

Phyton (Horn, Austria) Special issue: "Achenkirch II"	Vol. 36	Fasc. 4	[155]-[162]	1. 7. 1996
-------------------------------------------------------------	---------	---------	-------------	------------

Survey of Heavy Metal Deposition at the Schulterberg (Achenkirch Altitude Profiles) by Using Basidiomycetes as Bioindicators

By

U. PEINTNER¹⁾ & M. MOSER¹⁾

Key words: Basidiomycetes, heavy metals, lead, zinc, cadmium, copper, biomonitoring.

S u m m a r y

PEINTNER U. & MOSER M. 1996. Survey of heavy metal deposition at the Schulterberg (Achenkirch Altitude Profiles) by using basidiomycetes as bioindicators.- *Phyton* (Horn, Austria) 36 (4): 155 - 162.

In the course of an interdisciplinary study on the Schulterberg in Achenkirch (Tyrol, Austria), fleshy fruitbodies of 33 basidiomycetes species were collected, and their heavy metal concentrations (Cd, Cu, Pb, Zn) measured. The measured lead concentrations (range: 0.1-232 µg/g dry weight, median: 10 µg/g dw) were much higher than those reported from other investigations. The basidiomatal stipes showed the highest absorption of lead, i.e. a 2 to 8-fold increase of lead compared to the extractable soil content. The cadmium values varied between 0.1 and 85 µg/g dw (median 4.8 µg/g dw) and were remarkably high for alpine areas. Moreover this element was accumulated by the basidiomatal caps by the factor 10-35. Zinc levels (2-385 µg/g dw) seemed to be normal, this element was also enriched in the caps by factors between 15 and 20. Compared to soil copper is accumulated in the caps to a very high degree (700 fold). It is, however - compared to other results - still a relatively very low amount (1-121 µg/g dw).

Furthermore, different taxa were examined for their use as bioindicators. The results suggest that some species of the genus *Cortinarius* could be suitable for the bioindication of cadmium, zinc and copper. The specific accumulation patterns of the different taxa are still unclear. More detailed information about the constancy of their accumulation behaviour is required.

I n t r o d u c t i o n

Basidiomycetes are known to accumulate heavy metals in their mycelia and basidiomata (= fruit bodies) (TYLER 1982, DIETL 1987, SCHMITT 1987, GAST &

¹⁾ Institute of Microbiology; Leopold Franzens Universität Innsbruck, Technikerstr. 25, A-6020 Innsbruck, Austria.

al. 1988, ZABOVSKI & al. 1990). This property can be used for bioindication. The aim of this study was to survey the heavy metal situation in an Alpine valley and to find fungal taxa suitable for bioindication of heavy metal depositions especially in limestone areas.

Materials and Methods

Sampling of Basidiomycetes

Fungi were collected from September 1991 to October 1993 on the Schulterberg north slope from 840 m to 1685 m a.s.l. At the same locations soil samples were taken by MUTSCH 1995. For further descriptions of the sample sites see ENGLISCH 1992, MUTSCH 1994, ENGLISCH & STARLINGER 1995, MARGL 1995, PEINTNER & MOSER 1995a.

Fungal species functioning as bioindicators must be frequent, and form fleshy basidiomata, as at least 0,5 g dry weight (=dw) material is required for heavy metal analysis. Basing on the results of the mycological study performed in this area (PEINTNER & MOSER 1996) the heavy metal content of 33 species which fulfilled these requirements for potential bioindicators were determined by measuring 245 collections. Collections (at least 2 basidiomata) were made of fungi of the same species fruiting on an area of 1 m² maximum. Visible soil particels were cleaned off in the field. The samples were dried at 40°C.

Preparation of the samples

The collections were pulverized by crushing them between two sheets of clean paper. Then they were dried at 105°C again. Samples of 1 g dry weight were added to 5 ml of perchloric acid. The decomposition was effected by using a heating block at 185°C. The solutions were diluted to a volume of 50 ml.

Chemical analysis

The concentrations of Cd, Cu, Pb and Zn were determined by an atomic absorption spectrometer with a graphite furnace (Perkin Elmer HGA-400 2380) and/or a polarographic analyser (Metrohm 646 VA Processor). A comparison of the two methods showed corresponding results.

