ACTA UNIVERSITATIS CAROLINAE

European Journal of Environmental Sciences

VOLUME 7 / NUMBER 1
2017

CHARLES UNIVERSITY
KAROLINUM PRESS
2017

ISSN 1805-0174 (Print)
ISSN 2336-1964 (Online)

CONTENTS

ARBUSCULAR MYCORRHIZAL FUNGI ENHANCE GROWTH, PHYSIOLOGICAL PARAMETERS AND YIELD OF SALT STRESSED *PHASEOLUS MUNGO* (L.) HEPPER

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ABSTRACT

A pot experiment was conducted in a greenhouse to investigate the effect of two dominant indigenous arbuscular mycorrhizal fungi, viz. *Funneliformis mosseae* (F) and *Acaulospora laevis* (A), on the growth of *Phaseolus mungo* subjected to salinity levels of 4, 8 and 12 dS m^{-1}. Mycorrhizal fungi alone and in combination improved the growth of the plants at all the salinity levels over that of the untreated control plants. However, a combination of *F. mosseae* and *A. laevis* resulted in maximum root and shoot length, biomass, photosynthetic pigments, protein content, mycorrhization, nodulation, phosphatase activity, phosphorus uptake and yield at the 8 dS m^{-1} salinity level. Peroxidase activity and electrolyte leakage were minimum at the 8 dS m^{-1} salinity level due to improved water absorption as a result of the highest mycorrhization occurring at this level of salinity. Nitrogen and potassium uptake decreased with increase in salinity and highest uptake of these nutrient elements was recorded in the treatment with both mycorrhizal fungi at a salinity level of 4 dS m^{-1}. The results of the present experiment indicate *P. mungo* inoculated with *F. mosseae* and *A. laevis* can be successfully cultivated of at salinity level of 8 dS m^{-1}. Saline soils with an electrical conductivity of nearly 12 dS m^{-1} were not suitable for growing this legume.

Keywords: mycorrhizal fungi, *Phaseolus mungo*, salinity stress, nutrient uptake, peroxidase

Introduction

The ability of soil to provide necessary nutrients for plants determines its sustainable productivity. The scarcity of micronutrients is one of the factors limiting the stability, productivity and sustainability of soil (Bell and Dell 2008). Soil salinization is a major and increasing problem in different parts of world, especially in dry and semi-arid areas. Nearly 7% of the land surface in the world is occupied by salt affected soils (Sheng et al. 2011). The most common reasons for increasing land salinization include; excessive use of chemical fertilizers, inadequate drainage as well as irregular irrigation particularly in protected cultivation. Increased concentration of salts in soil disrupts its basic structure causing a reduction in soil porosity and consequently decreased aeration and water conductance (Cucci et al. 2015). In saline soils, plants suffer from different physiological disorders, which affect their overall growth and productivity due to increased osmotic pressure and the harmful effects of Na^+ and Cl^- ions. Increased accumulation of Na^+ as well as Cl^- ions in saline soils causes nutrient imbalance as excess of Na^+ restrains uptake of K^+ while excess of Cl^- ions slows down NO_3^- uptake (Turkmen et al. 2005). Salinity stress in plants is associated with increased production of reactive oxygen species, which causes oxidative damage resulting in the oxidation of lipids, proteins and chlorophyll causing membrane leakage as well as damage to nucleic acids. In response to this plants have complex antioxidant system including enzymes like catalase (CAT), superoxide dismutase (SOD) and peroxidase (POX) and some non-enzymatic molecules like glycine, proline, betaine, sorbitol and mannitol to protect them from oxidative damage due to salinity stress (Parvaiz and Satyawati 2008).

To cope up with the increasing problem of soil salinity, development of inbred crop plants that are tolerant of salinity stress and other physiochemical methods have been tried but have failed because of physiological or genetical trait complexity (Flowers and Flowers 2005; Munns 2005). The use of plant growth promoting microorganisms as a useful and practical way to ameliorate salinity stress has received much attention in recent years. Among the plant growth promoting microorganisms, the role of arbuscular mycorrhizal fungi in improving soil structure and alleviating salinity stress is well established (Ahanger et al. 2014). Mycorrhizal associations are widely recorded in saline soils and are able to utilize water and mineral salts more efficiently than roots of plants. These symbiotic fungi act as bio-alleviators of salinity stress by improving nutrient uptake, chlorophyll content, antioxidant enzyme activity, membrane stability, vegetative growth and phosphatase activity thus reducing the damage to plants caused by salinity stress (Sheng et al. 2011; Beltrano et al. 2013). Since legumes establish a tripartite association with rhizobacteria and AMF, it is recommended that legumes are inoculated with these microbes, which may assist phosphorus and nitrogen uptake resulting in improvement of growth and productivity under salinity stress.

Among the different food legumes, *Phaseolus mungo* (L.) Hepper pulses are highly nutritious containing 60% carbohydrate, 24% protein and 1.3% fat, plus minerals like calcium, phosphorus, potassium and vitamins like A, B and C (Sarwar et al. 2004). It is one of the most highly prized pulses in India. This country is the largest producer of *P. mungo* in the world. The hazardous effect of salt in the soil on the productivity of legumes is the major problem confronted by the farmers throughout

Sharma, N., Aggarwal, A., Yadav, K.: Arbuscular mycorrhizal fungi enhance growth, physiological parameters and yield of salt stressed *Phaseolus mungo* (L.) Hepper
European Journal of Environmental Sciences, Vol. 7, No. 1, pp. 5–13
https://doi.org/10.14712/23361964.2017.1

the world. Thus the ability of AMF to ameliorate salinity stress and improve the tolerance of *P. mungo* of salt could have important practical applications. The present experiment aimed to investigate the growth response of *P. mungo* grown at different levels of salinity and the role of AMF in improving growth and yield when grown in saline soils.

Materials and Methods

Growth Conditions

A pot experiment was conducted in a glass house at the Botany Department, Kurukshetra University, Kurukshetra, Haryana, India. The temperature was maintained at (30 ± 5 °C) and the relative humidity at 60–70%. Apart from sunlight, light was also provided for 16 hours each day by cool white fluorescent lamps. Soil used in this experiment consisted of 64.2% sand, 21.81% silt and 3.90% clay.

Mass Multiplication of Bioinoculants

In this experiment, two arbuscular mycorrhizal species viz. *Funneliformis mosseae* and *Acaulospora laevis* were used. They were isolated from the rhizosphere of *P. mungo* grown in the botanical garden of Kurukshetra University, Kurukshetra. After preparation of a starter inoculum using the Funnel Technique of Menge and Timmer (1982), these species were propagated using maize growing in standard pot culture as a host. Mass multiplication of *Trichoderma viride* was done using a modified wheat bran-saw dust medium (Mukhopadhyay et al. 1986) and the *Rhizobium* sp. (*Bradyrhizobium japonicum*) culture (procured from Department of Microbiology, CCS Haryana Agricultural University, India) was multiplied using a nutrient broth medium. Seeds of *Phaseolus mungo* were procured from CCS Haryana Agricultural University, Hisar, Haryana, India. The seeds were surface sterilized with 0.5% (v/v) sodium hypochlorite for 10 minutes and then washed with sterilized distilled water. Before seeds were sown in pots, 10 ml of a liquid suspension of *Bradyrhizobium* sp. was applied to each pot. Ten days after emergence the number of plants was reduced to 5 per pot.

Experimental Setup

The experiment was laid out in a randomized block design, with five replicates of each treatment. Soil from the experimental site was collected and mixed with sand in a ratio of 3:1 (soil : sand). This mixture was then sieved through 2-mm sieve and autoclaved at 121 °C for two hours for two consecutive days to render it free of naturally occurring microbes, including mycorrhizal fungi. After sterlization, the soil was then tested for the presence of microbes and it was found to be completely free of microbes. This was done to avoid the effect of other microbes on the growth response of *L. culinaris* under salinity stress. The Earthenware pots (24.5 × 25 cm) were selected and filled with 2.5 kg soil. Initially, the pots were saturated with three different levels of saline solution, i.e. 4, 8, and 12 dS m^{-1} (sodium chloride, calcium chloride and sodium sulphate in the ratio 7:2:1 w/v) as per Richards (1954). Then, pieces of maize root with 85% colonization by AM were chopped up and along with soil containing AM spores (620–650 per 100 g inoculum) were used as the AM inoculum. To each pot 10% (w/w), i.e. 200 g/pot inoculum of AM fungi alone and in combination was added to the soil before sowing the seeds. Pots were watered regularly with saline solution to maintain the required salinity level and were fertilized with a nutrient solution after 15 days (Weaver and Fredrick 1982), which contained half the recommended level of phosphorus and no nitrogen. For each level of salinity there were 4 treatments as outlined below:

1. Uninoculated (without AM inoculum but with *Bradyrhizobium* sp.)
2. *Funneliformismosseae* (F) with *Bradyrhizobium* sp.
3. *Acaulospora laevis* (A) with *Bradyrhizobium* sp.
4. F + A with *Bradyrhizobium* sp.

Plant Harvest and Analysis

After 120 days, plants were harvested by uprooting them and then various morphological and physiological parameters were measured. Plant height and root length were measured. For determining fresh and dry weight, roots and shoots were weighed after uprooting and then oven dried at 70 °C until a constant dry weight was obtained. Chlorophyll content was determined by using the method of Arnon (1949). Root and shoot phosphorus content was estimated using the 'Vanado-molybdo-phosphoric yellow colour method' (Jackson 1973) and nitrogen (N) content was determined using the Kjeldahl method (Kelplus nitrogen estimation system, supra-LX, Pelican Equipments, Chennai, India). Potassium content was analyzed using inductively coupled plasma analyzer-mass spectrometry (ICP-MS). Phosphatase activity was estimated using p-nitrophenyl phosphate (PNPP) as a substrate, which is hydrolyzed by the enzyme to p-nitrophenol (Tabatabi and Bremner 1969). Total protein was estimated using Bradford's (1976) method. Peroxidase activity was determined using Maehly's method (1954). Leaf area was measured using a leaf area meter (Systronics 21, Ahmedabad, India). Nodulation and yield in terms of number and weight of pods (g) per pot was recorded after 120 days.

Identification and Quantification of the Number and Colonization by AM Spores

Identification of AM spores (*F. mosseae* and *A. laevis*) was done using the identification manuals of Walker (1983); Scheneck and Perez (1990); Morton and Benny (1990) and Mukerji (1996). Quantification of AM spores was done using the Adholeya and Gaur 'Grid Line Intersect Method' (1994). 'Rapid Clearing and Staining' technique of Phillips and Hayman (1970) was used to estimate mycorrhizal colonization of roots. The latter was calculated using the formula: (Number of root segments colonized / number of root segments studied) × 100.

Electrolyte Leakage

To determine electrolyte leakage, fresh leaf samples (200 mg) were cut into small discs (i.e. 5 mm in diameter) and placed in test tubes containing 10 ml of distilled and deionized water. The tubes sealed with cotton plugs were placed in a water bath at a constant temperature of 32 ± 8 °C. After 2 h the initial electrical conductivity of the medium (EC1) was measured using an electrical conductivity meter. Afterwards the samples were autoclaved at 121 ± 8 °C for 20 minutes to kill the tissues and release all electrolytes. The samples were then cooled to 25 ± 8 °C and final electrical conductivity (EC2) was measured. The electrolyte leakage (EL) was calculated using the formula of Dionisio-Sese and Tobita:

$EL = EC1/EC2 \times 100$

Statistical Analysis

Data were subjected to an analysis of variance and means separated using the least significant difference test in the Statistical Package for Social Sciences (ver.11.5, Chicago, IL, USA).

Results

Growth

Plant Height and Shoot Biomass

In the present investigation, mycorrhizal plants were taller than non-mycorrhizal control plants (Table 1). Maximum height was recorded for the plants treated with the dual combination of F + A and growing in soil with a 8 dS m^{-1} (medium) salinity level followed by the same treatment but with plants growing in soil with a 4 dS m^{-1} (low) salinity level. In this experiment, untreated control plants subjected to a 12 dS m^{-1} salinity level were the smallest. This was reflected in the fresh and dry weights of the shoots. Highest shoot weight was recorded for F + A plants grown in soil with a medium salinity level (8 dS m^{-1}) followed by the same treatment at the low salinity level (4 dS m^{-1}). The shoot fresh weight of the control plants was the same when grown in soil with both a 12 dS m^{-1} and 4 dS m^{-1} salinity level. Inoculation of plants with *F. mosseae* proved to be more beneficial for increasing shoot fresh weight at a high salinity (12 dS m^{-1}) than a low salinity level (4 dS m^{-1}). In the un-inoculated plants, dry shoot weight decreased with increase in salinity while among the treated plants, inoculation with F + A at a medium salinity level (8 dS m^{-1}) gave the best results, as shown in Table 1.

Root length and root biomass

Root length and fresh and dry weights were highest for plants subjected to a medium salinity level (8 dS m^{-1}) followed by those subjected to a low salinity level (4 dS m^{-1}) and treatment F + A was the best of all the treatments (Table 1).

Leaf Area

Maximum leaf area was recorded for plants grown at the highest salinity level (12 dS m^{-1}) followed by those grown at the lowest salinity level (4 dS m^{-1}) when they

Table 1 Effect of AM fungi on the growth of *Phaseolus mungo* grown under different levels of salinity stress.

Salinity level	Parameters → Treatments ↓	Plant Height (cm)	Shoot weight (g)		Root Length (cm)	Root weight (g)	
			Fresh	Dry		Fresh	Dry
	C	31.58 ± 2.140^{f}	0.69 ± 0.019^{gh}	0.29 ± 0.006^{h}	04.74 ± 0.350^{g}	0.35 ± 0.121^{ef}	0.15 ± 0.044^{f}
4 dS m^{-1}	F	59.46 ± 1.611^{d}	6.37 ± 0.304^{d}	2.12 ± 0.246^{d}	12.76 ± 0.371^{d}	0.69 ± 0.114^{cd}	0.42 ± 0.277b^{cd}
	A	38.44 ± 1.849^{ef}	3.70 ± 0.248^{ef}	1.23 ± 0.027^{ef}	10.02 ± 0.370^{f}	0.55 ± 0.090^{de}	0.33 ± 0.167^{cde}
	F + A	78.10 ± 1.063^{b}	8.45 ± 0.043^{b}	2.90 ± 0.027^{c}	15.62 ± 0.238^{b}	0.82 ± 0.159^{bc}	0.45 ± 0.085^{bc}
	C	33.38 ± 1.583^{f}	0.84 ± 0.002^{g}	0.11 ± 0.003^{i}	05.28 ± 0.334^{g}	0.59 ± 0.231^{d}	0.18 ± 0.356^{ef}
8 dS m^{-1}	F	69.38 ± 1.344^{c}	7.92 ± 0.311^{c}	3.42 ± 0.340^{b}	14.16 ± 0.304^{c}	0.97 ± 0.246^{ab}	0.55 ± 0.211^{bf}
	A	37.70 ± 0.113^{ef}	4.43 ± 0.266^{e}	1.41 ± 0.024^{e}	11.24 ± 0.288^{e}	0.73 ± 0.090^{cd}	0.33 ± 0.137^{cde}
	F + A	86.40 ± 1.414^{a}	9.40 ± 0.218^{a}	4.14 ± 0.030^{a}	16.34 ± 0.397^{a}	1.07 ± 0.105^{a}	0.85 ± 0.071^{a}
	C	28.00 ± 1.046^{g}	0.69 ± 0.002^{gh}	0.06 ± 0.003^{i}	03.96 ± 0.336^{h}	0.18 ± 0.005^{f}	0.10 ± 0.036^{f}
12 dS m^{-1}	F	29.68 ± 1.948^{fg}	4.28 ± 0.173^{e}	1.16 ± 0.023^{ef}	11.68 ± 0.238^{e}	0.56 ± 0.245^{de}	0.15 ± 0.066^{f}
	A	37.86 ± 1.292^{ef}	2.16 ± 0.028^{f}	0.92 ± 0.003^{g}	09.30 ± 0.412^{f}	0.33 ± 0.156^{f}	0.12 ± 0.044^{f}
	F + A	40.04 ± 1.275^{e}	7.62 ± 0.023^{c}	1.58 ± 0.040^{e}	13.88 ± 0.303^{c}	0.64 ± 0.109^{cd}	0.27 ± 0.049^{def}
	L.S.D ($P \leq 0.05$)	906.3620	163.5300	488.9330	803.3010	13.9140	14.4890
	ANOVA $F_{(11,24)}$	1.9185	0.2239	0.1697	0.4238	0.1984	0.1631
F values	Salinity (S)	1271.0250	649.4820	507.2890	189.3410	35.8350	30.9380
	Treatments (T)	1799.3160	5.3780	1233.8870	2809.0000	26.8620	23.6350
	S × T	341.9970	91.9030	110.3380	5.1210	0.1330	4.4330

Legend: F†: *Funneliformis mosseae*, A: *Acaulospora laevis*, ‡: each value is the mean of five replicates, ± : standard deviation, AM: Arbuscular mycorrhizae, values in columns followed by the same letter are not significantly different, P ≤ 0.05, least significant difference test.

were treated with F + A (Table 5). Among the single inoculation treatments and control, the maximum leaf areas were recorded for plants grown in soil with a medium salinity level (8 dS m^{-1}).

Chlorophyll Content

Content of photosynthetic pigments recorded in AM treated plants grown in soils with different levels of salinity were higher than in un-inoculated control plants (Table 2). However, the highest total chlorophyll content was recorded for plants treated with a combination of *F. mosseae* and *A. laevis* followed by a single inoculation with *F. mosseae* and grown in soil with a salinity level of 8 dS m^{-1}. Further increase in salinity to 12 dS m^{-1} resulted in a decrease in chlorophyll content. Lowest concentration of photosynthetic pigments was recorded in untreated plants grown in soils with a 12 dS m^{-1} salinity level.

Protein Content

Regardless of mycorrhizal treatments, a salinity level of 8 dS m^{-1} resulted in remarkable increase in leaf protein content (Table 2). Further, increase in salinity to 12 dS m^{-1} had an adverse effect on leaf protein content. The protein content increased with increase in soil salinity up to 8 dS m^{-1}. Although, further increase in soil salinity resulted in a decrease in the content of protein in leaves; inoculation with *F. mosseae* and *A. laevis* increased protein content to the maximum level, followed by treatment with *A. laevis* alone, indicating its stimulatory effect on protein synthesis at all the levels of salinity used.

Mycorrhization

As evident from Table 2, the plants subjected to a medium salinity level (8 dS m^{-1}) had a greater number of AM spores and % root colonization as compared to higher and lower salinity levels. Beyond the medium salinity level, i.e. at (12 dS m^{-1}), there was a negative correlation between salinity and mycorrhization. Maximum mycorrhization was recorded in the combined treatment F + A of plants grown in soil with a salinity level of 8 dS m^{-1} followed by that recorded for plants treated only with *F. mosseae* , which indicates that *F. mosseae* is more tolerant of salinity than *A. laevis*.

Nodulation

In un-inoculated control plants, nodulation decreased with increase in salinity level but in plants treated with AM fungi, nodulation increased up to maximum level at a salinity level of 8 dS m^{-1} and was much lower at a salinity of 12 dS m^{-1} (Table 5).

Peroxidase Activity

Data presented in Table 3 reveals that peroxidase activity at a salinity level of 8 dS m^{-1} was less than at a salinity level of 4 dS m^{-1} due to more mycorrhization at a medium salinity level, which improved the water status of plants, which resulted in less osmotic stress. At salinity

Table 2 Effect of AM fungi on some physiological parameters and mycorrhization of *Phaseolus mungo* grown under different levels of salinity stress.

Salinity level	Parameters → Treatments ↓	Chlorophyll content (mg/g FW)			Protein content (mg/g FW)	AM spore number / 10 g of soil	AM Root colonization (%)
		Chl a	Chl b	Total Chl			
	C	0.714 ± 0.004^{k}	0.358 ± 0.019^{j}	1.0718 ± 0.012^{k}	0.191 ± 0.003^{k}	06.2 ± 2.86^{g}	1.98 ± 2.81^{i}
4 dS m^{-1}	F	1.067 ± 0.004^{d}	0.910 ± 0.006^{d}	1.978 ± 0.014^{d}	0.302 ± 0.002^{g}	63.8 ± 3.56^{c}	34.4 ± 4.72^{e}
	A	0.934 ± 0.003^{g}	0.681 ± 0.010^{g}	1.616 ± 0.014^{h}	0.466 ± 0.002^{d}	51.2 ± 3.70^{e}	29.9 ± 3.49^{f}
	F + A	1.264 ± 0.056^{c}	1.040 ± 0.005^{c}	2.304 ± 0.011^{c}	0.581 ± 0.004^{b}	72.6 ± 4.03^{c}	53.2 ± 2.86^{b}
	C	0.827 ± 0.005^{i}	0.411 ± 0.007^{h}	1.239 ± 0.023^{i}	0.206 ± 0.003^{j}	14.8 ± 3.83^{f}	03.0 ± 2.23^{i}
8 dS m^{-1}	F	1.627 ± 0.007^{b}	1.317 ± 0.006^{b}	2.944 ± 0.010^{b}	0.329 ± 0.002^{f}	77.6 ± 3.64^{a}	48.6 ± 3.49^{c}
	A	0.983 ± 0.006^{e}	0.921 ± 0.008^{d}	1.905 ± 0.013^{e}	0.574 ± 0.002^{c}	65.0 ± 4.00^{c}	37.6 ± 3.97^{de}
	F + A	1.753 ± 0.006^{a}	1.374 ± 0.007^{a}	3.126 ± 0.011^{a}	0.605 ± 0.006^{a}	78.6 ± 4.27^{a}	63.4 ± 3.84^{a}
	C	0.676 ± 0.004^{l}	0.356 ± 0.005^{j}	1.031 ± 0.010^{l}	0.179 ± 0.002^{l}	00.0 ± 0.00^{h}	00.0 ± 0.00^{i}
12 dS m^{-1}	F	0.880 ± 0.005^{h}	0.785 ± 0.011^{f}	1.665 ± 0.011^{g}	0.224 ± 0.003^{i}	56.4 ± 4.27^{d}	24.4 ± 3.20^{g}
	A	0.778 ± 0.006^{j}	0.376 ± 0.011^{i}	1.154 ± 0.004^{j}	0.286 ± 0.002^{h}	47.8 ± 3.34^{e}	16.8 ± 3.03^{h}
	F + A	0.957 ± 0.004^{f}	0.868 ± 0.012^{e}	1.826 ± 0.015^{f}	0.408 ± 0.007^{e}	59.2 ± 3.49^{d}	40.2 ± 4.32^{d}
	L.S.D ($P \leq 0.05$)	0.007	0.0138	0.0181	0.003	4.8759	4.596
	ANOVA $F_{(11,24)}$	185.540	625.5300	135.3400	176.240	291.9640	192.735
F values	Salinity (S)	36225.831	8310.8940	22637.2480	17434.234	128.7240	148.113
	Treatments (T)	33582.465	15861.2330	30146.8350	48110.463	979.4900	583.236
	S × T	5148.889	766.3310	2183.4160	2442.499	2.6230	12.358

Legend: F†: *Funneliformis mosseae*, A: *Acaulospora laevis*, ‡: each value is the mean of five replicates, ± : standard deviation, AM: Arbuscular mycorrhizae, FW: fresh weight, values in columns followed by the same letter are not significantly different, P ≤ 0.05, least significant difference test.

levels greater than 8 dS m^{-1} there was a marked increase in peroxidase activity associated with the stress induced damage. Single inoculation with *A. laevis* was less effective than a single inoculation with *F. mosseae* at all the salinity levels. Of the different treatments, the F + A combination resulted in the highest peroxidase activity at low and medium salinity levels, but inoculation with *F. mosseae* alone resulted in the maximum activity being recorded at the highest salinity level used.

Phosphatase Activity

Both in mycorrhizal and non-mycorrhizal control plants the maximum values of acid and alkaline phosphatase activity was recorded at a salinity level of 8 dS m^{-1}. At a salinity level of 12 dS m^{-1} the activity of both these enzymes decreased, however, the dual treatment F + A resulted in a greater increase in enzyme activity than the single treatment with either of the mycorrhizal fungi at all salinity levels (Table 3).

Electrolyte Leakage

In this study, increase in salt concentration in the soil above a salinity level of 8 dS m^{-1} resulted in a decrease in membrane stability. The double inoculation treatment with F + A at all the levels of salinity improved membrane stability, followed by a single treatment with *F. mosseae.* Less electrolyte leakage was recorded from mycorrhizal plants than non-mycorrhizal control plants at all the salinity levels used (Table 3).

Nutrient uptake

Phosphorus, Potassium and Nitrogen

In the present investigation, highest root and shoot phosphorus (P) content was recorded at the medium salinity level i.e. 8 dS m^{-1} in the double inoculation treatment F + A (Table 4). The combination of *F. mosseae* and *A. laevis* at all the salinity levels resulted in better root and shoot phosphorus contents than in the controls. Potassium (K) and nitrogen (N) uptake decreased with increase in salt concentration in the soil (Table 4). Mycorrhizal treatment increased potassium and nitrogen content in roots and shoots regardless of salt stress levels. Among the treated plants, treatment with *A. laevis* resulted in the least root and shoot potassium content at all salinity levels. Root potassium content was greater than that recorded in the shoots. Maximum shoot and root nitrogen uptake was recorded at 4 dS m^{-1} salinity level in treatment F + A. Shoots of *P. mungo* accumulated more nitrogen than the roots at all of the different levels of salinity used.

Yield

Since *P. mungo* is cultivated for its seeds, the effect of mycorrhizal inoculation on number and weight of pods under saline conditions is important. Mycorrhizal inoculation significantly increased yield of *P. mungo* compared to un-inoculated control plants at all the different levels of salinity used. At the 8 dS m^{-1} salinity level, maximum yield in terms of number and weight of pods per plant was recorded in the F + A treatment, followed that re-

Table 3 Effect of AM fungi on some biochemical parameters of *Phaseolus mungo* grown under different levels of salinity stress.

Salinity level	Parameters → Treatments ↓	Phosphatase activity (IU/g FW)		Peroxidase activity (mg protein / 10 min)	Electrolyte leakage (%)
		Acidic	Alkaline		
	C	0.030 ± 0.006^{c}	0.076 ± 0.008^{h}	0.242 ± 0.011^{j}	39.74 ± 0.476^{b}
4 dS m^{-1}	F	0.138 ± 0.010^{abc}	0.295 ± 0.010^{c}	0.599 ± 0.004^{d}	33.36 ± 0.192^{g}
	A	0.066 ± 0.006^{bc}	0.158 ± 0.007^{e}	0.486 ± 0.013^{f}	36.98 ± 0.503^{d}
	F + A	0.194 ± 0.008^{ab}	0.321 ± 0.005^{b}	0.812 ± 0.002^{b}	29.74 ± 0.252^{h}
	C	0.038 ± 0.007^{c}	0.082 ± 0.006^{gh}	0.161 ± 0.020^{k}	35.34 ± 0.315^{e}
8 dS m^{-1}	F	0.195 ± 0.007^{ab}	0.328 ± 0.008^{b}	0.315 ± 0.009^{h}	28.13 ± 0.208^{i}
	A	0.125 ± 0.006^{abc}	0.258 ± 0.007^{d}	0.243 ± 0.011^{j}	29.85 ± 0.194^{h}
	F + A	0.240 ± 0.008^{a}	0.387 ± 0.006^{a}	0.405 ± 0.006^{g}	25.76 ± 0.742^{j}
	C	0.024 ± 0.005^{c}	0.045 ± 0.006^{i}	0.297 ± 0.018^{i}	42.82 ± 1.550^{a}
12 dS m^{-1}	F	0.075 ± 0.006^{bc}	0.116 ± 0.008^{f}	0.856 ± 0.001^{a}	38.73 ± 0.396^{c}
	A	0.043 ± 0.008^{c}	0.090 ± 0.005^{g}	0.520 ± 0.008^{e}	40.29 ± 0.277^{b}
	F + A	0.153 ± 0.007^{abc}	0.288 ± 0.006^{c}	0.764 ± 0.004^{c}	34.62 ± 0.251^{f}
	L.S.D ($P \leq 0.05$)	0.1231	0.0101	0.015	0.733
	ANOVA $F_{(11,24)}$	3.7830	132.6300	233.530	499.604
F values	Salinity (S)	1.4230	1530.1650	4828.379	1485.808
	Parameter (T)	11.7500	3459.0210	4474.442	806.357
	S × T	0.5850	191.0380	433.999	17.493

Legend: F†: *Funneliformis mosseae*, A: *Acaulospora laevis*, ‡: each value is the mean of five replicates, ± : standard deviation, AM: Arbuscular mycorrhizae, FW: Fresh Weight, values in columns followed by the same letter are not significantly different, $P \leq 0.05$, least significant difference test.

corded for plants inoculated with same combination at the 4 dS m^{-1} salinity level (Table 5). Single treatment with *F. mosseae* also resulted in an increase in yield at all the different salinity levels compared to *A. laevis*. Soil salinity levels of 12 dS m^{-1} had a significant adverse effect on yield as the number and weight of pods were lower.

Discussion

Our results indicate that plant height, root length and biomass increased with increase in soil salinity up to 8 dS m^{-1}, but were all less at the 12 dS m^{-1}salinity level. In *Vicia faba* increase in plant height at medium and low sa-

Table 4 Effect of AM fungi on nutrient uptake of *Phaseolus mung*o grown under different levels of salinity stress.

Salinity level	Parameters → Treatments ↓	Phosphorus content (%)		Nitrogen content (%)		Potassium content (%)	
		Root	Shoot	Root	Shoot	Root	Shoot
	C	0.676 ± 0.011^{i}	0.449 ± 0.008^{j}	0.301 ± 0.0030^{def}	0.444 ± 0.0348^{h}	0.974 ± 0.038^{h}	0.708 ± O.0587^{g}
4 dS m^{-1}	F	1.269 ± 0.005^{de}	0.694 ± 0.007^{f}	1.212 ± 0.4022^{b}	1.678 ± 0.018^{b}	1.906 ± 0.0288^{c}	1.035 ± 0.0587^{c}
	A	1.133 ± 0.008^{fg}	0.643 ± 0.003^{g}	0.616 ± 0.0320^{c}	1.300 ± 0.0029^{c}	1.660 ± 0.0223^{e}	0.972 ± 0.0192^{d}
	F + A	2.181 ± 0.007^{b}	1.257 ± 0.005^{b}	1.430 ± 0.0246^{a}	1.931 ± 0.00273^{a}	2.324 ± 0.0207^{a}	1.232 ± 0.1041^{a}
	C	0.806 ± 0.007^{h}	0.507 ± 0.007^{i}	0.206 ± 0.0288^{fg}	0.298 ± 0.0225^{i}	0.828 ± 0.0238^{i}	0.576 ± 0.0240^{h}
8 dS m^{-1}	F	1.337 ± 0.008^{d}	0.846 ± 0.006^{d}	0.720 ± 0.0254^{c}	0.820 ± 0.0269^{e}	1.748 ± 0.0414^{d}	0.920 ± 0.0316^{de}
	A	1.295 ± 0.006^{de}	0.713 ± 0.006^{e}	0.448 ± 0.0319^{d}	0.670 ± 0.0247^{f}	1.538 ± 0.0319^{f}	0.840 ± 0.0314^{f}
	F + A	2.431 ± 0.286^{a}	1.400 ± 0.002^{a}	0.744 ± 0.0384^{c}	1.017 ± 0.0599^{d}	2.026 ± 0.1040^{b}	1.124 ± 0.0288^{b}
	C	0.623 ± 0.008^{i}	0.395 ± 0.005^{k}	0.124 ± 0.0230^{g}	0.148 ± 0.0258^{j}	0.644 ± 0.6270^{j}	0.432 ± 0.0286^{i}
12 dS m^{-1}	F	1.187 ± 0.008^{ef}	0.630 ± 0.011^{h}	0.389 ± 0.0320^{de}	0.524 ± 0.0303^{g}	1.636 ± 0.0270^{e}	0.898 ± 0.0238^{e}
	A	1.063 ± 0.007^{g}	0.513 ± 0.006^{i}	0.228 ± 0.0356^{efg}	0.4722 ± 0.0030^{h}	1.220 ± 0.6254^{g}	0.724 ± 0.0304^{g}
	F + A	1.858 ± 0.017^{c}	0.975 ± 0.006^{c}	0.684 ± 0.0336^{c}	0.791 ± 0.0028^{e}	1.946 ± 0.0201^{c}	1.044 ± 0.0384^{c}
	L.S.D ($P \leq 0.05$)	0.113	0.009	0.152	0.224	0.055	0.052
	ANOVA $F_{(11,24)}$	233.006	103.540	56.818	2112.724	795.512	133.440
F values	Salinity (S)	58.545	5894.968	109.260	5500.719	372.964	105.774
	Treatments(T)	800.891	32949.820	116.998	3412.981	2645.806	410.992
	S × T	7.218	532.687	10.913	333.264	11.217	3.894

Legend: F†: Funneliformis mosseae, A: Acaulospora laevis, ‡: each value is the mean of five replicates, ± : standard deviation AM: Arbuscular mycorrhizae, values in columns followed by the same letter are not significantly different, P ≤ 0.05, least significant difference test.

Table 5 Effect of AM fungi on leaf area, nodules and yield of *Phaseolus mung*o grown under different levels of salinity stress.

Salinity level	Parameters → Treatments ↓	Leaf Area	No of nodules (per pot)	Yield (per plant)	
				No. of pods	Weight of pods (g)
	C	08.22 ± 1.522^{fg}	07.8 ± 2.387^{e}	03.0 ± 1.581^{d}	1.294 ± 0.343^{fg}
4 dS m^{-1}	F	18.31 ± 1.598^{c}	12.8 ± 2.432^{cd}	05.6 ± 2.302^{abcd}	1.916 ± 0.379^{de}
	A	13.76 ± 1.842^{d}	10.2 ± 1.923^{de}	03.6 ± 2.073^{cd}	1.556 ± 0.438^{ef}
	F + A	25.50 ± 2.214^{b}	17.0 ± 2.915^{b}	07.8 ± 1.923^{ab}	3.040 ± 0.436^{b}
	C	10.34 ± 2.226^{f}	03.8 ± 2.387^{fg}	04.0 ± 2.549^{cd}	1.510 ± 0.395^{ef}
8 dS m^{-1}	F	20.78 ± 2.554^{c}	15.6 ± 3.209^{bc}	06.6 ± 2.408^{abc}	2.390 ± 0.382^{cd}
	A	15.45 ± 2.019^{d}	13.6 ± 3.209^{bcd}	04.6 ± 2.701^{bcd}	1.868 ± 0.366^{e}
	F + A	23.47 ± 1.917^{b}	21.0 ± 2.738^{a}	09.0 ± 3.162^{a}	3.772 ± 0.346^{a}
	C	06.76 ± 2.214^{g}	02.2 ± 1.788^{g}	02.6 ± 2.408^{d}	0.858 ± 0.277^{g}
12 dS m^{-1}	F	13.09 ± 1.770^{de}	09.0 ± 3.535^{e}	05.0 ± 3.162^{bcd}	1.228 ± 0.445^{fg}
	A	10.86 ± 2.245^{ef}	06.8 ± 2.387^{ef}	02.8 ± 1.303^{d}	1.034 ± 0.201^{fg}
	F + A	29.26 ± 2.414^{a}	14.2 ± 2.364^{bc}	05.2 ± 3.492^{bcd}	2.600 ± 0.351^{bc}
	L.S.D ($P \leq 0.05$)	2.7441	3.5661	3.3833	0.503
	ANOVA $F_{(11,24)}$	63.6630	21.8740	3.2330	28.063
F values	Salinity (S)	7.8950	22.3980	3.6940	33.374
	Treatments(T)	206.5570	60.0710	8.7870	79.060
	S ×T	10.8060	2.6010	0.3030	0.793

Legend: G†: *Funneliformis mosseae*, A: *Acaulospora laevis* ‡: each value is the mean of five replicates, ± : standard deviation, AM: Arbuscular mycorrhizae, values in columns followed by the same letter are not significantly different, P ≤ 0.05, least significant difference test.

linity level is recorded by Amira and Qados (2010) while in ornamental Purslane, Alam et al. (2015) records an increase in fresh and dry shoot weight at a salinity level of 8 dS m^{-1}. Our results confirm the findings of Pessarakli et al. (2015) who note an increase in root biomass of the *Distichlis spicata* at medium salinity levels compared to that recorded in high and low salinity level treatments. Under salinity stress, plant growth and biomass is limited by a lower availability of nutrients and the energy expenditure necessary to nullify the toxic effects of NaCl and other salts. Mycorrhization increases growth and biomass of the host plant due to AM mediated enhanced nutrient acquisition, especially a better P nutrition (Sharifi et al. 2007; Colla et al. 2008). Salinity stress lowered the concentration of photosynthetic pigments due to the toxic effects of salt on nitrogen and magnesium absorption, which are vital constituents of chlorophyll (Kaya et al. 2009). Another reason could be the increased activity of chlorophyllase due to salinity stress, which resulted in the destruction of photosynthetic pigments. The greater chlorophyll content of plants inoculated with mycorrhizal fungi could be due to the increased uptake of magnesium and nitrogen by AM hyphae (Abdel Latef and Chaoxing 2011) or an increase in the activity of enzymes required for the synthesis of chlorophyll (Murkute et al. 2006). Due to the higher concentration of photosynthetic pigments, photosynthesis in mycorrhizal plants subjected to salinity stress is higher than in un-inoculated stressed plants (Abdel Latef and Chaoxing 2011), which resulted in increased growth.

Salinity stress up to salinity level 8 dS m^{-1} resulted in an increase in leaf protein content. The reason may be due to an accumulation of salt stress proteins, which help in establishing a proper cellular ion and osmotic homeostasis (Amini et al. 2007; Garcia et al. 2008). These proteins act as nitrogen reserves for plants, which can be utilized later. Further, the decrease in leaf protein content with increase in salinity up to 12 dS m^{-1} is due to a decrease in uptake and utilization of nitrogen, which is an essential element for protein synthesis (Kusano et al. 2011). Mycorrhizal inoculation of plants improved leaf protein content regardless of the salinity. Our findings are in agreement with those of Datta and Kulkarni (2014) who also report an increase in protein content in mycorrhizal plants subjected to salinity stress.

A high soil salinity may not reduce mycorrhization, as increased mycorrhization under high saline conditions is reported by Aliasgharzadeh et al. (2001) and Yamato et al. (2008). The upper limit of the salinity tolerance of the AMF used in the experiment was 12 dS m^{-1}, the level at which spore number and mycorrhization were drastically reduced. Decreased mycorrhization in *P. mungo* plants at salinities above 8 dS m^{-1} could be due to the high pH associated with high salt concentrations inhibiting the germination of fungal spores. Even though high salinity caused a decrease in mycorrhization subsequent mycorrhizal dependency increased, which indicates that the symbiosis between roots and AM fungi strengthens once the association is established, which indicates the importance of this symbiosis for plant production under saline conditions (Rabie and Almadini 2005). There was a direct correlation between mycorrhization and nodulation in the present experiment indicating the stimulatory role of mycorrhizae on nodulation. In this experiment, mycorrhizal plants growing at all of the salinity levels used were less affected in terms of nodulation parameters than the control plants because the root exudation pattern was modified both quantitatively and qualitatively by AMF, which results in an increase in nodulation (Garg and Manchanda 2009).

Salinity stress in plants results in an increase in the production of ROS (Reactive Oxygen Species) and hence, oxidative stress, which has toxic effects on different biomolecules. As different antioxidant enzymes nullify the effect of damage induced by ROS, it is possible that this accounts for the high activity of peroxidase recorded at the highest salinity level i.e. 12 dS m^{-1}. Increase in antioxidant enzyme activity with increase in salinity is confirmed by Hashem et al. (2015). At all the salinity levels used, there was a higher peroxidase activity in the treatment inoculated with mycorrhizal fungi, which support the findings of Alqarawi et al. (2014) and Abd Allah et al. (2015). Estimates of phosphatase activity in plants help to assess phosphorus metabolism in mycorrhizal plants as this enzyme is present in the vacuoles of AM hyphae (Tisserent et al. 1993). Mycorrhizal inoculation positively affected phosphatase activity. Our results confirm the findings of Peng et al. (2011) who report an increase in alkaline phosphatase activity in mycorrhizal *Astragallus sinicus* under saline conditions. The major organelle in plants adversely affected by soil salinity stress is the cell membrane, as peroxidation of lipids causes the solutes to leak through the membrane decreasing its stability (Kaya et al. 2009). Mycorrhizal inoculation of plants improved membrane stability due to higher antioxidant activity and phosphorus uptake. The decrease in electrolyte leakage in mycorrhizal plants recorded in this experiment confirms the findings of Abd Allah et al. (2015).

Phosphorus uptake was negatively affected at a soil salinity level of 12 dS m^{-1} because of the precipitation of phosphate (H_2PO_4) ions by calcium, magnesium and zinc ions, which adversely affects the uptake of this element (Marshner 1994). Mycorrhizal fungi are able to solubilize the precipitated phosphorus, thus increasing the availability of this immobile element under saline conditions (Srividya et al. 2010). Another reason for the improved P uptake by mycorrhizal plants is the greater soil volume penetrated by their extra radical mycelium, which extends beyond nutrient depleted zones in the soil. In the present experiment, mycorrhizal inoculation of plants also improved potassium uptake under different salinity levels. Our results confirm the findings of Patel et al. (2010) and Abd Allah et al. (2015). The decrease in the uptake of potassium, with increase in salinity recorded in this experiment is due to a high concentration of sodium within the root zone, which has an antagonistic effect. The elevated concentration of sodium and chloride ions interfere with potassium ion channels in the plasma membrane of root cells causing a decrease in the uptake of this nutrient. A possible reason for the increased K uptake by mycorrhizal plants is their ability to store sodium in vacuoles of root cells as well as intra-radical hyphae (Cantrell and Linderman 2001). Increase in the tolerance of mycorrhizal plants to saline conditions may be attributed to their increased biomass due to enhanced nutrient uptake, which results in the dilution of the toxic effects of ions (Campanelli et al. 2012). Like potassium, nitrogen content in the plants also decreased with increase in soil salinity. Increased nitrogen uptake by mycorrhizal plants could be attributed to the ability of the extra-radical mycelium of mycorrhizal fungi to absorb nitrate and ammonium and translocate nitrogen in the form of arginine (Guether et al. 2009). Another reason could be the AM mediated increase in activity of urease in the soil, which may help in breaking down urea and in the liberation of NH_3^+ or NH_4^+ ions (Zhao et al. 2010). Higher nitrogen uptake by the mycorrhizal plants helps them to maintain a greater concentration of photosynthetic pigments, proteins and other non-protein amino acids like proline, which are important in osmotic adjustment as osmoprotectants (Evelin et al. 2009).

Maximum yield at the medium salinity level was recorded in this experiment. Highest mycorrhization at the 8 dS m^{-1} salinity level helped the plants to cope up with the deleterious effects of the salinity, as it resulted in an increase in growth, P uptake, phosphatase activity, chlorophyll and protein content and decrease in electrolyte leakage. A 12 dS m^{-1} soil salinity level had an adverse effect on yield, as the number and weight of pods produced was significantly lower. The positive effect of mycorrhizal inoculations on yield under saline conditions is confirmed by the results of Hajiboland et al. (2010).

Conclusion

With increase in soil salinity levels up to 12 dS m^{-1} electrolyte leakage and peroxidase activity increased, whereas, photosynthetic pigments, nutrient uptake, leaf protein content, phosphatase activity, mycorrhization, nodulation and all the morphological parameters measured decreased. Although, mycorrhization decreased at high salinity levels, the AM treatment positively affected photosynthetic pigments, nutrient uptake, leaf protein content, phosphatase activity, mycorrhization, nodulation, peroxidase activity and growth and decreased membrane damage. The results of the present experiment indicate that the growing of *P. mungo* at a 8 dS m^{-1} salinity level after inoculation with a combination of F + A should be recommended. The cultivation of this pulse legume, however, should be discouraged if the salinity level of the soil is nearly 12 dS m^{-1} or above.

Acknowledgement

Authors are thank the Chairman, Department of Botany, Kurukshetra University Kurukshetra for providing the infrastructure and laboratory facilities for carrying out this research.

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CHARACTERIZATION OF DUST SAMPLES FROM A COAL STRIP MINE USING A RESUSPENSION CHAMBER

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ABSTRACT

A metallic cylindrical resuspension chamber (V = 0.437 m^3, S = 0.35 m^2, S/V = 8.38) was constructed to disperse samples of soil and various kinds of dust. The chamber allows on-line determination of number/mass size distribution of aerosol particles formed by dispersion and subsequent sampling of size-segregated particles on filter media. The samples tested were lignite, power plant flue ash and overburden soil from the Nastup coal strip mine in Northern Bohemia. About 20 mg of the individual samples were pneumatically dispersed by 0.5 liter of pressurized air inside the chamber under defined temperature and humidity conditions. Then the dynamics of aerosol size distributions was recorded using an aerodynamic particle sizer with a frequency of 5 seconds. The lignite and flue ash contributed most to the mass of atmospheric aerosol particles smaller than 10/2.5 micrometer – PM_{10}/ $PM_{2.5}$. The re-suspended mass of the samples varied between 0.001% for overburden soil and 0.32% for mine road soil. The aerosolized lignite and flue ash samples, sampled by a Harvard Impactor and a Personal Cascade Impactor Sampler, revealed that the ash contained higher amounts of fine particles than the lignite and subsequent chemical analyses, carried out using SEM-EDX, reveals that the $PM_{2.5}$ fraction formed by dispersion of the ash samples had the highest content of sulphur, and PM_{10} was dominated by Si. PM_{10} was closest to mullite, while the $PM_{2.5}$ fraction contained sulphides, pyrites, pyrrhotites and polytypes of sulphide. The PM_1 fraction was dominated by quartz glass. The fractions of sizes 2.5–1 μm and 0.5–0.25 μm were dominated by Si and S, respectively.

Keywords: fugitive dust, fly ash, pulverized coal, aerosol wettability

Introduction

The atmospheric aerosol, the colloid formed by the dispersion of solids and/or liquids in the gaseous coat of the Earth, has been a subject of interest since the end of the 1930s. A key source of coarse particles in the atmospheric aerosol is the dust produced by numerous surfaces and processes such as deserts, agricultural areas, road surfaces, devastated landscapes, volcanic eruptions, building sites and industrial zones. Aerosols are also produced worldwide in mining and industrial combustion processes. But in contrast to combustion-related aerosols (Ning et al. 2010) the dynamics of atmospheric processes in the resuspension-related aerosol is much simpler. Atmospheric aerosol pollution, with concentrations often exceeding current health limits, is among the most serious problems in air quality both globally and locally (Thimmaiah et al. 2009) in the Czech Republic. While the effects of gaseous pollutants on human health have been sufficiently studied and recorded, much less is known about the origin, behaviour and effects of the aerosol particles. Many studies have shown that aerosol particles have a negative effect on human health and vegetation (Dockery et al. 1993; Pope et al. 1995). Most countries have converted from the original method of aerosol sampling, Total Suspended Particles (TSP), which does not differentiate between particles of different sizes, to the so-called thoracic fraction of the aerosol PM_{10}. This method uses a front-end device for measuring, with an efficiency of 50%, the particles on a filter that have an aerodynamic diameter of 10 μm. A study published in the 1990s reports that concentrations of $PM_{2.5}$ are more strongly correlated with the negative effects on human health than PM_{10} (Schwartz et al. 1996). This has led to the regulation of PM_x emissions and the setting of emission limits for aerosol particles in the USA and the EU. Globally, the current trend is to change to measuring the aerosol fraction $PM_{1.0}$ and to consider the relationship between the number of particles and their size.

The TSP and also the PM_{10} have been continually monitored for many years by the Czech Hydrometeorological Institute and the National Institute of Public Health. PM_{10} and $PM_{2.5}$ are emitted into the atmosphere primarily by high-temperature processes, secondarily by gas-particle conversion and thirdly by resuspension of existing, already deposited matter. While the first two processes add mostly to the fine fraction $PM_{2.5}$, the resuspension contributes to the coarse fraction. Resuspension is a significant source of aerosol for both local and intercontinental air masses.

The generation, capture and measurement of particles in a controlled laboratory environment are very important for determining the emission potentials of different sources of dust and for defining the physical characteristics, chemical composition and toxicological risk connected with the emission of particles into the atmosphere from specific locations or source materials.

