiety (T–A), confusion–bewilderment (C–B), depression–dejection (D–D), anger–hostility (A–H), fatigue–inertia (F–I), vigor–activity (V–A) and friendliness (F). Upon completion, raw scores were assessed and a total mood of disturbance (TMD) was calculated based on the following formula: $TMD = [(T-A) + (C-B) + (D-D) + (A-H) + (F-I)] - [(V-A)]$ [14]. Friendliness was considered separately, because it represents a mood state that may influence the severity of mood disturbance through interpersonal functioning. A high TMD indicates an unfavorable psychological state.

Additionally, participants had to answer the following open-ended question, in a given space-range of approximately 300 words: “Please describe any details you found interesting during the urban/ nature exposure. In order to properly answer this question please consider any visual, auditory, olfactory, gustatory or tactile stimulus that you experienced and provoked either euphoria or discomfort. Please describe that particular stimulus as well as the way it affected you”. This part of the survey focuses on the gaps of green space research as indicated by [79]. While forest walking is commonly applied as a practice for verifying the positive effects of green environments on human health, no insight has been provided regarding the senses that participants mostly use while “taking in” the forest atmosphere. So, by comparing and analyzing the results, conclusions were reached on whether a special sense prevails during natural experiences, and if it relates to positive or negative physiological and psychological states.