Results and Discussion

The accumulation or exclusion of heavy metals in basidiomata depends on two factors: the fungal species and the chemical element (Table 1).

Cadmium

The cadmium values of the 33 basidiomycetes varied between 0.1 and 85 µg/g dw with a median of 4.8 µg/g dw. Compared with the (for plants available) Cd concentrations of the soil (MUTSCH 1995) all 33 fungal taxa accumulated cadmium. This element was accumulated in the basidiomatal caps with a factor 10-35, that is twice as much as in the stipes. Species with the highest concentrations were *Tricholoma saponaceum*, *Cortinarius anomalus* and *C. infractus*.

Copper

The copper content varied from 1-121 $\mu\text{g/g dw}$ with a median of 29 $\mu\text{g/g dw}$. This element was accumulated to a very high degree (700-fold) noting that the concentrations in the fruit body caps had twice the concentration of the stipes. The highest concentrations of copper were measured in *Lycoperdon foetidum*, *Lactarius lignyotus* and *Cortinarius anomalus*.

Lead

Lead was the only element that showed higher values in the basidiomatal stipes than in the caps, where it was enriched by the factor two to 8. The measured lead content ranged from 0.1-232 $\mu\text{g/g dw}$ (median: 10 $\mu\text{g/g dw}$), the highest values were found in *Cortinarius venetus*, *C. odorifer* and *Tricholoma vaccinum*.

Zinc

The zinc concentrations of the 33 fungal species varied between 2-385 $\mu\text{g/g dw}$ (median 141 $\mu\text{g/g dw}$) in the caps; in this part of the fruitbodies the values were twice as high as in the stipes. The accumulation factor was 15-20-fold in the caps. *Lactarius lignyotus*, *Cortinarius anomalus* und *Tricholoma saponaceum* had the highest zinc values.

Table 1. Heavy metal content (mean value and median) of 33 basidiomycetes collected on the Schulterberg (Achenkirch, Austria). The Cd, Cu, Pb, Zn values are given for the basidiomatal cap and stipe, or for the whole fruit body (= fb). n = number of measured collections.

	(μg/g dry weight)	n	Cd		Cu		Pb		Zn		
			median	mean	median	mean	median	mean	median	mean	
1	<i>Armillaria borealis</i>	cap	1	10.0	10.0	15.0	15.0	7.8	7.8	89.0	89.0
	<i>A. borealis</i>	stipe	1			7.0	7.0			51.0	51.0
2	<i>Amanita muscaria</i>	cap	3	23.5	23.5	38.0	38.0	8.5	8.5	216.3	216.3
	<i>A. muscaria</i>	stipe	4	7.8	6.9	9.4	10.7	4.0	5.9	97.3	117.2
3	<i>Collybia butyracea</i>	cap	2			77.5	77.5	10.5	10.5	170.5	170.5
	<i>C. butyracea</i>	stipe	2			87.5	87.5	26.0	26.0	182.0	182.0
4	<i>Cystoderma amianthinum</i>	cap	1	10.2	10.2	29.0	29.0	4.0	4.0	38.5	38.5
	<i>C. amianthinum</i>	stipe	1	2.0	2.0	12.0	12.0	8.0	8.0	16.5	16.5
5	<i>C. carcharias</i>	cap	1	25.1	25.1	30.6	30.6	6.2	6.2	140.0	140.0
	<i>C. carcharias</i>	stipe	1	7.6	7.6	16.9	16.9	6.6	6.6	34.2	34.2
6	<i>Cortinarius anomalus</i>	cap	3	44.4	36.2	101.6	82.2	3.8	3.5	150.7	151.7
	<i>C. anomalus</i>	stipe	2	10.9	10.9	42.8	42.8	18.0	18.0	96.1	96.1
7	<i>C. infractus</i>	cap	17	26.7	31.1	33.9	41.7	4.4	4.7	171.9	181.2
	<i>C. infractus</i>	stipe	14	12.6	13.9	22.8	22.4	16.3	17.9	98.1	87.0
8	<i>C. odorifer</i>	cap	19	13.0	14.0	18.4	19.9	8.9	10.8	83.2	81.2
	<i>C. odorifer</i>	stipe	19	3.4	6.2	11.1	13.0	21.0	27.8	57.8	63.9
9	<i>C. sulphureus</i>	cap	8	11.5	14.2	20.2	33.4	5.4	7.9	161.5	153.3
	<i>C. sulphureus</i>	stipe	8	2.5	3.5	15.9	22.3	6.0	7.1	75.5	82.7
10	<i>C. varicolor</i>	cap	5	18.8	17.3	70.8	76.5	14.5	33.7	146.3	140.7
	<i>C. varicolor</i>	stipe	5	6.3	5.9	50.2	47.6	3.5	20.0	78.5	85.0