During the twentieth century, many researchers described devices for producing or re-suspending dust. These devices were designed for various purposes: e.g., for the simulation of contaminated air inside a dusty factory (Dahmann et al. 1997), the control of industrial pro-

Civiš, M., Thimmaiah, D., Hovorka, J.: Characterization of dust samples from a coal strip mine using a resuspension chamber
European Journal of Environmental Sciences, Vol. 7, No. 1, pp. 14–26
https://doi.org/10.14712/23361964.2017.2

cesses ASTM (1984), in the pharmaceutical industry for the development of dry inhalers that maximize the concentration of fine aerosol in order to achieve the longest possible path of the particles inside the respiratory tract after inhalation (Hindle et al. 1995; Concessio et al. 1997; Newman et al. 2002; Newman et al. 2004), for the exposure of laboratory animals to high concentrations of mineral aerosols in studies on respiratory diseases (Muhle et al. 1990), for the preparation of samples for chemical analyses (Morales et al. 1994), for the measurement of the ecophysiological effect of dust on leaves (Hirano et al. 1995) and simulating the penetration of solid particles into buildings (Lewis 1995; Chen et al. 1999). For many years, standard dusts were prepared, with different sizes of particles and different indices of light refraction. These standards were used in tests of the capture effectiveness of filtration devices and for testing optical devices measuring the dispersion of light by aerosol particles. The most widely known is Arizona Dust, which has been used since the 1940s (SAE Handbook 1943). However, few devices have been designed that specifically examine the contribution of dust or mineral aerosols to the total concentration of aerosol in the atmosphere.

One of the first laboratory devices for measuring dust production from different materials was designed and built by the German researchers Andreasen, Hofman-Bang and Rasmussen (1939). Their experiment used a long, thin-walled tube in which they measured the precipitation time of particles of various materials and deduced their sizes from Stokes Law. That study also reports the first measurements of the fractionation of particles from the parent material under different conditions of relative humidity and moisture. Their device is considered the precursor of the whole series of modern devices for producing dust and for observing the free fall of particles of various sizes (Cheng 1973; Sutter et al. 1982; Heitbrink et al. 1992; Lanning 1995). Subsequent dust formation devices used the principle of vibration screening (Deichman et al. 1944; Sonkin et al. 1946), or produce dust by means of a scraper that breaks the material mounted on a rotating cylinder (Graham et al. 1985).

The majority of laboratory methods dealing with the production of dust from a parent material or with resuspension in relation to the atmospheric aerosol (both in interior and exterior environments) use physical sampling on filtration media with a subsequent gravimetric analysis. The literature contains very few examples of other techniques, such as those based on optical sensors (Li et al. 1996) or a Tapered-Element Oscillating Microbalance (Busacca et al. 1997; Breum 1999).

In this work, we describe the construction of a resuspension chamber used for the dispersion of samples of soil, dust and standard aerosol materials found in the air, under clearly defined temperature-humidity conditions. The main aim of this work is to utilize the device for the dispersion of soil samples from the coal strip mine Nastup in Northern Bohemia and to determine the size distribution of particle number and particle mass in the individual samples.

Material and Methods

The resuspension chamber is made of hot-dip galvanized steel. Its volume (V) is 0.437 m^3, the inner surface area is 0.35 m^2 and the S/V ratio is 8.38. A detailed diagram is shown in Fig. 1.

Two asynchronous ventilators with a regulated power of 15 W, which can be used to create a turbulent environment, are positioned opposite each other in the center of the chamber walls. The ventilators are suspended on rotating heads, allowing the particles to be kept airborne (at 5 m s^{-1}), or to have their deposition increased in a turbulent environment. The ventilators were not used in our experiments.

Bushings of diameter 100 mm, with mechanically operated flaps, are located in the upper and lower bases. The upper bushing is joined to a flexible double-walled, heat-insulated aluminum hose, which supplies air from the humidifier and spiral heater at the desired humidity (20–80%) and temperature (15–40 °C). The lower bushing is connected to a similar hose which encloses the circuit, taking the air from the chamber back to the humidifier. This regulated air circulation allows the required temperature-humidity conditions to be reached inside the chamber. The air humidity is controlled by a commercial ultrasound humidifier using distilled water. The humidifier is positioned in a separate galvanized vessel with one outlet and two inlets. Each inlet is equipped with a regulated ventilator (15 W) and a manually operated flap. The dispersed water droplets are captured in a porous sponge. Some ultra-fine particles can, under certain conditions, leak through the sponge (Vincent et al. 1993), but in our case the dispersed water droplets had sufficient size to be captured with high efficiency. The capture efficiency reached near 100%, as tested by an aerosol spectrometer. The diagram of the humidifier vessel is shown in Fig. 2.

The air is cleaned by a front-end HEPA filter and the humidity of the incoming air is removed by silica gel. Fig. 1 shows the detailed schema of the resuspension chamber.

Prior to suspension, the samples were dried at a temperature of 40 °C and then sieved through a 0.037 mm mesh size Tyler screen. Then the samples were weighed into a special glass vial, which has 4 jets at the base. The vial is inserted into a bushing in the center of the upper part of the chamber. After the required temperature-humidity conditions are reached inside the chamber, the sample is pneumatically dispersed by 0.5 l of dried compressed air from a pressurized bottle (kept at 9–10 atm); the velocity of the flow through the jets reaches 6 m s^{-1}.

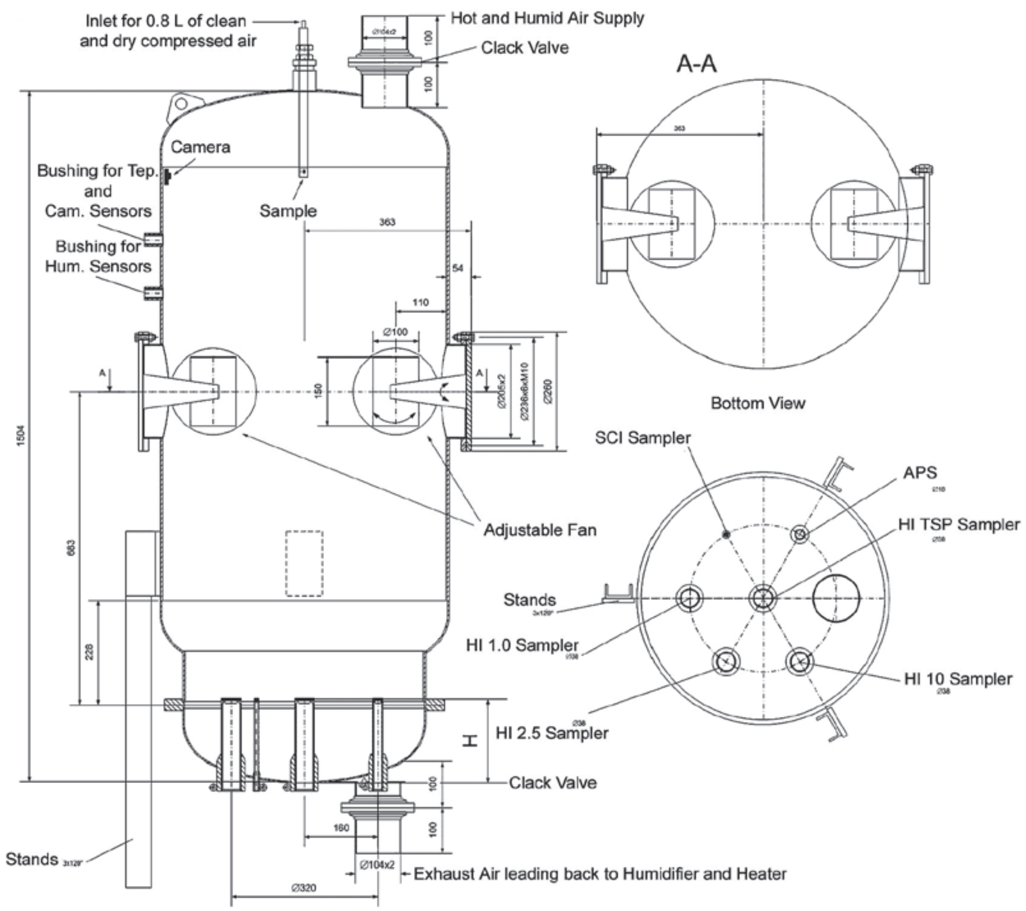

Fig. 1 Diagram of the resuspension chamber.

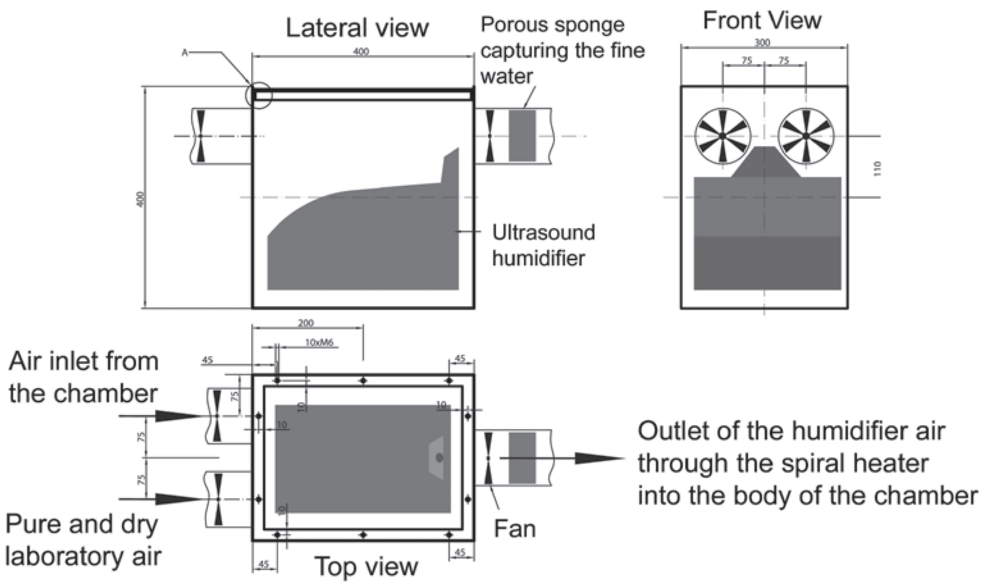

Fig. 2 Diagram of the peripheral vessel with the humidifier.

The process of suspension can be monitored using a camera, which is placed inside the chamber.

The lower base of the chamber is completely removable after loosening the screw joint, which is sealed with a rubber seal. The base can hold sampling heads of various diameters, depending on the measurement (see Fig. 3).

Devices Used in the Experiment

The aerosol produced by the dispersion of a sample was observed using an Aerodynamic Particle Sizer, APS model 3321, which monitored the size distributions of particles in the range of 0.524 to 20 μm. Depending on the suspendable quantities of the individual samples, the

Fig. 3 Sampling heads in the removable base of the resuspension chamber.

analyzed aerosol must be diluted with clean air before it enters the APS, so that the aerosol/air ratio is 1/6.7. The APS inlet has a side feed of diluting air with an adjustable flow rate and a front-end HEPA filter.

Selected samples were gravimetrically analyzed using Harvard impactors (Marple et al. 1987), TSP and a Personal Cascade Impactor Sampler, PCIS (Misra et al. 2002). The resulting size fractions and the air flow rate are given in Table 1. After the dispersion, the samples were separated and captured on one 37 mm and four 25 mm polytetrafluorethylene (PTFE) pre-weighed filters. The filters with deposited aerosol were chemically analyzed. Before each weighing, the filters were kept in a desiccator at close to 50% humidity and 21 °C (saturated KNO_3). During the gravimetric analyses, the exhausted air was replaced with new air with the required temperature-humidity characteristics. The laminar flow created by the gravimetric sampling reached velocities of around 0.003 m s^{-1}. If all gravimetric sampling devices were used, the studied air would be exhausted within 8 minutes. During the aerosol analysis using the APS, the analyzed air is highly diluted, the sampling flow rate is low (0.12 l min^{-1}) and therefore the influence of the dilution of the studied aerosol by the new air can be neglected. A large air flow rate through the chamber during sampling for gravimetric analyses and the determination of mass concentrations of the individual size fractions would require using the correction component for ventilation. However, in our case, gravimetric methods were used to measure changes in the mass of individual size fractions at different temperature-humidity conditions. The masses were evaluated as percentage ratios between TSP and mass values of the individual size fractions. In this way, the use of the aerosol dynamic equations could be avoided.

A scanning Electron Microscope, SEM (Hitachi S-4800) coupled Energy Dispersive Spectroscopy, EDS (Noran System Six software, Thermo Electron Corp., USA) was used to view and analyze aerosol on Teflon filters. A deposit area of 0.36 mm^2 on the filter (0.6 × 0.6 mm) was analyzed.

Table 1 List of impactors used.

	Size Fraction					Air Flow [l min^{-1}]
Harvard Impactor	TSP	PM10	PM2.5	PM1		10
Sioutas Cascade Impactor	>2.5 μm	2.5–1.0 μm	1.0–0.5 μm	0.5–0.25 μm	<0.25 μm	9
Aerodynamic Particle Sizer	0.5–20 μm (52 channels)					1

Table 2 List of samples collected at the Nastup coal strip in Northern Bohemia.

Sample	Locality	Position		Mass Density [kg m^{-3}]	% of mass after sieving	Mass for Suspension [mg]
1	Homogenized lignite	E 13.33299	N 50.40022	1.66	5.31	10
2	Coal stacker	E 13.33890	N 50.41228	2.65	0.96	10
3	Lignite mine bed	E 13.32273	N 50.42746	1.66	2.41	10
4	Coal dust from the road	E 13.32273	N 50.42746	1.66	16.81	10
5	Coal mine	E 13.32727	N 50.42886	1.66	1.05	10
6	Road in the coal mine	E 13.32727	N 50.42886	1.66	6.55	10
7	Dumping site for ash	E 13.28177	N 50.42273	2.22	10.32	10
8	Dumping site for gypsum	E 13.27959	N 50.41076	2.94	3.28	10
9	Ash-fresh	E 13.27959	N 50.41076	2.32	16.32	10
10	Capping soil	E 13.38563	N 50.40848	2.73	0.41	10
11	Ash from Tušimice powerplant	E 13.37946	N 50.38382	2.65	6.77	10

Field Sampling

1–2 kg samples were sampled at the coal strip mine Nastup in the Northern Bohemia (50.415232N, 13.343338E) from different parts of the mine such as mine roads, coal dump, mining locations, flue ash dump, gypsum dump and the soil used for covering flue ash and gypsum dumps. The aim was to find which parts of the mine contribute most to aerosol particle emission. Table 2 lists the samples, their geographical location, virtual density, percentage of mass remaining after 2 hours drying at 40 °C and subsequent sieving through a Tyler screen, and the sample weight used for suspension.

In total, five dispersions of each sample were carried out in the resuspension chamber at 20 °C and RH 50%. The individual size distributions of particle number and mass were measured with 2 s time resolution for the duration of 10 minutes. The first 5 minutes (150 distributions) of each dispersion were subsequently selected (5 dispersions of each sample in total). This selection provided us with data for calculating the average size distribution (from the total number of 750 distributions) of particle mass and number related to the individual samples.

The lignite samples were also studied by gravimetric analysis using the HI under varied time-humidity conditions. The lignite and flue ash samples were then analyzed at 20 °C and RH 50% using the HI and the PCIS, and their size distributions of mass were determined as ratios of individual fractions to the TSP. Elemental analysis using electron microscopy and X-ray energy dispersive spectrometry (EDS) was carried out using the flue ash deposited on the filters.

Results and Discussion

Figs 4 to 14 show the average size distributions of particle number and mass of the individual samples dispersed in the resuspension chamber. Tables 3 to 5 give the statistical values for the average size distributions of particle number and mass including the CMD (count median diameter) or MMD (mass median diameter), respectively, of the aerosol particles.

The data show that the highest average particle number and mass concentrations are those recorded for flue ash (Figs 10 and 14), which is normally disposed of as landfill in the exhausted parts of the surface mine. If the sieved sample (10 mg) becomes airborne in the chamber, the mass concentration would reach 22.88 mg cm^{-3} or as a percentage of the dispersed matter: 2.58% for sample 7 and 2.71% for sample 11 (Table 4 and 5). The effectively dispersed portions as percentages of the total mass of the dry samples are 0.26% for sample 7 and 0.19% for sample 11. The mass concentrations of dispersed matter reached by other samples were 0.34% (sample 8 – gypsum) and 1.88% (sample 4 – mine surface). The average number concentration for sample 7 reached as high as 70 particles per cm^3. The size distribution had two peaks, the first around 0.5 μm and the second around 1.3 μm. The average mass concentration reached by the flue ash sample was 0.59 mg m^{-3} and the size distribution had two peaks, the first at 4 μm and the second at 10 μm. The flue ash is transported by conveyor belt directly to the exhausted parts of the lignite mine from the Tusimice power plant. After reaching the end of belt, it is loosely poured onto the dump, where it should be subsequently covered with overburden soil. The ash samples were also gravimetrically analyzed using HI and PCIS.

The average number concentrations of road lignite dust (sample 4, Table 3) reached 60 particles per cm^3 with a peak around 1.6 μm (Fig. 9) and the mass concentrations were 0.43 mg per cm^3 with two peaks at 3.3 μm and 10 μm (Table 3). The mine roads are used by heavy machinery, which breaks up the lignite (sample 5 – Fig. 8) deposited on the road surfaces from particles with an

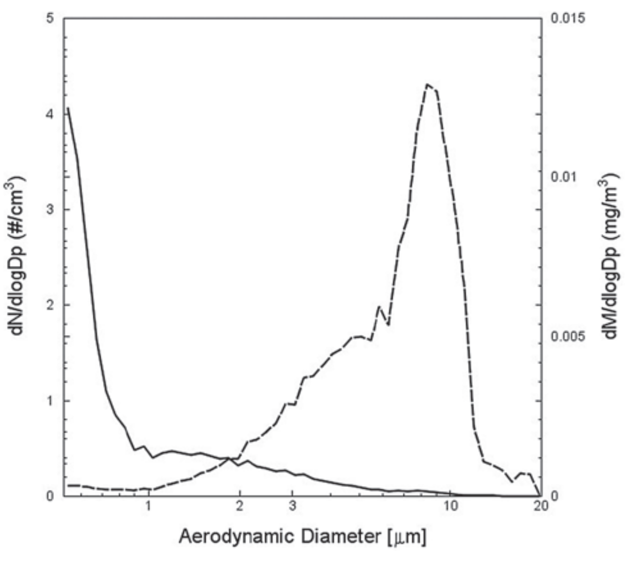

Fig. 4 Sample 1, Homogenized Lignite: continuous line – number size distribution, dashed line – mass size distribution.

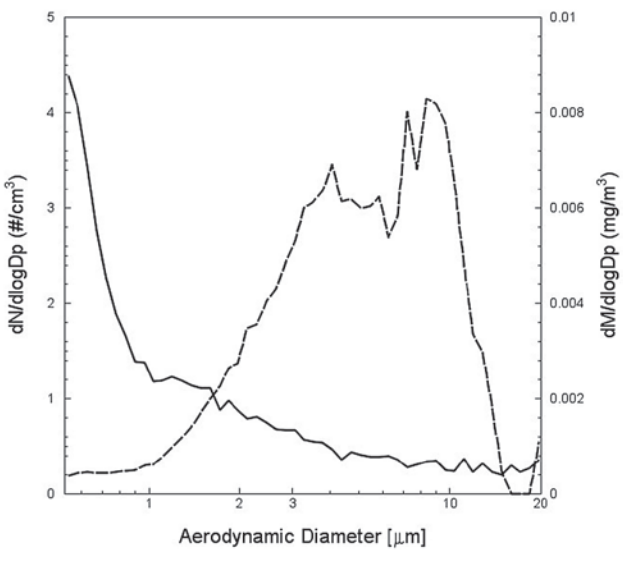

Fig. 5 Sample 2, Coal Stacker: continuous line – number size distribution, dashed line – mass size distribution.

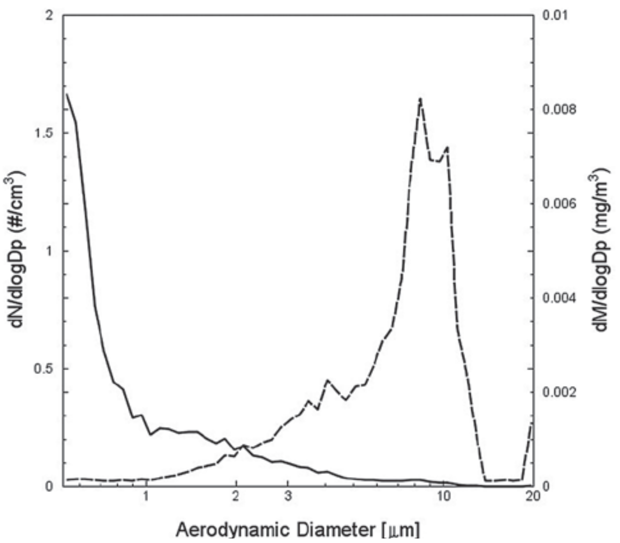

Fig. 6 Sample 3, Lignite Mine Bed: continuous line – number size distribution, dashed line – mass size distribution.

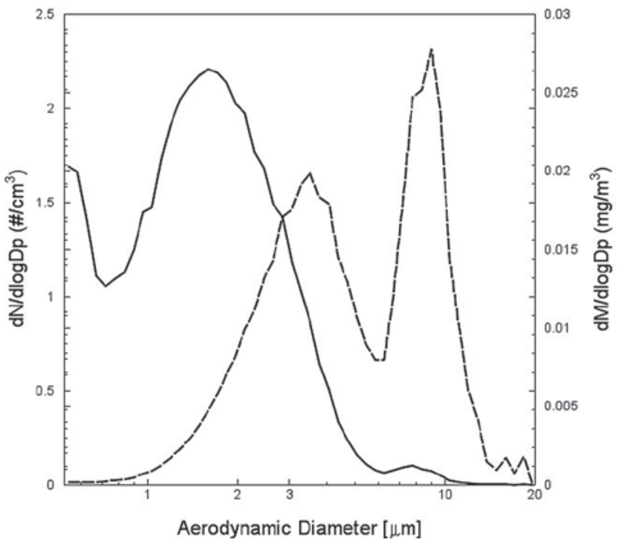

Fig. 7 Sample 4, Coal Dust from the Road: continuous line – number size distribution, dashed line – mass size distribution.

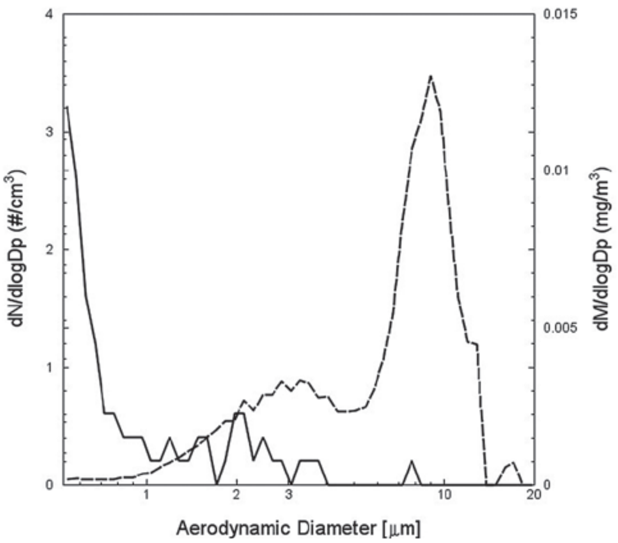

Fig. 8 Sample 5, Coal Mine: continuous line – number size distribution, dashed line – mass size distribution.

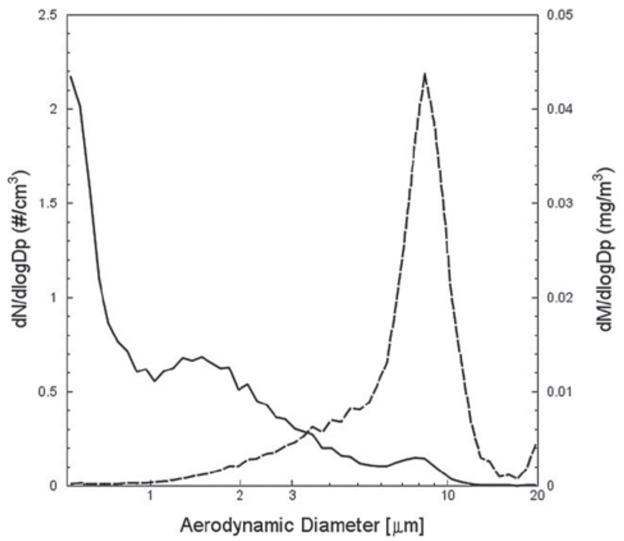

Fig. 9 Sample 6, Road in the Coal Mine: continuous line – number size distribution, dashed line – mass size distribution.

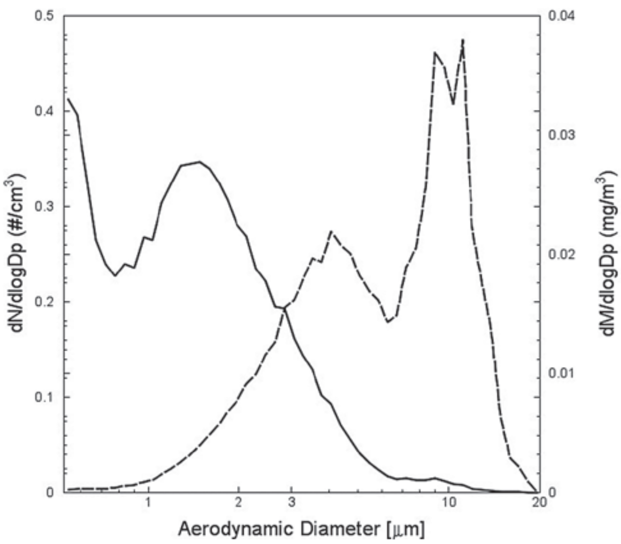

Fig. 10 Sample 7, Dumping Site for Ash: continuous line – number size distribution, dashed line – mass size distribution.

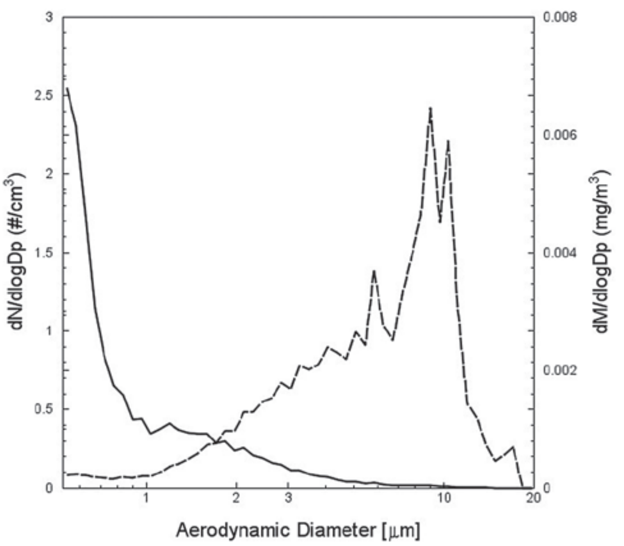

Fig. 11 Sample 8, Dumping Site for Gypsum: continuous line – number size distribution, dashed line – mass size distribution.

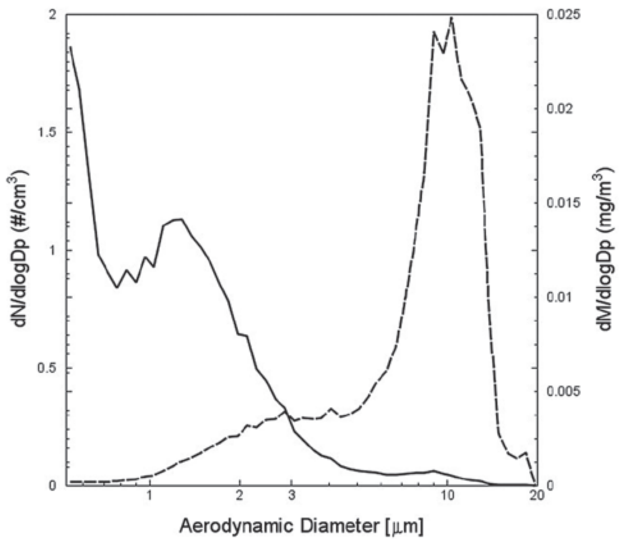

Fig. 12 Sample 9, Dumping Site for Fresh Ash: continuous line – number size distribution, dashed line – mass size distribution.

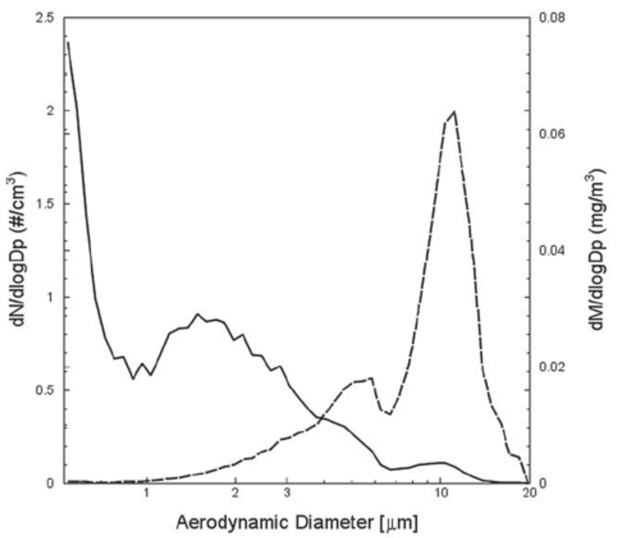

Fig. 14 Sample 11, Ash from Tušimice power plant: continuous line – number size distribution, dashed line – mass size distribution.

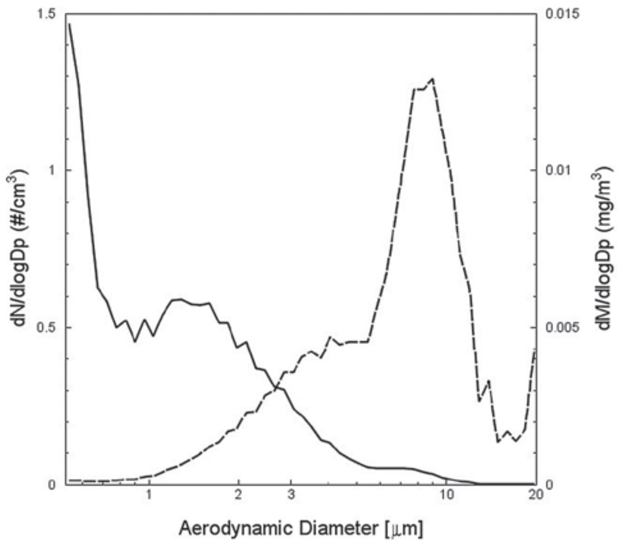

Fig. 13 Sample 10, Capping Soil: continuous line – number size distribution, dashed line – mass size distribution.

original size of around 10 μm into smaller particles with sizes around 3.3 μm (sample 4 – Fig. 7). The final result of this process is that the total mass concentration of atmospheric aerosol is largely affected by the presence of these particles from the mine roads (Fig. 7), rather than the particles originating from the direct mining process or the flue ash dump.

The smallest average mass concentration of particles suspended in the resuspension chamber was reached by the sample of gypsum used in the desulphurization process. The average mass concentration was only 0.08 mg m^{-3} (0.34% of the theoretically possible sample weight and only 0.01% of the total sample mass was dispersed). The gypsum dumps therefore have the least influence on the total mass concentration of atmospheric aerosol.

Fig. 15 shows a map of the North Bohemian lignite mine and indicates the sampling locations. The locations are colour-coded according to the percentage of dis-

Table 3 Sample 1–4.

Sample No	**1**		**2**		**3**		**4**	
Sample Name	**Homogenized Lignite**		**Coal Stacker**		**Lignite Mine Bed**		**Coal Dust from the Road**	
	Number [# cm^{-3}]	Mass [mg m^{-3}]	Number [# cm^{-3}]	Mass [mg m^{-3}]	Number [# cm^{-3}]	Mass [mg m^{-3}]	Number [# cm^{-3}]	Mass [mg m^{-3}]
Average	49.84	0.15	63.80	0.17	21.84	0.08	56.80	0.43
Median	49.84	0.13	67.93	0.14	21.90	0.06	58.48	0.42
Standard Deviation	7.74	6.73	19.14	0.10	2.56	0.07	19.09	0.19
Particle Diameter [μm] CMD/MMD	1.28	6.73	3.05	12.86	1.28	2.13	3.28	11.97
Average Particle Size [μm]	0.77	1.71	0.89	11.97	0.83	3.78	3.05	5.42
Maximum Particle Concentration	73.56	4.32	65.12	0.97	32.36	0.84	95.87	1.54
% of mass after sieving	n.a.	5.31	n.a.	0.96	n.a.	2.41	n.a.	16.81
Suspended % of total sample mass	n.a.	0.03	n.a.	0.01	n.a.	0.01	n.a.	0.32

n.a. = not applicable

Table 4 Sample 5–8.

Sample No	5		6		7		8	
Sample Name	Coal Mine		Road in the Coal Mine		Dumping Site for Ash		Dumping Site for Gypsum	
	Number [# cm^{-3}]	Mass [mg m^{-3}]	Number [# cm^{-3}]	Mass [mg m^{-3}]	Number [# cm^{-3}]	Mass [mg m^{-3}]	Number [# cm^{-3}]	Mass [mg m^{-3}]
Average	30.91	0.15	34.85	0.36	68.74	0.59	31.19	0.08
Median	30.35	0.12	35.77	0.34	67.93	0.54	30.98	0.06
Standard Deviation	4.92	0.10	7.44	0.19	17.79	0.25	7.46	0.07
Particle Diameter [μm] CMD/MMD	5.10	1.98	1.28	3.28	3.05	2.12	3.05	1.84
Average Particle Size [μm]	1.84	3.78	3.60	4.07	2.64	2.45	0.89	3.05
Maximum Particle Concentration	46.02	0.79	58.48	1.75	132.14	1.74	45.42	1.75
% of mass after sieving	n.a.	1.05	n.a.	6.55	n.a.	10.32	n.a.	3.28
Suspended % of total sample mass	n.a.	0.01	n.a.	0.10	n.a.	0.26	n.a.	0.01

n.a. = not applicable

Table 5 Sample 9–11.

Sample No	9		10		11	
Sample Name	Dumping Site for Fresh Ash		Capping Soil		Ash from Tušimice Powerplant	
	Number [# cm^{-3}]	Mass [mg m^{-3}]	Number [# cm^{-3}]	Mass [mg m^{-3}]	Number [# cm^{-3}]	Mass [mg m^{-3}]
Average	37.69	0.27	27.98	0.18	44.06	0.62
Median	37.98	0.22	27.73	0.14	27.73	0.53
Standard Deviation	16984	0.20	4.31	0.15	14.11	0.37
Particle Diameter [μm] CMD/MMD	3.05	2.64	3.05	12.86	3.28	2.64
Average Particle Size [μm]	2.64	2.46	1.17	2.83	0.89	4.07
Maximum Particle Concentration	107.14	1.62	41.19	1.44	80.39	2.83
% of mass after sieving	n.a.	16.32	n.a.	0.41	n.a.	6.77
Suspended % of total sample mass	n.a.	0.19	n.a.	0.00	n.a.	0.18

n.a. = not applicable

persed matter from the total mass of dry sample as it was found in the resuspension chamber experiments.

The APS analysis showed that the flue ash, after its suspension, reaches the highest number and mass concentrations. As far as the size distribution is concerned, it also contains very small particles, 1 μm and smaller. In the North Bohemian mine, therefore, flue ash represents the largest potential source of aerosol particles, although it is stored only in a small number of locations. If not quickly covered with overburden soil, the flue ash dumps turn into major sources of aerosol particles. Another significant aerosol source is the lignite dust deposited on the mine roads. Generally speaking, the potential of a given aerosol source depends on what part of the total area of the mine it occupies. From this point of view, the roads are most probably the largest source of aerosol pollution.

Fig. 16 shows the relation of the individual size fractions of lignite to temperature and humidity. The relation is expressed as the ratio of individual size fractions to the TSP (100%). The data was obtained using the HI gravimetric method. Five measurements were made for each set of temperature-humidity conditions. The sample weight for each dispersion was 10 mg. Fig. 16 shows the mass of lignite size fractions for different temperature and humidity conditions. The lignite aerosol mass slowly increased with relative humidity in comparison with inorganic aerosol (Hu et al 2010). The largest increase in mass was identified at 30 °C and a RH 80% for the fractions $PM_{2.5}$ and PM_1. The fraction PM_1 showed the highest sensitivity to humidity changes at 30 °C.

Fig. 17 shows the size distribution of particle mass expressed as ratios of individual fractions of deposited

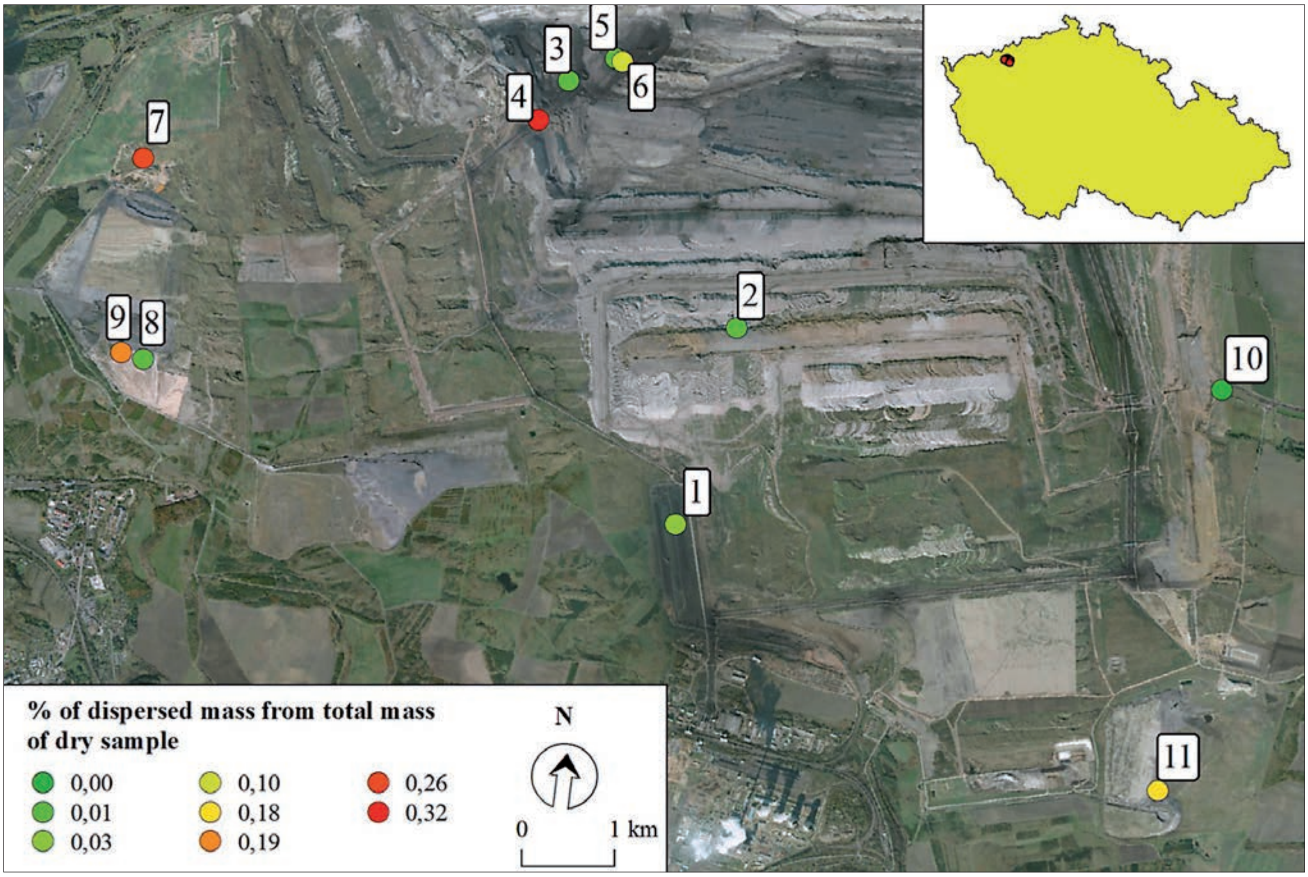

Fig. 15 Aerial view of the Nastup mine with sampling locations marked. 1 – homogenization dump, 2 – overburden damping machine, 3 – mine bed, 4 – dust deposited on the road surface, 5 – location of mining, 6 – dust deposited on the road surface, 7 – flue ash dump, 8 – gypsum dump, 9 – flue ash, 10 – overburden, 11 – Tusimice flue ash.

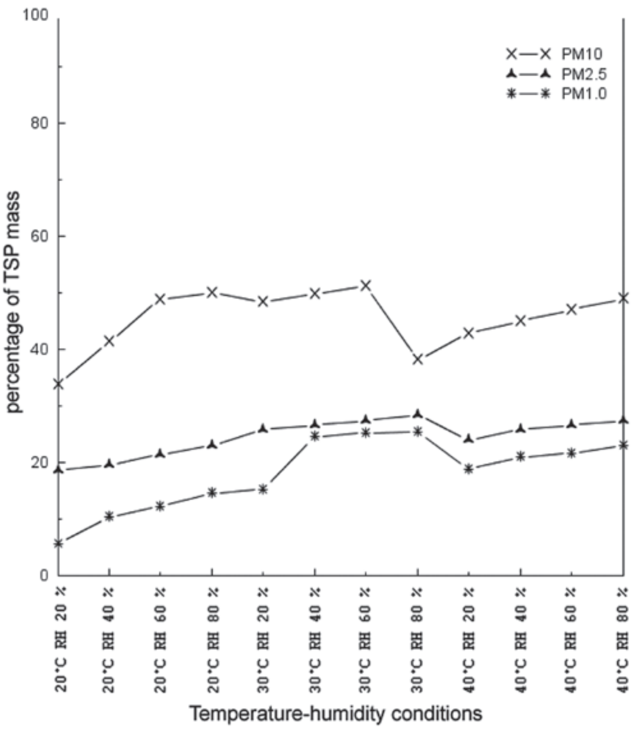

Fig. 16 Mass of the individual size fractions of lignite under different temperature-humidity conditions relative to TSP [%].

matter to the TSP using the data from the HI and PCIS at 20 °C and a RH 50%. The TSP from the PCIS was calculated as the sum of all mass values. Fraction HI $PM_{2.5}$ reached the value of 17% and PM_1 10%.

Graphs of size distributions of mass concentration of individual fractions of flue ash are shown in Fig. 18. They are expressed as ratios of the individual fractions to the TSP. Flue ash contains higher amounts of particles in size fractions $PM_{2.5}$ and PM_1. The difference of 2.5–1 µm reaches 10% and the PCIS fraction 2.5–1 µm also reaches 10%. Flue ash contains a higher proportion of the finer fractions 2.5–1, 1–0.5 and 0.5–0.25. These results indicate that flue ash is a greater health hazard for the population of the North Bohemian Lignite Basin than the lignite mining itself. Loosely stored flue ash can easily turn airborne during dry and windy summer periods and in this way becomes a hazardous source of aerosol if it is not quickly covered with soil.

Elemental analysis of samples of flue ash was carried out using SEM/EDS. The resulting images are shown in Figs 19 and 20 and elemental composition summarized in Table 6. The main component of the PM_{10} fraction is mullite $Al_6Al_4(O_3)(O_{½}, OH, F)Si_3O_{16}$, which is most frequently found in flue ashes. The $PM_{2.5}$ fraction was dominated by sulphides, pyrites, pyrrhotites and polytypes of

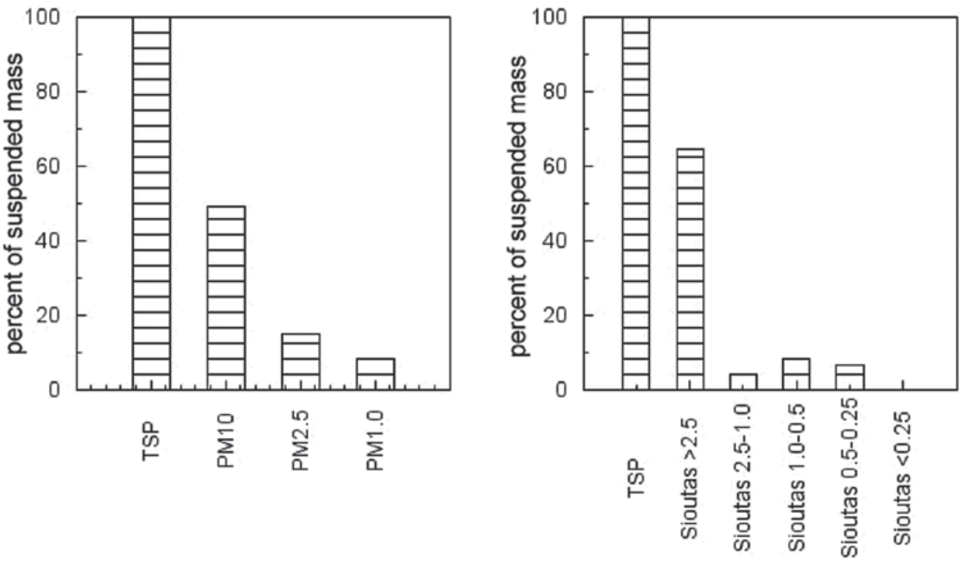

Fig. 17 Size distribution of particle mass in lignite sample, as a ratio of the individual fractions to the TSP, data from HI and PCIS (20 °C and RH 50%).

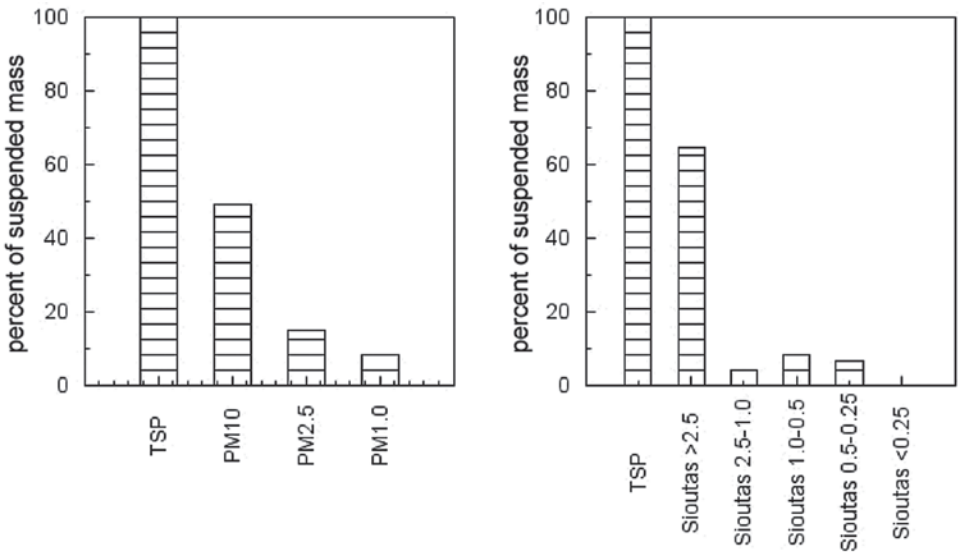

Fig. 18 Size distribution of particle mass in flue ash sample, as a ratio of the individual fractions to the TSP, data from HI and PCIS (20 °C and RH 50%).

sulphide. The PM_1 fraction contained the highest percentage of sodium, the most probable particle composition being sodium glass.

The analysis of the most frequent grains captured on the PCIS from fraction A (> 2.5 μm) identified mullite. The analysis of fractions PCIS B (2.5–1.0 μm) and PCIS C (1.0–0.5 μm) identified quartz. PCIS D (0.5–0.25 μm) contained mostly pyrites and SIO E (< 0.25 μm) mostly as sodium glass. Fig. 19 shows an SEM image of the filter with the deposit of aerosol of flue ash, size fraction B (2.5–1.0 μm). Agglomerations of particles on the filter are visible at the sites labelled 1, 2, 3 and 4.

Conclusions

A resuspension chamber was built as a part of the author‘s doctoral work. The chamber was designed to disperse loose solids in air under laboratory conditions of regulated temperature and relative humidity. The aerosol produced can be observed using APS or by a gravimetric method of sampling. The chamber enables us to simultaneously study the size distributions of atmospheric aerosol samples and their chemical composition.