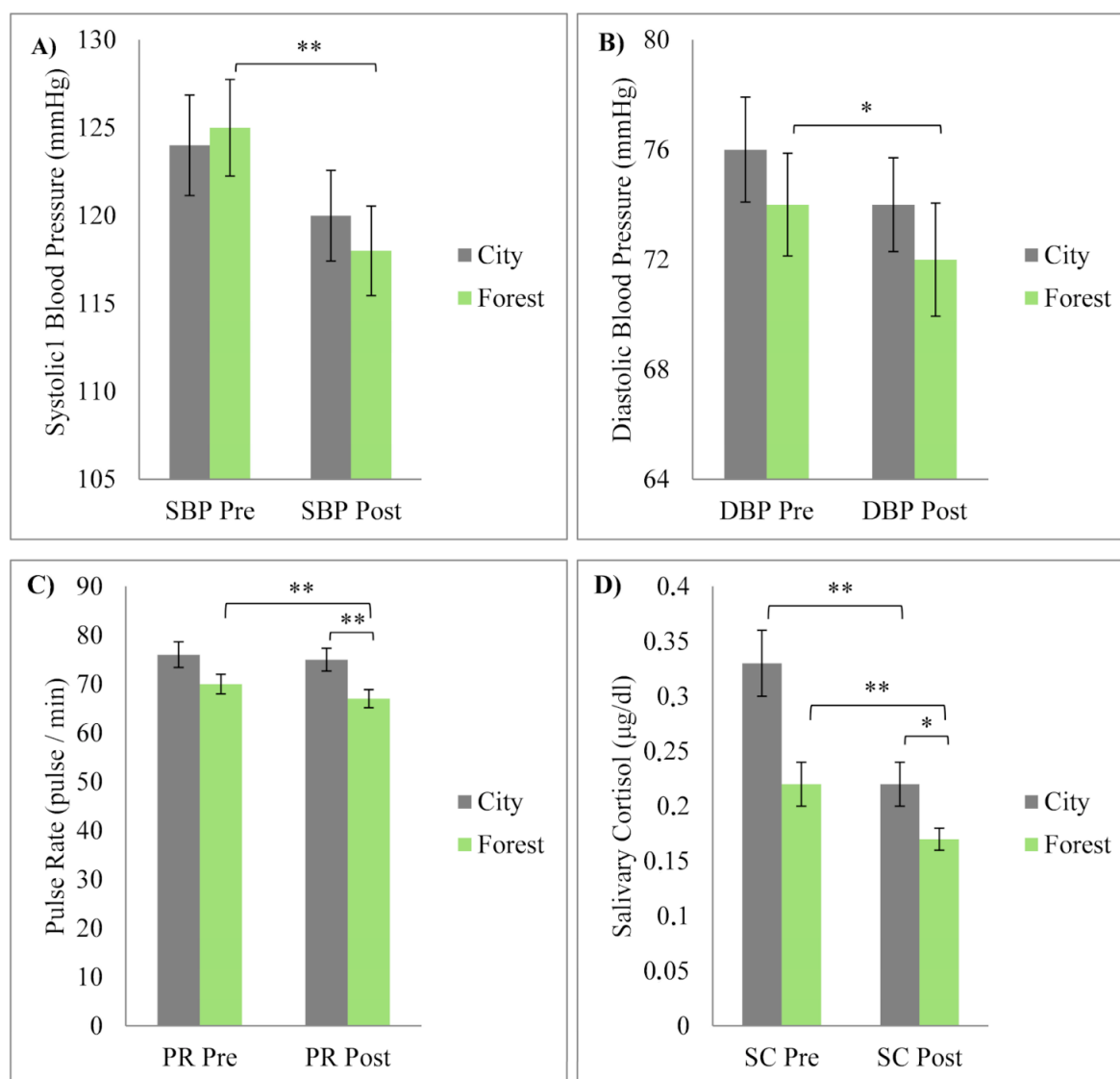
2.5. Data Analysis

Data process included a comparison between pre- and post-intervention scores as well as between post-results. Samples were initially analyzed using the One Sample Kolmogorov–Smirnov test. Since the physiological indexes' samples groups had a normal distribution, the paired samples *t*-test was used. Wilcoxon signed-rank test was used for psychological data (POMS). A *p*-value < 0.05 was determined as statistically important. Correlations between our dependent variables (differences between post/pre-results of SBP, DBP, PR, SC, TMD) on both environments and our eight predictor variables (age, BMI, gender, frequency of physical activity, medication, smoking status, perceived stress levels and environmental profile, a scale variable extracted by adding the scores of each question that determined the environmental attributes of each individual) were explored by multiple linear regression. The initial linear model was simplified with a backward elimination, and the significance of the predictor variables was adjusted using a Bonferroni correction at $0.05/8 = 0.006$. Results were considered reliable after examining the heteroscedasticity effects and the normality of the residuals. All answers from the open-ended question were sorted into six categories (five concerning human senses and one labeled as “other”) and a frequency analysis was applied. All values were expressed as mean ± one standard error of means (SE). Statistical analysis was processed with IBM SPSS Statistics Data Editor Version 19.

3. Results

Figure 3 shows the mean values for physiological indexes on each experimental site. SBP, DBP and PR were significantly decreased after the walk at the forest ($p < 0.01$, $p < 0.05$, $p < 0.01$), while no statistically significant differences were observed following exposure in the city environment. SC levels decreased in both environments ($p < 0.01$ in the city, $p < 0.01$ in the forest). The post-test comparison between the two settings showed that PR and cortisol were significantly lower in the forest environment than in the

urban environment ($p < 0.01$, $p < 0.05$). The exact values and statistics of Figure 3 are also presented in Supplementary Materials Table S1.



With regards to POMS subscales and TMD, all negative subscales (T–A $p < 0.05$, D–D $p < 0.05$, A–H $p < 0.01$, F–I $p < 0.05$) except for C–B were significantly increased in the urban environment, while the positive ones significantly declined in the post-exposure samples (V–A $p < 0.001$, F $p < 0.01$). Overall, post-walking TMD was worse in the urban environment ($p < 0.05$). Forest walking resulted in much lower scores of T–A ($p < 0.01$), D–D ($p < 0.01$), A–H ($p < 0.05$), and C–B ($p < 0.01$), and significantly higher scores for V–A ($p < 0.01$) and F ($p < 0.001$). A declining trend was also observed at F–I, but without statistical significance. Consequently, TMD was significantly improved following exposure to the forest environment ($p < 0.01$). Between sites, the post-test results for anxiety, depression, anger, fatigue, and confusion significantly decreased, while the values for vigor and friendliness significantly increased. Again, TMD had a major improvement in the forest environment (Figure 4).