(µg/g dry weight)		n	Cd		Cu		Pb		Zn		
			median	mean	median	mean	median	mean	median	mean	
11	<i>C. varius</i>	cap	1	7.3	7.3	59.0	59.0	10.0	10.0	95.5	95.5
	<i>C. varius</i>	stipe	1	3.6	3.6	17.0	17.0	11.9	11.9	69.0	69.0
12	<i>C. venetus</i>	cap	8	7.1	8.3	30.2	30.8	15.3	14.0	141.8	146.8
	<i>C. venetus</i>	stipe	9	2.5	2.4	14.0	18.9	32.0	33.5	84.0	77.4
13	<i>Hydnum repandum</i>	fb	3	2.1	2.1	24.4	26.1	6.3	6.3	53.0	55.3
14	<i>Hygrophorus chrysodon</i>	cap	1	6.0	6.0	24.0	24.0	11.0	11.0	133.0	133.0
	<i>H. chrysodon</i>	stipe	1	3.0	3.0	28.0	28.0	34.0	34.0	95.0	95.0
15	<i>H. eburneus</i>	cap	2	7.0	7.0	50.0	50.0	5.2	5.2	272.5	272.5
	<i>H. eburneus</i>	stipe	2	1.6	1.6	30.5	30.5	16.3	16.3	204.0	204.0
16	<i>H. lucorum</i>	fb	1			12.0	12.0	0.6	0.6	150.0	150.0
17	<i>Inocybe fraudans</i>	fb	1	1.6	1.6	80.8	80.8	18.9	18.9	165.0	165.0
18	<i>Lactarius acris</i>	cap	1			13.0	13.0	1.2	1.2	36.0	36.0
19	<i>L. blennius</i>	cap	1			19.0	19.0	1.1	1.1	90.0	90.0
	<i>L. blennius</i>	stipe	1			10.0	10.0	1.4	1.4	45.0	45.0
20	<i>L. deterrimus</i>	cap	12	4.6	5.6	12.0	20.4	2.7	5.0	150.9	163.5
	<i>L. deterrimus</i>	stipe	12	1.3	1.8	11.0	12.6	13.0	36.5	96.4	93.7
21	<i>L. lignyotus</i>	stipe	1	4.1	4.1	121.0	121.0	2.4	2.4	56.5	56.5
	<i>L. lignyotus</i>			3.2	3.2	41.0	41.0	5.0	5.0		
22	<i>L. picinus</i>	cap	4	1.2	1.9	16.0	12.5	13.0	15.0	49.8	59.3
	<i>L. picinus</i>	stipe	4	0.2	1.0	14.5	13.0	6.0	5.7	47.0	55.3
23	<i>L. porninsis</i>	cap	1			8.0	8.0	1.2	1.2	225.0	225.0
	<i>L. porninsis</i>	stipe	1			7.0	7.0	8.0	8.0	92.0	92.0
24	<i>L. salmonicolor</i>	cap	3	6.0	7.3	10.0	10.3	15.0	15.3	101.0	100.7
	<i>L. salmonicolor</i>	stipe	3	5.0	4.5	9.0	9.7	30.0	34.0	93.0	86.3
25	<i>L. scrobiculatus</i>	cap	8	2.0	3.2	16.0	15.0	7.3	13.1	55.0	76.6
	<i>L. scrobiculatus</i>	stipe	7	0.8	1.0	13.0	12.6	12.0	15.8	53.5	50.1
26	<i>Lepista muda</i>	fb	1	11.9	11.9	110.0	110.0	2.2	2.2	63.5	63.5
27	<i>Mycena pura</i>	cap	1	3.4	3.4	62.0	62.0	1.8	1.8	77.0	77.0
	<i>M. pura</i>	stipe	1			2.0	2.0	1.3	1.3	2.0	2.0
28	<i>Russula queletii</i>	cap	1			12.0	12.0	1.5	1.5	145.0	145.0
	<i>R. queletii</i>	stipe	1			12.0	12.0	11.0	11.0	87.0	87.0
29	<i>Sarcodon imbricatus</i>	cap	4	4.1	3.3	34.9	29.9	7.1	7.6	171.4	185.0
	<i>S. imbricatus</i>	stipe	4	0.9	0.8	30.8	28.6	9.5	12.8	221.9	234.6
30	<i>Tricholoma vaccinum</i>	cap	9	2.1	2.9	22.2	21.8	2.6	3.9	127.9	129.6
	<i>T. vaccinum</i>	stipe	10	0.6	0.7	7.0	8.1	19.1	15.9	64.7	69.1
31	<i>T. saponaceum</i>	cap	1	85.5	85.5	85.0	85.0	1.3	1.3	225.0	225.0
	<i>T. saponaceum</i>	stipe	1	20.9	20.9	32.0	32.0	1.7	1.7		
32	<i>Lycoperdon foetidum</i>	fb	2	3.0	3.0	158.2	158.2	13.0	13.0	175.0	175.0
33	<i>L. pyriforme</i>	fb	2			88.3	88.3	11.8	11.8	112.6	112.6