The resuspension chamber was used for the dispersion of samples at 20 °C and a RH 50% collected from various parts of a North Bohemian lignite mine. The samples were subsequently analyzed using APS with a time resolution of 5 s. Average profiles of size distributions of particle number and mass concentration were determined for the individual samples. It was found that flue ash reached the highest mass concentrations after the dispersion: up to 2.7% of the dispersed sample weight, and 0.26% of the total mass of the sample, became airborne. In contrast, gypsum reached only low mass concentrations after its dispersion in the resuspension

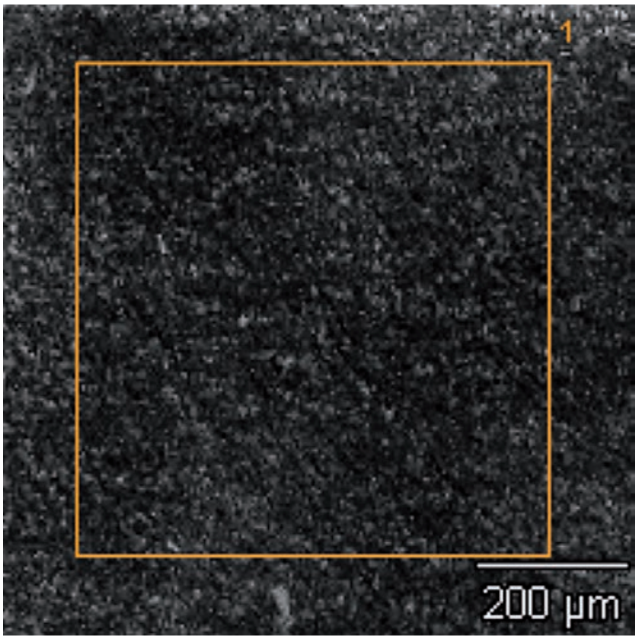

Fig. 19 Electron microscope image of the filter with deposit of flue ash aerosol, size fraction TSP.

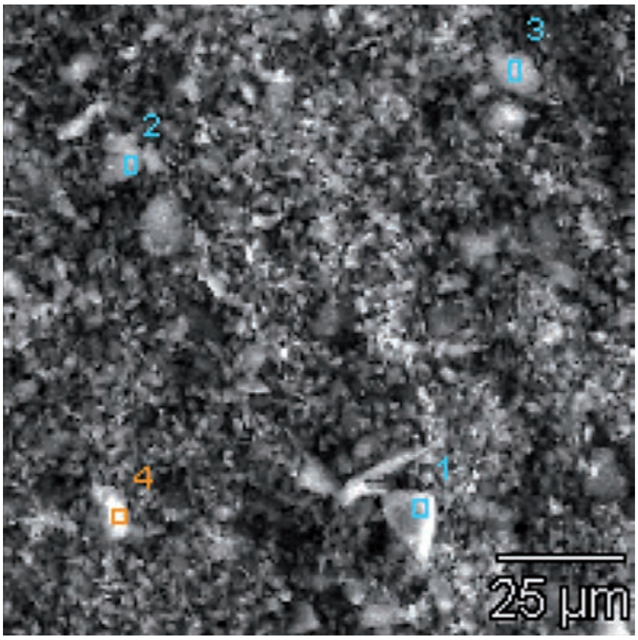

Fig. 20 Image of the filter with deposit of flue ash aerosol, size fraction PCIS B (2.5–1.0 µm).

Table 6 Percentage of elements in TSP, PM_{10}, $PM_{2.5}$, PM_1 and SIOUTAS > 2.5, 2.5–1.0, 1.0–0.5, 0.5–0.25 and < 0.25 µm.

	Atom (%)												Species
	O	Na	Mg	Al	Si	S	Cl	K	Ca	Ti	Fe	Cu	
TSP	73.35	0.26		2.37	20.20	2.24		0.07	0.30	0.08	1.12		All species
PM_{10}	71.40		0.35	3.27	23.14	0.94		0.13	0.15		0.62		Mullite
	75.11		0.81	4.32	16.08	2.09		0.13	0.48	0.11	0.88		Mullite
	67.32	0.44	0.76	7.77	20.74	1.08		0.48	0.35	0.10	0.85	0.11	Mullite
	68.07		0.69	7.70	20.42	0.93		0.50	0.16	0.11	1.30	0.14	Mullite
$PM_{2.5}$	71.51	1.35		2.73	11.43	10.4			1.72		0.89		Polytypes of sulphide
	74.85	1.15		1.79	11.30	8.03			0.55		2.33		Polytypes of sulphide
	61.35		0.73	8.13	21.91	2.98		0.72	0.54	0.10	3.35	0.18	Polytypes of sulphide
	77.11	4.35		1.23	12.96	1.45					0.91	2.00	Polytypes of sulphide
PM_1	77.18	3.24	0.91	4.10	10.72	1.77		0.15	0.22		1.70		Sodium glass
	73.34	10.50		3.93	4.34							7.89	Sodium glass
	74.88	7.46		2.97	10.59							4.10	Sodium glass
SIO A	72.68			1.41	22.70	0.39			2.61		0.21		Mullite
	71.48			0.59	27.59	0.33							Mullite
	81.48			1.94	16.58								Mullite
	74.95			0.42	24.27								Mullite
SIO B	65.90	0.31		0.63	32.03	0.49			0.34		0.31		Quartz
	72.06			0.77	26.35	0.36			0.23		0.24		Quartz
	57.10			1.32	40.88	0.4					0.29		Quartz
SIO C	67.23			1.64	28.23	0.46			0.34		2.11		Quartz
	72.51			0.65	26.84								Quartz
SIO D	58.92	28.70		1.42	6.13	3.09	0.41	0.08	0.57		0.6	0.11	Pyrites
	62.85	12.50		2.88	15.34	4.20	0.28	0.17	0.71		0.89	0.16	Pyrites
	64.45	16.70		2.55	13.35	1.68	0.20	0.11	0.32		0.62		Pyrites
	63.73	10.30		3.36	17.88	2.02	0.33	0.28	0.68		1.25		Pyrites
SIO E	82.25	1.88	0.60	3.98	9.22	2.07							Sodium glass
	79.67	7.06		2.24	6.12	1.31						3.59	Sodium glass
	70.29	0.53		8.68	13.32	3.00		0.16	1.41	0.17	2.41		Sodium glass
	75.81	1.84	0.85	5.92	9.47	1.02		0.23	0.34		4.53		Sodium glass

chamber: 0.34% of the dispersed sample weight (0.01% dispersed matter of the total mass of dry sample), and overburden soil showed the lowest mass concentrations after the suspension (only 0.001% dispersed matter from the total mass of the dry sample). Dust particles from the mine roads and currently exploited locations will probably contribute the most to the total mass concentration of the atmospheric aerosol in the region studied because the mass concentrations of dust reached up to 1.88% of the dispersed matter and the areas producing such dust form the largest part of the total area of the mine.

The lignite and flue ash samples were gravimetrically analyzed using HI under various temperature-humidity conditions. We found that the ratio of individual size fractions to TSP increased with increase in relative humidity, most significantly with the fraction PM_{10} at 30 °C and RH 60%. At 40 °C, the PM_{10} fraction increased in mass only slightly. The PM_1 fraction showed the greatest tendency to increase in mass at 30 °C and RH 40–80%.

A profile of the size distribution of particle mass was obtained from the gravimetric analysis of lignite and flue ash samples at 20 °C and RH 50%. The analysis revealed higher mass values at smaller fractions (2.5–1, 1–0.5 and 0.5–0.25 μm) of flue ash in comparison to the same fractions of lignite.

The filters with flue ash deposits were chemically analyzed using an electron microscope. The analysis showed elemental differences in the fractions studied. Dominant species were identified in the individual fractions (mullite, pyrites, polytypes of sulphide and sodium glass).

Acknowledgements

This work was funded by the Grants Agency of Charles University, Prague, under the project "Determination of the re-suspendable fraction in samples of soil and street dust using a resuspension chamber" No. 40307 and by the Czech Ministry of Environment under the project "Determination of the concentrations of atmospheric aerosol with high size and time resolution for the purpose of sources location and toxicity estimate" No. VaV SP/1a3/149/08.

The author would like to thank R. Nedbalek and M. Eng, who made it possible for us to collect samples at the Nastup coal strip mine in Northern Bohemia, and J. Brejcha and M. Eng for the density measurements on the samples.

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PROPOSAL FOR AN INDICATIVE METHOD FOR ASSESSING AND APPORTIONING THE SOURCE OF AIR POLLUTION

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ABSTRACT

The main objective was to provide a feasible approach for approximately apportioning the sources of air pollution based on simple calculations using measured concentrations of ambient air pollutants and meteorological data. The methods are based on dividing a monitored area into sectors using a common compass rose and obtaining hourly average concentrations of pollutants and relevant data on wind direction and speed over at least three seasons of a year. As a result, the relative contributions of all sources of air pollution in an area with a monitoring station are determined, together with the absolute contributions of single or groups of sources of pollution and the levels to which the emissions need to be reduced to meet the requirements of Directive 2008/50/ESt. The proposed methods are verified using data from measuring stations complying with that required by this Directive and are suitable for improving plans aimed at reducing air pollution as defined by the same document. This approach using data for a particular area revealed a total concentration of PM_{10} of 22.72 μg/m^3, with the maximum permissible concentration of 12.33 μg/m^3 this necessitates a reduction in concentration of the contributions from this selected group sources of 10.37 μg/m^3. When these simple methods are used, further and more accurate apportionments of the source could be made using more complex mathematical modelling. However, this is only necessary in areas with many sources of pollution. Although these methods cannot compete with disperse and other types of modelling they may be useful in providing a basic overview of the situation in a particular area.

Keywords: air pollution, PM_{10} concentration, source apportionment, Directive 2008/50/ES, pollutant monitoring, air quality improvement plans

Introduction

Air pollution is an important environmental risk factor with an unquestionable adverse effect on human health (Amodio et al. 2009; Ruiz et al. 2011). The fact that the levels of risk to health from air pollutants are not negligible is mainly due to the political and economic status of a country with a sharp contrast between social pressure toward an acceptable air quality and the financial and economic pressures for sustaining production and consumption. The policy of a democratic, law-abiding state influenced by these two opposing forces usually seeks (from a historical perspective, at least temporarily) an equilibrium as expressed in its legislation (DIRECTIVE 2008/50/ EC 2008). Such an equilibrium, however, may be easily disturbed by inadequate inspection or adherence to the adopted legal norms. Yet an apparent problem in many countries (Mijić et al. 2009; Masiol et al. 2010; Unal et al. 2011), including the Czech Republic, is non-adherence to legal limits concerning ambient air pollutants.

Health risks of ambient air pollutants acceptable for society are, among others, legislatively regulated by limit values for pollutants in the atmospheric boundary layer, particularly in residential areas or agglomerations (US EPA 2000). Legislation contains numerous requirements concerning acquisition and assessment of data on air pollution. Thus, it might be said that from a legislative point of view, the issue has been resolved. Unfortunately, the opposite is true since the regulations do not answer the fundamental questions of what is the contribution of individual sources of pollution to the overall pollution in a particular area, for which sources corrective measures are needed to improve air quality and the extent to which the regulations are not adhered to in that area. Such solutions should be primarily fair, reliable and simple so that they could be implemented using data that are collected as required by the above legislation and thus are immediately available.

Currently, the contribution to air pollution of individual sources is usually determined from data on sources of emission (Juda-Rezler et al. 2006; Srivastava et al. 2008; Viana et al. 2008; Thimmaiah et al. 2009; Mooibroek et al. 2011) using dispersion (diffusion) models (Perez-Roa et al. 2006). Given the fact that emissions are spread in the air by diffusion and flow of air and the relations describing these phenomena are relatively complicated (Cimorelli et al. 2004), dispersion models used to calculate air pollutant concentrations utilize many simplifications, leading to results different from the measured data. Although mathematical models are indispensable for predicting air pollution and additional calculations related to the measured data, this approach has other practical drawbacks. One example is the frequent unreliability of

Jiřík, V., Tomášková, H., Machaczka, O., Kissová, L., Břežná, B., Dalecká, A., Janout, V.: Proposal for an indicative method for assessing and apportioning the source of air pollution
European Journal of Environmental Sciences, Vol. 7, No. 1, pp. 27–34
https://doi.org/10.14712/23361964.2017.3

officially reported data on emissions, another the irrelevant results of dispersion studies due to unavailable data on some sources of pollution in the monitored area.

This article proposes methods that preferably use accurate data that are an increasingly reliable source of information on air pollution as compared with dispersion models and are thus, in accordance with valid legislation, a critical starting point for assessing higher emission loads, that is, those close to or beyond the limit values. The objective of the methods is to use as simple processing of the measured data as possible (Xiao et al. 2012) and additional dispersion models to estimate the contribution of individual sources of pollution in a particular area so that these data may be used as a starting point for adopting regional programs for reducing air polluting emissions, which determine mandatory corrective measures aimed at improving air quality and reducing risks to health.

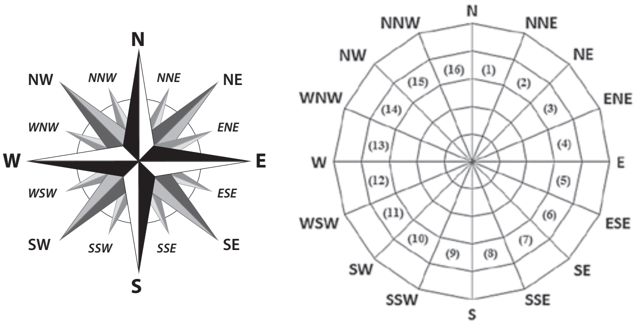

Fig. 1 Wind rose divided into 16 sectors.

Materials and Methods

Measurements

The input data set comprises hourly average concentrations (C_h) of ambient air pollutants. The approach is used for pollutants transported to a monitoring station in a particular area by diffusion and flow of air from all surrounding sources. Data on pollutant concentrations obtained from fixed monitoring stations are in accordance with Directive 2008/50/EC of the European Parliament and the Council (hereinafter the Directive) (Directive 2008/50/EC Chapter 5 2008) are, for the purposes of public health protection, considered valid and representative for the entire location.

Hourly data on pollutant concentrations (C_h) and wind direction and speed must be acquired over a longer time period (Hrust et al. 2009) to eliminate seasonal and yearly fluctuations and ensure that the average concentrations over the entire period are really representative for the area. The longer time period refers to the time for which the Average Exposure Indicator is calculated in accordance with the Directive, for example, 3 years.

A monitored area may be divided into sectors (*k*; directions as defined angles with vertices at a sampling point) according to the cardinal, intercardinal and secondary intercardinal points (Fig. 1).

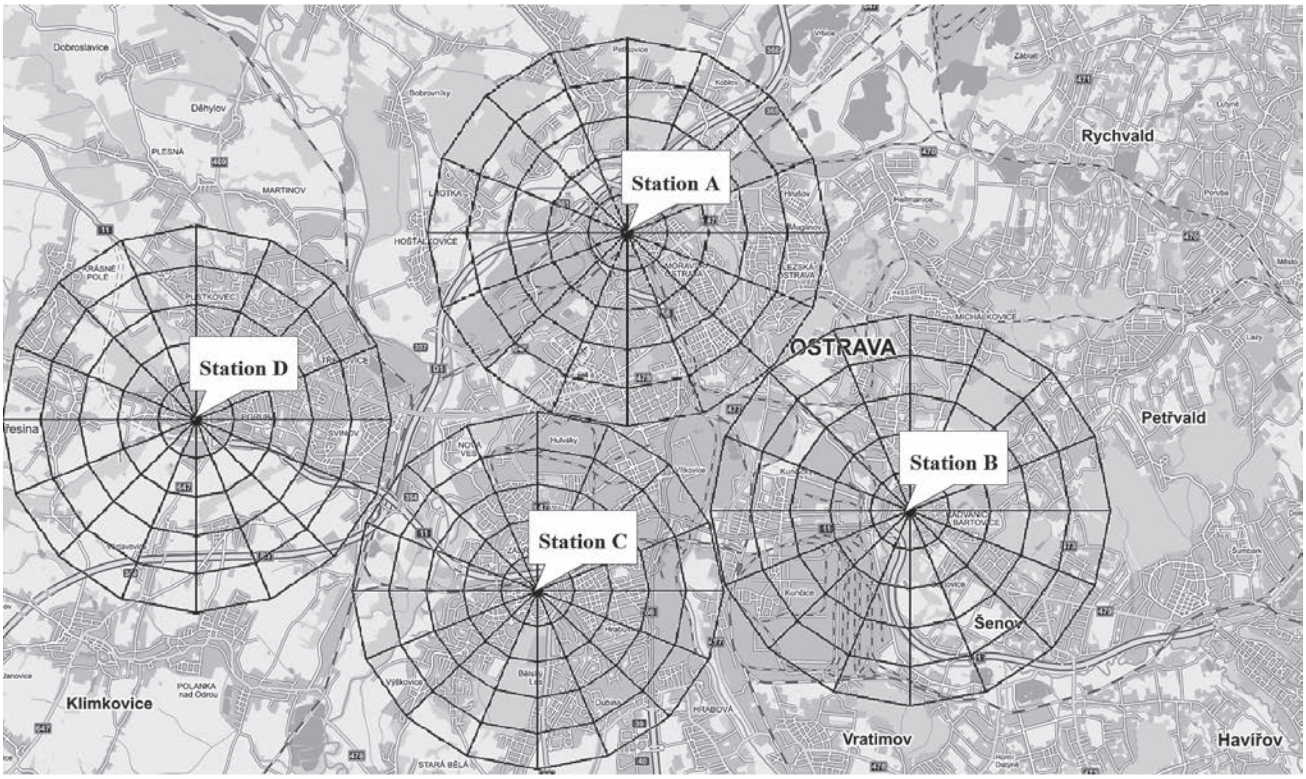

Fig. 2 An example of four monitoring stations and related wind roses.

Such division into sectors may be used for all monitored areas, with sectors having their vertices at the sampling points (Fig. 2).

The data on hourly average concentrations (C_h) of an air pollutant, hourly average wind direction and speed values may be used to calculate average concentrations (C_k) of the pollutant for individual sectors over the entire time period as follows:

$$C_k = \frac{\sum_{h=1}^{N_k} C_{kh}}{N_k} \text{ [µg m}^{-3}] \tag{1}$$

Where:
- k is the sector (each sector is an angle of 22.5°; see Fig. 1);
- h is the hourly value, or average value per hour;
- C_{kh} [µg m^{-3}] is the hourly average concentration of the pollutant in the air at an hourly average wind direction $u_k > 0.5$ m s^{-1} from sector k;
- N_k is the number (frequency) of hourly average wind directions (and measured concentrations C_{kh}) over the entire time period from sector k; and
- C_k [µg m^{-3}] is the average concentration of the pollutant in the air over the entire time period with an hourly average mean direction $u_k > 0.5$ m s^{-1} from sector k.

Authors' note: The average or median value of a set of data on concentrations of a pollutant should be calculated with regard to the statistical distribution of the data. When determining average concentrations in compliance with legislation valid in EU countries, this approach is not used and the law requires that median values are calculated as arithmetic means. Such an approach, however, is not statistically correct.

Thus, the average concentrations C_k represent partial concentrations of the pollutant at a sampling point in a monitored area carried by the air flow from a particular circular sector downwind if the wind speed is $\bar{u}_h > 0.5$ m s^{-1}. Under calm wind conditions, i.e. $\bar{u}_h \leq 0.5$ m s^{-1}, the average concentration at the sampling point (C_s) is calculated as follows:

$$C_s = \frac{\sum_{h=1}^{N_s} C_{sh}}{N_s} \text{ [µg m}^{-3}] \tag{2}$$

Where C_{sh} [µg m^{-3}] is the hourly average concentration of the pollutant under calm wind conditions, i.e. $u_k \leq 0.5$ m s^{-1} (US EPA 2000), and N_s is the number (frequency) of concentrations C_{sh} measured over the entire period. The lower and upper limits of the confidence interval (at a significance level of 95%) for the average concentrations C_k and C_s are referred to as C_{k95L}, C_{k95H}, C_{s95L} and C_{s95H}, respectively. These may be used to determine the significance of differences in concentrations between individual sectors or concentrations under calm wind conditions.

Relative frequencies R_k and R_s may be expressed as quotients:

$$R_k = \frac{N_k}{(\sum_{k=1}^{16} N_k) + N_s} \tag{3}$$

$$R_s = \frac{N_s}{(\sum_{k=1}^{16} N_k) + N_s} \tag{4}$$

with

$$(\textstyle\sum_{k=1}^{16} R_k) + R_s = 1 \tag{5}$$

Relative contributions P_k and P_s of the pollutant may be estimated from average concentrations C_k and C_s of the pollutant and relevant frequencies N_k and N_s, respectively:

$$P_k = \frac{N_k C_k}{(\sum_{k=1}^{16} N_k C_k) + N_s C_s} \tag{6}$$

$$P_s = \frac{N_s C_s}{(\sum_{k=1}^{16} N_k C_k) + N_s C_s} \tag{7}$$

with

$$(\textstyle\sum_{k=1}^{16} P_k) + P_s = 1 \tag{8}$$

Concentrations C_k and C_s and frequencies N_k and N_s may be used to calculate the average concentration C_m in the monitored area:

$$C_m = \frac{(\sum_{k=1}^{16} N_k C_k) + N_s C_s}{(\sum_{k=1}^{16} N_k) + N_s} \text{ [µg m}^{-3}] \tag{9}$$

Similarly, concentration contributions D_k and D_s may be calculated, using either relative contributions P_k and P_s, respectively, and the average concentration C_m or relative frequencies R_k and R_s, respectively, and the average concentration C_k:

$$D_k = P_k\, C_m = R_k\, C_k \text{ [µg m}^{-3}] \tag{10}$$

$$D_s = P_s\, C_m = R_s\, C_s \text{ [µg m}^{-3}] \tag{11}$$

with

$$(\textstyle\sum_{k=1}^{16} D_k) + D_s = C_m \tag{12}$$

This approach corresponds with that used in dispersion models where long-term (yearly) concentrations of a pollutant are the sum of contributions corresponding to concentrations for individual standardized meteorological situations multiplied by the average frequency of these situations (Bubník et al. 1998). As is the case with D_k and D_s, relative frequencies R_k and R_s and a selected limit value LV may be used to determine maximum permissible concentration contributions $D_{k,max}$ and $D_{s,max}$, respectively:

$$D_{k,max} = R_k\, LV \text{ [µg m}^{-3}] \tag{13}$$

$$D_{s,max} = R_s\, LV \text{ [µg m}^{-3}] \tag{14}$$

If concentration contributions D_k or D_s are greater than maximum permissible concentration contributions

$D_{k,max}$ and $D_{s,max}$, respectively, necessary reductions in concentration contributions in sectors k may be simply determined as follows:

$$\Delta D_k = D_k - D_{k,max} \text{ [µg m}^{-3}\text{]} \quad (15)$$

$$\Delta D_s = D_s - D_{s,max} \text{ [µg m}^{-3}\text{]} \quad (16)$$

No reductions in emission contributions are needed if $\Delta D_k \leq 0$ and $\Delta D_s \leq 0$. Concentration contributions D_k may be considered as minimum since they do not involve contributions from sources under calm wind conditions although they affect the area. This is expressed by concentration contribution D_s, a sum of all concentration contributions from all surrounding sources in the area under calm wind conditions that is unable to provide adequate information about contributions of sources in individual sectors k. This contribution may be apportioned among individual sectors or sources using approaches for calm wind periods in dispersion models (Bubník et al. 1998).

The total concentration contribution D_s over a calm wind period may be roughly apportioned among sectors k or individual sources j according to the following formula, assuming that the emission flow of the pollutant resembles a cylinder with radius X and height L:

$$D_{skj}(calc) \cong \frac{Q_{kj} \cdot CF \cdot T_S}{2\pi \cdot X_{kj}^2 \cdot L} \text{ [µg m}^{-3}\text{]} \quad (17)$$

with

$$\sum_{k=1}^{16} \sum_{j=1}^{N_{kj}} D_{skj} = D_s \quad (18)$$

In addition to emission flow of a source Q_{kj} [t/year] and its distance from a sampling point X_{kj} [m], such a concentration contribution is also dependent on duration T_s of the calm wind period and the average height of the atmospheric mixed layer L [m] (US EPA 1999). The conversion factor CF is 114,155.25 [year × µg / h × t]. Although this is only a rough estimate as it does not consider, for instance, the height of sources (a more accurate estimate requires the use of a dispersion model for calm wind conditions), it is sufficient for the purpose as seen from experimental data. The reliability of the estimate may be considerably increased if nearly all sources are considered and the fulfillment of the condition in formula (15) may be verified.

Thus, concentration contributions of sources j in sectors k are calculated as follows:

$$D_{k,tot} = D_k + \sum_{j=1}^{N_{kj}} D_{skj} \text{ [µg m}^{-3}\text{]} \quad (19)$$

where N_{kj} is the number of sources j in sector k.

Maximum permissible concentration contributions of individual sources under calm wind conditions may be roughly estimated in sixteen sectors k by evenly apportioning maximum permissible concentration contribution D_s among all the considered sources j:

$$D_{skj,max} = D_s / (N_{kj} \cdot 16) \quad (20)$$

with

$$\sum_{k=1}^{16} \sum_{j=1}^{N_{kj}} D_{skj,max} = D_{s,max} \quad (21)$$

Therefore, total maximum permissible contributions of sources in sectors k are calculated as follows:

$$D_{k,max,tot} = D_{k,max} + \sum_{j=1}^{N_{kj}} D_{skj,max} \text{ [µg m}^{-3}\text{]} \quad (22)$$

Total necessary reductions in concentration contributions of all sources in sectors k are calculated as:

$$\Delta D_{k,tot} = D_{k,tot} - D_{k,max,tot} \text{ [µg m}^{-3}\text{]} \quad (23)$$

If a selected sector k contains N_{kj} sources, an adequate dispersion model, i.e. calculation, may be used to determine the relevant concentration contributions $D_{kj}(calc)$ valid at the sampling point. In this case, data on concentrations are obtained not from measurements but from a dispersion model and may not therefore be valid (see the reasons stated in the Introduction). Validity of dispersion model results may be simply verified using the following formula for summing up concentration contributions $D_{kj}(calc)$:

$$\sum_{j=1}^{N_{kj}} D_{kj}(calc) = D_{k,tot} \text{ [µg m}^{-3}\text{]} \quad (24)$$

If the relation is not fulfilled, data on concentrations from the dispersion model do not correspond with the measured data and model calculation results have to be corrected. Necessary reductions in concentration contribution ΔD_{kj} of a source in the monitored area may be determined analogically to those in case of ΔD_k, as seen from formula (15):

$$\Delta D_{kj} = D_{kj}(calc) - \mathrm{D}_{k,max,tot} \text{ [µg m}^{-3}\text{]} \quad (25)$$

Additionally, a suitable dispersion model and the necessary reductions in concentration contributions in the particular area may be used to determine necessary reductions of emissions for each source, potentially leading to adherence to the adopted limit values.

Equipment and Software

To verify the methods, data from monitoring stations were processed with the statistical software Stata (Stata Corp., Release 9, College Station, Texas, USA) and the spreadsheet program Excel (Microsoft Corp., Worldwide, USA).

Results

The above methods were verified using experimental data from measuring stations in some boroughs of the city of Ostrava included in the national network consis-

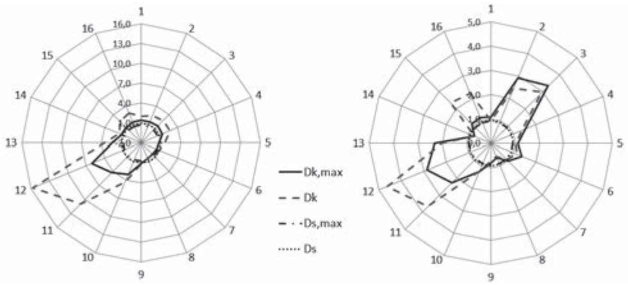

Fig. 3 Average concentrations of PM_{10} in the air obtained from measuring station B in winter and summer.

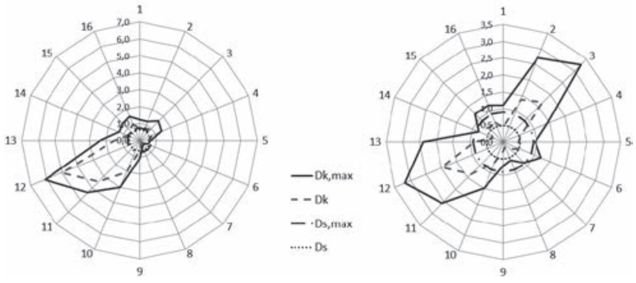

Fig. 4 Average concentrations of NO_2 in the air obtained from measuring station B in winter and summer.

tent with the Directive. The measuring station in one of the city boroughs (Radvanice and Bartovice) is referred to as B in Fig. 2. In this as well as other areas of the city, measuring stations recorded readings above limit values for pollutants, especially particulate matter. All hourly average concentrations and data on wind direction and speed were obtained over several years (all seasons over a period of six years).

Data used to verify the above method are graphically summarized in Fig. 3 for PM_{10} and in Fig. 4 for NO_2. The detailed results are listed only for PM_{10} in numerical form because of the possible scope of the article, as a demonstration of the above method.

Table 1 clearly shows that relative contributions of pollutants from sectors k = 10, 11 and 12 are $\Sigma P_k = 0.34$ and relative contributions from all sectors under calm wind conditions are $P_s = 0.30$. This corresponds with concentration contributions $\Sigma D_k = 20.28$ [μg/m³] and $D_s = 17.85$ [μg/m³]. If maximum permissible concentration contributions are $D_{k,max} = 9.91$ [μg/m³] for these three sources and $D_{s,max} = 12.88$ [μg/m³] for all sectors under calm wind conditions, the necessary reductions in concentration contributions are $\Delta D_{10,11,12} = 10.37$ [μg/m³] and $\Delta D_s = 4.97$ [μg/m³], respectively.

There are numerous important sources of pollution in Ostrava. To illustrate the application of the above meth-

Table 1 Parameters of the proposed method for PM_{10} obtained from the B measuring station.

PM_{10} Limit value [μg m⁻³] = 40									
Units	–	–	[μg m⁻³]	[μg m⁻³]	[μg m⁻³]	–	[μg m⁻³]	[μg m⁻³]	[μg m⁻³]
Sector *k*	N_k	R_k	C_{k95L}	C_{k95H}	C_k	P_k	D_k	$D_{k,max}$	ΔD_k
1	1301	0.0311	45.31	48.85	47.08	0.0243	1.47	1.25	0.22
2	2255	0.0540	43.96	46.90	45.43	0.0407	2.45	2.16	0.29
3	2588	0.0619	44.74	47.56	46.15	0.0474	2.86	2.48	0.38
4	1566	0.0375	51.76	57.04	54.40	0.0338	2.04	1.50	0.54
5	965	0.0231	53.00	58.68	55.84	0.0214	1.29	0.92	0.37
6	1013	0.0242	39.79	43.53	41.66	0.0168	1.01	0.97	0.04
7	724	0.0173	37.60	41.58	39.59	0.0114	0.69	0.69	–0.01
8	582	0.0139	36.34	40.72	38.53	0.0089	0.54	0.56	–0.02
9	981	0.0235	37.23	41.05	39.14	0.0152	0.92	0.94	–0.02
10	2265	0.0542	50.15	53.09	51.62	0.0464	2.80	2.17	0.63
11	3437	0.0822	87.15	90.51	88.83	0.1212	7.31	3.29	4.02
12	4655	0.1114	89.77	92.91	91.34	0.1688	10.17	4.46	5.72
13	2445	0.0585	46.14	49.00	47.57	0.0462	2.78	2.34	0.44
14	1017	0.0243	45.15	49.82	47.49	0.0192	1.16	0.97	0.18
15	1247	0.0298	77.02	85.08	81.05	0.0401	2.42	1.19	1.22
16	1290	0.0309	78.90	85.52	82.21	0.0421	2.54	1.23	1.30
Forwind	ΣN_k	ΣR_k	C_{w95L}	C_{w95H}	C_w	ΣP_k	ΣD_k	$\Sigma D_{k,max}$	$\Sigma \Delta D_k$
	28331	0.6779	60.67	64.50	62.58	0.7039	42.43	27.12	15.31
Windless	N_s	R_s	C_{s95L}	C_{s95H}	C_s	P_s	D_s	$D_{s,max}$	ΔD_s
	13461	0.3221	54.67	56.17	55.42	0.2961	17.85	12.88	4.97
Total	ΣN_k+N_s	ΣR_k+R_s	C_{m95L}	C_{m95H}	C_m	ΣP_k+P_s	ΣD_k+D_s	$\Sigma D_{k,max}+D_{s,max}$	$\Sigma \Delta D_k+\Delta D_s$
	41792	1.0000	58.74	61.81	60.28	1.0000	60.28	40.00	20.28

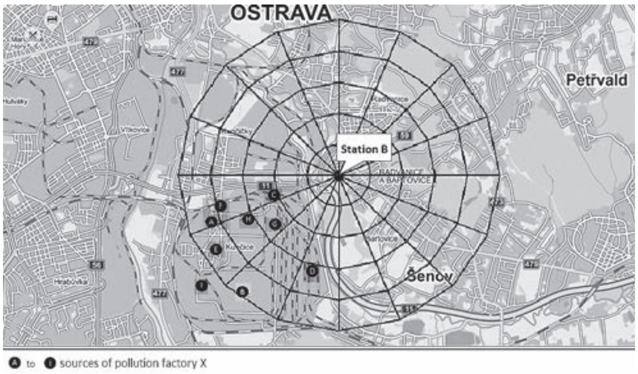

Fig. 5 Distribution of pollution sources around measuring station B.

od, several sources of pollution in Plant X were selected which are found in sectors k = 10, 11 and 12 (see Fig. 5).

Concentration contribution D_s and the relevant necessary reduction ΔD_s for all sources under calm wind conditions may be divided into concentration contributions for individual sources D_{skj} and necessary reductions in concentration contributions for individual sources under calm wind conditions ΔD_{skj} using formulae (17) to (22). For sources shown in Fig. 5, characterized by variables Q_{kj} = 1246.48 [t/year], X_{kj} = 2599 [m] for distances of sources from the sampling point from 1570 up to 3670 meters, T_s = 50 [h], L = 200 [m] (CHMI, 2008), are calculated: D_{skj} = 1.27 [μg/m³], $D_{skj,max}$ = 2,42 [μg/m³] and $\Delta D_{skj} = -1.14$[μg/m³].

Thus, the total concentration contribution for all sources in sectors k = 10, 11 and 12 is $D_{k,tot}$ = 20.28 + 1.27 = 21.55 [μg/m³], the maximum permissible concentration contribution is $D_{kmax,tot}$ = 9.91 + 2.42 = 12.33 [μg/m³] and $\Delta D_{k,tot}$ = 10.37 + (−1.14) = 21.55 − 12.33 = 9.22 [μg/m³].

This example is described in order to establish the validity of the above method.

Discussion

The above methods propose several parameters simply describe the estimation of concentration contributions of individual sources or groups of sources to pollution of a particular area. Such pollution, if approximately equal to or greater than the limit values, must be, in accordance with the Directive (DIRECTIVE 2008/50; EC 2008) assessed using measured data and not dispersion (Bubník 1998; Cimorelli et al. 2004) or receptor (Hopke et al. 2010; Zeng et al. 2010) modelling. This rule is respected by the proposed methods and the parameters are calculated exclusively from measured data and modelling may only be used to obtain more accurate results.

The basic parameter of the methods is relative contribution P_k of a selected pollutant brought to the monitored area from a particular direction (i.e. sector k; see formulae (1) to (6) in section Measurements, if the wind speed is >0.5 m/s (i.e. not under calm wind conditions; see below) (Donnelly et al. 2011; Henry et al. 2012). To determine this parameter, more detailed measured data should be used, such as hourly average concentrations and corresponding hourly average wind direction and speed values over a longer time period. Given the relatively large amount of such data (theoretically, 365 days × 24 hours = 8,760 hourly values), high statistical power of the results may be assumed; fluctuations in annual data (from all seasons) should be compensated for by using data obtained over three or more years (similar to the Average Exposure Indicator as defined by the Directive). In the present study, hourly values obtained over six years were used, with the number of values (sum of N_k) from a single measuring station exceeding 40,000 (see Table 1). Concentration contribution D_k of a pollutant is simply calculated from relative contribution P_k and average concentration in the area C_m, see formula (10).

The above parameters are not valid under calm wind conditions, that is, if wind speed exceeds 0.5 m/s. Although this value was also experimentally determined in this study, the results are beyond the scope of this article. Since the value found in the present study is consistent with that published by the US EPA (AERMOD, 2004), calm wind conditions were defined as wind speed ≤ 0.5 m/s. Given the fact that under calm wind conditions, all neighbouring sources contribute to pollution at the sampling site, parameters for all sources in the area together were first calculated, that is, relative contribution P_s using formula (7), concentration contribution D_s using formula (11) and maximum permissible concentration contribution D_{smax} using formula (14), and then apportioned among individual sources. For such apportionment, the following must be known: emission flow of sources of the pollutant Q_{kj}, distance of sources from the sampling point X_{kj}, height of the atmospheric boundary layer L and average duration of calm wind periods T_s. Although this is only a rough approximation that may not provide accurate results for areas and sources that differ considerably in height, our experiences have shown that it is likely to be applicable in most cases. However, it must be remembered that the calculation is only used for calm wind conditions, which are rather sporadic in some areas. In the area monitored in the present study, calm wind conditions accounted for approximately 30% of the 6-year period (see R_s in Table 1), that is, they were relatively very common (the monitored area is known for frequent and long periods of smog and calm wind). Yet, based on our experiences with monitoring and dispersion modelling, the results obtained with the aforementioned methods are not far from the truth. The average height of the atmospheric mixed layer L may be calculated or measured (US EPA 1999).

The proposed methods have been practically verified by calculations using accurate data. The article shows sample calculations related to several sources of pollution within a single large industrial Plant X on the outskirts of a city (a population of approximately 300,000) and a measuring station located in a residential area considerably affected by them. The long-term average concentration of PM_{10} was 60.3 μg/m³, with the limit value being set at 40 μg/m³. Thus, to meet the limit value, the amount of particulate matter emissions from the sources in the plant would have to be reduced by $\Delta D_k = 10.37$ [μg/m³] under windy conditions and by $\Delta D_{skj} = -1.14$ [μg/m³] under calm conditions at the site of the measuring station, that is, by a total of $\Delta D_{k,tot} = 9.22$ [μg/m³]. To achieve the total necessary reduction in concentration contributions $\Sigma\Delta D_k + \Delta D_s = 20.28$ [μg/m³], the other sources account for the remaining reduction by 11.06 [μg/m³].

Data on the necessary reduction of concentration contributions $\Delta D_{k,tot}$ may be used by the source operator to model the amount of pollutants emitted by the source to meet the limit values for pollutants in ambient air, that is, not only the emission limits. Moreover, these data should include national and regional action plans that, in accordance with the Directive, should ensure that the population is exposed to acceptable air pollutant levels over a defined period of time. If the plans for reducing concentrations of air pollutants only contain technical measures to reduce emissions and the unsatisfactory condition is not corrected over time, the source operator remains unpunished and the population continues to be exposed to increased health risks.

Conclusion

The presented approximate source apportionment is a simple mathematical application using measured data available from any measuring station compliant with Directive 2008/50/EC, that is, where limit values for ambient air pollutants are exceeded. Data on concentrations of ambient air pollutants, corresponding meteorological data and some available data on sources of air pollution and their groups are used to calculate concentration contributions of selected pollutants relevant to individual sources. Subsequently, necessary reduction of these concentrations is determined so that the total contribution of all sources does not exceed the limit values defined by the Directive. The methodology was tested using data relevant to a particular source of pollutants.

Although the above methods are only a first approximation for obtaining information on source apportionment in a monitored area they may be a sufficient and fair starting point for developing air quality plans in accordance with the Directive. Unfortunately, the document does not contain even minimal guidance on how to make individual source operators reduce their emissions. If air quality plans developed in accordance with the Directive comprised requirements for reduction of concentration contributions, the presented approximate methods could be used to determine maximum permissible concentration contributions of every source more accurately so that the limit values are adhered to. The presented calculations for a selected group of sources within a single large plant demonstrate the practical use of the methods. The total contribution of the plant adjacent to a residential area where a monitoring station is located was 20.28 μg/m³, being composed of a contribution under non-zero air flow conditions and a contribution under calm conditions. To ensure that the limit values as defined by the Directive are not exceed in the zone, when all sources of pollution are considered, the contribution would have to be reduced by 10.37 μg/m³. This requirement should be incorporated in the ambient air quality improvement plan so that reverse modelling could be used to define relevant reductions of emissions for each source and the feasibility or non-feasibility of corrective measures could be determined, potentially leading to additional decisions. After the time limit for applying corrective measures expires, fulfillment of the requirement implemented in the air quality plan may be checked using the same methods.

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THE CHANGES THAT OCCURRED IN LAND COVER IN POSTCOMMUNIST COUNTRIES IN CENTRAL EUROPE

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ABSTRACT

Central European landscape has undergone dramatic changes during the last decades. Big changes in the political and economic systems resulted in a large-scale transformation in land use, especially in the agricultural and silvicultural sectors. At the same time, significant changes in urban regions were recorded. In order to quantify and compare the most important processes in land cover changes, we analyzed so-called land cover flows within four post-communist countries in Central Europe – Czech Republic, Slovakia, Poland and Hungary – using CORINE Land Cover databases for 1990, 2000 and 2006. Contradictory processes in landscape change were recorded such as large scale agricultural extensification vs. intensification, or afforestation vs. deforestation. Moreover, there are significant regional differences in the changes in spatial patterns.

Keywords: land cover changes, Central Europe, typology

Introduction

There is no part of the Earth's surface that has not been influenced by human activities. Each type of landscape, natural, cultural and urbanized, is continuously changing and evolving. Understanding the causes, principles and possible aftermaths of these changes in land cover is especially important mainly for making effective decisions about landscape management and its use without causing irreversible loss of valuable environments.
Some of the key factors affecting the global environment are changes in land cover occurring at all levels from local to regional and global. Landscape changes are caused by both natural processes and human activities. The mutual interaction of these factors creates a cultural landscape. Landscape changes are usually considered as positive and/or negative. This depends on the overall context of the landscape. They can result in a decline in biodiversity and loss of the identity of the existing landscape. On the other hand, they generate very valuable habitats and ecosystems. In addition to completely new processes, the termination or temporary interruption of changes in landscape may also result in a transformation of the landscape – for example, abandonment of an agricultural landscape and leaving it to spontaneous development. This usually results in the spread of trees in open habitats, and ultimately to a climax forest. The most important factor here is an undisturbed process of natural succesion. Therefore, understanding landscape development is an important part of caring for landscapes.

Materials and Methods

The analysis of changes in land cover decribed in this paper is focused on four countries in the region of Central Europe, Czech Republic, Slovakia, Hungary and Poland. The changes in land cover were based on six elementary processes of changes in landscape that occur frequently in Central Europe: sub/urbanisation, intensification of agriculture, extensification of agriculture, afforestation, deforestation and construction of water bodies. For their precise definition see Romportl et al. (2010). Identification of changes in land cover was based on Feranec et al. (2010) – see Fig. 1.

For assessing the changes in land cover we used the database CORINE Land Cover (CLC) created by the European Environmental Agency (EEA) in cooperation with other European Union institutions responsible for environmental protection. CLC consists of detailed maps of land cover at a scale of 1:100,000. Land cover is divided into 44 classes (Büttner et al. 2012). Currently, databases for 3 horizons, 1990, 2000 and 2006, exist for individual states. Another types of data represent the change databases for the periods 1990–2000 and 2000–2006. They record increases or decreases in the area of different classes of land cover between two reference periods. The basis for the state databases CLC are Landsat satellite images with a spatial resolution of 25 meters. The minimum unit for land cover inventory is 25 ha. Change databases include only contiguous polygons with a minimum size of 5 ha (Büttner et al. 2012).

The result of the unification of all the changes in land cover was a total of 320 types of changes of land cover in the case of CLC 1990–2000, and 240 for CLC 2000–2006, respectively. Using the scheme shown in Fig. 1, they were ranked among the following six processes of land cover changes (LCF1-Sub/urbanisation, LCF2-Intensification of agriculture, LCF3-Extensification of agriculture, LCF4-Afforestation, LCF5-Deforestation, LCF6-Construction of water bodies). During the identification of the individual processes, there were some cases that could

Romportl, D., Kuna, P.: The changes that occurred in land cover in postcommunist countries in Central Europe
European Journal of Environmental Sciences, Vol. 7, No. 1, pp. 35–49
https://doi.org/10.14712/23361964.2017.4

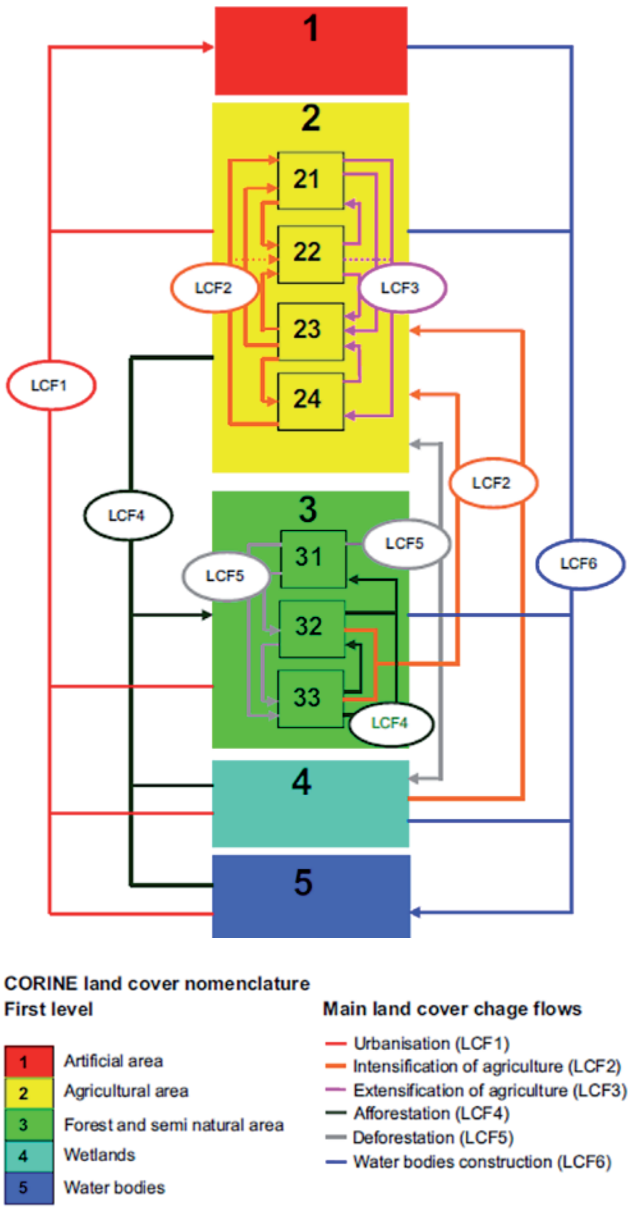

Fig. 1 Relation between changes in land cover and CORINE Land Cover classes (Feranec et al. 2010).

not be classified using this scheme. For example, the transformation of class 222-Fruit trees and berry plantations to class 221-Vineyards or that of class 213-Rice fields to class 211-Non-irrigated arable land. These changes in land cover could not be classified using any of the basic processes of changes in land cover. In addition, they constitute only a very small part of the total area. Therefore, they were excluded from the analysis. The result was thus 305 (CLC 1990–2000) and 233 (CLC 2000–2006) types of changes in land cover.

In addition to several characteristics (total area and proportion of each process in relation to other types of changes in land cover) the key point of analysis was the determination of the area of each change in land cover in terms of the surface of a regular square grid. EEA grid with a field size 100 km^2 was used as a reference. The regular square grid was limited by the shape of the area of interest, which caused the size of some fields to be reduced to a few square kilometers. Therefore, it was necessary to set a limit to field size, for which it was still possible to calculate specific characteristics. In this case, all fields with an area of less than 10 km^2 were excluded from the analysis. This step did not have a negative affect on the results of our analysis.

Maps are of areas where the proportion of the process of change in land cover on the surface of the grid is higher or lower than the average for the region or where the process is entirely absent.

The typology of changes in land cover was done by a using cluster analysis to assess the changes in landscape. It was based on identifying those landscapes showing marked changes in land cover. The analysis was made using function “cluster”. Clustering using k-means with 5 clusters was used. Consequently, it was necessary to determine which process of change in land cover was associated with each cluster. Always one cluster was associated with landscape where no changes occurred.

The six processes of changes in land cover based on the database CORINE Land Cover 1990–2000 and 2000–2006 are described below (see Romportl et al. 2010):

Sub/urbanisation (LCF 1) – assessed as an increase in urban areas (categories 111-Continuous urban fabric and 112-Discontinuous urban fabric) and Industrial, commercial and transport areas (categories 121-Industrial or commercial units, 122-Road and rail networks and associated land and 124-Airports) at the expense of other classes.

Intensification of agriculture (LCF 2) – Process of changes in landscape in terms of a change in any land cover category to either the class 211-Arable land or 221-Vineyards.

Extensification of agriculture (LCF 3) – Assessed as in terms of a change form categories 211-Arable land and 221-Vineyards to other land cover classes except urban, water and mining areas.