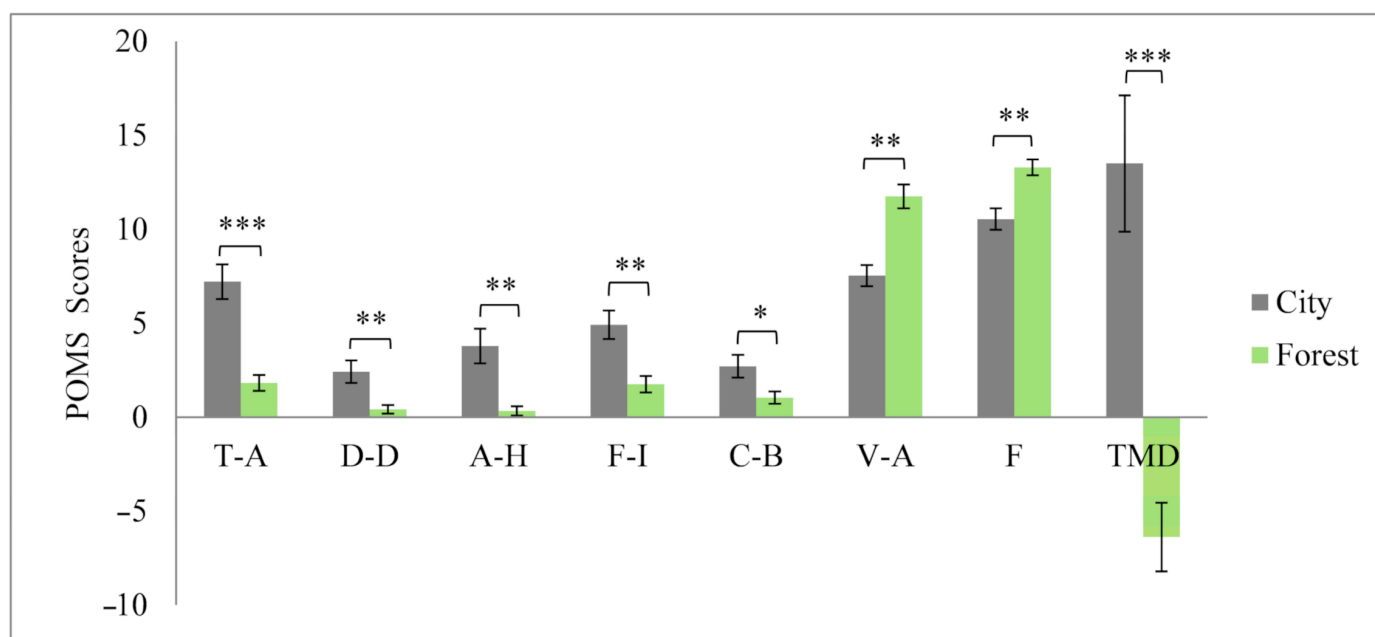


Figure 4. Post-walking POMS results. Values are shown as mean \pm standard error of means. T-A: Tension–Anxiety, D-D: Depression–Dejection, A-H: Anger–Hostility, F-I: Fatigue–Inertia, C-B: Confusion–Bewilderment, V-A: Vigor–Activity, F: Friendliness, TMD: Total Mood of Disturbance, N = 24, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, by Wilcoxon signed-rank test.

We observed a correlation between SC post- and pre-exposure in the forest environment, environmental profile and BMI. In fact, the environmental profile was negatively correlated with SC (a higher environmental profile leads to lower SC levels) and BMI was positively correlated with SC (a higher BMI is related to higher SC levels). These predictor variables explained 65% of the post-pre SC variation effect of forest walking on the participants. The multiple linear regression model did not identify any statistically significant effect of the predictor variables on the post-pre variation of SC in the city.

The open-ended question results are shown in Figure 5. In the city center, the majority of comments were related to vision, followed by hearing, olfaction and taste. Vision stimuli provoking negative feelings were dirt, traffic, lack of green space, gray color, homelessness, trash, and lack of sunlight. On the other hand, view of ancient ruins, shops and Christmas decorations were elements that triggered a pleasant sensation. Hearing was related to noise and traffic, therefore causing a disturbing feeling. Olfaction showed contradictory results: bad smells and pollution displeased 13 participants, while coffee and food smells had a positive effect on five individuals. Taste could only be implied through the “irritation” caused by the polluted atmosphere. The overall feeling of loneliness and desertedness was stated as a negative factor, while walking in the company of friends was widely acknowledged as pleasant.

