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# Carotenoids in a food chain along a pollution gradient

Saila Sillanpää[a,*], Juha-Pekka Salminen[b], Esa Lehikoinen[a], Eija Toivonen[a], Tapio Eeva[a]

[a]Section of Ecology, 20014 University of Turku, Finland
[b]Laboratory of Organic Chemistry and Chemical Biology, 20014 University of Turku, Finland

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ABSTRACT

Carotenoids are synthesized by plants, therefore insects and birds must obtain them from their diet. They function in pigmentation and as antioxidants. We studied the carotenoid profiles in a model food chain (plant–insect–bird) in an air pollution gradient to find out whether heavy metal pollution affects the transfer of carotenoids across the trophic levels. Birch leaves showed higher β-carotene and, one of the birch species (*Betula pendula*), higher total carotenoids levels in the polluted area. There was no difference in the lutein concentration of caterpillars' food source, birch leaves, between the study areas. Autumnal moth larvae accumulated lutein more efficiently than β-carotene while sawfly larvae accumulated β-carotene over lutein. Because of different antioxidant profiles in different leaf chewing insects their sensitivity to pollution stress may differ. The lutein concentration of plasma and feathers of Great tit nestlings did not differ along the pollution gradient. The lack of difference in lutein concentration of autumnal moth larvae along pollution gradient may partly explain the lutein concentrations of Great tit nestlings, since the abundance of autumnal moth larvae peak during the nestling phase of Great tit. The lutein concentration of autumnal moth larvae was positively associated to circulating plasma lutein level of Great tit indicating the importance of carotenoid rich diet during the nestling phase. In addition, the higher the plasma lutein concentration the more lutein was deposited to feathers, irrespective of the other possible functions of lutein in nestlings. We found that carotenoid levels differed between the polluted and the unpolluted area especially at lower levels of food chain: in birches and in caterpillars.

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## 1. Introduction

Carotenoids have many important physiological functions at all trophic levels (Krinsky, 1994; Olson and Owens, 1998; Fraser and Bramley, 2004; Škerget et al., 2005; Carrol and Berenbaum, 2006). Plants are able to produce carotenoids in chloroplasts, and also to regulate their amounts better than organisms at higher trophic levels. Carotenoids affect plant development and adaptation, which suggest that their synthesis is coordinated with other developmental processes (Young, 1991; Young and Britton, 1993; Castemiller and West, 1998; Fraser and Bramley, 2004). Organisms higher in food chain, such as insects and birds, are unable to synthesize carotenoids *de novo*, therefore they have to obtain carotenoids from their diet (McGraw and Hill, 2001) and thus are dependent of the carotenoid availability in their environment. The carotenoid content of an insect is dependent of the carotenoid content of its diet, but many insect species selectively absorb one carotenoid, lutein, over the others (Ahmad, 1992). Carotenoids also act in pigmentation of insects, e.g. protecting them from predation and light damage (Rothchild, 1978; Carrol and Berenbaum, 2006). Carotenoid depletion may further delay hatching of insect larvae (Sakamoto et al., 2003). In plants and insects carotenoids function as antioxidants against endogenous and exogenous oxidative stress (Ahmad, 1992; Bungard et al., 1999).

* *Corresponding author*. Tel.: +358 23335717; fax: +358 23336550.
*E-mail address:* saisil@utu.fi (S. Sillanpää).

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Similar functions are found in birds, which absorb carotenoids from their diet and transfer it via plasma to tissues (Parker, 1996; McGraw et al., 2003a). In passerine birds carotenoids and melanins are the most important pigments in feather coloration (Badyaev and Hill, 2000). In many bird species the most abundant carotenoid expressed in plumage coloration is lutein, often associated with its close molecular relative, zeaxanthin, or zeaxanthin derivative, dehydrolutein (Brush, 1978; McGraw et al., 2003b). Lutein may be selectively accumulated over other carotenoids into feathers (McGraw et al., 2003b).

Pollutants cause oxidative destruction of carotenoids (Bačkor and Vaczi, 2001) and may also increase and/or decrease the amount of primary (e.g. nutrients) and secondary compounds (e.g. phenolics) of plants and hence change the nutritive value of plants for herbivorous insects (Heliövaara and Väisänen, 1993; Riemer and Whittaker, 1989; Loponen et al., 2001). Insects, on the other hand, are the primary carotenoid source for many birds. For example, the Great tit (*Parus major* L.), an insectivorous passerine, prefers carotenoid rich caterpillars in its diet (Gosler, 1993). Matching nestling phase to insect larval period is crucial for caterpillar eating birds (Nager and van Noordwijk, 1995; van Noordwijk et al., 1995; Isaksson and Andersson, 2007). In addition to being a source of nutrients, energy and carotenoids, phytophagous insects are also a source of pollutants to birds, since they accumulate heavy metals from plants (Heliövaara et al., 1987; Heliövaara and Väisänen, 1990; Lindqvist, 1992; Kozlov et al., 2000; Dauwe et al., 2004a).