Afforestation (LCF 4) – Assessed in terms of a change of any land cover category to the classes 311-Deciduous forests, 312-Coniferous forests, 313-Mixed forests or 324-Low forest vegetation. There are two possible options for assessing the process of afforestation. The difference depends on initial state, which is or is not class 324-Low forest vegetation.

Deforestation (LCF 5) – Assessed in terms of a change from categories 311-Deciduous forests, 312-Coniferous forests and 313-Mixed forests to any other land cover classes, except class 324-Low forest vegetation.

Construction of water bodies (LCF 6) – Assessed in terms of a change in any land cover category to class 511-Water courses and 512-Water bodies.

Only the above-described basic processes of changes in land cover were included to the analysis. They are the most common cause of changes in landscape. Feranec et al. (2010) refers to other processes of changes in land cover as marginal. For example, human activities associated with the exploitation of natural resources (mining and subsequent recultivation of mining areas). These processes were not included in the analysis.

Results

The results of the analysis show that the area that underwent a change in land use was 13,738.37 km^2 during the first period 1990–2000 and was 6,793.64 km^2 in the second period 2000–2006. Detailed overview of all the processes in the changes in land cover is shown in Tables 1 and 2. While the largest change in land cover was due to afforestation during both periods, in second place was the extensification of agriculture in the first period and deforestation in the second period.

Immediately after the political changes in 1989, there were efforts to correct insensitive changes inflicted on the landscape during the communist era. The following period was characterized by new methods and principles in landscape management. According to the EEA Land Accounts for Europe 1990–2000 Report (EEA Technical Report no. 11/2006) and Feranec et al. (2000) the most significant processes were afforestation but also deforestation, extensification of agriculture in areas with less fertile soils, intensification of agriculture and urban sprawl connected with the growth in urban and industrial areas.

During the period 1990–2000, the largest area of change in land cover was detected in the Czech Republic. Changes in landscape affected an area of 5,095.16 km^2, which is 37.09% of total area subject to changes and 6.46% of the area of this country. In the period 2000–2006 the greatest area of changes in land cover occurred in Hungary (2,649.53 km^2). The percentage of the area affected by changes in land cover was 39.00%, which is 2.84% of the area of this country. Detailed overview of some characteristics is shown in Appendix 1–2.

Sub/urbanisation (LCF 1)

Another important change in the landscape that has occurred in Central Europe is urbanisation and suburbanisation. A common sign of this type of change in land cover is its close association with the largest cities and major transport routes in the region. The total area affected by this change in land cover was 581.6 km^2 in the period 1990–2000 and increased to 627.7 km^2 between 2000 and 2006.

During the period 1990–2000 the extent of the built-up area was the greatest in Poland (250.49 km^2). The process of sub/urbanisation was concentrated in the surroundings of big cities, such as Warszawa, Łódź, Poznań, Kutno, Kraków, Görlitz and Wroclaw. In the assessment of the sub/urbanisation process, two cities played a significant role, Gdańsk and Szczecin. Their indisputable importance lies in the fact that they are seaports with a link to international shipping. All these cities are major transport nodes with rapidly developing of built-up areas. Between 2000 and 2006, sub/urbanisation was also significant (254.81 km^2). First, it was the development

Table 1 Assessed processes of land cover change in period 1990–2000.

Processes of change in land cover (LCF)	Area [km^2]	Percentage of the area LCF [%]	Percentage of the region* [%]
Sub/urbanisation (LCF 1)	581.59	4.23	0.11
Intensification of agriculture (LCF 2)	1,466.87	10.68	0.28
Extensification of agriculture (LCF 3)	4,138.09	30.12	0.78
Afforestation (LCF 4)	4,564.44	33.22	0.86
Deforestation (LCF 5)	2,769.37	20.16	0.52
Artificial water bodies (LCF 6)	218.02	1.59	0.04
Total	13 738.38	100.00	2.58

* Total area of Central Europe is 532,932.66 km^2.

Table 2 Assessed processes of the changes in land cover in the period 2000–2006.

Processes of the changes in land cover (LCF)	Area [km^2]	Percentage of the area LCF [%]	Percentage of the region* [%]
Sub/urbanisation (LCF 1)	627.74	9.24	0.12
Intensification of agriculture (LCF 2)	717.19	10.56	0.14
Extensification of agriculture (LCF 3)	927.82	13.66	0.17
Afforestation (LCF 4)	2,267.36	33.38	0.43
Deforestation (LCF 5)	2,170.51	31.95	0.41
Artificial water bodies (LCF 6)	83.02	1.22	0.02
Total	6,793.64	100.00	1.28

* Total area of Central Europe is 532,932.66 km^2.

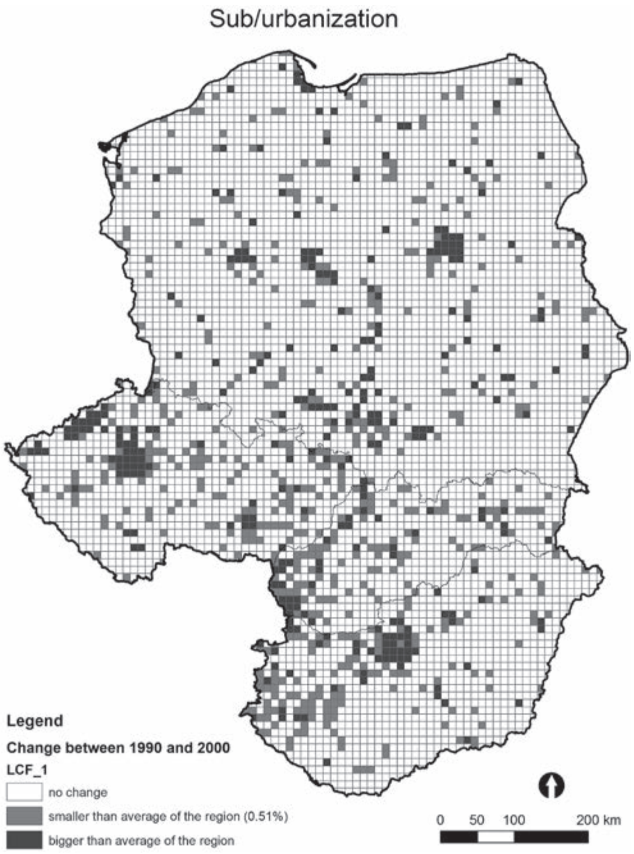

Fig. 2 Process of sub/urbanisation (1990–2000).

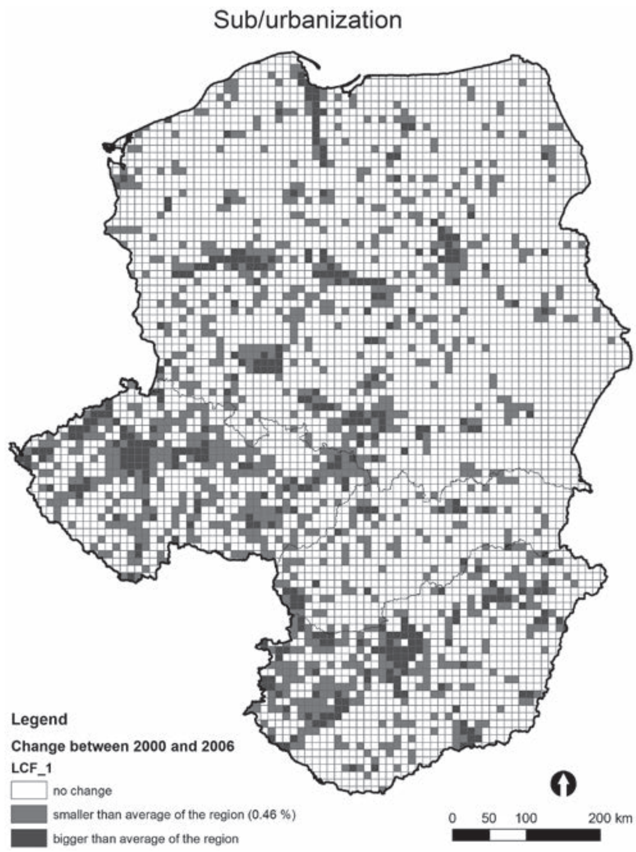

Fig. 3 Process of sub/urbanisation (2000–2006).

of transport networks and commercial and industrial zones within these cities. This type of sub/urbanisation was most distinct in Poland. For example, it was the A2 between Konin and Łódź, and A1 between Gdańsk and Łódź in central Poland, and the route between Konin and Wroclaw. In addition, within big cities the process of sub/urbanisation also continued.

In the Czech Republic, sub/urbanisation affected an area of 161.64 km^2. This type of change in land cover occurred mainly in big cities, in particular in Praha and in some regional capitals and other cities such as Liberec, Karlovy Vary and Sokolov, Plzeň, České Budějovice, Jihlava a Třebíč, Brno, Olomouc and Opava. On the other hand, Ostrava did not follow this trend because of this period there was a decrease in heavy industry and mining. Another area significantly affected by sub/urbanisation is the region of Podkrušnohoří (Chomutov, Most). In the Czech Republic, there was a decrease in the area in the period 2000–2006 (144.48 km^2). Significant changes in land cover were associated with both residential and commercial sub/urbanisation. The first of which was typical for regional capital cities (Ústí nad Labem, Karlovy Vary, Plzeň, Pardubice) and the surroundings of Praha, such as Kladno, Rakovník, Beroun with their large satelite residential areas.

A smaller area of sub/urbanisation was recorded between 1990 and 2000 in Hungary (110.36 km^2), which was mainly in Budapest, Székesfehérvár, Eger and Miskolc. In Hungary, sub/urbanisation affected a total area of 191.15 km^2 in the period 2000–2006. The most distinct developments were the residential and commercial buildings in the outskirts of cities (Budapest, Debrecen, Miskolc, Szeged and in the west of Györ, Sopron and Szombathely) and expansion along transport networks, such the following motorways: M5 connecting Budapest and Szeged, M1 between Budapest and Györ and M7 connecting Budapest and Nagykanizsa.

Changes in land cover associated with sub/urbanisation affected an area of 59.10 km^2 in Slovakia. The most significant development of built-up areas occurred in the western part in border areas, in particular in Bratislava, Nitra and Trnava, and also in Prešov, Košice, Poprad, Žilina, Trenčín, Banská Bystrica etc. In the period 2000–2006 the area affected by sub/urbanisation was 37.30 km^2 and occurred mainly in the immediate zone of Bratislava, Žilina, Poprad and Trnava, and adjacent to some important routes.

Intensification of agriculture (LCF 2)

Intensification of agriculture affected a total area 1,466.90 km^2 over the period 1990–2000. During the second period, the area was smaller (717.19 km^2). In Hungary, the area affected by the intensification of agriculture was 768.87 km^2, with the most signifiant changes occurring in fertile lowlands in the Szamos basin northeast of the city of Debrecen. Other regions affected were the

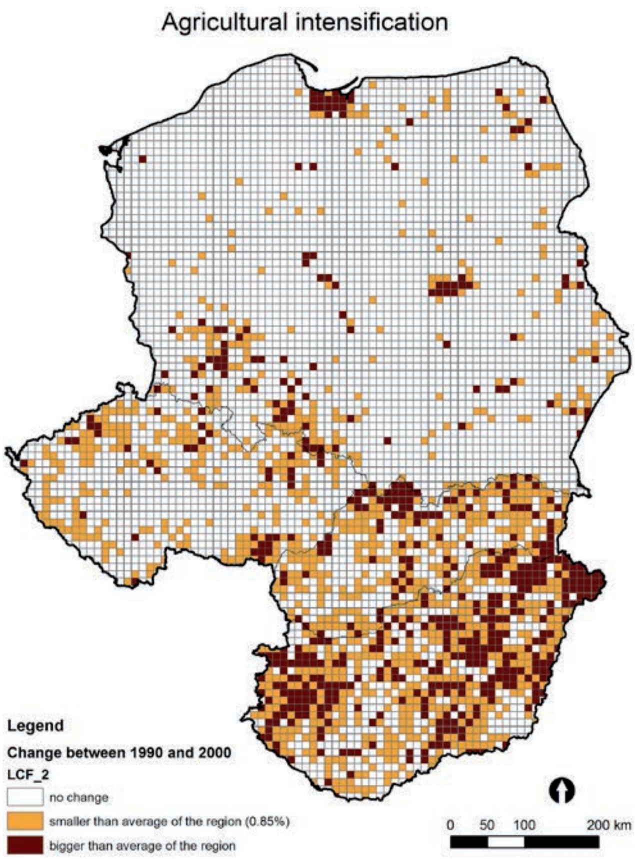

Fig. 4 Process of intensification of agriculture (1990–2000).

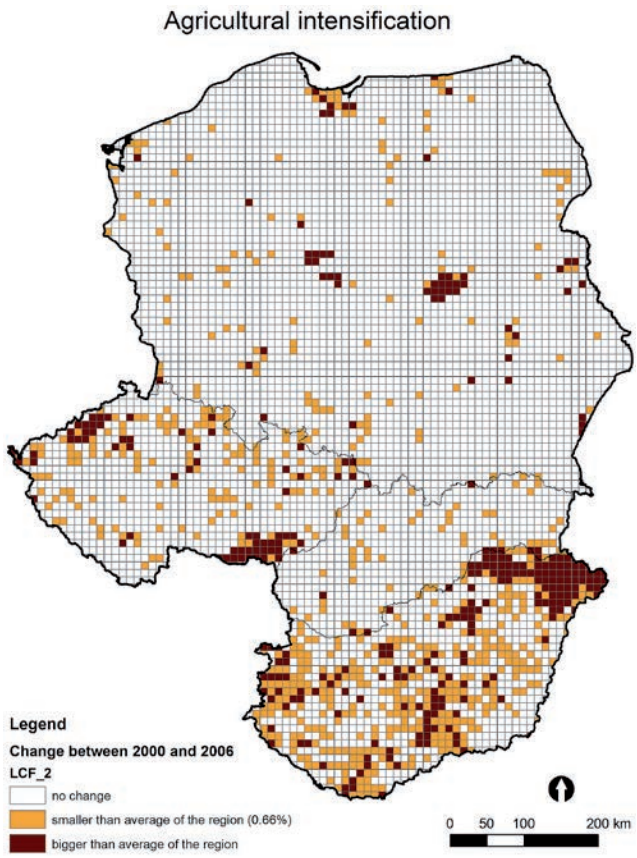

Fig. 5 Process of intensification of agriculture (2000–2006).

lowlands along the rivers Tisa and Körös, around Lake Balaton. During the second period, intensification of agriculture occurred mainly in lowlands in the Tisa and Szamos basins in the east of Hungary and in the Körös basin in the northeastern part of the Great Hungarian lowland.

In Poland it affected 314.45 km^2, with the most significant changes occurring in northern Poland in the region of Visla bay, on the eastern edge of the Pomořanská lake platform at the mouth of the river Visla in Gdańsk bay and the eastern part of the Mazurská lake platform, in large areas in the Mazovská lowlands in the Wisla basin, Slezská lowlands and edge of the Velkopolská lowland. In comparison with the previous period, between 2000 and 2006 there was a large decrease in area affected (132.59 km^2). This process continued in Central Poland, in particular, in the fertile lowlands in the Wisla basin in region of the Mazovská lake platform and on the eastern edge of the Velkopolská lowland. The most significant changes in land cover occured in northern Poland at mouth of Wisla on the Baltic Sea. In other regions, the area affected decreased.

In Slovakia, the area affected was 242.71 km^2, with the main areas affected in the sub-mountainous regions of Javorníky, Oravské Beskydy and Malá Fatra. Other areas where there were increases were in the Východoslovenská lowlands, foothills of Vihorlatské vrchy, highlands of Laborecká and Ondavská vrchovina and regions around Spiš. Between 2000 and 2006, the area affected decreased to only 10% of the value in previous period (24.64 km^2), with the changes recorded only in several small regions in Podunajská lowlands in the surrounding of Trnava and in the Východoslovenská lowlands.

The smallest area affected was recorded in the Czech Republic (140.84 km^2), with the level of intensification depending on natural conditions. It was concentrated in the warmest regions of Dyjskosvratecký úval and Dolnomoravský úval, and in lowlands in the region of Poohří and Polabí (lowlands of Žatecko, Lounsko), but significantly also in the colder foothills of the Šumava and Nízký Jeseník. Between 2000 and 2006, the area affected increased to 152.17 km^2, with he most significant increases in the warmest regions of fertile lowlands. As during the previous period, this process continued in the same regions and extended to regions in the Svratka basin, Litoměřicko and highlands in the České středohoří. In the regions of Chebská and Sokolovská pánev the changes were associated with the recultivation of former mining areas.

Extensification of agriculture (LCF 3)

While between 1990 and 2000 the area land subjected to this type of change in land use reached 4,138.10 km^2 and in the second period was 927.82 km^2. Generally, there was an overall decline in the area subjected to this type of land change in the whole of Central Europe. In

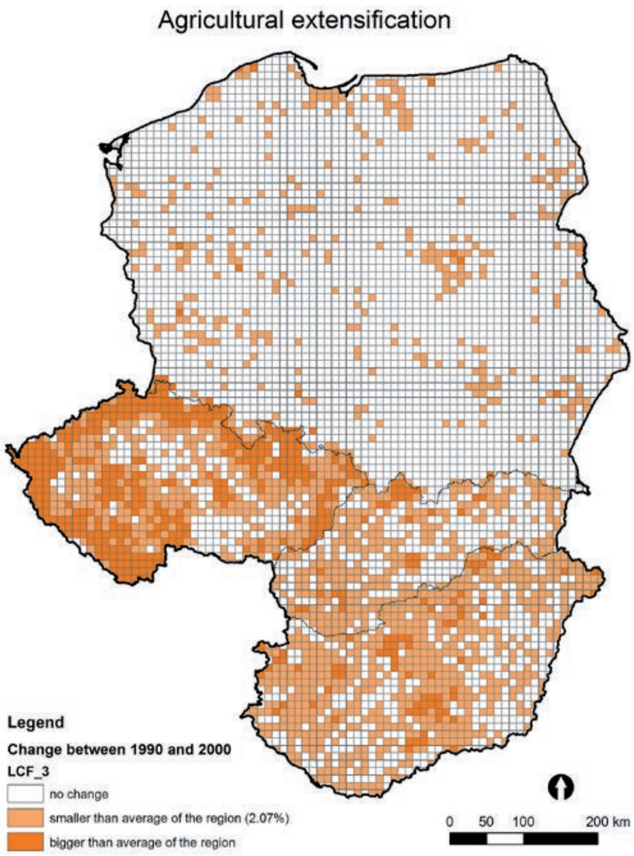

Fig. 6 Process of extensification of agriculture (1990–2000).

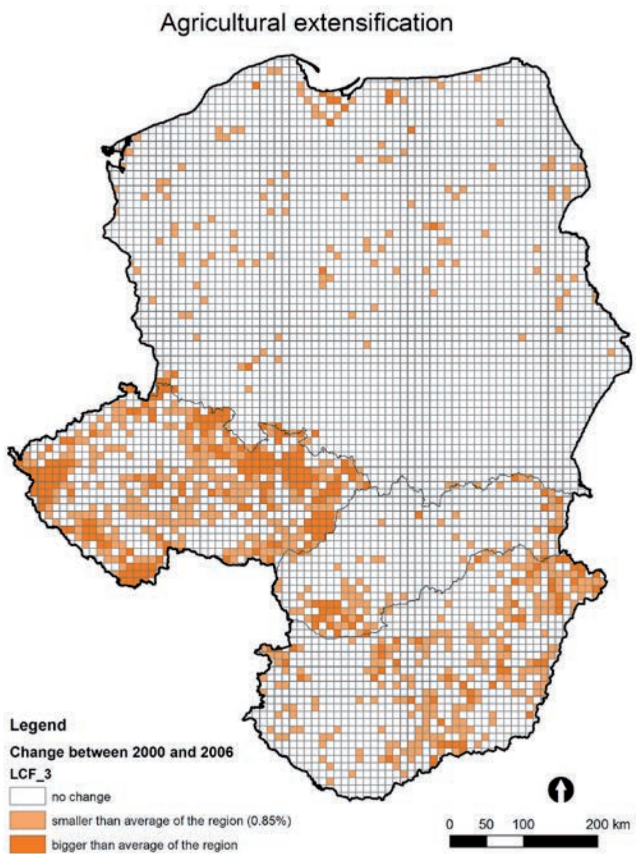

Fig. 7 Process of extensification of agriculture (2000–2006).

the previous period, large parts of the Czech Republic, Hungary and Slovakia were subjected to this type of land use. In the period 2000–2006, there were several separate centers of extensive agriculture. This was particularly common in the Czech Republic. During the first period, it covered an area of 2,936.44 km^2. The most significant changes in landscape associated with extensification of agriculture were mainly recorded in the mountains and foothills in border areas in the region of Novohradské hory, foothills of Šumava, Doupovské hory, Slavkovský les, Lužické hory and České středohoří, and to a lesser extent in the region of Králický Sněžník, Kladská kotlina, Hanušovická vrchovina and the mountains of Nízký and Hrubý Jeseník, highlands of Javořická vrchovina, Žďárské vrchy and Jihlavské vrchy. In comparison with the previous period, there was a significant decline in both the area (643.89 km^2) and the percentage of the area of the country from 3.72% (1990–2000) to 0.82% in the period 2000–2006. Landscape changes connected with extensification of agriculture were significant in regions mentioned above and the regions Smrčiny, Karlovarská vrchovina, Orlické hory, Broumovská vrchovina and Bílé Karpaty. In comparison with the Czech Republic, a significantly smaller area was affected by extensification of agriculture in other countries assessed. In Hungary, the area affected was 679.89 km^2 and mainly occurred in the Matra and Bukovské hory regions and in part of the Tisa basin. Between 2000 and 2006, the area affected was only 158.73 km^2. The process of extensification of agriculture was concentrated mainly in the border area with Romania, lowlands in the Tisa and Szamos basins and the whole area of lowlands in the basin of the Danube River. There were also land cover changes at a few separate localities on the Great Hungarian Plain.

In Slovakia, the area affected by the extensification of agriculture was only 295.56 km^2 and mainly occurred in the region of Oravské Beskydy and Oravská Magura. The intensity was comparable with the situation in the region Nízký Jeseník, but the area affected was smaller. The total area affected in the period 2000–2006 was 60.87 km^2. This type of landscape change occurred mainly at two locations but with a greater intensity. The first region consists of a belt stretching from Laborecká and Ondavská vrchovina through Vihorlatské vrchy to the north of the lowlands on the border with Ukraine. The second area was the lowlands in the Dudváh basin. In the Levočské hills this type of agriculture ceased.

The area of extensification of agriculture reached 226.20 km^2 in Poland. This analysis indicaters that in Poland the affect of the extensification of agriculture was marginal with minimal scope and intensity. In the period 2000–2006, the area of extensification of agriculture was 64.32 km^2 in Poland and the land cover changes were more scattered. Numerous relatively large areas were located in the lowlands. At higher altitudes, it occurred only in border regions, highlands of Frýdlantská pahorkatina,

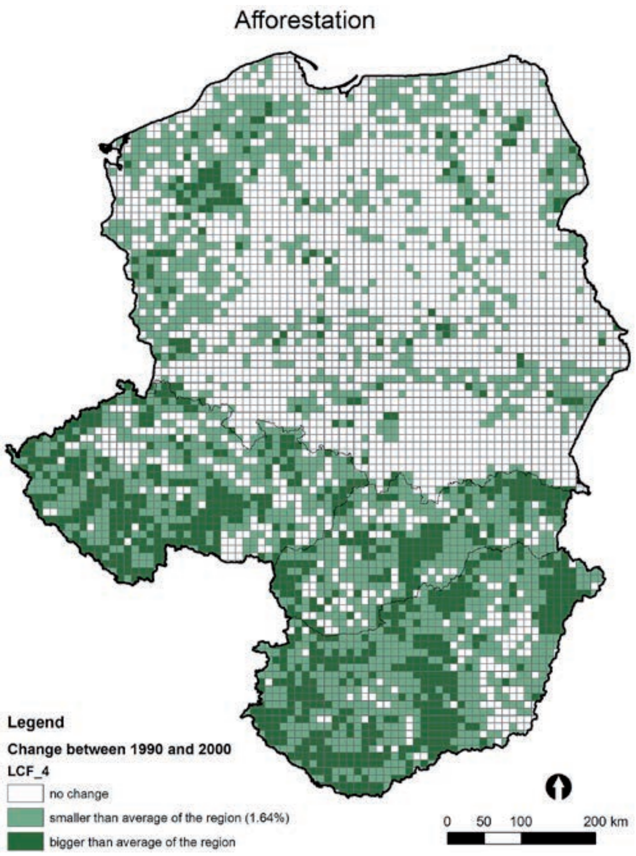

Fig. 8 Process of afforestation (1990–2000).

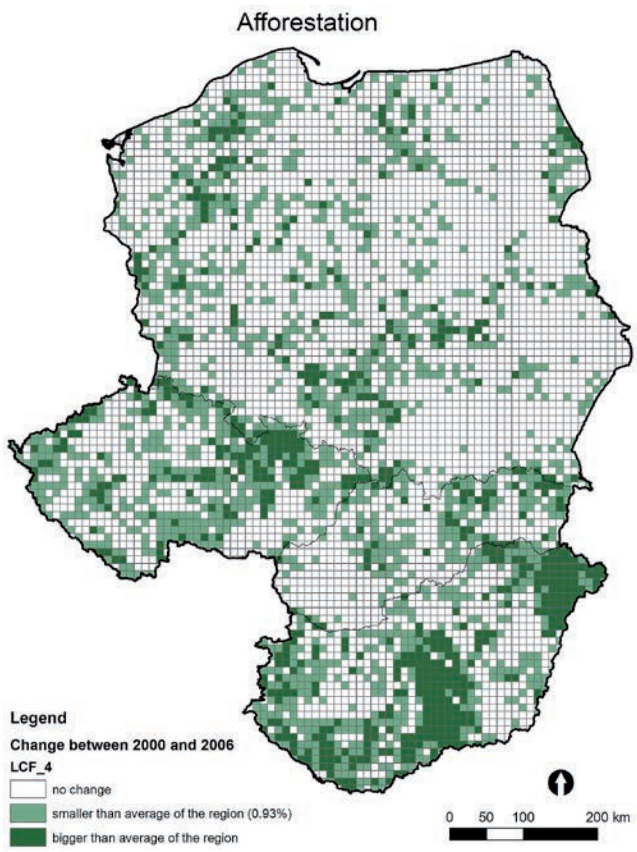

Fig. 9 Process of afforestation (2000–2006).

Zlatohorská pahorkatina, Žulovská pahorkatina or lowlands of Vidnavská. Significant changes in landscape due to extensification of agriculture occurred in the north of Poland in region of the mouth of the river in Gdansk Bay.

Afforestation (LCF 4)

The results of this analysis indicates that the area of afforestation was biggest (1,709.27 km^2) in Hungary between 1990 and 2000, mainly in two regions, northeastern region near the border with Romania and in central Hungary in the western part of the Great Hungarian Plain along the rivers Danube and Tisa and almost the entire western third of the country. Significant afforestation occurred also in the northern mountains (Bakoňský les, Vértes, Cserhát, Mátra, Bukovské hory). Between 2000 a 2006, the area of afforestation was 1,100.44 km^2. Although there was a decline in the area of afforestation, the percentage of the area affected by all the processes studied almost did not change. This means that the relative area of changes in landscape associated with the possitive process of afforestation increased at the expense of other process. Afforestation continued in the same regions as in the first period. Only the region between mountains Bakoňský les and Bukovské hory was not so important in terms of afforestation.

A smaller area of afforestation was recorded in the Czech Republic, where it was 1,289.11 km^2 and occurred mainly in the border regions in the mountains of Jizerské and Lužické hory, Krušné hory, Český les and in the region of Šumava and foothills of Šumava. There was also significant afforestation in the region of Rychlebské hory, Nízký and Hrubý Jeseník, Moravskoslezské Beskydy and Javorníky. Afforestation was also associated with recultivation of former mining areas in Podkrušnohoří and Ostravsko regions. Another distinct process of afforestation also occurred in former military training areas (Císařský les, Doupov, Libavá, Ralsko, Boletice). Between 2000 and 2006, the area of afforestation (432.21 km^2) was significantly smaller than in the previous period. Afforestation continued in some border areas, mountains of Nízký and Hrubý Jeseník, Moravskoslezské Beskydy, Český les, Šumava and foothills of Šumava, Krkonoše and Jizerské hory. Significant afforestation occurred in a belt stretching from the mountains and foothills of Orlické hory, through the highlands of Kladská kotlina, Hanušovická vrchovina and Zábřežská vrchovina to the lowlands of Hornomoravský úval and Vyškovská brána. In the highlands of Brdská vrchovina and Ralsko the afforestation continued especially in connection with the decline in activities in the military training areas.

In Poland, the area of afforestation was 793.15 km^2. The largest area of afforestation was on the western part of Pomořanská lake platform and Velkopolská lake platform, Krajenská jezerní oblast and in the Masovian lowlands and wetlands in the south of the Masurian

Lake District lowlands. Between 2000 and 2006 the area of afforestation reached 606.32 km^2 and continued on the western part of the Pomořanská lake platform with a continuous belt stretching from the Silesian border through the highlands of Malopolská vrchovina to Lublinská vrchovina.

In Slovakia afforestation affected an area of 772.92 km^2, mainly in the eastern part of the country (highlands of Laborecká vrchovina, Ondavská vrchovina and Vihorlatské vrchy) and to a lesser extent in the western part of Slovenské rudohorie and regions of Štiavnické vrchy, Tríbeč, Vtáčnik and Strážovské vrchy. In the second period the area affected by afforestation was smaller (128.40 km^2), with the largest area in the region of Laborecká and Ondavská vrchovina, Vihorlatské vrchy and Bukovské vrchy. Significant afforestation also occurred in the region of Spišská Magura.

Deforestation (LCF5)

During the period 1990–2000, there was a decline in the area of forest by an area of deforestation of 2,769.4 km^2. In the second period (2000–2006), deforestation affected almost the same area as afforestation. The total area of deforestation was 2,170.51 km^2. In comparison with the previous period, deforestation decreased by about one quarter (22%). Important indicator is the relative percentage of the area of all the landscape changes in Central Europe, which increased in case of deforestation from 20.16% in the period 1990–2000 to 31.95% in 2000–2006.

The total area of deforestation in Poland was 874.12 km^2 and was mainly occurred in southern Poland on the eastern edge of the Silesian Lowland and the Velkopolská lowland and to a lesser extent in the Odra basin, surroundings of Wroclaw and in the highlands of Malopolská vrchovina, Lublinská vrchovina and lowlands on the eastern and western parts of Velkopolská lake platform. In Poland, there was clear shift in the center of deforestation from high altitudes to the lowlands in the second period. The area was 719.81 km^2 and mainly occurred in the eastern regions of Velkopolská and Slezská lowland and Mazurská jezera.

A smaller area of deforestation was recorded in Hungary (788.87 km^2). Suprisingly significant deforestation occurred in areas where afforestation occurred. However, deforestation affected a much smaller area. Most deforestation occurred in eastern Hungary near the border with Romania and to lesser extent in the western part of Great Hungarian Plain, border areas in the west and in the region of Malá uherská lowland, Bakoňský les and highlands of Bukovské hory and Mátra. In the second period (2000–2006) area of deforestation was similar (difference of 28 km^2) 760.65 km^2. Deforestation was characterized by a similar spatial extension as in the previous

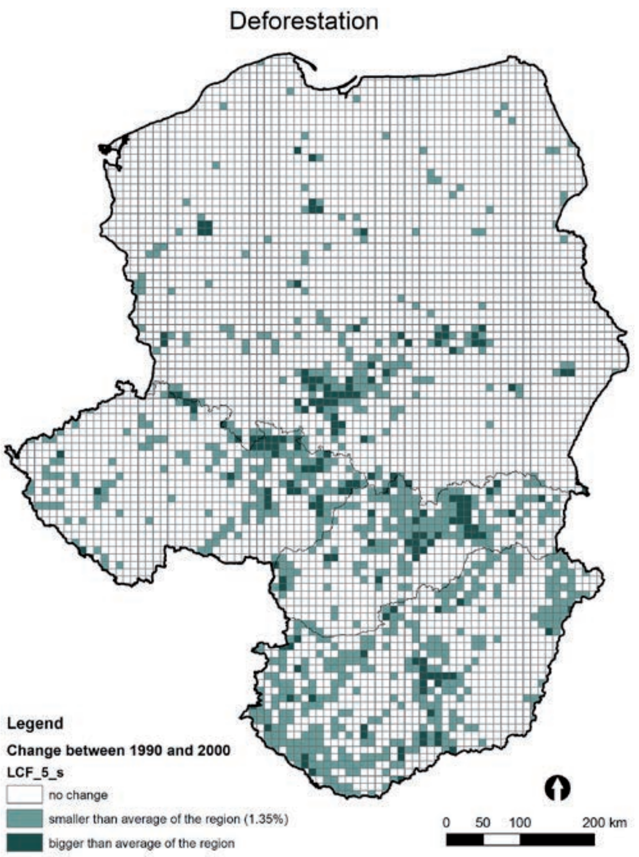

Fig. 10 Process of deforestation (1990–2000).

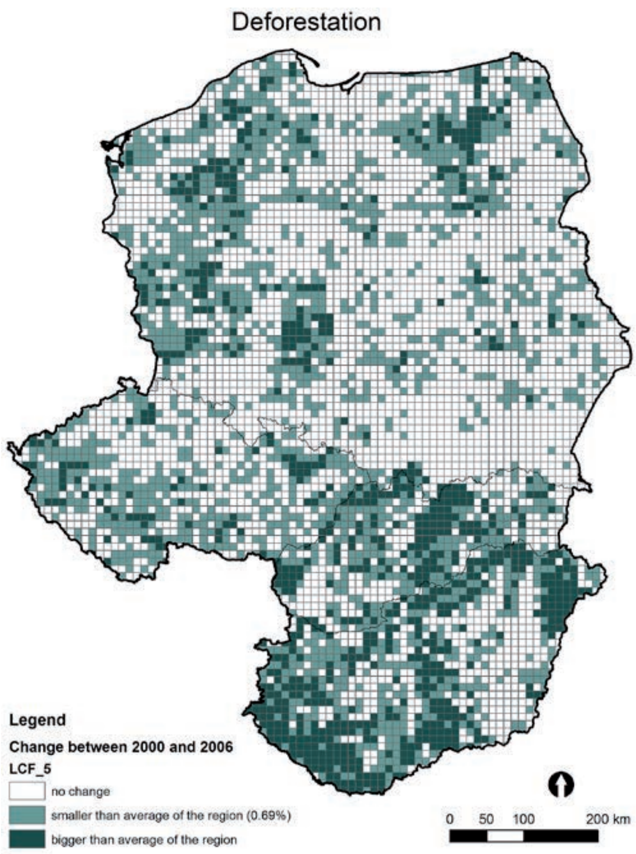

Fig. 11 Process of deforestation (2000–2006).

period and increase in intensity. The most signifiant increase was in the western border area and Drava basin in the northern part of the Malá uherská lowlands. A further increase in the extent occured in the lowlands in the Danube and Tisa basins. Deforestation continued in the Bakoňský les, Mátra, Bukové hory and eastern Hungary.

The area of deforested landscape in Slovakia was 557.99 km², which mainly occurred in the central part of Slovakia in the region of Levočské vrchy, Čergov, Spiš, Nízké Tatry, Slovenské rudohorie, Orava and Velká and Malá Fatra. In the second period (2000–2006) a further area of 49.36 km² was deforested. The process of deforestation continued in the region of Spišská Magura and Levočské vrchy. Significant deforestation also occured in large areas in the region of Nízké and Vysoké Tatry. Extensive deforestation was associated with the gale (storm) and subsequent tree felling in 2004. Other areas of deforestation were identified in the region of Oravské Beskydy, Javorníky, Bílé Karpaty, Laborecká vrchovina and in the lowlands of Morava River.

In the Czech Republic the area of the deforestation reached 548.39 km² most of which was in the region of Krkonoše, Doupovské hory, Šumava, Krušné hory and Brdská vrchovina or Polabská lowland. More significant deforestation occurred in northern Moravia in the region of Králický Sněžník, Kladská kotlina, Hrubý a Nízký Jeseník, Moravskoslezské Beskydy, Brněnská vrchovina and on the eastern edge of Českomoravská vrchovina. In the second period (2000–2006), the area of deforestation was the lowest in the Czech Republic with a total area of 198.69 km².

Artificial water bodies (LCF 6)

The last of the land cover changes included in the analysis was artificial water bodies. In comparison with other processess, it affected the landscape only marginally. Nevertheless, it had a significant effect on the landscape. The total area so affected in Central Europe in the period 1990–2000 was 218 km² and between 2000 and 2006 it was only 83.0 km² (Appendix 2).

In the period 1990–2000 the most significant area of construction of artificial water bodies occurred in Poland where reached a total area 86.06 km², mainly in the form of extensions to numerous lake basins, which occurred throughout the whole area of this country. Between 2000 and 2006 the area affected was 45.04 km² and involved only the construction of new water bodies, not watercourses and was concentrated in several centers in southern parts of Poland, mainly in the eastern part of the Slezská lowland (upper reaches of the Odra River).

Only a slightly smaller area was affected in Slovakia (67.03 km²) and that was mainly in the region of the Gabčíkovo dam on the river Danube, with smaller areas in the region of Orava in the north of Slovakia and signif-

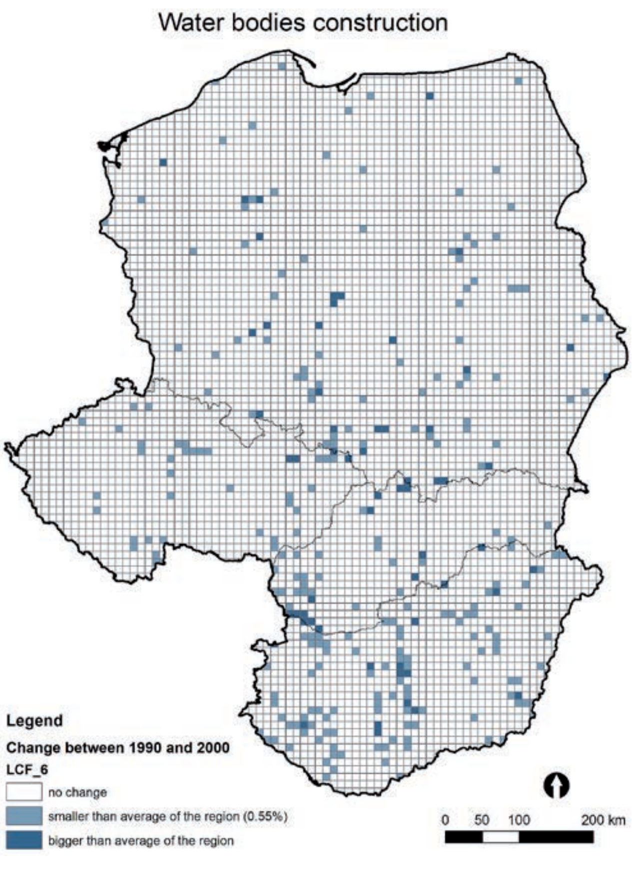

Fig. 12 Water body construction (1990–2000).

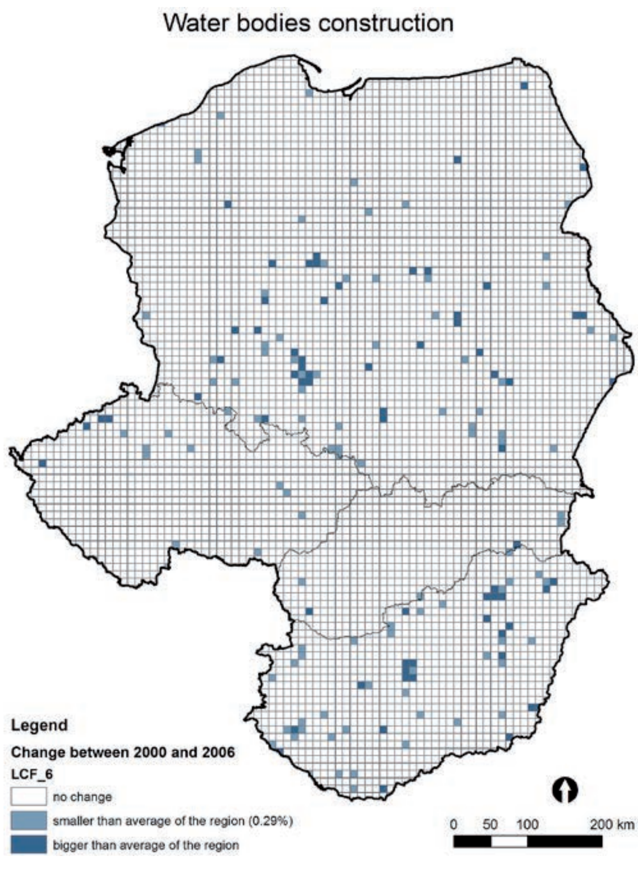

Fig. 13 Water body construction (2000–2006).

icant areas also in Central Slovakia. In the second period (2000–2006), it affected only 1.51 km^2 and in comparison with previous period the decrease was very rapid. There were only few small areas in the western and eastern parts of Slovakia.

The area affected in Hungary was even less, 46.18 km^2 (1990–2000) and mainly occurred in the lowlands throughout the country but most significantly in the Danube and Tisa basins in central Hungary and in the southwestern lowlands in region of the Malá uherská lowlands, with few localituies in the east. Between 2000 and 2006, there was a decrease in the extent of this change in landscape with the construction occurring mainly in the central part (lowlands of Danube River) and northeastern part (lowlands of Tisa River) of Hungary.

In the Czech Republic, the area affected was the lowest. In the period 1990–2000, the total area of 18.74 km^2 was mainly associated with the construction of water bodies in the north of Moravia. In 1997, the Slezská Harta dam was finished and started operating and numerus ponds were constructed in the south of Bohemia and in Central Bohemia in lowlands of the Labe River. A significantly lower area of 5.70 km^2 was affected in the second period as very few water bodies were constructed and only in small areas in the region of Mostecko and Sokolovsko and associated there with the recultivation a mining area. Other areas affected were in the lowlands of Morava River and in the region of Jindřichův Hradec and Mikulov.

Types of landscape (cluster analysis)

Based on the cluster analysis of both periods, 1990–2000 and 2000–2006, the following five types of landscape itemised below were identified. Their spatial extent in Central Europe is shown on the maps included in Figs. 14 and 15. In Appendix 3–4 there are graphs with the average values of the percentages of the land cover changes mentioned in the five clusters.

Types of landscape in Central Europe (1990–2000)

Type of landscape 1

In cluster 1 are the regions where sub/urbanisation and construction of water bodies were the most significant. Based on results of the cluster analysis this cluster includes the most important causes of changes in land cover, with sub/urbanisation more important than the construction of water bodies. In terms of natural conditions, the first type of landscape occurs in the regions of big cities and in their outskirts and the second in lowland floodplains of big rivers.

Type of landscape 2

Cluster 2 includes extensification of agriculture. While the average value for Central Europe was 2.07% the clus-

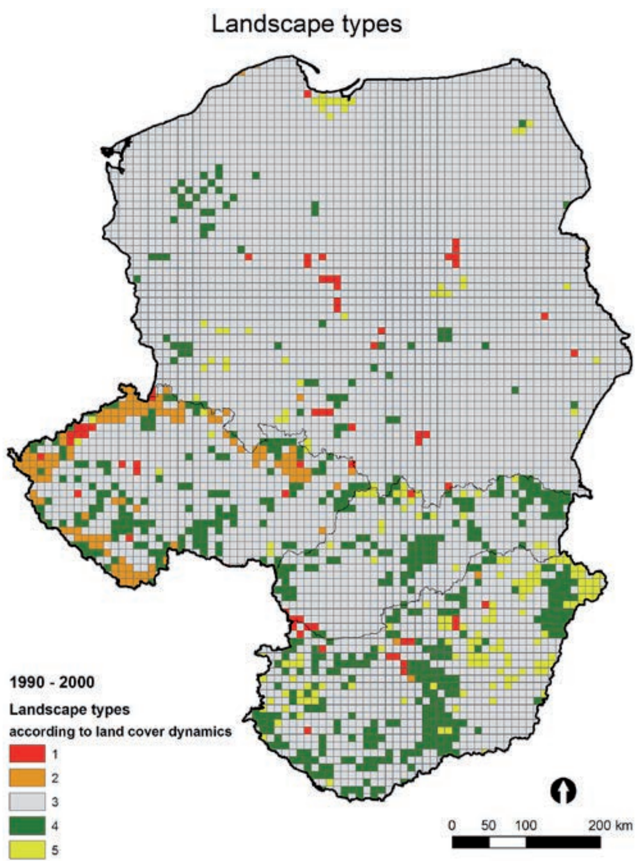

Fig. 14 Types of landscape (1990–2000).

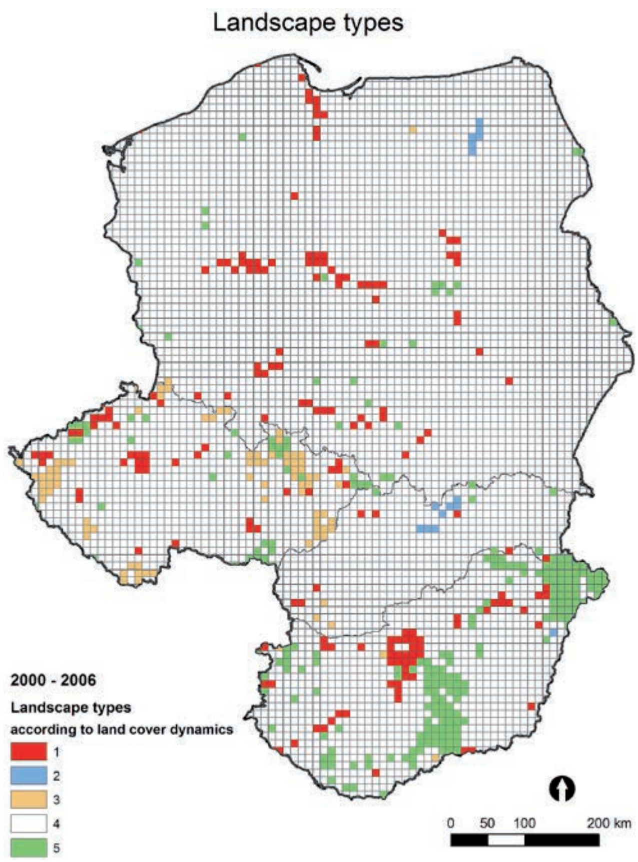

Fig. 15 Types of landscape (2000–2006).

ter mean value was 14.78%. This extraordinary value far above the average for the region assessed was not noted in any other cluster. Throughout the period 1990–2006 the extensification of agriculture was a phenomenon specific to the landscape of the Czech Republic. This kind of land cover changes did not occur anywhere else in Europe to the same extent as in the Czech Republic. The reasons are outlined in the previous chapter of this paper.

Type of landscape 3

Between 1990 and 2000 cluster 3 includes those regions where any kind of change in land cover occured. There were no landscape changes that were crucial for the formation of the landscape mosaic. This type of landscape is characterized by the fact that there were no significant changes in land cover. As expected, this type of landscape occupied the largest area in Central Europe in the period 1990–2000.

Type of landscape 4

Cluster 4 includes landscapes dominated by two contradictory changes in land cover, the massive afforestation associated with expanding forest stands in the landscape and deforestation in a significant part of this type of landscape.

Type of landscape 5

This type of landscape is significantly affected by the intensification of agriculture. The main occurence of this kind of change in land cover occurred mostly in the fertile lowlands and at lower altitudes in foothills.

Types of landscape in region of Central Europe (2000–2006)

Type of landscape 1

Based on results of the cluster analysis cluster 1 includes landscape in which sub/urbanisation is dominant and the construction of water bodies less so as it was not decisive for this type of landscape. Water bodies were constructed only in a few regions in Central Europe.

Type of landscape 2

Cluster 2 includes landscapes affected mainly by deforestation. In terms of effect of this on the landscape, other changes in land cover had less of an affect the landscape and no significant effect on the landscape mosaic. The type of landscape associated with deforestation occured only in two not very large regions in Slovakia and Poland (see Fig. 15).

Type of landscape 3

In case of cluster 3 the dominant change in land cover was associated with the extensification of agriculture. From the spatial distribution of extensification of agriculture it is clear that this type of landscape occurred in the Czech Republic and nowhere else. Between 2000 and 2006 the same situation prevailed as in 1990–2000. This type of landscape was concentrated mainly in the uplands in the border region.

