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The effect of calcium and magnesium on the radiosensitivity of *Wolffia arrhiza* (L.) Wimm.

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Abstract

After irradiation with gamma radiation from ^{60}Co, differences were found in the survival rates of *Wolffia arrhiza* (L.) Wimm., cultured *in vitro* with different amounts of calcium and magnesium. Radiation doses ranged from 80 to 3000 $mC \cdot kg^{-1}$. Magnesium and especially calcium increased the resistance of *Wolffia* to acute radiation. This was expressed by the survival of the plants cultured in mediums supplemented with calcium and magnesium after receiving doses of 1200 and 3000 $mC \cdot kg^{-1}$ while plants cultured in basic medium already showed absolute mortality (DL 100) at exposition to 800 $mC \cdot kg^{-1}$. Radiosensitivity of *Wolffia arrhiza* is high compared with other species from the *Lemnaceae* family.

Key words: irradiation, radiosensitivity, radioresistance, survival rate populations

INTRODUCTION

The radiobiological and radioecological studies done until now on plants from the *Lemnaceae* family have not included species from the genus *Wolffia*, among which is the rootless *Wolffia*, *Wolffia arrhiza* (L.) Wimm. found in Poland. It is possible that the reason for this is the relative scarcity of this plant.

Wolffia arrhiza distincly differs from other plants of the *Lemnaceae* family in its taxonomic features, among which is its number of chromosomes (Daubs 1965). It could be expected that it would show a species-specific radiosensitivity to ionizing radiation. The aim of this study was to determine the tolerance of *Wolffia arrhiza* to gamma radiation from ^{60}Co under experimental conditions. The question was studied if calcium and magnesium in the environment could have an effect, and in what degree, on the resistance of rootless *Wolffia* to acute radiation on the individual as well as the populational level. In this study the experimental *Wolffia* population came from clones from a natural *Wolffia* population found in a pond near Namysłów in Lower Silesia. The experimental population index necessitated the characterization of the natural population and its habitat conditions.

METHODS

CHEMICAL ANALYSIS OF WATER

The pH of the water from the pond in Namysłów, where *Wolffia arrhiza* grows, was determined potentiometrically. Its phosphorus, calcium, magnesium and chlorine content was assayed using methods described by Gomółka and Szypowski (1975). Potassium and sodium were assayed with a flame photometer according to the Johnson-Ulrich methods, total nitrogen by the Kjeldahl method and sulphates nephelometrically according to Just and Hermanowicz (1964). The analyses were done three times on samples taken in July, 1982.

BIOMETRICAL ANALYSIS

In the biometrical analysis of plants from the natural population, the following features were taken into account: area of the maternal frond, in mm^2; length and width of the maternal frond, in mm; top angle, in degrees; the shape index of the fronds, that is, the ratio of the lenght to the width of the maternal frond. Samples of plants were taken randomly from five places in the pond. The areas of 50 randomly chosen plants from each of the five samples were measured using a photographic-planimetric method (Czopek 1959). The measurements of the remaining plants were taken from photographs, with the scale having been strictly kept. The same kind of biometric analysis was done for the experimental *Wolffia arrhiza* population.

EXPERIMENTS

In radioecological studies, radiosensitivity of plants is determined by assesing the proper development reactions of the plants to radiation and their survival rate after exposition to a specified dose of it (Sparrow 1962).

Plants from the natural *Wolffia arrhiza* population were sterilized and cultured *in vitro* under constant lighting and temperature (26°C, 5000 lx) in the way described by Wożakowska-Natkaniec (1977a,b). The descendants of these plants were used in *in vitro* cultures for the experiments with radiation.

The experiment with different doses of radiation was to determine the survival rate of the plants and to establish the values of the lethal doses (DL 50 and DL 100), which characterize the radiosensitivity of the studied species. The vegetative multiplication rate (v) was also calculated using Landolt 's equation (1957):

$$v = 0.4343 \lg \frac{N_t}{N_0} \cdot \frac{1}{t} \cdot 1000$$

Where: N_o — initial number of fronds, N_t — final number of fronds after a given time, t.

