ACTA UNIVERSITATIS CAROLINAE

European Journal of Environmental Sciences

VOLUME 10 / NUMBER 1
2020

CHARLES UNIVERSITY
KAROLINUM PRESS

European Journal of Environmental Sciences is licensed under a Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

© Charles University, 2020
ISSN 1805-0174 (Print)
ISSN 2336-1964 (Online)

CONTENTS

MATING BEHAVIOUR OF THE PREDACEOUS LADYBIRD, *HARMONIA DIMIDIATA*

MONALISA, AHMAD PERVEZ*, and MUMTAJ JAHAN

Biocontrol Laboratory, Department of Zoology, Radhey Hari Govt. P. G. College, Kashipur,
Udham Singh Nagar – 244713, Uttarakhand, India
* Corresponding author: ahmadpervez@yahoo.com

ABSTRACT

We studied the mating behaviour of the predaceous ladybird beetle, *Harmonia dimidiata* (Fabricius) (Coleoptera: Coccinellidae). The courtship behaviour of the male involves the secretion from the tibio-femoral joints of its hind-legs of yellow coloured reflex blood containing the alkaloid harmonine, which is usually the first line of defence of this ladybird. In this case, this reflex blood also functions as a nuptial gift from the male, which is edible and facilitates mating. The amount of reflex blood offered as a nuptial gift decreases with each subsequent mating. Mating in *H. dimidiata* was prolonged and initially increased before subsequently decreasing with each subsequent mating. This information could be useful for the mass rearing of this species in the laboratory.

Keywords: *Harmonia dimidiata*; harmonine; mating; mating duration; tibio-femoral joint; reflex blood

Introduction

Harmonia (=Leis) dimidiata (Fabricius) is a thirteen-spot, multivoltine predaceous ladybird beetle (Coleoptera: Coccinellidae) that occurs in North America, India, Pakistan, Nepal, Bhutan, China, Taiwan and Japan (Yu et al. 2018). It was introduced into Far Eastern Russia from China. It is very effective in the biocontrol of aphids infesting melon, cucumber and peppers in greenhouses (Kuznetsov and Pang Hong 2002). It can be easily reared on the aphid species *Myzus persicae* (Sulzer) and *Schizaphis graminum* (Rondani) throughout the year, as the adults do not undergo diapause or migrate (Kuznetsov and Pang Hong 2002). It is an important predator of the apple aphid, *Aphis pomi* de Geer (Kumari 2018), mustard aphid, *Lipaphis erysimi* (Kalt.) (Singh and Singh 1986) and cotton aphid, *Aphis gossypii* (Glover) (Yu et al. 2018) and can consume more than 200 cotton aphids per day (Yu et al. 2013). The high net and daily consumption of *A. gossypii* of 13,050 and 200 aphids, respectively, reported for *H. dimidiata* (Yu et al. 2013) indicate its high foraging ability (Pervez and Yadav 2018). Female *H. dimidiata* mature earlier and tend to produce more eggs for longer when aphids are abundant (Agarwala et al. 2009). Thus, its high functional and numerical responses indicate its great aphid biocontrol potential (Pervez et al. 2018).

Despite its biocontrol potential, little is known about its reproduction, which is a prerequisite for mass rearing. Mating in ladybirds starts with a brief courtship by the male (Omkar and Pervez 2005). Behavioural studies on mating reveal that males spend most of their time searching for potential mates and on encountering a female they court her before copulating (Obata 1987; Omkar and Srivastava 2002; Omkar 2004; Pervez and Singh 2013).

Preliminary experiments revealed that males secrete reflex blood when courting (authors' personal observation). It is widely held that adult ladybirds release a yellowish orange liquid known as reflex blood as a defence when attacked (Zvereva and Kozlov 2016; Knapp et al. 2018). This reflex blood consists of haemolymph, which is exuded through joints in the exoskeleton in response to an attack by a predator (Majerus and Majerus 1997; Hodek et al. 2012; Knapp et al. 2018). In the present study, the possible role of this defensive exudate as a lure/nuptial gift in the mating behaviour of *H. dimidiata* is examined. Due to the paucity of literature on mating in *H. dimidiata*, laboratory experiments were designed to investigate the details of the courtship and mating behaviour in *H. dimidiata* and the effect of multiple mating on mating duration. The results increase our understanding of mating behaviour in *H. dimidiata* and help in mass rearing of this species.

Materials and Methods

Stock culture

Adults of *Harmonia dimidiata* occur in low numbers in local agricultural fields. These were collected and brought to the laboratory from an orchard at Kashipur, India, where they were feeding on the aphid, *Aphis gossypii* (Glover) infesting common wireweed, *Sida acuta* Burm. We cultured them in the laboratory by keeping pairs of adults in Petri dishes (9.0 cm diameter × 2.0 cm height) containing an *ad libitum* supply of the aphid, *A. gossypii* along with pieces of its host plant under constant conditions (27 ± 1 °C; 65 ± 5% RH; 14L:10D) in an Environmental Test Chamber (*Remi, Remi Instruments*). The adults were allowed to mate and the eggs they laid were reared from egg-hatch to adult-emergence in 500 ml glass beakers (11.0 cm high and 9.0 cm in diameter, prey as above). Newly emerged adults were isolated in Petri dishes (size and host as above) and reared until they attained sexual maturity. The F_1 generation adults were used in the mating experiments.

Monalisa, Pervez, A., Jahan, M.: Mating behaviour of the predaceous ladybird, *Harmonia dimidiata*
European Journal of Environmental Sciences, Vol. 10, No. 1, pp. 5–8
https://doi.org/10.14712/23361964.2020.1
© 2020 The Authors. This is an open-access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

(i) Courtship and mating behaviour of ladybirds

A ten-day-old unmated adult male of *H. dimidiata* was paired with a same-aged virgin female in a Petri dish (9.0 cm diameter × 2.0 cm deep) containing an *ad libitum* supply of the aphid, *A. gossypii* on pieces of its host plant. Their courtship and mating behaviour were carefully observed under a Trinocular Assembly *Lyzer* at 40× and 100× magnification until mating ceased. During courtship, the release of reflex blood by the adult male was also recorded. The experiment was replicated five times (n = 5).

(ii) Effect of multiple mating on mating duration

The mating pair in experiment 1 were continuously monitored for the next ten days between 1000 and 1800 hours and any subsequent mating recorded. The complete mating duration was recorded. The data on mating duration were subjected to Kolmogorov–Smirnov test and Bartlett's test to check for normal distribution and homogeneity of variances, respectively, using statistical software (SAS 2002). Thereafter, the data on mating duration were subjected to one-way ANOVA using SAS (2002). The data on mating duration in each subsequent mating was also subjected to regression analysis to determine, whether mating duration changed in each subsequent mating using SAS (2002).

Results and Discussion

Adult male *H. dimidiata* initiated courtship by approaching an adult female. The male ladybird paused for 1.6 ± 0.2 seconds and watched the female from a distance of 1.8 ± 0.3 cm. Similar male behaviour is reported for other species of ladybirds (Obata 1987; Omkar and Srivastava 2002; Omkar and Pervez 2005). The male examined (1.0 ± 0.5 sec) and then embraced the female, which is also reported by Omkar and Pervez (2005) for *P. dissecta*. The male attached his body to the latero-posterior side of the female. Thereafter, he moved counter-clockwise and approached the anterior end of the female. As the male circled the female from her head end (*i.e.* from the head and pronotum) and moved towards the posterior end he released a yellowish sticky substance from the tibio-femoral joint of his hind-legs almost in front of the mouth of female while touching her head with his aedeagus. *Harmonia* sp. release reflex blood as the first line of defence against attackers. Females licked this reflex blood and allowed the males to mount them (Fig. 1). Thereafter, the male mounted the female from the posterior end, made genital contact and attempted to mate. After prolonged mating, the process was terminated by the male circling on the elytra of the female. This behaviour was recorded for both previously unmated and mated males.

In the present study, we noticed that the quantity of reflex blood released by the male decreased with each subsequent mating. In addition, the females also behaved in strange way when her mate was not interested in copulating, she touched his abdomen with her antennae and ran away. It could be a way of inducing him to mate. Mating in *H. dimidiata* is characterized by the use of aposematic fluid as a nuptial gift. Ladybirds release defensive substances, when provoked. Adults bleed from the tibio-femoral joints and larvae from dorsal glands. It is sticky and coagulates quickly on exposure to air and may stick to predator's legs, antennae and mouthparts (Eisner et al. 1986). In *H. dimidiata* this defensive chemical also serves as a lure/nuptial gift, which stimulates or initiates mating. Harmonine [(17R,9Z)-1,17-diaminooctadec-9-ene] is a major constituent of reflex blood and haemolymph of *Harmonia sp.* and is mainly antimicrobial (Röhrich et al. 2011; Hodek et al. 2012). Reflex blood has negative effects on predators and pathogens. However, the insect pathogenic fungus, *Beauveria bassi-*

Fig. 1 Photographs showing (a) mating behaviour, and (b) reflex blood offered to female as nuptial gift.

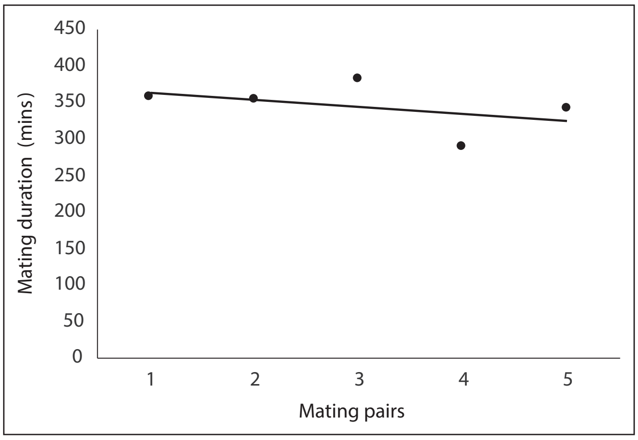

Fig. 2 Summary of mating duration on five occasions recorded over 10 consecutive days.

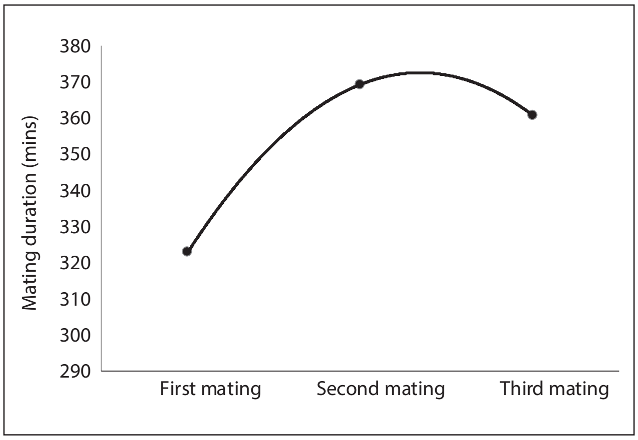

Fig. 3 Duration of mating recorded in the first, second and third mating of *H. dimidiata*.

ana (Balsamo) Vuillemin is resistant to this defence and attacks hibernating ladybirds (Roy and Cottrell 2008).

The results indicate that reflex bleeding in ladybirds has an additional function. It seems to be both palatable and sticks to the head of the female forming a blindfold. In certain insects, the nuptial gift is delivered *via* seminal ejaculations (Lewis and South 2012). For instance, in the ladybirds, *Adalia bipunctata* (L.) (Perry and Row 2008) and *Harmonia axyridis* (Pallas) (Obata and Hidaka 1987) the spermatophore that females ingest after mating is the nuptial gift. Similarly, male Bella moth, *Utetheisa ornatrix* (L.), gift females defensive alkaloids from a pair of extrusible brushes, known as coremata, which are used to protect her eggs and larvae from predation (Eisner and Meinwald 1995). In *H. dimidiata*, defensive alkaloids are sequestered as bait or a nuptial gift, which is exceptional and may account for the prolonged duration of mating. Although it is well known that ladybirds are aposematic and highly toxic to predators this is the first evidence that their chemical defence is a component of mate recognition and important for fecundity and the fitness of both male and female. Below the possible functions of reflex bleeding will be explored and discussed further.

Mating duration was not affected by previous mating, as mating duration did not differ statistically over time ($F = 0.68$; $P = 0.53$; d.f. = 2, 10; One-way ANOVA, Fig. 2). Although not significant, duration of mating of virgins was shorter than that of individuals that had previously mated (Fig. 3). The mating duration recorded for each successive mating, revealed an initial increase with the maximum duration recorded at the third mating after which it declined with each subsequent mating ($Y = -27.467X^2 + 128.8X + 221.47$; $r = -0.99$; $P < 0.0001$). The duration of each successive mating of five pairs was similar and predicted by the linear equation $y = -9.648X + 373.89$; $r = -0.44$; $P < 0.05$. The similar linear trend of the best-fit line reveals that all five experimental pairs exhibited similar behaviour in terms of mating duration (Fig. 2). This is not in accord with the results of Omkar and Pervez (2005) who report that virgins mated for longest (275.40 ± 12.23 min) and those previously mated the shortest (176.60 ± 5.60 min). Likewise, duration of mating in the zigzag ladybird, *Menochilus sexmaculatus* (Fabricius) was longest between virgins (133.0 ± 2.8 min) and shortest between those previously mated (95.0 ± 4.2 min) (Bind, 2007). In comparison, the duration of mating recorded for *H. dimidiate,* which provides a nuptial gift in the form of reflex blood, is much longer. This could result in a single mating per day and be a form of mate guarding.

Thus, it is concluded that: (i) Courtship in *H. dimidiata* is accompanied by unusual behaviour that has not been previously recorded in ladybirds, (ii) males provide a courtship gift in the form of reflex blood, which is readily accepted by the female, (iii) the amount offered decreases with each subsequent mating, (iv) mating in *H. dimidiata* is very long, with (v) the duration of mating of virgins the longest, after which it decreases with each subsequent mating.

Acknowledgements

Authors are thankful to the Science and Engineering Research Board, Department of Science and Technology, Government of India in the form of Major Research Project (EMR/2016/006296).

REFERENCES

Agarwala BK, Singh TK, Lokeshwari RK, Sharmila M (2009) Functional response and reproductive attributes of the aphidophagous ladybird beetle, *Harmonia dimidiata* (Fabricius) in oak trees of sericultural importance. J Asia Pacific Entomol 12: 179–182.

Bind RB (2007) Reproductive behaviour of a generalist aphidophagous ladybird beetle *Cheilomenes sexmaculatus* (Coleoptera: Coccinellidae). Int J Trop Ins Sci 27: 78–84.

Eisner T, Goetzs M, Aneshansley D, Ferstandig-Arnold G, Meinwald J (1986) Defensive alkaloid in blood of Mexican bean beetle (*Epilachna varivestis*). Experimentia 42: 204–207.

Eisner T, Meinwald J (1995) The chemistry of sexual selection. Proc Nat Acad Sci 92: 50–55.

Hodek I, van Emden HF, Honěk A (2012) Ecology and Behaviour of the Ladybird Beetles (Coccinellidae). Willey-Blackwell, Chichester.

Knapp M, Dobeš P, Řeřicha M, Hyršl P (2018) Puncture vs. reflex bleeding: Haemolymph composition reveals significant differences among ladybird species (Coleoptera: Coccinellidae), but not between sampling methods. Eur J Entomol 115: 1–6. https://doi.org/10.14411/eje.2018.001.

Kumari M (2018) Studies on the biology and predatory potential of *Harmonia dimidiata*, a major predator of *Aphis pomi* De Geer on apple host in India. Adv Entomol 6: 170–175.

Kuznetsov VN, Pang Hong (2002) Employment of Chinese Coccinellidae in biological control of aphids in greenhouse in Primorye. Far East Entomol 119: 1–5.

Lewis SM, South A (2012) The evolution of animal's nuptial gift. Adv Study Behav 44: 53–97.

Majerus M, O'Donald P, Weir J (1982) Evidence for preferential mating in *Adalia bipunctata*. Heredity 49: 37–49.

Obata S (1987) Mating behaviour and sperm transfer in ladybeetle, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae). Appl Entomol Zool 22: 434–442.

Obata S, Hidaka T (1987) Ejection and ingestion of the spermatophore by the female ladybird beetle, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae). Can Ent 119: 603–604.

Omkar (2004) Reproductive behaviour of two aphidophagous ladybird beetles, *Cheilomenes sexmaculatus* and *Coccinella transversalis*. Ent Sinica 11: 47–60.

Omkar, Pervez A (2005) Mating behavior of an aphidophagous ladybird beetle, *Propylea dissecta* (Mulsant). Insect Sci 12: 37–44.

Omkar, Srivastava S (2002) Reproductive behaviour of an aphidophagous ladybeetle, *Coccinella septempunctata* Linnaeus. Eur J Entomol 99: 465–470.

Perry JC, Row L (2008) Ingested spermatophores accelerate reproduction and increase mating resistance but are not a source of sexual conflict. Anim Behav 76: 993–1000.

Pervez A, Kumar R (2017) Preference of the aphidophagous ladybird, *Propylea dissecta* for two species of aphids reared on toxic host plants. Eur J Environ Sci 7: 130–134.

Pervez A, Singh S (2013). Mating patterns in the aphidophagous Ladybird, *Hippodamia variegata*, depend on body size. Eur J Environ Sci 3: 109–112.

Pervez A, Singh PP, Bozdogan H (2018) Ecological perspective of the diversity of functional responses. Eur J Environ Sci 8: 5–9. https://doi.org/10.14712/23361964.2018.13.

Pervez A, Yadav M (2018) Foraging behaviour of predaceous ladybird beetles: a review. Eur J Environ Sci 8: 10–16. https://doi.org/10.14712/23361964.2018.14.

Röhrich CR, Ngwa CJ, Wiesner J, Schmidtberg H, Degenkolb T, Kollewe C, Vilcinskas A (2011) Harmonine, a defence compound from the harlequin ladybird, inhibits mycobacterial growth and demonstrates multi-stage antimalarial activity. Biol Lett 8: 308–311.

Roy H, Cottrell TE (2008) Forgotten natural enemies: interactions between coccinellids and insect – parasitic fungi. Eur J Entomol 105: 391–398.

Singh KC, Singh TK (1986) Aphidophagous coccinellids of northeastern India: Manipur-I. Entomon 14: 291–296.

Yu JZ, Chi H, Chen BH (2013) Comparison of the life tables and predation rates of *Harmonia dimidiata* (F.) (Coleoptera: Coccinellidae) fed on *Aphis gossypii* Glover (Hemiptera: Aphididae) at different temperatures. Biol Control 64: 1–9.

Yu JZ, Chen BH, Güncan A, Atlihan R, Gökçe A, Smith C, Gümüş E, Chi H (2018) Demography and Mass-Rearing *Harmonia dimidiata* (Coleoptera: Coccinellidae) Using *Aphis gossypii* (Hemiptera: Aphididae) and Eggs of *Bactrocera dorsalis* (Diptera: Tephritidae). J Econ Entomol 2: 1–8.

Zvereva EL, Kozlov MV (2016) The costs and effectiveness of chemical defenses in herbivorous insects: a meta-analysis. Ecol Monogr 86: 107–124.

COMPOSTING AND VERMICOMPOSTING USED TO BREAK DOWN AND REMOVE POLLUTANTS FROM ORGANIC WASTE: A MINI REVIEW

ALENA GRASSEROVÁ[1,2], ALEŠ HANČ[3], PETRA INNEMANOVÁ[1,4], and TOMÁŠ CAJTHAML[1,2,*]

[1] Institute of Microbiology of the Czech Academy of Sciences, Vídeňská 1083, 14220, Prague 4, Czech Republic
[2] Institute for Environmental Studies, Faculty of Science, Charles University, Benátská 2, 12801, Prague 2, Czech Republic
[3] Department of Agro-Environmental Chemistry and Plant Nutrition, Czech University of Life Sciences Prague, Kamýcká 129, 16500, Prague 6, Czech Republic
[4] DEKONTA a.s., Dřetovice 109, 273 42 Stehelčeves, Czech Republic
* Corresponding author: cajthaml@biomed.cas.cz

ABSTRACT

The advantages of combining composting and vermicomposting to break down and remove pollutants from organic waste are reviewed. This mini-review aims to present the benefits of combining these methods and the outcome of specific cases of environmental remediation.

Keywords: composting; earthworms; heavy metals; organic pollutants; sewage sludge

Introduction

Organic substances occur in nature due to human activities and natural processes (Luo et al. 2014). These compounds occur throughout the environment at low trace concentrations; nevertheless, their negative effects on organisms including humans are well known. Such organic substances include pesticides, pharmaceuticals, personal care products, endocrine disruptors and industrial chemicals. Organic contaminants and heavy metals are not completely removed by wastewater treatment plants (WWTPs). The by-product, WWTP sludge, is rich in nutrients and therefore used as an agricultural fertilizer (Clarke and Smith 2011). In order to remove micropollutants from bio solids and at the same time maintain their valuable properties, green technologies called bioremediations are being developed (Hickman and Reid 2008). Typical biotechnologies for this purpose are composting or vermicomposting, which are environmentally friendly, low-maintenance and low-cost methods. When these two processes are combined, even better outcomes can be achieved in terms of breaking down organic matter and removal of pollutants from bio solids (Lim et al. 2016). The objective of this paper is to review the advantages of combining composting and vermicomposting in terms of both the properties of the end product and removal of pollutants.

Composting

During thermogenic composting, the organic matter is decomposed by microorganisms (Finstein and Morris 1975), so it is important to aerate the compost, in order to replenish the oxygen. Under optimal conditions, the thermal phase takes around one month (de Bertoldi et al. 1983). The main decomposers are bacteria, fungi, actinomycetes or protozoa. Over many years, composting has also been used for the bioremediation of polluted substrates (Cajthaml et al. 2002; Cai et al. 2007; Covino et al. 2016; Iranzo et al. 2018; Guo et al. 2020; Wei et al. 2020).

Vermicomposting

Vermicomposting is a process in which earthworms are used to break down organic matter (Domínguez 2004). The decomposition starts in the gizzards of the earthworms' after which the organic matter is digested by enzymes and microorganism in their guts. Product of vermicomposting is rich in nutrients and can be re-used as organic fertilizer (Yadav and Garg 2019). If a pollutant is removed from the soil via vermicomposting, the process is called vermiremediation (Rodriguez-Campos et al. 2014). Shi et al. (2019) recently defined vermiremediation as "an earthworm-based bioremediation technology that makes use of the earthworm's life cycle (i.e., feeding, burrowing, metabolism, secretion) or their interaction with other abiotic and biotic factors to accumulate and extract, transform, or degrade contaminants in the soil environment". Processes involved in vermiremediation are vermiaccumulation, vermiextraction, vermitransformation and drilodegradation (Shi et al. 2019). Earthworms can successfully remove organic micropollutants (Chachina et al. 2016; Chevillot et al. 2017; Havranek et al. 2017; Rorat et al. 2017; Lin et al. 2019; Owagboriaye et al. 2020) and heavy metals (Azizi et al. 2013a; Suthar et al. 2014; He et al. 2016).

Grasserová, A., Hanč, A., Innemanová, P., Cajthaml, T.: Composting and vermicomposting used to break down and remove pollutants from organic waste: a mini review
European Journal of Environmental Sciences, Vol. 10, No. 1, pp. 9–14
https://doi.org/10.14712/23361964.2020.2
© 2020 The Authors. This is an open-access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Combination of composting and vermicomposting used to break down organic waste

Composting and vermicomposting alone are very successful methods for decomposing organic waste. However, each has its drawbacks, which can be overcome by combining the two techniques (Lim et al. 2016). Temperatures during self-heating of the compost can increase to 80 °C (Finstein and Morris 1975). These elevated temperatures (higher than 55 °C) are necessary to suppress pathogens in sludge (Grewal et al. 2006) but at the same time are lethal for earthworms (Domínguez 2004). It is therefore reasonable to start with composting, during which the pathogens are killed and the decomposition process begins. Ndegwa and Thompson (2001) confirm that by combining composting-vermicomposting eliminates pathogens. On the contrary, starting with vermicomposting, followed by composting, results in that the system does not reach temperatures high enough to kill the pathogens.

When the thermophilic phase is completed, earthworms are added to continue the decomposition and facilitate aeration of the material. The earthworms disturb the organic material and produce very small particles with favourable agrochemical properties resulting in high concentrations of available nitrogen and phosphorus (Hanc and Dreslova 2016). The size of the particles is crucial, as small particles have a large total surface area, which makes it easier for the microbes to access the material. Tognetti et al. (2005) report another benefit of the composting-vermicomposting method. As mentioned above, the thermophilic phase is detrimental to earthworms, therefore large areas are needed for spreading the material to prevent overheating. However, if the waste is subjected to the thermophilic phase prior to vermicomposting, the latter can be initiated in the surface layer, which reduces the demands on space. The same authors report a difference between compost and pre-composted vermicompost in terms of nutrient content. The pre-composted vermicompost has higher nutrient concentrations and an enhanced microbial activity resulting in a higher yield of ryegrass when applied as a fertiliser. However, these authors also point out that the quality of the product is not only dependent on the technology used, but also on the starting material, that is, the nature of the waste and bulking agent.

Table 1 gives examples of composting-vermicomposting using different kinds of organic waste. It is apparent that the incubation time for composting and vermicomposting is not the same. Composting usually takes around two to four weeks depending on the starting material and duration of the thermophilic phase. For instance, Nair et al. (2006) suggest for producing pathogen free compost from kitchen waste 9 days of pre-composting followed by 2.5 months of vermicomposting. Thermocomposting reduces both the time and area needed for vermicomposting by reducing the volume of material to be processed.

Composting-vermicomposting used to remove pollutants

Earthworms can accumulate heavy metals and organic pollutants (Sinha et al. 2008). Moreover, they increase their availability for microorganisms by grinding the waste into smaller particles. Earthworms generally improve soil microbial activity by stimulating the growth of bacteria and fungi both in their intestine and their faeces (Dendooven et al. 2011). Increase in activity of the detoxification enzymes cytochrome P450 and glutathione-S-transferase in earthworms are reported when they ingest different kinds of pollutants, which indicates earthworms are also able to degrade pollutants (Achazi et al. 1998; Zhang et al. 2009; Zhao et al. 2020). Earthworms are great accumulators of metals, especially zinc and cadmium, which are incorporated into their soft tissues. In that sense, earthworms can also act as indicators of metal pollution. Metals can also be transformed to a valent state inside earthworms, which makes them more available for plants.

Pollutants are not always completely removed from WWTP (Luo et al. 2014), in which case the WWTP sludge should not be used as a fertiliser, as it would contaminate field plants and the whole food chain. Composting and vermicomposting are both proven to be successful methods for removing pollutants (Poulsen and Bester 2010). However, vermicomposting is not suitable for the immediate remediation of WWTP sewage sludge due to the toxicity of NH_3 and CH_4 (Awiszus et al. 2018). Pre-composting with a nutrient-rich bulking agent, such as cow manure or green waste, stabilizes sewage sludge (Kaushik and Garg 2003; Hanc and Dreslova 2016). It not only reduces its toxicity to earthworms, but also adds nutrients to the final product.

Vermiremediation of sewage sludge or contaminated soil using pre-composting has been investigated (see Table 2). However, there is no detailed comparison of pre-composting-vermicomposting with composting and vermicomposting in terms of pollutant removal. Composting followed by vermiremediation is studied mainly for its efficiency in removing heavy metals and polycyclic aromatic hydrocarbons. Maňáková et al. (2014) report that combining these processes results in a greater reduction in the mobility and bioavailability of arsenic. The mobile arsenic pool is reduced to 4/9 of its initial value due to bioaccumulation. Soobhany et al. (2015) confirm the vermiaccumulation of other heavy metals with decrease in the bioaccumulation factors (BCFs) as follows: Cd > Ni > Cu > Co > Cr > Zn. In contrast, composting without earthworms results in a progressive increase in heavy metal concentrations due to the reduction in the volume of compost due to decomposition. Rorat et al.

Table 1 Summary of the results of composting-vermicomposting of organic wastes.

Organic waste	Bulking agent (amendment)	Composting duration (days)	Vermicomposting duration (days)	Earthworms used	Notes/Findings	Reference
Municipal sewage sludge digestate	Green waste, spent mushroom compost, wheat straw, biochar	43	90	*Eisenia fetida*	Similar outcomes as conventional composting, but kinetin concentration was two times higher.	Rékási et al. 2019
Vinasse	Bagasse, cow manure, zeolite	21	60	*Eisenia fetida*	Lower content of vinasse and higher content of zeolite resulted in better quality compost.	Alavi et al. 2017
Sewage sludge	Municipal solid waste, grass clippings, sawdust	30	45	*Eisenia andrei, Eisenia fetida, Dendrobaena veneta*	*Eisenia* species of earthworms exhibited stronger defence and higher ability to accumulate heavy metals.	Suleiman et al. 2017
Press mud	Cow dung, green manure plants	21	50	*Eudrilus eugeniae*	Ratio 2:1:1 (pressmud : cow dung : green manure plants) resulted in the high quality compost.	Balachandar et al. 2020
Garden waste	Cattle manure, spent mushroom substrate	21	70	*Eisenia fetida*	Ratio 2:1:1 (garden waste : cattle manure : spent mushroom substrate) resulted in high quality compost.	Gong et al. 2019
Pistachio waste	Cow dung	45	45	*Eisenia fetida*	Ratio 1:3 (pistachio waste : cow dung) resulted in high quality compost.	Esmaeili et al. 2020
Vegetable waste	Cow dung, saw dust, dried leaves	8	20	*Eisenia fetida, Eudrilus eugeniae*	Stabilized end product within a short period of time using rotary drum.	Varma and Kalamdhad 2016
Sugarcane press mud	Bagasse, sugarcane trash	30	40	*Drawida willsi*	Composting-vermicomposting method reduced the time required for composting.	Kumar et al. 2010
Rice straw, paper waste	Cow dung	21	105	*Eisenia fetida*	High fragmentation and homogeneity of vermicompost based on SEM pictures.	Sharma and Garg 2018
Press mud sludge	Cattle dung	15	135	*Eisenia fetida*	Ratio 1:3 (compressed sludge : cattle dung) resulted in good growth and fecundity of earthworms.	Bhat et al. 2016
Sewage sludge, vinasse	Rabbit manure	21	56	*Eisenia fetida*	Rabbit manure enhanced the reproduction and weight of earthworms.	Molina et al. 2013
Tomato crop residues	Almond shells	63	198	*Eisenia andrei, Eisenia fetida*	Vermicompost and pre-composted vermicompost had similar properties.	Fornes et al. 2012

(2017) also report vermiaccumulation of heavy metals, with decreases in BCFs as follows: Cd > Cu > Zn > Ni >Cr > Pb. Kharrazi et al. (2014), on the other hand, report increases in heavy metals concentrations in compost produced by the composting-vermicomposting process. These authors discuss possible reasons for this e.g. mineralization making metals more available or loss of the overall mass due to decomposition. They did not study the vermiaccumulation of heavy metals. Suleiman et al. (2017) report the accumulation of heavy metals by three species of earthworm, namely *Eisenia andrei, Eisenia fetida* and *Dendrobaena veneta*. BCFs were ranked as follows: Cd > Co > Cu > Zn > Ni > Pb > Cr. Of the earthworms studied, the *Eisenia* species exhibit the highest ability to vermiaccumulate heavy metals.

The fate of polycyclic aromatic hydrocarbons (PAHs) during vermicomposting is also reported. Rorat et al. (2017) report a significant reduction in 16 priority PAHs after 30 days of composting followed by 35 days of vermicomposting. Total amount of PAHs is reduced by up to 85.75%, with the reduction in naphthalene, acenaphtylene, phenanthrene and benzo(g,h,i)perylene the most marked. In addition to vermiaccumulation, degradation is reported, namely that of 5-rings PAHs to 3- and 4-rings PAHs. Composting alone is efficient when degrading PAHs (Cajthaml and Šašek 2005). However, degradation occurs in the final maturation phase, which can take up to 300 days. Composting-vermicomposting could therefore potentially decrease the time required to remove PAHs.

Table 2 Summary of the results of pre-composting followed by vermiremediation of pollutants.

Pollutant	Matrix	Bulking agent (amendment)	Composting duration (days)	Vermicom-posting duration (days)	Earthworms used	Notes/Findings	Reference
Arsenic	Sewage sludge	Horse manure, sawdust, grass clippings	90	90	*Eisenia fetida*	Decrease in mobility to 4/9.	Maňáková et al. 2014
Heavy metals	Municipal solid waste	Food waste, paper waste, yard waste, cow dung	17	53	*Eudrilus eugeniae*	BCFs: Cd > Ni > Cu > Co > Cr > Zn.	Soobhany et al. 2015
Heavy metals	Sewage sludge	*Miscanthus* green waste, market waste, organic fraction of municipal solid waste	30	35	*Eisenia andrei*	BCFs: Cd > Cu > Zn > Ni > Cr > Pb.	Rorat et al. 2017
Heavy metals	Sewage sludge	Corn waste, cow dung, compost, paper	30	40	*Eisenia fetida*	Increase in heavy metal content due to the decrease in overall mass.	Kharrazi et al. 2014
Heavy metals	Pig manure	Rice straw	15	45	*Eisenia fetida*	Increase in the Cu and Zn availability after vermicomposting.	Zhu et al. 2014
Heavy metals	Sewage sludge	Spent mushroom compost	21	105	*Lumbricus rubellus*	90–98.7% removal of Cr, Cd and Pb.	Azizi et al. 2013a
Heavy metals	Sewage sludge	Municipal solid wastes, grass clippings, sawdust	30	45	*Eisenia andrei, Eisenia fetida, Dendrobaena veneta*	BCFs: Cd > Co > Cu > Zn > Ni > Pb > Cr.	Suleiman et al. 2017
Petroleum hydrocarbons	Soil	Compost	x (compost as amendment)	15	*Eisenia fetida*	Enrichment of microorganisms after adding compost as an amendment.	Ceccanti et al. 2006
16 priority PAHs	Sewage sludge	*Miscanthus* green waste, markets waste, organic fraction of municipal solid waste	30	35	*Eisenia andrei*	Degradation of 5-ring PAHs to 3- and 4-ring PAHs is reported.	Rorat et al. 2017
Anthracene, phenanthrene, benzo(α)pyrene	Soil, sewage sludge	x	21	60	*Lumbricus rubellus*	99.99% PAHs removed.	Azizi et al. 2013b
Asphaltenes	Heavy fuel oil	Cow bedding, rice husks, seaweed extracts, potato peelings	112	183	*Eisenia fetida*	Microorganisms obtained carbon and energy from asphaltenes.	Martín-Gil et al. 2008

Conclusion

Pre-composting is an important step when decomposing organic waste by vermicomposting. It facilitates its breakdown, suppresses pathogens and decomposes toxic compounds, which could harm the earthworms. Moreover, as it results in a reduction in mass, less time and space is needed for vermicomposting. Pre-composting can be also used prior to the vermiremediation of WWTPs sludge and contaminated soil. A combination of composting and vermicomposting has been successfully used for removing polycyclic aromatic hydrocarbons and heavy metals. However, no research has been done on using this method for removing other organic pollutants, such as pharmaceuticals or endocrine disruptors. This mini-review indicates that composting-vermicomposting is a promising low-cost and environmentally friendly way of treating contaminated WWTP sludge.

Acknowledgements

This work was supported by The Ministry of Agriculture of the Czech Republic (grant number QK1910095).

REFERENCES

Achazi RK, Flenner C, Livingstone DR, Peters LD, Schaub K, Scheiwe E (1998) Cytochrome P450 and dependent activities in unexposed and PAH-exposed terrestrial annelids. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 121: 339–350.