Fungi as food

Most people appreciate fungi as part of their food. Neither in Austria, nor in Germany legal limits concerning maximal heavy metal concentrations of "wild mushrooms" exist. According to the Austrian legal papers "Erlässe des Bundesministeriums für Gesundheit, Sport und Konsumentenschutz" the recommended values for commercially produced mushrooms are 1 µg/g dw lead and 0.5 µg/g dw cadmium. These recommended values can be exceeded to 100 %.

Only 5 of the 33 investigated fungal species showed concentrations below this tolerated values. However most of the basidiomycetes contained much higher amounts of lead or cadmium. Therefore we would like to draw attention to the fact that excessive consumption of "wild mushrooms" can be hazardous to health.

Comparing the results with heavy metal measurements in other European areas

Measuring the heavy metal content of basidiomata can be a suitable method of surveying the impact of pollution, on condition that at least 8-10 different fungal species are investigated with enough samples (GAST & al. 1988). Comparison of the Achenkirch data with data available about other European areas showed: the lead concentrations measured in the Schulterberg area were very high compared to values from Italy (BORELLA & al. 1991, BERTANI & al. 1990), Sweden (TYLER 1980, 1982), Switzerland (IRLET & RIEDER 1985), Poland (GRZYBEK 1991-92), Belgium (GAST & al. 1988) Tschechoslovakia (TURNAU 1991), and to international standard values (SCHMITT 1987). The cadmium values were high too, especially for alpine environments: only the cadmium content of fungi from Belgium and The Netherlands were slightly higher. The zinc values in the Achenkirch area can be regarded as normal, while the copper values are the lowest values known so far in Europe.

Heavy metals in fungi compared with other measurements of heavy metals in the Achenkirch area

As part of the "Studies of Ecosystems in the Limestone Alps" other investigations on heavy metal pollution in the Achenkirch area have been carried out (SMIDT & al. 1994).

The measurements in fungi indicate an evident pollution with lead and a comparably high amount of cadmium. Other investigations carried out in the Achenkirch area showed corresponding results:

MUTSCH 1995 studied the same sample sites where our mushrooms were collected measuring the heavy metal content of the soil. The high amount of mobile lead in the soil indicated inputs from atmospheric far-distance transport. No significant altitudinal gradient could be found.

HERMAN 1995 examined the concentrations of Pb and Cd in spruce needles. Their values of lead did not indicate a pollution. Also the cadmium concentrations were predominantly low, only in the needles of the uppermost sample areas in Achenkirch a beginning impact could be assumed.

The concentrations of lead and cadmium in the bark of spruce (HERMAN 1992) varied greatly and showed signs of pollution, particularly in the uppermost sample plots of the Christlum Altitude Profile.

The concentrations of heavy metals in mosses, measured by ZECHMEISTER 1995 in the area showed a marked increase in the deposited amounts of heavy

metals with increasing altitude. The mean concentrations were clearly higher than those of other Austrian sample sites.