The plants were irradiated with the following doses: 80, 250, 500, 800, 1200 and 3000 $mC \cdot kg^{-1}$. The variants of the experiment were plants given different doses of radiation, cultured on the basic medium — A (modified Hoagland's medium, its composition is qiven by Wożakowska-Natkaniec 1977b), on the basic medium with a doubled content of magnesium salts, that is, 0.986 $g \cdot dm^{-3}$ $MgSO_4 \cdot 7H_2O$ — called medium B. The third variant was constituted by plants cultured in the basic medium with a double content of calcium (0.504 $g \cdot dm^{-3}$ $Ca(H_2PO_4)_2 \cdot H_2O$ and 3.306 $mg \cdot dm^{-3}$ $Ca(NO_3)_2 \cdot 4H_2O$). The experiments were carried out in 5 repetitions. Each repetition consisted of 10 *Wolffia arrhiza* plants in an Erlenmeyer flask. During the time of the experiments (14 days) the fronds were counted and the mortality rate determined. Observations of the plants were also made.

STATISTICAL AND MATHEMATICAL ANALYSIS

In working up the empirical data, variance analysis with two and three variables was used. The significance of differentating the population traits anv erimental results was determined using the F-Snedecor test, with the probability of 0.05 (Scheffe 1959).

RESULTS

CHARACTERISTICS OF THE HABITAT OF THE NATURAL *WOLFFIA ARRHIZA* POPULATION

The ponad near Namysłów, where the *Wolffia arrhiza* comes from, is a natural one. It had once been a part of the Widawa River bed. *Wolffia arrhiza* is part of the association belonging to the class *Lemneta* W. Koch et Tx. 1954, order *Lemnetalia,* from the union *Lemnion minoris* (Scamoni 1967). Aside from *Wolffia,* also found here are plants from the ecological

Table 1

Characteristics of habitats of *Wolffia arrhiza* populations

pH	Contents in $mg \cdot dm^{-3}$							
	N	PO_4^{-3}	K	Na	Ca	Mg	SO_4^{-2}	Cl^-
7.6	3.25	0.14	11.5	16	194.8	17.5	40.8	50

Table 2

Characteristics of natural and experimental populations of *Wolffia arrhiza*

Type of population		Medium	Area of fronds, mm^2	Frond, mm		Top angle of frond, grade	Shape index
				length	width		
Natural			1.78	1.45	1.20	126	1.20
Experimental		A	1.77	1.45	1.20	126	1.20
		B	1.74	1.39	1.13	124.9	1.23
		C	1.80	1.46	1.22	132.2	1.20
Irradiated	$80\ mC \cdot kg^{-1}$	A	1.82	1.35	1.21	141.7	1.12
		B	1.84	1.38	1.26	143.2	1.10
		C	1.86	1.42	1.34	149.8	1.06
	$800\ mC \cdot kg^{-1}$	A	1.32	1.10	0.92	120	1.20
		B	1.46	1.18	0.98	122.4	1.20
		C	1.54	1.20	1.00	128	1.20
LSD*			0.02	0.038	0.018	1.6	0.03
F-Snedecor test			87.7	34.83	54.26	45.2	11.0

* LSD — least significant difference.

limno-pleustonic group, such as *Lemna minor, Lemna trisulca, Spirodela polyrrhiza, Riccocarpus natans* as well as plants rooted at the bottom of the pond from the order *Potametalia*. This is an eutrophic habitat. The chemical characteristics of the water in the pond near Namysłów are presented in Table 1. The concentration values for each of the elements in the water from that pond are characteristic for surface waters of a slight degree of eutrophization (Gomółka and Szypowski 1975). The water in the pond is clear and greater amounts of biogenic substances are not observed during the summer or autumn.

CHARACTERISTICS OF THE NATURAL *WOLFFIA ARRHIZA* POPULATION

A massive incidence of *Wolffia arrhiza* near the adge of the pond was seen at the begining of June. If filled the surface of the water not covered by duckweed (*Lemna* sp. sp.) or *Spirodela polyrrhiza*. At that time the density of the *Wolffia arrhiza* population was 213-260 plants on a surface of 10 cm^2.

Fully grown plants from the natural population of *Wolffia arrhiza* are characterized by the following average values: 1.78 mm^2-area of fronds; 1.45 mm in lenght and 1.2 mm in width (Table 2). It was found that the average values of the lenght and width of the maternal *Wolffia arrhiza* fronds from Namysłów are greater than the average values for these traits given by Daubs (1965) in the taxonomic characteristics of this species.