Alavi N, Daneshpajou M, Shirmardi M, Goudarzi G, Neisi A, Babaei AA (2017) Investigating the efficiency of co-composting and vermicomposting of vinasse with the mixture of cow manure wastes, bagasse, and natural zeolite. Waste Manag 69: 117–126.

Awiszus S, Meissner K, Reyer S, Müller J (2018) Ammonia and methane emissions during drying of dewatered biogas digestate in a two-belt conveyor dryer. Bioresour Technol 247: 419–425.

Azizi AB, Lim MPM, Noor ZM, Abdullah N (2013a) Vermiremoval of heavy metal in sewage sludge by utilising *Lumbricus rubellus*. Ecotoxicol Environ Saf 90: 13–20.

Azizi AB, Liew KY, Noor ZM, Abdullah N (2013b) Vermiremediation and mycoremediation of polycyclic aromatic hydrocarbons in soil and sewage sludge mixture: a comparative study. Int J Environ Sci Dev 4: 565–568.

Balachandar R, Baskaran L, Yuvaraj A, Thangaraj R, Subbaiya R, Ravindran B, Chang SW, Karmegam N (2020) Enriched pressmud vermicompost production with green manure plants using *Eudrilus eugeniae*. Bioresour Technol 299: 122578.

Bhat SA, Singh J, Vig AP (2016) Effect on growth of earthworm and chemical parameters during vermicomposting of pressmud sludge mixed with cattle dung mixture. Procedia Environ Sci 35: 425–434.

Cai Q-Y, Mo C-H, Wu Q-T, Zeng Q-Y, Katsoyiannis A, Ferard J-F (2007) Bioremediation of polycyclic aromatic hydrocarbons (PAHs)-contaminated sewage sludge by different composting processes. J Hazard Mater 142: 535–542.

Cajthaml T, Bhatt M, Šašek V, Matějů V (2002) Bioremediation of PAH-contaminated soil by composting: A case study. Folia Microbiol 47: 696–700.

Cajthaml T, Šašek V (2005) Application of supercritical fluid extraction (SFE) to predict bioremediation efficacy of long-term composting of PAH-contaminated soil. Environ Sci Technol 39: 8448–8452.

Ceccanti B, Masciandaro G, Garcia C, Macci C, Doni S (2006) Soil bioremediation: combination of earthworms and compost for the ecological remediation of a hydrocarbon polluted soil. Water Air Soil Pollut 177: 383–397.

Chachina SB, Voronkova NA, Baklanova ON (2016) Biological remediation of the petroleum and diesel contaminated soil with earthworms *Eisenia fetida*. Procedia Eng 152: 122–133.

Chevillot F, Convert Y, Desrosiers M, Cadoret N, Veilleux É, Cabana H, Bellenger JP (2017) Selective bioaccumulation of neonicotinoids and sub-lethal effects in the earthworm *Eisenia andrei* exposed to environmental concentrations in an artificial soil. Chemosphere 186: 839–847.

Clarke BO, Smith SR (2011) Review of 'emerging' organic contaminants in biosolids and assessment of international research priorities for the agricultural use of biosolids. Environ Int 37: 226–247.

Covino S, Fabianová T, Křesinová Z, Čvančarová M, Burianová E, Filipová A, Vořísková J, Baldrian P, Cajthaml T (2016) Polycyclic aromatic hydrocarbons degradation and microbial community shifts during co-composting of creosote-treated wood. J Hazard Mater 301: 17–26.

de Bertoldi M, Vallini G, Pera A (1983) The biology of composting: A review. Waste Manag Res 1: 157–176.

Dendooven L, Alvarez-Bernal D, Contreras-Ramos SM (2011) Earthworms, a means to accelerate removal of hydrocarbons (PAHs) from soil? A mini-review. Pedobiologia 54: 187–192.

Domínguez J (2004) State-of-the-art and new perspectives on vermicomposting research. In: Earthworm Ecology (2nd Edition). CRC Press, USA, pp 401–424.

Esmaeili A, Khoram MR, Gholami M, Eslami H (2020) Pistachio waste management using combined composting-vermicomposting technique: physico-chemical changes and worm growth analysis. J Clean Prod 242: 118523.

Finstein MS, Morris ML (1975) Microbiology of municipal solid waste composting. Adv Appl Microbiol 19: 113–151.

Fornes F, Mendoza-Hernández D, García-de-la-Fuente R, Abad M, Belda RM (2012) Composting versus vermicomposting: a comparative study of organic matter evolution through straight and combined processes. Bioresour Technol 118: 296–305.

Gong X, Li S, Carson MA, Chang SX, Wu Q, Wang L, An Z, Sun X (2019) Spent mushroom substrate and cattle manure amendments enhance the transformation of garden waste into vermicomposts using the earthworm *Eisenia fetida*. J Environ Manage 248: 109263.

Grewal SK, Rajeev S, Sreevatsan S, Michel FC (2006) Persistence of Mycobacterium avium subsp. *paratuberculosis* and other zoonotic pathogens during simulated composting, manure packing, and liquid storage of dairy manure. Appl Env Microbiol 72: 565–574.

Guo Y, Rene ER, Wang J, Ma W (2020) Biodegradation of polyaromatic hydrocarbons and the influence of environmental factors during the co-composting of sewage sludge and green forest waste. Bioresour Technol 297: 122434.

Hanc A, Dreslova M (2016) Effect of composting and vermicomposting on properties of particle size fractions. Bioresour Technol 217: 186–189.

Havranek I, Coutris C, Norli HR, Rivier PA, Joner EJ (2017) Uptake and elimination kinetics of the biocide triclosan and the synthetic musks galaxolide and tonalide in the earthworm *Dendrobaena veneta* when exposed to sewage sludge. Environ Toxicol Chem 36: 2068–2073.

He X, Zhang Y, Shen M, Zeng G, Zhou M, Li M (2016) Effect of vermicomposting on concentration and speciation of heavy metals in sewage sludge with additive materials. Bioresour Technol 218: 867–873.

Hickman ZA, Reid BJ (2008) Earthworm assisted bioremediation of organic contaminants. Environ Int 34: 1072–1081.

Iranzo M, Gamón M, Boluda R, Mormeneo S (2018) Analysis of pharmaceutical biodegradation of WWTP sludge using composting and identification of certain microorganisms involved in the process. Sci Total Environ 640–641: 840–848.

Kaushik P, Garg VK (2003) Vermicomposting of mixed solid textile mill sludge and cow dung with the epigeic earthworm *Eisenia foetida*. Bioresour Technol 90: 311–316.

Kharrazi SM, Younesi H, Abedini-Torghabeh J (2014) Microbial biodegradation of waste materials for nutrients enrichment and heavy metals removal: An integrated composting-vermicomposting process. Int Biodeterior Biodegrad 92: 41–48.

Kumar R, Verma D, Singh BL, Kumar U (2010) Composting of sugar-cane waste by-products through treatment with microorganisms and subsequent vermicomposting. Bioresour Technol 101: 6707–6711.

Lim SL, Lee LH, Wu TY (2016) Sustainability of using composting and vermicomposting technologies for organic solid waste biotransformation: recent overview, greenhouse gases emissions and economic analysis. J Clean Prod 111: 262–278.

Lin Z, Zhen Z, Liang Y, Li J, Yang J, Zhong L, Zhao L, Li Y, Luo C, Ren L (2019) Changes in atrazine speciation and the degradation pathway in red soil during the vermiremediation process. J Hazard Mater 364: 710–719.

Luo Y, Guo W, Ngo HH, Nghiem LD, Hai FI, Zhang J, Liang S, Wang XC (2014) A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Sci Total Environ 473: 619–641.

Maňáková B, Kotyza J, Svobodová M, Hofman J (2014) Effects of combined composting and vermicomposting of waste sludge on arsenic fate and bioavailability. J Hazard Mater 280: 544–551.

Martín-Gil J, Navas-Gracia LM, Gómez-Sobrino E, Correa-Guimaraes A, Hernández-Navarro S, Sánchez-Báscones M, Ramos-Sánchez MdC (2008) Composting and vermicomposting experiences in the treatment and bioconversion of asphaltens from the Prestige oil spill. Bioresour Technol 99: 1821–1829.

Molina MJ, Soriano MD, Ingelmo F, Llinares J (2013) Stabilisation of sewage sludge and vinasse bio-wastes by vermicomposting with rabbit manure using *Eisenia fetida*. Bioresour Technol 137: 88–97.

Nair J, Sekiozoic V, Anda M (2006) Effect of pre-composting on vermicomposting of kitchen waste. Bioresour Technol 97: 2091–2095.

Ndegwa PM, Thompson SA (2001) Integrating composting and vermicomposting in the treatment and bioconversion of biosolids. Bioresour Technol 76: 107–112.

Owagboriaye F, Dedeke G, Bamidele J, Aladesida A, Isibor P, Feyisola R, Adeleke M (2020) Biochemical response and vermiremediation assessment of three earthworm species (Alma *millsoni*, *Eudrilus eugeniae* and *Libyodrilus violaceus*) in soil contaminated with a glyphosate-based herbicide. Ecol Indic 108: 105678.

Poulsen TG, Bester K (2010) Organic micropollutant degradation in sewage sludge during composting under thermophilic conditions. Environ Sci Technol 44: 5086–5091.

Rékási M, Mazsu N, Draskovits E, Bernhardt B, Szabó A, Rivier P-A, Farkas C, Borsányi B, Pirkó B, Molnár S (2019) Comparing the agrochemical properties of compost and vermicomposts produced from municipal sewage sludge digestate. Bioresour Technol 291: 121861.

Rodriguez-Campos J, Dendooven L, Alvarez-Bernal D, Contreras-Ramos SM (2014) Potential of earthworms to accelerate removal of organic contaminants from soil: A review. Appl Soil Ecol 79: 10–25.

Rorat A, Wloka D, Grobelak A, Grosser A, Sosnecka A, Milczarek M, Jelonek P, Vandenbulcke F, Kacprzak M (2017) Vermiremediation of polycyclic aromatic hydrocarbons and heavy metals in sewage sludge composting process. J Environ Manage 187: 347–353.

Sharma K, Garg VK (2018) Comparative analysis of vermicompost quality produced from rice straw and paper waste employing earthworm *Eisenia fetida* (Sav.). Bioresour Technol 250: 708–715.

Shi Z, Liu J, Tang Z, Zhao Y, Wang C (2019) Vermiremediation of organically contaminated soils: Concepts, current status, and future perspectives. Appl Soil Ecol 147: 103377.

Sinha RK, Bharambe G, Ryan D (2008) Converting wasteland into wonderland by earthworms – a low-cost nature's technology for soil remediation: a case study of vermiremediation of PAHs contaminated soil. Environmentalist 28: 466–475.

Soobhany N, Mohee R, Garg VK (2015) Comparative assessment of heavy metals content during the composting and vermicomposting of municipal solid waste employing *Eudrilus eugeniae*. Waste Manag 39: 130–145.

Suleiman H, Rorat A, Grobelak A, Grosser A, Milczarek M, Płytycz, B, Kacprzak M, Vandenbulcke F (2017) Determination of the performance of vermicomposting process applied to sewage sludge by monitoring of the compost quality and immune responses in three earthworm species: *Eisenia fetida*, *Eisenia andrei* and *Dendrobaena venet*a. Bioresour Technol 241: 103–112.

Suthar S, Sajwan P, Kumar K (2014) Vermiremediation of heavy metals in wastewater sludge from paper and pulp industry using earthworm *Eisenia fetida*. Ecotoxicol Environ Saf 109: 177–184.

Tognetti C, Laos F, Mazzarino MJ, Hernández MT (2005) Composting vs. vermicomposting: a comparison of end product quality. Compost Sci Util 13: 6–13.

Varma VS, Kalamdhad AS (2016) Efficiency of rotary drum composting for stabilizing vegetable waste during pre-composting and vermicomposting. Environ Process 3: 829–841.

Wei Y, Zhao Y, Zhao X, Gao X, Zheng Y, Zuo H, Wei Z (2020) Roles of different humin and heavy-metal resistant bacteria from composting on heavy metal removal. Bioresour Technol 296: 122375.

Yadav A, Garg VK (2019) Biotransformation of bakery industry sludge into valuable product using vermicomposting. Bioresour Technol 274: 512–517.

Zhang X, Lu Y, Shi Y, Chen C, Yang Z, Li Y, Feng, Y (2009) Antioxidant and metabolic responses induced by cadmium and pyrene in the earthworm *Eisenia fetida* in two different systems: contact and soil tests. Chem Ecol 25: 205–215.

Zhao S, Wang B, Zhong Z, Liu T, Liang T, Zhan J (2020) Contributions of enzymes and gut microbes to biotransformation of perfluorooctane sulfonamide in earthworms (*Eisenia fetida*). Chemosphere 238: 124619.

Zhu W, Yao W, Zhang Z, Wu Y (2014) Heavy metal behavior and dissolved organic matter (DOM) characterization of vermicomposted pig manure amended with rice straw. Environ Sci Pollut Res 21: 12684–12692.

SPATIAL ANALYSIS OF THE ACCESSIBILITY OF URBAN GREENSPACE AT THE CITY LEVEL

PANAGIOTIS – TSAMPIKOS A. KOLIOTSIS*
and MARIA P. PAPADOPOULOU

Laboratory of Physical Geography and Environmental Impacts, School of Rural and Surveying Engineering, National Technical Univesity of Athens (NTUA), 9 Iroon Polytechniou, University Campus, Zografou 15780 GR
* Corresponding author: panagiotis.koliotsis@gmail.com

ABSTRACT

The aim of this study was to analyse the access people have to urban greenspaces at the city level (Athens) using a combination and by comparing different methods. These two approaches are the Accessible Natural Greenspace Standards (ANGSt) Model and selected urban greenspace indices. According to the results, the accessibility of areas of urban greenspace is sufficient in most of Athens, which indicates that the majority of its residents have access to urban greenspaces. The correlation of accessibility with urban greenspace indices provided a better classification for Athens, in terms of citizens' quality of life, as 20% of the Municipalities have a higher value for greenspace than that recommended by the World Health Organization of 9 m^2. If this percentage is expressed as a population equivalent, only 13.3% of the population of Athens has a higher value than the minimum recommended. In addition, 21% of the population has a much smaller value and, in particular, it does not exceed 2 m^2 of greenspace per capita.

Keywords: accessible natural greenspace standards model; Athens; climate change; multicriteria analysis; urban greenspace indices

Introduction

The intensive urbanization in the 20th century was among the major causes of the significant reduction in urban greenspaces in modern cities. The existing greenspaces were fragmented, because of the required planning ordinaces, without creating an urban network (Aravantinos 1997). Urban greenspace improves the residents' quality of life, particularly in densely populated cities. The multiple benefits that arise from the existence of greenspaces in cities are in the area's microclimatic conditions, such as temperature, filtering of solar radiation and improvement in the neighbourhood's social relations. The causes of Climate Change are divided into two main categories: a) external causes, primarily changes in the global energy balance and in the amount of energy received from the sun, and b) internal causes associated with changes in the composition of the atmosphere as well as the earth's surface and land use – factors directly related to human activities and the corresponding greenhouse gas emissions (GHGs) (USGCRP 2014). According to the United Nations Framework Convention on Climate Change (UNFCCC), Climate Change (CC) is defined as the change in climate that is caused directly or indirectly by human activities (United Nations 1992).

Thus, the appropriate adaptation strategies and necessary actions and measures have to be considered at local, regional, national and continental levels. In order to increase a city's resilience and positively contribute to its sustainability, urban greenspace should be properly designed. In other words, the people should be provided with a means of accessing these areas (Wooley 2003). The quality of public urban greenspace is directly related to the living standards, health and quality of life of a city's inhabitants (Iliadou et al. 2012). Greenspaces can also significantly contribute to the promotion of environmental, social and economic benefits of Green Infrastructure as a planning tool (Papageorgiou and Gementzi 2018).

In Greek cities, urban greenspaces generally arise as surplus land during reconstruction and not as the result of urban planning, which explains the low level of greenspace per capita in comparison to other European metropolitan cities (Pournara 2013). Pozokidou (2018) attempted to model the dynamics of land use and transportaton infrastructure using a simplified urban model for the periurban area of east Thessaloniki considering all factors contributing to urban growth including greenspace.

The term "urban greenspace" has prevailed as a term used to characterize an area that is put aside during the development of a city and remains free of buildings and hosts different forms of vegetation. Urban and suburban greenspace is a sustainability index of the urban fabric. In addition, a main factor in the planning of urban greenspace, apart from its existence, is the accessibility or proximity of these areas. A key indicator of accessibility is proximity of greenspace to a residence or neighbourhood (World Health Organization 2016). However, there are many definitions and research studies on the accessibility of urban greenspace. The most common view is that the accessibility of urban greenspace should be based on its proximity and size (Mougiakou and Photis 2014). For example, as the size of the urban greenspace increases, the area over which it is considered to be accessible also increases.

To better understand the interdependency of urban spaces and human life, an uninterrupted observation and analysis is required. Nowadays, Geographic Information

Koliotsis, P.-T. A., Papadopoulou, M. P.: Spatial analysis of the accessibility of urban greenspace at the city level
European Journal of Environmental Sciences, Vol. 10, No. 1, pp. 15–21
https://doi.org/10.14712/23361964.2020.3
© 2020 The Authors. This is an open-access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Systems (GIS) are a valuable tool used by engineers, planners and policy makers to analyze descriptive information at any spatial resolution. GIS is designed to capture, analyse and process spatial data related to climatic conditions and geomorphology as well as the demography and characteristics of building environments (Goodchild 1985). GIS can easily update maps by incorporating new data that can simply be added to the existing database or map.

Case study: Athens

In this analysis, the area of interest is the metropolitan area of Athens, the capital of Greece, which is one of the most densely populated cities in the country. During the last century, the urban core of Athens developed in a self-contained region called the "Attica Basin", between the Penteli, Parnitha, Ymittos and Aigaleo mountains and the Saronikos Gulf. The "Attica Basin" coincides with the Athens-Piraeus Spatial Unity and is divided into 5 spatial subsections: a) Central Athens, b) South Athens, c) North Athens, d) West Athens and e) Piraeus.

The urban greenspace sustainability index is quite low in Athens, because of the compact urban fabric and high population density (OECD 2014). According to the annual statistical publication of OECD, Athens is 4th from the bottom with only 0.96 m^2 of green area per person (OECD 2014). Urban greenspaces differ in the five Spatial Subsections and also among the municipalities within the same Subsection. Both the network and amount of greenspace per resident are different in each Subsection, indicating a significant variability in the living standards in the "Attica Basin" (Table 1).

An analysis of the financial profiles of the spatial subsections indicates that a high income per capita is accompanied by a high percentage of greenspace per capita, or the residents want to move to areas with a higher percentage of greenspace (Kalavrytinos and Damigos 2006). This is because the marital or personal financial status of people leads them to initiate changes within their city, as they move to areas where there is a better quality of life. In the case of Athens, this fact led to the relocation to the suburbs of a large number of people (Asimakopoulos et al. 2011).

Methods

The Accessible Natural Greenspace Standards (ANGSt) model

The accessibility of greenspace to citizens within a city varies from country to country and from city to city. The Accessible Natural Greenspace Standards (ANGSt) model was developed in 1990 to estimate the access to a natural area within the urban fabric by defining the minimum distance that a citizen has to walk to reach an area of urban greenspace (Buell 2009). Following this approach, an urban greenspace network is classified into the following classes, in terms of there should be at least:

- one accessible 2 ha greenspace within 300 m distance,
- one accessible 20 ha greenspace within 2 km distance,
- one accessible 100 ha greenspace within 5 km distance,
- one accessible 500 ha greenspace within 10 km distance.

Natural urban green areas and greenways, such as public parks, are included in this study as greenspace. Thus, "green" parks, groves and hills within the urban fabric are taken into account.

The cartographic visualization and analysis, in terms of the spatial location and areas characterized as areas of public greenspace, were obtained using GIS. First, a spatial database for the GIS environment was developed. More specifically, the accessibility of urban greenspace was defined based on an influence zone created around the green areas (buffer), which is the distance of a service or means of access. For example, around a 2 ha area of urban greenspace, a buffer zone of 300 m radius is created. Similarly, around a 20 ha and 100 ha area of greenspace, buffer zones of 2 km and 5 km radius, respectively, are created (Fig. 1).

In order to estimate the number of citizens that have access to areas of urban greenspace in Athens, the population, housing, and postcode data in the 2011 Census was obtained from the Hellenic Statistical Authority and analysed (Hellenic Statistics Authority 2016). By joining the population data with the postcodes (city block number), a spatial mapping of the people who have access to an area of greenspace in the corresponding influence zones was obtained. A city block was the spatial unit of the analysis used to estimate the number of people who have access to a corresponding area, because a block is the lowest level of information on the population. The influence zone (buffer) was created in order to define the population served by a specific area of greenspace, regardless of its extent within the limits of the region.

Urban greenspace indices – multicriteria analysis

Multicriteria Definition Analysis (MCDA) is a general framework used in complex decision making situations, with multiple and conflicting objectives. The basic idea of MCDA is to evaluate the performance of alternatives with respect to the criteria that determine the dimensions of the decision making process (Montibeller et al. 2010). MCDA allows decision making to include a range of social, environmental and technical criteria. MCDA provides, also, techniques or algorithms for comparing and ranking different outcomes, even though different indicators are used.

For maintaining the robustness of this analysis, a multicriteria analysis (MCDA) was also used to evaluate the results. The MCDA was done using DEFINITE software that supports decisions based on a finite set of alternatives. By defining the various subsections studied, the criteria under which the evaluation was carried out, the cor-

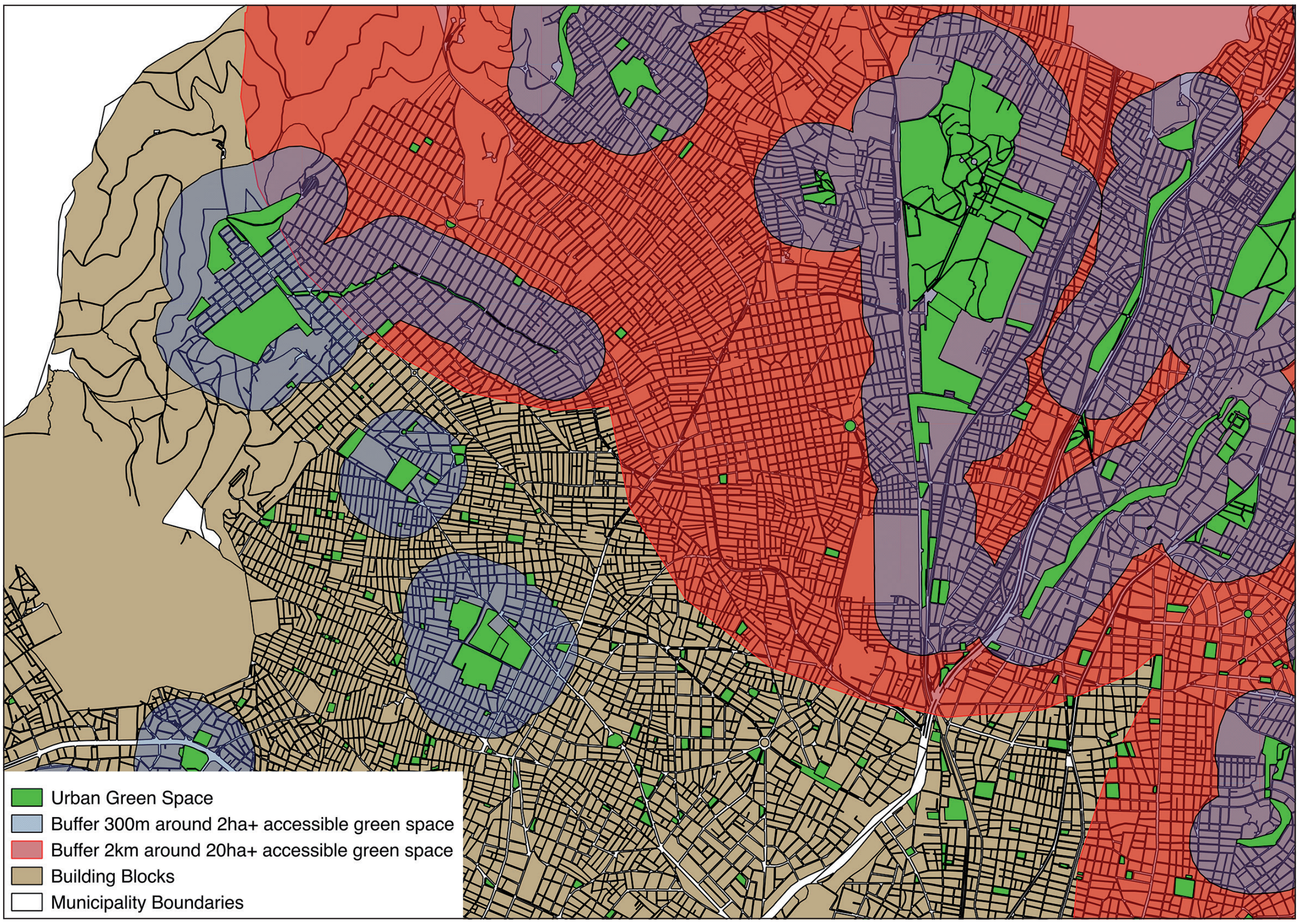

Fig. 1 Buffer zones of 300 m and 2 km, respectively, around 2 ha+ and 20 ha+ greenspace areas.

responding weights that express the relative importance of each criterion were properly defined. In this analysis, all the criteria were considered to be of equal importance.

As the lowest amount of urban greenspace recommended by the World Health Organization is 9 m^2 per capita (OECD 2014), the present analysis of accessible urban greenspace in Athens not only considers public but also private areas of greenspace.

For the term "accessibility", a geometric number based on the Euclidean distance is defined. In addition, a private greenspace index is determined for each Municipality in Athens, based on the building coverage ratio (BCR) that determines the limit of building coverage on a plot. Essentially, it acts as a complementary number for private areas of greenspace coverage. The correlation of accessibility with these two indices allowed a better classification of Athens in terms of its citizens' quality of life. In Fig. 2, a classification of the Municipalities in Athens is shown, based on the percentage of private greenspace obtained from the building coverage ratio. In Fig. 3, a similar classification based on the minimum percentage of urban greenspace per capita in the Municipalities of Athens, as defined by the World Health Organization, is presented.

Based on this approach, only 20% of the Municipalities have a higher value of greenspace than 9 m^2. If this percentage is expressed as a population equivalent, only 13.3% of the population in Athens have a higher value than the minimum recommended. In addition, 21% of the population has a much smaller value and, in particular, it does not exceed 2 m^2 of greenspace per capita.

Furthermore, regarding private greenspace, in 40% of the Municipalities of Athens the coverage of this index exceeds the building coverage, meaning that the building coverage ratio is less than 0.5. That is, 72.9% of the population live on plots of land where the building coverage is greater than the private greenspace coverage.

Results

In this section, the results of the two analyses, ANGSt Analysis and Multicriteria Defintion Analysis, are shown.

Results based on ANGSt analysis

The overall accessibility to urban greenspaces consist of the union of 3 of the 4 greenspace classes: 2 ha, 20 ha and 100 ha, since there are no areas of greenspace of 500 ha or more in Athens. Table 1 summarizes the total population (Hellenic Statistics Authority 2016), the population that has access to greenspace, the percentage population coverage and the index of urban greenspace per capita in the 5 spatial subsections of Athens.

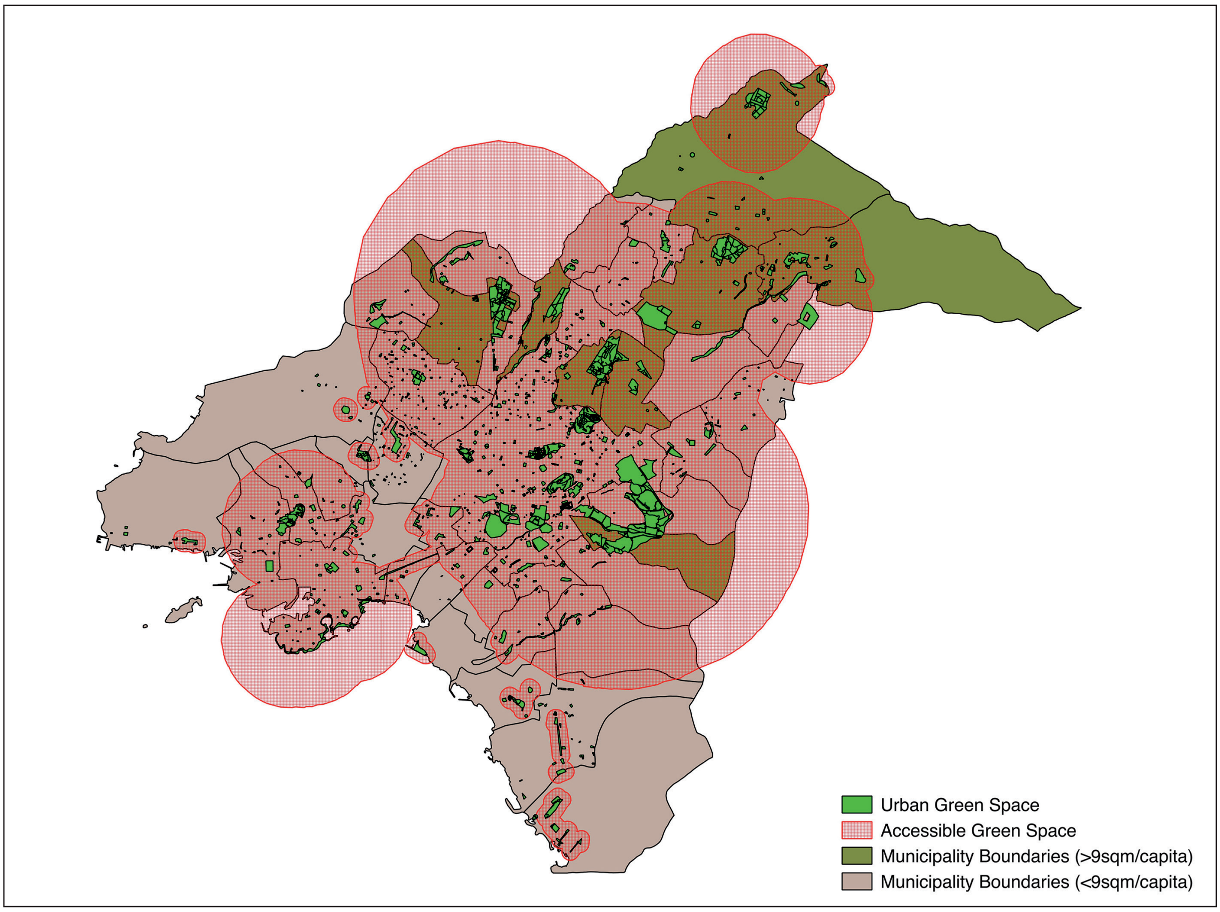

Fig. 2 Classification of Municipalities in terms of the minimum % of urban greenspace per capita.

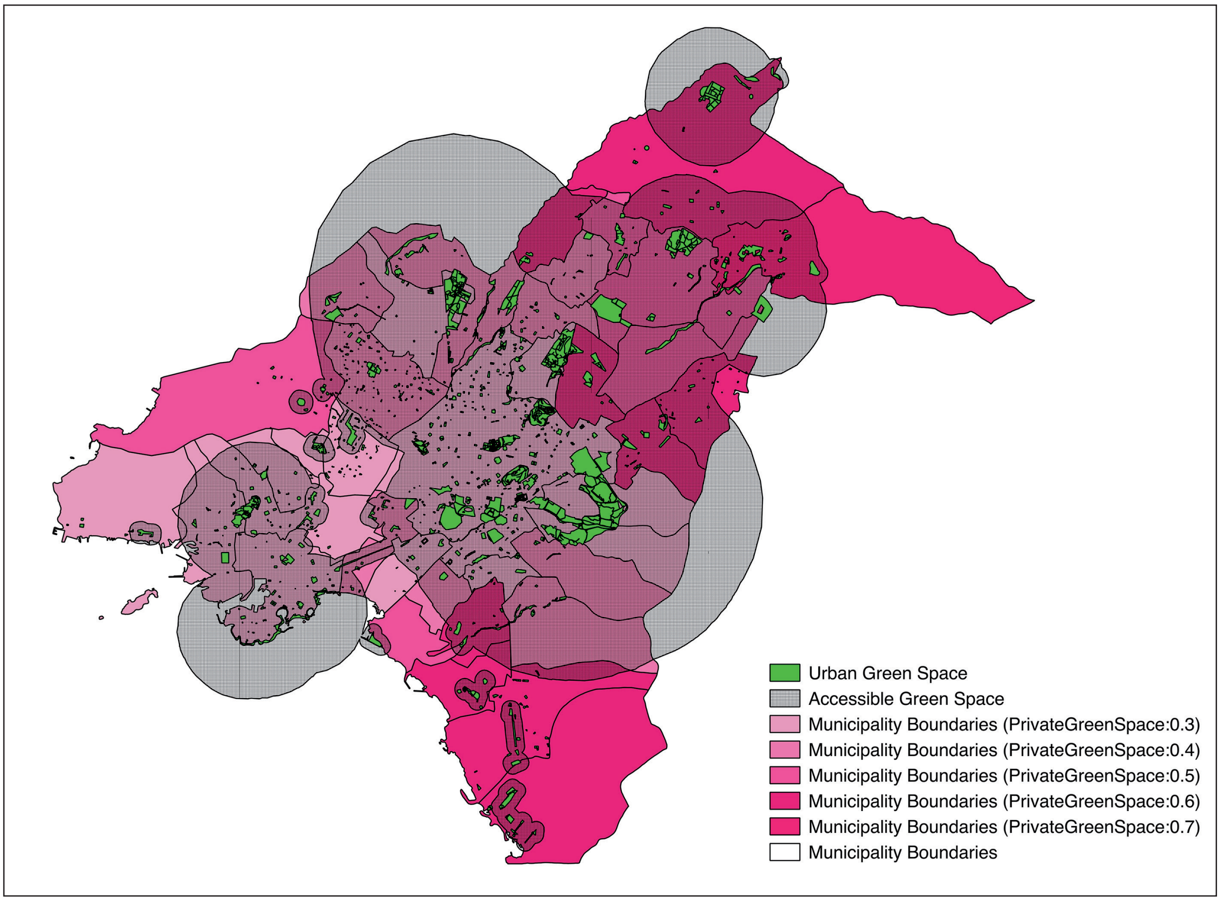

Fig. 3 Classification of the private greenspace index recorded in the different Municipalities of Athens.