Fungi - today widely accepted as separate regnum - usually accumulate heavy metals to a much higher degree than plants do: Basidiomycetes showed twice the zinc concentrations. 4-7 times the copper concentrations and up to 10 times the cadmium concentration found in mosses collected at the same site. On the other hand lead is accumulated in mosses 2-4 times more than in fungi. Spruce needles and bark seem not to accumulate lead and cadmium to a degree comparable to mosses or fungi.

Thus, fungi can be regarded as very sensitive accumulating bioindicators for cadmium, zinc and copper; lead is - compared to the extractable soil content - accumulated 2-8 times only. Mushrooms are suitable organisms for a survey of heavy metal deposition.

Basidiomycetes as bioindicators for heavy metals

Bioindicators for cadmium and lead should be those fungal species which accumulate both elements at a high degree. A surprising result of the present investigation was, that of the 33 studied taxa particularly mycorrhiza forming fungi of the genus *Cortinarius* showed this abilities. *Cortinarius anomalus*, *C. venetus*, *C. infractus*, *C. varicolor* and *C. odorifer* could be suitable bioindicators.

But more detailed information about the mechanisms and constancy of their accumulation behaviour is required. The present state of knowledge is, that the accumulation patterns of different heavy metals vary within different fungal species. For the following reasons little is known yet about the accumulation behaviour of basidiomycetes: heavy metals are, like nutrients, absorbed by the mycelia growing in the soil. The mechanisms by which some species accumulate certain elements but exclude others are still unclear. As the mycelium in its natural habitat usually cannot be observed, we only can measure the heavy metal concentrations of the basidiomata produced by this mycelium. Therefore it is impossible to establish the period, in which heavy metals have been accumulated by the mycelium, as we usually do not know anything about its age and extension. Maybe just exactly for this reason - namely that mycelia accumulate heavy metals over a wide area and a relatively long time - fungi have the advantage to be very sensitive accumulation indicators.

A c k k n o w l e d g e m e n t s

This project was supported by the Bundesministerium für Land und Forstwirtschaft. We would like to thank F. MUTSCH, T. PÜMPEL and B. STOJANOVIC for their assistance and collaboration in the heavy metal analysis of the samples.

References

- BERTANI P.L., BOCCHI A., BRACCHI P.G. & TASSI PELATI L. 1990. Applicazione della potenziometria di stripaggio anodico (P.S.A) alla determinazione del piombo e del cadmio in alcune specie fungine raccolte nella provincia di Parma. *Fungi atque Loci Natura.*- Atti del IV convegno Internazionale di Micologia. 27-30/IX/1987. Borgo Tarò. Italy.
- BORELLA P., QUAGLIO G.P., CASELGRANDI E. & CASSINADRI M. 1991. I funghi accumulano cadmio rame e piombo in concentrazioni significative.- *Hygiene moderna* 95: 9-25.
- DIETL G. 1987. Abhängigkeit der Schwermetallaufnahme höherer Pilze von der Substratzusammensetzung und von Standortfaktoren.- *Bibliotheca Mycologica*. Band 110.
- ENGLISCH M. 1992. Standortliche Grundlagen im Bereich der Höhenprofile Achenkirch.- *FBVA-Berichte* (Federal Forest Research Centre. ed.) 70: 13-18.
- ENGLISCH M. & STARLINGER F. 1995. Waldgesellschaften und Standorte im Bereich der Waldstandorte in Achenkirch.- *FBVA-Berichte* (Federal Forest Research Centre. ed.) 87: 25-54.
- GAST C.H., JANSEN E. & BIERLING J. 1988. Heavy metals in mushrooms and their relationship with soil characteristics.- *Chemosphere* 17: 789-799.
- GRZYBEK J. 1991-1992. Ozanaczenie zwartosci ołowiu, kamu I niklu zu pomoca spektroskopii adsorbpcij atomowej w suchych grzyzbów wielkooecnikowych rosnacych w Polsce.- *Acta Mycologica XXVII* (2): 213-220.
- HERMAN F. 1992. Nährstoffgehalte von Fichtennadeln sowie Schadstoffgehalte in Fichtennadel- und Borkenproben des Untersuchungsgebietes Achenkirch.- *FBVA-Berichte* (Federal Forest Research Centre. ed.) 70: 83-90.
- 1994. Nutrient content of spruce needles and content of pollutants in spruce needles and bark from the Achenkirch investigation area.- *Phyton* (Horn. Austria) 34 (3): 85-96.
- IRLET B. & RIEDER K. 1985. Cadmium und Blei in den Pilzen aus der alpinen Stufe der Schweizer Alpen.- *Mycol. Helv.* 1 (6): 393-399.
- KREHAN H. & Tomiczek C. 1992. Forstliche Beschreibung der Probeflächen und der Kronenzustände der Probestämme an den Höhenprofilen Achenkirch.- *FBVA-Berichte* (Federal Forest Research Centre. ed.) 70: 19-24.
- LEPSOVA A. 1988. Vyznam studia plodnic macromycetu pro biomonitorovani zmen v lesnim ekosystemu. 152 p. (cand. diss.; depon. in Dept. of Botany. Charles University Prague)
- MARGL H. 1994. Klimadaten aus dem Raum Achenkirch.- *FBVA-Berichte* (Federal Forest Research Centre. ed.) 78: 17-25.
- MUTSCH 1995. Einstufung der Böden im Raum Achenkirch nach chemischen Parametern.- *FBVA-Berichte* (Federal Forest Research Centre. ed.) 87: 55-69
- PEINTNER U. & MOSER M. 1995 a. Artenvielfalt und Abundanz von Basidiomyceten im Projektgebiet Achenkirch.- *FBVA-Berichte* (Federal Forest Research Centre. ed.) 87: 69-95.
- PEINTNER U. & MOSER M. 1995 b. Schwermetallgehalte von Basidiomyceten und deren Eignung als Bioindikatoren.- *FBVA-Berichte* (Federal Forest Research Centre. ed.) 87: 161-169.
- PEINTNER U. & MOSER M. 1996. The Mycobiota of an Alpine Valley.- *Phyton* (Horn. Austria) 36 (4): 65-82.
- RANGGER und INSAM 1995. Mikrobielle Aktivitäten und Biomasse entlang eines Höhenprofils in den Nordtiroler Kalkalpen.- *FBVA-Berichte* (Federal Forest Research Centre. ed.) 87: 95-104.
- SCHMITT J.A. 1987. Fungi and heavy metals. Centro studi per la flora mediterranea Borgo Val di Tarò.- Atti del IV Convegno internazionale di Micologia. 27-30/IX/1987.