Type of landscape 4

Between 2000 and 2006, cluster 4 included landscapes where none of the changes in land cover assessed occured. As in the previous period, this type of landscape was the most common in Central Europe.

Type of landscape 5

Cluster 5 includes two types of changes in landscape, the intensification of agriculture associated with the expansion of arable land and afforestation. In some regions, this type of landscape was made up of mainly abandoned and less fertile soils left to spontaneous natural succesion. In other regions, the intensification of agriculture was more pronounced and resulted in changes in land cover.

Discussion

As the results of the analysis show there have been very specific changes in land cover in Central Europe. Changes in landscape occured and are still occurring in in relatively different context than in the rest of Europe. Nevertheless, it is possible to identify similar trends. While the paper by Feranec et al. (2010) shows that the European landscape was significantly shaped by the six basic processes of changes in land cover it is clear that some processes were more effective and widespread while others were not so significant. Some changes in land cover were not as extensive as others were, or occurred only in some regions, or some countries. A typical example is the extensification of agriculture, which occurred solely in the Czech Republic during both periods, 1990–2000 and 2000–2006.

The expectation is that the six basic changes in land cover described for the whole Europe also occurred in Central Europe and shaped six corresponding types of landscape, within each of which there would be one dominant process resulting in change in land cover (sub/urbanisation, intensification of agriculture, extensification of agriculture, afforestation, deforestation and construction of water bodies). The actual situation is quite different.

Based on the cluster analysis five types of landscape were identified in each of the periods. One type of landscape is characterized by none of the fundamental processes that result in changes in land cover applying to the extent that they could be considered as determining this type of landscape. The remaining four types of landscape include one dominant or a combination of processes resulting in change in land cover. Based on the results of the cluster analysis it is clear that landscapes were primarily formed by extensification of agriculture and afforestation, intensification of agriculture, sub/urbanisation and deforestation in period 1990–2000. In period 2000–2006, the landscapes were primarily formed by deforestation and to a lesser extent by extensification of agriculture, afforestation, intensification of agriculture and sub/urbanisation. From results of this analysis it is also evident that the construction of water bodies completely ceased to be important in shaping the landscape. In comparison with other processes resulting in changes in land cover it had only a marginal effect and therefore was not decisive in forming any of the given types of landscape. While Feranec et al. (2010) report that the area used for constructing water bodies increased by more then 50 km^2 only in Slovakia and Poland in period 1990–2000 (Feranec et al. 2010), the results of our analysis indicates that the value was almost the same in Hungary (more than 46 km^2).

From the results it is also evident that the changes associated with extensification of agriculture resulted in a unique type of transformation in the landscape, which occurred exclusively in the Czech Republic. Similarly, Feranec et al. (2010) highlight the leading position of the Czech Republic in the list of European countries based on the area subject to an extensification of agriculture. According to Feranec et al. (2010), the area was 2,961.15 km^2 in period 1990–2000, which is only slightly different from that identified by this analysis (2,936.44 km^2). Between 1990 and 2000 extensification of agriculture occured in 3.5% of the Czech Republic (Feranec et al. 2010). In the assessment of the changes landscape as part of the typology, the value was even higher (3.72% of the territory of the Czech Republic in period 1990–2000). The situation in Poland was very different as it affected less than 0.1% of the country (Feranec et al. 2010). According to the results of this analysis, the percentage of extensification of agriculture in this country was 0.07% in the period 1990–2000 and 0.02% between 2000 and 2006.

Conclusion

The results of the analysis of changes in landscape are described in detail using maps (Figs. 2 – 15). Based on the database CORINE Land Cover, 305 types of changes in land cover were identified in the period 1990–2000 and 233 in the period 2000 and 2006. Subsequently, these changes in land cover were reclassified into six basic processes of changes in land cover that have resulted in most of the changes in landscape that have occurred in Central Europe. They are: Sub/urbanisation (LCF1), Intensification of agriculture (LCF2), Extensification of agriculture (LCF3), Afforestation (LCF4), Deforestation (LCF5) and Construction of water bodies (LCF6). The basic process used to identify the processes is that used by Feranec et al. (2010) (see Fig. 1). The spatial distribution of the processes resulting in changes in landscape was primarily determined by natural conditions and changes in land cover that occurred during the communist period.

While the Czech Republic was the country with the largest area of changes in land cover (5,095 km^2 and 6.5% of the area of this country) in the period 1990–2000, during the second period 2000–2006 it was Hungary (2,649 km^2 and 2.8% of the area of this country). During the period 1990–2006, the most significant change in land cover was associated with afforestation. In terms of the total area of afforestation it resulted in the largest change in land cover in Hungary (2,809 km^2) and the Czech Republic (1,721 km^2) between 1990 and 2006.

Landscape changes associated with extensification of agriculture resulted in the second and the third largest changes in land cover. It can be considered as a unique process in the formation of the landscape in the Czech Republic. Nowhere else in Europe did any other process have the same effect. The main reason was the need to repair the damage caused by the collectivization of the countryside. The most widespread type of change in land cover associated with the extensification of agriculture was the transformation of intensively used arable land into extensively used meadows and pastures. This type of change in landscape accounts for more than 81% of the total area of extensification of agriculture during the period 1990–2006. Primarily it was the abandonment of arable land in less fertile sloping sub-mountain regions and their subsequent grassing. Linked to this there was also the restoration of permanent landscape structures (hedgerows, woods, etc.) that significantly improve the ecological stability of a landscape.

Another significant change in land cover was that due to deforestation resulting in a distinct increase in the percentage of all other changes in land cover. In the majority of the cases (97.7% and 99.8%, respectively) of deforestation was concentrated in regions with continuous forest, which at the end of each period were classified as class 324-Low forest vegetation. This indicates that immediately after deforestation the process of afforestation began.

Sub/urbanisation was the only one of the basic causes of changes in land cover that increased between the two periods assessed. Mainly it resulted from the transformation of rural areas into urbanized areas (70% and 75%, resperctively of all the changes in landscape associated with sub/urbanisation). The process of sub/urbanisation is defined as an increase in the numbers of two types of buildings. The first is called residential sub/urbanisation and consists of satelite residential areas in the immediate surroundings of big cities (the so-called "urban sprawl"). During both periods it was the most significant type of sub/urbanisation (292 km^2 between 1990–2006). The second type of sub/urbanisation is the expansion or building of new industrial and commercial buildings (logistic centers, warehouses, buildings for the trans-shipment of goods, etc.) close to mainly international roads.

Based on these results, five types of landscape were identified by using the cluster analysis in software Statistica 10. Always there was one type of landscape where none of the basic changes in land cover occurred. Between 1990 and 2000, the first type of landscape in terms of area was characterized by a dominance suburbanisation and urbanisation and to a lesser extent by the construction of water bodies. In the next type of landscape in order of area was characterized by extensification of agriculture. The third type of landscape included those regions with no changes in land cover. The penultimate type of landscape was characterized by a combination of afforestation and deforestation. The last type of landscape identified intensification of agriculture as dominant. Spatial distribution of the different types is shown on the map in Fig. 14. In the period 2000–2006 the composition in terms of the types of landscape was different. Only the area of the first type of landscape was the same as in the previous period but the extent and construction of water bodies was less. The second type of landscape was that in which deforestation prevailed. In the third type of landscape, the most significant process was the extensification of agriculture. The fourth type included regions where no changes in land cover occurred. The last type of landscape was where there were two processes resulting in changes in the landscape: intensification of agriculture and afforestation.

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Appendix 1 Area of changes in land cover in Central Europe (1990–2000).

	Czech Republic			**Slovakia**		
LCF	**Area of LCF [km²]**	**Percentage of all LCF [%]**	**Percentage of the country* [%]**	**Area of LCF [km²]**	**Percentage of all LCF [%]**	**Percentage of the country* [%]**
Sub/urbanisation	161.64	3.17	0.205	59.10	2.96	0.121
Intensification of agriculture	140.84	2.76	0.179	242.71	12.16	0.495
Extensification of agriculture	2,936.44	57.63	3.723	295.56	14.81	0.603
Afforestation	1,289.11	25.30	1.634	772.92	38.74	1.577
Deforestation	548.39	10.76	0.695	557.99	27.97	1.138
Construction of water bodies	18.74	0.37	0.024	67.03	3.36	0.137
	5,09516		6.460	1,995.31		4.071
	Poland			**Hungary**		
LCF	**Area of LCF [km²]**	**Percentage of all LCF [%]**	**Percentage of the country* [%]**	**Area of LCF [km²]**	**Percentage of all LCF [%]**	**Percentage of the country* [%]**
Sub/urbanisation	250.49	9.85	0.080	110.36	2.69	0.118
Intensification of agriculture	314.45	12.36	0.101	768.87	18.74	0.825
Extensification of agriculture	226.20	8.89	0.073	679.89	16.57	0.730
Afforestation	793.15	31.17	0.254	1,709.27	41.66	1.835
Deforestation	874.12	34.35	0.280	788.87	19.23	0.847
Construction of water bodies	86.06	3.38	0.028	46.19	1.13	0.050
	2,544.47		0.816	4,103.45		4.405

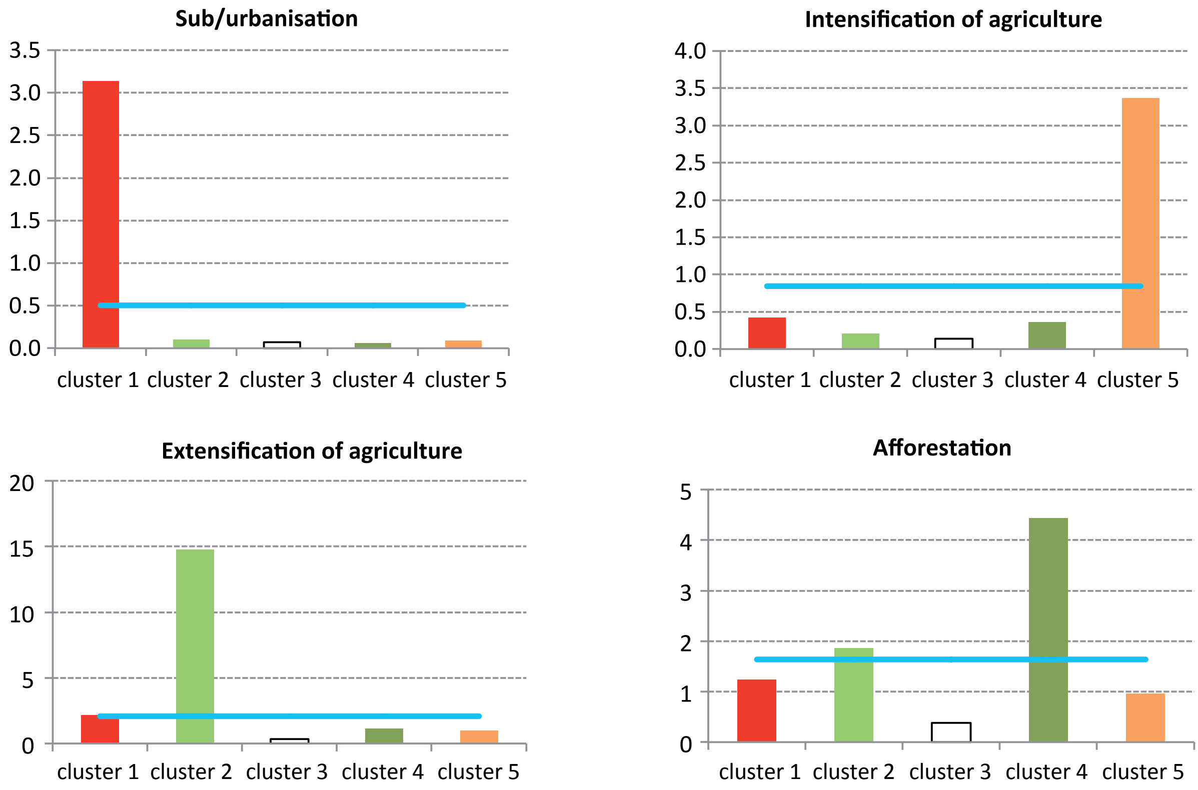
* Area of countries in region of Central Europe: Czech Republic – 78,876.29 km²; Slovakia – 49,012.99 km²; Poland – 311,878.56 km²; Hungary – 93,164.82 km².

Appendix 2 Area of changes in land cover in Central Europe (2000–2006).

	Czech Republic			Slovakia		
LCF	**Area of LCF [km²]**	**Percentage of all LCF [%]**	**Percentage of the country* [%]**	**Area of LCF [km²]**	**Percentage of all LCF [%]**	**Percentage of the country* [%]**
Sub/urbanisation	144.48	9.16	0.183	37.30	5.01	0.076
Intensification of agriculture	152.17	9.65	0.193	24.64	3.31	0.050
Extensification of agriculture	643.89	40.83	0.816	60.87	8.18	0.124
Afforestation	432.21	27.41	0.548	128.40	17.26	0.262
Deforestation	198.69	12.59	0.252	491.36	66.04	1.003
Construction of water bodies	5.69	0.36	0.007	1.51	0.21	0.003
	1,577.13		1.999	744.08		1.518
	Poland			**Hungary**		
LCF	**Area of LCF [km²]**	**Percentage of all LCF [%]**	**Percentage of the country* [%]**	**Area of LCF [km²]**	**Percentage of all LCF [%]**	**Percentage of the country* [%]**
Sub/urbanisation	254.81	13.98	0.082	191.15	7.22	0.205
Intensification of agriculture	132.59	7.27	0.043	407.79	15.39	0.438
Extensification of agriculture	64.32	3.53	0.021	158.73	5.99	0.170
Afforestation	606.32	33.26	0.194	1,100.44	41.53	1.181
Deforestation	719.81	39.49	0.231	760.65	28.71	0.816
Construction of water bodies	45.04	2.47	0.014	30.78	1.16	0.033
	1,822.89		0.584	2,649.54		2.844

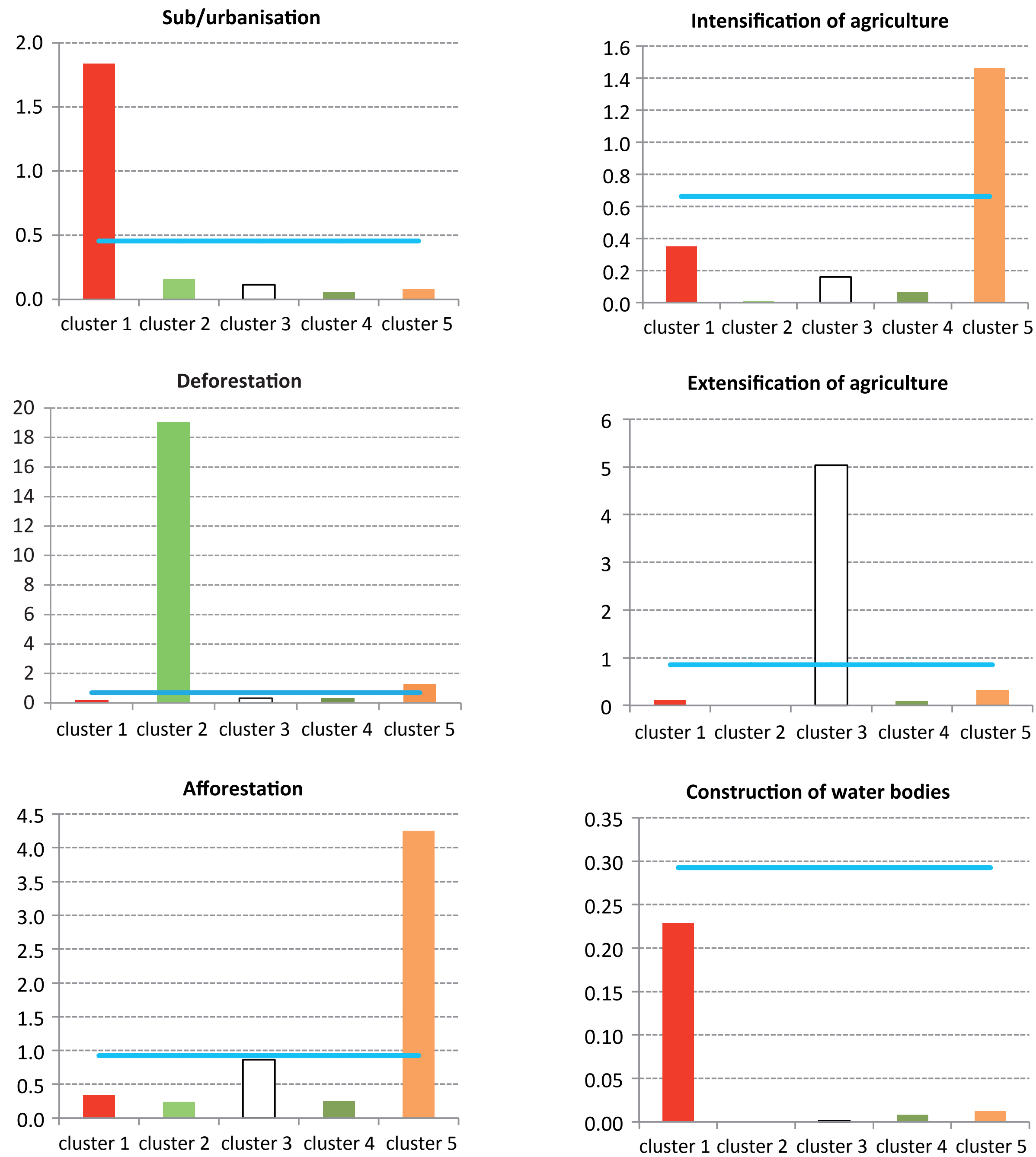
* Areas of the countries in Central Europe: Czech Republic – 78,876.29 km²; Slovakia – 49,012.99 km²; Poland – 311,878.56 km²; Hungary – 93,164.82 km².

Appendix 3 Proportion of causes of changes in land cover in the different clusters (1990–2000)*.



* Horizontal line indicates the average proportion of non-zero values for each of the processes in the different clusters.

Appendix 4 Proportion of the causes of the changes in land cover in the different clusters (2000–2006).



* Horizontal line on the graphs indicates the average proportion of non-zero values for each of the process in the different clusters.

CURRENT DISTRIBUTION AND HABITAT PREFERENCES OF RED DEER AND EURASIAN ELK IN THE CZECH REPUBLIC

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ABSTRACT

Here we determine the distribution, numbers and habitat preferences of two of the largest species in the family Cervidae present in the Czech Republic, red deer and Eurasian elk. Red deer occurs predominantly in vast areas of forest, i.e. mainly in the mountains bordering this country and several large forest units in the interior. The range of this species has been increasing along with the size of its population. Areas of its permanent occurrence may be generally characterized as regions largely covered with deciduous and coniferous forests and pastures, and regions with a more diverse landscape. Red deer does not occur in areas that are mainly arable or urban, or in areas covered with extensive water bodies and wetlands. As these animals prefer large forests, they occur mainly at high altitudes where the terrain is rugged. The Eurasian elk permanently occurs in the Czech Republic in a single area located between the state border and the right bank of the Lipno Dam. Its home range has been diminishing, presumably along with its numbers. The area of its permanent occurrence is characterized by an abundance of coniferous trees, some pastures and water bodies. The Eurasian elk does not occur in areas covered with arable and urban land but also surprisingly in areas with mainly deciduous forest. Both species prefer high altitudes, but Eurasian elk prefers areas with little difference in the terrain vertically.

Keywords: habitat preferences, red deer, Eurasian elk

Introduction

Monitoring the home ranges of various species and their potential changes, induced by natural population, environmental factors, or by man, is one of the essential conditions for understanding their ecology, habitat requirements, or the threats to their survival. The present study addresses current knowledge on the distribution and habitat requirements of two of the largest species of deer in this country. Its main objective is to summarize and refine the existing data on the occurrence of red deer and Eurasian elk in the Czech Republic and to describe the connections between the current state of the landscape and the actual occurrence of the focal species. These two species were selected as representatives of large mammals with relatively high requirements for space, vegetation cover and a quiet environment.

Material and Methods

Red Deer (*Cervus elaphus*)

Red deer are currently patchily distributed throughout most of Eurasia. It reaches Ireland and extends over Central Asia, the Himalayas and China to Primorsky Krai and Korea. It also occurs in the northwest of Africa and North America (Anděra and Horáček 2005; Grubb 2005). In Central Europe, it can be found at various altitudes ranging from the lowest to approximately 2,500 m a.s.l. in the Alps (Koubek and Zima 1999).

In the past, red deer supposedly occupied most of our forests. Its populations became fragmented due to the extensive deforestation that has occurred mainly in the interior of the country (Anděra and Červený 2009).

The current distribution of red deer is closely associated with large forest complexes in the mountains and highlands, and its populations are subject to game management practices. In the Czech Republic, it continuously occupies border mountain areas and their adjacent foothills. These mountain ranges actually form a ring running from the Moravian part of the Carpathians, over the Sudetes in the north, to the Krušné hory Mts., further to the Šumava and reaching the Novohradské hory Mts. Inside the country, this species occurs at Brdy, Křivoklátsko and in the Českomoravská vrchovina and Drahanská vrchovina uplands. Because of deforestation, it is rarely seen in the lowlands, with the exception of floodplain forests such as Soutok near Lanžhot (Anděra and Hanzal 1995; Hlaváč and Anděl 2001; Anděra and Červený 2009).

At present, red deer predominantly occurs in areas with continuous forest cover (coniferous, mixed and

Romportl, D., Bláhová, A., Andreas, M., Chumanová, E., Anděra, M., Červený, J.: Current distribution and habitat preferences of red deer and Eurasian elk in the Czech Republic
European Journal of Environmental Sciences, Vol. 7, No. 1, pp. 50–62
https://doi.org/10.14712/23361964.2017.5

deciduous), but with numerous clear-cuts and pastures. They prefer clearings that have a rich layer of herbaceous plants (Welch et al. 1990; Anděra and Červený 2009). Where the Eurasian lynx is likely to occur, the red deer will seek open young growth providing both sufficient food and a safe hiding place (Čejka 2001). In autumn, animals leave their summer resting places at high altitudes and move to lower areas where they spend winter. Along with the melting snow in spring, they return back to the mountain areas (Mysterud 1999; Šustr 2007).

Eurasian Elk (*Alces alces*)

The distribution of the Eurasian elk extends from Norway across Sweden into Finland, Russia, Baltic countries, Belarus, Poland, and Ukraine to Siberia, reaching as far as the Yenisei River. However, this species is extinct in Western and a large part of Central Europe (Corbet 1978; Bauer and Nygrén 1999; Grubb 2005).

The Eurasian elk has never been abundant in the Czech Republic. The last records before its local extinction date back to the 16th century (Anděra and Kokeš 1978). Remnants of these animals can be found dating from the Early Middle Ages (approx. until the year 1200) at archaeological sites in the lowlands along the Labe and Ohře rivers, and in southern Moravia; there are no archaeological findings dated to a later period (Peške 1995; Kyselý 2005). Thus, it may be presumed that elks were rare in this country after the High Middle Ages. Nevertheless, migrating individuals were recorded in 1957 (Anděra and Kokeš 1978) and the first calf to be born here was recorded in the region of Jindřichův Hradec in 1974 (Andreska 1988).

Two core areas of this species' permanent occurrence were gradually established in this country (Anděra and Hanzal 1995). The first was located in the eastern part of the region of Třeboň and its surroundings, i.e. in a 20 km wide strip running from the Novohradské hory Mts. to the Středočeská vrchovina uplands. In winter, elks occurred quite regularly in pine forests in the region of Bechyně, in the surroundings of the Borkovická blata, in the region of Jindřichův Hradec and between Příbraz and Mirochov. In summer, they migrated to the territory of PLA Třeboňsko, the wetlands of the Nová řeka Canal and the adjacent pond basin (Homolka 2000). This population died out at the turn of the first decade of the 21st century (Šustr, Kašperské Hory, pers. comm. 2010). The last area in the Czech Republic where the Eurasian elk is considered to have occurred permanently is located on the right side of the Lipno Dam and was usually delimited by the border with Austria in the south, by the right bank of the dam in the north, by the former village of Kapličky in the east, and by a settlement called Svatý Tomáš in the west. This area extends over approximately 100 km^2 and elks shelter here throughout the entire year (Homolka 1998; Homolka 2000; Anděra and Červený 2009).

Sufficient feeding grounds and minimum disturbance are existential conditions for the occurrence of the Eurasian elk. The animals prefer wet marshy forests in lowlands and uplands, but avoid steep slopes. The carrying capacity of a given area is one of the limiting factors. Most important tree species for them are the goat willow (*Salix caprea*), alder buckthorn (*Frangula alnus*) and Scots pine (*Pinus silvestris*) (Homolka 1998). There is competition for food between elks and deer, especially the red deer, whose presence may be another limiting factor for the occurrence of Eurasian elk (Homolka 2000).

Data Collection

Questionnaires are the main source of data on the occurrence of Eurasian elk and red deer. In the periods 1991–1992 and 2005–2006, and for the elk also in 2009–2010, in cooperation with the Ministry of the Environment of the Czech Republic, these were submitted to traditional hunting associations within the Czech-Moravian Hunting Association, leased hunting districts of LČR, owners and tenants of other hunting districts including Vojenské lesy a statky ČR (Military Forests and Farms of the Czech Republic), regional offices of AOPK ČR (Agency for Nature Conservation and Landscape Protection of the Czech Republic), and administrative autorities of large-scale protected areas (PLAs and NPs). In case of the elk, further sources of information involved literature, observations by various zoologists and game keepers, and observations made within Mapping of Mammals in the Czech Republic conducted by www.biolib.cz (Anděra and Hanzal 1995; Anděra and Červený 2009). The data from the sources mentioned above were interpreted using a basic map of hunting districts. The structure and form of the questionnaire gave three options for the occurrence of the focal species: permanent occurrence, occasional occurrence and rare occurrence. Occurrence of the Eurasian elk was divided solely into permanent and occasional, the latter also involving observations of migrating individuals. For this reason, the analysis used data on elk from 2005–2009, which are sufficiently comprehensive and still relatively recent. Data on the absence (not to be confused with "no data") of the focal species in a given area are available for red deer from 2005–2009 and for Eurasian elk from 2006–2009. Questionnaires from 2005 and complementary data from the period 2005–2009 covered 78%, while questionnaires on the Eurasian elk from 2009 covered merely 26% of the territory. Hunting districts where questionnaires were not completed and submitted were excluded in order to prevent them distorting the analysis. Polygons of hunting districts were used as the basic spatial units for assessing the spatial distribution of the focal species. As opposed to hunting districts, military areas and national parks are significantly larger. To keep the sizes of all the units assessed comparable, military areas and national parks were divided by their areas. The data on the occurrence of both focal species were expressed in a total 6056 polygons. Each polygon in a hunting district was also assigned basic characteristics of its conditions, which were used to assess the requirements and preferences of the species. The following variables were selected as appropriate indicators of the quality of these conditions:

Abiotic Factors

Altitude – expressed as the mean altitude above sea level of hunting districts (source data DEM SRTM 100 × 100 m)

Vertical heterogeneity – expressed as a standard deviation of altitude within hunting districts (source data DEM SRTM 100 × 100 m)

Habitat Factors

Type of habitat – expressed as the percentage of individual classes of land cover according to CORINE Land Cover 2006 (EEA 2009) within hunting districts. The classes of land cover subject to assessment were aggregated into the following categories:

1. urban land
2. orchards and gardens
3. arable land
4. meadows and pastures
5. heterogeneous agricultural land
6. coniferous forests
7. deciduous and mixed forests
8. natural non-forested areas
9. wetlands
10. water bodies

Factors of Anthropogenic Disturbance

Road density, expressed as the length of all communications per hectare within a hunting district.

Factors of Landscape Structure

Land cover diversity – expressed using Shannon diversity index.

Heterogeneity of land cover – expressed as the number of patches of land cover.

Hunting Data

Further data were provided by the Czech Statistical Office, which was acquired as part of the statistical research carried out by the Ministry of Agriculture (annual report on hunting districts, on populations of game, and hunting) and the Ministry of the Environment (annual report on hunting districts, populations of game and hunting in national parks). The mentioned annual reports on hunting districts, populations of game and hunting supplied information on game management in the given period (1 April of a given year – 31 March in the following year). To assess the sizes of game populations, the present study counts were compared with the values of the minimum viable population of game in spring.

Statistical Analysis

We determined how the hunting districts with particular occurrence statuses differed in terms of altitude,

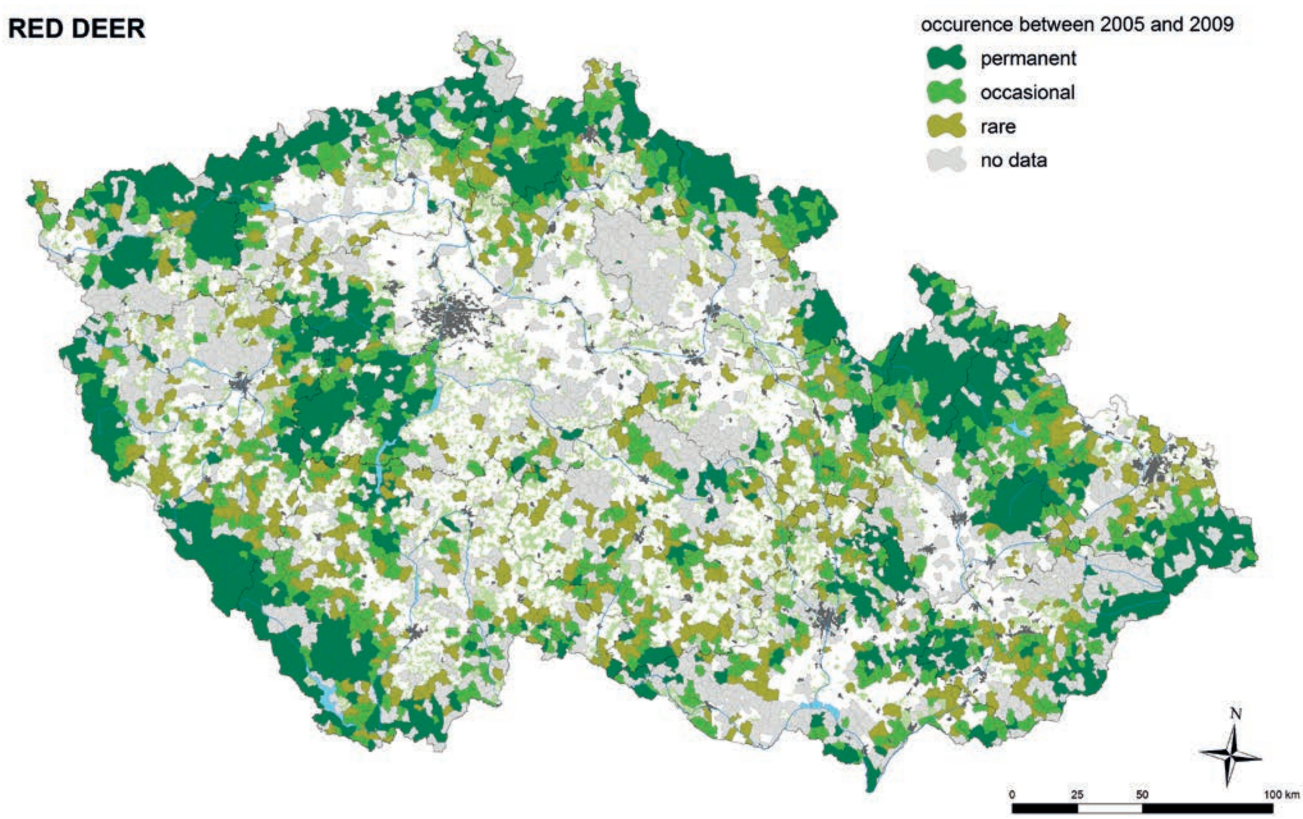

Fig. 1 Distribution of red deer in the Czech Republic between 2005 and 2009.

vertical heterogeneity, road density, number of land cover categories and Shannon diversity index, using Kruskal-Wallis ANOVA in STATISTICA program. Kruskal-Wallis ANOVA is a nonparametric method used to compare many groups (StatSoft 2001).

To find the relationship between the percentages of the different land cover categories in hunting districts and the recorded occurrence status we used canonical correspondence analysis (CCA) computed using CANOCO for Windows (Ter Braak and Šmilauer 2002). CCA is a direct (constrained) method of unimodal ordination directly displaying the relationship between the variables analyzed (Lepš and Šmilauer 2003).

Results

Red Deer

According to the data based on questionnaires from 2005, the red deer occupies an area 42.42% of the size of this country (see Fig. 1). As the questionnaires were completed and submitted for assessment from only 77.8% of the country, the number is not entirely accurate. Quantifying this species occurrence only in areas for which data are available, we reach a figure of 54.52% of the area of this country. Permanent occurrence is reported in 24.03% of the area covered and 18.69% of the total area of this country, occasional occurrence in 17.16% (13.35% respectively), and rare occurrence in 13.33% (10.37% respectively).

The most significant areas for this species are in border mountains. In the interior of this country, a population of red deer inhabits the region of the Brdy, Žďárské vrchy, Drahanská vrchovina Uplands, Ždánický les and Chřiby. Another population in the lowlands of Soutok near Lanžhot is also significant. The resulting map more precisely delimits the range of this species in this country than graticule based zoological mapping.

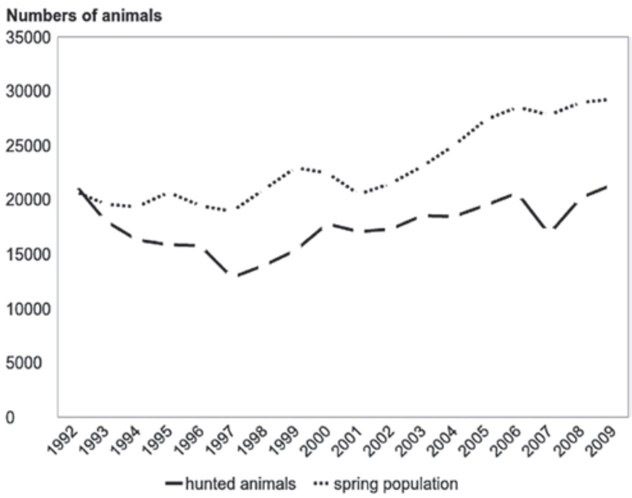

Fig. 2 Red deer (*Cervus elaphus*) game management results for 1992–2010 (source: Czech Statistical Office). Numbers of animals killed and estimated minimum viable population in spring.

The census of minimum viable populations in spring reports 20,841 animals in 1992, compared to 26,824 animals in 2005 (data from March 2006), and 29,895 animals in 2009 (data from March 2010). The populations are obviously increasing (by 11.4% since 2005 and even 43.4% since 1992) (Fig. 2). As the data were supplied by hunting associations, they are considered to be rough estimates, both of the trend and the actual numbers.

Habitat Preferences

The core areas for red deer populations are in forested mountain ranges. The analyses of habitat preferences depict the trend of a more frequent permanent occurrence

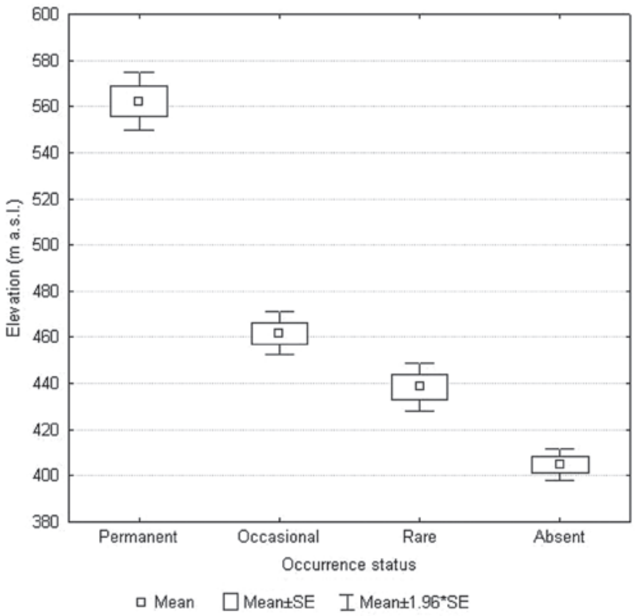

Fig. 3 Mean altitudes of hunting districts with particular categories of the red deer occurrence displayed as boxplots.

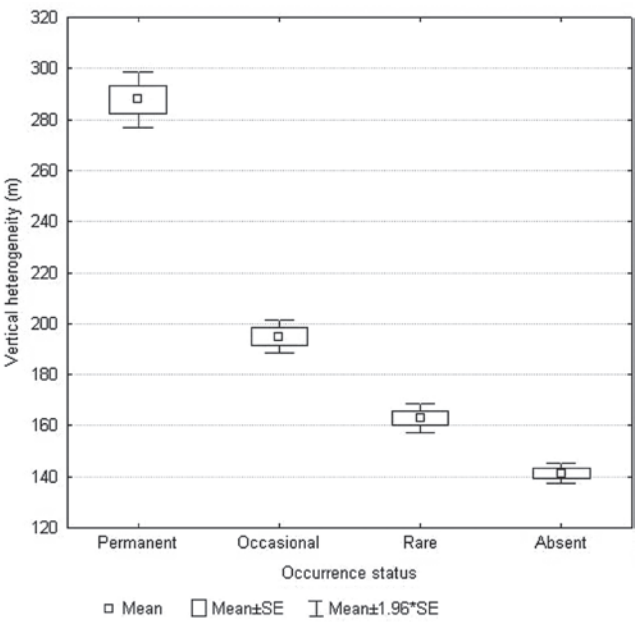

Fig. 4 Mean vertical heterogeneity of hunting districts with particular categories of the red deer occurrence displayed as boxplots.

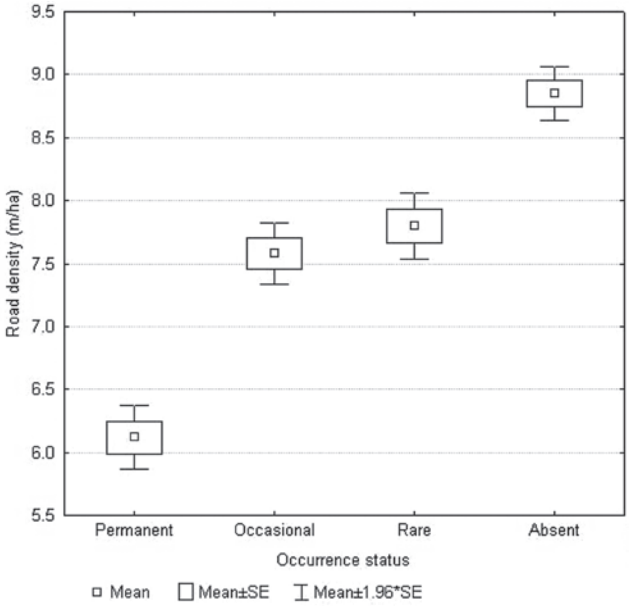

Fig. 5 Mean lengths of roads (meters per hectare) in hunting districts with particular categories of the red deer occurrence displayed as boxplots.

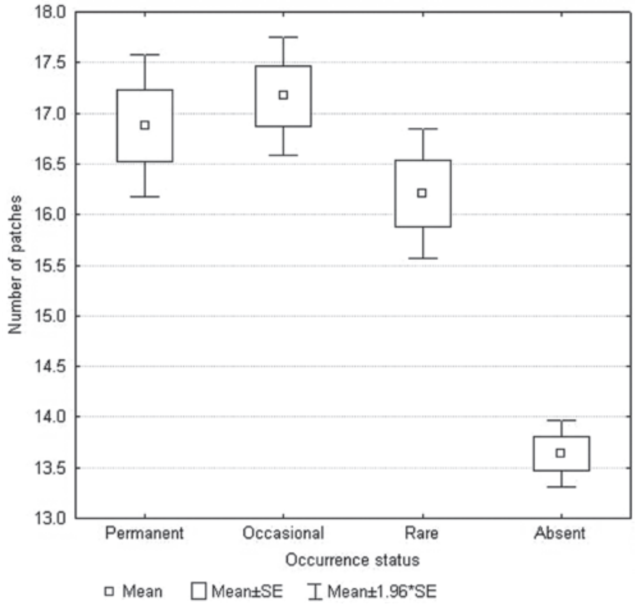

Fig. 6 Land cover heterogeneity expressed in terms of the mean number of patches in hunting districts with particular categories of red deer occurrence displayed as boxplots.

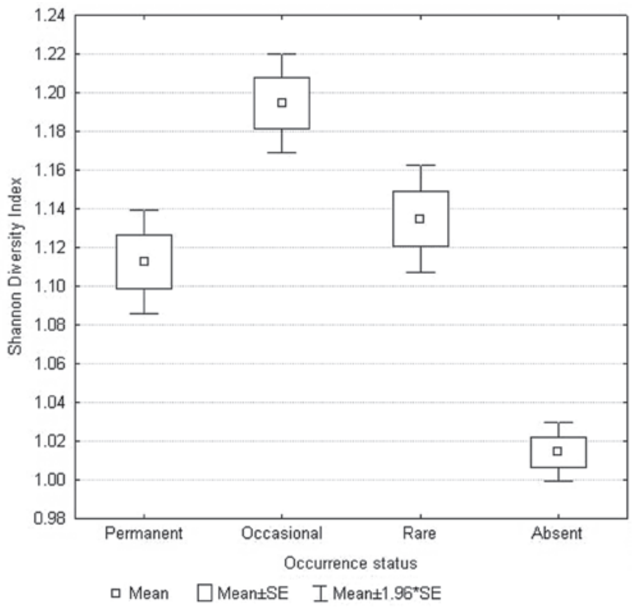

Fig. 7 Land cover diversity (expressed in terms of the Shannon diversity index) in hunting districts with particular categories of red deer occurrence displayed as boxplots.

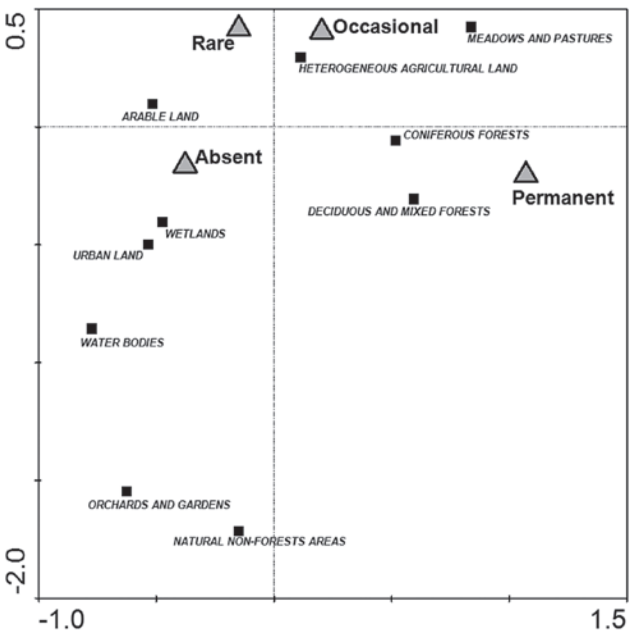

Fig. 8 Canonical correspondence analysis of land cover classes at locations where the occurrence status of red deer differ.

at high altitudes (Kruskal-Wallis test: H (3, N = 4566) = 512.5707, ***P < 0.001) (Fig. 3).

Mountain areas where red deer permanently occur in the Czech Republic have a pronounced vertical heterogeneity (Kruskal-Wallis test: H (3, N = 4566) = 899.1497, ***P < 0.001) (Fig. 4).

Permanent occurrence of red deer is typically associated with low anthropogenic disturbance measured in terms of a low density of roads (m/ha) (Kruskal-Wallis test: H (3, N = 4566) = 241.0102, ***P < 0.001) (Fig. 5).

Areas with red deer occurrence are typically the more heterogeneous hunting districts with a larger number of patches (Kruskal-Wallis test: H (3, N = 4566) = 199.3514, ***P ≤ 0.001) (see Fig. 6), more land cover classes (Kruskal-Wallis test: H (3, N = 4566) = 165.346, ***P ≤ 0.001) and generally higher diversity of different kinds of land cover expressed in terms the Shannon diversity index SHDI (Kruskal-Wallis test: H (3, N = 4566) = 170.8695, ***P ≤ 0.001) (Fig. 7).

The canonical correspondence analysis (Fig. 8) revealed significant differences in the share of individual land cover classes in hunting districts with different categories of red deer occurrence (**P < 0.01). Areas with permanent occurrence of red deer are generally characterized as those with a larger cover of deciduous and coniferous forests and meadows. Areas where the species is absent are mainly arable or urban land, or mainly covered by water bodies or wetlands.

Eurasian Elk

In the last period under review (2006–2009), the occasional occurrence of elk was reported inn 1.49% and permanent occurrence in 0.69% of this country's area (Fig. 11). Questionnaires relating to the period 2006–2009 were completed and submitted from only a quarter of the country (26.52%) and thus are fragmented. For this reason, the data for 1993–2005 is the most precise information on the current distribution of this species as these were collected from 77.8% of the country's area. During 1993–2005, the Eurasian elk was recorded occurring permanently on 0.41% and occasionally or migrating in 5.73% of the country (Fig. 10). Compared to the data for 1985–1992, when permanent occurrence was recorded in 0.33% and occasional or migratory in 9.75% of the country (Fig. 9), the total area of permanent occurrence of this species increased by 24%, whereas, the area of occasional and migratory occurrence dropped by 41.2%. The data for 2006–2009 imply, however, that the area of Eurasian elk permanent occurrence increased by 68.3% over that in the preceding period (doubled compared to 1992). This increase mainly reflects the extended home range of the population in the Šumava Mts. As the Eurasian elk in the region of Třeboň became extinct, the above numbers are currently not valid.

Migrating individuals occurred frequently anywhere in the past. Their numbers though have dropped significantly. In recent years, elks occupied only the right bank of the Lipno Dam and PLA Třeboňsko, but this population died out in 2010. In the east of the Šumava Mts., this species currently occurs in the surroundings of the village Dolní Drkolná. The range of its permanent occurrence spreads over the entire right bank of the dam and reaches its left bank in the south, particularly in the surroundings of Frymburk. Compared to previous years, its distribution has changed range most significantly in terms of expanding to the south in NP Šumava, where elks occur in an area extending to the village of Stožec. In the recent past, these animals could also be seen in some areas adjacent to those of permanent occurrence both in the Šumava and in the region of Jindřichův Hradec. This was most probably due to individuals leaving permanent populations. There are also records of elk occurring in the Českomoravská vrchovina Uplands and the north of Bohemia. However, these could relate to migrating animals. Maps in Figs. 9–11 record elk occurrences in two periods that are comparable in terms the time and intensity of data collection. They indicate a decrease in the

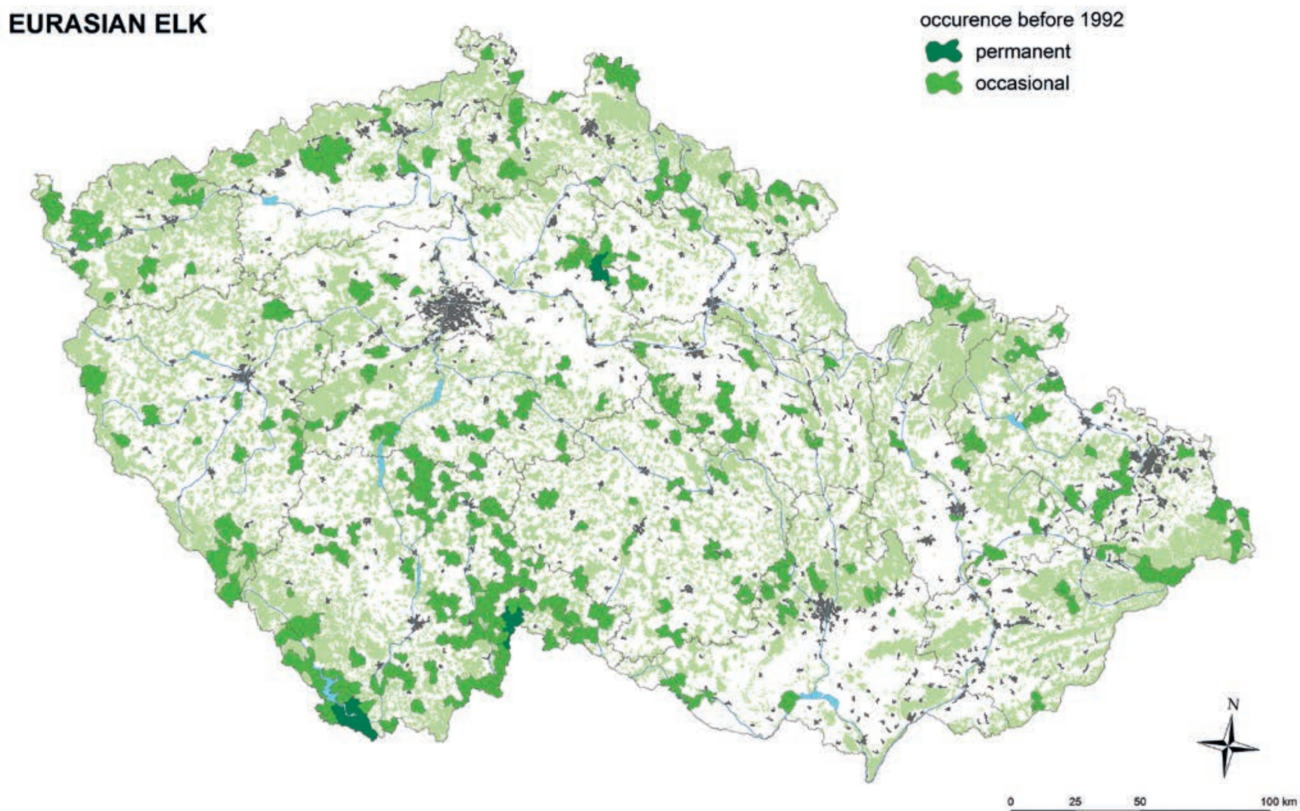

Fig. 9 Map showing the distribution of Eurasian elk in the Czech Republic in 1985–1992.