In terms of the natural environment, out of the five senses, hearing is the one prevailing, mostly provoking positive reactions (from the sound of birds, streams, rustling of the leaves, serenity, lack of noise), and limited negative ones (due to the sound of motocross bikes and passing airplanes). In addition, odors from pines and wet soil and inhaling “fresh air” were related to “refreshing” feelings, while the smell of animal excrement was disturbing for one participant. The landscape, trees, plants, greenness, and a “giant” mushroom were recorded as positive, but the lack of wildlife and the view of occasional feces had a reverse effect on two subjects. Six participants noted having positive reactions from touching trees and other plants, and one from tasting nuts/fruits.

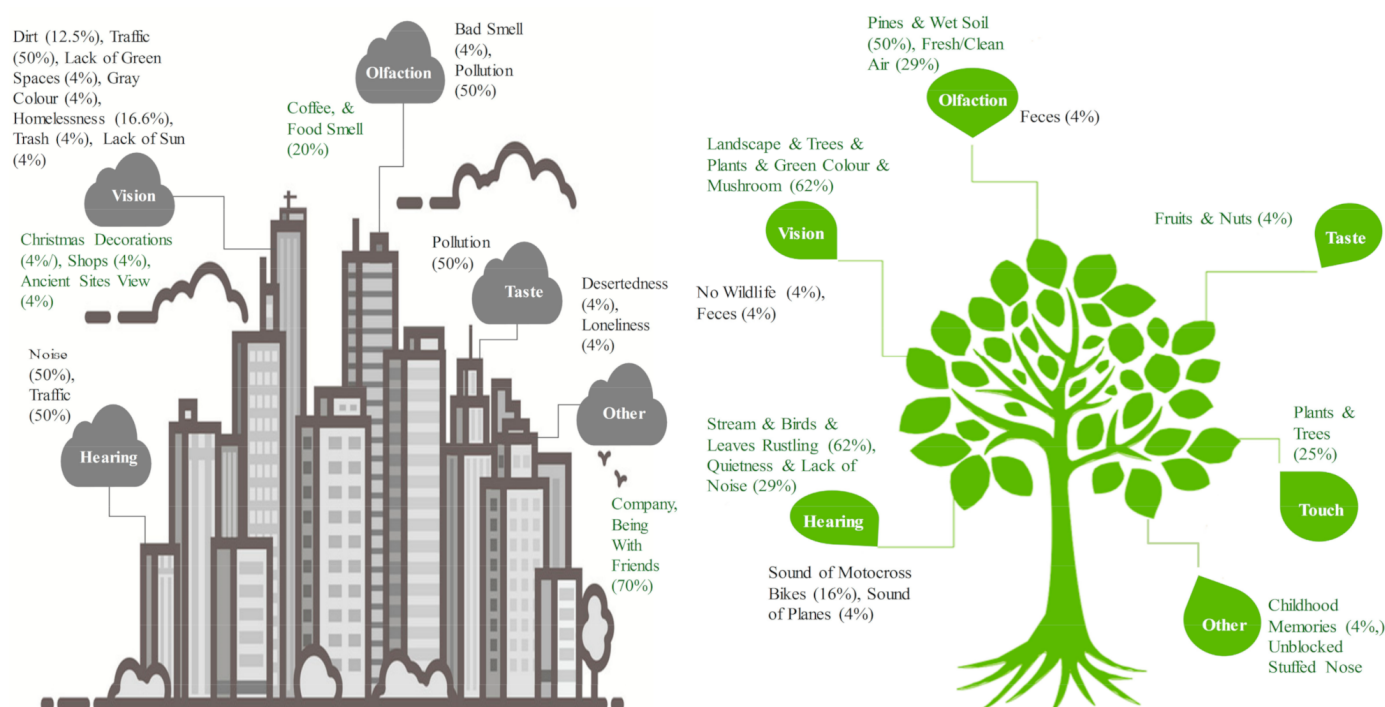


Figure 5. Open-ended question results: city center (left) and forest (right). Positive comments are indicated by green color and negative comments by black color (N = 24). City center illustration copyright of <https://www.vectorstock.com/royalty-free-vector/line-city-vector-12659602> and forest illustration copyright of <https://www.edrawsoft.com/infographics/editable-infographic-templates.html> (accessed on 17 December 2020).