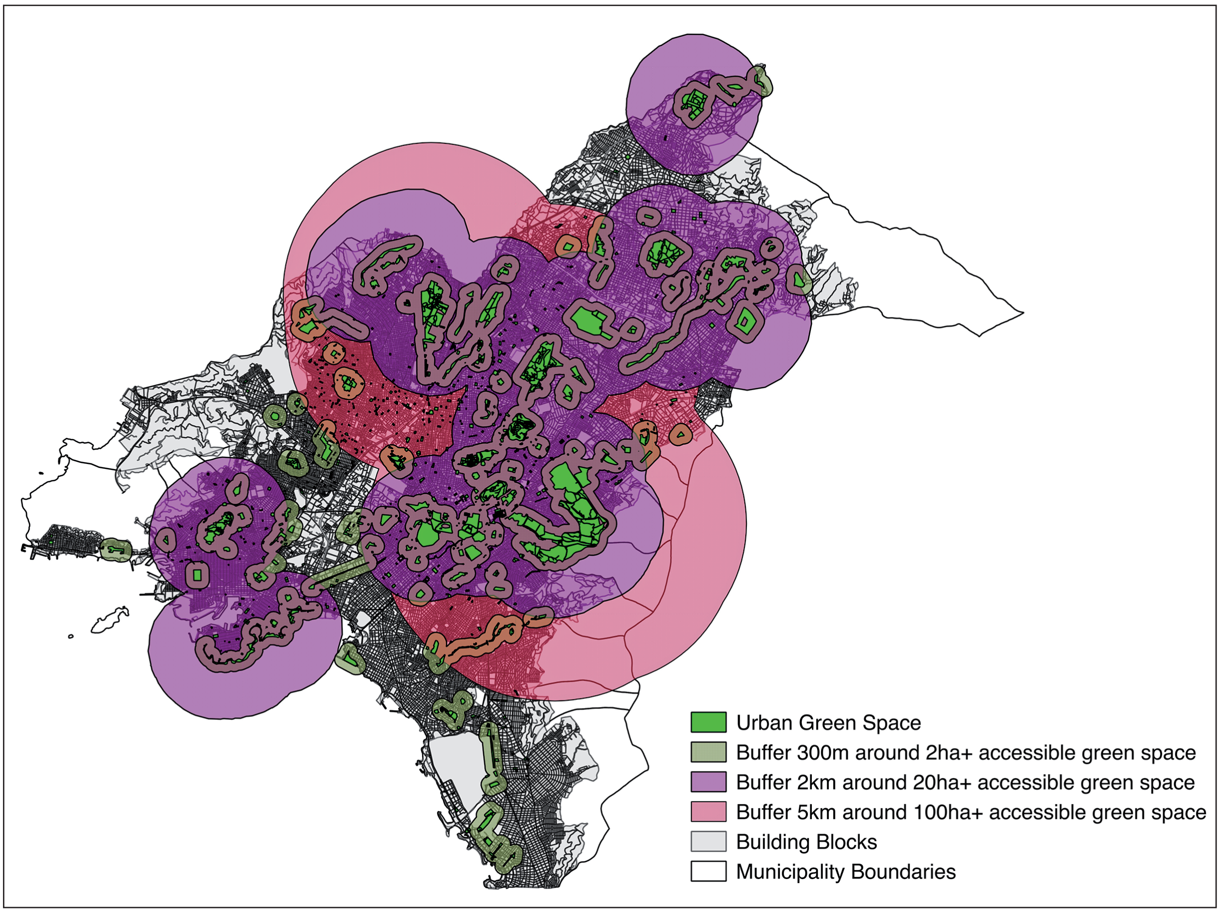

Fig. 4 Athens greenspace accessibility based on the ANGSt analysis.

Central Athens has access to areas of urban greenspace due to the existence of significant groves and hills that have not been built on. North and West Athens also have quite a high percentage of greenspace coverage. Apart from the existence of areas of urban greenspace, the high density of buildings in these three subsections of Athens and the short distance to existing urban greenspaces are the main reasons for the high percentages of accessibility. On the other hand, in the coastal area of South Athens, little urban greenspace exist, except in Piraeus, because the built up area there is far from Athens "green" core (Koliotsis and Papadopoulou 2017). The same trend is recorded for the index of greenspace per capita, which is derived from the General Urban Plan of each municipality (Organization of Planning and Environmental Protection of Athens 2017).

In Fig. 4, the limits of the accessible urban greenspace per class is shown. According to the extracted data on accessible limits, the limit of an area of 2 ha within 300 m means that almost none of the households meet all the ANGSt requirements. In addition, in Central, North and West Athens, most households are within the 20 ha and 100 ha accessible urban greenspace areas, which are mainly located in Central Athens and not in the wider network of greenways. The same pattern is recorded in Piraeus in which there are few areas of greenspace. Finally, in South Athens, there are only a few areas of greenspace and, as a result, the accessibility there is very low.

A key finding of this analysis is that only 19% of the citizens of Athens do not meet the ANGSt requirements and 23% do. In terms of all greenspace area classes, Central Athens had a better coverage. It is remarkable that population coverage and the extent of areas of urban greenspace did not increase accordingly (Table 2). This is due to the spatial location of urban green areas. In other words, accessibility is independent of administrative boundaries and depends only on the size and spatial extent of areas of urban greenspace. This fact explains, for instance, the significant reduction in the population coverage of 20 ha greenspace areas in South Athens, as compared to the corresponding coverage of 2 ha greenspace areas.

Results based on MCDA

In Fig. 5, the overall results of the multi-criteria analysis, with respect to the performance of each Spatial Subsection in relation to each criterion, are shown. As far as the urban greenspace per capita value goes, North Athens is best (1.00) followed by Central Athens (0.99), while the worst is South Athens (0.35). Regarding private greenspace, North Athens again is best followed by South Athens (0.93), while the worst is Piraeus (0.56). The overall results show that, considering the equal importance

Table 1 The Accessibility of Urban Greenspaces in Athens.

Spatial Subsections	Population (Census 2011)	Population (AGNSt model)	**AGNSt Population Coverage (%)**	**Urban Greenspace (m²/cap)**
North Athens	592,490	523,698	88.4	8.30
West Athens	489,675	397,825	81.2	5.48
Central Athens	1029,520	1029,520	100.0	8.25
South Athens	529,826	205,792	38.9	2.93
Piraeus	448,997	342,928	76.4	3.24
Total	**3090,508**	**2499,763**		
Mean Value			80.9	5.64

Table 2 AGNSt model key findings.

Spatial Subsections	**% of households**		
	within 300 m of 2 ha	**within 2 km of 20 ha**	**within 5 km of 100 ha**
North Athens	33	75	73
West Athens	33	52	73
Central Athens	49	80	100
South Athens	16	2	32
Piraeus	44	58	0
Mean Value	**35**	**53**	**56**

criteria, which assumes that the relation of urban greenspace to the quality of peoples' life is independent of ownership, the best area is North Athens (1.00), followed by Central Athens (0.81), while the worst is Piraeus (0.47).

ANGSt analysis vs. MCDA

The ANGSt analysis of the accessibility to urban greenspaces did not include urban greenspace per capita or private greenspace. Only the actual existence, the spatial location and the proximity to these areas were considered. In Table 3, the overall results and subsection hierarchy obtained from the ANGSt analysis and the urban greenspace indices analysis are presented, which indicate a different ranking. This is explained by the fact that in the first analysis, only the Euclidean distance was considered. Whereas, in the latter analysis, the population-related data was also included in the estimates of urban greenspace indices.

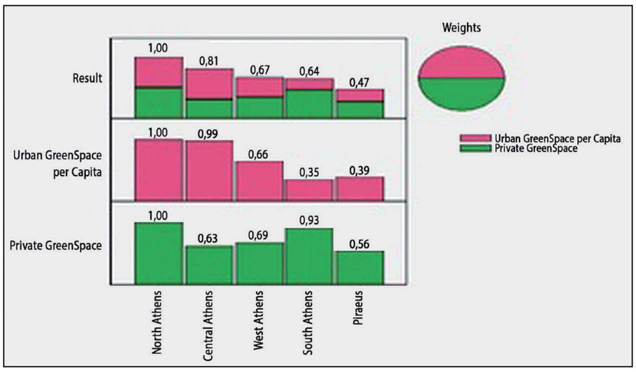

Fig. 5 Results of the multicriteria analysis.

Table 3 Results of the ANGSt model and Urban Greenspace Indices analysis.

Spatial Subsections	**ANGSt Model**		**Urban GreenSpace Indices**	
	Population Coverage (%)	**Hierarchy – Ranking**	**MultiCriteria Analysis Score**	**Hierarchy – Ranking**
North Athens	88.4	2	1.00	1
West Athens	81.2	3	0.67	3
Central Athens	100.0	1	0.81	2
South Athens	38.9	5	0.64	4
Piraeus	76.4	4	0.47	5

Conclusion

The present analysis highlights the accessibility of urban greenspaces in Athens based on the Accessible Natural Greenspace Standards (ANGSt) model. Subsequently, based on this analysis of the data, an estimate of the accessibility of areas of urban greenspace was produced, which gives an indication of the people's ability to access natural greenspace. Based on the results, the majority of the residents in Athens have access to areas of urban greenspace.

The term "accessibility" is poorly understood by many and the concept should be better promoted in order to support the actions of local authorities to improve accessibility and the daily interplay between residents and areas of greenspace. Despite the accessibility being satisfactory, the percentage of greenspace area per resident in Athens is quite low compared with other European cities. This indicates an adequate area of greenspace is as important as its accessibility and the combination of these two indices, among others, can provide a better way of evaluating the quality of the urban environment at the city level. The spatial distribution of urban greenspaces not only affect their accessibility to residents in a particular subsection, but also those in neighbouring subsections.

It is difficult to quantify private greenspaces because the design of such greenspaces is entirely based on each owner. The approach, based on the building coverage ratio, was considered sufficient, given the qualitative differentiation within the urban fabric among the Municipalities.

In this analysis, various methods were used. However, a combination of these methods gave a better estimate of the contribution of urban greenspaces to the quality of the citizens' everyday life. The ANGSt model proved to be a reliable, useful, and effective tool for assessing the current levels of accessibility to greenspaces and comparison of accessibility to areas of greenspace areas within different urban patterns, among different cities and countries. It also provides a standard way of evaluating the accessibility of areas of greenspace and, if necessary, how it can be improved. On the other hand, the multi-criteria analysis also proved to be a reliable tool for evaluating alternative solutions of complex problems and a measure of the extent to which the various alternatives may achieve the objectives. Finally, GIS proved to be a useful spatial analysis tool for mapping and visualizing the spatial data associated with both the assessment of accessibility and the spatial distribution of areas of urban greenspace.

REFERENCES

Aravantinos A (1997) Urban Planning: For the Sustainable Development of Urban Space. Symmetria, Athens.

Asimakopoulos D, Santamouris M, Farrou I, Laskari M, Saliari M, Zanis G, Tigas K, Giannakidis G (2011) Risks and impacts of climate change on the built environment. Bank of Greece, Athens.

Buell S (2009) Analysis of Accessible Natural GreenSpace Provision for Essex, including Southend-on-Sea and Thurrock Unitary Authorities. Essex Wildlife Trust, England.

Goodchild MF (1985) Geographic Information Systems in undergraduate geography: A contemporary dilemma. The Operational Geographer 8: 34–38.

Hellenic Statistics Authority (2016) http://www.statistics.gr. Accessed 10 October 2016.

Iliadou E, Papachatzis A, Kotrotsiou St (2012) Effect of urban environment of human health. Interscientific Health Care 4: 43–48.

Kalavrytinos N, Damigos D (2006) The economic value of urban green spaces in the Attica Basin. Technika Chronika Scientific Journal of the TCG 1: 7–21 (in Greek).

Koliotsis PT, Papadopoulou MP (2017) The contribution of accessible urban greenspace in the quality of residents' life in the Attica Basin – A spatial analysis. E-proceedings 15th International Conference on Environmental Science and Technology, Rhodes, Greece.

Montibeller G, Franco A (2010) Multi-Criteria Decision Analysis for Strategic Decision Making. In: Zopounidis C, Pardalos P (eds) Handbook of Multicriteria Analysis, Applied Optimization. Springer, Berlin, Heidelberg, pp 25–48.

Mougiakou E, Photis Y (2014) Urban green space network evaluation and planning: optimizing accessibility based on connectivity and raster GIS analysis. Eur J Geogr 4: 19–46.

OECD (2014) Economic Environmental and Social Statistics. OECD Publishing. http://dx.doi.org/10.1787/factbook-2014-en. Accessed 15 September 2017.

Organization of Planning and Environmental Protection of Athens (2017) http://geodata.gov.gr/en/organization/orsa?page=2. Accessed 15 September 2017.

Papageorgiou M, Gementzi G (2018) Setting the grounds for the green infrastructure in the metropolitan areas of Athens and Thessaloniki: The role of green space. Eur J Environ Sci 8: pp 83–92.

Pournara S (2013) Public Urban Greenspace. http://www.arch.ntua.gr/sites/default/files/project/6947_/astikoi_koinohristoi_horoi_prasinoy.pdf. Accessed 10 September 2017.

Pozokidou G (2018) Modeling Urban Dynamics: The case of peri-urban development in East Thessaloniki. Eur J Environ Sci 8: pp 23–30.

United Nations (1992) United Nations Framework Convention on Climate Change, FCCC/INFORMAL/84 GE.05-62220 (E) 200705.

USGCRP (2014) Climate Change impacts in the United States. In: Melillo JM, Richmond TC, Yohe GW (eds) The Third National Climate Assessment. U.S. Global Change Research Program. doi:10.7930/J0Z31WJ2.

World Health Organization (2016) Urban green spaces and health: A review of evidence. http://www.euro.who.int/__data/assets/pdf_file/0005/321971/Urban-green-spaces-and-health-review-evidence.pdf?ua=1. Accessed 20 August 2017.

Wooley H (2003) Urban Open Spaces. Spon Press, London, United Kingdom.

QUANTITATIVE ASSESSMENT OF FOREST ECOSYSTEM STRESS CAUSED BY CEMENT PLANT POLLUTION USING IN SITU MEASUREMENTS AND SENTINEL-2 SATELLITE DATA IN A PART OF THE UNESCO WORLD HERITAGE SITE

ALI ASHARFI[1], YOUSEF ERFANIFARD[1,*], FARSHAD AMIRASLANI[1], ALI DARVISHI BOLOORANI[1], and ALIJAFAR MOUSIVAND[2]

[1] Department of Remote Sensing and GIS, Faculty of Geography, University of Tehran, Tehran, Iran
[2] Department of Remote Sensing and GIS, Tarbiat Modares University, Tehran, Iran
* Corresponding author: erfanifard@ut.ac.ir

ABSTRACT

Anthropogenic industrial dust decreases productivity and slows down the growth of plants. Quantifying the effects of industrial dust on vegetation and determining the distance over which factories scatter dust are of paramount importance for biodiversity conservation and sustaining ecosystem services. This study aims at quantifying the effect of dust emitted by the Neka cement plant (NCP), Mazandaran province, northern Iran, on the surrounding Hyrcanian forests based on an analysis of the Leaf Area Index (LAI) retrieved from Sentinel-2 imagery. An Inductively Coupled Plasma Mass Spectrometer (ICP-MS) was used to quantify the concentrations of cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), calcium (Ca), magnesium (Mg), sodium (Na), silicon (Si) and zinc (Zn) in leaves of the dominant Chestnut-leaved Oak (*Quercus castaneifolia*). A feed-forward neural network algorithm and field measurements were used to retrieve the leaf area index (LAI) from Sentinel-2 data with a RMSE of 0.42 (m^2/m^2). MODIS-NDVI and EVI time series spanning 17 years (2000 to 2017) were analysed to ensure the independence of the variation in the condition of the vegetation from drought or other environmental factors. The results indicate that Sentinel-2 data can be used to map degradation due to pollution from the cement plant and quantify the effect of the dust spatially. Dust from the cement plant (dust source) was carried approximately 4700 meters in the direction of the prevailing wind. A significant correlation of 0.849 was recorded between LAI and distance from the NCP. It is concluded that dust from the NCP had adverse ecological effects on the neighbouring forest ecosystems recently designated a UNESCO World Heritage Site.

Keywords: BPNN parameter retrival; drought analysis; Hyrcanian forest; industrial dust; leaf area index (LAI); sentinel-2

Introduction

Dust deposited on leaves of plants reduces their ability to photosynthesize and tolerance of drought and other environmental stresses (Shepherd et al. 2016). The adverse effects can be either direct by causing physical and chemical damage to the plants or indirect due to increase in pests and diseases (Manning and Feder 1980). The effect of dust deposition on vegetation depends on the size, accumulation and chemical properties of the dust particles (Yamaguchi et al. 2017). For instance, dust particles larger than stomatal openings (i.e., 8–10 micron) can block stomata and smaller particles may enter into the leaf tissue (Bell et al. 2002). Therefore, dust particles emitted from industrial factories may have significant negative effects on the photosynthesis, growth, and productivity of vegetation.

Dust particles may be of either natural or anthropogenic origin. The manufacture of cement, an anthropogenic source of dust, is the primary source of calcareous particles (Darley 1966). Its manufacture is closely monitored nowadays because of the environmental pollution it causes and also its contribution (approximately 5–7%) to the total anthropogenic emission of CO_2 (Chen et al. 2010). Although different mechanical systems are used to control and collect dust in a cement plant, a considerable amount of dust is still generated and dispersed in the environment during the extraction and crushing of the raw material. Several studies report the emission of major greenhouse gases and atmospheric pollutants, such as CO_2 and SO_2, by the cement industry (e.g. (Capros et al. 2001; Gartner 2004; Josa et al. 2007). Similarly, there are studies of the effects of natural and industrial dust deposition on different types of vegetation based on field measurements (Lal and Ambasht 1982; Prasad and Inamdar 1990; Iqbal and Shafüg 2000; Joshi and Swami 2009; Zia-Khan et al. 2015; Shepherd et al. 2016). Cement dust particles cause a reduction in plant photosynthesis (Darley 1966; Borka 1980; Zia-Khan et al. 2015), plant transpiration (Joshi and Swami 2009) and results in a reduction in chlorophyll content and increase in water loss in plant (Eveling 1969; Chaurasia 2013; Zia-Khan et al. 2015). It is, therefore, critical to quantify accurately the spatial scale over which dust is carried and affects the functioning and productivity of adjacent ecosystems. Among the biochemical and biophysical properties of vegetation that are affected by industrial dust, Leaf Area Index (LAI) is critical in terms of the productivity and ecosystem process of forests and crops at various spatial and temporal scales (Fang et al. 2003). LAI's response to deposition of dust gives a good indication of forest stress (Madejón et al. 2006; Suciu et al. 2008). As a quantitative indicator, LAI indicates the amount of green foliage, photosynthetic capacity and gas-water exchange in an ecosystem (Mousivand 2015). Ground-based measurements of LAI

Asharfi, A., Erfanifard, Y., Amiraslani, F., Boloorani, A. D., Mousivand, A.: Quantitative assessment of forest ecosystem stress caused by cement plant pollution using in situ measurements and Sentinel-2 satellite data in a part of the UNESCO World Heritage Site
European Journal of Environmental Sciences, Vol. 10, No. 1, pp. 22–31
https://doi.org/10.14712/23361964.2020.4
© 2020 The Authors. This is an open-access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

are time-consuming and tedious and often not practical when investigating large areas. Remote sensing, however, provides a faster, non-destructive and an affordable way of estimating LAI at regional to global scales with reliable spatial and temporal resolution (Verstraete et al. 1996; Zheng et al. 2009). Remotely sensed data are frequently used for studying forest biophysical properties and disturbance caused by pests, disease, fire and drought (Entcheva 2000; Frolking et al. 2009; Kennedy et al. 2010; Chen and Meentemeyer 2016; Abdi et al. 2019). There are very few studies assessing the effects of natural and industrial dust on the functioning of vegetation using remote sensing (Toutoubalina and Rees 1999; Persson 2014) and no studies to our knowledge on the use of satellite images to quantify the effects of pollution from cement plants on surrounding forests. There is an increasing interest in understanding and monitoring forests surrounding industrial factories in order to determine the adverse effect of industrial dust on these ecosystems. Because of their widespread distribution and ease of sampling trees are ideal for assessing the effects of pollution (Kardel et al. 2010). However, using conventional ground surveying techniques to do this can be tedious and expensive. Alternatively, remote sensing offers a suitable tool for quantifying and assessing forest stress due to dust deposition.

Hyrcanian forests are a remnant of old-growth forest dating back more than 25 million years. The forests stretch almost 850 km along the northern slopes of the Alborz Mountains on the southern coast of the Caspian Sea and have a rich faunal and floral biodiversity. The critical role of Hyrcanian forests as a valuable native habitat of more than 3,200 species of vascular plants and 240 species of animals recently resulted in registration of these forests as a UNESCO World Heritage Site (July 2019). The Neka cement plant (NCP) is located at the northern edge of the Hyrcanian mixed forests in northern Iran. Field surveys and Visual interpretation of satellite imagery along with public reports indicate that this industry has had an adverse effect on these forests (e.g. https://financialtribune.com). Nevertheless, the adverse effects of dust deposition on the surrounding forest have not yet been studied.

Therefore, the current study uses remotely sensed data to assess the adverse effects of NCP on the neighbouring Hyrcanian forests in northern Iran. The main objective of this study is to evaluate the potential of Sentinel-2 satellite imagery for estimating LAI of forest stands adjacent to the NCP. This study provides information about the reliability of remote sensing technologies for assessing the stress caused by dust emitted during the manufacture of cement to forests and for efficient decision-making for close-to-nature forest management.

Material and Methods

Study area

The Hyrcanian forests on the south side of the Caspian Sea, cover 500 km^2 along 850 km of the mountains from southeast Azerbaijan to the province of Golestan in Iran. These forests have existed here for 25 to 50 million years. Refugia for broad-leaf forests, which was the dominant species in the North Temperate Zone 25-50 million years ago in the early Cenozoic era, existed in this area (Ramezaniet al. 2008). The area studied is located 36.60 to 36.66 N and 53.36 to 53.52 E, in the east of Mazandaran province, at the northern edge of the Hyrcanian mixed forests near the southern shore of the Caspian Sea in Iran. This area includes the NCP, which was established in 1981 (Fig. 1). The minimum, maximum, and average altitudes in the study area are 80, 600 and 300 meters above sea level, respectively. The climate is semi-Mediterranean characterized by mild and humid winters and hot and humid summers. The dominant species of trees in this region are Chestnut-leaved oak (*Quercus castaneifolia*) (hereafter oak), Persian ironwood (*Parrotia persica*), hornbeam (*Carpinus betulus*) and Siberian elm (*Zelkova carpinifolia*). In addition, trees such as maple (*Acer* sp.) and alder (*Alnus* sp.), among others, grow in the valleys and along the rivers. The NCP covers an area of 67 ha. Some of the dust comes from vehicles transporting raw and depositing waste materials, while the rest is emitted from the chimneys of the cement factory. Two chimneys are used for cooling, four are associated with the cement mill and three others with the milling of materials.

Field measurements

To adequately model the accumulation of industrial dust and its effect on vegetation, it is essential to measure the concentration of dust particles and their mineralogical-chemical composition along with plant biophysical properties. To do so, we have quantified the heavy metals on tree leaves by collecting samples and analysing them using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS). Moreover, *in situ,* LAIs were measured to quantitatively estimate the available total area of leaves per unit ground area. Field measurements were made from 17 to 20 September 2017, which corresponds to the vigorous growth period of deciduous trees in the area studied. Leaf samples were collected at 2–3 m above the ground in the four cardinal directions (i.e., north, south, east and west) from the canopies of the trees used for the ICP-MS measurements, with minimum contact with the surface of the leaves.

ICP-MS data

ICP-MS was used to quantify concentrations of cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), calcium (Ca), magnesium (Mg), sodium (Na), silicon (Si) and zinc (Zn) in leaves of the dominant oak trees. ICP-MS is a powerful tool for tracing many elements and their isotopes with high sensitivity and reliable precision (Bulska and Wagner 2016). The area was divided into 20 squares plots of 250 × 250 m and categorized into different classes according to vegetation and distance

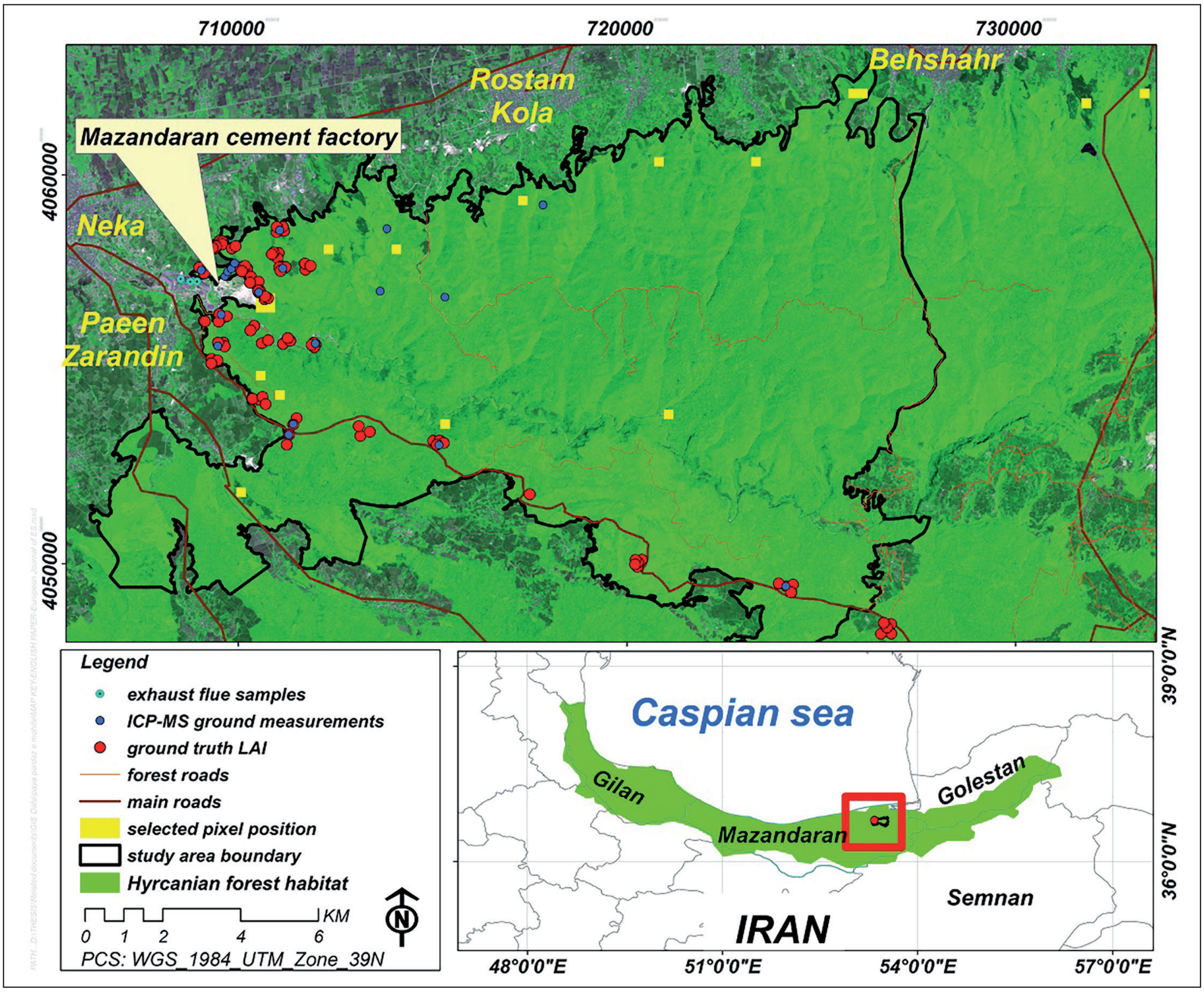

Fig. 1 Map showing locations where ICP-MS samples (blue dots), ground measurements of LAI (red dots), exhaust flue samples (cyan coloured dots) were collected and selected similar pixels (yellow polygons), Hyrcanian forests recently designated a UNESCO World Heritage Site is located along the south coast of the Caspian Sea (light green), study area (rectangular red box) are presented on a true-colour composite (RGB) of Sentinel-2B.

from the NCP (Fig. 1). The detached leaves were stored in labelled plastic bags in a cooling package and transferred to the laboratory. In the laboratory, the leaves were placed in beakers, covered with watch glasses and dried for 48 hours in an oven at 70 °C. Later, these samples were ground and then digested in a closed system using ultra-pure nitric acid and hydrogen peroxide in a microwave oven. ICP-MS multi-element standard solutions were used to determine the concentrations of different heavy metals, which ranged from 0.1 ppb to 100 ppm.

LAI field measurements

Hemispherical (fisheye) photography was used to estimate forest LAI. This technique has been widely and successfully used for estimating the LAI and canopy structures of plants over the past decades (Macfarlane et al. 2000; Chen et al. 2006; Demarez et al. 2008; Wang et al. 2018). Hemispherical images record the spectral and spatial characteristics of the forest canopy, from which direct and scattered light regimes can be estimated (Frazer et al. 2001). A Canon EOS 6D camera, equipped with the Canon lens EF 8–18 mm f/4L, and a spider tripod, equipped with a lever, were used for hemispherical photography. The images were processed by the Gap Light Analyser (GLA) software and used to estimate LAI (Frazer et al. 1999; Olivas et al. 2013; Sadeghi et al. 2016; Fournier and Hall 2017). The GLA is acknowledged to be good and reliable way of estimating LAI.

The measurements were made under a uniformly overcast sky or a clear sky within 2 hours of sunset or sunrise (Breda 2003). To select representative samples, the area studied was divided into several zones based on the distance from the dust source, and random sampling was used in each zone. Measurements were made in the field in four cardinal directions from the NCP as the dust source. A total of 105 measurements were collected from an area of about 30 × 15 km around the factory (Fig. 1). The LAI measurements ranged between 0.94 and 4.5 with a mean of 2.85 and a standard deviation of 1.17.

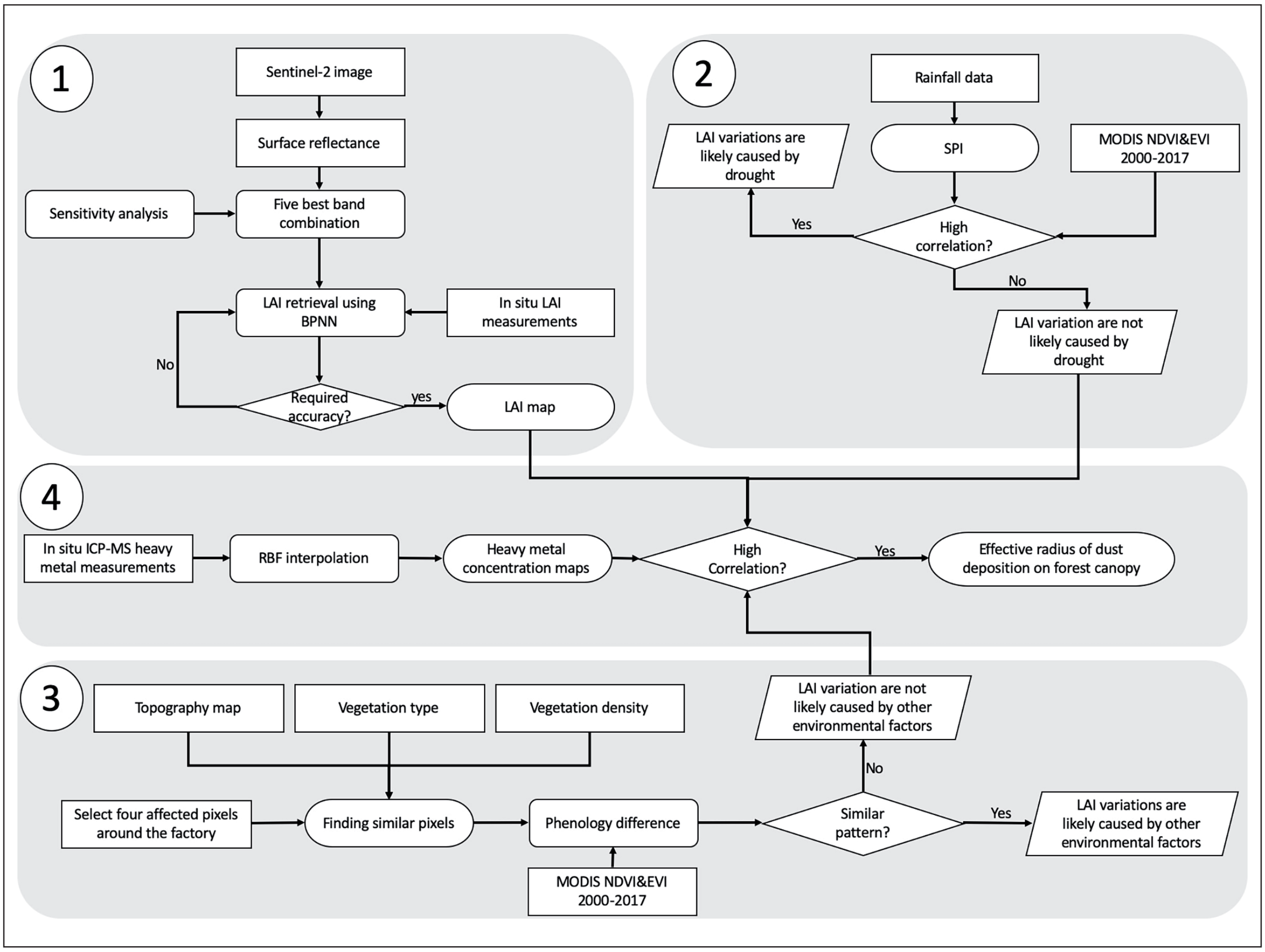

Fig. 2 Flowchart of the methodology. LAI retrieval from Sentinel-2 image of September 22, 2017 (1); was used to check whether variations in LAI were caused by drought (2); or other environmental factors (3); which determine the effective radius of the deposition of dust on the canopy of the forest (4).

Methods

As the main objective of this study was to quantify the effect of dust emitted by NCP on surrounding forests, we first investigated, whether there was any relationship between the deposition of dust particles and health of vegetation in polluted areas based on variation in LAI. We then determined, whether the variation in LAI was the result of contamination with dust, drought or other environmental factors. Finally, the effective radius of dust deposition impact was determined. This analysis required spatially continuous maps of LAI and dust contamination in the area studied. The flowchart of the methods used in this study is given in Fig. 2.

A three-layer Back Propagation Neural Network (BPNN) with one input layer, ten hidden layers and one output layer was used to retrieve LAI from Sentinel-2 satellite images (Foody and Atkinson 2002; Fang et al. 2003). The hyperbolic tangent sigmoid was chosen as the transfer function between the input and hidden layers. The combination of tangent and linear functions is adequate for fitting different kinds of functions for retrieving LAI (Combal et al. 2003; Verger et al. 2011). The satellite image was converted to surface reflectance using the "Sen2Cor" plugin, embedded in SNAP software to remove unwanted atmospheric influences on the recorded signals. Sentinel-2 spectral bands with spatial resolutions of 10 m and 20 m were used where the ten m-spatial resolution bands were resampled into 20 m spatial resolution bands. A sensitivity analysis was used to determine the Sentinel-2 spectral bands most sensitive to LAI (see Mousivand et al. 2014). Trial and error analysis were carried out to determine the most efficient combination of bands for LAI retrieval. Consequently, the five spectral bands most sensitive to LAI were selected for further retrieval. The LAI field measurements were split into 70% training and 30% validation data. For training the BPNN, 10-fold cross-validation was used, in which the training data were divided into ten subsamples. For each, nine subsamples were used to train the model and the remaining subsample as a test set. The final model validation was computed using the independent validation dataset (i.e., 30%). To ensure that the variation in LAI within the area studied was not caused by drought, a drought analysis was done. Firstly, the Standardized Precipitation Index (SPI)

(1-, 2-, 3-, 6- and 12-month SPI) was computed using rainfall data obtained from the meteorological station closest to the dust source for the period 2000-2017. This index is widely used to characterize drought over a range of timescales (e.g., Guttman 1998; Łabędzki 2007). In addition, the relationships between SPI and the different vegetation indices, such as NDVI and EVI, extracted from the MODIS data, were determined for the period 2000-2017. Monthly NDVIs and EVIs were retrieved from 16-day NDVI and EVI MODIS products and their correlations with SPIs were determined for the period of 2000-2017. A close relationship between NDVI and SPI is used as an indicator for determining drought severity and duration of the cover of vegetation (Ji and Peters 2003).