Heavy metals cause oxidative stress (Kaminski et al., 2007) and thus affect different physiological functions of birds, for example the ability of bird to mount an immune response (Hõrak et al., 2004). Oxidative stress by pollutants is caused when there are more oxidants than antioxidants present in the target organism or cell (see Ahmad, 1992). Environmental pollution and urbanization can also affect the carotenoid-based coloration of feathers in insectivorous birds (Eeva et al., 1998; Hõrak et al., 2001; Isaksson et al., 2005). One possible explanation to pale plumage coloration is the scarcity of carotenoid rich food items in polluted and urban areas. Alternatively pollution-related oxidative stress might lead to depletion of carotenoids in the body. Great tits suffer higher oxidative stress in urban (e.g. higher air pollution) than in rural populations (Isaksson et al., 2005). However, the association between carotenoid coloration (which may act as indicator of pollution stress) and oxidative stress is unclear (Isaksson et al., 2005). Isaksson et al. (2007) have shown that plumage carotenoid coloration may not be reliable signal of current oxidative stress and it is possible that plasma carotenoids are not active as free radical scavengers.

Heavy metals pass through the food chain from bottom till top, highest concentrations being usually found at higher trophic levels (Laersen et al., 1994; Niecke et al., 1999). This may also cause higher need of antioxidants for detoxification at higher levels of a food chain. On the other hand, carotenoids, which function as antioxidants, are synthesized in the basal part of food chain, in plants (Fraser and Bramley, 2004). Therefore, carotenoids are thought to be scarce and costly resources for organisms higher in the food chain (see Olson and Owens, 1998).

We studied the transmission of carotenoids in a model food chain, plant–insect–bird, in the surroundings of a well known pollution gradient around a copper smelter. Our interest was to study carotenoid concentrations in three different trophic levels. We measured carotenoid profiles along the whole food chain during the nestling phase of an insectivorous passerine, the Great tit. Our main interest among carotenoids was lutein since it is exclusively the dominant carotenoid in egg yolk, plasma and feathers of many bird species, including the Great tit (Saino et al., 2002; McGraw et al., 2003a,b; Hargitai et al., 2006). Accordingly, we studied lutein and β-carotene concentration of two birch species: silver birch (*Betula pendula*) and downy birch (*Betula pubescens*), two insect groups, i.e. autumnal moth (*Epirrita autumnata*, Geometridae) and phytophagous sawflies (Hymenoptera: Symphyta). Finally we studied pollution effects on lutein concentration of Great tit nestling plasma and feathers. With pollution effect we mean both direct and indirect effects that result from environmental changes due to pollution. Isaksson and Andersson (2007) studied carotenoid availability by diet quantity and quality and feeding behaviour of parent Great tits. Partali et al. (1985) studied carotenoids in a terrestrial food chain. However, this is to our knowledge the first study that takes to account carotenoid profiles along a pollution gradient in a terrestrial food chain: plant–insect–bird.

## 2. Materials and methods

### 2.1. Study organisms

The Great tit is a territorial and resident species that is susceptible to pollution in the study area all the year round (see Gosler, 1993; Eeva, 1996). Though Great tit is omnivorous, it prefers insects especially when feeding nestlings. Depending on their availability, phytophagous caterpillars make up to 60–95% of nestling diet (Gosler, 1993; Eeva, 1996).

We concentrated on two important food sources for tits: sawfly (Hymenoptera: Symphyta) and autumnal moth (*Epirrita autumnata*, Geometridae) larvae. Sawflies are a group of insects, in which larval stage spreads over the growing season from the burst of leaves of deciduous trees till leaf senescence depending on the species (Riipi, 2004). We treated all sawfly species as a group. Two unidentified species of sawflies were the main species feeding on birch leaves in the polluted and in the unpolluted area during the nestling phase of Great tit. The autumnal moth hatches in spring at leaf flush (at our study area early May). The larval period of autumnal moth lasts approximately one month in southern Finland and consists of five distinct instars (Tenow, 1972).

Birch leaves are a food resource for larvae of autumnal moths and many sawfly species. Phytophagous insects obtain carotenoids as well as heavy metals from the leaves they feed upon. Birches are also good objects for studying pollution effects since they tolerate pollution exposure relatively well and readily colonize polluted areas (Denny and Wilkins, 1987; Eltrop et al., 1991). We measured carotenoid profiles along the pollution gradient in two closely related birch species, downy birch (*Betula pubescens*) and silver birch (*Betula pendula*). In our study area these two common birch species form mixed stands with Scotch pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*).

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