4. Discussion

The present study assessed the effects of walking in two different environments (natural and urban) on physiological (SBP, DBP and PR) and psychological (POMS) indexes, with emphasis on stress levels, as expressed by the variance of salivary cortisol. Our findings are in agreement with previous studies, that forest walking improves stress levels and overall human health [80–84]. Following the intervention in the forest area, all physiological indices were significantly reduced. On the contrary, in the city apart from SC, all other indices were relatively stable. The SC drop in both environments exceeded the expected percentage of 11.7%/hour [85]. The PR and SC values were also significantly lower when walking in the forest environment in comparison with walking in the urban environment, although no significant differences were observed in the mean values of SBP and DBP [56].

The drop of SC in the city environment can be partially explained as an activity-induced effect, and/or due to the socialization between the subjects [86]. Furthermore, the much lower values of SC in the Tatoi area could be attributed to the relaxation properties of *Pinus halepensis* and *Juniperus oxycedrus* phytoncides and terpenes. The first one primarily emits α -pinene, myrcene and Δ^3 carene, caryophyllene and β -pinene [87,88], while the second one emits limonene [89]. Though current research on the mental health effects of these volatile organic compounds is mostly focused on animal experiments, the results indicate that these terpenes have antidepressant/anxiolytic functions and contribute to mood regulation [89–94]. However, mechanisms and pathways through which terpenes contribute to human health remain unclear [82,95].

These results are in agreement with the use of forests in healthcare programs development in various countries: from the “forest recreation program” in the United States in the 1960s to the Kneipp therapy in Germany, and from “Shinrin-yoku” (1982) to the officially established project on the “Therapeutic effects of forests plan” in 2005 in Japan [95,96]. Natural ecosystems provide a series of services to human communities, comprising, inter

alia, cultural services (based on typology of [97]) that include recreation and health benefits. For example, urban and peri-urban forests have been found to have recreational value and provide numerous psychophysical and psychosocial health benefits [98–100] relative to urban gray environments [101]. In addition, forests provide provisioning services such as medicines. Plant secondary metabolites that are synthesized by the plants to defend themselves from various sources of biotic and abiotic stress [102] are the dominant source for drug discovery and development to treat various kinds of human diseases [90].

The correlations extracted by the multiple linear regression indicated that environmental profile and BMI are associated with the variance of SC during the forest intervention. More specifically, a higher environmental profile leads to lower SC levels, while a higher BMI is related to higher SC. These associations indicate that participants with a greater appreciation for the natural environment, a stronger environmental conscience, and life experiences from natural settings, were able to relax and expel feelings of intensity while being in the forest area. Therefore, it is expected that forest walking reduced their stress levels. On the other hand, the positive association between BMI and SC has been previously shown in studies from around the world and is consistent with the current knowledge establishing a link between psychosocial stress, including perceived stress, and BMI, even at the level of obesity [103–105]. POMS analysis further illustrated that forest environments can facilitate psychological benefits [56]. After walking in the forest, POMS negative subscales were significantly decreased, while the positive ones increased. A comparison among the post-exposure results also showed a significantly lower TMD at the natural setting, when related to the city center.

Supplementary evidence was obtained by the open-ended question: urban space was connected to negative reactions and the natural environment to positive. Vision, hearing and olfaction were described as the dominant senses in participants' feedback. During the intervention in the city, references were made for stimuli triggered by vision, hearing, olfaction and touch, but in the forest, gustatory experiences were also recorded. As far as hearing was concerned, natural noises from the forest setting resulted in a tranquility effect, while man-made noise in the city center increased the levels of annoyance. We can, therefore, assume that forest environments help us experience our surroundings with all senses [96], and that the combination of visual, auditory and olfactory factors in green spaces can lead to health and well-being benefits [106–110]. This was also observed in our study, whereby, while walking in the forest, participants concentrated their attention on their surroundings and were not interacting with each other as much as in the urban environment.

Physical conditions such as temperature, humidity and noise [65] were not significantly different between the two environments. However, we noticed that noise was differentiated in terms of quality. Thus, green spaces can also be suggested as tools for achieving noise mitigation [111]. Air pollution levels in the green area were significantly lower than in the city center ($p < 0.001$). Previous research has found evidence on the threat that ultrafine particles pose to human health. More importantly, researchers have investigated these factors—along with physical activity, social interactions and conceptualized stress—as pathways explaining the relations between GSs and improved psychological states [53,83]. In our study, air pollution and noise were considered as characteristics of the urban and nature environments. Particularly, the lack of primary and traffic-related pollutants, as well as artificial sound, were expected to deliver different findings regarding stress levels and mood states in the two field locations. Thus, our findings about the beneficial effects of the natural environment on stress may also be described by the limited presence of air pollutants in the forest setting.