In addition, time series of NDVIs and EVIs were analysed for pixels with similar conditions to ensure that the variations in LAI are not affected by other environmental factors. First, four MODIS pixels around the dust source that were affected by deposition of dust were selected manually. Then, an algorithm was used to find the pixels that share similar characteristics (such as average altitude, slope, aspect, type of vegetation and density) in the east and south directions around the factory. The NDVI and EVI time series were used to interpret phenological differences between contaminated pixels and similar uncontaminated ones and determine if the variations in LAI are due to dust deposition.

Radial basis function (RBF) interpolation was used to generate a map of the area contaminated with heavy metals. This algorithm can be effectively used for interpolation of scattered measurements regardless of the dimensions (Fornberg and Flyer 2005). The interpolated maps provide a graphical representation of spatial variability in the deposition of heavy metals within the area studied. This indicates where the concentration of a specific heavy metal is high and at least where there is a deposition of dust on tree leaves. Finally, the correlation between LAI and deposition of dust was calculated and the spatial scale, at which dust from the NCP has affected the canopy of the forest. Three statistical metrics were used for evaluating the performance of the estimations, including Root Mean Square Error (RMSE), coefficient of determination (R^2) and Mean Absolute Error (MAE).

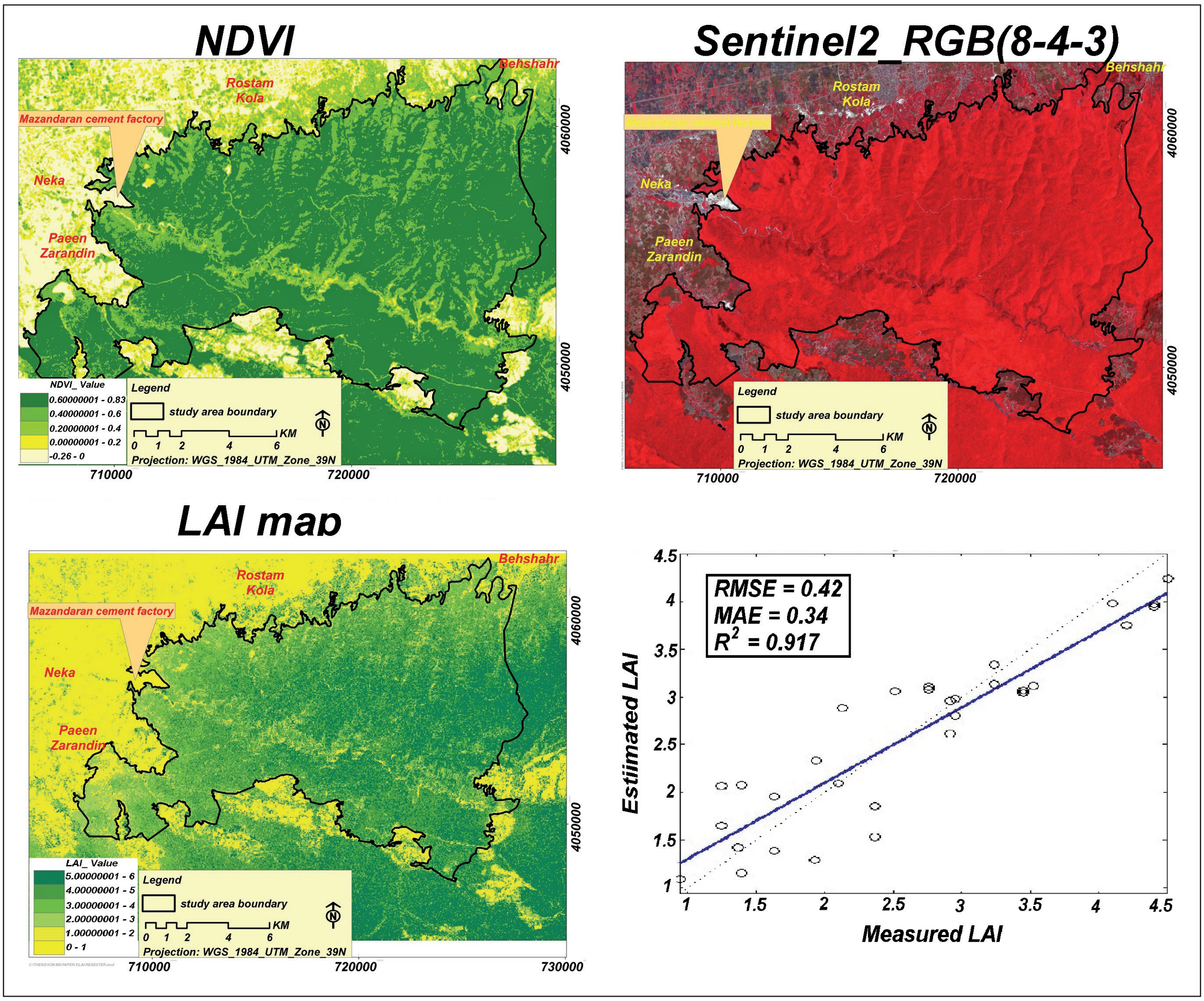

Fig. 3 Estimates of LAI based on Sentinel-2B imagery, NDVI and Sentinel-2B RGB imagery.

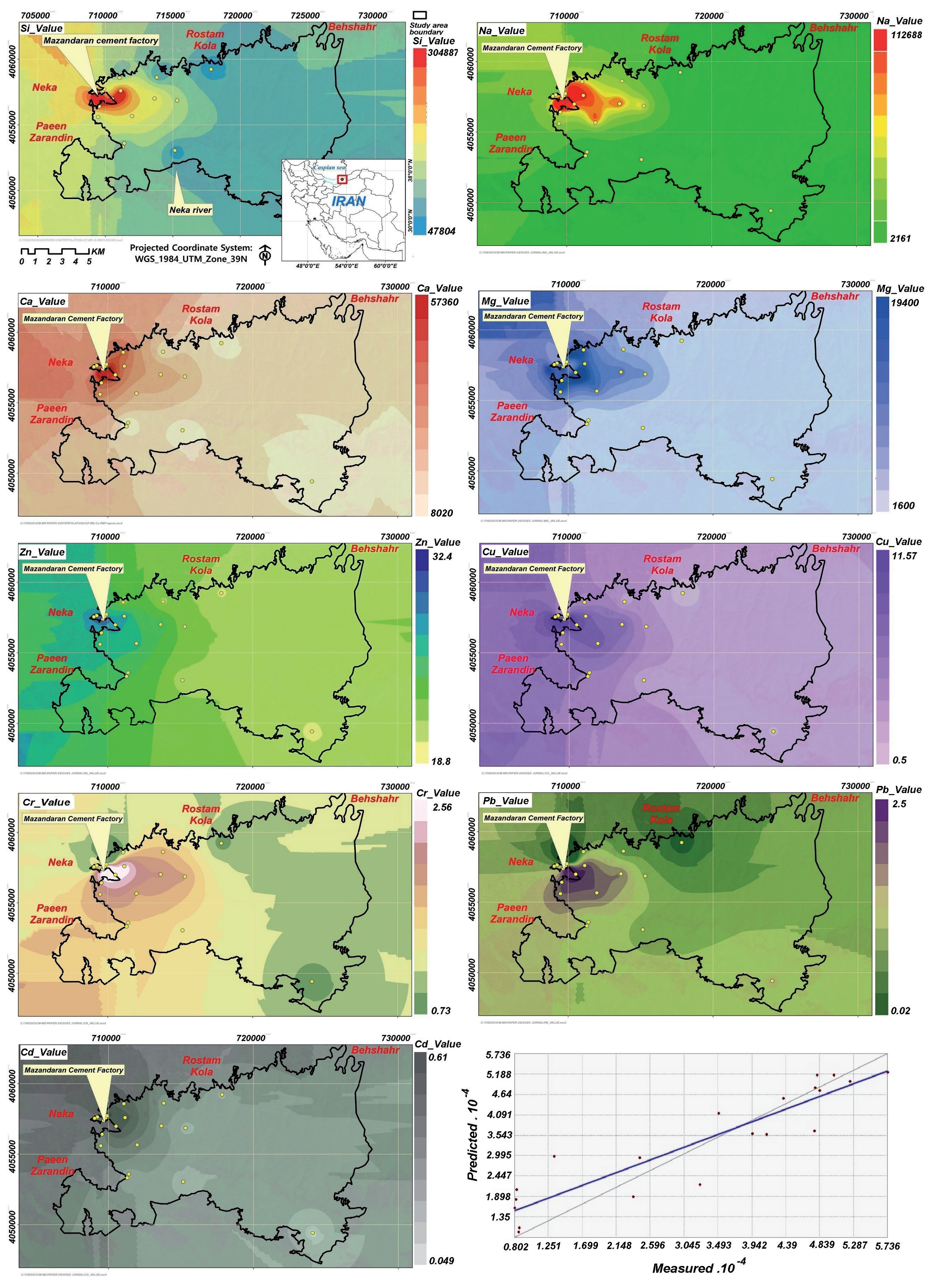

Fig. 4 The spatial distribution of the concentrations of nine heavy metals: cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), calcium (Ca), magnesium (Mg), sodium (Na), silicon (Si) and zinc (Zn), in leaves of oak trees. Lower right: validation of interpolated results for Ca using the RBF method.

Fig. 5 Examples of deposition of dust on the leaves of oak (Right) and Persian ironwood (Left) growing close to the Neka cement plant.

Results and Discussion

LAI estimation

Accurate estimates of LAI based on satellite data is a prerequisite for quantifying the effects of the deposition of industrial dust on plant health and productivity. To check the performance of the retrieval algorithm, estimates of LAI were validated using an independent dataset consisting of 30% of *in situ* measurements of LAI. The estimate of LAI using RMSE was 0.42 (m^2/m^2) and from MAE based on Sentinel-2 imagery it was 0.34 (m^2/m^2) (Fig. 3). The high value of the coefficient of determination ($R^2 = 0.917$) and low values of RMSE indicated that the linear relationship between the estimated and measured LAI values was strong. In general, a good agreement was found between the predicted and field measurements of LAI, with no systematic discrepancies. These results revealed no major over- or underestimation and, therefore, the retrieval model provided a reliable estimate of LAI. This is in line with results presented in previous studies (e.g., Delegido et al. 2011; Majasalmi et al. 2016; Bochenek et al. 2017; Clevers et al. 2017; Zhang et al. 2017), which report strong relationships between field estimates of LAI and those based on Sentinel-2. This is partly due to the Sentinel-2 red-edge spectral bands for biophysical parameter retrieval (Delegido et al. 2013).

Fig. 3 shows the LAI map derived from Sentinel-2 imagery. The map displays LAIs ranging from 0 (light yellow) to more than 6 (dark green) for areas covered with a wide range of crops and different forest trees. While the lowest LAI was recorded for bare soil, built-up areas and water bodies, the maximum LAI was recorded for closed-canopy forests with nearly complete interception of solar radiation. The NDVI and original Sentinel-2 RGB image are also provided for the sake of comparison and visual interpretation. The estimates of LAI were based on the types of land cover depicted in Fig. 1 and changes in cover of vegetation depicted in the NDVI map.

Spatial distribution of heavy metals

The spatial distribution of nine heavy metals: Cd, Cr, Cu, Pb, Ca, Mg, Na, Si and Zn, are shown in Fig. 4. These maps provide a graphical representation of the spatial variability in the deposition of heavy metals within the area studied and indicate where the concentration of specific heavy metal is highest (or lowest). These maps were generated using RBF spatial interpolation of measurements of heavy metal accumulation in the leaves of oak trees. The RBF interpolation model was compared with other conventional methods such as Inverse Distance Weighted (IDW), Kriging and cubic spline interpolation. Results indicate the RBF method is accurate for almost all heavy metals. Fig. 4 provides an example of a cross-validation for interpolating Ca using the RBF method. The NRMSE value (0.14 for Ca) was the lowest compared with values obtained using other methods. The concentration ranges (all data in mg/kg d.m.) of the different heavy metals were: Cd (0.049–0.61), Cr (0.731–2.56), Cu (0.5–11.57), Pb (0.02–2.455), Zn (18.823–32.357), Ca (8000–57000), Mg (1600–19400), Na (2150–113000) and Si (48000–305000). The spatial distribution of heavy metals was clearly greatly affected by topography and distance from NCP.

The highest accumulation of heavy metals was recorded close to the factory and then decreased with distance from the factory. Similar patterns in the distribution of heavy metals and hazardous gases produced during the manufacture of cement are reported by Zhang et al. (2015) and Atamaleki et al. (2015). However, there was a tendency for the effect to be more significant in the east (Fig. 4) and in the immediate surrounding of the cement factory, which is a major heavy metal concentration hotspot. Large and heavy dust particles are mainly deposited close to the source. Therefore, the accumulation of dust was greatest close to the source. Two examples of leaves collected close to the factory are shown in Fig. 5.

It is noteworthy that oak leaves are rich in Ca, Mg, Na and Si, which is why the recorded concentration of these elements was so high. However, there are no similar studies on the natural concentration of heavy metals in the leaves of trees in the area studied for comparison, however, the average concentrations of Zn, Cr, Cu, Pb and Cd in oak leaves collected from trees located east and south were higher than in those collected from trees located southwest of the cement plant.

Correlation between LAI and dust deposition

The maps depicted in Fig. 4 indicate high concentrations of the heavy metals around the NCP, which decrease with increase in distance from the dust source. However, to obtain a better picture of the effects of the deposition of dust, variations in the vegetation associated with the deposition of dust was measured quantitatively.

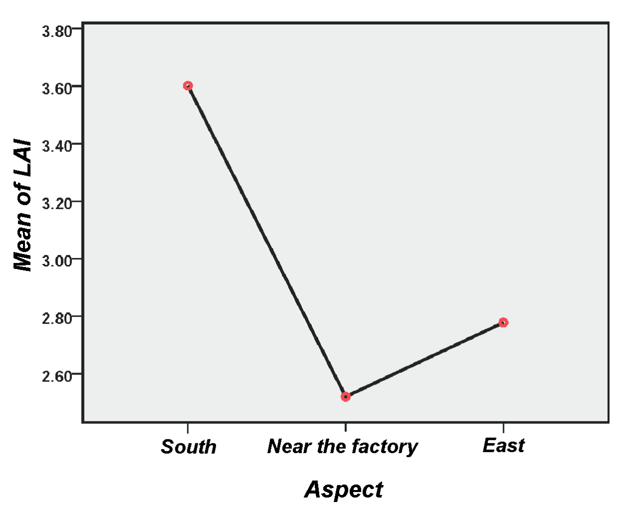

Fig. 6 Average LAI of similar pixels in the south, east and near the Neka cement plant.

Although the plot of the variation in LAI with distance from the factory in different directions quantifies the effect in homogenous areas, it does not accurately quantify the stress in the area studied where the topography and vegetation is very complex. The analysis, therefore, was restricted to a limited number of similar minute areas. Some of the areas around the factory were identified as affected during the field survey. Other minute areas that were similar in terms of slope, aspect and type and density of vegetation, allowing for a variation of up to 5% in in the different factors, were also selected. A total of 3125 similar areas were identified in the area studied.

North and west of NCP was agricultural land, which was not included in this study, the analysis only included areas in the south, east and close to NCP. A distance of 500 m around the factory was considered to be close to NCP. Based on the heavy metal contamination recorded during the field survey three regions were characterized with average LAIs of 2.55, 2.80 and 3.63 close to, east and south of NCP, respectively (Fig. 6). Since similar pixels in terms of LAI, were carefully selected the lowest LAI close to the NCP and to the east of NCP are likely to be linked to a significant effect of the dust on forest LAI. A significant effect was recorded close to the NCP, where a difference of 1.08 was recorded compared to less affected LAIs in the south. These results are in good agreement with the concentration maps, which indicates that the dust particles are mainly carried by the prevailing wind. Although average LAI reflects the overall effect of dust, it does not provide information on the distribution and extent of the variation. Therefore, measures of dispersion including variance, skewness and kurtosis were also considered and normality of the data tested. From the results of the p-p plot test, skewness, and kurtosis values, it is concluded that LAI values are normally distributed. Furthermore, a statistical hypothesis test was used to assess the statistical significance of the difference between the properties (average and variance) of LAI in different areas. The results of hypothesis tests revealed a significant relationship between LAI and the direction from the factory with a confidence level of 5%. Moreover, a significant correlation (0.849) was recorded between LAI (dependent variable) and distance from the NCP (independent variable). This relationship increased for the pixels within 4700 meters of the source of the pollution, after which they ceased increasing and became almost stable. Likewise, a strong correlation was found between Cr, Cu, Cd, Pb, Mg, and Na concentrations and distance from the factory with a similar increase up to 4700 m. However, concentrations of Zn, Ca and Si did not show consistent increase with distance. We therefore conclude that this distance is the spatial scale over which dust particles cause meaningful changes in LAI and reduce functioning of the vegetation.

Analysis of possible effects of drought

In addition, the correlations between NDVIs and EVIs, and SPIs were assessed to ensure that the variations in LAI were not caused by drought or other environmental stresses. The relationship between NDVI/EVI and SPI within the area studied was low (<0.2) with lags of up to 12 months, which indicates there is no evidence of drought. Therefore, it was concluded that the variations in LAI in the area studied was not due to drought. In addition, the 17 year time series of NDVI and EVI were compared to check whether the changes in LAI were due to local anomalies or other local stresses, but no evidence was found to support such a claim.

Conclusions

The main aim of this study was to quantify the response of the Hyrcanian mixed forest to stress caused by industrial dust produced during the manufacture of cement. To do so, ground-based measurements of LAI, chlorophyll, and spectroscopy were made concurrently with the satellite observations. Moreover, leaf samples were collected to measure the concentration of nine heavy metals using ICP-MS. A MODIS-LAI time series was used to test whether the stress was caused by drought or other environmental factors.

The significant finding of this study was that the adverse effect of industrial dust deposition on the leaves of the trees in the surrounding forest could be quantitatively mapped using the LAI extracted from Sentinel-2 satellite data. To the best of our knowledge, there is no study on the quantification of the effect of industrial dust using satellite data. We also recorded strong relationships between ground measurements of LAI and those obtained using Sentinel-2 data. The results are promising because the relationship between the variation in LAI and distance from the dust source, i.e., NCP, was strong and associated with heavy metal concentrations. We found that the dust produced by the cement plant caused an average decrease in LAI of 1.08 in the immediate surroundings of the factory and 0.83 in the easterly direction In contrast, south of the factory was less affected. This highlighted the role of prevailing wind and topography in spreading dust and dust deposition. In this study, we quantitatively mapped the dust deposition over a distance of 4700 m both east and south of the factory. This study revealed that the dust reduced the growth of oak trees and caused them to shed their leaves, which is likely to account for the low LAI values recorded. Drought analysis using the time series SPI and NDVI/EVI revealed no clear evidence for the variations in LAI being due to drought or other environmental factors. Consequently, the variation in LAI is inferred to be due to the direct effect of the dust produced during the manufacture of cement.

Based on this study, changes in forests due to dust deposition can be closely monitored using remote sensing. This study provides evidence that dust from industrial processes, specifically cement manufacture, is deposited on the surrounding environment. The results further our understanding of the interaction between a forest ecosystem and deposition of industrial dust produced by the manufacture of cement and reveal that the new generation of multispectral satellite data, such as Sentinel-2, can be used to detect the effect of pollution on forests.

REFERENCES

Abdi O (2019) Climate–Triggered Insect Defoliators and Forest Fires Using Multitemporal Landsat and TerraClimate Data in NE Iran: An Application of GEOBIA TreeNet and Panel Data Analysis. Sensors 19: 3965, https://doi.org/10.3390/s19183965.

Atamaleki A, Zarandi S M, Fakhri Y, Mehrizi E A, Hesam G, Faramarzi M, Darbandi M (2019) Estimation of air pollutants emission (PM10, CO, SO_2, and NO_x) during development of the industry using AUSTAL 2000 model: A new method for sustainable development, MethodsX 6: 1581–1590, https://doi.org/10.1016/j.mex.2019.06.010.

Bell JN, Bell N, Treshow M (eds) (2002) Air pollution and plant life. John Wiley and Sons.

Bochenek Z, Dąbrowska-Zielińska K, Gurdak R, Niro F, Bartold M, Grzybowski P (2017) Validation of the LAI biophysical product derived from Sentinel-2 and Proba-V images for winter wheat in western Poland. Geoinf Issues 9: 15–26.

Borka G (1980) The effects of cement dust pollution on growth and metabolism of Helianthus annuus. Environ Pollut 22: 75–79.

Breda NJJ (2003) Ground-based measurements of leaf area index: a review of methods, instruments, and current controversies. J Exp Bot 54: 2403–2417, https://doi.org/10.1093/jxb/erg263.

Bulska E, Wagner B (2016) Quantitative aspects of inductively coupled plasma mass spectrometry. Philos Trans R Soc A Math Phys Eng Sci 374:20150369, https://doi.org/10.1098/rsta.2015.0369.

Capros P, Kouvaritakis N, Mantzos L (2001) Economic Evaluation of Sectoral Emission Reduction Objectives for Climate Change. Top-down Analysis of Greenhouse Gas Emission Reduction. European Commission.

Chaurasia S (2013) Effect of cement industry pollution on chlorophyll content of some crops at Kodinar, Gujarat, India. Proc Int Acad Ecol Environ Sci 3: 288–295.

Chen C, Habert G, Bouzidi Y, Jullien A (2010) Environmental impact of cement production: detail of the different processes and cement plant variability evaluation. J Clean Prod 18: 478–485.

Chen G, Meentemeyer RK (2016) Remote sensing of forest damage by diseases and insects. In: Remote Sens Sustain, Weng Q (eds) CRC Press, Taylor and Francis Group, Boca Raton, Florida, pp 145–162.

Chen JM, Govind A, Sonnentag O, Zhang Y, Barr A, Amiro B (2006) Leaf area index measurements at Fluxnet-Canada forest sites. Agric For Meteorol 140: 257–268.

Clevers J, Kooistra L, van den Brande M (2017) Using Sentinel-2 Data for Retrieving LAI and Leaf and Canopy Chlorophyll Content of a Potato Crop. Remote Sens 9: 405–420, https://doi.org/10.3390/rs9050405.

Combal B, Baret F, Weiss M, Trubuil A, Macé D, Pragnère A, Myneni R, Knyazikhin Y, Wang L (2003) Retrieval of canopy biophysical variables from bidirectional reflectance using prior information to solve the ill-posed inverse problem. Remote Sens Environ 84: 1–15, https://doi.org/10.1016/S0034-4257(02)00035-4.

Darley EF (1966) Studies on the effect of cement-kiln dust on vegetation. J Air Pollut Control Assoc 16: 145–150, https://doi.org/10.1080/00022470.1966.10468456.

Delegido J, Verrelst J, Meza CM, Rivera J P, Alonso L, Moreno J (2013) A red-edge spectral index for remote sensing estimation of green LAI over agroecosystems. Eur J Agron 46: 42–52, https://doi.org/10.1016/J.EJA.2012.12.001.

Delegido Jesús, Verrelst J, Alonso L, Moreno J (2011) Evaluation of Sentinel-2 Red-Edge Bands for Empirical Estimation of Green LAI and Chlorophyll Content. Sensors 11: 7063–7081, https://doi.org/10.3390/s110707063.

Demarez V, Duthoit S, Baret F, Weiss M, Dedieu G (2008) Estimation of leaf area and clumping indexes of crops with hemispherical photographs. Agric For Meteorol 148: 644–655, https://doi.org/10.1016/j.agrformet.2007.11.015.

Entcheva PK (2000) Remote sensing of forest damage in the Czech Republic using hyperspectral methods. PhD Thesis, New Hampshire University, Durham.

Eveling DW (1969) Effects of spraying plants with suspensions of inert dusts. Ann Appl Biol 64: 139–151, https://doi.org/10.1111/j.1744-7348.1969.tb02864.x.

Fang H, Liang S, Kuusk A (2003) Retrieving leaf area index using a genetic algorithm with a canopy radiative transfer model. Remote Sens Environ 85: 257–270, https://doi.org/10.1016/S0034-4257(03)00005-1.

Foody GM, Atkinson PM (eds) (2002) Uncertainty in remote sensing and GIS, John Wiley and Sons, Hoboken, NJ, https://doi.org/10.1111/j.0031-868X.2004.282_2.x.

Fornberg B, Flyer N (2005) Accuracy of radial basis function interpolation and derivative approximations on 1-D infinite grids. Adv Comput Math 23: 5–20, https://doi.org/10.1007/s10444-004-1812-x.

Fournier RA, Hall RJ (eds)(2017) Hemispherical Photography in Forest Science: Theory, Methods, Applications, Springer, Dordrecht, Netherlands, https://doi.org/10.1007/978-94-024-1098-3.

Frazer GW, Canham CD, Lertzman KP (1999) Gap Light Analyzer (GLA), Version 2.0: Imaging software to extract canopy structure and gap light transmission indices from true-colour fisheye photographs. Simon Fraser University, Burnaby, British Columbia, and the Institute of Ecosystem Studies, Millbrook, New York.

Frazer Gordon W, Fournier RA, Trofymow JA, Hall RJ (2001) A comparison of digital and film fisheye photography for analysis of forest canopy structure and gap light transmission. Agric

For Meteorol 109: 249–263, https://doi.org/10.1016/S0168-1923(01)00274-X.

Frolking S, Palace MW, Clark DB, Chambers JQ, Shugart HH, Hurtt GC (2009) Forest disturbance and recovery: A general review in the context of spaceborne remote sensing of impacts on aboveground biomass and canopy structure. J Geophys Res Biogeosciences 114: 1–27, https://doi.org/10.1029/2008JG000911.

Gartner E (2004) Industrially interesting approaches to "low-CO_2" cements. Cem Concr Res 34: 1489–1498.

Guttman NB (1998) Comparing the palmer drought index and the standardized precipitation index. J Am Water Resour Assoc 34: 113–121, https://doi.org/10.1111/j.1752-1688.1998.tb05964.x.

Iqbal MZ, Shafüg M (2000) Periodical Effect of Cement Dust Pollution on the Growth of Some Plant Species. Turk J Botany 25: 19–24.

Ji L, Peters AJ (2003) Assessing vegetation response to drought in the northern Great Plains using vegetation and drought indices. Remote Sens Environ 87: 85–98.

Josa A, Aguado A, Cardim A, Byars E (2007) Comparative analysis of the life cycle impact assessment of available cement inventories in the EU. Cem Concr Res 37: 781–788.

Joshi PC, Swami A (2009) Air pollution-induced changes in the photosynthetic pigments of selected plant species. J Environ Biol 30: 295–298.

Kardel F, Wuyts K, Babanezhad M, Vitharana UWA, Wuytack T, Potters G, Samson R (2010) Assessing urban habitat quality based on specific leaf area and stomatal characteristics of Plantago lanceolata L. Environ Pollut 158: 788–794, https://doi.org/10.1016/j.envpol.2009.10.006.

Kennedy RE, Yang Z, Cohen WB (2010) Detecting trends in forest disturbance and recovery using yearly Landsat time series: 1. LandTrendr - Temporal segmentation algorithms. Remote Sens Environ 114: 2897–2910.

Łabędzki L (2007) Estimation of local drought frequency in central Poland using the standardized precipitation index SPI. Irrig Drain 56: 67–77, https://doi.org/10.1002/ird.285.

Lal B, Ambasht RSS (1982) Impact of cement dust on the mineral and energy concentration of Psidium guayava. Environ Pollut Ser A Ecol Biol 29: 241–247, https://doi.org/10.1016/0143-1471(82)90065-4.

Macfarlane C, Coote M, White DA, Adams MA (2000) Photographic exposure affects indirect estimation of leaf area in plantations of Eucalyptus globulus Labill. Agric For Meteorol 100: 155–168.

Madejón P, Marañón T, Murillo JM (2006) Biomonitoring of trace elements in the leaves and fruits of wild olive and holm oak trees. Sci Total Environ 355: 187–203.

Majasalmi T, Rautiainen M (2016) The potential of Sentinel-2 data for estimating biophysical variables in a boreal forest: a simulation study. Remote Sens Lett 7: 427–436, https://doi.org/10.1080/2150704X.2016.1149251.

Manning WJ, Feder WA (1980) Biomonitoring air pollutants with plants. Applied Science Publishers London, England.

Mousivand A (2015) Retrieval of vegetation properties using Top of Atmosphere radiometric data A multi-sensor approach. PhD Thesis, Delft University of Technology, The Netherlands.

Mousivand AJ, Menenti M, Gorte B, Verhoef W (2014) Global sensitivity analysis of the spectral radiance of a soil-vegetation system. Remote Sens Environ 145: 131–144, https://doi.org/10.1016/j.rse.2014.01.023.

Olivas PC, Oberbauer SF, Clark DB, Clark DA, Ryan MG, O'Brien JJ, Ordoñez H (2013) Comparison of direct and indirect methods for assessing leaf area index across a tropical rain forest landscape. Agric For Meteorol 177: 110–116, https://doi.org/10.1016/j.agrformet.2013.04.010.

Prasad MSV, Inamdar JA (1990) Effect of cement kiln dust pollution on groundnut (Arachis hypogaea). Indian Bot contactor 7: 159–162.

Persson S (2014) Estimating leaf area index from satellite data in deciduous forests of southern Sweden. Master's Degree, Lund University, Sweden.

Ramezani, E, Marvie Mohadjer MR, Knapp HD, Ahmadi H, Joosten H (2008) The late-Holocene vegetation history of the Central Caspian (Hyrcanian) forests of northern Iran. The Holocene 18: 307–321, https://doi.org/10.1177/0959683607086768.

Sadeghi SMM, Attarod P, Van Stan JT, Pypker TG (2016) The importance of considering rainfall partitioning in afforestation initiatives in semiarid climates: A comparison of common planted tree species in Tehran, Iran. Sci Total Environ 568: 845–855, https://doi.org/10.1016/j.scitotenv.2016.06.048.

Shepherd G, Terradellas E, Baklanov A, Kang U, Sprigg W, Nickovic S, et al. (2016) Global Assessment of Sand and Dust Storms. United Nations Environment Programme, Nairobi.

Suciu I, Cosma C, Todică M, Bolboacă SD, Jäntschi L (2008) Analysis of soil heavy metal pollution and pattern in central Transylvania. Int J Mol Sci 9: 434–453, https://doi.org/10.3390/ijms9040434.

Toutoubalina OV, Rees WG (1999) Remote sensing of industrial impact on Arctic vegetation around Noril'sk, northern Siberia: Preliminary results. Int J Remote Sens 20: 2979–2990, https://doi.org/10.1080/014311699211561.

Verger A, Baret F, Camacho F (2011) Optimal modalities for radiative transfer-neural network estimation of canopy biophysical characteristics: Evaluation over an agricultural area with CHRIS/PROBA observations. Remote Sens Environ 115: 415–426, https://doi.org/10.1016/j.rse.2010.09.012.

Verstraete MM, Pinty B, Myneni RB (1996) Potential and limitations of information extraction on the terrestrial biosphere from satellite remote sensing. Remote Sens Environ 58: 201–214, https://doi.org/10.1016/S0034-4257(96)00069-7.

Wang J, Xiong Q, Lin Q, Huang H (2018) Feasibility of using mobile phone to estimate forest Leaf Area Index: a case study in Yunnan Pine. Remote Sens Lett 9: 180–188, https://doi.org/10.1080/2150704X.2017.1399470.

Yamaguchi T, Watanabe M, Noguchi I, Koike T (2017) Tree Decline at the Somma of Lake Mashu in Northern Japan. In: Takeshi I (ed) Air Pollution Impacts on Plants in East Asia. Springer, Tokyo, pp. 135–150.

Zheng G, Moskal LM (2009) Retrieving Leaf Area Index (LAI) Using Remote Sensing: Theories, Methods, and Sensors. Sensors 9: 2719–2745, https://doi.org/10.3390/s90402719.

Zhang C, Pattey E, Liu J, Cai H, Shang J, Dong T (2017) Retrieving leaf and canopy water content of winter wheat using vegetation water indices. Appl Earth Obs Remote Sens 11: 12–126.

Zhang S, Worrell E, Crijns-Graus W (2015) Cutting air pollution by improving energy efficiency of China's cement industry, energy procedia, Elsevier 83: pp 10–20, https://doi.org/10.1016/j.egypro.2015.12.191.

Zia-Khan S, Spreer W, Pengnian Y, Zhao X, Othmanli H, He X, Müller J (2015) Effect of dust deposition on stomatal conductance and leaf temperature of cotton in Northwest China. Water (Switzerland) 7: 116–131, https://doi.org/10.3390/w7010116.

DOES ARTIFICIAL SNOW FERTILISE THE SOIL OF MOUNTAIN MEADOWS IN THE KRKONOŠE NATIONAL PARK?

ZDENKA KŘENOVÁ[1,2,*], ONDŘEJ BÍLEK[3], and VLADIMÍR ZÝVAL[3]

[1] Global Change Research Institute of the Czech Academy of Sciences, Bělidla 4a, CZ-60200 Brno, Czech Republic
[2] Institute for Environmental Studies, Faculty of Science, Charles University, Benátská 2, CZ-12900 Prague, Czech Republic
[3] Geo Vision s.r.o., Brojova 2113/16, 326 00 Plzeň 2 – Slovany, Czech Republic
* Corresponding author: krenova.z@czechglobe.cz

ABSTRACT

There are no high mountains in the Czech Republic and only few of them are higher than 1500 m a.s.l. Nevertheless, skiing is one of the most popular winter sports in this country and has a long history and tradition. During the last two decades, climate change, big differences in snow cover from year to year and unusual warm winter periods causing the snow to melt resulted in visitors to Czech ski resorts going to the Alps. Managers of ski resorts facing this challenge recognised that artificial snow enables skiing throughout the entire season and overcomes the risk posed by climate to the skiing business. However, many ski resorts are located in protected areas and it is difficult to negotiate changes in the rules for preparing and applying artificial snow with conservationists, who are fearful of the negative effects of snowmaking on rare and protected species and habitats. This paper presents results of a case study conducted in the SkiResort ČERNÁ HORA – PEC in the Krkonoše National Park throughout the 2019 season. The seasonal changes in the water quality in two reservoirs and six creeks, from which water is used for making artificial snow, were determined in order to assess the risk of this snow adding fertiliser to the meadows on ski slopes. We found that the nutrients recorded in two reservoirs and six creeks were very low. Water quality parameters did not exceed the limits of permissible pollution of surface and drinking water. Several episodic increases in the parameters measured were recorded and the causes discussed. We did not measure the direct effects of artificial snow on grassland communities. However, the use of water from these reservoirs and creeks for snowmaking does not pose a significant risk in terms of adding fertiliser to the meadows on ski slopes. To eliminate these risks and unusual events, several management measures for improving the water regime in the area studied are proposed. To better understand the effect of artificial snow on mountain meadows, permanent plots and long-term monitoring of vegetation, soil invertebrates and soil chemistry are recommended.

Keywords: artificial water reservoirs; climate change; management of protected areas; snow-making; water resources

Introduction

Skiing is one of the most popular winter sports in the Czech Republic and has a long history and tradition; the first ski club was founded in 1887 by Josef Rössler-Orovský. In 1893, the first ski races took place at Jilemnice (Krkokonoše – Giant Mountain) and in 1903 the Association of Skiers in the Czech Kingdom was founded, which was the first ski association in the world (www.ahscr.cz). In recent decades, there has been a significant development in skiing techniques as well as an increased demand for skiing-related services. In spite of the much lower altitudes than in the Alps, there is a large number of ski resorts all over the Czech Republic (about 200 ski areas with a total of almost 800 ski lifts), all of them located between 900 and 1,450 m a.s.l. (only 3 ski areas are at over 1,300 m a.s.l.).

