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Ecologic-Biological Effects of Cobalt, Cuprum, Copper Oxide Nano-Powders and Humic Acids on Wheat Seeds

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Abstract

We have determined the comparative efficacy of the effect of cuprum and cobalt nano-particles (30-40 nm), humic acids in an ultra-fine state and mineral salts of these metals used as micro-fertilizers on plants growth and development at concentration from 0.01 till 1000 gr. per hectare norm of viability (gr./h.n.v.). We have conducted some bio-chemical investigations and studied activity of peroxydase and superoxide dismutase ferments in wheat roots and seedlings.

The lab tests have determined concentrations when spring wheat plantlets growth and development slows down: for cuprum nano-particles – 500 gr./h.n.v., cobalt – 400 gr./h.n.v., copper sulphate and cobalt chloride – 100 gr./h.n.v. The concentrations of 0.5-10 gr./h.n.v. cuprum and cobalt nano-powders have a well defined stimulating effect. The effect of using the metals nano-particles has been 8-12 % higher than that of the humic acids. Nano-particles have energy to activate metabolic cell processes of plants and concentrations up to 10 gr./h.n.v. can be recommended for the use in agrotechnology that is proved by field tests: wheat yield has increased under the humic acids per 7 %, cuprum and cobalt nano-particles per 17 % and 14 % correspondingly as compared with the control.

Cuprum and copper oxide nano-powders (of different chemical substance) at concentrations 0.1 gr./h.n.v.-100 gr./h.n.v. have influenced activity of phytohormones of cytokinin, gibberellin, abscisic acid and indole acetic acids in wheat seedlings. The increase of cuprum nano-powder concentration in 100 times has not caused the decrease of wheat physiologic processes. For copper oxide nano-powders at concentration of 100 gr./ha the hormones activity changes from 22 to 53 % and the use of copper oxide at concentrations higher than 100 gr./h.n.v. is unreasonable. The wheat seeds treatment with 200 nm particles before planting has not changed morpho-physiologic and biochemical indexes as related to the control.

Keywords: Nano-particles, wheat, ferments, hormones, yield

1. Introduction

Nano-technologies as one of the main directions of modern science and technology development can bring the results comparable to those achieved for the recent decades in the nearest future (Foster, 2008). Among the spheres of nano-technologies achievements application one can name construction materials, microelectronics, optics, power engineering, military art, transport, biotechnologies, medicine, environment protection, agriculture. The objects of nano-technologies are nano-materials like nano-tubes, nano-composites, foam materials, fullerenes, ultra-dispersed powders and so on.

In comparison with ordinary substances nano-materials can possess totally different physical and chemical characteristics and biological activity (Gogos, 2012; Folmanis, 1999; Churilov, 2010; Polishchuk, 2014). The results of the researches have shown that nano-particles due to their structure, small size (Eduardo, 2009), high-surface area and low concentration create favorable conditions for plants more intensive growth and development resulting in both vegetative and reproductive mass accumulation and the biochemical composition change (Schwab, 2011; Churilov, 2013; Nazarova, 2013; Churilov, 2012).

The results of the synthesized nano-materials toxicity research are rather contradictory and give rise to many new questions. But the absence of toxicological data should not lead to the preventive delay of nano-technological research. Conversely, it is necessary to strive for moderate balance between further development of nano-technology and research to determine the potential danger.

1.1 Materials and Methods

1.1.1 Study Area

The integrated study of nano-materials use as ecologically safe stimulators of agricultural crops growth presupposes the study of physiological and biochemical processes in plants cells. At that phonological observations and biocenosis' changes agro-ecological investigations play a significant role.

1.1.2 Sampling

The samplings are the seeds of spring wheat breed "Lada".

1.1.3 Sample Treatment and Analysis

We have analyzed sustainability (germination energy, germinability) and morphological-physiological indexes (sprout and root length and mass) of wheat plantlets at the laboratory of the Center of nanotechnologies and nano-materials for AIC at Ryazan State Agrotechnological University Named after P.A. Kostychev.