- SMIDT S., HERMAN F., GRILL D. & GUTTENBERGER H. (eds.) 1994. *Phyton* (Horn, Austria) 34.
- TURNAU K. & KOZLOWSKA H. 1991. The influence of industrial dust on the heavy metal content of fungi. *Zesz nauk uniw Jagiellonsk Pr. Bot.*: 135-144. Inst. Botany, Jagiellonian Univ. Dep. Plant Taxonomy Phytogeography, ul Lubicz 46. 31-512 Krakow. Pol.
- TYLER G. 1980. Metals in sporophores of basidiomycetes.- *Trans Br. Mycol. Soc.* 74: 41-49.
- TYLER G. 1982. Accumulation and exclusion of metals in *Collybia peronata* and *Amanita rubescens*.- *Trans. Br. Mycol. Soc.* 79: 239-245.
- ZABOVSKI D., ZASOSKI R. J., LITKE W. & AMMIRATI J. 1990. Metal content of fungal sporocarps from urban, rural, and sludge-treated sites.- *J. Environ. Qual.* 19.
- ZECHMEISTER H. 1993. Die Erfassung der atmosphärischen Schwermetalldeposition mittels Bryophyten entlang des Schulterberg-Profiles.- *FBVA-Berichte* (Federal Forest Research Centre, ed.) 78: 41-47.
- ZECHMEISTER H. 1995. Survey of heavy metal deposition by using Bryophytes as biomonitors at the Schulterberg profile.- *Phyton* (Horn, Austria) 34 (3): 73-70.

ZOBODAT - www.zobodat.at

Zoologisch-Botanische Datenbank/Zoological-Botanical Database

Digitale Literatur/Digital Literature

Zeitschrift/Journal: [Phyton, Annales Rei Botanicae, Horn](#)

Jahr/Year: 1996

Band/Volume: [36_4](#)

Autor(en)/Author(s): Peintner Ursula, Moser M.

Artikel/Article: [Survey of Heavy Metal Deposition at the Schulterberg \(Achenkirch Altitude Profiles\) by Using Basidiomycetes as Bioindicators. 155-162](#)