CHARACTERISTICS OF THE EXPERIMENTAL *WOLFFIA* POPULATION

The characteristics of the fully-grown *Wolffia arrhiza* plants from the experimental populations cultivated in different mediums (A, B, C) are significantly differentiated, which is shown by the variance analysis of the measurements of the studied features (Table 2). The experimental population cultivated on the basic medium (A) does not differ from the natural population from Namysłów in the area, lenght and width of the fronds, the shape index and top angle of the frond. The experimental *Wolffia* populations cultivated on mediums modified by increasing the magnesium salt (B) and calcium salt (C) content differ from the natural population in the dimensions of the analysed traits. Plants cultivated in the medium with increased magnesium are characterized by smaller dimensions and ellipsoidal fronds. Whereas *Wolffia* cultivated on the medium enriched with calcium are the largest and have orbicular fronds.

RADIOSENSITIVITY OF *WOLFFIA ARRHIZA*

In the experiments, exposition to gamma radiation at a dose of 80 $mC \cdot kg^{-1}$ did not cause mortality of the *Wolffia* in any of the mediums (A, B, C) but had a signifficant effect on its developmental features that is, on the size and shape of the plants (Table 2). Plants which received this dose developed a greater area, width and top angle of the fronds, that is, were almost round in shape (suborbicular).

Total mortality was found in two experimental variants (on medium A and B) at a dose of 800 $mC \cdot kg^{-1}$ (DL 100, Table 3). However, the complete dying out of the plants took place at different times from exposition, depending on the medium. Plants cultured on the basic medium (A) died earlier — after 14 days, those on the medium enriched with magnesium (B), after 21 days. Plants cultured on the medium enriched with calcium (C) died after 28 days and only after having received a dose of 1200 $mC \cdot kg^{-1}$ (DL 100, Table 3).

Tabela 3

The effect of gamma irradiation (^{60}Co) on the population of *Wolffia arrhiza* depending on the medium and time of cultivation

Time of cultivation, days	Medium	Dose, $mC \cdot kg^{-1}$								LSD*	$F_{emp.}$
		0	80	250	500	800	1200	3000			
5	A	2.50	2.29	2.00	1.60	1.20	1.02	1.00	1.51	0.078	576.4
	B	2.52	2.30	2.09	1.90	1.20	1.12	1.47	1.74	0.44	18.9
	C	2.82	2.51	2.40	2.00	1.51	1.26	1.07	1.93	0.064	214.3
7	A	3.98	3.46	2.88	2.00	1.20	1.05	0	1.80	0.19	25.6
	B	4.36	3.62	3.18	2.50	1.26	1.18	1.07	2.44	0.24	275.9
	C	4.50	3.98	3.48	2.70	1.91	1.31	1.08	2.71	0.17	456.3
9	A	6.00	4.78	3.98	2.75	1.26	1.07	0	2.84	0.45	45.9
	B	6.68	5.76	4.78	3.80	1.52	1.18	1.10	3.54	0.62	15.9
	C	7.50	6.30	5.24	3.85	2.40	1.41	1.15	3.98	0.81	132.1
11	A	9.10	7.10	5.90	3.56	1.00	0	0	3.81	0.21	280.2
	B	9.90	9.10	7.00	4.20	1.66	1.21	1.13	4.87	0.02	1024.2
	C	12.00	10.80	8.9	7.00	5.00	1.41	1.17	6.68	0.74	124.2
14	A	16.80	13.10	10.20	5.10	1.00	0	0	6.50	0.11	1230.6
	B	19.90	18.20	14.10	6.60	1.52	1.00	0	8.70	0.14	1424.2
	C	24.00	22.40	19.70	12.90	9.80	1.52	1.05	13.05	0.61	219.2
$\bar{x}$		8.92	7.70	6.83	4.18	2.21	1.046	0.72			$F_{tab} = 2.6$

		LSD*	F-Snedecor test	
			$F_{emp.}$	$F_{tab.}$
Variabilities	dose	0.14	4120.9	2.14
	medium	0.159	1197.4	3.04
	time	0.118	6142.8	2.41
Interactions	dose x time	0.313	687.1	1.57
	dose x medium	0.243	50.1	1.80
	time x medium	0.0215	298.0	1.98
	dose x medium x time	0.543	23.65	1.42

* LSD — least significant difference.