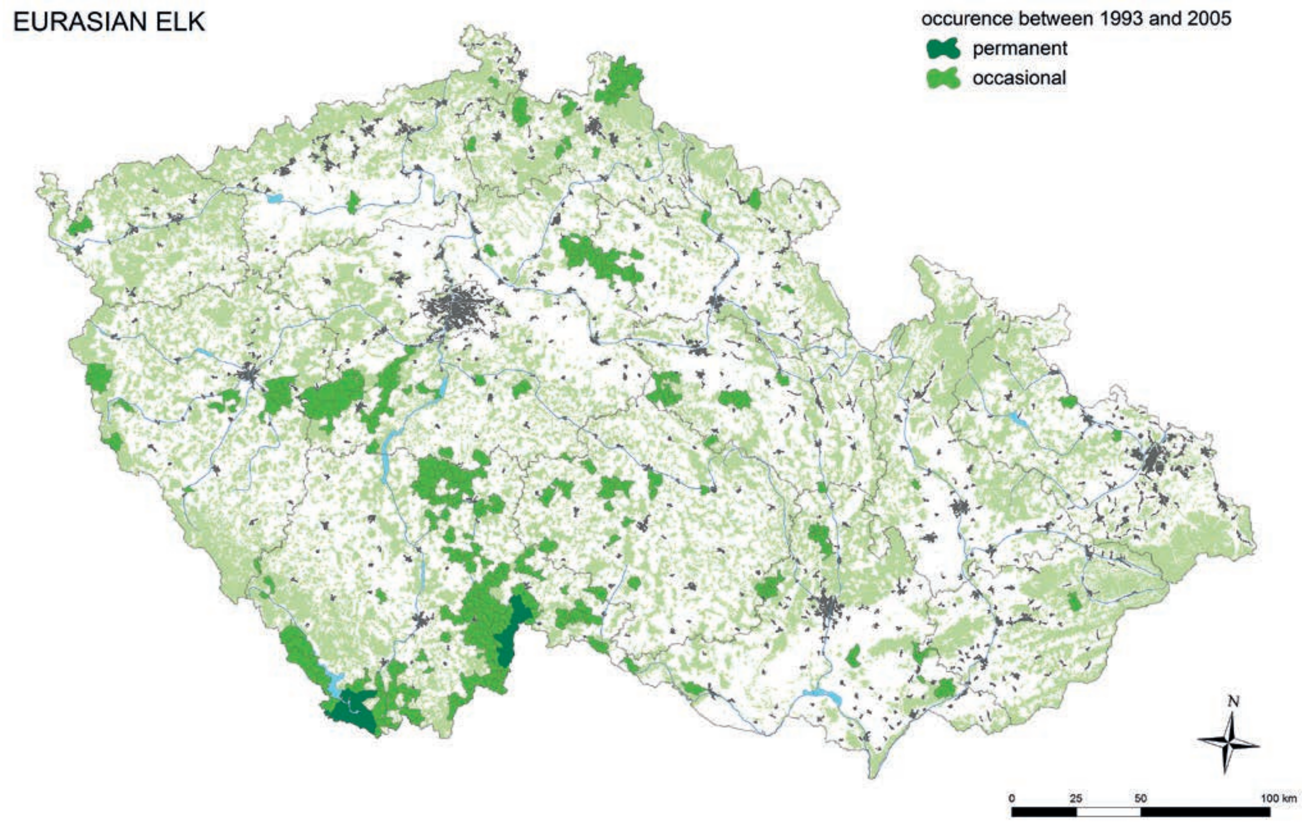

Fig. 10 Map showing the distribution of Eurasian elk in the Czech Republic in 1993–2005.

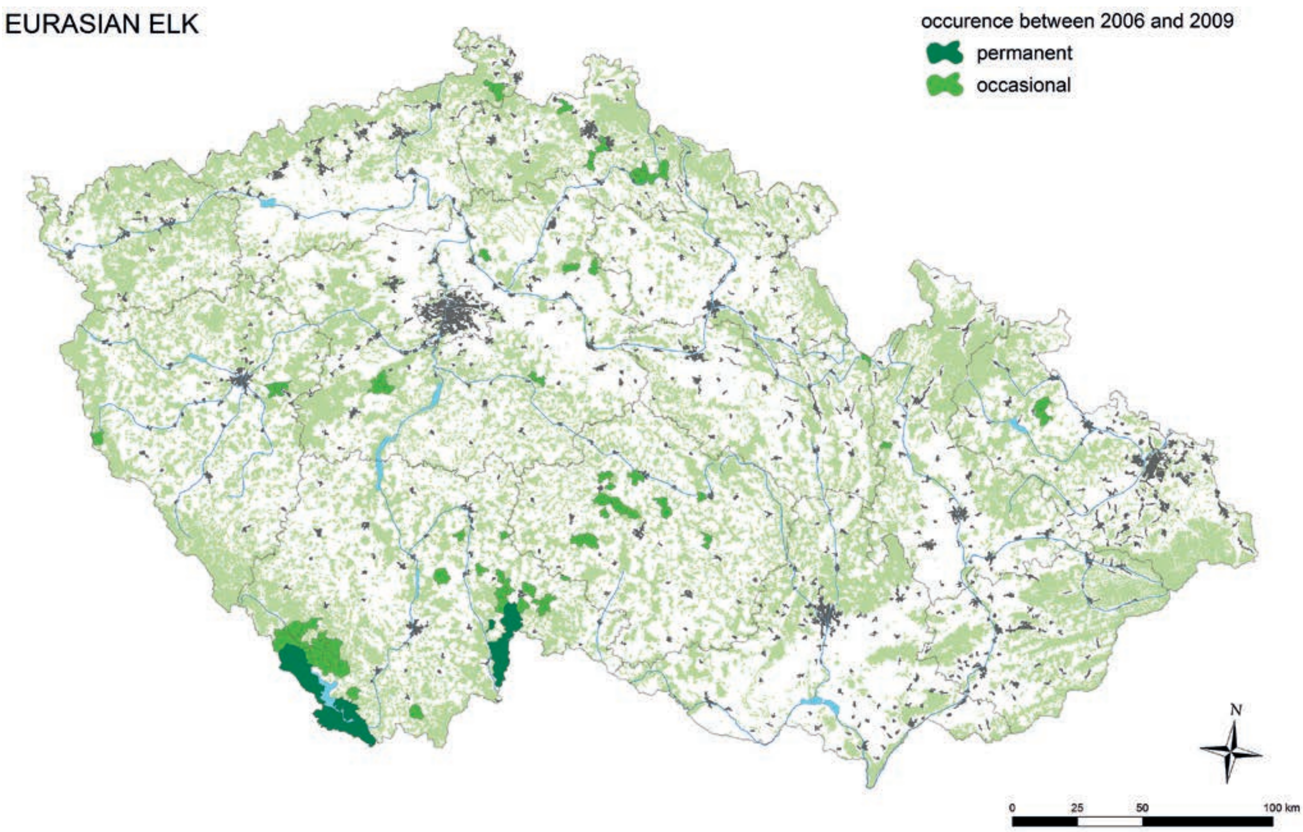

Fig. 11 Map showing the distribution of Eurasian elk in the Czech Republic in 2006–2009.

number of animals observed migrating, mainly at Podkrušnohoří and in the north of Moravia. In addition, a permanent micropopulation in the region of Nymburk has also disappeared. These negative trends continued in the last period reviewed (2005–2009) and resulted in the disappearance of the permanent population in the region of Třeboň in 2010.

From 1993, the number of elks recorded in the spring minimum viable population (published by the Czech Statistical Office) in the Czech Republic has varied between 13 and 60 animals. On average, the population of Eurasian elk in the Czech Republic is 28 animals. This data, however, is very likely to be inaccurate, unreliable and of low informative value. The number of 50 to 60 animals is completely misleading.

Habitat Preference

Hunting districts with the permanent occurrence of Eurasian elk are mostly located at high altitudes (Kruskal-Wallis test: H (2, N = 6056) = 53.06290, ***$P < 0.001$).

There is a strong correlation between vertical heterogeneity of the terrain and altitude ($r = 0.574$; N = 6056, *$P < 0.05$), which is reflected in the occurrence of red deer (Fig. 3). The situation with Eurasian elk is obviously distinct (Fig. 12), which implies that, within the range of its distribution, elk prefers a rather flat terrain, which is not the case for red deer. The vertical heterogeneity at locations at high altitudes with permanent or occasional occurrence of Eurasian elk is not high, which is reflected vertical heterogeneity of the districts where elk are hunted being lower than what is normal for locations at that altitude (Fig. 13).

Areas with permanent occurrence of the Eurasian elk are characteristic with low anthropogenic disturbance expressed as road density (Kruskal-Wallis test: H (2, N = 6056) = 10.67066, **$P < 0.01$) (Fig. 14).

Areas of permanent occurrence of the Eurasian elk have a significantly lower land cover diversity (expressed in terms of Shannon's diversity index) (Kruskal-Wallis test: H (2, N = 6056) = 12.18477, **$P < 0.01$) (Fig. 15). Similarly, the heterogeneity of land cover in hunting districts with the permanent occurrence of this species, expressed as an average number of patches and average number of land cover classes, is also lower in these areas (Kruskal-Wallis test: H (2, N = 6056) = 26.54428; ***$P < 0.0001$, resp. Kruskal-Wallis test: H (2, N = 6056)

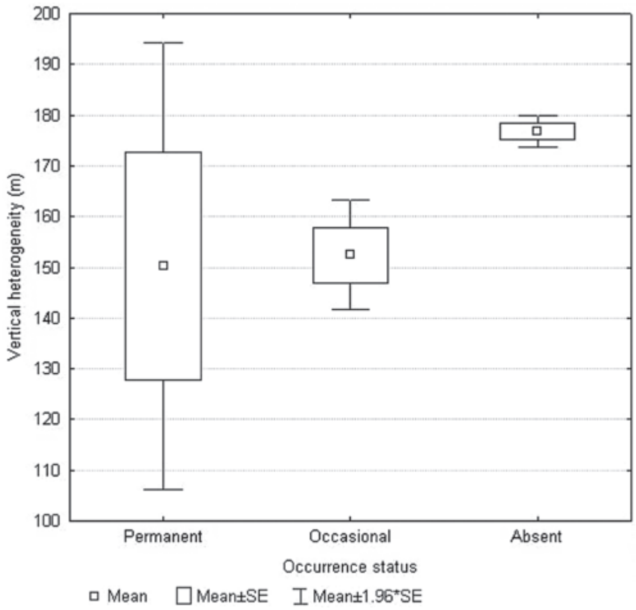

Fig. 13 Mean vertical heterogeneity in hunting districts with particular categories of Eurasian elk occurrence displayed as boxplots.

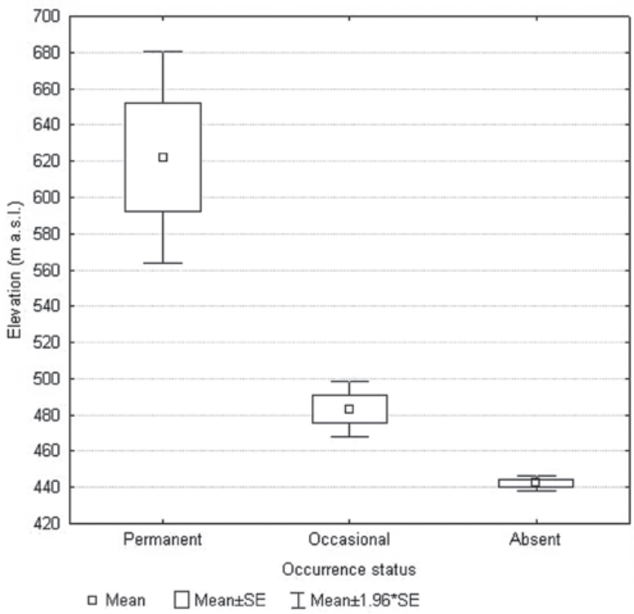

Fig. 12 Mean altitude of the hunting districts with particular categories of Eurasian elk occurrence displayed as boxplots.

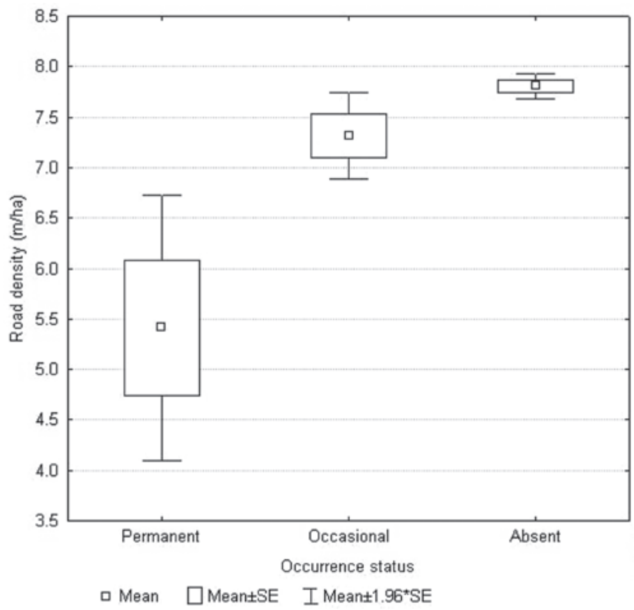

Fig. 14 Mean road density (meters per hectare) in hunting districts with particular categories of Eurasian elk occurrence displayed as boxplots.

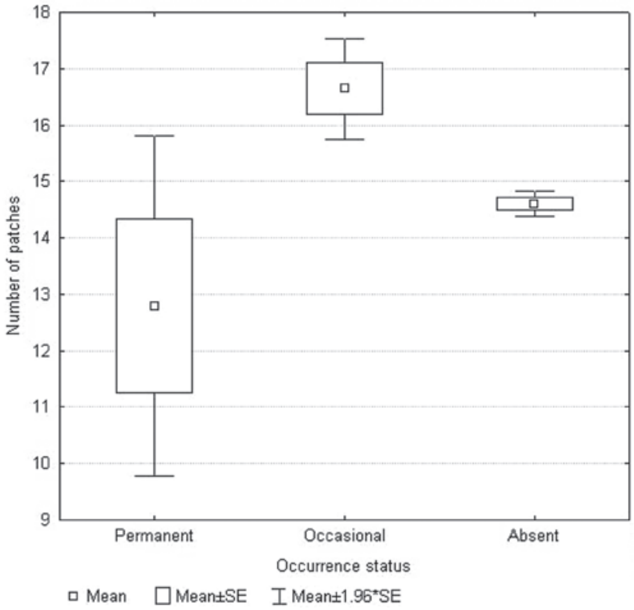

Fig. 15 Land cover heterogeneity expressed as the mean number of patches in hunting districts with particular categories of Eurasian elk occurrence displayed as boxplots.

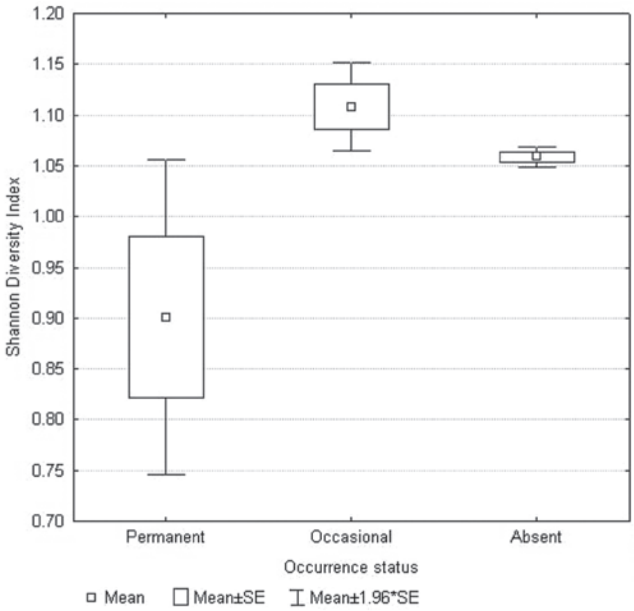

Fig. 16 Land cover diversity (expressed in terms of Shannon's diversity index) in hunting districts with particular categories of Eurasian elk occurrence displayed as boxplots.

= 52.36012; ***P < 0.0001) (Fig. 16)). Thus, Eurasian elk are markedly different from red deer, which prefer areas with high heterogeneity and diversity of land cover.

Canonical correspondence analysis (Fig. 17) revealed significant difference in the share of individual classes of land cover in hunting districts with different occurrences of Eurasian elk (**P < 0.01). Areas with permanent occurrence characteristically have a higher proportion of coniferous forests, pastures and water bodies, whereas those where the species is absent typically have a high proportion of arable and urban land, and deciduous forest.

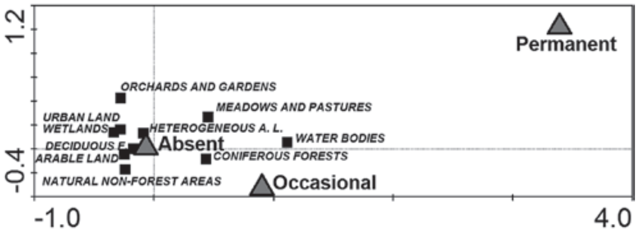

Fig. 17 Canonical correspondence analysis of land cover classes at locations with different occurrence of Eurasian elk. (Abbreviated HETEROGENEOUS A. L. = heterogeneous agriculture land, DECIDUOUS F. = deciduous and mixed forests).

Discussion

Red Deer

Maps of red deer abundance in the Czech Republic based on the distribution of this species in hunting districts indicater substantially lower numbers than the data of Anděra and Červený (2009), which are based on graticule based maps, which are significantly more precise. Nevertheless, these data indicate a gradual increase in the distribution of this species.

Two publications, Anděra and Hanzal (1995) and Anděra and Červený (2009), who use a graticule for the spatial division of their map, provide the best data on the distribution of red deer in the Czech Republic over the past 20 years. The Czech Republic is well covered in both cases. Questionnaires on the distribution of this species were completed and submitted from 82.2% of the territory in 1991–1992 and 93% in 2005–2006. The data for the first period records 306 squares of permanent occurrence and 214 of occasional occurrence of the red deer. Two squares of rare occurrence were excluded from data processing in the first period as the data were not considered reliable. The figures for the second period (2005–2006) records 317 squares of permanent and 142 squares of occasional occurrence of the red deer. This indicates no dramatic changes in either the extent or the geographic distribution. In the period 2005–2006 there was a decrease in the number of squares of occasional occurrence by more than a third (Anděra and Červený 2009). It should be noted, however, that the original data for the second period also included rare occurrences of this species, recorded in 104 squares of the grid, which were excluded from the final assessment. The exclusion of the two squares with unreliable data in the period 1991–1992 is considered justified. In the second period, however, there were 104 squares of rare occurrence (data submitted by approximately 600 hunting associations). Data coming from such a large number of squares are less likely to be unreliable and their exclusion from the assessment significantly distorts the distribution of red deer in the Czech Republic. If we include data on rare occurrences then a comparison of both periods under review gives a different result concerning the distribution of red deer in the Czech Republic: no substantial changes in the areas of permanent occurrence of this species with them permanently occurring in 46.6% of this country's area; areas of occasional occurrence decreases by approximately a third to 20.9%; but the number of squares with reported rare occurrence increases 52 times from 0.29% in 1991–1992 to 15.3%. If the figures on occasional and rare occurrence are taken as a single category, the area of such a category increases by 13.9% between the two periods and red deer occurs occasionally or rarely in 36.2% of the country. Generally, the area of any occurrence of this species has been increasing. This assumption is also supported by growing numbers of practically all wild even-toed ungulates subject to game management. Red deer is currently even considered as overpopulated, which is, according to a number of authors, due to inappropriate processing of hunting statistics and hunting plans (Kamler 2010; Kamler and Plhal 2010; Marada 2010; Pfeifer 2010). The growing numbers of red deer are also apparent from the data provided by the Czech Statistical Office (Fig. 2), which are considered by certain sources as 30% underestimated (Pfeifer 2010). The Ministry of the Environment of the Czech Republic has already responded to the situation by issuing Guidelines for State Administration Bodies to Reduce Populations of Hoofed Game in the Czech Republic of 4 July 2008 (Ref. No. 23485/2008 – 16200). It is thus not surprising that red deer migrate to new areas and often makes use of agricultural land with a higher proportion of fields and roads. A mature maize field surprisingly offers a quieter environment with an even better food supply than a forest full of mushroom pickers (Pfeifer 2010).

Red Deer Habitat Preferences

The fact that this study indicates that the red deer occur mainly at high altitudes in the Czech Republic is not determined by their preference for a montane or sub-montane environment. Forest vegetation providing sufficient cover for game often is a more important factor than food supply in determining the actual distribution of this species (Borkowski 2004). High altitude areas are covered with extensive forests in this country. The red deer population density is highest in lowland forests as they have a higher carrying capacity (Prokešová et al. 2006), which proves that high altitude areas are not its naturally preferred habitat. As the population density is largely affected by game management practices, the interpreted data should be approached with certain prudence. Still, altitude and the related choice of habitats may locally play a role. Čejka (1998) reports that the population density of red deer increases with increase in altitude, possibly because this species prefers the southern sides of mountain ridges as they provide a more favourable microclimate and a good view that enables them to respond faster to potential threats.

More frequent permanent occurrence at high altitudes is also related to the occurrence there of a more pronounced vertical heterogeneity, which is highly correlated with altitude ($r = 0.574$; $N = 6056$, $^*P < 0.05$). The vertical heterogeneity in hunting districts with a permanent occurrence of red deer at a given altitude is slightly higher than what we might expect. This suggests that this species, unlike Eurasian elk, does not avoid vertically heterogeneous terrain.

The distribution range of red deer is obviously of a relict or anthroporelict character in Central Europe as this species has been forced out into marginal and less inhabited parts of the country where there are still large areas of forest and a lower road density. The data do not allow a direct assessment of the negative effects of road density on red deer populations. Nevertheless, the negative effects of road density and the related drop in habitat accessibility are generally accepted (Frair et al. 2008).

The type of vegetation and land cover is important for red deer both as a hiding place and for feeding. This species is generally and naturally understood as forest game (Anděra and Horáček 2005). Non-forest habitats are also important for this species. In Norway, for example, the weight of the deer is positively correlated with the proportion of meadows in the landscape (Mysterud et al. 2002). Suitable habitats with a sufficient carrying capacity affect the fitness of animals even in subsequent generations (McLoughlin et al. 2008). That red deer favour pastures is also reported by Godvik et al. 2009. These authors demonstrate that, in forest habitats containing some forage, there is both an increase in the selection of pastures (i.e., not proportional) and a reduction in the time spent in pastures (i.e., not constant time use) with a decrease in the availability of pastures within the home range. Similarly, Jones and Hudson (2002) report that this species uses the grass/meadow habitat more than expected, while all other habitats are used in proportion to their availability.

In the Czech Republic, the red deer predominantly occurs at high altitudes and from the agricultural point of view less important areas, where the proportion covered by pastures has significant increased in the past 20 years (Romportl et al. 2010). This may be one of the factors causing the mentioned increase in the red deer population (see Fig. 2 – game management in 1992–2009).

Higher SHDI values in areas with reported occurrence of red deer support the presumption that it is an ecotonal species adapted to feeding on a mixed diet of browse and graze (Clutton-Brock et al. 1982).

Eurasian Elk

The number of migrating individuals is currently much lower than in the past, when they could be observed practically anywhere (Anděra and Horáček 2005). At the end of the 1990s, Homolka (1998) stated that 30–50 animals permanently occurred in the Czech Republic. Later, the area of occasional occurrence of Eurasian elk diminished nearly by 85% as opposed to the turn of 1980s, which presumably relates to the predicted negative development of the population in Poland (Raczyński 2008), where most of the migrants came from. It is reflected in the fact that there has been a significant reduction in the number of migrating animals in Moravia, in the area of the Krušné hory Mts. and the České středohoří uplands. Nevertheless, elks still migrate from the area of the Orlické hory Mts. moving south-westwards into south Bohemia. Migration of these animals from Poland may be related to their tendency to follow significant topographic elements in the landscape, which in this case may be the Nysa Kłodzka River (Andersen 1991; Ball et al. 2001; Rolandsen et al. 2010). The lower numbers of occasional and migratory occurrences are, to a certain extent, affected by incomplete data acquired within the last reporting period, but may still indicate a certain development.

In the early 1990s, when the Czech Republic opened its border with neighbouring countries, elks found a new way to the south and began to spread and search for new areas to settle. Austrian administration responded to the presence of this new species by issuing permits to shoot three animals (Ševčík 1994; Mrlík 1998). The administration of PLA Třeboňsko responded immediately to the issuing of these permits by sending a letter to the Provincial Government of Lower Austria, pleading for assistance and addressed various nature conservation and zoological institutions. This resulted in a number of negotiations the outcome of which was that elk would only be hunted in Austria in exceptional circumstances or prevented altogether (Ševčík 1994). Homolka and Heroldová (1997) state that the Eurasian elk is already listed in Austria as game subject to protection throughout the year. A permit may be granted to hunt this animal if it can be proved it caused damage. In Germany, the Eurasian elk is considered as a species not natural to the cultural landscape and is hunted (Homolka and Heroldová 1997). This species has always been protected all year round in the Czech Republic under Act No. 114/1922 Coll., where it is listed in the category of specially protected species as a strongly threatened species. Hunting it is still possible under a Decree of the Ministry of Agriculture No. 134/1996 Coll. in the period from 1 August to 31 December, but only upon a permit issued by the Ministry of the Environment. No such permit has ever been issued in the Czech Republic. However, at least 5 animals have been illegally hunted since 1976 (Anděra and Kokeš 1978). Poaching is generally reported as one of the most frequent causes of death in Eurasian elk (based on data from Bohemia, Slovakia, Austria and Germany) and makes up 36% of the death rate (Homolka 2000; Hutr 2004). The first rank in these sad statistics is held by road kills, i.e. 38% (Anděra and Kokeš 1978; Homolka 2000; Hutr 2004). Three cases were reported from Bavaria in 2007 (Märtl 2009). Also in the Czech Republic an elk was hit by a car on expressway I/35 near Hodkovice nad Mohelkou on 7 April 2009 (Suchánková 2009).

The increasing number of areas of permanent occurrence is interesting. It may have been due to a reduced number of individuals migrating to Austria. Elks began to spread into Austria in the early 1990s, when the barriers along the Czech borders were removed. This invasion only lasted for two years. As the animals did not find favourable conditions there, they left, except from the area adjacent to the region of Jindřichův Hradec) (Homolka and Heroldová 1997; Mrlík 1998).

The area of this species' permanent occurrence grew in size mainly on the right bank of the Vltava River and the Lipno Dam, where the number of animals is similar to that in the past. This is the last permanent population of this species in this country. It settled here at the turn of the 1970s and there were 13 animals in 1995 (Homolka 1998); Anděra and Červený (1994) mention 10 to 20 animals. The records based on trails indicate a minimum of 11 animals in 1996 (Homolka 1998). The Lipno population is currently estimated at 10–15 animals; the most accurate census was conducted by game wardens in the Forest District Vyšší Brod who reported 9 individuals (Märtl 2009; Šustr 2010). Extension of the area of permanent occurrence of Eurasian elk into the National Park Šumava is undoubtedly the most significant change. It is documented, for example, by Šustr (2010) and supported by data on animals hit by cars in neighbouring Bavaria (Märtl 2009), but which most probably came from NP Šumava.

Until recently, the region of Třeboň was the second area of this species' permanent occurrence in the Czech Republic. The maximum size of this population was estimated at 10–20 animals in the second half of the 1980s (Ševčík 1994). In 1993, the estimates were 3–5 animals (Ševčík 1994) in this population; the same as that estimated by Kuchyňka (1994). Ševčík (1995) further added that the total number of animals occupying the area might have been slightly higher as some animals might not have been observed, particularly in marginal areas. Homolka and Heroldová (1997) estimate the elk population in the region of Třeboň to be 10 animals. Unfortunately, more precise data on the occurrence of individual animals is missing for more recent years and permanent occurrence is reported only at PLA Třeboňsko. The last animal probably lived here in the first decade of the 21st century and died in the second half of 2010 (Šustr, Kašperské Hory, pers. comm. 2010).

Other permanent "micropopulations" were recorded in the past, particularly in areas adjacent to Třeboň. Mrlík (1996) report about 18 animals on average every year in a large area between Nové Hrady and Veselí nad Lužnicí between 1990 and 1994. From 1991, elk calves were observed in the region of Bechyně, where the number of animals varied between 3 and 5 throughout the year. The number recorded in the Jistebnice region was around 6–8 animals and 5 were reported in the region of Jindřichův Hradec (Homolka and Heroldová 1997). Homolka (2000) also describes permanent occurrence of this species in an area extending from the Novohradské hory Mts. to the Středočeská vrchovina uplands, for which he cites approximately 30 animals. However, there is no more detail on their movements.

There was a micropopulation of elk in the region of Nymburk, where 1–2 females with calves and a male occupied the area of Forest Districts Dymokury, Kněžice and Rožďalovice (Homolka and Heroldová 1997). Data from the first decade of the 21st century state, however, indicates that this population died out before 2004 (Anděra and Červený 2009). There are also areas where permanent occurrence was not considered but females with calves were observed. At the beginning of the new millennium, Hutr (2004) reports several records of this species in the surroundings of Příbram and Brdy, including a female with a calf. The preference of elks for forested areas is presumably related to their timidity. As is the case of the red deer, the Eurasian elk primarily seeks environments that provide good cover. At the home range scale, elks select early seral habitats that provide both food and cover, or primarily cover (MacCraken et al. 1997).

This species' tendency of avoid areas subject to disturbance is clearly indicated in this study by the small percentage of urban land in areas of permanent occurrence and is also reported in substantially less inhabited Finland (Nikula et al. 2004).

Preference for a vertically less heterogeneous terrain is probably due the animal's physical proportions. With its huge body, it is better adapted to flat lake areas in Fennoscandia, northern Russia and Siberia. This preference for less steep terrain compared to the red deer is also reported by Gillingham and Parker (2008).

The macrohabitat analysis revealed that Eurasian elk occurs in areas with a high percentage of coniferous forests in the Czech Republic, which is in accordance with its food requirements, i.e. leaves and shoots of certain tree species, such as Goat Willow (*Salix caprea*) and Alder Buckthorn (*Frangula alnus*) (Homolka and Heroldová 1997; Homolka 1998). These trees usually do not form vast and continuous stands but grow in patches in successive stages in meadows, abandoned agricultural land and waterlogged sites. This is where elks seek their food (Homolka 1998). In winter, elks in the Czech Republic prevailingly feed on annual shoots of previously mentioned trees and, among conifers, on pine stands (Homolka 1998). The same food strategy is frequently recorded in other parts of this species' home range (Courtois et al. 2002). Unproven preference for wetlands and deciduous forests is a surprising result. In both cases, this fact may be due to by the nature of the CORINE Land Cover habitat data. The minimum size of a mapping unit determined by the spatially extensive classification of satellite images is 25 ha, which exceeds the typical size of wetlands in the Czech Republic. Wetlands are mostly minor enclaves inside a larger matrix and thus are not recorded in the data used. For this reason, this type of habitat is not typically included as an individual class of land cover but more frequently as part of larger coniferous or mixed forest stands or heterogeneous areas with agricultural land and a natural cover of woody species.

Acknowledgements

The authors would like to acknowledge and express their gratitude to E. Chumanová (The Silva Tarouca Research Institute for Landscape and Ornamental Gardening, Průhonice) for consultations on statistical analyses and J. Pondělíček and J. Růžička (Ministry of Agriculture, Prague) and J. Císař (Forest Management Institute, Brandýs nad Labem) for providing a digital map of the hunting districts in the Czech Republic.

This research project was supported by the Ministry of the Environment of the Czech Republic from projects No. MSM 6293359101 and VaV SP/2d4/36/08.

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CAN ALGAL BIOTECHNOLOGY BRING EFFECTIVE SOLUTION FOR CLOSING THE PHOSPHORUS CYCLE? USE OF ALGAE FOR NUTRIENT REMOVAL – REVIEW OF PAST TRENDS AND FUTURE PERSPECTIVES IN THE CONTEXT OF NUTRIENT RECOVERY

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ABSTRACT

Eutrophication of water by nutrient pollution is a global environmental issue. Biological methods for removing nutrients are environmentally friendly and sustainable. Therefore, this article summarizes main trends in the use of algae for removing nutrients from wastewater using both suspended and attached algal-based systems. A wide variety of algal species and experimental approaches has been tested to date. Researchers report that algae are able to effectively remove a variety of pollutants and nutrients. This review also discusses the potential of algal-based technology for nutrient, especially phosphorus, recovery. Despite the fact that effective nutrient removal has been demonstrated, there are still many challenges to be overcome in the development of succesfull technologies.

Keywords: wastewater treatment, algae, nutrients removal, phosphorus recovery

Introduction

Pollution of surface water due to high concentrations of nutrients is a global issue affecting all countries worldwide. Eutrophication is a term, which was used in limnology already at the beginning of the 20th century (Weber 1907). But this term was used only for describing the effects of pollution on ecosystems without a deeper understanding of the causes. Since the mid-20th century, mass presence of algal blooms in reservoirs, growth of macrophyta and periodic killing of fish were symptoms, which were not possible to ignore (Schindler 2006). R. A. Vollenweider was the first scientist who linked high nutrient input into lakes with eutrophication. He performed a comprehensive analysis of available data and of systematic scientific studies. From the results, he deduced that a reduction in the input of phosphorus (P) and in some cases also nitrogen (N) would avoid eutrophication and its symptoms in lakes (Vollenweider 1968). Vollenweider's results were supported by W. T. Edmonson, who published a six-year study of Lake Washington. He found a strong correlation between P concentrations and algal standing crops. When the nutrient load was reduced by diverting sewage away from the lake, it rapidly recovered (Edmonson 1970). In other studies P was also determined as the limiting factor for growth of phototrophic organisms in lakes and reservoirs (e.g. Schindler 1977; Ahlgren 1978; Holtan 1981). Numerous studies focused on causes and effects of high nutrient concentrations on water ecosystems were carried out starting from seventies of 20th century (e.g. Hutchinson 1973; Ahlgren 1978; Howarth 1988; Jeppesen et al. 2002).

The high input of P and N into surface water originated from variety of sources (Smith 1998). Main *point sources* of nutrient pollution are mostly wastewater effluent (municipal and industrial), runoff and leachate from waste disposal sites, runoff and infiltration from animal feedlots, runoff from mines and unsewered industrial sites and overflows of combined storm and sanitary sewers (Novotny and Olem 1994). *Point sources* are more easily monitored than *nonpoint sources,* which are diffuse. *Nonpoint sources* of nutrients include runoff from agriculture, runoff from unsewered areas, septic tank leachate and runoff from failed septic systems and atmospheric deposition over water surfaces (Carpenter et al. 1998). High concentration of P and N in water results in an abundant growth of algae, cyanobacteria and macrophytes. Nutrient pollution also causes shifts in the dominant species towards cyanobacteria, which are potential producers of toxic compounds (Skulberg et al. 1984). The abundant growth of algal biomass starts a cascade of negative processes in water ecosystems. Dense algal mats reduce the quality of the living conditions for other organisms such as invertebrates and fish. Decomposition of large amounts of algal biomass causes diel fluctuations in pH and in dissolved oxygen concentrations, which is harmful for fish. Decomposition of biomass can also cause taste and odour problems. Worse water quality also results in restrictions on recreation and swimming in polluted water (Quinn 1991).

Monitoring and control of nutrient load is an essential part of water management (Daniel et al. 1994; EU Water Frame Directive 2000/60/EU). The problem of nutrient pressures on water resources are also included in the 7th Environment Action Programme (Decision No 1386/2013/EU of the European Parliament, 2013). Despite all efforts, control of nutrient pollution remains one of the most important environmental issues (Jarvie et al. 2013).

The next serious topic inseparably connected with eutrophication, is the P recycling. P is an essential ele-

Sukačová, K., Červený, J.: Can algal biotechnology bring effective solution for closing the phosphorus cycle? Use of algae for nutrient removal
European Journal of Environmental Sciences, Vol. 7, No. 1, pp. 63–72
https://doi.org/10.14712/23361964.2017.6

ment for all living organisms. P rocks are mined only in a few regions in the world. The currently known reserves are concentrated in few countries, particularly Morocco (Scholz et al. 2013). The resources of P rocks are very small in Europe, especially bearing in mind the high demand. The study "Phosphorus flows and balances of the European Union Member States" describe an unbalanced economy in terms of P. On the one hand European countries are fully dependent on imports of P rock, on the other hand there are a great losses of P to wastewater and food waste (van Dijk et al. 2016). Big losses of imported P to the environment cause serious environmental problems. This unsustainable management of non-renewable resources needs to be changed. For the above reasons, it is necessary to focus on the development of new technologies for phosphorus removal and recovery.

Biological methods of nutrient removal from wastewater are considered to be low cost and environmental-friendly technologies (Mantzavinos et al. 2005). Different groups of microorganisms can be used for removing nutrients (Bashan and Bashan 2004). Many studies demonstrate the high ability of microalgae to reduce the nutrient content of wastewater (Christenson and Sims 2011; Whitton et al. 2015). Moreover, this method can bring several benefits because it does not generate additional waste, such as activated sludge, does not require the use chemical substances for phosphorus reduction and provides an opportunity for efficiently recovering nutrients (Mantzavinos et al. 2005; Pittman et al. 2011). Therefore, the objectives of the present study are to summarize past development and recent progress in nutrient removal technologies using microalgae in the context of nutrient recovery.

The Algae and their Role in Biotechnology

The cyanobacteria and algae are highly heterogeneous groups of organisms including both small unicellular species and large freshwater and marine organisms of sizes more than 1 m with a multicellular organization. The algae, like plants, are photosynthetic organisms but have a simpler cellular organization. The algae have no roots, stems, leaves or complex vascular networks. They occur as single cells, multicellular colonies, simple or branched filamentous, leafy or blade forms without a high degree of cell differentiation (Barsanti and Gualtieri 2006). The cyanobacteria and algae can colonize all biotopes. They can live as planktonic organisms in the euphotic zone of lakes, water reservoirs or in the sea. They also colonize firm surfaces submerged in water and live attached on sediments, stones, plants etc. (Stevenson 1996). Microscopic species are called "microalgae". Large species with complex cellular organization are called "macroalgae". The term "microalgae" is used in a wide sense in applied phycology. It includes both prokaryotic cyanobacteria and eukaryotic algae (Masojídek and Prášil 2010).

Algae are used in several areas of biotechnology. Primarily, it is the commercial production of microalgae for dietary supplements, cosmetic products and nutrition for aquaculture (Becker 2004). Microalgae are characterized by a high content of valuable compounds such as proteins, amino acids, essential unsaturated fatty acids and vitamins. Commercially produced genera are mainly *Chlorella*, *Arthrospira* (*Spirulina*), *Dunaliella*, *Nannochloropsis* and *Haematococcus* (Spolaore et al. 2006; Mimouni et al. 2012).

Microalgae were identified as an important source of lipids with potential use as feedstock for biofuel production. Microalgae can produce different types of lipids, for example unsaturated fatty acids (eicosapentanoic acid or docosahexanoic acid) and neutral lipids including triacylglycerids (Markou and Nerantzis 2013). They can be a suitable feedstock for biofuel production after conversion of the lipids to fatty acid methyl esters.

Research on the potential for using algae for bioremediation is currently an important issue in microalgal biotechnology. Microalgae can be used in wastewater treatment for the removal of different pollutants. Reduction in chemical and biological oxygen demands are mainly studied together with the removal of N and P in agricultural, domestic or municipal wastewater (e.g. Shelef et al. 1980; Fallowfield and Garret 1985; Arcila and Buitrón 2016). Algae are also an effective bio-sorbent for removing heavy metals because their cell surfaces are negatively charged and they have large cell surface to volume ratios (Filip and Peters 1979; Wilde and Benemann 1993; Roberts et al. 2013; Li et al. 2015).

As mentioned above, many studies have shown the ability of algae to grow in wastewater and to reduce nutrient concentrations in laboratory-scale studies (e.g. Proulx et al. 1993; Chevalier et al. 2000; Doria et al. 2012). For this purposes, the microalgae can be cultivated in suspension or attached to a firm surface. Much attention has been paid to the cultivation of algae in wastewater treatment ponds and natural attached algal-based systems (Adey et al. 2011; Park et al. 2011). The possibility

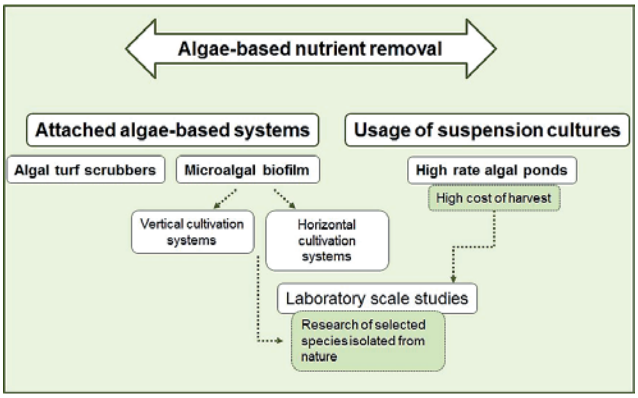

Fig. 1 Summary of main trends in nutrient removal technologies using algae.

of nutrient removal coupled with production of biofuels has been studied. The connection of these two processes would bring important economic benefits, including a reduction in the cost producing biofuels (Chinnasamy et al. 2010; Christenson and Sims 2011).

Wastewater Treatment Using Suspended Algae

Wastewater Treatment of High Rate Algal Ponds (WWT HRAP)

The first studies that focused on the potential of algae for wastewater treatment were published in the middle of the 20th century (Oswald and Gotaas 1957; Bogan et al. 1961). At that time, the concept of WWT HRAP was established. High rate algal ponds (HRAP) are shallow oxidation ponds mixed by means of a paddle wheel. They are used for the treatment of municipal, industrial and agricultural wastewater (Park et al. 2011). This technology was tested in South Africa in the 70s. Its efficiency in treating industrial wastewater with high concentrations of N was studied over a long period of time. Parameters of HRAP were optimized to achieve better light conditions in cultivation suspension and to minimize evaporation of water from the HRAP. Density of cell culture was set at 0.5 g DW l^{-1}. Simultaneously, a sufficient reduction in nitrogenous substances was achieved (Bossman and Hendricks 1980). HRAP was tested for different kinds of wastewater, but the most often investigated treatment of liquid wastes was those from agriculture. Repeated reductions in BOD, concentrations of N and P are recorded in agriculture wastewater (Shelef et al. 1980; Picot et al. 1991). Reduction of N substances in municipal wastewater was studied in Spain. During a pilot study, algal cultures in HRAP was able to remove about 70% of the N (Garcia et al. 2000). Predictive models of algal growth, oxygen production and reduction in pollutants were developed based on long-term studies (Kroon et al. 1989; Sukenik et al. 1991). Typical biomass production in these systems ranged from 8–35 g m^{-2} d^{-1} (Shelef et al. 1980).

No special algal species were selected for inoculating HRAP, instead the algal assemblage developed naturally in the ponds. Therefore, many studies were focused on the ecology and succession of these specific algal assemblages. Palmer (1974) studied species composition in WWT HRAP. He found that the most frequently recorded species were green algae, especially *Chlorella*, *Ankistrodesmus*, *Scenedesmus*, *Chlamydomonas*, *Micractinium*, *Euglena* and Cyanobacteria, genus *Oscillatoria*. Erganshev and Tajiev (1986) report similar species in six lagoons in central Asia. Sim and Goh (1988) also report an algal assemblage dominated by green algae in HRAP containing agriculture wastewater in Singapore.

The main disadvantage of HRAP is that it is difficult and expensive to harvest the algal biomass, which is necessary for effective wastewater treatment (Cromar et al. 1992). HRAP is mostly criticized because of their low productivity due to light limitation, high dissolved oxygen levels and loss of biomass to grazers (Chisti 2007; Mata et al. 2010; Park et al. 2011). Recently, researchers have focused on optimizing the operating parameters such as hydraulic retention time, mixing, CO_2 availability and cultivation mode and controlling grazers (Park and Craggs 2011; Park et al. 2013). New progress was recently presented in the potential usage of WWT HRAP for low-cost biofuel production (Mehrabadi et al. 2015; Arcila and Buitrón 2016).

Laboratory-Scale Studies Using Suspension Cultures

While HRAP are naturally colonized by algal assemblages, many studies since the 90s have evaluated the effectiveness of particular species of algae for N and P removal. In particular, great attention was paid to those species that were easy to harvest in order to reduce the costs of harvesting. Proulx et al. (1993) studied the growth of the cyanobacterium *Phormidium bohneri* in secondary effluent. These species are able to remove 83% of the N and 81% of the P from municipal wastewater, moreover, they also have a high ability to aggregate and settle in ponds. Several evaluations of the nutrient removal capacity under different conditions for several benthic cyanobacteria are published. Arctic species *Phormidium tenue* and *Oscillatoria sp.* were tested to develop technology suitable for the cool climate in Canada (Talbot and de la Noüe 1993; Chevalier et al. 2000).

Doria et al. (2012) isolated the microalga *Scenedesmus acutus* from municipal wastewater and recorded its biomass production coupled with reduction in nutrients during growth in a tubular bioreactor (50 l). She reports a biomass production of 0.24 g DW l^{-1} d^{-1} and complete removal of N from wastewater. The disadvantage was that it was necessary to add microelements (Fe, Mg) to the wastewater. Different species of green microalgae are repeatedly used in various types of wastewater. The genus *Scenedesmus* is able to remove 94% of organic N and 66% of P from municipal wastewater (de Alva et al. 2013). Similarly, Ren et al. (2015) report that *Scenedesmus* isolated from soil reduced the concentration of COD and nutrients in starch wastewater. Other species used are for example *Monoraphidium sp.*, *Chlorella ellipsoidea*, *Chlorella vulgaris*, *Neochloris oleoabundans* or *Desmodesmus sp*. All these species are able to effectively remove nutrients from wastewater (Wang et al. 2011; Arbib et al. 2014; Holbrook et al. 2014; Fang et al. 2015).

In addition to monocultures, algal consortia were also tested. Chinnasamy et al. (2010) developed algal consortia and determined their capacity for nutrient absorption. A consortium including the green microalgae *Chlorella sp.*, *Chlamydomonas sp.*, *Scenedesmus sp.*, *Gloeocystis sp.* and cyanobacteria *Anabaena sp*. and *Limnothrix sp.* was cultivated in wastewater mainly from carpet mills. This consortium removed 96% of the nutrients. Similarly, Renuka et al. (2013) used four consortia dominated by *Chlorella sp.*, *Scenedesmus sp.*, *Chlorococcum sp.* and cyanobacteria *Phormidium sp.*, *Limnothrix sp*. and *Anabaena sp*. and report that the highest nutrient removal was achieved by a consortium dominated by filamentous cyanobacteria. Generally, algal consortia are able to survive environmental fluctuations and are resistent to invasion by other species (Subashchandrabose et al. 2011).

Recently, the research on the use of algae for wastewater treatment has included an evaluation of the energy content of the algal biomass produced, lipid production and production of biofuels (Fang et al. 2015; Kim et al. 2015; Ren et al. 2015). This topic is also connected with the development of new technologies and new devices for cultivating algae.

Wastewater Treatment Using Attached Algae-based Systems

The harvest of algal biomass from suspension in wastewater is technically difficult and accounts for 20–30% of the costs connected with cultivating algae (Liu and Vyverman 2015). This led to a greater interest in solutions using algae attached to submerged surfaces (Hoffmann 1998).