In the Czech Republic, as well as in many other Eastern European countries, skiing is popular (Vanat 2019), however, the numbers skiing has not increased to the extent predicted in the first decade of this century. Even though Poland, Slovakia and the Czech Republic modernised their resorts, attendance remained stagnant. Of course, there can be various socio-economic reasons for this (incl. quality and price of the services offered in ski resorts) or climate change affecting the amount and quality of snow can play a role. Climate change, big differences in snow cover from year to year and previously unusual warm winter periods causing snow to melt, results in skiers going to the Alps rather than Czech ski resorts. Obviously, the ski industry in the Czech Republic faces the same problems as most other ski resorts. Attendance at Czech ski resorts has been stagnant or even declining for several years (Vanat 2019). In winter 2018/19, when there was an abundance of natural snow, there was a 3% improvement in attendance, which reached the highest recorded over the last 10 years (AHS 2019). However, winter 2019/2020 was not good in terms of the amount of snow and attendance at Czech ski resorts was very low. There is high probability that winter tourism will have to deal with the adverse effects of global warming more often (e.g., Koenig and Abegg 1997; Breiling and Charamza 1999; Elsasser and Bürki 2002).

Managers of ski resorts know that snowmaking enables skiing throughout the entire season and mitigates the risks of global warming to the ski business. However, many ski resorts are located in protected areas and there are strict rules about the preparation and application of artificial snow devised by conservationists who fear it will have negative effects on rare and protected species and habitats. Scientific knowledge and recommendations are essential for the appropriate management of ski resorts and mitigation of their negative effects on mountain ecosystems, especially in national parks and other protected areas. During the last two decades, many papers report the effects of skiing and artificial snow on vege-

Křenová, Z., Bílek, O., Zýval, V.: Does artificial snow fertilise the soil of mountain meadows in the Krkonoše National Park?
European Journal of Environmental Sciences, Vol. 10, No. 1, pp. 32–41
https://doi.org/10.14712/23361964.2020.5
© 2020 The Authors. This is an open-access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

tation (e.g. Jones and Devarennes 1995; Kammer 2002; Wipf 2005; Kocková 2011; Zeidler et al. 2016), soil invertebrates or small mammals (Negro et al. 2009, 2010), birds (Zeitler and Glanzer 1998; Baines and Richardson 2007; Thiel et al. 2008), soil quality (e.g. Freppaz et al. 2013) and the water regime (e.g. Treml et al. 2012; Fuksa 2016; Hruška 2017).

There have been occasional studies on the ecological aspects of downhill skiing in the Krkonoše National Park since the 1970s. Štursa (2007) states that downhill skiing has resulted in a lot of ecological problems for the management of the Krkonoše National Park mainly in terms of: (1) permanent decrease in total forest area and serious negative effects on the ecological stability of forest habitats adjacent to downhill slopes, (2) soil erosion on deforested slopes, (3) permanent changes in the character of the landscape and (4) the biotechnological management of downhill slopes. Ten years later, a new review of the effect of skiing on the nature in the Krkonoše/Giant Mts (Czech Republic) was published (Flousek 2016), which describes the particular effects of different activities associated with the construction and maintenance of new ski areas, operation and modernisation of ski areas and winter sports. He also considers the predicted effects of climate change on the future of the ski industry in mountain areas and discusses the increasing use of artificial snow. Hruška (2017) monitored the quality of water in the Labská Dam (17 km NW from our study area) as a potential source for the production of artificial snow in ski resorts in the Špindlerův Mlýn region and reports very low concentrations of nutrients in the water. In addition, Hruška et al. (2017) report that using water from this dam for making artificial snow results in the critical load for nitrogen deposition being exceeded at all the localities monitored. However, currently it is lower than it was in the 1990s, because between 2000 and 2016 atmospheric nitrogen deposition in the Krkonoše Mountains decreased by 22%. This roughly corresponds to the increase that is likely to result from using water from the Labská Dam for artificial snow. Finally, possible effects of the high concentrations of Ca and Mg in artificial snow produced using water from this dam on acidified forest and grassland habitats are discussed by Hruška (2017).

Currently, there is an active research project of the Technology Agency of the Czech Republic No. TH02030080 entitled 'Support of long-term planning in the area of water management in the Krkonoše National Park'. The first results of this project dealing with the effect of artificial snow on selected streams in terms of changes in flow rates and differences between natural snow and the snow lying on the slope in terms of snow density and associated runoff characteristics have been published by Treml (2019). He reports that water abstraction from medium and large streams for snowmaking does not pose a serious problem during a normal winter. Small streams are more vulnerable due to their greater fluctuations in flow rate. In winter there are two critical periods – the beginning of winter and periods of severe frost. To overcome this, he recommends the use of artificial reservoirs that are filled when flow rates are high.

Our study focused on the quality of the water in the artificial reservoirs used for snowmaking at the Ski Resort ČERNÁ HORA – PEC in terms of the seasonal variability in the quality of the water. The results were used to suggest possible ways of avoiding adding fertiliser to the meadows on ski slopes when applying artificial snow.

Methods

Study sites and sampling of water

The Krkonoše National Park (KRNAP, established in May 1962, area 363.27 km^2), is the oldest Czech national park. KRNAP is located in the Giant Mountains (Krkonoše in Czech; the highest Czech geomorphological complex with the highest summit – Sněžka peak: 1,603 m a.s.l.). For decades, this area has been a very popular destination for winter sports, especially downhill and cross-country skiing. There are about 170 kilometres of ski pistes (www.krkonose.eu), many of which use artificial snow. SkiResort ČERNÁ HORA – PEC is the largest resort in this region (Fig. 1). Annually, about 850,000 visitors enjoy more than 50 km of slopes with 6 cableways and 21 lifts (www.skiresort.cz). Thanks to artificial snow, the season usually lasts from the beginning of December to the middle of April (or at least the end of March). This resort uses water from several creeks, two reservoirs and the Úpa River for preparing artificial snow.

The monitoring of water quality in the two reservoirs, the water of which is used for the preparation of artificial snow for SkiResort ČERNÁ HORA – PEC, was carried out during 2019. In particular, the no-flow reservoir located at the upper cableway station Černá hora (designated as site S1, Fig. 2) and a flow through reservoir near the lower cableway station at Janské Lázně (site S2, Fig. 3) were studied. These reservoirs were monitored over 15–30 day periods during the 2019 season. Monitoring started on April 3, 2019 and ended on January 7, 2020, i.e. at the time of the highest annual occupancy of accommodation facilities and when the water in the reservoirs had been used several times for snowmaking.

To compare the water quality in the reservoirs, several selected creeks occurring in study area and used for artificial snow production (S3–S8 sites) were sampled and chemical analyses conducted. Detailed descriptions of the sites monitored, S1–S8, together with the dates sampled are given in Table 1.

Samples of surface water from reservoirs were collected using a sampler at a depth of about 0.5–1.0 m and from creeks samples were collected from a depth of up to 0.2 m below the surface. Simultaneously, the physicochemical parameters of the water (pH, conductivity, surface water temperature) were measured using pH and conductivity meters Hanna HI98129. The OPR meter Hanna HI98120

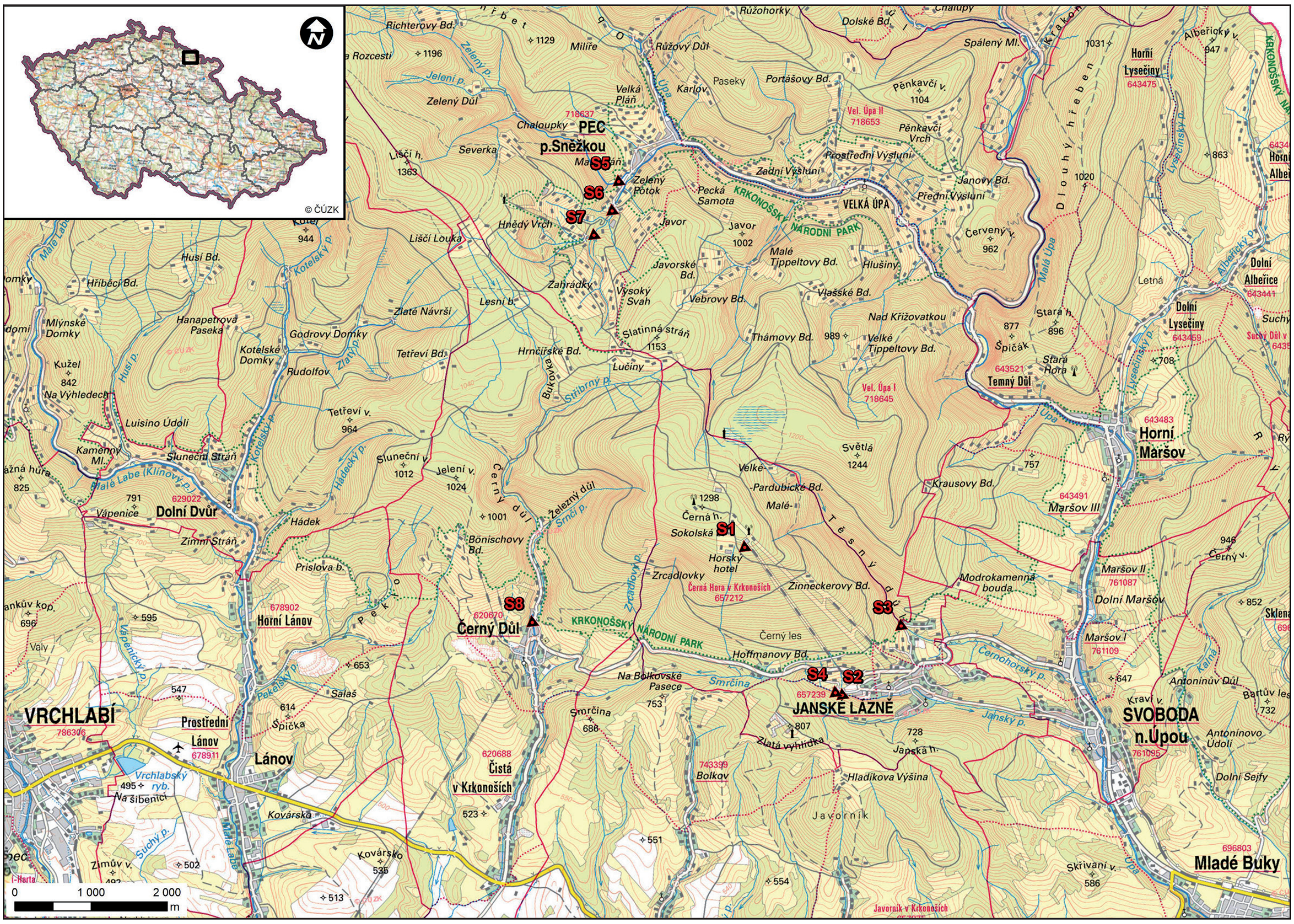

Fig. 1 Map of the study area.

was used to measure the redox potential – Eh. Both instruments were calibrated according to the manufacturer's instructions prior to measurement. After collection, the samples were stored in a portable refrigerated box and submitted for laboratory processing within a maximum of 36 hours.

Chemical and data analyses

Samples collected from the reservoirs and creeks were transferred to a certified laboratory – LABTECH Brno Ldt. – Hygienic Laboratories Klatovy. The following analyses were carried out according to the standard operating procedures of the accredited laboratory (accreditation of the Czech Accreditation Institute No. 1147) on all samples: pH, conductivity, DOC, P_{total}, PO_4^{3-}, DON, N_{total}, NO_3^-, NH_4^+, Ca and Mg. Chlorophyll was determined only in samples from sites S1 and S2. All determined hydrochemical parameters are given in Table 2.

We used the analysis of variation in STATISTICA 12 (Anonymous 2012) to compare the reservoirs and creeks. We also tested the effect of time and compared the quality of water in the reservoirs based on 13 samples using the Repeated Measurement in Split-plot ANOVA in the STATISTICA 12 program.

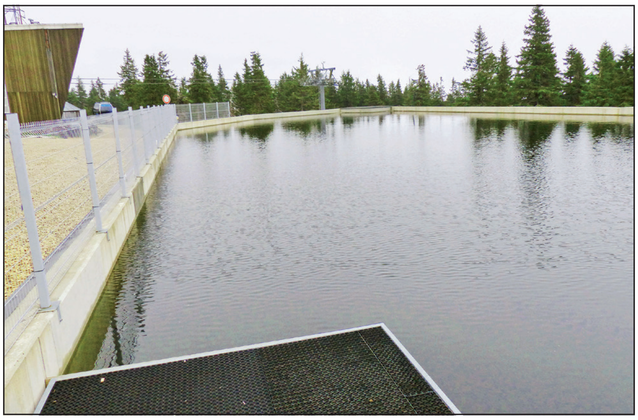

Fig. 2 The upper reservoir at study site S1.

Fig. 3 The lower reservoir at study site S2.

Table 1 Sites monitored and date sampled.
Sample sites S3-S8 were located where water is collected for making artificial snow: S3 – the upper reservoir; S4 – the lower reservoir (and partly also from the upper reservoir); S5 – ski slope Javor in Pec pod Sněžkou; S6 – ski slope Hnědý vrch; S7 – an alternative site for ski slope Hnědý vrch, a new reservoir is under consideration for this site; S8 – ski resort Černý Důl.

	name	GPS coordinates Y|X		site description	sampling*
S1	**Upper reservoir – Černá hora**	50°38´51.753"N	15°45´4.057"E	An impermeable artificial reservoir (capacity 10,790 m^3) close to the upper station of cableway Janské Lázně – Černá hora, 1250 m a.s.l. Built in 2011. No fish. This reservoir is filled from Černohorský Creek, which comes from the Černohorské Peat Bogs and from the Janský Creek (approximately in the ratio 2:1).	K01, K02, K03, K04, K05, K06, K07, K08, K09, K10, K11, K12, K14
S2	**Lower reservoir – Janské Lázně**	50°37´48.548"N	15°46´8.349"E	An artificial reservoir (capacity 2,060 m^3) close to the lower station of cableway Janské Lázně – Černá hora, 670 m a.s.l. Built in 2006. Population of brook trout lives in this reservoir. This flow through reservoir gets water from Janský Creek.	K01, K02, K03, K04, K05, K06, K07, K08, K09, K10, K11, K12, K14
S3	**Černohorský Creek – Janské Lázně**	50°38´17.183"N	15°46´49.227"E	Springs from the Černohorské Peat Bogs. Samples were collected along 2.7 km of this creek, on the outskirts of Janské Lázně.	K01, K02, K03, K04, K09, K13, K14
S4	**Janský Creek – Janské Lázně**	50°37´49.381"N	15°46´5.529"E	On the southern hillside of Černá hora. Samples were collected along 3.95 km of this creek, near the lower reservoir (S2).	K01, K02, K03, K04, K09, K13, K14
S5	**Zelený Creek – Pec p. Sněžkou**	50°41´27.513"N	15°43´41.863"E	On the eastern hillside of Zadní Planina Mt. Samples were collected along 1.1 km of this creek.	K01, K02, K03, K04, K09, K13, K14
S6	**Vlčí Creek 1 – Pec p. Sněžkou**	50°41´14.519"N	15°43´37.296"E	On the south-eastern hillside of Liščí Mt. Samples were collected along 0.65 km of this creek.	K01, K02, K03, K04, K09, K13, K14
S7	**Vlčí Creek 2 – Pec p. Sněžkou**	50°41´4.266"N	15°43´24.849"E	On the south-eastern hillside of Liščí Mt. Samples were collected along 1.1 km of this creek.	K01, K02, K03, K04, K09, K13, K14
S8	**Čistá Creek – Černý Důl**	50°38´20.078"N	15°42´41.740"E	On the east-south hillside of Liščí Mt. Samples were collected along 15.9 km of this creek, in the outskirts of Černý Důl village.	K01, K02, K03, K04, K09, K13, K14

*codes and dates when sampled:
K01 – 3/4/2019; K02 – 4/5/2019; K03 – 17/5/2019; K04 – 3/6/2019; K05 – 19/6/2019; K06 – 2/7/2019; K07 – 16/7/2019; K08 – 7/8/2019; K09 – 22/8/2019; K10 – 3/9/2019; K11 – 29/9/2019; K12 – 30/10/2019; K13 – 4/12/2019; K14 – 6/1/2020
Samples K13 were not collected from sites S1 and S2 because of lack of water due to artificial snow preparation at the beginning of the season.

Table 2 Water quality parameters recorded for samples collected from the upper (S1) and lower (S2) artificial reservoirs during 2019. Listed are basic statistical characteristics and limits of permissible pollution of surface and drinking water.

parameters	average		median		min		max		permissible pollution of surface water a)	permissible pollution of surface water used for supplying water b)	permissible pollution of drinking water c)
site:	S1	S2	S1	S2	S1	S2	S1	S2			
pH	7.35	7.36	7.49	7.18	6.37	6.72	8.56	8.97	5–9		6.5–9.5
conductivity [mS/m]	5.02	7.93	4.89	7.91	4.23	5.52	6.51	10.80			125
NH_4^+ [mg/l]	0.03	0.11	0.02	0.05	<0.02	<0.02	0.13	0.32	0.23		0.5
NO_3^- [mg/l]	0.68	2.03	0.25	2.20	<0.50	<0.50	1.55	4.20	5.4		50
N total [mg/l]	0.59	1.20	0.40	1.01	0.10	<0.50	2.14	4.74	6		
DON – N organic [mg/l]	0.36	0.65	0.19	0.37	0.01	0.11	2.12	4.49			
PO_4^{3-} [mg/l]	0.03	0.04	0.03	0.02	< 0.01	< 0.03	0.07	0.25			
P total [mg/l]	under LOF: 0.10 mg/l								0.15	0.05	
DOC [mg/l]	4.17	2.47	3.95	1.94	3.38	1.61	5.83	4.87			
Ca [mg/l]	7.83	10.00	8.16	10.5	4.25	6.23	11.20	13.8	120		30
Mg [mg/l]	0.99	1.74	1.04	1.74	0.47	1.08	1.3	2.38	190		10
chlorophyll A [mg/l]	4.57	67.90	3.60	5.90	<1	<1	10	680			

a) Limits of permissible pollution of surface water under the Decree No. 401/2015 Coll. – annual averages
b) Limits of permissible pollution of surface water used for water supply under the Decree No. 401/2015 Coll. – annual averages
c) Limits of permissible pollution of drinking water under the Decree No. 252/2004 Coll., with updates No: 187/2005 Coll., 293/2006 Coll., 83/2014 Coll., 70/2018 Coll. – the highest acceptable values

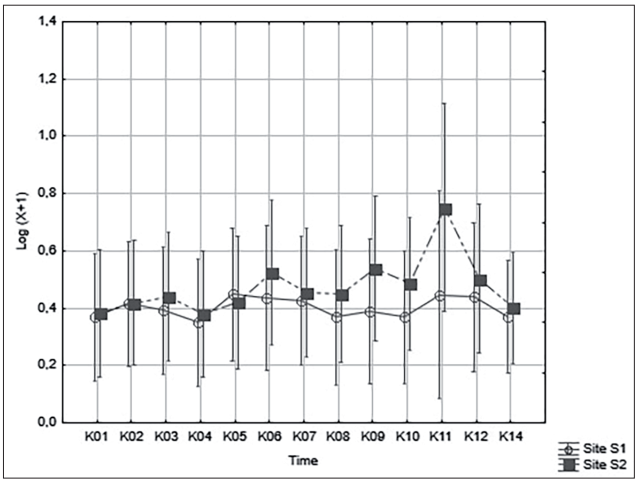

Fig. 4 The quality of the water slightly fluctuated during the season (dates sampled: K01-K14). However, both artificial reservoirs (S1 and S2 sites) showed similar trends. Vertical bars denote 0.95 confidence intervals.

Logarithmic transformation log(x + 1) was used to standardize some data. Data preparation and visualizations of our results were done in Microsoft Excel.

Results

Water quality in artificial reservoirs

The quality of the water in the reservoirs fluctuated during the season. Using Repeated Measurement in Split-plot ANOVA, statistically significant differences between sample dates (ANOVA, $p < 0.001$) were found. The first samples K1 and last samples K14 had similar parameters. They were collected immediately after filling the reservoirs on April 3, 2019 and January 6, 2020, respectively. Values of many of the parameters increased at the end of summer and during autumn. Similar trends were recorded in the upper (S1) and lower (S2) reservoirs. There were no significant differences between sites S1 and S2 ($F_{(12, 216)} = 1.4256$, $p = 0.156$; Fig. 4).

During 2019, the pH values of the water in the upper (S1) reservoir, which is mainly filled from Černohorský Creek, were slightly lower (Table 2, Fig. 5). In this reservoir, the highest pH value of 8.56 was recorded on July 2, 2019, but it decreased to 7.77 only 14 days later and subsequently fluctuated around 7.5 until the end of 2019. A significant decrease was recorded in sample K14, sampled on January 7, 2020. In the lower reservoir (S2), pH values increased slightly during the summer and the highest value of 8.97 was recorded on September 3, 2019.

The conductivity increased in both reservoirs during the season (Fig. 6). Slightly higher, but not statistically significant values were recorded in the lower S2 reservoir. The highest values were recorded in the lower S1 reservoir at the end of September and in October 2019.

The nutrients, i.e. nitrogen and phosphate ions, as well as their organic forms and total amounts of these elements, were very low in both reservoirs during 2019 (Table 2). They were under the legal limits determined by the Czech standards on the quality of surface water (CZ Decree No. 401/2015 Coll.) and drinking water (CZ Decree No. 70/2018 Coll.). Concentrations of total phosphorus were below the level of detectability. Also, the concentrations of phosphate ions were very low during the study period (Fig. 7), only higher values were recorded on July 16, 2019. Nearly a ten times higher value of PO_4^{3-} ions than usual were recorded in the lower (S2) reservoir on that date.

Samples from the lower (S2) reservoir had higher nitrate and total nitrogen concentrations throughout the observation period (Fig. 8), however, a statistically

Fig. 5 Seasonal changes in pH recorded in the upper (S1) and lower (S2) reservoirs. The numbers above the coloured columns are the pH values of samples K01–K14 (for dates sampled see Table 1).

Fig. 6 Seasonal changes in conductivity recorded in the upper (S1) and lower (S2) reservoirs. The numbers above the coloured columns are the conductivity values [mS/m] of samples K01–K14 (for dates sampled see Table 1).

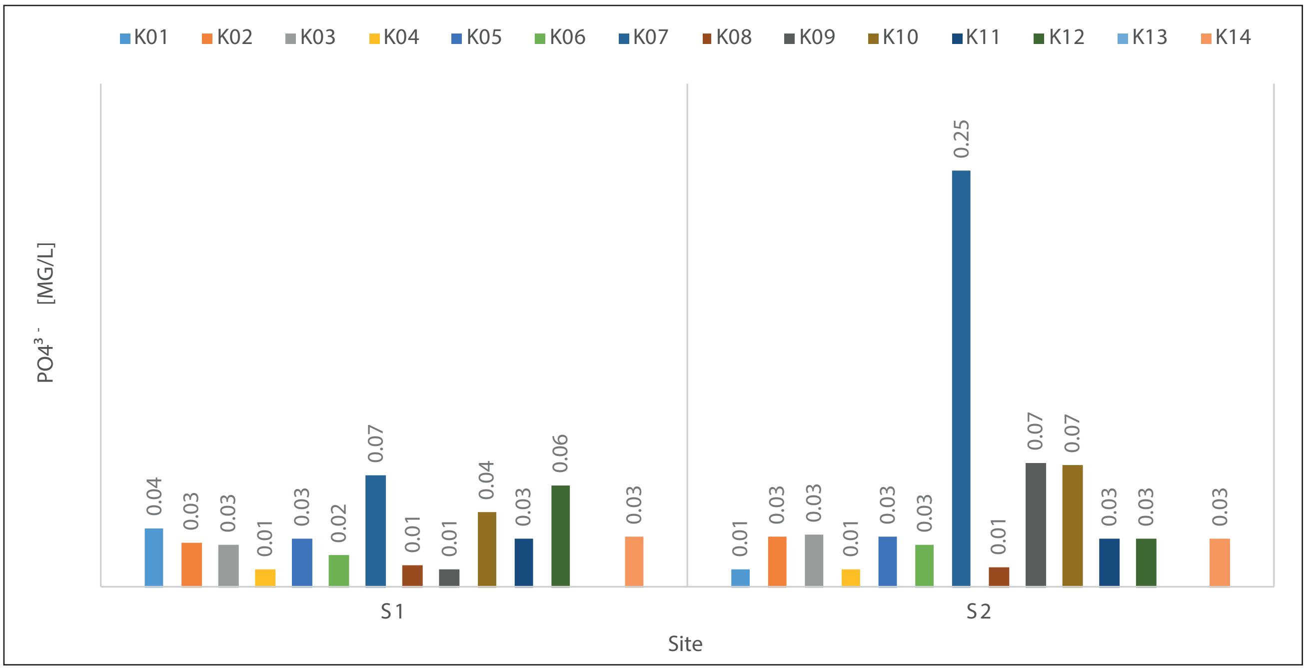

Fig. 7 Seasonal changes in the concentration of phosphate ions (PO_4^{3-}) recorded in the upper (S1) and lower (S2) reservoirs. The numbers above the coloured columns are the PO_4^{3-} ion concentrations [mg/l] of samples K01–K14 (for dates sampled see Table 1).

significant difference between the upper (S1) and lower (S2) reservoirs was confirmed only for nitrates (ANOVA, $p < 0.001$). The sample K11 taken from the lower reservoir (S2; sampled on September 29, 2019) contained much higher concentrations of organic and total nitrogen. Also, the concentration of chlorophyll A was very high at that time (Table 3, Fig. 9), which was associated with a high abundance of algae. At the same time, there were also increases in organic and total nitrogen concentrations, but at much lower values, in the upper (S1) reservoir (Fig. 8). Concentrations of nitrates in the lower reservoir decreased during the season and reached its lowest values by the end of September 2019.

The water in the upper (S1) reservoir contained statistically less magnesium and calcium than that in the lower (S2) reservoir (ANOVA, $p < 0.001$; Fig. 10).

Comparison of water quality in reservoirs and small creeks

Quality of the water in the reservoirs (S1, S2) and particular small creeks (S3–S8) was compared and sta-

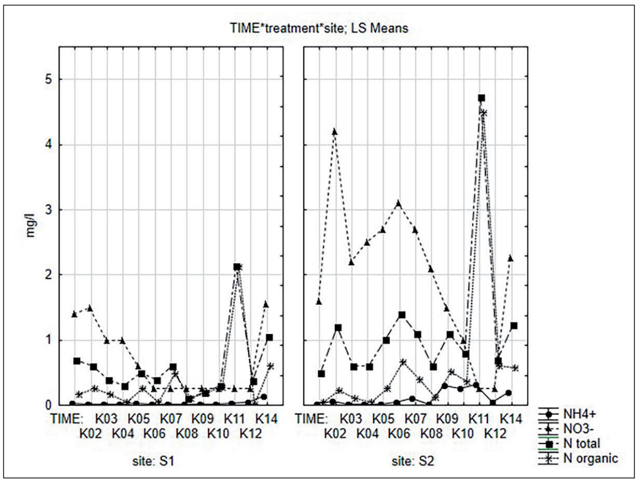

Fig. 8 Seasonal changes in the concentrations of nutrients recorded in the upper (S1) and lower (S2) reservoirs in samples K01–K14 (for dates sampled see Table 1). NH_4^+ – ammonium ions, NO_3^- – nitrate ions, N total – total nitrogen, N organic – organic nitrogen.

tistically significant differences in pH values (Fig. 11) and conductivity (Fig. 12) were recorded (ANOVA, $p < 0.001$). The lowest pH values were repeatedly recorded in samples from the Černohorský Creek (S3, Fig. 11).

Phosphate values, as in reservoirs, were very low in the creeks. In addition to the significantly higher value recorded in sample K07 from the lower reservoir (site S2, collected on 16 July 2019), a higher concentration of phosphate ions was also recorded in sample K09 from Janský Creek (site S4, collected on 22 August, 2019). Also, other K09 samples, collected from other creeks (S3 and S5–S8) on 22 August, 2019, had slightly higher concentrations of phosphate ions compared to the rest of the season. Total phosphorus values in the reservoirs were lower than the limit of detection.

Statistically significant differences (ANOVA, $p < 0.001$) were recorded for nitrate concentrations (Fig. 13). Samples from the upper (S1) reservoir and Černohorský Creek (S3) had much lower concentrations of nitrates than other sites. Concentrations of ammonium ions, organic and total nitrogen were very low in the samples from creeks (S3-S8) and reservoirs (S1, S2) throughout the season and none differed significantly. Only samples K11 collected from reservoirs (S1 and S2) on 29 September, 2019, had higher concentrations of total and organic nitrogen, as mentioned above.

Statistically significant differences (ANOVA, $p < 0.001$) were recorded between reservoirs and creeks in the concentrations of magnesium (Fig. 14) and calcium (Fig. 15). The highest concentrations of both elements were recorded in samples from Čistá Creek (S8) and the lowest in samples from the Černohorský Creek (S3). The samples from Zelený Creek (S5) also had lower concentrations of magnesium and calcium than the others.

Discussion

Throughout 2019, water nutrition parameters in the two reservoirs and six creeks were very low and similar to that reported by Hruška (2017) for Labská Dam and several creeks in the Špindlerův Mlýn region. Similar to his results, the concentrations of nutrients we recorded did not exceed the limits of permissible pollution of surface and drinking water defined by Czech legislation (CZ Decree No. 401/2015 Coll., CZ Decree No. 70/2018 Coll.). The upper (S1) reservoir was two thirds full of very clear and nutritionally poor water from the Černohorský Peat Bogs and Janský Creek, respectively, and one third from the

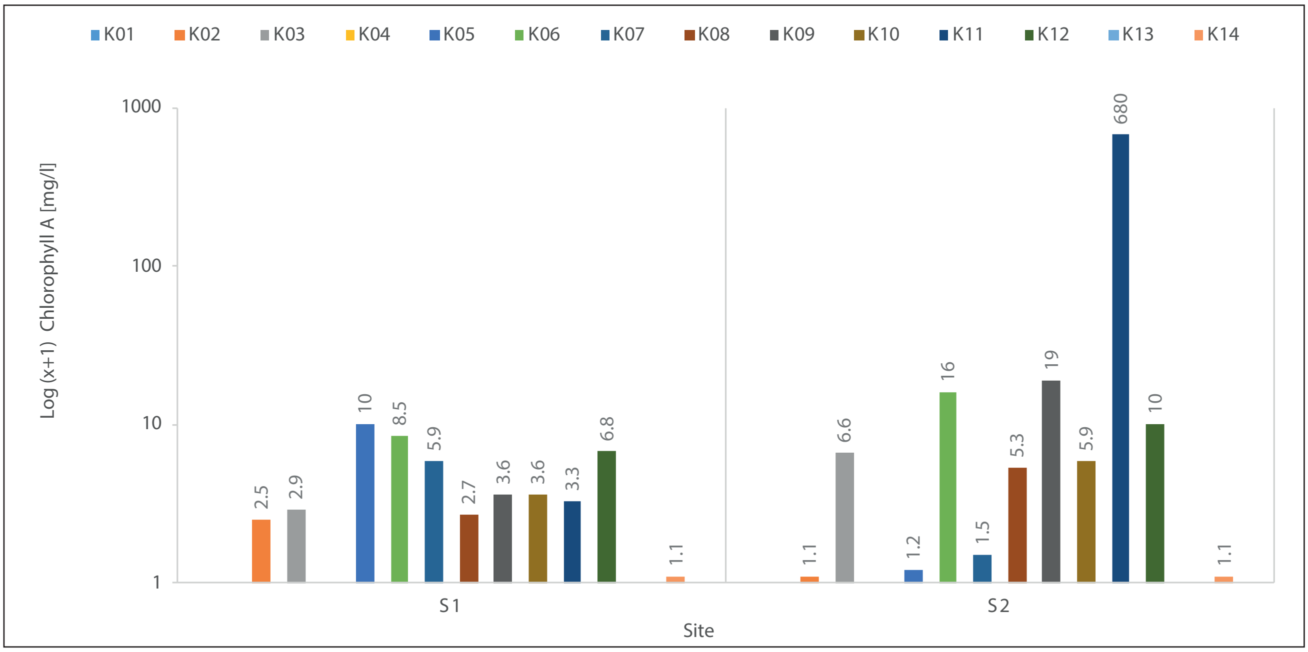

Fig. 9 Seasonal changes in the concentration of chlorophyll A recorded in the upper (S1) and lower (S2) reservoirs. The numbers above the coloured columns are logarithmic scale log(x + 1) chlorophyll A concentrations [mg/l] for samples K01–K14 (for dates sampled see Table 1).

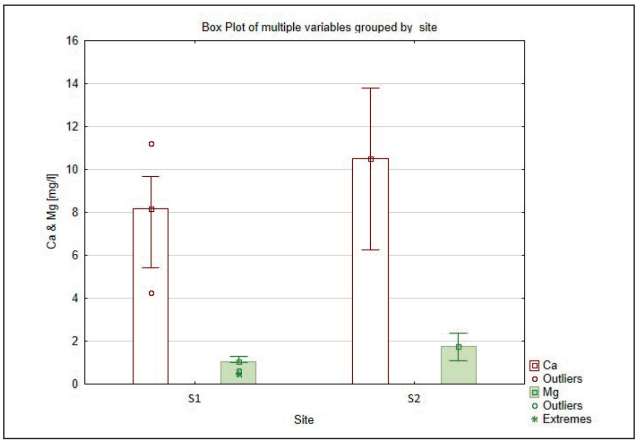

Fig. 10 Concentrations of magnesium (Mg) and calcium (Ca) recorded in samples from the upper (S1) and the lower (S2) reservoirs.

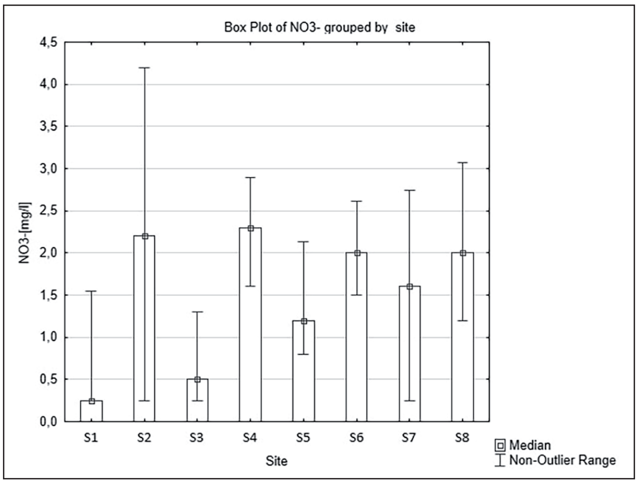

Fig. 13 Concentrations of nitrate ions (NO_3^-) recorded in samples from two reservoirs (S1, S2) and six creeks (S3–S8).

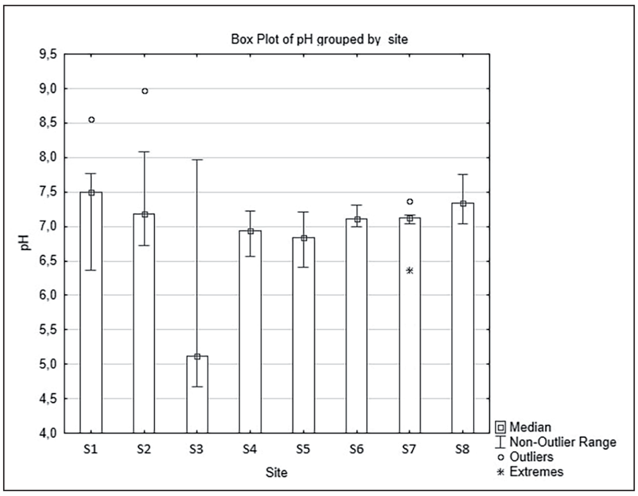

Fig. 11 pH values recorded in samples from two reservoirs (S1, S2) and six creeks (S3–S8).