Mortality of the plants was seen the earliest among those cultured on the basic medium (A), from the fifth day after irradiation (Table 3). On the seventh day after irradiation approximately 50% mortality of the plants cultured in the medium enriched with magnesium (B) was found. Plants cultured on the calcium-enriched medium (C) began to die the latest — on the 11th day after irradiation. These figures are for the lower doses of radiation used in the experiments. The mortality of *Wolffia* in time increased with increasing doses of radiation. Plants on the basic medium (A) began to die on the second day after irradiation with doses of 1200 and 3000 $mC \cdot kg^{-1}$, on the magnesium-enriched medium, on the third day. Those on the calcium-enriched medium (C) began to die the latest and a small per cent of them lived to 14 days after receiving the highest dose used in the experiment, 3000 $mC \cdot kg^{-1}$.

Strating from 500 $mC \cdot kg^{-1}$, which is a lethal dose (DL 50), and with all of the remaining higher doses used in these experiments, developmental anomalies were observed. These anomalies intensified with increasing doses of radiation. *Wolffia arrhiza* plants showed the following symptoms of stroke after irradiation: conglomeration of fronds, point chlorosis which deepended with time, inhibition of growth and development of fronds, change of shape of fronds, sporadically hypertrophy.

DYNAMICS OF THE POST-IRRADIATION POPULATIONS OF *WOLFFIA ARRHIZA*

The dynamics of a population is expressed by the change in the number of plants in time and by the multiplication rate. *Wolffia arrhiza* displays a different sensitivity to increasing doses of ^{60}Co radiation when different

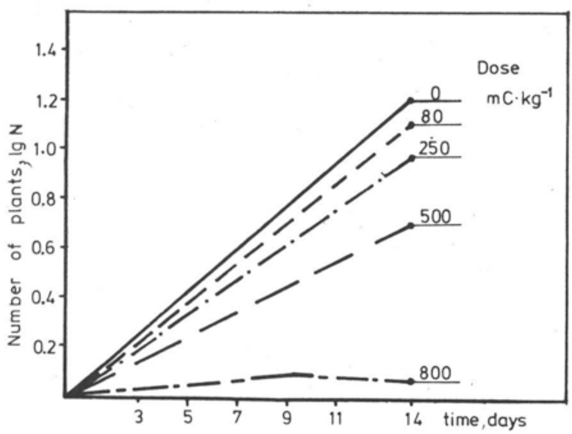

Fig. 1. The effect of gamma irradiation (^{60}Co) on the number of fronds and multiplication rate of *Wolffia arrhiza* populations cultivated on basic medium A

amounts of calcium and magnesium are used in the medium (Table 3). All of the doses of radiation lessened the increases in the number of plants by limiting their vegetative reproduction (Table 4). The dynamics

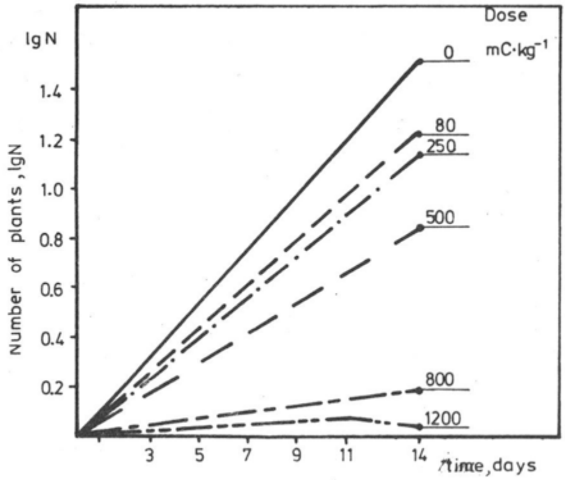

Fig. 2. The effect of gamma irradiation (^{60}Co) on the number of fronds and multiplication rate of *Wolffia arrhiza* populations cultivated on medium B