To analyze nano-materials influence on fodder crops in biological indexes we have used gel-like (on the basis of polysaccharides) cultivating environment as a substrate. We have chosen a polysaccharide got from alga (agar "Difco" or home-grown microbiological agar) as a gel-forming component for agar environment. Some chemical parameters of the micro-biological agar are as follows: sulfates ≤ 1 %; calcium ≤ 0.4 %; magnesium ≤ 0.2 %; total nitrogen ≤ 0.25 %; chilling point ≥ 36°C; fusing point of 1.2 % gel ≤ 5°C; pH (1.2 % gel) differs from ≤ 6.1 to ≥5.7 after autoclaving. We have been couching the seeds according to GOST 12038-84 with the help of a thermostat TCO-1M with a temperature range from 0° C to 60° C; permissible temperature variations ±1° C.

Metals nano-particles (NP) have had free particles form, high-surface area (up to 25 m2/g). We have prepared the suspension according to TU 931800-4270760-96 in an ultra-wave bath (model PSB-5735-5). They have established earlier (Kutskir, 2012; Polishchuk, 2014; Kutskir, 2012; Ilyichev, 2011) their high reactivity and catalytic reactivity in plants' and animals' cells and tissues. They have studied the nano-powders action in a concentration range 0.001 – 1000 g of powder per hectare norm of seeds planting (g/gr.n.v.).

We have conducted the biochemical investigations at Ryazan State Medical University Named after I.P. Pavlov. To determine the peroxidase and superoxide dismutase activity in plant tissues we have used the photometric kinetic test with the help of photoelectric colorimeter КFК-3-01-"ZOMZ". We have estimated cytokinins and abscisic acid by method of highly effective fluidal chromatography (VEZHH) of firm Biotronic. The conditions for chromatographing were as follows: ultraviolet detector (model VT 3030), wave length 268 nm, station Lichrosorb RP-18, 6 mkm, 4x150. Mobile phase: ethane nitrile-water-acetic acid (V/V - 55:44:1), flow velocity 0.8 ml/min, hold-up time – 10 min.

We have determined the biological activity of gibberellins with Francland and Warring method. The conditions for chromatography were as follows: детектор fluorescent detector RF-350 (Shimadzu), Em-350 nm, Ex-280 nm, station Lichrosorb RP-18, 6 mkm, 4x250. Mobile phase – 40 % water solution of methanol, flow velocity 0.5 ml/min, hold-up time – 12 min. The minimal registered concentration in all cases was 2.0 ngr in trial aliquot (20 mkl).

2. Results

2.1 Effect of Cuprum, Cobalt and Their Salts Nano-Particles on Germinating Energy and Spring Wheat Germinating Ability

Cuprum and cuprum sulfate nano-particles at concentration range 0.01; 0.10; 1.00 and 10.00 g per hectare norm of viability (g/h.n.v.) have stimulated the plantlets growth and development; the germinating energy under the influence of cuprum nano-particles exceeded the control correspondingly per 6 %, 7.8 %; 9 % and 7 %, under cuprum sulfate influence per 7 %, 8 %, 7 % and 3.8 % (Table 1).

Table 1. Germinative Energy and Viability of Wheat Seeds while Interacting with Cuprum, Cobalt, and Cuprum Sulfate Nano-Powders and Cobalt Chloride

Variants	Germinative Energy, %	Viability, %	Variants	Germinative Energy, %	Viability, %
Control	89.0±3.4	96.6±2.9	Control	89.0±3.4	96.6±2.9
NP-Cu 0.01	95.0±2.8	97.0±1.8	$CuSO_4$ 0.01	96.0±1.7	96.6±2.3
NP-Cu 0.10	96.8±2.1	98.8±1.0	$CuSO_4$ 0.10	97.0±2.9	98.4±1.1
NP-Cu 1.00	98.0±1.1	98.0±1.2	$CuSO_4$ 1.00	96.0±3.2	97.0±1.7
NP-Cu 10.00	96.0±1.3	97.3±1.1	$CuSO_4$ 10.00	92.8±3.7	96.0±1.9
NP-Cu 20.00	94.0±1.2	94.5±1.1	$CuSO_4$ 20.00	82.3±3.5	93.2±1.7
NP-Cu 100.00	85.2±1.4	96.8±2.5	$CuSO_4$ 100.00	83.2±3.9	92.4±2.8
NP-Cu 500.00	76.5±1.1	96.4±1.2	$CuSO_4$ 500.00	3.2±0.2	32.4±0.7
NP-Cu 1000	37.6±3.3	87.6±2.2	$CuSO_4$ 1000	2.0±0.2	16.0±3.9
NP-Cu 2000	35.6±1.6	84.6±2.8	$CuSO_4$ 2000	2.0±0.1	16.4±3.9
NP-Cu 5000	39.6±2.3	78.8±2.6	$CuSO_4$ 5000	2.0±0.1	4.8±1.1
NP-Co 0.10	94.6±1.2	96.0±0.9	$CoCl_2$ 0.10	92.5±1.6	97.0±1.1
NP-Co10.00	93.6±2.2	94.0±1.7	$CoCl_2$ 10.00	94.5±2.2	96.5±1.7
NP-Co 100.00	95.5±1.7	95.6±1.6	$CoCl_2$ 100.00	93.0±1.5	94.0±1.9
NP-Co 200.00	86.0±1.7	96.0±2.1	$CoCl_2$ 200.00	90.0±1.8	93.5±1.8
NP-Co 400.00	71.6±1.6	96.6±1.1	$CoCl_2$ 400.00	74.6±2.3	93.0±1.9
NP-Co 600.00	93.0±1.1	95.0±1.5	$CoCl_2$ 600.00	6.6±0.5	89.0±2.1
NP-Co 800.00	93.0±1.3	95.0±2.0	$CoCl_2$ 800.00	4.6±0.4	67.8±1.1