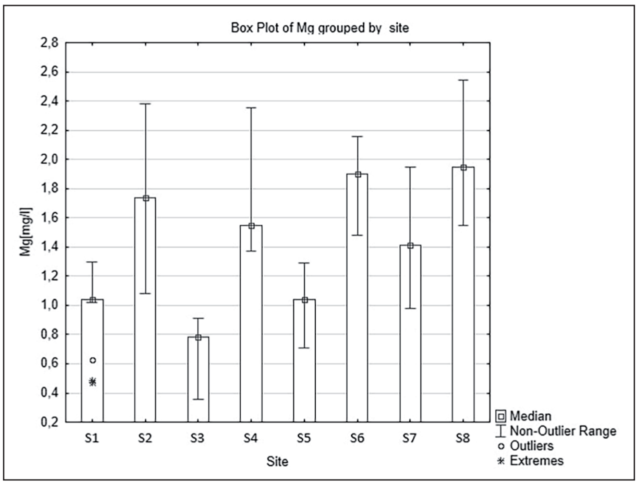

Fig. 14 Concentrations of magnesium (Mg) recorded in samples from two reservoirs (S1, S2) and six creeks (S3–S8).

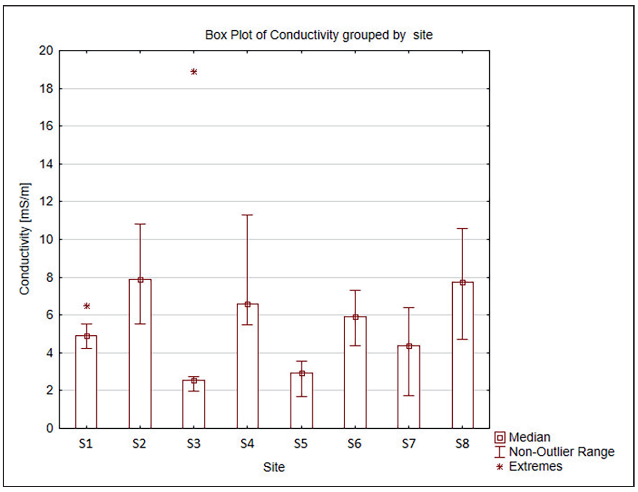

Fig. 12 Conductivity recorded in samples from two reservoirs (S1, S2) and six creeks (S3–S8).

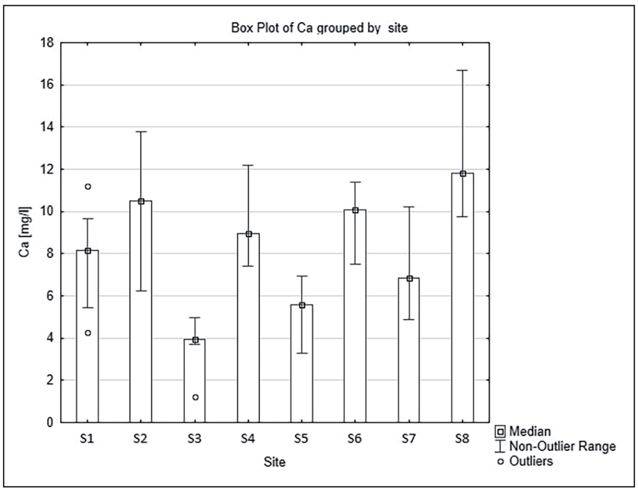

Fig. 15 Concentrations of calcium (Ca) recorded in samples from two reservoirs (S1, S2) and six creeks (S3–S8).

lower reservoir in spring. It then remained the same until November 2019 when production of artificial snow started. The pH and conductivity of the peaty water in S1 were lower than recorded in the lower reservoir (S2), which is permanently connected with Janský Creek. Samples from S2 had a slightly higher nutrient contents but there were no significant differences between the two reservoirs. Repeated measures analyses showed significant differences between sample dates. Partly water sharing between the lower and upper reservoir (i.e. about one third of the water in the upper reservoir came from the lower one) could explain similar trends in both reservoirs. Some nutrients from the lower reservoir were transferred to the upper reservoir. Concentrations of nutrients increased slightly during summer and the highest values were recorded at the end September. It is likely that natural seasonal dynamics in hydrochemical parameters together with those of water microorganisms (e.g. Kopáček et al. 2011; Fott 2013) account for these seasonal increases in nutrients.

The concentrations of nitrates and phosphate ions, organic forms and total volumes of phosphorus and nitrogen were very low in both reservoirs throughout season. Although low nutrient concentrations were recorded, some fluctuations in nutrient and chlorophyll A concentrations were recorded during summer. Higher concentrations and greater fluctuations were recorded in the lower reservoir (S2). The quality of water in this reservoir may be affected by the flushing of nutrients from around the reservoir or inflow of polluted water from Janský Creek, because samples from this creek (S4) repeatedly had higher concentrations of some nutrients. It is likely that these increases are associated with increases in the occupation of hotels and apartment houses in the small catchment area of Janský Creek above the reservoir.

High values of phosphates were recorded in sample K7 from the lower reservoir (S2; collected on July 16, 2019), when it was nearly ten times higher than the average value. At that time, the catchment of a small un-named creek entering the lower reservoir was landscaped and large amounts of soil moved, which resulted in large amounts of soil and other organic material contaminating the water. Also, high summer temperatures could have resulted in a mixing of the water in this reservoir, which was established in 2006, resulting in some of the phosphate in bottom sediments being circulated. In addition, the large population of brook trout may have eaten most of the invertebrates resulting in a massive increase in phytoplankton. The various factors and their combinations can differ from year to year. These risks can be eliminated or at least significantly reduced by proper water management in the area. A slight increase in phosphates was recorded in summer in the upper reservoir (S1), but this reservoir was recently cleaned so it contains very little sediment. Significantly lower concentrations of magnesium and calcium were recorded in the upper reservoir (S1), which was two-thirds filled with peaty bog water from Černohorský Creek whose source is the Černohorský Peat Bogs. Like bog water this water contains low concentrations of magnesium and calcium (Bourbonniere 2009; Špaček 2017).

Comparing data from reservoirs and several local creeks we found that water from Černohorský Creek (S3) had the lowest pH. This corresponds with results of Špaček (2017), who recognized Černohorský Creek as the most acidic creek in the Úpa River catchment, with a $pH_{min} = 4.3$ and $pH_{avg} = 5.7$. Samples from Černohorský Creek (S3) and the upper reservoir (S1), which is filled from this creek, also had significantly lower concentration of nitrates, magnesium and calcium, than samples from other sites. Samples from all creeks and both reservoirs had very low concentrations of ammonium ions, organic and total nitrogen throughout the season and did not differ significantly. Samples collected from Čistá Creek (S8) had the highest concentrations of magnesium and calcium, which reflects the occurrence erlan, a highly metamorphic limestone, in the area around the source of this creek (https://mapy.geology.cz).

Conclusions and Recommendations

We can conclude that the contents of nutrients recorded in two reservoirs and six creeks were very low throughout 2019. They did not exceed the limits legally permissible in surface and drinking water. Using water from these reservoirs and creeks for making snow does not result in a significant risk of adding fertiliser to the meadows on ski slopes. This study did not measure the direct effects of artificial snow on grassland, but using water with low concentrations of nutrients is unlikely to increase the fertility or change the species composition of mountain meadows. However, the results of our study cannot exclude that some other aspects of snowmaking can affect mountain meadows.

To eliminate the risks, we recommend: (i) fill reservoirs with water in spring when the runoff water is usually high and of very good quality; (ii) do not add water and avoid disturbing the sediment in the reservoirs in summer; (iii) avoid adding fish, sewage water or other sources of contamination to reservoirs.

To better understand the effect of artificial snow on the ecology of mountain meadows a set of permanent plots should be established and long-term monitoring of vegetation, soil invertebrates and soil chemistry undertaken. Long-term monitoring is essential, because experiences from different locations (e.g. Wipf 2002) show that significant changes occur over long periods, especially in ski resorts above the tree line, where natural alpine meadows occur. Monitored plots should be on ski slopes with and without artificial snow.

Acknowledgements

We thank the following institutions and persons: P. Hynek and Ski Resort staff for initiating this research and providing logistical support. LABTECH Brno Ldt. – Hygienic Laboratories Klatovy for chemical analyses and A. F. G. Dixon for revising the language. This work was supported by the Global Change Research Institute of the Czech Academy of Sciences.

REFERENCES

AHS (2019) České hory za sebou mají vydařenou sezónu. TZ 11. 4. 2019; Available from: https://www.ahscr.cz/-2/tiskove-zpravy/.

Anonymous (2012) STATISTICA, v. 12, StatSoft, Inc, Tulsa, OK, USA; Available from: http://www.statsoft.com.

Bourbonniere RA (2009) Review of Water Chemistry Research in Natural and Disturbed Peatlands. Can Water Resour J/Revue canadienne des ressources hydriques. 34: 393–414.

Breiling M, Charamza P (1999) The impact of global warming on winter tourism and skiing: a regionalised model for Austrian snow conditions. Reg Environ Change 1: 4–14.

Elsasser H, Bürki R (2002) Climate change as a threat to tourism in the Alps. Clim Res 20: 253–257.

Flousek J (2016) Vliv lyžování na horskou přírodu: shrnutí současných poznatků a stav v Krkonoších. Opera Corcontica 53: 15–60.

Fott J (ed) (2013) Limnology of Mountain Lakes. Springer Science and Business Media.

Freppaz M, Filippa G, Corti G, Cocco S, Williams MW, Zanini E (2013) Soil properties on ski-runs. In: Rixen C, Rolando A (eds) The impacts of skiing and related winter recreational activities on mountain environments. Bentham Science Publishers Bussum, pp 45–64.

Fuksa JK (2016) Umělé zasněžování – vliv na vodní toky a ekosystémy. Newsletter Adaptace měst na klimatickou změnu 5/2016, Sbor. přísp. konf. Tvoříme klima pro budoucnost, Liberec, 26.–27. 1. 2016: 22–24.

Hruška J (2017) Propojení areálů Špindlerův Mlýn: Stanovení změny překročení kritických zátěží dusíku použitím povrchové vody z přehrady Labská k umělému zasněžování. [Interconnection of ski resorts of Špindlerův Mlýn region: Determination of the change of critical nitrogen loads exceeded by the use of surface water from the Labská Dam for technical snow]. Global Change Research Institute CAS, Brno, Czech Republic; Available from: https://portal.cenia.cz/eiasea/detail/EIA_HKK906.

Hruška J, Hošek J, Hofmeise, J, Čížek L (2017) Kritické zátěže síry a dusíku pro lesní ekosystémy NP Krkonoše a jejich překročení atmosférickou depozicí v roce 2016. Ms. depon In: Správa KRNAP, Vrchlabí.

Jones HG, Devarennes G (1995) The chemistry of artificial snow and its influence on the germination of mountain flora. IAHS Publications-Series of Proceedings and Reports 228: 355–362.

Kammer PM (2002) Floristic changes in subalpine grasslands after 22 years of artificial snowing. J Nat Conserv 10: 109–123.

Kocková J (2011) Srovnání vegetace sjezdových tratí s umělým a přírodním sněhem v CHKO Bílé Karpaty a v CHKO Beskydy. [Comparison of vegetation on ski slopes with artificial or natural snow in CHKO Bílé Karpaty and CHKO Beskydy. Mgr. Thesis, in Czech]. Faculty of Sciences, University of South Bohemia, České Budějovice, Czech Republic.

Koenig U, Abegg B (1997) Impacts of climate change on winter tourism in the Swiss Alps. J Sustain Tour 5: 46–58.

Kopáček J, Hejzlar J, Vrba J, Stuchlík E (2011) Phosphorus loading of mountain lakes: Terrestrial export and atmospheric deposition. Limnol Oceanogr 54: 1343–1354.

Negro M, Isaia M, Palestrini C, Rolando A (2009) The impact of forest ski-pistes on diversity of ground-dwelling arthropods and small mammals in the Alps. Biodivers Conserv 18: 2799–2821.

Negro M, Isaia M, Palestrini C, Schoenhofer A, Rolando A (2010) The impact of high-altitude ski pistes on ground-dwelling arthropods in the Alps. Biodivers Conserv 19: 1853–1870.

Špaček J (2017) Vyhodnocení acidifikace a aktuálního ekologického stavu vodních toků. In: Beranová et al. (eds) Aktuální stav lesních a vodních ekosystémů na území evropsky významné lokality Krkonoše. Výstup projektu č. EHP-CZ02-OV-1-023-2015. Správa KRNAP. Vrchlabí. pp 35–44.

Štursa J (2007) Ekologické aspekty sjezdového lyžování v Krkonoších. In: Štursa J, Knapik R (eds) Geoekologické problémy Krkonoš. Sborn Mez Věd Konf, říjen 2006, Svoboda n. Úpou. Opera Corcontica 44: 603–616.

Thiel D, Jenni-Eiermann S, Braunisch V, Palme R, Jenni L (2008) Ski tourism affects habitat use and evokes a physiological stress response in capercaillie *Tetrao urogallus*: a new methodological approach. J Appl Ecol 45: 845–853.

Treml P (2019) Dopad technického zasněžování na toky v Krkonoších. VTEI 61: 20–30.

Treml P, Hanel M, Kašpárek L, Novický O, Březina S (2012) Vliv odběrů vody pro technické zasněžování na odtokovou výšku hlavních toků v Krkonoších. Opera Corcontica 49: 73–87.

Vanat L (2019) 2019 International Report on Snow & Mountain Tourism. Overview of the key industry figures for ski resorts and mountain tourism. Geneve [online] [cit. 4. 4. 2020]. Available from: http://www.vanat.ch/tarif-WR-data-2019.pdf.

Wipf S, Rixen C, Fischer M, Schmid B, Stoeckli V (2005) Effect of ski piste preparation on alpine vegetation. J Appl Ecol 42: 306–316.

Zeidler M, Duchoslav M, Banaš M (2016) How alpine heathlands response to the snow cover change on the ski slope? Long-lasting ski slope impact case study from the Hrubý Jeseník Mts. (Central Europe). Acta Soc Bot Pol 85: 1–13.

Zeitler A, Glanzer U (1998) Skiing and grouse in the Bavarian Alps. Grouse News 15: 8–12.

BIOMARKERS AS A TOOL FOR ASSESSING DIFFUSE CONTAMINATION OF COASTAL WETLAND

SILVIA VILLAR[1,2,3,*]

[1] Sistema Nacional de Investigadores, Agencia Nacional de Investigación e Innovación (SNI. ANII), Montevideo, Uruguay
[2] Instituto Iberoamericano de Posgrado (IBEP), España-Uruguay
[3] Administración Nacional de Educación Pública, Montevideo, Uruguay
* Corresponding author: environscience21@gmail.com

ABSTRACT

Playa Penino is a natural reserve located on the south coast of Uruguay. It hosts 244 species of birds (more than 50% of the bird biodiversity recorded for Uruguay). The area is included in the International *BirdLife* Program for biodiversity preservation. Urbanization and pollution generally have affected the quality of the water and biodiversity in wetlands. The analysis of the major wetland of Playa Penino revealed high levels of organic compounds. The biomonitoring was done using the caviomorph rodent *Ctenomys pearsoni* (commonly known as tuco tuco) as an indicator species because they inhabit burrows around the wetland. Genetic effects were determined using the comet assay and micronucleus test. The significant correlations between chemical and microbiological parameters and genetic damage might indicate that macronutrients from sewage could be one of the causes of the genetic damage. There is an urgent need to conserve the biodiversity of this natural area by introducing sewage treatment, cesspool control and by controlling settlement in the area etc.

Keywords: biomarkers; comet assay; *Ctenomys pearsoni*; micronucleus test; Playa Penino; wetland

Introduction

The net cumulative effect of humanity has been to reduce biodiversity and most societies have decreased it. The direct and indirect effects of humans on the environment are ubiquitous and almost all biodiversity research and conservation must take the possibilities of human influence into consideration (Kopnina 2016). Communities that live in the proximity of protected areas have exceeded the carrying capacity of their natural environment and are unintentionally depleting resources (Sponsel 2014).

Many wetlands harbour highly diverse biological communities and provide extensive ecosystem services. However, these important ecological features are being altered, degraded and destroyed around the world (Kopnina 2016; Sievers et al. 2017). Our current lack of knowledge of individual-level responses may therefore limit our capacity to manage wetland ecosystems effectively (Sponsel 2014; Kopnina 2016).

In South America, many wetlands are remote and far from agricultural and urban pressures. However, coastal areas have been subjected to severe anthropogenic effects (Kopnina 2016; Sievers et al. 2017). Notwithstanding their critical importance, many freshwater ecosystems are considered useless. Widespread ignorance about the important benefits that wetlands provide human societies has contributed to this notion and has promoted their destruction and degradation (Kopnina 2016; Sievers et al. 2017).

Playa Penino is a natural reserve located in the south of Uruguay. It hosts 244 species of birds (more than 50% of bird biodiversity recorded in this country); 69% are residents and 31% are migratory species, some are vulnerable or close to threat (Arballo and Bresso 2007). It is part of the International *BirdLife* Program for biodiversity preservation (CFPP 2013).

Although, Aldabe et al. (2009) mention the effect of urbanization and pollution in this area, currently the landscape has been completely altered by anthropogenic activities such as; overpopulation, irregular settlements, deforestation, introduction of livestock, invasion of exotic species, sand mining and habitat fragmentation (Rocha 1999; CFPP 2013; MVOTMA 2018). Part of the livelihood of the residents is dependent on the natural resources of the Playa Penino wetlands, such as juncus, totora, shells, sand and fish (Arballo and Bresso 2007). Although local authorities declared the area a nature reserve, protected under the category of "ecological beach" and is part of the National Protected Areas System (Arballo and Bresso 2007; MVOTMA 2018), there is no effective control over the area. Under this area is the Raigón aquifer, whose depth is less than 40 meters and is the sole source of water for more than 15,000 people, including towns, industry and rural areas (Martino et al. 2008). The shallow aquifer in sandy subsoil is severely affected by wastewater discharges and the absence of a sanitary network (MVOTMA 2018). Domestic effluents empty into filtration chambers or discharged directly into ditches next to houses.

GeoUruguay report (Martino et al. 2008) indicates that the major ecological problems are erosion, mining sand for construction and loss of biodiversity due to urban expansion and pollution of natural areas. Despite its ecological importance, there are no field studies on the levels of contamination.

Villar, S.: Biomarkers as a tool for assessing diffuse contamination of coastal wetland
European Journal of Environmental Sciences, Vol. 10, No. 1, pp. 42–50
https://doi.org/10.14712/23361964.2020.6
© 2020 The Author. This is an open-access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

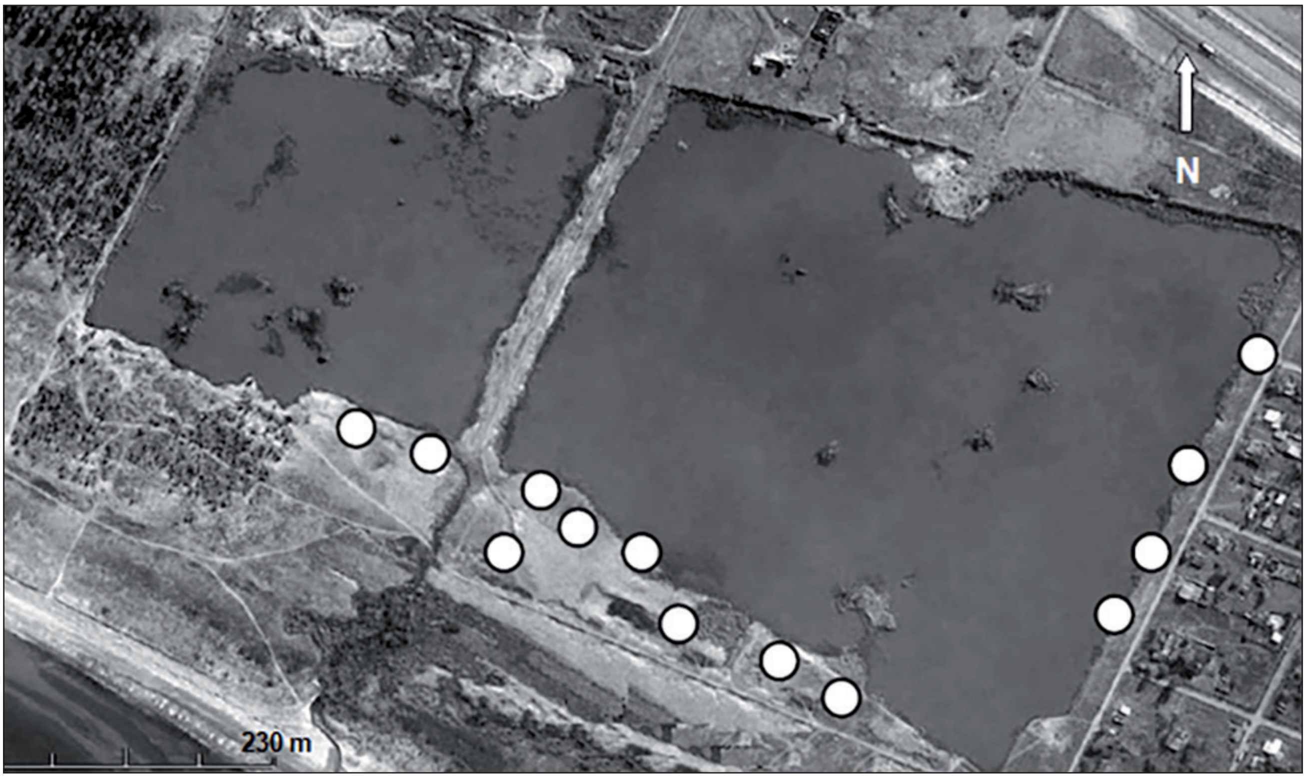

Fig. 1 Aerial photograph of the main wetland of Playa Penino with the sites, where the indicator species were captured, are indicated by the white circles. The number of circles does not represent the number of individuals captured. Modified version of the photograph in Google Earth.

A biomarker is a molecular or cellular indicator of the effects of a particular environment on the cells of an indicator species (Mudry and Carballo 2006). This makes it possible to assess the environmental conditions affect an organism. The indicator species is an organism that inhabits the ecosystem studied and has some important features. For example, it is widely distributed in the area studied and its biology is well-known, and could have a key role in the comprehension of the consequences of exposure to a xenobiotic. Identifying incipient deterioration in the indicator species indicates potential dangerous situations at sub-lethal levels, either for a point or diffuse source of a xenobiotic (Ribeiro et al. 2003; Mudry and Carballo 2006).

In environmental biomonitoring, genotoxic biomarkers are used to assess the risks to living organisms that inhabit a particular environment that is thought to be exposed to mutagenic substances (Clemente et al. 2004; Machella et al. 2006; Emmanouil et al. 2007; Yin et al. 2009; Kumar et al. 2010; Capriglione et al. 2011; Mohanty et al. 2011; Parolini et al. 2011).

The aim of this study was to determine the degree of contamination of wetland by measuring certain chemical and microbiological parameters and whether they negatively affect the site (Playa Penino) and its fauna. For the latter, we used an indicator organism, which could be affected at the molecular level by different levels of contamination: *Ctenomys pearsoni* (Lessa and Langguth 1983), commonly known as tuco tuco, which was chosen because they inhabit burrows around the wetland. They imbibe water in the form of raw sap from the roots of wetlands plants and rarely leave their burrows, so we do not have to consider the effects of air pollution. Genetic biomarkers of genetic damage were identified by using the comet assay and micronucleus test.

Methods

Playa Penino is a natural reserve, located in the Department of San Jose (34°47′S 56°24′W; south of Uruguay). It extends for 7 km along the coast. The area studied is located in the wetland of Playa Penino (route 1 South, kilometer 30) (Fig. 1). Locations where the indicator animals were captured are indicated by white circles. Animals were captured using live traps. Blood was obtained without sedation by cutting the base of a rear claw. To avoid stress, each animal was placed in an opaque tube leaving only its rear end exposed. Adult individuals were selected and sexed. Following disinfection of the cut, the animals were returned to their burrows. Blood samples were collected by dripping and placed in tubes containing heparin (da Silva et al. 2000). Individuals were collected in four months of each year (February, May, August and October), which includes the widest range in seasonal variability (i.e. wet and dry months) a total of 25 females / 25 males in 2013, 23 females / 20 males in 2015 and 22 females/21 males in 2017.

Control

Thirteen individuals (6 males and 7 female) of *Ctenomys pearsoni* from Playa Penino were also maintained in captivity in temperature-controlled conditions, in tanks containing sand and vegetables sufficient for a month, as negative controls. After a month, they were released back into the burrows that they previously inhabited, which were marked by flags. Blood was obtained from them during the last two days of their captivity and kept in liquid nitrogen until analysed.

Genetic biomarkers

The comet assay, or single cell gel electrophoresis (SCGE), is a standard method for determining Geno toxicity *in vivo* and *in vitro,* which records the genetic damage caused by clastogenic agents by measuring breaks in the DNA chains in specific cells (Ribeiro et al. 2003; Mudry and Carballo 2006). This characteristic and its sensitivity, makes it especially well-suited for Eco toxicological studies, both in terrestrial and aquatic environments (Ribeiro et al. 2003; Mudry and Carballo 2006; De Lapuente et al. 2015). Micronuclei are a portion or whole chromosomes that are not in the nucleus during anaphase. Their presence implies the loss of a part or an entire chromosome in daughter cells (Mudry and Carballo 2006; Luzhna et al. 2013). More than one micronucleus can be produced during cell division depending on the extent of the genetic damage (Luzhna et al. 2013).

Comet assay

The genotoxic evaluation was done using the comet assay. The standard procedure described by Hartmann et al. (2003) was used. Blood samples were centrifuged, washed in PBS and re-suspended in low melting agarose. Samples were incubated for 30 min at 37 °C and slides were prepared as outlined by Hartmann et al. (2003). After incubation, 100 µl of each sample was added to each slide, covered with normal agarose and subjected to lysis for 1 h in a lysis solution (NaCl 2.5 M, EDTA 100 mM, Tris 10 mM, Triton X100 1% and DMSO 10%, pH 10). The DNA was denaturalized in electrophoresis buffer for 20 min and after that subjected to electrophoresis for 30 min (20 V, 250 mA). Then the slides were neutralized in Tris 0.4 M, pH 7.5 for 15 minutes and stained with propidium iodide (PI) (10 µg/ml). The comet visualization and measurements were done using Comet Score^{TM} software (Tritek) and 100 cells for each individual were analysed under a fluorescence microscope (Shiner XY-FL^{TM}). To assess cytotoxicity, one slide was removed from lysis after 1 hour and neutralized without undergoing electrophoresis. Air dried slides were stained with PI and 100 cells per slide were scored visually. Cells were classified as normal, if their DNA was mostly condensed (normal cells) and apoptotic cells were visualized as diffused or condensed DNA without the round normal shape. Samples with a percentage of apoptosis lower than 15% were used for this study as recommended in the protocols (Hartmann et al. 2003; Mudry and Carballo 2006).

Micronucleus test

Following the procedure described by Fenech (2007) with modifications, smears were used to determine the number of micro nucleated polychromatic erythrocytes (MN-PCE) and total percentage of micronuclei (‰ MN) in more than 2,000 cells. A micronucleus was 1/16 to 1/3 of the size of the main nucleus and not attached to it (Garriott et al. 2002; Norppa and Falck 2003; Zalacain et al. 2005). Frequencies were analysed using parametric and non-parametric statistics (the latter for cases, in which the conditions of normality and homoscedasticity were not met using Centurion Statgraphics XVI.I software). The observations were made under a fluorescence microscope and slides were stained with propidium iodide (PI) (10 µg/ml).

Chemical analysis

Water samples (one per season) were analysed in the field using the kit colour (Merckmillipore^{TM}) for ammonium (NH_{4+}) and test strips (Hach^{TM}) for nitrates (NO_3-N) and phosphates (reactive orthophosphate PO_4) following the procedures indicated by the providers. Samples were from the surface along the shoreline of the wetland. Superficial water was collected in 500 ml sterilized plastic bottles at five locations in the wetland. Then, they were mixed in sterilized plastic container for the analyses. Samples were obtained when the indicator animals were captured.

Faecal coliform bacteria

3M™ Petrifilm™ Enterobacteriaceae plates were used following the procedures indicated by the provider and the samples described in the chemical analysis section. Water samples were obtained along with the indicator animals using the same procedure as in the previous section.

Statistical analyses

The normality and homoscedasticity of data obtained using biomarkers of genetic damage (comet assay and MN test) were tested by methods based on Kolmogorov-Smirnov for normality and Levene and Tukey for homoscedasticity (Zar 2010). To compare the data on these variables, analysis of variance (ANOVA) for parametric data and Kruskal-Wallis test for nonparametric data were used. Results of all the analyses are listed in tables along with the relationships between variables analysed using multiple correlation analysis (Statgraphic Centurion XVII software). The variables that were not normally distributed were transformed prior to correlation.

Agrochemical analyses

Samples of water (obtained as already described), soil (from the burrows of the captured animals) and the

Table 1 Values of % damaged DNA and OTM per month/year. Control values were: % damaged DNA 5.1; OTM 0.24.

Month[1]/Year	% DNA damaged	OTM
February 2013	31.40	9.08
May 2013	17.11	2.06
August 2013	44.97	7.45
October 2013	51.91	11.99
Annual average	**36.35**	**7.65**
February 2015	34.80	8.78
May 2015	23.10	1.66
August 2015	46.00	7.15
October 2015	59.50	11.19
Annual average	**40.85**	**7.20**
February 2017	33.20	10.21
May 2017	18.90	2.34
August 2017	50.14	8.05
October 2017	59.30	12.14
Annual average	**40.39**	**8.19**

[1] February corresponds to summer, May to autumn, August to winter and October to spring. This is valid for all tables.

Table 2 Values of PCE-MN and ‰ MN per month/year. *Significantly different from the other months. Control values were: MN-PCEs 0.03; ‰ MN 0.04.

Month/Year	MN-PCEs	‰ MN
February 2013	2.4	2.8
May 2013	1.5	1.7
August 2013	2.0	2.6
October 2013	5.6*	5.9*
Annual average	**2.9**	**3.3**
February 2015	2.3	3.2
May 2015	1.6	1.8
August 2015	2.1	2.7
October 2015	5.7*	6.2*
Annual average	**3.0**	**3.5**
February 2017	2.2	2.6
May 2017	1.6	1.8
August 2017	2.1	2.5
October 2017	5.3*	5.5*
Annual average	**2.8**	**3.1**

plants they consumed were tested for the presence of agrochemicals by LATU (Laboratorio Tecnológico del Uruguay) in every season of the year 2013. Gas exchange chromatography was used for the analyses, which is accredited by the United Kingdom Accreditation Agency, UKAS. The detection limit was 1 ng/l.

The presence or absence of the following pesticides was recorded: aldrin (chlorinated insecticide), carbaryl (insecticide, carbamate), cypermethrin (insecticide, pyrethroid), chlorothalonil (fungicide and chlorinated broad-spectrum insecticide), chlorpyriphos (organophosphate insecticide), dieldrin (chlorinated insecticide, prohibited use), α, β and λ endosulfan (organochlorine insecticide and acaricide, prohibited), malathion (organophosphate insecticide), methylchlorpyriphos (insecticide, organophosphate), methyl parathion (organophosphate, prohibited) (insecticide, pyrethroid), procymidone (chlorinated fungicide).

Results

Average values for the percentage of DNA damaged (% DNA damaged) and Olive tail moment (OTM) in the lymphocytes of *Ctenomys* did not differ between males and females (Table 1), Mann-Whitney U test ($p = 0.08$).

Data provided is for all the individuals sampled. Both variables are normally distributed according to Kolmogorov-Smirnov and homoscedastic according to Levene and Tukey tests. ANOVAs show highly significant differences ($p < 0.01$) between samples in all months in each year in both variables and the control samples, and no significant differences between the annual values for the three years ($p < 0.05$).

In Table 2, values for polychromatic erythrocytes (PCE) with one or more micronuclei (MN-PCEs) and ‰ MN are shown. There are no significant differences between males and females according to Mann-Whitney U test ($p = 0.075$). Data provided is for all the total individuals sampled. The MN-PCEs counted were 2000 (2 smears per individual) and values were averaged. Only polychromatic erythrocytes were counted as the spleen of *Ctenomys* removes abnormal erythrocytes from its blood, therefore normochromatic erythrocytes (NCE) were not detected. According the Kruskal-Wallis tests, there are significant differences ($p < 0.05$) between the October samples for each year and the other months analysed for both variables (MN-PCEs and ‰ MN). All samples showed significant differences relative to the controls ($p < 0.05$). There were no significant differences between the annual values of the three years ($p < 0.05$).

Phosphate reached its highest values in May and February in each year and nitrates increased in May only in 2013. Values for ammonium, on the other hand, were lowest in May and high in the February and October samples, which is coincident with the increase in the ammonium values. Faecal coliform bacteria (FC) also showed an increase in the months of February and October in each year (Table 3).

FC concentration was positively and significantly correlated with OTM. The concentration of ammonia was positively correlated ($p < 0.05$) with the biomarkers of genotoxicity (% damaged DNA and OTM). The percentage FC correlates positively and significantly with the concentration of ammonia in water (0.985, data not

Table 3 Values of the chemical and microbiological parameters of the compound samples from the wetland for each month/year.

Month/Year	Phosphates (mg/l)	Nitrates (mg/l)	Ammonium (mg/l)	Faecal coliform bacteria (CFU/100 ml)
February 2013	1.0	0	1.0	1300
May 2013	1.0	0.15	0	300
August 2013	0	0	0	800
October 2013	0	0	1.0	1500
Annual average	**0.5**	**0.004**	**0.5**	**975**
February 2015	1.0	0.15	1.0	1300
May 2015	0.5	0.15	0.5	300
August 2015	0	0	0	800
October 2015	0	0	1.0	1700
Annual average	**0.4**	**0.08**	**0.6**	**1025**
February 2017	1.0	0.15	1.0	1200
May 2017	0.5	0.3	0	300
August 2017	0	0	0.5	700
October 2017	0	0	1.0	1600
Annual average	**0.4**	**0.11**	**0.6**	**950**

shown in the Table). Correlation coefficient between phosphates and ‰ MN was moderate (Table 4).

There were no agrochemicals present in the water, soil and plants in any season of the year when they were recorded.

Discussion

Genetic damage

The biomarkers used revealed higher levels of genetic damage compared to other studies using the same genus (*Ctenomys*). The percentages of MN-PCEs and ‰ MN reveal the existence of serious genetic damage in the indicator species. The MN-PCEs values exceed those reported by Heuser et al. (2002) in *Ctenomys minutus* exposed to elevated levels of hydrocarbons and heavy metals (MN-PCEs value = 0.76) at locations along route 30 (coast of Rio Grande do Sul, Brazil). They are also higher than the maximum values of MN-PCEs (value = 2.8) obtained by da Silva et al. (2000) in *Ctenomys torquatus* for individuals exposed to polycyclic aromatic hydrocarbons (PAHs)

Table 4 Multiple correlation test showing significant coefficients at $p < 0.05$. NS = not significant.