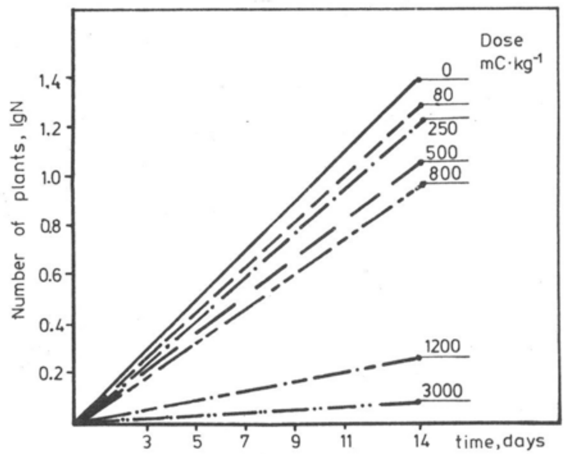

Fig. 3. The effect of gamma irradiation (^{60}Co) on the number of fronds and multiplication rate of *Wolffia arrhiza* populations cultivated on medium C

of the post-irradiation populations of *Wolffia* are shown on graphs (Figs. 1, 2, 3). After 14 days of cultivation on the basic medium, a dose of 80 mC·kg^{-1} causes a 20% fall in the number of plants compared with the control; higher doses cause a further fall to 50% at a dose of 500 mC··kg^{-1}, and the highest doses used — a 100% fall. The population count and multiplication rate of *Wolffia arrhiza* is higher on the magnesium-enriched medium (B) compared with the populations cultivated on the basic medium (A). This is so in respect to both control and irradiated populations

Table 4

Multiplication rates (V) of *Wolffia arrhiza* populations in different cultivation mediums after gamma irradiation (^{60}Co)

Time of cultivation, days	Medium	Dose, mC·kg^{-1}							$\bar{x}$
		0	80	250	500	800	1200	3000	
5	A	34.7	31.3	26.1	18.2	6.9	0.8	0	16.90
	B	34.9	31.4	27.8	24.2	6.9	4.3	1.7	18.73
	C	39.1	34.7	33.0	26.1	15.6	8.7	2.6	22.83
7	A	37.2	33.5	28.5	18.6	5.0	1.1	0	16.28
	B	39.7	34.7	31.0	24.8	6.2	4.3	1.8	20.38
	C	40.3	37.2	33.5	26.7	17.4	7.4	2.1	23.50
9	A	37.6	32.8	28.9	21.2	4.8	1.4	0	18.10
	B	39.8	36.7	32.8	24.1	8.7	3.4	1.99	21.05
	C	42.0	38.6	34.7	28.0	18.3	7.2	2.93	24.40
11	A	37.9	33.6	30.4	21.8	0	0	0	17.67
	B	39.3	37.9	33.4	24.6	8.7	3.2	2.09	21.31
	C	42.6	40.8	37.5	33.4	27.6	5.9	2.69	27.21
14	A	38.0	34.7	31.3	21.7	0	0	0	17.98
	B	40.3	39.1	35.7	25.4	5.6	0	0	20.87
	C	42.8	41.9	40.2	34.5	30.7	5.6	0.66	28.05

cultured on medium B. In this case the post-irradiation survival time of the plants is longer. *Wolffia arrhiza* achieves the highest population count compared with populations cultivated on mediums A and B, both for the control and irradiated plants, on the calcium-enriched medium (C). After a radiation dose of 800 mC·kg^{-1}, 40% of the population in medium C survives, whereas this dose is lethal (DL 100) for the remaining populations (A and B). This post-irradiation survival is the result of the high multiplication rate of this population — $v = 30.7$ after 14 days of culture (Table 4, Fig. 3).

DISCUSSION

The natural population of *Wolffia arrhiza* from Namysłów is characterized by larger dimensions than those given by Daubs (1965) as the upper limit. When the plants are transferred to culture they retain their size, therefore this is not a case of ecotypic differentiation. The differences between the size of the maternal frond of the studied population and the average size for the species should be attributed to environmental variability. May be the West European *Wolffia arrhiza* population studied by Daubs

(1965) and the Lower Silesia one are separate ecotypes, similar to the ecotypic differentiation of natural populations of this species in Italy.