Differences between average indexes are valid for $P \geq 0.95$.

Beginning with concentration 20 g/h.n.v. the germinating energy in a case with cuprum sulfate has gone down and at concentration 500 and 1000 g/h.n.v. it has been from 3.2 to 2.0 %, i.e. the seeds germination has practically been absent. The germinating energy in a case with cuprum nano-particles has declined only from the concentration 500 g/h.n.v., but at higher concentrations the seeds germinability remained on the level of 96.4 %.

The lab wheat viability under the influence of cuprum nano-particles (NP) practically in all variants was higher than that of the control. Only at concentration 1000 g/h.n.v. the viability was lower than the control one per 9.0 %. In a case of cuprum sulfate usage the seeds viability has increased maximum per 1.4 %, i.e. differed slightly from the control one. At concentration 500 g/h.n.v. the viability has declined per 64.2 % as compared with the control one. In a case of further cuprum sulfate concentration increase the total growth oppression of spring wheat has taken place.

When adding cobalt chloride at concentration from 0.1 to 100 g/h.n.v. (Table 1) to the cultivated land, the germinating energy of spring wheat seeds has been 3.5-5.5 % higher than that of the control one and at high concentrations (400-800 g/h.n.v.) the germination has been depressed (the germinating energy has been more than 10 times lower than that of the control one) that proves high toxicity of cobalt chloride at these concentrations.

The germinating energy in all the interval of cobalt NP concentrations has been higher the control one achieving the maximum value 6.5 % at 100 g/h.n.v. The lab viability of spring wheat under the influence of cobalt NP has corresponded to the control data in all the interval of concentrations that proves the normal seeds viability. In a case of using cobalt chloride the spring wheat lab viability at low concentrations (0.1 g/h.n.v.) has been at the control level and starting with concentration 100 g/h.n.v. it has declined consistently and authentically achieving the minimal data at concentration 800 g/h.n.v. (-84.4 % as compared with control). At that they have offended the seedlings development: the roots were “wire-like”, under the light microscopic examination one could see weak development of the root adsorbing portion, the number of root fibrils was definitely lower as compared with the normally germinated seeds. Consequently, cobalt salts concentration higher than 100 g/h.n.v. oppresses seeds germination.

2.2 Cuprum, Cobalt and Their Salts Nano-Particles Effect on Growth and Development of Spring Wheat Seedlings

At cuprum nano-particles concentration 1.0 g/h.n.v. the seedling length (Table 2) has been maximum and exceeded the control per 51.0 % and remained 49 % - 40 % higher up to concentration 100 g/h.n.v., that proves high biological activity of cuprum nano-particles.