Attached algal communities are traditionally called "periphyton", a term that was introduced for the first time in 1928 (Sládečková 1962). Later, the names "phytobentos" or "microphytobenthos" were adopted by hydrobiologists. Over the last few decades, the term "algal biofilm" for attached algae has become more widely accepted in algal biotechnology (Wetzel 2001). Research on periphyton was conducted intensively from 60s. Special interest was particularly directed to studies on the community structure and primary productivity in streams and rivers. Artificial shallow channels were also used for research on nutritional conditions (e.g. McIntire 1968). The effect of high concentrations of P and N on the primary productivity of algae is reported in many publications (e.g. Whitford and Schumacher 1961; Lowe et al. 1986; Davison 1991; McCormick 1996). As one of the first, Bush et al. (1963) report using algae attached in a raceway pond for removing nutrients. Hemens and Mason (1968) evaluate wastewater tertiary treatment in an outdoor shallow stream. Sládečková et al. (1983) proposed using artificial streams fitted with nylon mesh to remove nutrients from polluted water. Vymazal et al. (1988) further continued this concept and tested periphyton growth and rate of nutrient uptake in an outdoor artificial channel (5 m long) with artificial substrates for algal growth. The algal assemblage that developed spontaneously on the substrata came from the upper part of stream, which served as a source of water for the channel. In both experiments there were reductions in the concentrations of phosphorus and nitrogen together with an abundance of algal growth.

The Algal Turf Scrubbers (ATS) – Ecologically Engineered, Algal Based System

Simultaneously with Czech researchers, the American scientist Walter Adey and co-workers examined options for improving the artificial channel concept (Adey et al. 1993). They were inspired by coral reefs. The algal turfs growing on coral reefs are characterized by high primary production due to regular flooding by waves. Scientists designed pulsing hydraulic system to mimic the wave action on coral reefs (Adey et al. 2011). This ATS system consisted of an attached algal community in the form of a "turf" growing on polyethylene screens. The algal turfs grew in a shallow slopping raceway into which water was pumped from a water body. After the biological uptake of nutrients by algae, the water was released at the end of the raceway back into the water body. The algal biomass was regularly harvested (Craggs et al. 1996). The algal assemblage on turfs consisted mostly of filamentous green algae *Spirogyra sp.*, *Microspora sp.*, *Ulothrix sp.*, *Rhizoclonium sp.* and *Oedogonium sp*. These dominant species were accompanied by the cyanobacteria *Phormidium sp.* and *Oscillatoria sp.* and benthic diatoms. These algal turfs are a heterogeneous community with a high growth rate and high ability of regenerating (Craggs et al. 1996; Mulbry et al. 2008; Sandefur et al. 2011). Maximum values of the rate of P and N uptake were 0.73 and 1.58 g m^{-2} d^{-1} respectively. Harvests (including trapped organic particulates) varied from 5 to 60 g DW m^{-2} d^{-1} (Craggs et al. 1996; Mulbry et al. 2008; Kangas and Mulbry 2014).

This ecologically engineered, algal-based technology has been developed for more than 30 years in USA and was patented as an Algal Turf Scrubber (ATS^{TM}). The nutrient reduction potential of ATS systems have been assessed for both point and non-point sources of pollution. For example, the treatment of dairy manure effluent in central Maryland (USA) and agricultural wastewater in the Florida Everglades (Adey et al. 2011). Commercialization of this technology is under active development by HydroMentia Inc., which builds and operates ATS mainly in Florida. Recently, research to improve the performance of ATS has continued, with tests involving new applications and evaluations of the harvested biomass (Adey et al. 2011; Valeta and Verdegem 2015).

The Algal Biofilms

The ATS technology is an ecologically engineered design of a controlled ecosystem for nutrient removal. The success of this technology has depended mainly on the construction of hydraulic system with a specific water regime (Adey et al. 2011). But the assemblage of periphytic organisms was not manipulated to favour more desirable species with a higher ability to remove nutrients. A novel approach is to use microalgal biofilms consisting of selected species. These species are selected based on a high ability to reduce nutrients (Sukačová et al. 2015). This approach is a shift from ecological engineering to the design of biotechnology applications.

The term microalgal biofilm was introduced for microalgal assemblages that consist of microalgae that colonize illuminated surfaces submerged in water (Jarvie et al. 2002). The use of term microalgal biofilm overlaps with name periphyton in hydrobiology as described above. In aquatic ecosystems, the growth of microalgal biofilms starts with the colonization of a submerged surface by pioneer species. At first, the surface is colonized by diatoms, which are followed by coccal green microalgae. The growth of filamentous microalgae and cyanobacteria after one month is the last phase of species succession (McIntire 1968; Komárek and Sukačová 2004). Development of microalgal biofilms depend mainly on water temperature and trophic conditions (Johnson et al. 1997). The growth of algal biomass is exponential at the beginning and then decreases depending on the thickness of the biofilm. Biomass losses are caused by respiration, cell death, parasitism and grazing by invertebrates (Biggs 1996). In watercourses, the algal biofilm plays a key role in biogeochemical cycles and transformation of carbon, N and P (Allan and Castillo 2007).

In the context of algal biotechnology, the research on algal biofilms is motivated by two factors. The first is the cultivation of biotechnologically important species in the form of a biofilm in order to reduce the cost of harvesting the biomass. The evaluation of the potential of algal biofilms for nutrient removal is the second reason. The research is closely connected with the design of different cultivation devices. The microalgal biofilm cultivation systems can be constructed as panels from different materials placed vertically or horizontally with a slight slope.

The vertically constructed system "Twin Layer" bioreactor using filter paper attached to a glass plate as the area for biofilm growth. Cultivation medium flows down the glass plate and keeps the filter paper moist. This system is placed in an aquarium and is aerated with air enriched with CO_2. Production potential of such a bioreactor ranges from 3 to 18 g DW m^{-2} d^{-1} depending on the intensity of illumination (Liu et al. 2013). A similar system was used for nutrient removal from municipal wastewater. *Hallochlorella rubescens* CCAC 0126 growing on a nylon membrane fixed to a metallic frame was situated vertically and wastewater from different treatment stages flowed down the membrane. The average uptake rate of PO_4-P varied from 0.8 to 1.5 mg l^{-1} d^{-1}, the removal of P from wastewater during a two-day cycle of bioreactor operation was 78.9% and 85%, respectively, and the average microalgal growth was 6.3 g DW m^{-2} d^{-1} (Shi et al. 2014).

Other carrier materials used for biofilm cultivation are radially flexible PVC fillers placed in plexiglass chambers. Biofilm consisted of a mixture of several species: *Chlorella pyrenoidosa*, *Scenedesmus obliquus*, *Anabaena flos-aquae*, *Synechococcus elongatus* and *Microcystis aeruginosa*. P and N removal efficiency was about 95% and 84%, respectively from simulated wastewater during a four-day cycle.

Guzzon et al. (2008) focus on basic research on P removal using biofilms. The growth was measured in a horizontal incubator with four separated lanes under different conditions (Zippel et al. 2007). Polycarbonate slides inside lanes served as cultivation areas. Guzzon and co-workers describe the influence of different parameters such as light intensity, temperature and flow rate on biofilm growth and P removal. They report positive correlations between algal biomass production and its P content with light intensity. They also describe the occurrence of polyphosphate granules inside the cells of the algae on the biofilms.

In several studies, polystyrene foam was found to be a suitable material for algal biofilm cultivation. Johnson and Wen (2010) determined the growth of *Chlorella* sp. attached to polystyrene foam in dairy manure wastewater and evaluated the algal biomass for producing biodiesel. This revealed that this technology potentially can provide a less expensive method of growing and harvesting algal production. Posadas et al. (2013) constructed a horizontal cultivation system with polystyrene foam as the carrier material for the biofilm, which was inoculated with a microalgal-bacterial assemblage from HRAP treated municipal wastewater. They compared the removal efficiency of N, P and organic compounds by the microalgal biofilm with that of a bacterial biofilm in the same cultivation system. The microalgal biofilm was the most effective in nutrient removal and the reduction of organic compounds was the same in both systems.

The comprehensive research on new wastewater treatment technologies using microalgal biofilm was done in the European center of excellence for sustainable technology (WETSUS) in the Netherlands. Boelee et al. (2011) determined whether microalgal biofilms were suitable for the post-treatment of municipal wastewater. Reduction in P and N was measured in a laboratory-scale system. Microalgal biofilm dominated by filamentous cyanobacteria (*Phormidium* and *Pseudanabaena*) and coccal green algae (*Scenedesmus sp.*) were cultivated on PVC plastic sheets. Wastewater circulated over the biofilm, which absorbed nutrients. Maximum rate of P and N uptake under continuous illumination was 0.13 g m^{-2} d^{-1} and 1 g m^{-2} d^{-1}, respectively. Subsequent studies of Boelee and co-workers focused on the evaluation of the frequency of harvesting in relation to biomass production and nutrient reduction. They report that the same biomass was harvested on the second, fourth and seventh day. The premise of this study that there would be a reduction in the biomass produced as the biofilm thickened was not confirmed (Boelee et al. 2014).

The work mentioned above was done in a laboratory. There are very few pilot studies that evaluate removal efficiency of biofilms. Sukačová et al. (2015) report the rate of uptake of P by an algal biofilm assemblage that

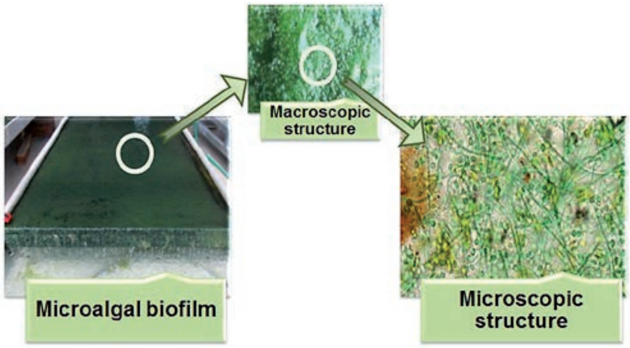

Fig. 2 Macroscopic and microscopic structure of algal biofilm. The filamentous cyanobacteria form a net that traps unicellular green algae.

consisted of filamentous cyanobacteria and coccal green algae, which grew on concrete panels with total area of 8 m^2 inclined at a slight angle. The wastewater was retained within the system for 24 hours. The average rate of uptake of P was about 0.16 g m^{-2} d^{-1}. The algal biofilm removed about 97% of total P from municipal wastewater after 24 hours.

The studies cited indicate that algal biofilms are very efficient at removing nutrients. Compared with ATS, the use of phototrophic biofilms on a large-scale is still uncommon and needs further improvement before it can be used for treating wastewater (Kesaano and Sims 2014; Whitton et al. 2015).

Matrix-Immobilized Algae

There are several laboratory studies on phycoremediation technologies using immobilized algae (e.g. Lau et al. 1997; de Bashan et al. 2002; Zhang et al. 2008). The main advantages of this technique is the separation of microalgae from wastewater and the production of a usable biomass. The immobilization method involves encapsulation of microalgae in beads. The material used for the immobilization must be very permeable, of low toxicity and highly transparent. The most often used material is alginate (Lau et al. 1997; Zhang et al. 2008). Various strains of microalgae have been immobilized, including the green algae *Chlorella vulgaris*, *C. pyrenoidosa*, *C. sorokiniana*, *Scenedesmus bicellularis*, *S. quadricauda* and cyanoprocaryota *Phormidium* (De la Noüe and Proulx 1988; Kaya and Picard 1996; Filippino et al. 2015). Immobilized microalgae are highly efficient at removing nitrogen and phosphorus from secondary effluents (De la Noüe and Proulx 1988; Kaya and Picard 1996). However, over the last few decades there has been little attention given to developing algal immobilization techniques for the treatment of tertiary wastewater (Filippino et al. 2015). Recently, research has started to focus on the optimization of growth of immobilized algae and increasing the efficiency of nutrient removal in the laboratory (Filippino et al. 2015).

Nutrient Recovery Potential

Considering the future need to recover nutrients, the utilization of nutrients by microalgae is an important issue in the nutrient removal process. The algal biomass that develops in wastewater can be utilised in several ways (Pittman et al. 2011). However, the presence of heavy metals, micropollutants or pathogens can reduce the possibility of reusing the nutrients.

One option is to use the algal biomass as a biofertilizers. The use of blue green algae for soil conditioning and as a biofertilizer in rice production is reported (Metting et al. 1990; Metting 1996). Mulbry et al. (2005) have used the algal biomass that developed during the treatment of cow manure treatment as a slow release fertilizer. They compared seedling growth using a commercial potting soil amended with either ATS biomass or a roughly comparable commercial fertilizer and report that plant growth was similar in both. Roberts et al. (2015) report that algae growing in bioremediation ponds at a coal-fired power station sequester metals from the wastewater. The algal biomass, which consists of the filamentous alga *Oedogonium,* can be converted to algal biochar for soil amelioration. When this biochar is added to a low-quality soil, it improves its retention of nutrients from fertilizer, which resulted in a better growth of radishes of 35–40% (Roberts et al. 2015). Although biochar is currently used to improve soil by restoring the carbon pool and providing essential trace elements, we hypothesize that algal biomass rich in phosphorus can also be effectively converted to biochar for enriching soils with phosphorus.

Algae are a good supplementary feed for livestock because they have a high protein content (Spolaore et al. 2006). However, the potential for using algae produced during wastewater treatment for feeding animals has not yet been studied. The algal biomass would have to meet the standards required for animal feed, which means that the feed source has to be free of pathogens and harmful substances.

Nutrient recovery using the algal biomass from wastewater treatment presents many challenges that remain to be overcome. Many algal species have been successfully used for removing nutrients in laboratory cultivation systems (e.g. Chinnasamy et al. 2010; Johnson and Wen 2010; Boelee et al. 2011; Fang et al. 2015), however, there are very few large-scale applications (Craggs et al. 1996). The lack of large-scale systems is limiting research on its potential for producing a phosphorus rich algal biomass. One of the few studies on the production of algal biomass as a biofertlizer is still that of Mulbry et al. (2005), which was published more than ten years ago. However, the results of this research indicates that algal biomass produced during the treatment of wastewater has very high potential for use as a biofertilizer.

Conclusions

The recovery of nutrients, especially P, seems to be necessary for the sustainable development of agriculture and the environment in the future. Many studies demonstrate the high ability of algae to remove nutrients from wastewater. Fewer studies have also shown the high potential of algae for nutrient recycling. Several steps are needed to overcome the problem of successfully developing algal biotechnologies for nutrient recycling. Primarily, it is the optimization of current technologies for more efficient sequestration of nutrients. These efforts should be focused especially on the traditional usage of HRAP for wastewater treatment. The adaptation of new methods developed in the laboratory for large scale use is also important. This step includes selection of suitable cultivation systems for specific species with a high ability of nutrient removal. The large-scale cultivation of microalgae in wastewater using closed photobioreactors is rarely reported. However, the optimization of energy inputs into the cultivation process and new technologies for harvesting could bring progress in this area. The next stage of the research will be the utilization of nutrient rich algal biomass obtained during the wastewater treatment process. The application of different kinds of biomass to soil connected with the investigation of nutrient release and the utilization by plants are only a few of the issues, but they are very complex. An effective solution could close the nutrient cycle. Despite the high potential of microalgae for nutrient recovery, there is still little attention paid to their use for nutrient removal in water management.

Acknowledgements

This work was supported by the Ministry of Education, Youth and Sports of CR within the National Sustainability Program I (NPU I), grant number LO1415.

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ABUNDANCE AND THREATS TO THE SURVIVAL OF THE SNOW LEOPARD – A REVIEW

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ABSTRACT

Snow leopard (*Panthera uncia*) is an endangered species and its population size is steadily declining. This review attempts to introduce and analyse the main factors threatening its survival in each of the countries in which it occurs: China, Bhutan, Nepal, Pakistan, Afghanistan, Tajikistan, Uzbekistan, Kyrgyzstan, Kazakhstan, Russia and Mongolia. To conserve the remaining snow leopard populations, it is necessary to determine where it occurs in the various areas. Here, recent data on its worldwide distribution are presented. Snow leopard has a very secretive lifestyle, which makes it difficult to estimate its abundance. Therefore, I also present an overview of the methods, such as searching for signs of its presence, capture-recapture, predator : prey biomass ratios, photographic-capture rate and genetic analyses, used for estimating the abundance of snow leopard in different studies and discuss their advantages and disadvantages.

Keywords: snow leopard, abundance, threats, worldwide distribution, conservation

Introduction

Snow leopard, *Panthera uncia* (Schreber 1775), *syn. Uncia uncia* is a member of the genus *Panthera* in the family *Felidae*. It is most closely related to the tiger, *Panthera tigris* (Jackson et al. 2008). The snow leopard is whitish-grey (tinged with yellow) in colour, and patterned with dark grey rosettes and spots (McCarthy and Chapron 2003). Unlike other big cats, it has under-developed fibro-elastic tissue in its vocal apparatus (Rajput 2009), so it is not able to make a full deep roar. In general, snow leopards live solitarily, though groups of up to six snow leopards are reported (McCarthy and Chapron 2003). In the 1972 IUCN's Red List of Threatened Animals it is listed as an "endangered" species (EN) due to its small population worldwide.

Its main habitat consists of alpine and sub-alpine regions with rugged and steep terrain separated by ridges, cliffs, rocky outcrops and gullies (Schaller 1977; Jackson and Ahlborn 1989; Ale et al. 2014). Nevertheless, it occurs even in flat and rolling terrain in Tibet and Mongolia, wherever there is ample cover in which they can hide (Jackson et al. 2008). Usually it inhabits areas at altitudes of from 3,000–4,500 m. However, at the northern limits of its range it occurs at much lower altitudes: 900–2,500 m (McCarthy et al. 2003). Its home range for instance in Nepal varies between 10 to 40 km^2 (Jackson and Ahlborn 1989). However, it may reach up to 140 km^2, in Mongolia, due to the lower prey densities and open terrain (McCarthy et al. 2005). Snow leopard density ranges from 0.1 to 10 or even more individuals per 100 km^2 (Jackson et al. 2008).

Snow leopard's main natural prey includes ibex (*Capra ibex*), argali (*Ovis ammon*) and bharal or blue sheep (*Pseudois nayaur*) (Jackson et al. 2008; McCarthy et al. 2008). The natural distribution of the snow leopard is closely correlated with that of its prey. One snow leopard needs about 20 to 30 adult blue sheep annually (Jackson et al. 2008). For instance, the habitat of blue sheep is spaced out in mountainous terrain from the Qilian Mountains in the north to the Himalayas in the south (Aryal et al. 2014), which includes Nepal, India, China, Pakistan and Mongolia. Unfortunately, there are no accurate estimates of blue sheep abundance (Schaller 1977; Oli et al. 1993). Its other prey species include pika (*Ochotona spp.*), hares (*Lepus spp.*), marmot (*Marmota spp.*), game birds, and small rodents (Jackson et al. 2008). Domestic livestock is a potential prey of snow leopard, e.g., in many cases snow leopard opportunistically kills more domestic livestock than wild prey. Our knowledge of its diet range and especially of the relative importance of individual species in its diet is still anecdotal and therefore, it would be appropriate to conduct additional surveys to better understand the trophic interactions in leopard-prey systems (Aryal et al. 2014).

Threats to the Snow Leopard

The main threats to the snow leopard include illegal trade, conflict with locals (human-snow leopard conflict), lack of conservation, awareness and policy, and climate change (Jackson et al. 2008).

Human-Snow Leopard Conflict

Conflict with local people is the most significant threat to snow leopards. This conflict is caused by snow leopards killing livestock, which leads to retaliatory killing (Jackson et al. 2008). Snow leopard home ranges overlap extensive agro-pastoral land, which is located inside and outside protected areas. The rapid increase in the killing of livestock is caused by the decline in the abundance of wild prey due to low primary productivity, competition for forage with livestock and the hunting of ungulates for meat (Bagchi and Mishra 2006; Jackson et al. 2008). Snow leopard's population density is positively correlated with

Valentová, K. A.: Abundance and threats to the survival of the snow leopard – a review
European Journal of Environmental Sciences, Vol. 7, No. 1, pp. 73–93
https://doi.org/10.14712/23361964.2017.7

the density of its prey (McCarthy et al. 2003). Livestock is an important food source for snow leopards; in some areas it makes up 58% of their food (Jackson et al. 2008).

Even though killing snow leopard is prohibited, cases of retaliatory killings of snow leopard by the herders are still reported, for instance in Nepal in the Kanchenjunga Conservation Area (KCA) (Ikeda 2014). Most herders have a negative attitude to snow leopard conservation programmes because of insufficient compensation for the damage they cause to their livestock and the un-realistic procedure for verifying that livestock has been killed by a snow leopard (Ikeda 2004). The economic damage is much greater than the compensation the herders get from the government. There are a few methods for estimating the monetary value of damage to livestock that were used in previous studies, such as half of the per capita average income in Himachal Pradesh, India (Mishra 1997) or a quarter of the per capita average annual income in Nepal (Oli et al. 1994). Ikeda in the KCA estimated the monetary value of damage to livestock in terms of local herder's economy in the Ghunsa Valley, Nepal (Ikeda 2014). The economic effect of loss of a herder's livestock differs between areas. For example, in western Nepal, herders possess sheep, goats, yaks and yak hybrids, while in eastern Nepal, yak and yak hybrid pastoralism is more important, so in general they have fewer animals in contrast with the hundreds of goats and large flocks of sheep in western Nepal. Therefore, the effect of losing one animal is greater in eastern than in western Nepal (Ikeda 2004). However, occasionally more than 100 sheep or goats are killed by snow leopard (so-called surplus or mass killing), which may cause a huge economic loss to local herders (Shrestha, pers. comm.) For households with the average herd size (36.6 head in KCA) the annual damage by snow leopards does not have such a big effect on their livelihood. However, in the worst scenario, for households with medium or small-sized herds (<40 head) the risk becomes unsustainable and they have to cease depending on yak pastoralism (Ikeda 2004).

Killing of snow leopards in retaliation for their killing livestock and the reduction in the abundance of natural prey is inherently very challenging in the Himalayan region (India, Nepal, Bhutan, Tibetan Plateau and other southern China), Karakorum and Hindu Kush (southwest China, Pakistan and Afghanistan). The abundance of the natural prey of snow leopard has been greatly reduced by illegal hunting in the Commonwealth of Independent States and western China (Kyrgyzstan, Kazakhstan, Xinjiang province of China, Uzbekistan and Tajikistan) (Jackson et al. 2008).

Illegal Trade

Illegal trade in snow leopards is also a serious threat to their survival. Snow leopards are killed not just for killing livestock but also for commercial purposes. There is a big demand for snow leopard pelts, followed by their claws, meat, male organs and bones as substitutes for tiger bones in Chinese medicine (Theile 2003). Because of the strong Chinese economy the illegal trade in snow leopards increases, for instance with adjoining Mongolia (Wingard and Zahler 2006) and Afghanistan, where it is difficult to stop because of the current military situation there (Habibi 2004). With the fall of the Soviet Union in the 1900s, in Kyrgyzstan and other recently independent states, increased unemployment and corruption in mountainous regions led to a growth in the black market trading in wildlife products (McCarthy et al. 2010). For instance, in Kyrgyzstan people living in villages close to protected areas are poorly paid and in some cases have to resort to poaching wildlife within the park boundaries (McCarthy et al. 2010). Nowadays some of the former socialist republics continue to promote a sustainable developmental agenda (McCarthy et al. 2010). Today, the level of poaching is lower, but in many of the former Soviet republics it continues.

Lack of Awareness and Policy

The general lack of awareness, at both local and national levels, for the need to conserve wildlife, especially predators, further hinders conservation efforts (Jackson et al. 2008).

To estimate abundance of snow leopard in some cases, e.g. when genetic analyses are used, it is necessary to transport samples across countries. Impossibility of transporting samples between countries complicates such surveys, especially in areas adjacent to politically sensitive international borders. In the IUCN's research, lack of trans-boundary cooperation is challenging at almost every location where snow leopards occur such as the Himalayan region and Commonwealth of Independent States and western China (Jackson et al. 2008). For instance, in Nepal, there is only one laboratory in Kathmandu specializing in wildlife, which is insufficient (Bikram Shrestha, pers. comm.). The majority of herders in Nepal complain about insufficient project management in terms of reliability and transparency (Ikeda 2004).

In some areas, e.g., in Kyrgyzstan, there is an insufficient legislative system for protecting reserves. McCarthy et al. (2010) compared the composition of species in an unprotected area which is used for hunting by foreign companies and a strictly protected national park (Sary Chat) in the Tien Shan Mountains in eastern Kyrgyzstan. Even though hunting is not permitted at Sary Chat, cases of poaching by rangers and local villagers are reported (Koshkarev and Vyrypaev 2000). On the other hand, after the breakup of the Soviet Union, Jangart was established as a foreign currency hunting reserve hosting non-nationals who come to Kyrgyzstan to hunt ungulates (McCarthy et al. 2010). Unexpectedly, the numbers of photographs of ungulates recorded by camera traps in the unprotected area (Jangart) were higher than in the protected area for most species (McCarthy et al. 2010). A possible explanation is that Jangart is more isolated from local villages than Sary Chat, where rangers and their families have settled along the edges of the park (McCarthy et al. 2010).

This reflects a fundamental problem with the reserves in Kyrgyzstan – a deficiency in auxiliary enterprises for local people. The government replaced park staff and a nongovernmental organization (CBF = Community Business Forum) and the International Snow Leopard Trust were involved, but nonetheless there is still evidence of continuing poaching of some carnivores in and around the reserve (McCarthy et al. 2010).

Ineffective enforcement of the law and institutional incapacity are problems mainly along the northern edge of the distribution of snow leopards (Russia, Mongolia, Tien Shan ranges and Altai in China), Karakhorum and Hindu Kush (Afghanistan, southwest China and Pakistan) (Jackson et al. 2008).

Military conflict has also affected snow leopards, primarily by destroying their habitats (landmines), secondarily by encouraging trade in wildlife (Jackson et al. 2008). This is a serious problem in the Himalayan region and Commonwealth of Independent States and western China.

Climate Change

Climate change is a serious threat to biodiversity (McCarty et al. 2001; Thomas et al. 2004; Beaumont et al. 2011). In general, climate change destabilizes systems and their management, such as the balance between resource use by locals and wildlife biodiversity (Comiso 2003; Mishra et al. 2004; Namgail et al. 2007; Sharma and Tsering 2009). The United Nations Intergovernmental Panel on Climate Change forecasts that the global temperatures will increase by something between 1.4 and 5.8 °C by 2100 (IPCC 2001; Locky and Mackey 2009). Most areas in snow leopard home ranges, such as high altitudes and cold deserts in the Trans-Himalayan region, are among the most vulnerable ecosystems in terms of the effects of climate change (Christensen and Heilmann-Clausen 2009; Dong et al. 2009; Sharma and Tsering 2009; Xu et al. 2009; Aryal et al. 2012a,b).

The tendency of snow leopards to move to lower altitudes will increase due to the movement of their prey, such as blue sheep in the Trans-Himalayas, due to substantial changes in vegetation communities; grasses and many species of shrubs are no longer found in sufficient abundance at high altitudes and consequently blue sheep move to forage at low altitudes where they more likely to encounter and kill livestock (Table 1, Aryal et al. 2013). Table 1 shows the predicted effect of climate change on the livelihood of the people, blue sheep and snow leopard, which are interrelated. According to the predictions based on bioclimatic models, about 30% of snow leopard habitat may be lost in the Himalayas due to a shifting treeline and consequent shrinking in the alpine zone, mostly along the southern edge of the range and in river valleys (Forrest et al. 2012). In most of the snow leopard's range, people practice rotational grazing in which corrals are located at different altitudes. Therefore, livestock killing is correlated with herding practices (Shrestha pers. comm.). Increased crop raiding by blue sheep and the killing of livestock by snow leopards have adversely affected the livelihood of local people (Aryal et al. 2013).

Methods of Estimating Snow Leopard Abundance

To optimize the management of snow leopard, it is necessary to know its distribution within an area and relative abundance in different habitats (Sheng et al. 2010). Its secretive lifestyle makes estimating its abundance quite difficult. Methods used for estimating snow leopard abundance include search for signs of their presence, capture-recapture, predator : prey biomass ratios, pho-

Table 1 Overall effect of climate change on four parameters. Source: Interview with local people (n = 221). Data are the percentages of the 221 respondents that agreed with the statement (Source: Aryal et al. 2013).

Rangelands (%)	Livelihood (%)	Blue sheep (%)	Snow leopard (%)
Reduction in water sources (18)	Reduction in food and drier farmland (9)	Reduction in numbers (38)	Reduction in numbers and fewer sightings (41)
Reduction in grass (33)	Reduction in livestock (4)	Crop raiding (16)	Increase in attacks on livestock due to decrease in natural prey (24)
Drier (12)	Health problems with new diseases (3)	Movement downwards towards farmland (14)	Approaching villages (13)
Reduction in snowfall (27)	Reduction in drinking water and fewer irrigation channels (36)	Sightings near villages (4)	Change in use of habitat and increase in their tendency to kill domestic livestock rather than natural prey (22)
Move towards desertification (10)	Changes in the timing and cultivation of agricultural crops (27) Increase in wind speed Economic crisis (7) Water seepage and damage to traditional houses (14)	Reduction in grazing land (20) Change in habitats use (8)	

tographic-capture rate and genetic analyses (McCarthy et al. 2008). No photograph of a wild snow leopard existed until 1980 (Schaller 1980). Now camera traps are available they have replaced live-capture (direct capture of an animal), which is almost impossible to use because of their very low rate of encounter: about 3/1,000 trap-nights (McCarthy et al. 2008).

Sign Surveys (SLIMS)

Snow Leopard Information Management System (SLIMS) was developed recently to monitor the abundance of snow leopards and their prey (Jackson and Hunter 1996). The system is based on standardized sign surveys, which are used regularly. Sign surveys result in indices of relative abundance of snow leopards, which can be used to compare areas with similar topographies (Ale et al. 2014). This method can be used to monitor trends in abundance in the same area over a long time scale, so long as it is complemented by additional methods such as genetic analyses (e.g., Janecka et al. 2008) or remote cameras (e.g., McCarthy et al. 2008). SLIMS is cheap, has a minimal impact on the species studied and, therefore, is most commonly used for monitoring snow leopards (Schaller 1977; Schaller 1998; Wilson and Delahay 2001; Wolf and Ale 2009). As the majority of ecological problems can be tackled using only indices of density, absolute estimates of density are unnecessary luxuries (Caughley 1977), SLIMS may be sufficient in most cases.

The guesstimate of Snow Leopard numbers based on sign abundance follows Jackson and Hunter (1996): 20 signs per kilometre indicates 10 individuals per 100 km^2 – a crude, quick and easy-to-use method, which has been useful in conservation planning in countries where resources are scarce (Ale et al. 2014). It is appropriate to space out the cameras: install at least one camera per approximately 25 km^2, as this is assessed to be the minimum home range of a female adult snow leopard (Jackson and Hunter 1996; Ale et al. 2014).

To determine where to locate transects for sign survey, it is necessary to hike through the region and detect all sites where suitable habitat and terrain exist for snow leopard and where its prey occur. As Ale at al. (2014) did in Nepal, it is important to identify sites, which are often used by snow leopard to move around in its home range such as narrow valleys, trails, ridgelines and cliff-edges (Jackson and Hunter 1996). To locate these sites it is helpful to use 1:50,000 topographic maps as used by Ale et al. (2014) or the 1:100,000 topographic maps used by McCarthy et al. (2008) in Kyrgyzstan and China.

After locating transects (sites with high probability of snow leopard occurrence) they are searched for signs of snow leopards. In addition, to provide useful information for comparison with that gathered along sign transects, it is possible to record signs between transect lines (McCarthy et. al. 2008). Signs demonstrating snow leopard presence include scent (or spray) marks, scrapes, faeces, pugmarks (footprints) and rocks or boulders that snow leopards use to deposit their scent or cheek-rub (Ale et al. 2014). Scrapes and scent marks are more expensive to detect than faeces and pugmarks. However, they may give us more biological and ecological information (Schaller 1977, 1998).

According to surveys conducted in the Mustang District of Nepal's Annapurna Conservation Area (Table 2), scats and scrapes are detected most frequently, scent sprays and pugmarks less often. Ale et al. (2014) report that the probability of finding signs is highest in spring (10.2 signs/km) and lowest in summer (2.1 signs/km), which may be due to snow leopard and its prey, blue sheep, moving to higher locations, which are less accessible to the observers (Jackson and Hunter 1996; Oli and Rogers 1996). Another possible explanation is that snow leopard signs are obliterated by livestock in summer (Ale et al. 2014). For a better comparison of the effectiveness of sign surveys in different seasons it is also necessary to conduct sign surveys during summer and spring in

Table 2 Signs indicating the abundance of snow leopards at Mustang, Annapurna (Source: Ale et al. 2014).

	Transect	Length (km)	Feces	Pugmark	Scrape	Spray	Hair	Total	Total sign/km	Sign sites	Sign sites/km	Scrape/ km
Season												
Autumn	9	7.2	19	10	33	4		66	9.2	46	6.4	4.6
Spring	15	11.0	15	6	90		1	112	10.2	38	3.5	8.2
Summer	27	19.4	22	3	16			41	2.1	23	1.2	0.8
Total		37.6	56	19	139	4	1	219	5.8	107	2.8	3.7
Study area												
Lower Mustang	3	24.4	45	15	56	4	1	121	5.0	77	3.2	2.3
Upper Mustang	18	13.2	11	4	83			98	7.4	30	2.3	6.3
Total		37.6	56	19	139	4	1	219	5.8	107	2.8	3.7

other countries. If such surveys show that the probability of finding signs is consistently higher in spring, then it might be sufficient to conduct just spring surveys.

For estimates of predator numbers using SLIMS a standard methodology should be used, e.g., for comparison of sign transects between various areas, unit transect lengths should be compared (Ale et al. 2014). It is also necessary to reduce observation bias. One of the options is that all observers involved obtain the same training to avoid disagreements over what constitutes a snow leopard scrape, which can lead to erroneous results (McCarthy et al. 2008). Environmental conditions (e.g. snow cover) and accessibility of the terrain can also affect the results. With respect to the latter, in Qinghai (China), where snow leopards mark the bases of hills flanking broad valleys where their travel routes are less accessible (Schaller et al. 1988; Ale et al. 2014). Also in the Himalayas, where wide U-shaped valleys and broad ridges are common, it is difficult to find signs of snow leopard (Jackson and Hunter 1996).

Predator : Prey Biomass Ratio

Predator-prey models of population dynamics predict that there is a negative feedback between prey and predator biomass (Fuller and Sievert 2001; Carbone and Gittleman 2002). This is indirectly supported by an observation at Sary Chat in Kyrgyzstan where a decrease in snow leopard abundance was followed by an increase in that of ungulates (McCarthy et al. 2008). This provides the basis for another method of estimating the size of snow leopard populations, the predator : prey biomass ratio, from which the abundance of snow leopard can be estimated if one knows its prey biomass (Fuller and Sievert 2001; Carbone and Gittleman 2002), which is estimated using SLIMS (Jackson and Hunter 1996).

For measuring the number of snow leopard prey it is necessary to select a favourable point at each site, from where one does not disturb the animals and from where it is possible to localise and determine group size, age and sex of each individual (McCarthy et al. 2008). For observations, it is appropriate to use binoculars and spotting scopes. After this, one calculates the total prey biomass by multiplying the number of animals observed by the average prey weight (Fedosenko and Blank 2001). Ungulate biomass per 100 km^2 can then be recalculated to leopard biomass by a simplified conversion factor of 10,000 kg prey for 90 kg of predator (Carbone and Gittleman 2002), which can be converted to numbers using an average weight for snow leopard of 50 kilograms (McCarthy et al. 2008). The predator:prey ratio may be, however, biased due to competition for food with other species of predator such as wolves (McCarthy et al. 2008).

Another, more sophisticated method proposed by Aryal et al. (2014) is that of the maximum number of snow leopards that can be supported by the prey available (its carrying capacity, K), which is calculated as

$$K = A / (ESSR \times AHRS)$$

with:

$$ESSR = PBY / (PD \times SUF \times BD \times EHD)$$

where:

A – area;

AHRS – average home range size (about 22.6 km^2/individual);

ESSR – ecological sustainable stocking rate;

PBY – prey biomass /snow leopard/year (~548kg/year, 1.5kg/day – Schaller 1977);

PD – total prey biomass/km^2;

SUF – safe use factor – the total biomass production of the ecological site that is available for use by animals with the remaining biomass available for ecological sustainability (Alberta Sustainable Resource Development 2004; Aryal 2007; Aryal et al. 2014); in Nepal, Aryal et al. (2014) used 25% for SUF due to the presence of other predators (lynx, red fox, jackal, wolf); it means that snow leopards can consume just 25% of the total sheep population and the rest is available to other predators;

BD – birth : death, for instance for blue sheep in Nepal it is presumed to be 2:1, based on the estimate that 50% of blue sheep die between birth and 2 years of age in the Dhorpatan Hunting Reserve (Schaller 1977; Wegge 1979);

EHD – environment and human disturbance factors in the habitat (poaching, livestock grazing) where the grassland is that sustains the prey : predator population; e.g., the Upper Mustang in Nepal has a lower young-to-old male ratio than Lower Mustang due to its lower productivity (Ale et al. 2014); productive grasslands are expected to have a higher proportion of young males while the opposite would be the case for ungulate populations occupying degraded grasslands (Ale et al. 2014).

Genetic Analyses

Genetic analysis of faecal DNA is a promising method of estimating the abundance of snow leopard (McCarthy et al. 2008) and with a better developed format for scat collection, it would provide a better understanding of territoriality or marking behaviour. Genetic analysis is the only method that provides information about genetic relationships, including the source of dispersers (Gese 2001; McCarthy et al. 2008). The other advantage is that the estimates are not subject to observer bias as they based on using specialized equipment and prior training (McCarthy et al. 2008). Genotyping of faeces may generate a higher number of known individuals than visual discrimination based on photographs and provide minimum population estimates. For instance, for Sary Chat, Jangart, and Tomur the estimates based on photographs were 3, 5 and 9 individuals respectively, while those based on genotypes were 9, 9 and 17 (McCarthy et al. 2008).

Nevertheless, one major disadvantage of these methods is the price (about USD 50–225 for one sample) and also logistics of transporting faecal material between countries (McCarthy et al. 2008). One of the alternatives for reducing costs could be in-country laboratories for processing the genetic data. Shrestha, who is conducting studies in Nepal, processes samples in a laboratory in Kathmandu and cooperates with his colleagues in Prague for further analyses. It would, however, be more effective if it were possible to export the samples between countries.

To utilize genetic analyses for monitoring snow leopards, first samples must be collected. For instance, McCarthy et al. (2008) in China and Kyrgyzstan received their samples (suspected snow leopard faeces) from study areas which they determined using SLIMS. Scent pads or hair samples from cheek rubbing (Weaver et al. 2005) can also be used to create a more accurate method of sampling. It is important to minimize samples which do not belong to snow leopard by selecting them according to their shape, location and size. McCarthy et al. (2008) avoided contamination by collecting faecal samples wearing latex gloves and using plastic spoons and then storing in individual 5-ml transport tubes containing 4 ml of 90% ethanol. In general, to avoid errors in scat collection, it is appropriate to obtain samples of scrapes to increase the confidence that it is a snow leopard sign. After collection of samples, DNA extraction is carried out in a laboratory and the polymerase chain reaction (PCR) is set for a low-quantity of DNA in the samples. For DNA extraction it is possible to use stool kits, e.g., the Qiagen stool kit (Qiagen Inc., Valencia, CA) and protocols inclusive of negative controls to monitor for contamination. After PCR, the sample of an approximately 160 base-pair section of the cytochrome B gene of the mitochondrial DNA control region is sequenced (McCarthy et al. 2008). To identify which species deposited each faecal sample, stated primers and formerly published methods are used (Farrell et al. 2000; Onorato et al. 2006). To distinguish individuals of snow leopard, it is appropriate to have as many primers (polymorphic microsatellite loci) as possible. McCarthy et al. (2008) used 10 polymorphic microsatellite loci to identify individual snow leopards.

Camera Traps

Camera trapping has a wide use ranging from birds to mammals (Cutler and Swann 1999). It is used to estimate presence/absence (Foster and Humphrey 1995; Whitefield 1998), population characteristics (Karanth 1995; Karanth and Nichols 1998) daily activity patterns (Pei 1998; Azlan and Sharma 2006; Sathyakumar et al. 2011), and abundance (Carbone et al. 2001; O'Brien et al. 2003; Rowcliffe et al. 2008) of animals. Camera trapping is considered to be a modern non-invasive method (Mace et al. 1994; Karanth 1995; Karanth and Nichols 1998; Carbone et al. 2001; Mackenzie and Royle 2005) for monitoring cryptically living animals, such as snow leopard, and for population studies of species whose individuals can be recognized by marks (Karanth 1995; Carbone et al. 2001; Sathyakumar et al. 2011). It is more dependable than other methods when sample sizes are small and species are scarce (Carbone et al. 2001; Sathyakumar et al. 2011). Karanth et al. (2002) and Henschel and Ray (2003) provide detailed methods for using camera traps for estimating the densities of tigers (*Panthera tigris*) and leopards (*Panthera pardus*). Camera traps are also more suitable for local teams, including staff of protected areas, so that they can carry out these surveys independently and sustainably over a long period (Alexander et al. 2015).

However, there are also constraints. They are difficult to use in areas that are difficult to access due to dense vegetation, steepness or are located at great distances (Sathyakumar et al. 2011). For instance, the high sensitivity of infrared sensor camera units used by Sathyakumar et al. (2011) resulted in them capturing wind-caused movement of vegetation, which resulted in many photographs being taken of vegetation. In bad weather, such as high rainfall or extremely low temperature, the cameras sometimes fail.

We define a "photo event" as any photograph or set of photographs of a snow leopard at photo-trap site even though it is not possible to identify the individual (McCarthy et al. 2008). Wilson and Anderson (1985) define photographic rate as an index of relative abundance (RAI), calculated as the number of photographs of a species divided by the number of trap-days per site (in most cases 100 trap-nights). To obtain more accurate results, it is appropriate to count photographs of individuals captured more than once within one hour by the same camera as one photograph (Bowkett et al. 2007; Sathyakumar et al. 2011). The number of trap-nights depends on the number of cameras and on the number of days they are operated. For instance, McCarthy et al. (2008) conducted a survey spanning 1,078 to 1,180 trap-nights in each area. In most cases, cameras are oriented towards the south or north so that they are less affected by sun light. In general, the first choice for camera trapping are marking sites along suspected snow leopard trails, e.g., along high, well-defined and narrow ridgelines or valley bottoms or immediately adjacent to frequently scent-sprayed rocks and scrapes (Jackson et al. 2006; Ale et al. 2014). Camera sites are then usually arranged about 2 km from each other in a circular pattern, 45–50 cm above the ground, with a 90-second delay between photographs, as in McCarthy at al. (2008). An infrared sensor is an advantage (Sathyakumar et al. 2011).

Capture-Recapture Method

The camera capture-recapture method is considered to be a feasible way of estimating densities of individually recognizable animals with large home ranges and low densities, so it is especially suitable for snow leopard (Silver et al. 2004; McCarthy et al. 2008). However, when the densities are very low, this method can be vulnerable to logistical constraints (McCarthy et al. 2008).

There are several theories on how to create a spatial buffer of potential capture locations to cover the total sampling area (Fig. 1, McCarthy et al. 2008). One of them is the mean-maximum-distance-between-recaptures, formulated on the basis of capture–recapture of small mammal populations (Wilson and Anderson 1985). This theory is often criticised in the literature (McCarthy et al. 2008) because its dependability is thought to decrease when trap rate declines and home range size increases (Wilson and Anderson 1985). Another possibility is to use maximum distance between recaptures for the buffer (O'Brien et al. 2003). The former theory is not well supported in the literature and often criticized (McCarthy et al. 2008), as it is suspected that the dependability decreases when trap rate declines and home range size increases (Wilson and Anderson 1985). A third theory or method is the use of the average minimum reported home-range size or average home-range size of the species (Otis et al. 1978). It is not that easy to estimate the size of the home range because it depends on the accessible food biomass. Standard home range size is about 22.6 km²/individual. Another issue is that data are not available for some areas of snow leopard occurrence. The Tien Shan Mountains are a good example (McCarthy et al. 2008). Home range size of carnivores is often inversely correlated with prey biomass (Fuller and Sievert 2001). Therefore, it is appropriate to take into account ungulate densities (Table 1) and fit the data by a linear regression. McCarthy et al. (2008) calculate the effective study area according to the methods described above (Fig. 1): mean maximum distance moved between recaptures, half mean maximum distance moved by recaptured animals, radius of the average minimum home range or average home range and radius of the estimated home range from ungulate densities (McCarthy et al. 2008). By choosing one of these

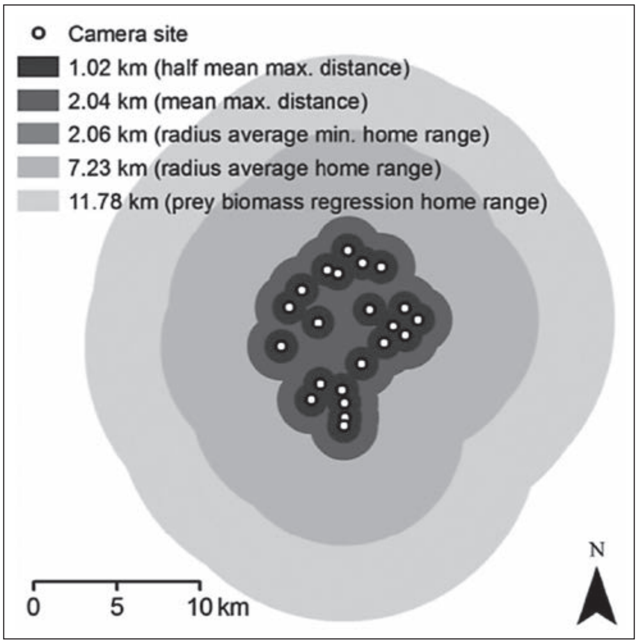

Fig. 1 Effective snow leopard study-area buffers around camera-trap sites in the Jangart huting reserve, Kyrgyzstan, 2005 (Source: McCarthy et al. 2008).

methods, the density estimates can be altered considerably (McCarthy et al. 2008). Total area covered by cameras and size of the effective area of buffer circles are used for density calculations (McCarthy et al. 2008).

Biotelemetry (Global Positioning System, Collaring and Radio Collaring)

Biotelemetry is a method for obtaining detailed information about animals that are not easily observed (Jackson et al. 2004). Thanks to biotelemetry, it is possible to better understand the patterns of movement and factors affecting the distributions of animals, their home range, patterns of habitat utilization, social organization and habitat preferences (Jackson 1996; Schofield et al. 2007). This knowledge is important for behavioural ecology and the management and conservation of protected areas (Schofield et al. 2007). For snow leopard tracking, radio and satellite transmitters (Global Positioning System, GPS) are used. Both methods are based on receiving data or signals from the transmitter attached to a collar around the animal's neck.

For fitting the collar, the animal must be trapped, which requires special skills and can sometimes be dangerous for the animal, therefore both these methods are considered to be invasive (Jackson 1996; Jackson et al. 2004). According to Jackson (1996) the most effective trapping locations are places where vegetation, boulders and other physical structures constrain the movement of snow leopards to a natural trail less than 0.5 m wide and where an abundance of fresh snow leopard scrapes and related signs indicate recent visits and ongoing marking activity. The immobilized animals are usually weighed, measured, tattooed inside one ear with an identifying number and fitted with a radio-collar (Jackson 1996).

Telemetry is about one order of magnitude more costly than camera trapping due to the manpower and equipment needed (Jackson et al. 2004). Their use is often forbidden along national borders and in other politically sensitive areas, which coincide with a large proportion of the snow leopard's range (Schofield et al. 2007).

Radio tracking is based on the transmission of pulses of radio frequency by radar systems and measuring the time that they take to be reflected back (McEwan 1995). This time is a measure of the distance from the radar unit to the reflecting objects (McEwan 1995). Highly directional antennas allow such transmissions and signal reflections back to be narrowly focused, so that the direction of such reflective objects can also be estimated (McEwan 1995). Radio telemetry has been used in several parts of Nepal (Oli 1994), India (Chundawat 1989, 1990, 1992) and Mongolia (Schaller et al. 1994). Results of these studies are limited by small sample size (1–3 individuals) or periods of monitoring that never last longer than 3 months (Jackson et al. 2004). First in-depth study using radio telemetry was conducted by Rodney Jackson in Nepal in the 1980s. During 1994–1997, McCarthy et al. (2005) surveyed snow leopard movements and activities on the basis of year-round radio-monitoring in the Altai Mountains in southwestern Mongolia. Home ranges were determined to be at least of 13–11 km^2 in size (McCarthy et al. 2005). The disadvantage of this method is that a mountainous, rocky terrain may affect radio-wave propagation and the range of reception due to radio-wave attenuation, signal bounce and deflection (Amlaner and MacDonald 1980; Jackson 1996).