Correlation coefficients p < 0.05				
	Phosphates	**Nitrates**	**Ammonium**	**Faecal coliforms**
% damaged DNA	NS	NS	0.980	NS
OMT	NS	NS	0.969	0.975
PCE-MN	NS	NS	NS	NS
‰ MN	NS	0.531	NS	NS

in a coal mining area located at Candiota in southern Brazil. The values of MN-PCEs also are higher than the maximum value obtained by Ayla et al. (2005) for rats exposed to lead acetate (a solvent used in the production of cosmetics and furniture) (value = 2.41).

The average % of DNA damaged ranged from 36 to 41%. Kopjar et al. (2008) report values of 0.85–7.37 for % DNA damaged and 0.11–0.86 for OTM, in peripheral blood of fish from rivers that were contaminated with urban effluents (nitrates, nitrites, and ammonium). Festa et al. (2003) report maximum values for % of DNA damaged of 32.8 and Tail Moment of 38.28 in *Mus spretus* blood cells during a toxic spill at the Aznalcollar mine in Doñana National Park, which dumped acidic water, mud full of toxic metals and arsenic into the Guadiamar River.

The highest values recorded in this study indicate the presence of the genotoxic agents lead, hydrocarbons, heavy metals and arsenic in the Playa Penino wetland. It is likely that these xenobiotics are entering the blood system of the indicator species via the plants it consumes.

Effects of the chemicals and bacteria

There were no registered agrochemicals in the samples of water, soil and plants analysed. Although antibiotics were not tested for in the water, it is important to point out that Rodríguez (2010) reports values for these substances of less than 1 μg/l in surface waters in areas with larger populations than at Playa Penino. This value is the minimum that results in adverse effects on microorganisms *in vitro* (Rodríguez 2010). Moreover, the concentration of microorganisms should be affected by the presence of antibiotics in the water, so that the high concentrations of enterobacteria recorded in this study is not consistent with the presence of toxic levels of antibiotics (Rodríguez 2010).

Even though the presence of detergents was not recorded, many of them include phosphate, which would generate a very big increase in the phosphate values of water samples (Romero 2006).

According to ordinance 253/79 governing the quality of water in Uruguay (MVOTMA 1979), the Playa Penino wetland corresponds to class 2 b, which includes "*waters intended for recreation by direct contact with the human body for many residents and visitors of the area*" or class 3 "*waters destined to the preservation of fish in general and other members of the water flora and fauna, or also waters destined to the irrigation of crops whose product is not consumed in a natural way or in those cases that being consumed in natural way irrigation systems are applied that do not cause the product to wet*". For either class, the value for ammonia exceeded the allowable limits in all samples (allowable limit 0.02 mg/l). In contrast, nitrates are below the limits set by this ordinance (10 mg/l).

Regarding the nitrogen-related contributions, the average annual values ranged from 0.5 to 0.6 mg/l for ammonium. For nitrates, the average annual values ranged from 0.004 to 0.11 mg/l. The average annual nitrate values and their maximum values (0.11 mg NO_3/l) were below the national and international standards (Arocena et al. 2008). The maximum average ammonium values (0.6 mg NH_4/l) exceeded not only these ranges, but the international values for the preservation of aquatic life and drinking water (Arocena et al. 2008). Ammonia is not toxic, but its presence even in low concentrations may mean the presence of faecal bacteria and pathogenic microorganisms in the water. Ammonia is produced during the first inorganic stage of the bacterial breakdown of urea and proteins, and it is a good indirect chemical indicator of the faecal contamination of water (Laws 1993; Rodríguez et al. 2006).

The average values for phosphate (PO_4) ranged from 0.4 to 0.5/l, which is greater than the internationally recommended level (Delgadillo et al. 2010; Putz 2010). Phosphates are directly related to the eutrophication of rivers, and especially lakes and reservoirs, considering that only one gram of phosphate (PO_4) causes the growth of up to 100 g of algae (Delgadillo et al. 2010; Putz 2010). Directive UE 91/271/EEC indicates that the critical concentrations for incipient eutrophication are between 0.1–0.2 mg/l PO_4 in running water and between 0.005–0.01 mg/l PO_4 in still water (De Azevedo and Da Matta 2004; Márquez et al. 2007; Putz 2010).

Phosphorus, unlike nitrogen, is not obtained from the atmosphere; it comes from the excreta of living organisms and is mainly contained in fertilizers, pesticides, detergents and sewage (Márquez et al. 2007). Rainfall is higher from January to May than in other months (110 mm/month) in the San José Department (climate-data.org), which may account for the higher levels of phosphate in water being a product of runoff and filtration from rudimentary septic chambers.

Nitrogen and phosphorus are essential for all living organisms, as they are a basic part of molecules such as proteins and nucleic acids (Spiro and Stigliani 2004), and play a key role in their growth. Under normal conditions, these substances come mainly from external natural contributions and the decomposition of dead organic matter (recycling). When the environmental conditions are altered, however, the additional contributions come mostly from the discharge of urban waste and certain industrial processes, as well as from the increasing use of fertilisers and pesticides in agriculture (De Azevedo and Da Matta 2004; Spiro and Stigliani 2004; Cicerone et al. 2007).

In urban or domestic wastewater effluents, nitrogen is present primarily as organic nitrogen or ammonium and, to a lesser extent, as nitrites and nitrates (De Azevedo and Da Matta 2004; Spiro and Stigliani 2004; Cicerone et al. 2007; Delgadillo et al. 2010). The reduced nitrogen is converted into nitrite (nitrification) and subsequently into nitrate (De Azevedo and Da Matta 2004; Cicerone et al. 2007; Newman and Clements 2008; Delgadillo et al. 2010).

Ammonium particularly inhibits the symbiotic fixation of nitrogen by microorganisms associated with plants, which are in close contact with water in the wetland environment (Newman and Clements 2008; Delgadillo et al. 2010). This process is indirectly affected by the increase in the cytoplasmic concentration of ammonium in the roots exceeding their assimilative capacity (Newman and Clements 2008; Delgadillo et al. 2010). The chemical compounds detected in the wetland at Playa Penino might be affecting the genome of the indicator species, which lives in burrows, in the immediate proximity of water, consumes the roots of the vegetation associated with the wetland, absorbing the sap and pollutants present especially in those species with a high water content (*Equisetum sp*., *Cynodon dactylon* and *Paspalum nicorae*).

In an aquatic environment, the presence of nitrogen in the form of ammonia is regulated by a chemical equilibrium that determines the coexistence of two forms: ammonia (NH_3) and ammonium ions (NH_4^+). The relative proportion of each is determined by pH, temperature and ionic strength (Delgadillo et al. 2010). The concentration of ammonia increases with increase in pH and temperature and decreases with increase in ionic strength; the ammonium ion predominates at pH values lower than 7 (Pepe and Lombardi 2003). The toxicity of ammonia for organisms is related to the non-ionized form (ammonia), mainly because it is highly soluble in lipids, which facilitates its passage through biological membranes, causing damage to respiratory surfaces. In fish, for example, the distal parts of the fins lose their original colour and turn white or transparent (Pepe and Lombardi 2003; Spiro and Stigliani 2004). Ammonium salts induce skin secretions and destroy gills (Pepe and Lombardi 2003; Spiro and Stigliani 2004). Relevant to this, the mass death of fish recorded in 2013 and 2015 in Playa Penino could have resulted from the process described above.

In the digestive system of mammals, nitrogen compounds (ammonium and nitrates) are transformed into nitrites by microbes. An excess of nitrite induces the formation of methaemoglobin (Khademikia et al. 2013), which blocks haemoglobin transporting oxygen. In addition, these nitrogen compounds (nitrates and nitrites) in water can be transformed into nitrosamines, whose teratogenic and carcinogenic potential is recognized (Camargo and Alonso 2006). As mentioned before, tuco tuco obtains water from the roots of the plants (raw sap, mostly from grass in this area), so they may be accumulating these compounds, as they are in the water and their metabolic transformation could occur later, in other parts of the plants. However, this needs to be tested under controlled conditions in the laboratory.

The ability of faecal bacteria to reproduce outside the intestine of mammals is favoured by suitable environmental conditions (Eaton and Franson 2005). Their presence is interpreted as an indication that pathogenic organisms may be present and their absence that the water or food is free of disease-producing organisms (Eaton and Franson 2005). Uruguayan ordinance 253/79

(MVOTMA 1979) establishes a maximum limit of 1000 cfu/100 ml for faecal coliform bacteria (FC) for class 3 water and a maximum limit of 500–1000 cfu/100 ml for class 2 water. The microbiological contamination by FC recorded in this study exceeded the guide levels for these classes of water, as well as for drinking water (according to national and international regulations) and are a risk even for the recreational use of water (Delgadillo et al. 2010). The values of FC recorded in this study were similar (May) or higher (October and February) to those recorded in sections of the Traiguén IX Region river in Chile (540 cfu/ml; 1100 cfu/ml; 920 cfu/ml) (Rivera et al. 2009). Pérez et al. (2004) report FC values for the water in Lake Izabal and Río Dulce, in Guatemala, which are lower than those recorded in this study (Lake Izabal 454 cfu/100 ml and Río Dulce 62 cfu/100 ml). These authors mention that these levels of contamination pose a health hazard for the population inhabiting the margins of these environments. Something similar occurs in the Playa Penino wetland, which is also used for irrigation, fishing and recreational uses by the surrounding population.

Pollution by both bacteria and chemicals pose a high risk to human health, affect the crops and reduce the availability and quality of fish, which are a source of food for communities where the incidence of poverty is high.

The multiple correlation analysis (MCA) revealed significant positive correlations between the concentration of ammonia and the presence of enterobacteria, and the genetic damage recorded in the blood cells of *Ctenomys*. Although positive these relationships do not prove there is causal connection between the genetic and environmental variables. It might indicate that the discharge of sewage has a negative effect on the wetland. This requires additional *in vivo* and *in vitro* studies.

Squatter settlements around Playa Penino along with the lack of an urban sanitation system has resulted in an increase in nutrients to above the maximum allowed by current national and international regulations, which has disturbed natural nutrient cycling.

Conservation implications

The results of this study might indicate the decrease in biodiversity at Playa Penino is due to an acute and chronic exposure of the genetic material of the organisms there to pollutants that in the long-term might reduce their fitness and survival.

An indicator species was used to detect a potentially dangerous situation associated with the presence and action of pollutants, which to some extent can be extrapolated to humans (Alonso and Tabor 2004; Den Besten and Munawar 2005; Bischoff et al. 2010; Fernández-Tajes et al. 2011). The extent of the genetic damage to individual cells measured using OTM and percentage of DNA damaged revealed acute and recent exposure to xenobiotics, which may be associated with the discharge of domestic wastewater. The MN test also revealed the chronic exposure of the animals showing genetic damage to clastogenic and/or aneugenic events (Mudry and Carballo 2006).

It is essential to maintain the ecosystem integrity of Playa Penino and ensure the protection of the health of-humans by a strict surveillance and control of septic tanks and design of an adequate sanitation system. The regularization of the squatter settlements through a housing plan that contemplates the socio-economic possibilities of the inhabitants, would reduce the pollutant load from sewage.

The restoration of the physical-chemical balances between the soil, water and sediment will also be beneficial, as is described in the GeoUruguay report (Martino et al. 2008).

REFERENCES

Aldabe J, Mejía P, Morena V (2009) Propuesta de proyecto de selección y delimitación del área Humedales del Santa Lucía para su ingreso al Sistema Nacional de Áreas Protegidas. MVOTMA. Uruguay.

Alonso A, Tabor G (2004) Special Section: Marine Sentinel Species. Introduction: Marine Vertebrates as Sentinels of Marine Ecosystem Health. Eco Health 1: 236–238.

Arballo E, Bresso A (2007) Reserva Natural Playa Penino. Fondo de las Américas, Intendencia Municipal de San José, DINAMA. Uruguay.

Arocena R, Chalar G, Fabián D, de León L, Brugnoli E, Silva M, Rodó E, Machado I, Pacheco J, Castigioni R, Gabito L (2008) Evaluación ecológica de aguas y biomonitoreo. DINAMA. Uruguay.

Ayla C, Oya O, Ulkü C (2005) The evaluation of micronucleus frequency by acridine orange fluorescent staining in peripheral blood of rats treated with lead acetate. Mutagenesis 20: 411–415.

Bischoff K, Priest H, Mount-Long A (2010) Animals as Sentinels for Human Lead Exposure: A Case Report. J Med Toxicol 6: 185–189.

Camargo J, Alonso A (2006) Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. Environ Int 32: 831–849.

Capriglione T, De Iorio S, Gay F, Capaldo A, Vaccaro M, Morescalchi M, Laforgia V (2011) Genotoxic effects of the fungicide ethiophanate-methylon *Podarcis sicula* assessed by micronucleus test, comet assay and chromosome analysis. Ecotoxicology 20: 885–891.

Cicerone D, Sánchez-Proaño P, Reich S (2007) Contaminación y medioambiente. Eudeba, Argentina.

Clemente B, Devaux A, Perrodin Y, Danjean M, Ghidini-fatus M (2004) Assessment of sediment ecotoxicity and genotoxicity in freshwater laboratory microcosms. Ecotoxicology 12: 323–333.

Comisión de Fomento Playa Penino (CFPP) (2013) Reserva Natural Playa Penino. Proyecto de Gestión Ambiental: Pobladores de Autódromo y Penino unidos en la restauración y recuperación ambiental de la faja costera aledaña a la Playa, como medida de mitigación y adaptación al Cambio Climático. MVOTMA/PPD/FMAM/PNUD, Uruguay.

da Silva J; Freitas T; Heuser V; Marinho J, Erdtmann B (2000) Genotoxicity biomonitoring in coal regions using wild rodent *Ctenomys torquatus* by comet assay and micronucleus test. Environ Mol Mutag 35: 270–278.

De Azevedo F, Da Matta A (2004) As bases toxicológicas da ecotoxicologia. Intertox. RIMA. Brasil.

De Lapuente J, Lourenço J, Mendo Sónia A, Borràs M, Martins M, Costa P, Pacheco M (2015) The Comet Assay and its applications in the field of ecotoxicology: a mature tool that continues to expand its perspectives. Front Genet 6: 180.

Delgadillo O, Camacho A, Pérez L, Andrade M (2010) Depuración de aguas residuales por medio de humedales artificiales. In: Durán N (ed) Centro Andino para la Gestión y Uso del Agua (Centro AGUA). Universidad Mayor de San Simón, Bolivia.

Den Besten J, Munawar M (2005) Ecotoxicological testing of marine and freshwater ecosystems: emerging techniques, trends, and strategies. CRC Press, Taylor and Francis Group.

Eaton A, Franson M (2005) Standard methods for the examination of water and wastewater. American Public Health Association.

Emmanouil C, Sheehan T, Chipmana J (2007) Macromolecule oxidation and DNA repair in mussel (*Mytilus edulis* L.) gill following exposure to Cd and Cr (VI). Aquat Toxicol 82: 27–35.

Fenech M (2007) Cytokinesis-block micronucleus cytome assay. Nat Protoc 2: 1084–1104.

Fernández-Tajes J, Flórez F, Pereira S, Rábade T, Laffon B, Méndez J (2011). Use of three bivalve species for biomonitoring a polluted estuarine environment. Environ Monit Assess 177: 289–300.

Festa F, Cristaldi M, Ieradi L, Moreno S, Cozzi R (2003) The comet assay for the detection of DNA damage in *Mus spretus* from Doñana National Park. Environ Res 91: 54–61.

Garriott M, Phelps J, Hoffman W (2002) A protocol for the in vitro micronucleus test. I. Contributions to the development of a protocol suitable for regulatory submissions from an examination of 16 chemicals with different mechanisms of action and different levels of activity. Mutat Res 517: 123–134.

Hartmann A, Agurell E, Beevers C, Brendler-Schwaab S, Burlinson B, Clay P, Collins A, Smith A, Speit G, Thybaud B, Tice R (2003) Recommendations for conducting the in vivo alkaline Comet assay. Mutagenesis 18: 45–51.

Heuser V, da Silva J, Moriske H, Dias J, Yoneama M, Freitas T (2002) Genotoxicity biomonitoring in regions exposed to vehicle emissions using the comet assay and the micronucleus test in native rodent *Ctenomys minutus*. Environ Mol Mutag 40: 227–235.

Khademikia S, Rafiee Z, Amin M, Poursafa P, Mansourian M, Modaberi A (2013) Association of nitrate, nitrite, and total organic carbon (TOC) in drinking water and gastrointestinal disease. J Environ Public Health, https://www.doi.org/10.1155/2013/603468.

Kopnina, N (2016) Forest Ecosystem Services Commodification of natural resources and forest ecosystem services: examining implications for forest protection. Environ Conserv, https://www.doi.org/10.1017/S0376892916000436.

Kopjar N, Mustafic P, Zanella D, Buj I, Caleta M, Marcic Z, Milic M, Zdravko D, Mrakovcic M (2008) Assessment of DNA integrity in erythrocytes of *Cobitis elongate* affected by water pollution: the alkaline comet assay study. Folia Zool 57: 120–130.

Kumar R, Nagpure N, Kushwaha B, Srivastava S, Lakra W (2010) Investigation of the genotoxicity of malathion to freshwater teleost fish *Channa punctatus* (Bloch), using the micronucleus test and comet assay. Arch Environ Contam Toxicol 58: 123–130.

Laws E (1993) Aquatic pollution: an introductory text. John Wiley, New York.

Lessa EP, Langguth A (1983) *Ctenomys pearsoni* n. sp. (Rodentia, Octodontidae) del Uruguay. Res. Comun. Jorn. Cienc. Nat 3: 86–88.

Luzhna L, Kathiria P; Kovalchuk O (2013) Micronuclei in genotoxicity assessment: from genetics to epigenetics and beyond. Front Genet 4: 131.

Machella N, Battino M, Pisanelli B, Regoli F (2006) Influence of the SCGE Protocol on the Amount of Basal DNA Damage Detected in the Mediterranean Mussel, *Mytilus galloprovincialis*. Environ Mol Mutagen 47: 579–586.

Márquez A, Senior W, Martínez G, González A (2007) Concentraciones de nitrógeno y fósforo en sedimentos recientes de la Laguna Los Patos, Estado Sucre, Venezuela. Bol Inst Oceanogr 46: 137–145.

Martino D, Buonomo M, Villalba C (2008) GeoUruguay: Informe del estado del ambiente. CLAES, PNUMA, DINAMA. Uruguay.

Ministerio de Vivienda, Ordenamiento Territorial y Medioambiente (MVOTMA) (2018) Plan de aguas urbanas, Ciudad del Plata, Uruguay.

Mohanty G, Mohanty J, Nayak A, Mohanty S, Dutta S (2011) Application of comet assay in the study of DNA damage and recovery in rohu (*Labeo rohita*) fingerlings after an exposure to phorate an organophosphate pesticide. Ecotoxicology 20: 283–292.

Mudry M, Carballo M (2006) Genética Toxicológica. Ed. De los 4 Vientos. Argentina.

MVOTMA (1979) Código de aguas. www.mvotma.gub.uy/decretos-ministerio/item/10010048-decreto-253-979.

Newman MC, Clements WH (2008) Ecotoxicology: a comprehensive treatment. CRC Press, Taylor and Francis Group, Boca Raton, London and New York.

Norppa H, Falck G (2003) What do human micronuclei contain? Mutagenesis. 18: 221–233.

Parolini M, Binelli A, Provini A (2011) Assessment of the potential cyto–genotoxicity of the nonsteroidal antiinflammatory drug (NSAID) diclofenac on the zebra mussel (*Dreissena polymorpha*). Water Air Soil Pollut 217: 589–601.

Pepe U, Lombardi M (2003) Tratamiento alternativo de efluentes cloacales individuales. 13° Congreso Argentino de saneamiento y medioambiente. Buenos Aires.

Pérez F, Oliva B, Herrera K, Juárez J, Galindo C (2004) Contaminación físicoquímica y bacteriológica del Río Dulce y lago de Izabal. Final report, Proj. DIGI-USAC, Guatemala.

Putz P (2010) Eliminación y determinación del fosfato. Hach Lange www.interempresas.net/Quimica.

Ribeiro L, Salvadori D, Marques E (2003) Mutagenese Ambiental. Ed. ULBRA. Brasil.

Rivera N, Encina F, Palma R, Mejías P (2009) La Calidad de las Aguas en el Curso Superior y Medio del Río Traiguén. IX Región-Chile. Información Tecnológica 20: 75–84.

Rocha G (1999) Playa Penino y la conservación de los hábitats costeros del Uruguay. Cotinga 11: 68–70.

Rodríguez R (2010). Influencia de los fármacos presentes en el agua residual sobre la resistencia de la bacteria *Escherichia coli* y su eliminación por oxidación avanzada. PhD Thesis, CONACYT, México.

Rodríguez A, Arpajón Y, Castaño Z, Marsán R, Lugo D, Roevros N, Chou L, Rojas N, Heydrich M (2006) Análisis de contaminantes inorgánicos tóxicos en las aguas del río Almendares, Cuba. ENMA, CONYMA.

Romero R (2006) Detergentes con o sin fosfatos. Técnica Industrial, España.

Sievers M, Hale R, Parris K, Swearer S (2017) Impacts of human induced environmental change in wetlands on aquatic animals. Biol Rev, https://www.doi.org/10.1111/brv.12358.

Spiro T, Stigliani W (2004) Química medioambiental. Pearson-Prentice HallPearson, Madrid.

Sponsel L (2014) Human impact on biodiversity: overview. In: Levin SA, Waltham MA (eds) Encyclopedia of Biodiversity. Academic Press. pp 137–152.

Yin X, Zhu G, Li X, Liu S (2009) Genotoxicity evaluation of chlorpyrifos to amphibian Chinese toad (Amphibian: Anura) by Comet assay and Micronucleus test. Mutat Res-Gen Tox En 680: 2–6.

Zalacain M, Sierrasesúmaga L, Patiño A (2005) El ensayo de micronúcleos como medida de inestabilidad genética inducida por agentes genotóxicos. Revisión 28, https://www.doi.org/10.4321/S1137-66272005000300007.

Zar J (2010) Biostatistical Analysis. Pearson Education. London.

LARGE SCALE MANIPULATION OF THE INTERACTIONS BETWEEN KEY ECOSYSTEM PROCESSES AT MULTIPLE SCALES: WHY AND HOW THE FALCON ARRAY OF ARTIFICIAL CATCHMENTS WAS BUILT

JAN FROUZ[1,2,*], MARTIN BARTUŠKA[1,2], JAN HOŠEK[3], JIŘÍ KUČERA[4], JIŘÍ LEITGEB[5], ZBYNĚK NOVÁK[6], MARTIN ŠANDA[7], and TOMÁŠ VITVAR[7]

[1] Charles University, Faculty of Science, Institute for Environmental Studies, Benátská 2, 12800, Praha 2, Czech Republic
[2] Biology Centre CAS, ISB & SoWa, Na Sádkách 7, 37005, České Budějovice, Czech Republic
[3] Ekologické služby, Tichá 784/4, 268 01 Hořovice, Czech Republic
[4] EMS Brno, Turistická 5, 62100 Brno, Czech Republic
[5] Arch 93, Majakovského 29, 360 05 Karlovy Vary – Rybáře, Czech Republic
[6] ENVItech Bohemia s.r.o., Ovocná 34, 161 00, Praha 6, Czech Republic
[7] Department of Landscape Water Conservation, Czech Technical University in Prague, Faculty of Civil Engineering, Thákurova 7, 166 29 Praha 6, Czech Republic
* Corresponding author: jan.frouz@natur.cuni.cz

ABSTRACT

Understanding how natural processes arise from complex interactions between particular processes at small spatiotemporal scales and in turn how these processes form patterns at large spatiotemporal scales is one of the current principal questions in environmental science. The problem is very complicated, as in many cases, key processes are often studied by researchers in separate disciplines such as ecology, soil science or hydrology. One of the major obstacles is that the processes at a landscape scale are difficult to manipulate and, in many cases, even measure. In particular, the belowground processes are in many cases overlooked or at least understudied. Here we briefly describe a methodological solution used to cope with this problem and describe artificial catchments designed for experimental manipulation at the level of a landscape, called FALCON. This array has two treatments: one mimics a site reclaimed using an alder plantation and the other was left to unassisted primary succession. For each treatment, there were two replicates in four similar catchments. Individual catchments are hydrologically isolated from the environment and equipped with instruments, so that all the main processes and all significant flows of substances and energy in the ecosystem can be monitored, including the cycling of water, nutrients and gas between the ecosystem and the atmosphere. In addition, in each catchment there are sets of lysimeters, which allow the study of small-scale processes and how these can be extrapolated to the catchment scale. In addition, two lysimetric fields exist alongside the catchments for monitoring the effects of the experimental manipulation.

Keywords: carbon storage; downscaling; energy budget; runoff; soil development; upscaling; water budget

Concept of large-scale manipulation experiments and how they can help us with upscaling and downscaling

Issues of scale are present in virtually all environmental disciplines (Wu et al. 2006). While natural processes are generally driven by hotspots and transport between hotspots, they are significantly affected by particular features at several spatiotemporal scales. For example, several previous hydrological studies (e.g. Klemeš 1983; Dooge 1986; Blöschl and Sivapalan 1995; Merz et al. 2009) reveal that small-scale processes and approaches cannot be easily extrapolated to large spatiotemporal scales. Similar examples involving the dynamics of organic matter in soil (O'Rourke et al. 2015) reveal that processes at the scale of particles and aggregates may be driven by factors that are different from those driving key processes at large scales. For example, establishment of tree seedlings in rain forest depends on their interaction with pathogens at a microscale and animals that transport their seeds at the landscape scale. The common feature of all these examples is that although processes at large scales are the product of small-scale processes, the complexity at large scales often cannot be predicted from that at small spatiotemporal scales, and in turn, processes and approaches at large scales cannot easily be downscaled. Understanding the interactions between the processes determining the overall output of major ecosystems, is therefore one of the key problems of modern environmental science. In a recent study, Blöschl et al. (2019) highlight the need to address issues of scale as one of the crucial tasks of hydrology. Similarly, scaling is an issue in various aspects of soil science (O'Rourke et al. 2015; Pachepsky and Hill 2017) ecology (Urban 2005) and many other environmental disciplines (Wu et al. 2006). Thinking about interactions between scales involves a related aspect, system boundaries. Boundaries are ubiquitous at all scales (Cadenasso et al. 2003). Large systems can often be reduced to a set of subsystems or units with natural boundaries, in respect to soil the horizons can be reduced to soil aggregates etc.

Frouz, J., Bartuška, M., Hošek, J., Kučera, J., Leitgeb, J., Novák, Z., Šanda, M., Vitvar, T.:
Large scale manipulation of the interactions between key ecosystem processes at multiple scales: why and how the falcon array of artificial catchments was built
European Journal of Environmental Sciences, Vol. 10, No. 1, pp. 51–60
https://doi.org/10.14712/23361964.2020.7
© 2020 The Authors. This is an open-access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Units defined by natural boundaries often have their own set of drivers that determine the processes in that unit, such as processes at the level of a pedon or soil aggregate, if one only considers soil (O'Rourke et al. 2015).

The complexity of processes across spatiotemporal scales also implies uncertainties in the observation and monitoring of processes. The assessment of large-scale processes is often based on several partial processes or indicators, from which the overall outcome is estimated using various statistical and modelling approaches. This is inevitable, because at some scales we cannot directly measure the whole process and instead measure the rate of a process, a sample or an indicator. These values, however, are biased not only by common measurement errors, but also by uncertainties about whether the sample or indicator includes the spatiotemporal variability of a given parameter, how well the given indicator describes the whole process or if all key processes are covered (Wu et al. 2006). This difficulty in determining the parameters at large scales is one of the reasons why it is difficult to make comparison across scales.

The most important way of testing hypotheses in environmental science is to use manipulation experiments. In this way, it is possible to control all key variables, keep most of them constant, and manipulate one or few. This allows the testing of a hypothesis about the effect of a particular factor. However, there is no guarantee that the factor manipulated affects the process in the real world. In contrast, formulating hypotheses based on the observation of individual factors is dependent on how they correspond with reality. However, one can never be sure if the observed pattern is not driven by interactions with factors not observed in the study. Manipulation experiments mostly involve well controlled small-scale "bottle experiments" (Kareiva 1989). Many of the basic theoretical concepts of environmental science are based on experiments done at micro and mesoscales. However, as explained earlier, upscaling from understanding at a small scale is difficult and, in many cases, hardly possible due to the great complexity at large scales. In addition, small-scale processes are often modulated in large-scale settings. That is, a particular small-scale manipulation experiment often mimics a particular large-scale setting, which limits the generality of these experiments. One way of overcoming the limitations of small-scale manipulation and the observation approach may be by using controlled manipulation experiments at a large scale (Lawton 1996) or even at a landscape scale. There are numerous manipulation experiments at large scales in the literature, for example, manipulation of patch quality and disturbance of connectivity and other properties (Jenerette and Schen 2012). While these manipulation experiments may provide some understanding of what happens at large scales, they are seldom designed to explore the interaction between processes at various spatiotemporal scales or those between various domains such as soil, vegetation etc. A way forward is a transplant experiment in which a small part of an ecosystem is placed in another context (Jenerette and Schen 2012). These experiments indicate how small-scale processes are modified at large scales.

Promising methods for exploring the interaction of processes across scales include a combination of large-scale manipulation with observation, and/or manipulation of the conditions at the smallest spatiotemporal scales, as in catchment-scale manipulation experiments (Cosby et al. 1996). These experiments address the changes that occur in the whole catchment in response to experimental manipulation. This can be accompanied by detailed studies and/or manipulation experiments at small spatiotemporal scales.

As already mentioned, concept of scales is associated with boundaries (Cadenasso et al. 2003). At each spatial scale, there are parts of the system that are natural units with well-defined natural boundaries. An example of such well-defined units, which are a part of large system, are individual trees in a forest, soil aggregates in a particular soil layer or, at a landscape scale, catchments that are a natural unit of water movement in the landscape etc. On the other hand, some components of an ecosystem are more "continuous" and definition of boundaries are then somewhat arbitrary (Cadenasso et al. 2003). When experimenting, we can define the experimental unit. We may use natural units, with natural boundaries, such as individual trees, or arbitrarily set boundaries, such as an area of forest with several trees. This choice will depend on the question, method and other factors, such as the tradition of a particular field of research. This choice has many consequences. Some research questions are best addressed using individual, natural units, others using arbitrary boundaries. Moreover, individual units (trees) in a forest may be very variable. This variability is likely to mask the effect of the treatment. We may choose similar trees, but then the result applies only to trees with similar attributes. We may also study groups of trees and choose a size of group that includes a particular range of variation of the natural unit (in each group, trees will be variable, and may even include the range of variability occurring in the forest). However, at the same time, we make sure that variability of our arbitrary chosen unit (group of trees) is low. Often, we use a combination of both, natural and arbitrary boundaries; for example, we may use a natural boundary of one plant as an experimental unit, but arbitrarily define boundary by the plot in which the plant is grown. Boundaries can also be manipulated. Many landscape-scale experiments involve manipulating patch size or spatial arrangement etc. (Jenerette and Schen 2012), which is done by manipulating boundaries.

Studies of ecosystems often involve interactions between various processes, namely energy flow and the cycling of water, carbon and other nutrients, which are closely interlinked (Raffaelli 2010). Despite the close linkage of some of the key processes, for which it is easy to find natural units at the landscape scale, for others it is more practical to work with arbitrary units. Catchment-level experiments (Cosby et al. 1996) typically include a natural unit, the water cycle, so suspended or dissolved matter can be quantified and budgeted for. Manipulation experiments at a catchment scale are suitable, when focussing on water and nutrient budgets at the landscape scale. Manipulating natural catchments is particularly difficult, as it is impossible to replicate the experiments. This means that the natural variability within a catchment and between catchments masks the effect of the manipulation. On the other hand, studies on primary production and soil C storage in forests consisting of different species of trees may be replicated by planting blocks of different species of trees side by side in the form of a common garden experiment. This will enable, after some time, the determination of the biomass produced in individual blocks, the properties of the soil in the blocks with different species of trees, and if the initial soil conditions are known, how much of certain elements, such as soil C, was sequestered, or lost (Vesterdal et al. 1998; Frouz et al. 2013). The advantage of this approach is that it is possible to choose areas that are relatively homogeneous for planting the trees, which will result in very good statistical power, when evaluating the effect of the treatments on the target variable, such as C storage in plant biomass or C storage in soil. It may also be possible to manipulate or record what happens in these large-scale treatments, in order to determine, how it corresponds with processes at smaller spatial scales (Frouz et al. 2013). However, when linking the effect of individual treatments in common garden experiments to water movement in a landscape, direct budgeting would be extremely difficult. It would be necessary, therefore, to study individual components of the water cycle or their indirect indicators and try to upscale budgeting by modelling bearing in mind all the uncertainties, such an approach entail.

Concept of manipulation experiments using artificial catchments

Many of the drawbacks of natural catchments arise from their natural variability and/or peculiarities, which make the direct measurement of some components difficult to budget for, such as, subsurface water flow. These drawbacks can be overcome by using artificial catchments, which are built in a way that reduces spatial heterogeneity and allows a more precise budget for water at a large scale. These artificial catchments have been used for several decades to help conceptualize the runoff pathways on small hillslopes. These studies opened the way for the development of a variety of new concepts namely in hydrology and runoff, on natural or artificial hillslopes (Laine-Kaulio et al. 2014; Gabrieli and McDonnell 2018). The new generation of artificial and/or artificially managed catchments were developed to address complex processes between atmosphere, vegetation, soil and water, known as the "critical zone". The pioneering artificial catchments in this context are the Hydrohill catchment in China (Kendall et al. 2001) and the Chicken Creek catchment in Germany (Gerwin et al. 2009), which form a part of the worldwide Critical Zone Exploration Network (Lin et al. 2011; Brantley et al. 2017). The grassland hillslope Hydrohill catchment (490 m^2) was established in order to compare the artificial Hydrohill with the adjacent natural forested micro-catchment Nandadish within the Chuzhou Hydrology Laboratory (Gu et al. 2018) and in particular to constrain the principal surface and near subsurface runoff on hillslopes (Beven 2012; van Verseveld et al. 2017). In contrast to the vegetated Hydrohill, the Chicken Creek catchment (6 ha) built in 2005 is a landscape designed to determine the initial phase of ecosystem evolution. The Chicken Creek catchment is an attempt to link various disciplines in ecology and environmental sciences, including botany, zoology, hydrology and soil science. Studies on these artificial catchments have resulted in substantial progress in the development of new concepts in hydrology, ecosystem ecology and other related fields in aspects, such as runoff, erosion or relationship between ecosystem development, water budget and other ecosystem properties.

Artificial catchments are systems that are easy to monitor, which makes it easier to determine water and solute flow. However, individual artificial catchments are nevertheless case studies similar to those on natural catchments described above. However, a combination of artificial catchments and large-scale experimental manipulations (e.g. common garden approach) can overcome this. By developing several artificial catchments in the same manner side by side, will enable treatments to be replicated (e.g., manage plots differently or plant them with different species of trees) and at the same time accurately determine all key ecosystem processes, namely energy flow and cycles of water, carbon and other nutrients. We can study the response of these processes to different treatments and at the same time, using small-scale observations or manipulation can downscale and observe mechanisms driving these processes at small spatial scales. This will enable the development of new ways of studying the connection between key ecosystem processes, such as soil C sequestration, water storage and many others.

This is similar to the various recently developed ecotrone facilities, which consist of sets of mesocosms in the form of large lysimeters. The conditions in these facilities can be manipulated, but at a much smaller scale than in open-air artificial catchments. The largest lysimeter includes shrubs or even small trees. A big advantage of these facilities is that they are highly controlled and the treatments can be replicated. A disadvantage is their relatively small size (several tens of m^2 containing around 10 tons of soil) (Barry et al. 2019; Rosher et al. 2019) compared to the above-mentioned artificial catchments of 10^2–10^4 m^2. Their small size means they cannot be used for studying even small-scale landscape processes, such as runoff, erosion and sedimentation, or competition between woody species etc.