Table 2. Wheat Seedlings Length while Interacting with Cuprum, Cobalt, and Cuprum Sulfate Nano-Powders and Cobalt Chloride

Variants	Spring Wheat (7-days) Aerial Portion, mm	Spring Wheat (7-days) Underground Portion, mm	Variants	Spring Wheat (7-days) Aerial Portion, mm	Spring Wheat (7-days) Underground Portion, mm
Control	31.1±3.4	142.8±2.4	Control	31.1±2.3	142.8±2.2
NP-Cu 0.01	44.6±3.2	164.5±4.5	$CuSO_4$ 0.01	33.6±1.4	134.8±4.2
NP-Cu 0.1	46.0±2.5	176.6±3.2	$CuSO_4$ 0.1	31.9±4.3	132.8±2.3
NP-Cu 1.0	46.9±3.0	132.4±1.9	$CuSO_4$ 1.0	40.6±2.3	127.6±2.2
NP-Cu 10.0	45.0±1.2	178.4±3.2	$CuSO_4$ 10.0	42.9±3.2	129.1±2.3
NP-Cu 20.0	45.1±3.2	178.5±3.8	$CuSO_4$ 20.0	41.0±3.6	129.0±3.8
NP-Cu 100.0	46.4±3.6	165.5±3.4	$CuSO_4$100.0	32.2±3.7	112.6±3.6
NP-Cu 500.0	40.6±2.4	166.2±2.5	$CuSO_4$ 500.0	17.2±2.5	1.0±3.4
NP-Cu 1000.0	25.6±1.4	40.3±3.2	$CuSO_4$ 1000.0	23.3±2.7	1.0±2.3
NP-Co 0.1	33.1±2.4	175.1 ±2.7	$CoCl_2$ 0.1	33.1±1.1	161.1±1.6
NP-Co 10.0	32.6±2.4	177.5±2.9	$CoCl_2$ 10.0	30.4±2.4	172.5±4.1
NP-Co 100.0	28.3±1.9	154.1±2.2	$CoCl_2$100.0	32.6±2.7	113.6±3.7
NP-Co 200.0	30.2±2.0	160.9±1.8	$CoCl_2$ 200.0	31.2±1.9	114.9±6.3
NP-Co 400.0	33.0±2.0	135.1±1.4	$CoCl_2$ 400.0	24.9±2.0	82.0±1.7
NP-Co 600.0	29.8±1.9	147.0±2.4	$CoCl_2$ 600.0	17.2±1.9	12.7±0.5
NP-Co 800.0	22.9±1.0	138.8±1.2	$CoCl_2$ 800.0	15.2±1.5	12.5±1.4

Differences are valid for P≥0.95.

At cuprum nano-particles concentration 0.1 g/h.n.v. the total root length has exceeded the control per 23.7 % till concentration 500 g/h.n.v. that shows the growth processes stimulation. We have determined the cuprum nano-particles oppressing effect only at concentration 1000 g/h.n.v.. The seedling aerial portion length was 17.6 % less than that of the control one.

In spite of the apparent oppression at higher concentrations (1000 g/h.n.v.) one can see that the seeds treated with cuprum nano-powder have given the number of root fibrils significantly more than the ones from the control group and the plants treated with cuprum sulfate.

In the presence of cuprum sulfate at concentrations 0.01 g - 100 g/h.n.v. one could see the seedling aerial portion development stimulation (per 8.6 % - 37.5 %). But practically in all experimental variants the length of the seedling underground portion was less than that of the control one. At concentrations 500 and 1000 gr/h.n.v. the roots of the seedlings treated with cuprum sulfate were practically absent (Table 2).

While using cobalt nano-particles the length changes of both the seedlings and the root portion of germinants have had positive dynamics. The length of the aerial portion even at concentration 400 gr/h.n.v. has exceeded the control number per 6.1 %. At that the roots number in all variants has been equal to 4 items and more and the number of root fibrils has been optimal according to plants biological peculiarities. Cobalt penetrating into plants' root system, probably, has become active in tissues, stimulated bio-chemical processes connected with plant hormone synthesis and activated enzyme systems determining growth processes.

The use of cobalt chloride at low concentrations (0.1-10.0 g/h.n.v.) has promoted spring wheat roots and sprouts development (per 6.4-4.8 %). At higher concentrations of cobalt chloride (400-800 g/h.n.v.) one could see stable oppression of sprouts' length from 19.9 to 51.1 % and roots length from 20.5 % to 91.5 % as compared with the control one.

Lab researches have made it possible to determine concentrations when the growth and development of spring wheat seedlings slows. That comprised 500 g/h.n.v. for cuprum NP, 100 g/h.n.v. for cuprum sulfate, 400 g/h.n.v. for cobalt NP, 100 g/h.n.v. for cobalt chloride. At concentrations 0.1 - 10 g/h.n.v. cuprum and cobalt nano-particles possess strongly marked stimulating effect up to 70 % exceeding these metals mineral salts action (Table 2). At concentrations of cuprum sulfate from 100 to 5000 gr/h.n.v. the root system development has been disturbed and the seedlings were abnormally long and "fleshy" with low mass (90.3 % lower than control).