The radiosensitivity of *Wolffia arrhiza*, as has been shown experimentally, is very high in comparison with other species from the *Lemnaceae* family. The lethal radiation dose (DL 100) for the studied *Wolffia* population is 800 $mC \cdot kg^{-1}$; in comparison, the DL 100 for *Lemna minor* is 1806 $mC \cdot kg^{-1}$ (Kasinov 1966), for *Spirodela polyrrhiza,* 2580 $mC \cdot kg^{-1}$ (Wożakowska-Natkaniec 1977b). According to Sparrow (1962) *Wolffia's* lower resistance to hard gamma radiation for ^{60}Co (its higher radiosensitivity) should be attributed to the higher number of chromosomes in this species than in other species from the *Lemnaceae* family. Daubs (1965) gives the following data: *Lemna minor* has 42, *Spirodela polyrrhiza* — 40, *Wolffia arrhiza* — 50 chromosomes. The survival of *Wolffia arrhiza* found a short time after irradiation with doses higher than 800 $mC \cdot kg^{-1}$ (DL 100) can be explained in agreement with Grodzienski (1969) by the population effect. It can be assumed that the irradiated populations are heterogenous in respect to radiosensitivity, which is understandable taking into account the high number of plants in the population.

The developmental anomalies of *Wolffia arrhiza* caused by gamma radiation which were found in this experiment are not specific for this species. They have already been described in other species of the *Lemnaceae* family (Posner and Hillman 1960, Kasinov 1966, Wożakowska-Natkaniec 1977b). The anomalies described here are not found even sporadiacally in the natural *Wolffia arrhiza* population from Namysłów. Gamma radiation is, then, a specific factor limiting the development of this species.

The delayed mortality of the experimental *Wolffia arrhiza* population in the calcium-enriched medium indicates a role for calcium in protection against radiation. According to Sharkovskiy et al. (1970), calcium may be an important factor in the post-irradiation repair of a population. The finding of a greater multiplication rate of *Wolffia arrhiza* after irradiation with critical doses (800 and 1200 $mC \cdot kg^{-1}$, Table 4) in the calcium-enriched medium in our experiments may be compared to the ecological radiosensitivity of *Lemna minor* determined by the naturally higher level of calcium in its habitat (Wożakowska-Natkaniec 1977b). The resistance of *Lemna minor* and *Spirodela polyrrhiza* increases with the water calcium content in their habitats increasing to 93.07 $mg \cdot dm^{-3}$. A similar, but a much smaller however, effect of resistance of *Wolffia arrhiza* was found when the medium was enriched with magnesium (B). It can then be assumed that *Wolffia arrhiza* shows an ecological radiosensitivity depending on the chemical properties of the water in its habitat. This is understandable in view of the studies by Zimmermann (1981). This author studied the effect of calcium and magnesium on the growth and multiplication of plants from the *Lemnaceae* family cultured *in vitro*. It should be added that various other bivalent metals can affect the resistance of plants to ionizing radiation (Ilina et al. 1970, Kalam 1970).

The wide geographical range over which *Wolffia arrhiza* appears allows the assumption that populations from ecologically different habitats, that is, from bodies of water of different chemical compositions, may show ecological radiosensitivity as the experimental populations showed a different tolerance to radiation depending on the calcium or magnesium content of the medium,

1. Among plants from the *Lemnaceae* family, *Wolffia arrhiza*, which is characterized by the greatest radiosensitivity and therefore the lowest resistance to ionizing radiation, may be the first to be expelled from the competitive limno-pleustonic system in case of post-radiative disturbances of its structure.

2. Calcium and magnesium in the environment increase the resitance of *Wolffia arrhiza* to acute radiation and may be used for post-radiative population repair.

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Wpływ wapnia i magnezu na promienioczułość Wolffia arrhiza (L.) Wimm.

Streszczenie

Stwierdzono różnice w przeżywalności roślin *Wolffia arrhiza* (L.) Wimm. pod wpływem promieniowania gamma z ^{60}Co w kulturach *in vitro* przy różnych ilościach wapnia i magnezu w pożywkach. Dawki promieniowania zróżnicowano od 80 do 3000 $mC \cdot kg^{-1}$. Magnez, a zwłaszcza wapń zwiększają odporność wolffii na promieniowanie ostre. Wyraża się to przeżywalnością roślin przy dawkach 1200 i 3000 $mC \cdot kg^{-1}$ na pożywkach wzbogaconych w wapń lub magnez, podczas gdy na pożywce podstawowej całkowita śmiertelność roślin (DL 100) następuje już przy dawce ekspozycji 800 $mC \cdot kg^{-1}$. Promienioczułość *Wolffia arrhiza* jest duża w stosunku do innych gatunków roślin z rodziny *Lemnaceae*.