Another complementary way of quantifying landscape ecosystem processes at the scale of at least 10^2–10^4 m^2 is the eddy covariance method for assessing the exchange of gases (CO_2, water vapour and methane) between ecosystems and the atmosphere (Baldocchi et al. 2003). Thus, the next logical step could be a replicated manipulation experiment consisting of several adjacent artificial catchments, designed for studying the exchange of gases between ecosystems and the atmosphere.

An example of application of manipulation experiments using experimental catchments

As explained above, using hydrologically isolated artificial catchments as a unit in a large-scale manipulation experiment brings many advantages in terms of linking various ecosystem processes at large spatiotemporal scales. However, it is difficult to carry out a replicated manipulation experiment that includes more than one artificial catchment. This is because of the necessity to balance the number of units with the number of treatments and replicates. Based on the processes that should be assessed, the smallest size of an artificial catchment needs to be close to existing experimental natural catchments, which is 10^2 m^2 (Gu et al. 2018). This is sufficient for using the eddy covariance technique, in which one tower serves two neighbouring systems with the possibility of including additional units. The large size of this unit also indicates it would be useful for exploring processes at large spatial scales. Consequently, this setup will be more suitable for studies over long periods. This is even more enhanced by the complexity of the construction, during which isolating the catchment hydrologically involves excavating large quantities of soil and backfilling or dumping it elsewhere. It is therefore an advantage if the excavation and backfilling or spreading the soil mimics processes that occur in a natural catchment and can be part of the experiment. In the past, experimental sites in previously mined areas such as Chicken Creek, were used to explore the early stages in the development of an ecosystem. Because of the complexity, manipulation experiments can only include a limited number of treatments. Here we propose a meaningful scientific question that can be addressed using two treatments. In addition, we look for treatments that are similar to an existing manipulation experiment, so we can benefit from pre-existing knowledge in formulating a hypothesis. Practical applications of the results of manipulation experiments are a useful asset. The general question proposed is how the growing of N fixing plants affects ecosystem development differently compared to unassisted ecosystem development. As the planting of nitrogen-fixing plants is a popular reclamation strategy, this also has practical implications. Nitrogen is the most limiting nutrient in temperate ecosystems (Vitousek and Howarth 1991). This is particularly true for sites in the early stages of primary succession, including post-mining sites. That is why planting N_2-fixing trees is often recommended to speed up ecosystem development (Parkinson 1978; Mikola et al. 1983). Exploring the effect of increasing nitrogen in an ecosystem is likely to result in a better understanding of the recent global shift in ecosystem behaviour that has recently resulted in an increase in the availability of nitrogen. After global climate change, the addition of nitrogen to ecosystems has resulted in is the next-largest effect of man on world ecosystems. Over the past few decades, by various measures, such as anthropogenic N fixation, burning of fossil fuel and promoting N-fixing plants, man has substantially increased N input into ecosystems. (Vitousek and Howarth 1991) The consequences of this increased N input include substantially increased primary productivity, promotion of fast-growing tall species of plants, reduction of plant diversity, soil acidification, affected the mycorrhiza association and – site specifically – lower C sequestration in soil. Understanding how the addition of N-fixing plants alters the long-term development of ecosystems, compared to unassisted primary succession is a question that relates to many key issues in ecosystem ecology, soil science and other related disciplines.

Another reason for the choice of this experimental system is that well-studied Chrono sequences of reclaimed alder plantations and unreclaimed sites are available near the experimental catchment. Open cast mines often operate for decades. During that time, they generate very similar sites of the same sort of substrate using similar technologies. These sites are ideal for studying ecosystem development using Chrono sequences. A Chrono sequence is a set of similar sites with the same trajectory of development. Extensively studied Chrono sequences are available for post mining sites, which were either reclaimed by planting alder or left to unassisted ecosystem development. These Chrono sequences reveal that sites with different N inputs differ substantially in terms of soil organic matter storage and soil profile development (Frouz et al. 2001; Frouz and Nováková 2005; Šourková et al 2005; Frouz and Kalčík 2006; Frouz et al. 2008; Frouz et al. 2013) (Fig. 1). These differences result from complex interactions between plant production, plant litter inputs and soil biota (Frouz et al. 2007, 2008, 2013). Sites with nitrogen-fixing plants harbour a richer soil fauna, namely more earthworms. Earthworms, due to intensive bioturbation, incorporate plant litter into soil, which results in a faster initial soil C storage and formation of the organo-mineral A horizon in the soil. In contrast, at unreclaimed sites initial soil formation is slow, earthworms are absent, soil fauna caused bioturbation is weak and most litter accumulates in a litter and fermentation (Oe) layer. It has been also reported that these interactions

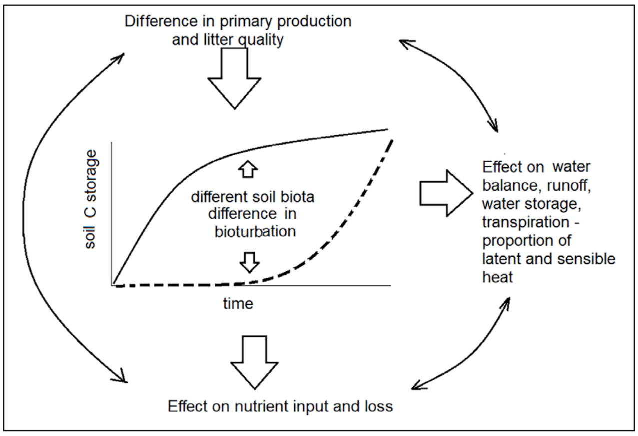

Fig. 1 Scheme of major interactions, between key ecosystem components, which will be studied using FALCON array of artificial catchments. In the middle there is schematic graph of expected dynamics of soil C storage in the catchment based on previous work on chronosequences of similar sites in vicinity (Šourková et al. 2005; Frouz and Kalčík 2006). In this diagram reclaimed alder plantation are represented by solid line, unreclaimed sites with succession are represented by dashed line.

affect the development of plant communities (Frouz et al. 2008). Moreover, plants that increase the amount of organic matter in soil produce soils that have a high water field capacity and are able to store more water (Frouz 2018). At a mesoscale, it is reported that bioturbation affects soil porosity. Availability of light and nitrogen affects understory vegetation and this again affects the earthworm community (Roubíčková and Frouz 2014). In addition to soil carbon storage, earthworms affect soil conditions, nutrient availability, interaction between arbuscular mycorrhiza and ectomycorrhiza, which also affect plant communities (Frouz et al. 2008). Consequently, this model offers numerous possibilities for exploring the interaction between traits of dominant plants, soil and ecosystem carbon storage and the water budget. This triangle of interactions needs to be understood in order to be able to effectively address ongoing climate change by manipulating land use. Despite many studies of the individual linkages (Frouz et al. 2007, 2008, 2013; Cejpek et al. 2018) the complexity of interactions is largely underexplored. Moreover, this triangle of interactions is closely linked to other processes, such as plant primary production, which drives litter input, nutrient dynamics, soil biota developments etc. Because primary production and C storage is so different at reclaimed and unreclaimed sites (Fig. 1), it should be possible to determine the effect of different soil C sequestration on different key ecosystem properties and partition, using statistics and modelling, how individual small-scale processes contribute to large-scale patterns. Then, we will be able to manipulate them at a microscale and so test our hypothesis. We believe that the ability to link precise budgeting and detailed observation in two-contrasting treatments over time at a large scale and by small-scale experimental manipulation will help us link key ecosystem processes across scales.

These Chrono sequence studies also indicate that the observed effects at large spatiotemporal scales, such as runoff, water storage and carbon storage, are outcomes of processes that occur at the level of soil aggregates or between them, further interacting with various processes at the mesoscale. One of the key objectives of the FALCON project is therefore to understand how these small-scale processes interact and form large-scale patterns. One of the tools that should help us answer this question is a set of small lysimeters embedded in the catchment or in its buffer area. About half of them will be used for monitoring how an observation at a small-scale relates to the large-scale picture and the other half can be used for experimental manipulation. In addition, it is likely that a set of mesoscale facilities will be developed in the future for markedly manipulating key factors, such as, the traits of dominant species of trees.

The experimental catchment FALCON

Site description

The FALCON experimental catchment was established on a post-mining site near Sokolov (Czech Republic). The name Sokolov (in local language) is derived from that of a bird of prey – a falcon. It complements the Hydrohill and Chicken Creek catchments, the only two currently monitored artificial catchments worldwide. Unlike the existing experimental catchments, which were designed for particular case studies, there are two treatments in FALCON catchment that mimic a reclaimed alder plantation and unreclaimed site left to primary succession, respectively. There are two replicates of each treatment resulting in four parallel catchment plots. They are hydrologically isolated from the environment and equipped with instruments to monitor the main ecosystem processes: the cycling of water, nutrients and gas between the ecosystem and the atmosphere, that is, all the significant fluxes in the ecosystem. The parameters monitored include rainfall, surface and subsurface outflow, chemical and isotopic composition of precipitation, pore water and runoff components at a high spatial resolution. Sets of soil lysimeters in each plot will measure the small-scale water balance, which can be extrapolated to the scale of the entire experimental catchment. Two lysimetric fields are located outside the catchment area, which can be used for manipulating the rainfall regime and composition of the aggregate.

The catchment is located at a post mining site near Sokolov, Czech Republic (50.2218908N, 12.7071839E), which is part of LTER Sokolov post-mining ecosystems in an area owned by Sokolovská Uhelná, a.s. Mean annual precipitation is 650 mm, mean annual temperature 6.8 °C and altitude 428 m a.s.l. The installation is located on an inner spoil tip of overburden from the open cast mine Jiří, which consists of Miocene sediments that contain up to 70% clay, in which the dominant minerals are

Kaolinite, Illite and Montmorillonite. The clay, however, is impregnated with carbonates (calcite and siderite) and fossil organic matter (kerogen) and in the tip forms large solid blocks (mudstones) that over time break down into smaller pieces the size of sand or gravel. Initially the clay is slightly alkaline, pH ~8. The catchment area is located on a gentle slope (~0.6%) facing southwest. Previous research in this area indicates that plants grow well on this material at reclaimed sites and those left to natural succession (Frouz et al. 2001, 2008).

Experimental setup

The whole array consists of four separate catchments 40 m wide and 60 m long, located side-by-side (Fig. 2) and facing northwest with the longer axis in a southeast-northwest direction. The catchments are surrounded in the north and west by a channel that drains the individual catchments and prevents water flowing from the upper parts of the slope into the catchments. Another drainage channel prevents water inflow from the upper parts of the hill and is located on the southeast side of the area. Individual catchments include small channels that prevent surface inflow from upper parts of the catchment area. The boundary of each partial catchment is sealed by compacted clay that acts as an insulator of very low saturated hydraulic conductivity due to artificial compaction ($K_s = 1.1 \times 10^{-10}$ m s^{-1}), which is 5–10 cm thick at the bottom and along the sides of the catchments. This pool was then backfilled with broken claystone over the sealant layer, which is initially likely to increase the hydraulic conductivity (as high as $K_s = 10^{-4}$ m s^{-1}), which will then gradually decrease over time to values typical for consolidated clays in this region (K_s at the range of 10^{-5} to 10^{-7} m s^{-1}; Cejpek et al. 2018). Each catchment drains to the southwest, where there is a water gate measuring the subsurface outflow from the catchment. Surface water is collected in a concrete ditch with a water gate to measure surface runoff, which then enters the main channel by which water leaves the catchment (Fig. 2 and 3). Surface of each catchment as well as bottom of the sealing layer is shaped like an open book, being lowest close to the centre of the catchment and highest at the edges, with an inclination similar to that of the slope of the catchment. This generates a flow of surface and subsurface water towards the central part of the western edge of the catchment where the outlets for surface and subsurface water are located. For construction reasons, the deepest point for surface and subsurface water flow are not exactly above each other, but are about 3 m apart along the western edge.

The different stages in the construction of a catchment are visible in Fig. 4, starting with the excavation and sealing of the area, installing drainage, backfilling and reshaping ten areas, and installing a surface drainage system (Fig. 3). This enables the measuring of surface and subsurface runoff in four separate catchments. Two of the catchments were levelled and planted with alder (c. 10 000 seedlings per ha) as in other parts of the area. The other two were made to look like spoil tips and left to natural succession. The two levelled and two spoil tips are located side by side (Fig. 2) and the eddy tower is located in the northeast corner of the two catchments. The positions of the catchments and tower are arranged so that a diagonal line across the two-neighbouring catchments (i.e. two level or two curved catchments) is more than 100 m with over 90 m of target habitat facing in the direction of the prevailing wind. We modified the gap between the two catchments, so that it has the same vegetation as the catchments and will be included in eddy measurement, although not a part of the catchment per se. The equipment installed in the catchment is listed in Table 1 and its position depicted in Fig. 2. Below we describe in more detail the instrumentation that will be used to measure water flow, radiation and carbon storage and other processes in the catchments.

Table 1 Instrumentation.

Parameters measured	number of units
Surface runoff + water samples	one per catchment
Subsurface runoff + water samples	one per catchment
Subsurface water level and temperature	10 per catchment
Soil moisture and temperature at depths of 20 and 80 cm	10 per catchment
Observation shafts	7 per catchment
Vacuum ceramic cups	5 per catchment
Percolation lysimeters	6 per catchment
Bucket lysimeters	10 per catchment
Global radiation	one per two catchments
Meteorological station	one per catchment
Rainfall collector	10 per catchment
Eddy tower	one per two catchments
Soil respiration multiplexor	one per two catchments

Monitoring of key ecosystem processes

The equipment will be used to quantify the water (and matter) fluxes (including dissolved or suspended material) between the catchments and their surroundings and simultaneously quantify the distribution and quality of the water in different parts of the catchments. The input via atmospheric deposition is measured using precipitation (rain and snow) and deposition samplers (used for determining the chemistry of solutions) and automatic rain gauges for quantifying the volume. Soil solution is sampled by three types of lysimeters.

Surface runoff is collected in shallow concrete channels that come together at the end of the catchment and discharge into a reservoir where it is measured using a Thomson type overflow weir. The water level is continually monitored by an ultrasonic sensor. The outflow is calculated using an experimentally derived rating curve (height of water level/discharge), with a measurement

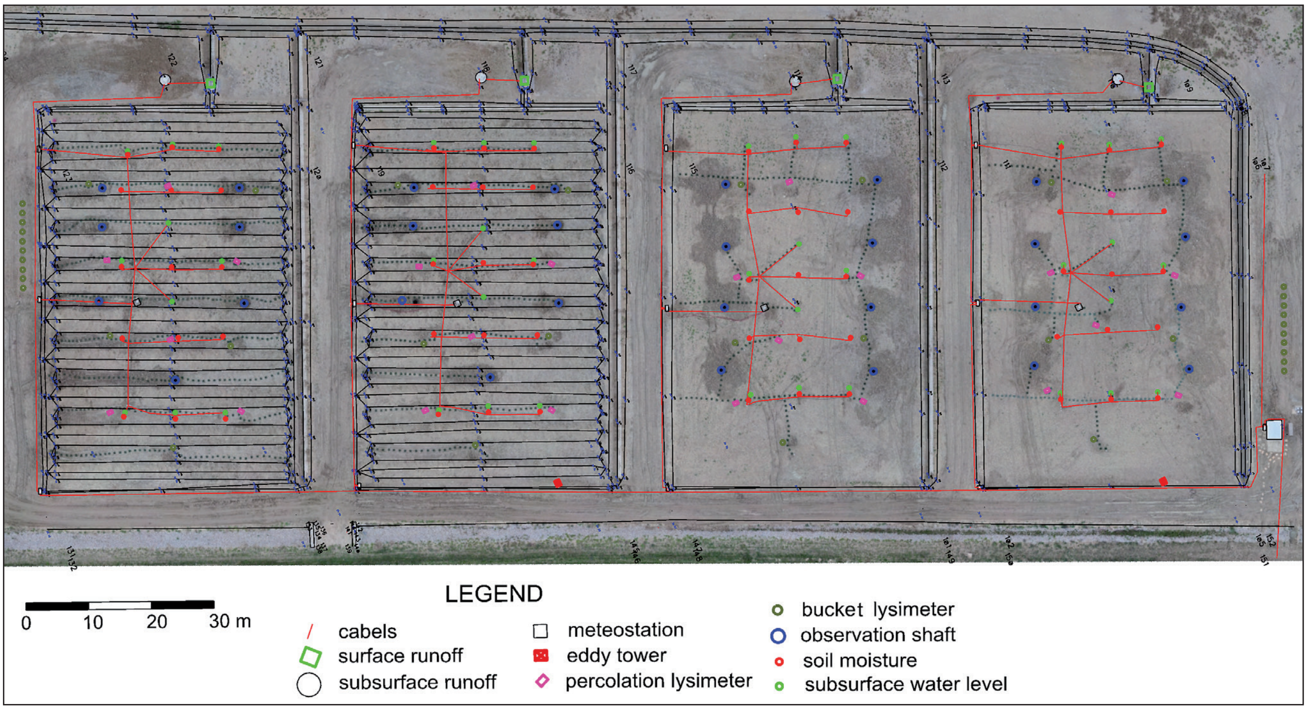

Fig. 2 Aerial photo of whole catchment array and location of instrumentation in one of the catchments.

range between 0.02 and 40 l s^{-1}. The recorded measurements are stored in a local data logger and simultaneously sent to the central data store. The surface outflow is also sampled using the automatic sampler HACH AS950, which samples a volume that is proportional to the quantity of surface outflow over a particular interval of time. As there are several sample bottles it is possible to collect surface outflow over particular periods or even collect several (separate) samples for a particular period. All this equipment is located in the underground shaft next to the overflow weir.

To measure subsurface runoff, the outflow from the bottom of the catchment passes through the boundary wall of the catchment via plastic pipes to the reservoir of Thomson's weir where it is measured. The level is continually monitored by an ultrasonic sensor and the outflow calculated as above, but in this case with a measurement range of between 0.01 and 13 l s^{-1} and stored on the data logger. The drainage outflow is sampled by an electronically controlled solenoid valve, which collects samples at user-specified intervals, as described above by equipment in the underground shaft (Fig. 5).

Soil water measurements and sampling. Soil solution is monitored and sampled using three different types of lysimeter:

1. Gravitation lysimeters (pan type) collect water mainly from large soil pores when it is saturated following rain and snowmelt. We used flat square shaped plastic lysimeters filled with inert quartz sand, with a capture area of 471.5 cm^2, which drain into an underground container in a plastic box. Depth of installation is 20 cm.
2. Suction lysimeters collect capillary water in between soil particles. We used the approved Teflon coated quartz vacuum lysimeter Prenart. Suction cups are connected by tubing to bottles that are regularly emptied using a vacuum pump. Standard depth of installation is 20 cm at selected points along with a second one at a depth of 60 cm.
3. Balance lysimeters measure different components of the soil solution by sampling it after percolating through soil with a well-defined surface area. It consists of two plastic cylindrical containers. Outer container is a watertight protective case permanently fixed in the ground. The inner container is placed inside this

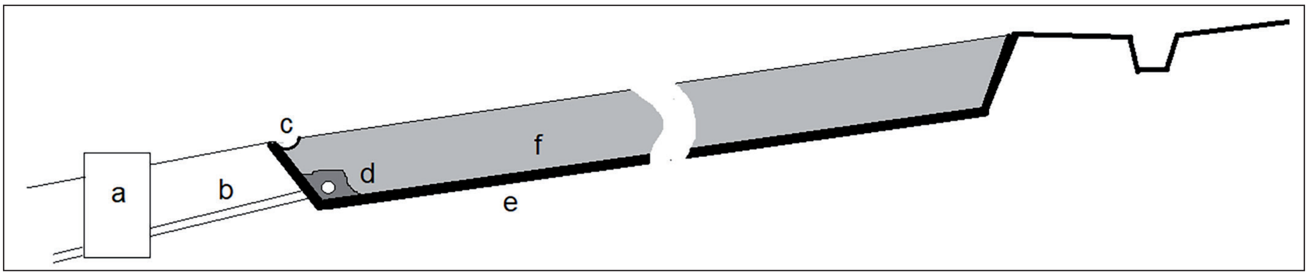

Fig. 3 Scheme of the individual catchment transection: a) measurement shaft for subsurface runoff measurement (see Fig. 4 for details), b) pipe bringing subsurface runoff from drainage system, c) surface runoff collecting ditch, d) drainage, e) clay sealing, f) backfill.

Fig. 4 Key steps of catchment construction a) excavation, b,c) construction of sealing, d) construction of drainage system, e) detail of surface runoff collecting ditch, f) overall view on finished array.

case and filled with soil. In this way, the percolating soil solution is filtered and subsequently collected and stored in the tank. The device is constructed, so that the inner container can be weighted using a portable balance and samples of the soil solution collected. The surface area of the lysimeter is 688 cm^2 and the depth in the soil 60 cm and it can weight changes with an accuracy equivalent to a rainfall of 1 to 2 mm. Soil Moisture sensors (Campbell) regularly measure soil moisture at depths of 20 and 80 cm in grid covering the catchment (Fig. 2). Wells with a pressure transducer in a stainless-steel case regularly located in the grid covering the catchment are used for water-level measurements.

To monitor the exchange of CO_2 and H_2O between the catchment and surrounding atmosphere, two adja-

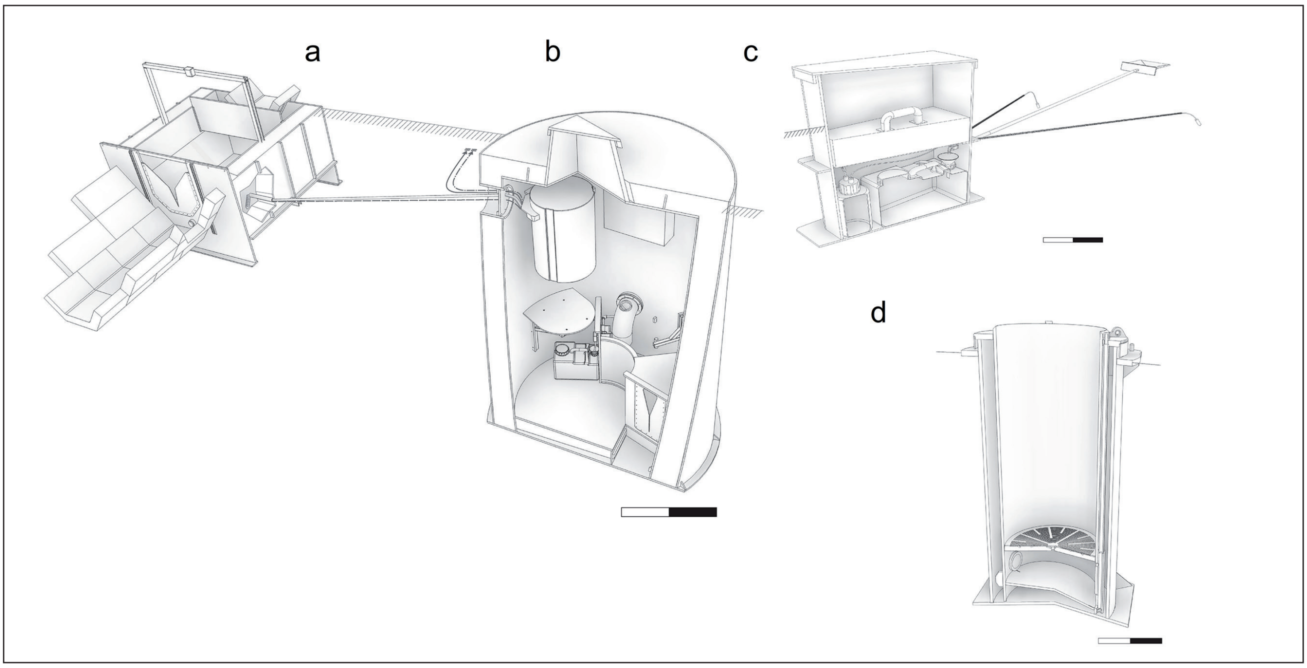

Fig. 5 Major devices used for water monitoring: a) device for surface runoff measurement, b) shaft for subsurface measurement, c) gravitation and suction lysimeters, d) balance (bucked) lysimeters.

cent of the catchment will be grouped in pairs in order to obtain a sufficient area for eddy covariance measurements, as explained above. Based on previous experience and preliminary measurements of wind direction, the tower was positioned so that it captured the prevailing winds coming from the basin. This will allow the total CO_2 exchange between the ecosystem and the atmosphere to be quantified. The eddy tower is equipped with a LI-7500DS Open Path Analyzer and Gill WindMaster anemometer. At the same time, eight fixed sites (2 in each river basin) will be installed to measure soil respiration using a LI-8100A Automated Soil Gas Flux System. Each pair of catchments is equipped with a unit for measuring total radiation and radiation balance.

In addition to equipment for monitoring water flow and gas exchange, an access shaft to a rhizotron will be located in each catchment. These are plastic shafts equipped with observation windows and predestined places, where it is possible to install additional devices. This will allow the easy and non-disturbing installation of virtually any device for monitoring soil development and nutrient flow. Each shaft is equipped with two observation windows (10 cm in diameter), located at depths of 0–10 cm and 20–30 cm for monitoring root growth and soil profile development. Data collected by loggers are stored in a central data depository in the catchment area, which is equipped with in-house software the universal measuring and evaluating system for environmental measurements WIN-I-MAG. The basis of which is time proven architecture for measuring and evaluating ENVItech systems, which are very reliable even under the most demanding conditions. The kernel of the system consists of a Run-time system that ensures parallel multi-tasking of several processes: measuring, archiving, processing, data export and various pictorial and printed outputs. All this is done in real time, without loss of data. Edges of each catchment are permanently marked by points that make it easier to monitor the catchment from drones using remote sensing. A network of erosion points is located in each catchment. Five permanent plots of vegetation and five neighbouring plots are available for collecting soil samples and following the development of the vegetation, soil chemical properties and soil biota.

Cooperative possibilities

This facility was built by a consortium led by the Biology Centre, which consists of Charles University, South Bohemian University and Czech Geological Survey, in addition to members of many other institutions, among which the Czech Technical University and Brandenburg Technical University are particularly noteworthy in terms of the planning and operation of the array. This facility was designed as an open access facility. Researchers who want to use the array to answer specific scientific questions, using various methods, including the use of existing data, measurement of additional parameters, lysimeter experimental manipulation, modelling approaches or any other research that might benefit from the existing array are warmly welcome. Interested researchers should contact the first author of this article to discuss their particular project.

Acknowledgements

FALCON catchment was built with the support of the project EF16_013/0001782, Research of key soil-water ecosystem interactions at the SoWa Research Infrastructure, Initial monitoring of the catchment was partly supported by project 8I20001 (both awarded by Ministry of Education Youth and Sport). Catchment planning, construction and operation received significant material and logistic support from Sokolovská Uhelná a.s. coal mining company. Arch 93 atelier did excellent work in planning the technical details of the catchment array. The principal contractor of the work was ENVITECH, other key sub-contractors who contributed substantially to the success of the project were Rekultivační výstavba Most, EMS Brno and Ekologické služby.

REFERENCES

Baldocchi DD (2003) Assessing the eddy covariance technique for evaluating carbon dioxide exchange rates of ecosystems: past, present and future. Glob Chang Biol 9: 479–492.

Barry KE, Mommer L, Ruijven J, Wirth C, Wright A et al. (2019) The Future of Complementarity: Disentangling Causes from Consequences. Trends Ecol Evol 34: 167–180.

Beven K (2012) Rainfall–Runoff Modelling: The Primer. 2nd ed John Wiley and Sons, Chichester, UK.

Blöschl G, Bierkens MFP, Chambel A, Cudennec C, Destouni G, Fiori A Zhang Y (2019) Twenty-three Unsolved Problems in Hydrology (UPH): a community perspective. Hydrol Sci J 64: 1141–1158.

Blöschl G, Sivapalan M (1995) Scale issues in hydrological modeling: a review. Hydrol Process 9: 251–290, https://www.doi.org/10.1002/hyp.3360090305.

Brantley SL, McDowell WH, Dietrich WE, White TS, Kumar P, Anderson SP, Chorover J, Lohse KA, Bales RC, Richter DD, Grant G, Gaillardet J (2017) Designing a network of critical zone observatories to explore the living skin of the terrestrial Earth. Earth Surf Dyn 5: 841–860.

Cadenasso M, Pickett STA, Weathers KC, Jones CG (2003) A Framework for a Theory of Ecological Boundaries. BioScience 53: 750–758.

Cejpek J, Kuráž V, Vindušková O, Frouz J (2018) Water regime of reclaimed and unreclaimed post-mining sites. Ecohydrology 11: e1911.

Cosby BJ, Norton SA, S. Kahl JS (1996) Using a paired-catchment manipulation experiment to evaluate a catchment-scale biogeochemical model. Sci Total Environ 183: 49–66.

Dooge JC (1986) Scale problems in hydrology, Kiesel Memorial Lecture, University of Arizona, February 1986. Reprinted in: Buras (ed) Reflections in hydrology. Washington, DC, American Geophysical Union, 1997, pp 84–145.

Frouz J (2018) Changes of Water Budget during Ecosystem Development in Post-Mining Sites at Various Spatiotemporal Scales: The Need for Controlled Systems. In: Liu J-F, Gu W-Z (eds) Hydrology of Artificial and Controlled Experiments. IntechOpen.

Frouz J, Elhottová D, Pižl V, Tajovský K, Šourková M, Picek T, Malý S (2007) The effect of litter quality and soil faunal composition on organic matter dynamics in post-mining soil: A laboratory study. Appl Soil Ecol 37: 72–80.

Frouz J, Kalčík J (2006) Accumulation of soil organic carbon in relation to other soil characteristic during spontaneous succession in non reclaimed colliery spoil heaps after brown coal mining near Sokolov (the Czech Republic). Ekologia 25: 388–397.

Frouz J, Keplin B, Pižl V, Tajovský K, Starý J, Lukešová A, Nováková A, Balík V, Háněl L, Materna J, Düker C, Chalupský J, Rusek J, Heinkele T (2001) Soil biota and upper soil layers development in two contrasting post-mining chronosequences. Ecol Eng 17: 275–284.

Frouz J, Livečková M, Albrechtová J, Chroňáková A, Cajthaml T, Pižl V, Háněl L, Starý J, Baldrian P, Lhotáková Z, Šimáčková H, Cepáková Š (2013) Is the effect of trees on soil properties mediated by soil fauna? A case study from post-mining sites. For Ecol Manage 309: 87–95.

Frouz J, Nováková A (2005) Development of soil microbial properties in topsoil layer during spontaneous succession in heaps after brown coal mining in relation to humus microstructure development. Geoderma 129: 54–64.

Frouz J, Prach K, Pižl V, Háněl L, Starý J, Tajovský K, Materna J, Balík V, Kalčík J, Řehounková K (2008) Interactions between soil development, vegetation and soil fauna during spontaneous succession in post mining sites. Eur J Soil Biol 44: 109–121.

Gabrielli CP, McDonnell JJ (2018) No direct linkage between event based runoff and groundwater recharge on the Maimai hillslope. Water Resour Res 54: 8718–8733.

Gerwin W, Raab T, Biemelt D, Bens O, Hüttl RF (2009) The artificial water catchment "Chicken Creek" as an observatory for critical zone processes and structures, Hydrol Earth Syst Sci Discuss 6: 1769–1795.

Gu WZ, Liu JF, Lin H, Lin J, Liu HW, Liao AM, Wang N, Wang WZ, Ma T, Yang N, Li GX, Zhuo P, Cai Z (2018) Why hydrological maze: The hydropedological trigger? Review of experiments at Chuzhou Hydrology Laboratory. Vadose Zone J 17: 170–174.

Jenerette D, Shen W (2012) Experimental landscape ecology. Landsc Ecol 27: 1237–1248.

Kareiva P (1989) Renewing the dialogue between theory and experiments in population ecology. In: Roughgarden J, May RM, Levin SA (eds) Perspectives in Ecological Theory, Princeton University Press, Princeton, NJ, pp 68–88.

Kendall C, McDonnell JJ, Gu W (2001) A look inside "black box" hydrograph separation models: A study at the Hydrohill artificial catchment, US-Japan Watershed Hydrology Special Issue, Hydrologic Processes 15: 1877–1902.

Klemeš V (1983) Conceptualization and scale in hydrology. J Hydrol 65: 1–23, https://www.doi.org/10.1016/0022-1694(83)90208-1.

Laine-Kaulio H, Backnas S, Karvonen T, Koivusalo H, McDonnell JJ (2014) Lateral subsurface stormflow and solute transport in a forested Hillslope: a combined measurement and modelling approach. Water Resour Res 50: 8159–8178.

Lawton JH (1996) The Ecotron Facility at Silwood Park: The Value of "Big Bottle" Experiments. Ecology 77: 665–669.

Lin H, Hopmans JW, Richter DD (2011) Interdisciplinary sciences in a global network of critical zone observatories. Vadose Zone J 10: 781–785.

Merz R, Parajka J, Blöschl G (2009) Scale effects in conceptual hydrological modelling. Water Resour Res 45, W09405: 1–15, https://www.doi.org/10.1029/2009WR007872.

Mikola P, Uomala P, Mälkönen E (1983) Application of biological nitrogen fixation in European silviculture. Forestry Sci 9: 279–294.

O'Rourke SM, Angers DA, Holden NM, McBratney AB (2015) Soil organic carbon across scales. Glob Change Biol 21: 3561–3574.

Pachepsky Y, Hill RL (2017) Scale and scaling in soils. Geoderma 287: 4–30.

Parkinson D (1978) The restoration of soil productivity. In: Holdgate MW,Woodman MJ (eds) The Breakdown and Restoration of Ecosystems. Plenum Publishing Company, New York, pp 213–229.

Raffaelli DG (2010) Ecosystem ekology new synthesis. Cambridge University press, Cambridge.

Roscher C, Karlowsky S, Milcu A, Gessler A, Bachmann D, Jesch A, Lange M, Mellado-Vázquez P, Strecker T, Landais D, Ravel O, Buchmann N, Roy J, Gleixner G (2019) Functional composition has stronger impact than species richness on carbon gain and allocation in experimental grasslands. PLoS ONE 14: e0204715.

Roubíčková A, Frouz J (2014) Performance of the earthworm Aporrectodea caliginosa on unreclaimed spoil heaps at different successional stages. Eur J Soil Biol 65: 57–61.

Šourková M, Frouz J, Šantrůčková H (2005) Accumulation of carbon, nitrogen and phosphorus during soil formation on alder spoil heaps after brown-coal mining, near Sokolov (Czech Republic). Geoderma 124: 203–214.

Urban DL (2005) Modeling ecological proceses across scales. Ecology 86: 1996–2006.

van Verseveld W, Barnard H, Graham C, McDonnell JJ, Brooks R, Weiler M (2017) A sprinkling experiment to quantify celerity-velocity differences at the hillslope cale. Hydrol Earth Syst Sc 21: 5891–5910, https://www.doi.org/10.5194/hess-21-5891-2017.

Vesterdal L, Raulund-Rasmussen K (1998) Forest floor chemistry under seven tree species along a soil fertility gradient. Can J For Res 28: 1636–1647.

Vitousek MP, Howarth RW (1991) Nitrogen limitation on land and in the sea: how can it occur? Biogeochemistry 13: 87–115.

Wu J, Jones, K, Li H, Loucks O (2006) Scaling and Uncertainty Analysis in Ecology: Methods and Applications. Springer-Verlag New York.