Nowadays, the GPS method is used more frequently than radio telemetry due to its greater accuracy. By matching the animal's GPS coordinates with a habitat map using satellite images or aerial photographs and ground-truthing, the researcher is able to determine habitat features or conditions that are most important for feeding, resting or breeding (Jackson et al. 2004). Along with data on average home range size and prey densities, scientists are able to better estimate snow leopard population size and density (Jackson et al. 2004). In 2013 in Mongolia, for the first time ever a mother snow leopard and its cubs were located using GPS collars and remote camera traps (Noras 2015). The Snow Leopard Trust team collared a female snow leopard and its sub-adult offspring and thanks to that it is possible to monitor the movement of the mother and cub, and observe when and how the young cat becomes independent (Noras 2015).

Comparison of the Methods Used for Estimating the Population Density of Snow Leopards

For a rigorous comparison of the abundance of snow leopards it is important to obtain data for all of its habitats. Such data does not exist. Studies conducted in different snow leopard habitats are not methodologically consistent. The reasons may be objective, e.g., differences between habitats can cause difficulties in collecting data, or the differences in snow leopard densities may not allow the use of the same method. At high densities (4–8 individuals/100 km^2), lower standard errors and an area that can be studied for a long period of time make it feasible to use the camera capture-recapture method as in the Hemis National Park, India (Jackson et al. 2006; McCarthy et al. 2008). In areas with very low densities and little prior knowledge of snow leopard behaviour, it may prove impossible to obtain sufficient data for viable capture–recapture modelling within a short (usually about the 7-weeks) time frame (Karanth et al. 2002; McCarthy et al. 2008). This leads us to suggest that the camera capture-recapture method is unreliable, when used where home ranges fluctuate in size and the capture rate very low (McCarthy et al. 2008). Biotelemetry is also a valuable method for obtaining detailed information on the spatial dynamics of individuals. Nowadays, it is more usual to use GPS collars than radio collars, which are less reliable. However, a huge problem with GPS tracking is its very high price and logistic challenges. From this point of view, use of this method is only possible in countries with a high per capita income or with external support from international organizations.

When an exact determination of the densities is not needed, it is possible to use sign surveys or photo rates. According to McCarthy et al. (2008) these two methods provide a valid index of snow leopard abundance because of their similarity with genetic results. On the contrary, the estimates resulting from predator : prey biomass ratios and capture-recapture disagree with other estimates of abundance. For obtaining exact estimates of densities, it would be more appropriate to use non-invasive genetic analyses, as mentioned above. Their results are not subject to observer bias, as are other methods, for instance erroneous identification of scats. For example in the genetic study by Janecka et al. (2008) in Mongolia, up to 60% of all scats that were attributed to snow leopard in fact belonged to red fox (*Vulpes vulpes*). From my point of view, the best way of estimating snow leopard abundance is by comparing the results of sign surveys with those of other methods (predator : prey biomass ratios, genetic analyses, camera trapping and camera capture-recapture).

Worldwide Distribution

Snow leopards are restricted to sub-alpine regions in South and Central Asia in a total of 12 countries (Fig. 2): China, Bhutan, Nepal, India, Pakistan, Afghanistan, Tajikistan, Uzbekistan, Kyrgyzstan, Kazakhstan, Russia and Mongolia (McCarthy and Chapron 2003). The total size of its habitat is approximately 1,835,000 km^2 and the total population is between 4,510 – 7,350 individuals (Fox 1994). Based on the range-wide model (Fig. 3) it's area of distribution is potentially larger: 3,024,728 km^2 (McCarthy and Chapron 2003). This may be an over estimate, however, because the range-wide model only uses the geographic habitat and neglects other parameters such

Fig. 2 Map showing the extent of the distribution of snow leopards (Source: Jackson et al. 2004).

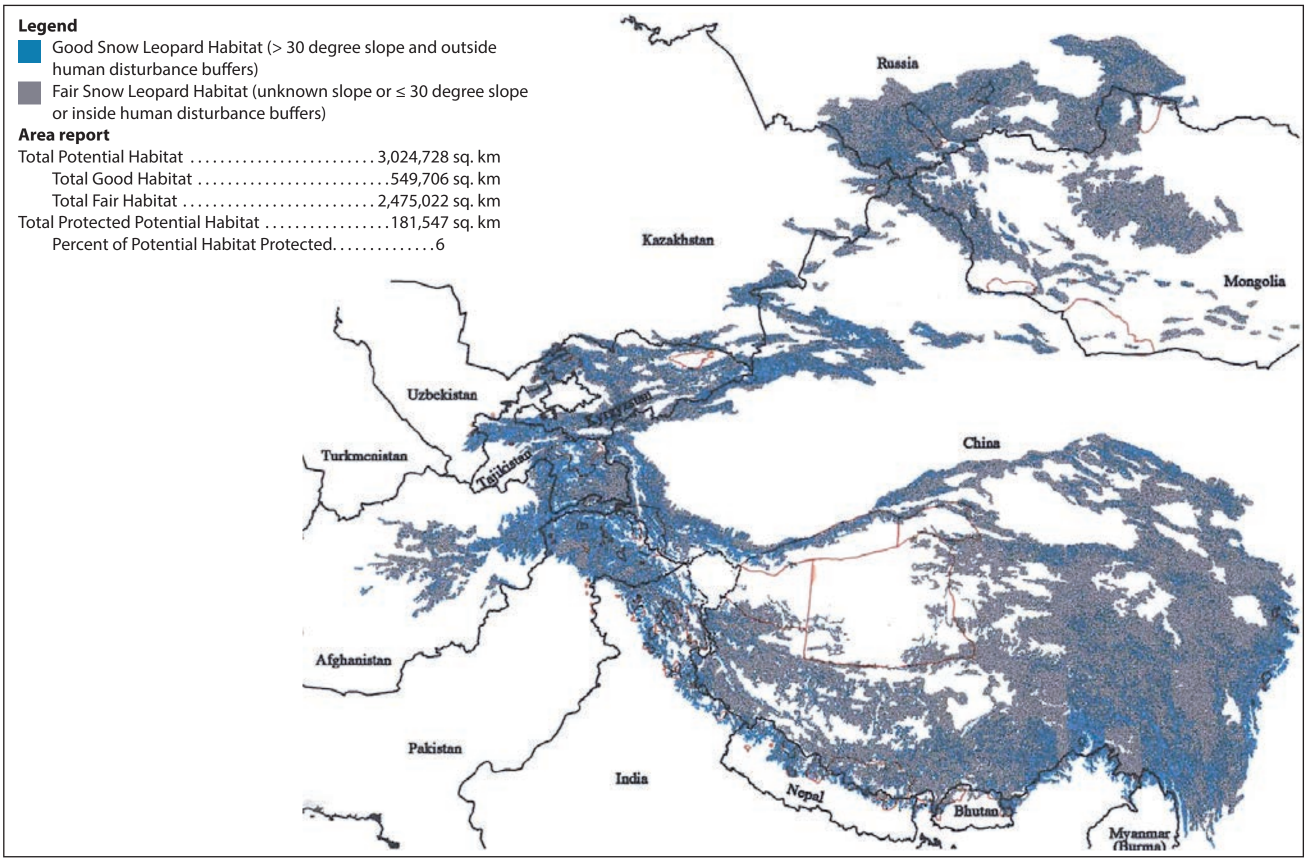

Fig. 3 Range wide model of potential snow leopard habitat (Source: McCarthy and Chapron 2003).

Table 3 Distribution, population estimates and population density of snow leopards (Source: Schaller 1976; Schaller 1977; Green 1988; Chundawat et al. 1988; Koshkarev 1989; Annenkov 1990; Jackson and Ahlborn 1990; Schaller 1990; Zhirjakov 1990; Fox et al. 1991; Jackson 1992; Schaller et al. 1994; Hunter and Jackson 1997; Jackson and Fox 1997b; Koshkarev 2000; McCarthy 2000a; Bykova et al. 2002; Kreuzberg-Mukhina et al. 2002; Poyarkov and Subbotin 2002; Hussain 2003; McCarthy and Chapron 2003).

Locality	Area of habitat (km^2)	Estimated population	Population density of snow leopards (individuals/area)
China	1,100,000	2,000–2,500	1/250–300 km^2 ≐ 0.3–0.4/100 km^2
Mongolia	101,000	500–1,000	?
Nepal	30,000	300–500	0.1–10/100 km^2
India	75,000	200–600	1/110–190 km^2 ≐ 0.5–0.9/100 km^2
Pakistan	80,000	200–420	1/250 km^2 ≐ 0.4/100 km^2
Tajikistan	100,000	180–220	?
Kazakhstan	71,079	180–200	?
Kyrgyzstan	105,000	150–500	2.35/100 km^2
Russia	130,000	150–200	?
Bhutan	15,000	100–200	1/100 km^2
Afghanistan	50,000	100–200	?
Uzbekistan	10,000	20–50	?

as competition, distribution of prey and grazing pressure (McCarthy and Chapron 2003).

Results of the range-wide model are presented in Fig. 3. Blue coloured areas are "good" sites with more than 30 degree slopes outside areas subject to human disturbance. Violet coloured areas are "fair" sites of unknown slope or less than 30 degrees or inside areas subject to human disturbance. Population densities of snow leopard in "good" areas are greater because they have a strong preference for irregular slopes in excess of 40° (McCarthy and Chapron 2003). The 109 protected areas with total size of 276,123 km^2, identified by Green and Zhimbiev (1997), are marked in red in Fig. 3. Table 3 shows estimated areas of snow leopard habitat in km^2 and the estimated populations and densities for individual countries. Table 3 contains all the information that I was able to find, nevertheless some data are missing. According to Table 3, the largest populations of snow leopard are in China (2,000–2,500 individuals), Mongolia (500–1,000 individuals), Nepal (300–500 individuals) and India with 200–600 individuals. The greatest population density of snow leopards is in Nepal (0.1–10/100 km^2), Kyrgyzstan (2.35/100 km^2) and India (0.5–0.9/100 km^2). Although the area of snow leopard habitat in Bhutan is the lowest (15,000 km^2), the population density of snow leopards there is considerable (1/100 km^2). There is an urgent need to determine the exact population density of snow leopards in Mongolia, Tajikistan, Kazakhstan, Russia, Afghanistan and Uzbekistan.

Nepal, India, Bhutan, (Myanmar)

The population of snow leopards in Nepal is estimated to be 150–300 individuals (Jackson 1979, unpub. data). However, a computerized habitat suitability model (Jackson and Ahlborn 1990) predicts a population of about 350 to 500 animals located in an area of approximately 30,000 km² (Table 3, McCarthy and Chapron 2003). The largest populations occur in the western parts of Nepal: Mustang, Mugu, Dolpo and Humla districts (Jackson 1979). The population density of snow leopards in Nepal (0.1–10 individuals/100 km², Table 3) is one of the highest ones in the world. For instance, in the Langu Valley in western Nepal, it is 8–10 individuals/100 km² (Jackson and Ahlborn 1989). Nepalese Himalayas are a good habitat for both snow leopard and its most important species of prey, blue sheep (Oli and Rogers 1991; Oli et al. 1993; Lovari et al. 2009; Aryal et al. 2010a).

Table 4 provides estimates of the numbers of animals monitored, number of days monitored, mean home-range size and ungulate population density for a few countries (India, Nepal and Mongolia). The exact size and shape of the home range of snow leopard in Nepal is not well known. The sizes of the home ranges of five individuals in Nepal range from 12 to 39 km² (Table 4) and they overlap each other (McCarthy and Chapron 2003). This home range size is larger than, e.g., in Mongolia, where the terrain is more open and prey is less abundant than in Nepal (Table 4, 140–400 km²/individual).

Jackson and Ahlborn (1990) suggest that it is likely that a large proportion of Nepalese snow leopards live outside protected areas, where they are at greater risk of interacting with humans. A study carried out in Nepal indicates that 42–60% of the use of the home range oc-

Table 4 Estimates of snow leopard home-ranges (km²) and related ungulate population density (no./km²) reported in published studies for India 1990, Mongolia 1992, 2005, Nepal 1994, 1996, 1997 (Source: McCarthy et al. 2008).

Location	No. animals monitored	No. days monitored	Mean home-range size	Ungulate population density	References
India	1	70	19.0	3.0–3.5	Chundawat 1990
Mongolia	1	41	12.0	1.7–2.3	Schaller et al. 1992
Nepal	3	Winter	19.0	6.6–10.2	Oli 1994, 1997
Nepal	5	120–450	19.4	4–8	Jackson et al. 1989
Mongolia	4	207	451.0	0.9	McCarthy et al. 2005

Table 5 Methods used and the estimates of snow leopard abundance obtained.

Locality	Sign survey (all signs/km), (scrapes/km)	Genetic analyses (minimum population size)	Predator: prey biomass ratios (snow leopard/ 100 km²)	Photo-capture rates (photos/100 trap nights)	Total carrying capacity (total number of snow leopards/km²)	Photo capture-recapture (snow leopards/100 km², n = identified s.l./ photo)
Mustang region, Nepal	5.8, 3.7		1.6	2.3	19, 1.6/km²	
Mt. Everest, Nepal	4.5, 3.2	4				
Langu Valley, Nepal	3.6 all signs/km					
Rolwaling, Nepal	3.2, <1					
northern Pakistan	2.4 all signs/km					
Tomur, China		9	1.1	2.37		0.74 (n = 5/6)
Zongjia Township, China		11				
Nuimuhong Township, China		5				
Suojia Township, China		5				
Qilianshan Nature Reserve of Gansu Province, China						3.52
Kunlun Mountains, China	0.16, 0.13					
Sary Chat, Kyrgystan		3	8.7	0.09		0.15 (n = 1/1)
Jangart, Kyrgyzstan		5	1.0	0.93		0.87 (n = 4/13)
Hemis NP, India				8.9, 5.6		
Khangchendzonga BR, India				0.257 ± 0.16		

curs within only 14–23% of the animal's total home area, indicating strong preference particular core areas. Core areas are marked significantly more than non–core areas, which indicates that social marking plays an important role in spacing out individuals (Jackson and Hunter 1996). The core zones in Nepal include Annapurna Conservation Area with approximately 350–500 snow leopards (Jackson and Ahlborn 1990). Almost the whole home range of snow leopards is surveyed for signs as mentioned above (Bikram Shrestha, pers. comm.). Sign density (Table 5) recorded at Mustang, which is in the Annapurna Conservation Area, is 5.8 signs/km, which includes 3.7 scrapes/km (Ale 2007). These results are comparable for those reported for Mt. Everest (4.5 signs/km, 3.2. scrapes/km). Based on sign surveys, the highest abundance of snow leopards is in north-western Nepal in the Langu Valley with 36 signs/km. In the north-eastern part of Nepal at Rolwaling the sign density is lower (3.2. signs/km, <1 scrape/km, Ale et al. 2010) than in the northwest. Sign density in Nepal is much higher than in northern Pakistan (2.4 signs/km, Hussain 2003) or Ladakh, India (2.6 scrapes/km, Fox et al. 2001). Based on genetic analyses (Table 5) 4 cats were detected in the Mt. Everest region in 2004–2006 (Lovari et al. 2009), which corresponds to the results from camera trapping, which indicate a minimum number of three individuals (capture rate; 2.3 individuals/100 camera trap nights) at Lower Mustang. Even though the sign density at Rolwaling is much lower than in Mt. Everest region, an unpublished report based on genotyping revealed occurrence there of three snow leopards (Karmacharya et al. 2012). In the upper Mustang region Aryal et al. (2014) estimate the biomass of blue sheep to be about 38,925 kg, which could support roughly 19 snow leopards (Table 5, 1.6 snow leopards/100 km^2).

Charles University in Prague also participates in data collecting on snow leopard in Nepal. In Prague, Pavel Hulva, Dušan Romportl, Tereza Marešová, Pavel Kindlmann and Bikram Shrestha, who is collecting data in Nepal, are cooperating. Shrestha et al. use genetic analyses and camera trapping. Shrestha has been studying snow leopards since 2004 in the Sagarmatha National Park in eastern Nepal and from 2010 to 2016 in the Annapurna Conservation Area, specifically in Lower Mustang and Upper Manang (Bikram Shrestha, pers. comm.). For camera trapping, the Bushnell model is used to estimate population size at different locations, identify individual snow leopards and determine gender. Although almost the whole Nepal is covered by sign surveys, a few areas lack detailed surveys. To obtain more exact information on the abundance of snow leopard, it would be appropriate to conduct surveys in those areas (e.g., Annapurna Base Camp), where prey is sufficiently abundant to support the presence of snow leopards.

Estimated population size of snow leopard in India is about 200–600 individuals in an area of 75,000 km^2 (Table 3, Chundawat et al. 1988; Fox et al. 1991). Counts of snow leopards are derived from an average density of one animal/110 km^2 for good habitat along the north slopes of the Himalaya with area of 30,000 km^2 and one animal/190 km^2 for lower quality habitat along the southern slopes of Himalaya with area of 22,000 km^2 (Table 3, Fox et al. 1991). Chundawat et al. (1988) suggested Ladakh as a core area of snow leopard (72,000 km^2). Snow leopard may occur in the following protected areas: Himachal Pradesh State (e.g., Pin Valley National Park, Khokhan Wildlife Sanctuary or Rupi Bhaba Wildlife Sanctuary), Uttaranchal State (e.g., Nanda Devi National Park, Nanda Devi National Park or Yamunotri Wildlife Sanctuar), Arunachal Pradesh State (e.g., Dibang Valley), Sikkim State (e.g., Kangchendzonga National Park, Dzongri Wildlife Sanctuary, and Tolung Wildlife Sanctuary) and Jammu and Kashmir State with 12 areas (e.g., Hemis National Park, Dachigam National Park or Lungnag Wildlife Sanctuary). The presence of snow leopard in many of these areas is uncertain (McCarthy and Chapron 2003). Northwest India hosts approximately 400 snow leopards with largest densities in the trans-Himalayan ranges in Ladakh. Therefore, new parks and reserves are being established there (Fox et al. 1991). The only one protected area where the density of snow leopard is known is the Hemis National Park of Ladakh region located in the Jammu and Kashmir State. Mallon and Bacha (1989) estimated 75–120 snow leopards in a 1,200 km^2 area to be living there. Jackson et al. (2006) reported 66 and 49 capture events (capture success 8.9 and 5.6 per 100 trap-nights, Table 5) in two consecutive years of 2003 and 2004 in the Hemis National Park. In the Khangchendzonga Biosphere Reserve in the eastern Himalayan region (Sikkim), Sathyakumar et al. (2011) conducted the first survey to obtain basic information on abundance of mammals including snow leopard. They proved the presence of snow leopard based on photo capture, scat/dung, tracking and information from locals. Photo capture rate of snow leopard was 0.257 photos/100 trap nights (Table 5, Sathyakumar et al. 2011). Sathyakumar et al. (2011) recommend that surveys are also carried out in other watersheds of the Khangchendzonga BR. In India, it is necessary to cover the whole area by sign surveys and after that also conduct detailed studies.

In northern Bhutan, along the high Himalayas, in accord with area-based estimates, the confirmed presence of snow leopard is about 100–200 individuals (Table 3, Fox 1989). The density of snow leopard is assumed to be 1/100 km^2. The suitable habitats are above 3,000 m in an area of about 15,000 km^2 (Fox 1994). In Jirgme Dorje National Park, sign surveys were conducted, suggesting a lower occurrence of snow leopards than in adjacent Shey Phoksundo National Park in Nepal, although there is a larger abundance of its prey in the former (Jackson and Fox 1997b; Jackson et al. 2000). In a part of the protected area (Torsa Strict Nature Reserve, Kulong Chhu Wildlife Sanctuary, Sakteng Wildlife Sanctuary) the occurrence of snow leopard is still not confirmed.

China and the Former Soviet Union

China, the largest state where snow leopard occurs, contains as much as 60% of its potential habitat: about 1,824,316 km^2 (Fig. 3, Hunter and Jackson 1997; McCarthy and Chapron 2003). Area of snow leopard presence is estimated as 1,100,000 km^2 with approximately 2,000–2,500 individuals (Table 3, Fox 1994). Due to irregular distribution of its prey, the mean density is 1 snow leopard/250–300 km^2 (Table 3, McCarthy and Chapron 2003).

Snow leopard habitat is located in six provinces in China (Qinghai, Gansu, Sichuan, Yunnan, Xinjiang and Xizang or Tibet) and in the seventh one (Inner Mongolia) it is nearly extinct (McCarthy and Chapron 2003). In almost every province, there is a lack of status surveys, such as in Sichuan Provence, Yunnan Province and in Tibet Autonomous Region (TAR). In the TAR, at several sites blue sheep are abundant. Areas with the highest priority for status surveys are the Nayainqentanglha, Taniantaweng and Ningjing Shan mountains in eastern and south-eastern Tibet, western Nepal, the mountains bordering Uttar Pradesh in India and the Nganlang Kangri mountains bordering Ladakh (McCarthy and Chapron 2003). Snow leopards are likely to occur on the northern slopes of the Himalayas close to border with Nepal and on mountain ranges bisecting the Tibetan Plateau (McCarthy and Chapron 2003). Jackson (1994a) report up to 100 snow leopards in the Qomolangma Nature Preserve, a 33,910 km^2 area along the main Himalayan and Nepalese border, centered around Mt. Everest. In the area including the Sanjiangyuan National Nature Reserve (Qinghai Province), Qiangtang National Nature Reserve (TAR) and Nanshan area (Danghe, Gansu Province) 89 samples were identified as indicating snow leopard and the presence of 48 individuals (Zhou et al. 2014). In the Tomur National Nature Reserve (Figure 4) in Xinjiang Autonomous Region there have been 20 SLIMS sign surveys (McCarthy et al. 2008). By counting the snow leopard's prey, ibex and argali, the snow leopard potential density was estimated to be 1.1 snow leopards/100 km^2 (Table 5, McCarthy et. al. 2008). Photo-capture rates in Tomur were 2.37 photos/100 trap-nights (Table 5). According to the results of the photo capture-recapture method (0.74 individuals/100 km^2, n = 5/6), it is estimated that about 6 snow leopards occur in Tomur, whereas the genetic analyses indicates at least 9 individuals (McCarthy et al. 2008). Home range of snow leopard in the Qinghai Province is highly fragmented (Liao 1994). Zhang et al. (2009) identified 11, 5 and 5 (Table 5) snow leopards using genetic analyses in Zongjia Township (ZJ) and Nuomuhong Township (NMH) in Dulan County, and Suojia Township (SJ) in Zhiduo County, respectively.

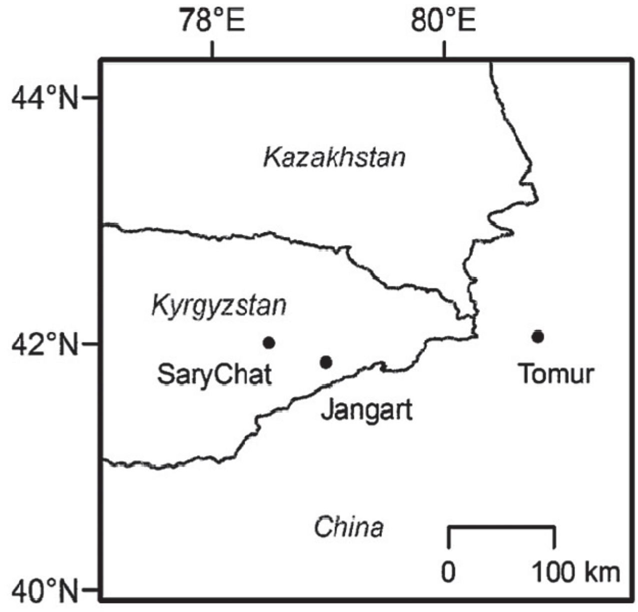

Fig. 4 Diagram of the study area showing the locations of 3 snow leopard camera capture-recapture study sites, Sary Chat, Jangart and Tomur, in the Tien Shan Mountains in Kyrgyzstan and China, 2005 (Source: McCarthy et al. 2008).

Apart from these areas with snow leopard occurrence, Schaller et al. (2008) identified three "hotspots" in Qinghai Province: North Zadoi, South Zadoi and Yushe, where the population density of snow leopard was estimated to be 1 individual per 25–35 km^2. In adjacent Gansu Provence northeast from Qinghai Province, 17–19 individuals were identified using the camera capture-recapture method. In total, 251 snow leopard captures were recorded over the 7,133 trap-days, which is an average of 3.52 captures per 100 trap-days (Table 5, Alexander et al. 2016).

The Gouli Region (Fig. 5), also in Qinghai Province, is considered to be one of the core zones of snow leopard in China. Sign surveys conducted there along transects of total length of approximately 440 km, recorded 72 signs and 60 snow leopard scrapes (Xu et al. 2008), which is 0.16 signs/km and 0.13 scrapes/km (Table 5). As mentioned there is no information on on the abundance of snow leopards in China and results of the few studies carried out there are not consistent with those from other countries.

Before the breakup of the USSR (1990) it was reported that there were 1,000 to 2,000 snow leopards there (Braden 1982; Bannikov 1984). Most of them (75%) were in Kyrgyzstan and Tajikistan (Koshkarev and Vyrypaev 2000). According to these authors and Bannikov (1984), there were 150–200 snow leopards in the Russian Union Republic, 100 in Uzbekistan and 180–200 in Kazakhstan, making a total of about 2,000 individuals. Koshkarev (1989) estimates the population in Tien Shan and Dzhungarsky Alatau to be about 400–500 individuals. After the disintegration of the USSR, the populations in Kazachstan and Kyrgyzstan decreased by at least 50% due to poaching of snow leopards and ungulates (McCarthy and Chapron 2003). The current legal and management status of many reserves is unknown.

Snow leopards are reported in Russia (Table 3, Table 6, Fig. 2) in the Altai and Sayan ranges on the border with

Fig. 5 Map showing the area surveyed in the Gouli Region in East Burhanbuda Mountain, Kunlun Mountains, China, and indicating the locations of leopard signs and camera traps (Source: Xu et al. 2008).

the People's Republic of Mongolia, and in southern Siberia, including the Tuvan and Buryat mountain ranges (Paltsyn et al. 2012). The mean population density in the Altai Mountains is estimated as 0.75–1.5/100 km^2 with approximately 40 individuals (Sopin 1977). In accordance with Table 5, in the Chikhachev Ridge located in the Altai Republic, Tuva Republic and Mongolia, about 5–7 snow leopards occur, or 10–15 if we include those in Mongolia. Snow leopard population size in the Sayan region is about 20–30 individuals (Koshkarev 1996) and in Sayano-Shushensky Nature Reserve in the Sayan region about 9–10 (Table 6). In southern Siberia, snow leopards possibly occur on the Okinsky and Tunkinsky Ridges (Table 6, Paltsyn et al. 2012). Smirnov et al. (1990) estimate that about 80 snow leopards reside in southern Siberia including animals that stray into Mongolia. As mentioned in Table 5, more research needs to be done in this area. In general for Russia, detailed data are lacking or outdated.

In Kyrgyzstan, there is insufficient information on wildlife in terms of the post-Soviet distribution and status of many species (McCarthy et al. 2010). On the other hand, the Government of the Kyrgyz Republic has increased the enforcement and development of protected areas (Dexel 2002; Chapron 2005). Snow leopards occur in the Talasskiy Alatau and Ferganskiy mountains and in Tien Shan bordering on China and Kazakhstan (Fig. 2, Braden 1982; Koshkarev 1989). Koshkarev (1989) estimates a population of 113–157, with an average population density of 2.35 snow leopards/100 km^2 (Table 3). McCarthy et al. (2008) report snow leopard abundance at Jangart and Sary Chat Ertash in the Tien Shan Mountains in eastern Kyrgyzstan (Table 5). Sixteen sign surveys in the Sary Chat with total transect length of 8.2 km and 13 surveys with 8.6 km transect length in the Jangart revealed 7 snow leopards in the Jangart. According to photo capture-recapture 0.15 (n = 1 identified individual/1 photograph) were recorded in the Sary Chat and 0.87 (n = 4/13) in the Jangart (Table 5). By counting the snow leopard prey: ibex and argali, snow leopard potential density was estimated as 8.7 snow leopards/100 km^2 in Sary Chat and 1.0 snow leopard/100 km^2 in Jangart.

According to Hunter and Jackson (1997), the estimated size of potential snow leopard habitat in Kazakhstan is 71,079 km^2. Its estimated population size is approximately 180–200 individuals (Table 3, Annenkov 1990; Zhirjakov 1990). In the south of Kazakhstan, snow leopards occur in the Khigizskiy Range and Tasskiy Alatau bordering Kyrgystan, in the Sarytau Mountains near Alma Ata and bordering China in the Dzungarsky Alatau (McCarthy and Chapron 2003). According to Zhirjakov (1990) there are about 20 snow leopards in Zailiskiy Alatau and northern Tien Shan. The presence of snow leopard in protected areas is confirmed for the Aksu Dzhabagliy State Reserve and Alma Atinskiy Nature Reserve (McCarthy and Chapron 2003).

There is no data on the current status and distribution of snow leopard in Tajikistan. They are said to occur

Table 6 Habitat area of snow leopards and estimates of their abundance in Russia (Source: Paltsyn et al. 2012).

Location	Habitat area, km²	Estimate population (# of animals)	Notes
Chikhachev Ridge	1000	5–7	Total population of this trans-boundary group, including Mongolia, is 10–15 animals
Tsagan-Shibetu Ridge, southern Shap-shalsky Ridge, western side of Western Tannu-Ola Ridge	2500	15–18	Total population of this trans-boundary group, including Mongolia, is 20–25 animals
Sayano-Shushensky Nature Reserve, its buffer zone, and adjacent parts of Khemchiksky and Kurturshubinsky Ridges	No more than 200–500	9–10	
Sengelen Ridge	2000	7–10	
Okinsky and Tunkinsky Ridges, possibly	5000–6000	15–20	This area requires additional research
Total	6000 (possibly 11,000–12,000 if Okinsky and Tunkinsky Ridges are included)	36–45 (possibly 50–65 if Okinsky and Tunkinsky Ridge populations are included)	

in the central and western parts in the Zeravshanskiy, Gissarskiy, Karateginskiy and Petr Pervyi mountains, in the Hazratishog and Darvaskiy Mountains and the Gorno-Badahshansk area, including the Pamirs (McCarthy and Chapron 2003). Bykova et al. (2002) estimate a total of 180–220 snow leopards occur there (Table 3). Occurrence of snow leopard is confirmed mainly in reserves and protected areas such as in the Great Pamir National Park (Hunter and Jackson 1997), Ramit State Reserve, Dashti-Dzhumskiy Reserve (Sokov 1990), Iskanderskul'skiy lake reserve, Muzkul'skiy, Pamisskiy and Sangvorskiy Zakazniki reserves (McCarthy and Chapron 2003). In 2003, Rodney Jackson conducted a survey in order to determine whether it was possible to promote wildlife conservation in Tajikistan. He trained local staff and herders to monitor snow leopards and Marco Polo sheep using basic survey methods based on transects (Jackson et al. 2004).

Uzbekistan is at the western edge of snow leopard's home range. They occur in the Turkestanskiy, Chatkalskiy and Gissarskiy ranges bordering Tajikistan and Kyrgyzstan (Braden 1982), where the total population is estimated to be 50 animals (Sludskiy 1973, cited in Braden 1982). Recently Kreuzberg-Mukhina et al. (2002) estimated the population of snow leopard to be about 20–50 in an area of 10,000 km² (Table 3). As in Tajikistan, snow leopard presence is confirmed in protected areas, for instance in Zaaminskiy State Reserve, Uzbek National Park, Gissarskiy State Reserve and the Chatkal'skiy State Reserve (McCarthy and Chapron 2003).

Pakistan, Afghanistan and Mongolia

The potential snow leopard habitat in Pakistan covers 80,000 km² (Table 3, Fox 1994) with about 200–420 individuals (Schaller 1977; Hussain 2003). Assuming a mean density of 1 snow leopard/250 km², the total number of snow leopards would be approximately 320 (McCarthy and Chapron 2003). Its occurrence is verified in the Northwest Frontier Provinces, Chitral District and in the Karakorum Range in the Northern Areas in the Gilgit, Hunza and Baltistan districts (McCarthy and Chapron 2003). Hussain (2003) surveyed the Baltistan district between 1998 and 2001 and estimates that approximately 36–50 snow leopards live there. With respect to the availability of its prey and suitable habitat he suggests 90–120 snow leopards occur in the whole Baltistan. Its presence in Azad Kashmir Province remains unconfirmed (Roberts 1977). Snow leopard occurrence is confirmed in the following protected areas: North-West Frontier Province (Chitral Gol National Park, Agram Besti Game Reserve, Goleen Gol Game Reserve, Gahriat Gol Game Reserve) and in northern parts (Khunjerab National Park, Baltistan Wildlife Sanctuary, Kargah Wildlife Sanctuary, Nazbar Nallah Game Reserve). In many protected areas, there are potential habitats for snow leopard, but there presence there has not been verified: Parit Gol Game Reserve, Tirichmir and Qashqar Conservancies, Kilik/ Mintaka Game Reserve, Naz/Ghoro Game Reserve, Sherquillah Game Reserve, Askor Nullah Game Reserve, Astore Wildlife Sanctuary, Chassi/Bowshdar Game Reserve, Danyor Nallah Game Reserve, Pakora Game Reserve, Machiara National Park and Ghamot Game Reserve (McCarthy and Chapron 2003). Snow leopards are also likely to be present in the Nanga Parbat Conservancy (McCarthy and Chapron 2003).

The numbers of snow leopards in Afghanistan remain to be determined. The estimates of the area of potential habitat in Afghanistan differ from each other. Fox (1989) estimates 80,000 km² and subsequently 50,000 km² (Fox 1994), and Hunter and Jackson (1997) suggest 117,653 km² (Table 3). Snow leopard occurrence is confirmed in the Hindu Kush and Pamir mountains in north-eastern Afghanistan (Habibi 1977; Petocz 1978; Sayer 1980). Snow leopards occur at Zedak in the southern part of Badakhshan (McCarthy and Chapron 2003). Due to a long history of many wars in Afghanistan, wildlife laws are not enforced there (McCarthy and Chapron 2003). The actual status of snow leopards at many locations is unknown (McCarthy and Chapron 2003). The latest information on the occurrence of snow leopards in Wakhan District in Badakhshan, was obtained by the Wildlife Conservation Society (WCS) and National Environmental protection Agency who installed remote camera traps in 2009 and recorded over 1300 images of snow leopard at 20 sites (Noras 2015). Three individuals were captured and equipped with satellite collars in 2012. Thanks to the confirmation of the presence of snow leopards in that area, the whole of Wakhan Distict, with area of 10,000 km^2, was declared a National park in 2014 (Noras 2015).

Mongolia is the state with the second largest population of snow leopards, estimated at 500–1,000 individuals in an area of approximately 101,000 km^2 (Green 1988) (Table 3, Fig. 2, Schaller et al. 1994; McCarthy 2000). It occurs in at least 10 protected areas: the Transaltay Gobi Strictly Protected Area or SPA, Khokh Serkh SPA, Otgontenger SPA, Tsagaan Shuvuut SPA, Turgen Uul SPA, Gobi Gurvansaikhan National Conservation Park, Altai Tavaan Bogd NCP, The Burhan Buudai Nature Reserve, Alag Khairkhan Nature Reserve and Eej Uul National Monuments (McCarthy and Chapron 2003). The main populations occur in the Altay and Transaltai Gobi mountain ranges, with smaller populations in the Khangai, Hanhohiy Uul and Harkhyra Uul ranges (McCarthy and Chapron 2003). Bold and Dorzhzunduy (1976) estimate that there are 170–230 snow leopards in the southern Gobi region in Mongolia. During 1994–1997 McCarthy et al. (2005) recorded snow leopard movements and activities based on year-round radio-monitoring in the Altai Mountains kin south western Mongolia. Home ranges determined by standard telemetry techniques are at least of 11–13 km^2 (McCarthy et al. 2005). In the area of Burhan Budai in the Altay, Schaller et al. (1994) found signs of at least 10 cats within 200 km^2. This population density is one of the highest estimated in the whole of its habitat. From 2008, the Snow Leopard Trust (SLT) and Panthera, in co-operation with the Mongolian government, started a 10 year program. Hitherto they captured and radio collared 20 individuals. To date, in the Altai Mountains there are conservation programs involve more than 400 herder families (Noras 2015). In 2014 in Tsagaan Shuvuut Strictly Protected Areas, at a transboundary site in Mongolia and Russia, a Mongolian-Russian team, headed by Dr. B. Munkhtsog captured a female snow leopard and fitted her with a North star satellite collar provided by the Snow Leopard Conservancy.

Conservation of Snow Leopard

Conservation of snow leopard is a very complex problem because its habitat is very rugged, it has a large home range and frequently comes into conflict with humans (Li 2013). Below is a list of some possible ways of protecting it by means of legislation, international cooperation, financial support, education and awareness.

Legislation

Legislation relating to the conservation of snow leopards is based on the designation of nature reserves by governments and supporting programs led by nongovernmental organizations (McCarthy and Chapron 2003; Mishra et al. 2003). However, their abilities are limited (Li 2013). There is a little information on the current management status of protected areas or their role in sustaining snow leopard populations (Green 1992, 1994; Fox 1994; Green and Zhimbiev 1997). To date, the areas of snow leopard habitat covered by nature reserves are just 0.3–27% in 11 of 12 countries (Li 2013). The only exception is Bhutan with 57% of the area of snow leopard home range protected (Hunter and Jackson 1997).

To preclude the threats to snow leopard such as poaching and illegal trade, it is necessary to implement legislation and conservation policies and prevent the hunting, killing, possession, sale and trade in snow leopards including all its body parts and derivatives at local, regional and national levels (Theile 2003; Aryal et al. 2013). It would help if governments could be assisted and given advice on how to penalize people who break the law, and to consider implementing “whistle-blower” policies to provide incentives for report illegal activities (Theile 2003; Aryal et al. 2013). Theile (2003) also recommend the setting up of “antipoaching” teams, which would monitor main markets and trade centres in order obtain information on illegal killing. For a better understanding of the factors affecting the effectiveness of protected areas for the conservation of diversity, long-term and detailed research and the evaluation of the interactions between populations outside and inside protected areas are needed (Gaston et al. 2008).

For successful conservation it is important to have the support of the government in each state. Four countries already have national action plans: Nepal, Pakistan, Mongolia and Russia (McCarthy et al. 2003). The government of India initiated Project Snow Leopard (PSL), a national governmental program, on 20 January, 2009. The goal of that project is to conserve snow leopard together with other species living at high-altitudes in five states in the Himalayas: Jammu and Kashmir, Himachal Pradesh, Uttarakhan, Sikkim and Arunachal Pradesh (Rajput 2009). The PSL is based on knowledge-based adaptive wildlife management policies and actions, law enforcement and promotion of awareness, and education on wildlife conservation (Rajput 2009). The PSL was constructed at a national conference at Ladakh in 2006 thanks to the collaboration of the International Snow Leopard Trust (ISLT), the Nature Conservation Foundation (NCF), the State Governments, the Ministry of Environment and Forests (Government of India), Wildlife Institute of India (WII), the Snow Leopard Network, local communities and certain NGOs (Rajput 2009).

International Cooperation

Snow leopard home range spans 12 countries in South and Central Asia. Therefore, cooperation of all these states is necessary, using internationally valid acts such as Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), which regulates the export or import of animals or their body parts (Nowell 2007). Protected areas play an important role in sustaining the overall snow leopard population because the home range of snow leopards includes areas that constitute international borders (McCarthy and Chapron 2003). The Snow Leopard Network (SLN 2008) unifies individuals and organizations such as the Snow Leopard Conservancy and the International Snow Leopard Trust. The function of the SLN is to coordinate, cooperate and share information. International conferences try to identify locations for snow leopard conservation, name Snow Leopard Conservation Units and provide a framework for the development of national action plans (Jackson et al. 2008). Recently, e.g., an International Conference on Range-wide Conservation Planning for Snow Leopards was held in Beijing, China in March 2008. Another function of international societies, such as the World Wildlife Fund (WWF), is to secure with governments transparent and clear funding for compensatory programmes, for instance with the Nepalese government (Ikeda 2004).

Snow leopard is legally protected from hunting by national legislation in most of the 12 states in its home range (McCarthy et al. 2003). Afghanistan has recently afforded snow leopards legal protection, after listing this species on the country's first Protected Species List in 2009. This act bans all hunting and trading of snow leopards within Afghanistan. Except for Tajikistan, the rest of the countries are signatories to the CITES agreement (Jackson 2014).

Education and Awareness

It is important to educate and increase awareness of snow leopard conservation among local communities, national governments and international audiences (Jackson et al. 2008). The effectiveness of conservation management is increased by using community-based programs that involve local people such as livestock herders, trekking guides, farmers and former hunters (Sathyakumar et al. 2011). Various ways of supporting cultural, social and religious beliefs of locals exist such as education of the public by monks in monasteries or involving locals in some studies, e.g., measurement of changes in climate (Jackson et al. 2008).

The Snow Leopard Survival Strategy (McCarthy et al. 2003) may include the management of grazing in order to diminish its effect on native wildlife (e.g. large ungulates) and support for husbandry practices that reduce livestock vulnerability to snow leopard predation and improve efficiency and yield (Jackson et al. 2008). As mentioned above, herders of small and medium-sized livestock are notably vulnerable to losses of livestock, which can be reduced corralling the livestock at night. Understanding what determines the carrying capacity (based on the distribution and population structure) of predators such as snow leopard will simplify the management of the human-wildlife conflict (Aryal et al. 2014). One of the major issues is the protection of prey populations (e.g. blue sheep) as a resource for snow leopards in order to reduce their need to kill livestock and so inflame the human-predator conflict (Aryal et al. 2014). It is also appropriate to provide a legal mechanism, which enables herders to kill snow leopards that repeatedly kill their livestock (McCarthy and Chapron 2003). In prior studies, physical precautions, such as installation of predator-proof livestock corrals or guard dogs or formation of core areas, for snow leopard conservation were recommended (Jackson and Hunter 1996; McCarthy and Chapron 2003). Another issue is to decrease the suspicion of herders that snow leopard abundance will increase unduly in the future, by more adaptable conservation policies that provide a better compromise between livestock rearing and wildlife protection (Ikeda 2004).

One of the ways of protecting snow leopards is a monastery-based conservation. In the Sanjiangyuan region in China's Qinghai Province and Mustang in Nepal, Buddhist monasteries play an important role in snow leopard conservation (Li 2013; Ale et al. 2014). This method may prove very efficient and by establishing it in other Tibetan Buddhist regions, it could result in the extension of protection to about 80% of the snow leopard habitat (Li 2013). It should be possible to decrease the killing of snow leopards by adhering to Buddhist tenets such as respect, love and compassion for all living beings. Thus, the 336 monasteries located in the Sanjiangyuan region could protect more snow leopard habitat (8,342 km^2) through social norms and active patrols than the core zones of the nature reserves (Li 2013).

As mentioned above, climate change is another serious issue (Threats to the Snow Leopard – Climate change). It is important to implement strategies that mitigate and adapt conservation management at the local level in order to reduce the effect of climate change (Jackson et al. 2008). This strategy may include plantations on private land and in local areas, use of solar energy for cooking and heating, spread of the seeding of native grasses around the area, development of water holes in areas where long distances need to be covered, storage of rainfall water for agriculture, construction of reservoirs for winter and times of water shortage, control of poaching and continued monitoring of the distribution of trees (Aryal et al. 2013). For conservation planning, bioclimatic models, used to predict the persistence of species populations and habitats resulting from climate change, are impractical because their reliability and scope are limited (Heikkinen et al. 2006; Lawler et al. 2006). Therefore, the use of individual-species climate models as guidelines for climate-integrated conservation planning may be more appropriate. They are more reliable than community-based or assemblage models (Hannah et al. 2002a; Pearson and Dawson 2003; Thuiller 2007).

Financial Support

For efficient conservation it is essential to understand the economic situation of local herders and to find how to avoid conflicts between wildlife conservation and livestock rearing in countries where incomes are low (Ikeda 2004). It is necessary to obtain detailed ecological and socio-economic information in order to design a system, which will function successfully (Mishra et al. 2003). Except for the Annapurna Conservation Area and the Spiti Region in Himachal Pradesh in India, where these surveys were conducted by Oli et al. (1994), Jackson et al. (1996), and Mishra (1997), there is a lack of such data for the rest of the snow leopard habitat (Ikeda 2004).

One option for improving the compensatory mechanism, is to involve herders in ecotourism activities (Schellhom and Simmons 2000), as in Baltistan (Tibet) and Pakistan (Hussain 2000). This made not be possible in Nepal because of the unstable situation in the country due to the lack of foreign trekkers (Hussain 2000). Other alternative financial incentives recommended by the Snow Leopard Survival Strategy (McCarthy et al. 2003) are the establishment of cottage industries, e.g., village-made handicrafts, or a well-structured ungulate trophy hunting program (Mishra et al. 2003; Jackson et al. 2008). This innovative program is already operating in Kyrgyzstan (Mishra et al 2003; McCarthy et al. 2010).

Conclusions

In this review, I have summarized the distribution of snow leopard in each of the countries in which it occurs based on the estimates available (Table 3 and 5). Despite all efforts, it was impossible to compare results obtained by using different methods, mainly due to inconsistent results. According to my survey, the largest populations of snow leopard are in China (2,000–2,500 individuals), Mongolia (500–1,000 individuals), Nepal (300–500 individuals) and India with 200–600 individuals (Table 3). On the other hand, the smallest populations are in Russia (150–200), Bhutan (100–200), Afghanistan (100–200) and in Uzbekistan (20–50). Based on the available data, the greatest population density of snow leopard per 100 km^2 of suitable habitat is in Nepal (0.1–10/100 km^2), Kyrgyzstan (2.35/100 km^2) and India (0.5–0.9/100 km^2). Although the area of habitat in Bhutan is one of the lowest (15,000 km^2), the population density of snow leopards there is very high (1/100 km^2). There is a need to determine the population density of snow leopards in Mongolia, Tajikistan, Kazakhstan, Russia, Afghanistan and Uzbekistan.

In the section Comparison of Methods Used for Snow Leopard Density Estimation, I compared the use of different methods to estimate snow leopard abundance. From my point of view, the best way is first to carry out a survey of signs, which can serve as a pilot study for choosing the appropriate method(s) and then comparing the results of all the methods used with those of other methods (predator : prey biomass ratios, genetic analyses, camera trapping and camera capture-recapture methods).

I have analysed the general threats to snow leopards in various regions, which revealed that the main threats come from conflict with locals (human-snow leopard conflict), lack of a conservation capacity, illegal trade, poor awareness and policy, and climate change. Killing snow leopards for killing livestock and the decline in the abundance of their natural prey is inherently challenging in the Himalayan region (India, Nepal, Bhutan, Tibetan Plateau and southern China), Karakorum and Hindu Kush (southwest China, Pakistan and Afghanistan). The issue of military conflict is also a problem in the Himalayan region and Commonwealth of Independent States and western China.

In order to improve conservation management for increasing the likelihood of snow leopards surviving, it would be useful to obtain detailed information on the current management status and its role in sustaining snow leopard populations in protected areas for which such information is currently unavailable.

Introduction of "antipoaching" teams may decrease poaching and illegal trade in areas where snow leopards are present. To improve international cooperation, the signature of Tajikistan, the last non-signatory country, to the CITES agreement is needed. Also, trans-boundary cooperation should be increased in areas of the snow leopard home range in order to decrease logistic constraints, e.g. transport of samples for genetic analyses, or the building of more laboratories in each country. Action plans now exist only for Nepal, Pakistan, Mongolia and Russia. It would be advisable to create national action plans or governmental programs in the remaining countries.

Based on my survey, the most successful conservation is that based on community-based programs involving local people, such as monastery-based conservation, protecting snow leopard through the adoption of social norms and active patrols. Thus it may be effective to spread this method to other Tibetan Buddhist regions. To improve the financial aspect of conservation management of snow leopards, it is important to understand the economic situation of local herders (up to now this is only available for the Annapurna Conservation Area in Nepal and Spiti Region in Himachal Pradesh in India) and estimate the monetary value of damage to livestock in order to determine the correct level of compensation. It is also important to improve the procedure for verifying the damage to livestock caused by snow leopards. The compensatory system may involve, e.g., ecotourism or cottage industry.

Another possible improvement to snow leopard conservation would be a decrease in the human-snow leopard conflict. One of the possible ways of doing this is to provide a legal mechanism by which herders can remove those snow leopards that persistently attack their livestock.

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