Our study has several strengths. It is the first one conducted in Greece. The crossover element enhances the solidity of the study for two reasons: it entails the advantage of comparing control to reference measurements permitting a thorough assessment of environmental impact on human health, as expressed by stress levels, and reduces confounding covariates' influence, because each participant serves as their own control. Stress levels

were assessed not only based on participants' perceptions, but also by human biology measures (salivary cortisol) [18,20,75,80,81,84,86,96,112–119]. Last, our experimental design included air pollution [14,58,81,114,119,120] and noise measurements [14,58,80,81,119,121], which are largely understudied covariates in green space studies. Our findings highlight the recreational value of forests and their potential to be a source of health and could contribute to development plans for achievement of sustainable development goals (SDG) 3 (good health and well-being) and 11 (sustainable cities and communities) [122]. Investigation of the role and use of green spaces is crucial, taking into consideration that the global health crisis due to the coronavirus COVID-19 pandemic is expected to bring changes in the human–public space relationship [123].

However, several limitations are noted. First of all, the sample size of our pilot study is relatively small, and we were only able to assess short-term effects of urban and forest exposure of about 60 min. For this reason, future studies should include a larger population size. This would help establish a generalization of the final results and avoid negative impacts of extreme values on statistical analysis [124]. Second, the experimental design offered opportunities for controlling for anxiety levels during the field exposure either by keeping participants in a seated position or by prohibiting conversations while physiological and psychological measurements were made. As a result, individuals remained calm and any activity-related effect was eliminated. Nonetheless, other factors that may affect salivary cortisol and subjective stress levels (e.g., waking time) outside the experimental protocol were not controlled for. In addition, our study lacks information on how the same order of exposure for all participants could affect our findings, since the crossover design was not random.

As there is still little insight regarding the senses mostly used during exposure to forest and urban environments, future research should focus on examining reactive effects of particular interventions towards each sense separately. The effect of terpenes on SC in humans is another area that should be further explored. Another issue to be considered refers to whether physiological or psychological indexes should be measured first, as there is insufficient information on whether sampling order and typology can affect subjects' mood and stress levels. Furthermore, as limited studies engage physical conditions, it is recommended that physiological and psychological responses, as well as weather conditions, be further examined. In the same context, additional mediators should be incorporated in future studies, such as biodiversity, seasonality and perception of individuals for GS, to see any possible impact on the natural exposure outcomes. The possibility of extending these correlations to different types of environments, such as blue spaces or different types of forests, such as broad-leaved forests, should also be examined in future research [125].

5. Conclusions

This pilot study showed that forest walking has positive effects on both physical and mental health, thus contact with nature can be proposed as an alternative method for stress alleviation and mood improvement. Walking itself has generally beneficiary effects, so it is suggested as a stress reduction activity for urban environments as well. Additionally, forest exposure has apparent short-termed positive effects on human health and well-being, especially when compared to the same intervention in an urban environment. Finally, our findings suggest that forest walking relates to positive reactions and feelings, unlike urban settings that provoke opposite results.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/su13137322/s1>, Figure S1: The walking course in Athens. Map provided by Google Map, Figure S2: The walking course in Tatoi forest. Map provided by Google Map, Table S1: Pre- and post-walking results for the city and forest environment, for SBP, DBP, PR, and SC. SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, PR: Pulse Rate, SC: Salivary Cortisol, N = 24, * $p < 0.05$, ** $p < 0.01$ by paired samples t -tests.

Author Contributions: Conceptualization, A.A.K. and O.-I.K.; methodology, A.A.K., N.M.F., G.P.C. and O.-I.K.; formal analysis, A.A.K. and N.M.F.; investigation, A.A.K. and O.-I.K.; resources, O.-I.K.; writing—original draft preparation, A.A.K., P.G.D., N.M.F., G.P.C. and O.-I.K.; writing—review and editing, A.A.K., P.G.D., N.M.F., G.P.C. and O.-I.K.; supervision, P.G.D. and O.-I.K.; project management: O.-I.K. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the 1964 Declaration of Helsinki and its later amendments.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data is available upon request from the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

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