2.3 Cuprum and Cuprum Sulfate Effect on Activity of Spring Wheat Seedlings Oxidation-Reduction Ferments

Provided conclusions are proved by the ferments activity analysis (peroxydase and superoxide dismutase). Oxidative stress leads to changing metabolism and regulation processes, appearance of morphologic symptoms pointing anabolic and catabolic ways. In the process of stress development the maximum activation of mechanisms including tolerance, abience and detoxication is achieved by means of opening stress and escape portions.

Peroxydase activity in plants correlates with development of plants immunity to stress. Unfavorable factors of environment negatively influence plants growth and development that occur in considerable changes of peroxydase activity the specter of that change under the influence of biologic and non-biologic agents. Superoxide dismutase (SD) is also attributed to the group of antioxidant ferments. Under the effect of unfavorable factors oxygen active forms increase including radicals. SD activity at that changes in different directions. Sometimes one can see its increase, in other cases decrease. It depends on the intensity of the stress factor effect (intensity and length of stress) as well as on the organism's sensibility and development stage and so on.

Researches (Churilov, 2012) have proved that peroxydase activity changes in a case of different nano-particles relative to control should not exceed deviations for more than 30 %.

The results of the experiment have shown that peroxydase activity depends on additives type and differs in location: the roots or sprouts in a case of the same concentration of compounds.

Peroxydase content in roots of the spring wheat experimental examples differs from that of the control group plants. At low concentrations of cuprum nano-powder from 0.01 to 10.0 g/h.n.v. one can see the 13.9 % increase of peroxydase activity as compared with that of control and at higher concentrations (from 500 до 1000 g/h.n.v.) the ferment's activity insignificantly decreases (per 0.2 %). The peroxydase activity in sprouts gradually decreases from 10.8 % to 36.6 % with the increase of cuprum nano-powder at concentrations from 0.01 to 1000 g/h.n.v. . The ferments activity change in spring wheat plantlets under the influence of different forms of cuprum micro-fertilizers is presented in Table 3. Peroxydase activity in spring wheat roots in a case with cuprum sulfate is higher as compared with the control group and with the increase of sulfate concentration it increases from 14.3 % (if CuSO4 0.01 g/h.n.v.) to 23,57% (if CuSO4 1000 g/h.n.v.). Peroxydase activity in sprouts in a case of increasing sulfate concentration gradually decreases as compared with that of the control group per 13.1 % (0.01 g/h.n.v.) to – 44.9 % (100 g/h.n.v.).

Table 3. Spring Wheat Activity of Peroxydase (optical density unit / fresh weight(g)•sec) and Superoxide Dismutase (activity conventional unit/ fresh weight(g))

Variant	Peroxydase				Superoxide Dismutase			
	Roots		Seedlings		Roots		Seedlings	
	Absolute Value	% to Control	Absolute Value	% to Control	Absolute Value	% to Control	Absolute Value	% to Control
Control	14.21±0.94	-	19.42±1.17	-	115.50±0.13	-	260.10±0.67	-
NP-Cu 0.01	14.80±0.89	+3.98	17.30±1.13	-10.80	116.00±0.78	+0.43	280.10±0.82	+7.00
NP-Cu 0.10	15.20±0.99	+6.97	16.20±1.09	-16.58	116.50±0.42	+0.86	294.4±0.97	+13.19
NP-Cu 1.00	15.88±0.78	+11.75	14.60±1.98	-24.82	117.01±0.67	+1.40	300.10±0.76	+15.38
NP-Cu 10	16.19±1.01	+13.90	15.07±1.20	-22.40	117.65±0.84	+ 1.86	313.34±0.54	+20.47
NP-Cu 100	15.18±0.94	+7.00	15.24±1.06	-21.51	96.85±0.75	- 6.14	291.30±0.92	+ 2.00
NP-Cu 500	13.86±0.86	- 7.39	14.44±1.09	-25.64	100.01±0.41	-13.41	231.20±0.46	-11.12
NP-Cu 1000	13.90±0.93	-7.20	12.30±1.13	-36.66	89.91±0.94	-28.00	204.80±0.65	- 2.,3
$CuSO_4$ 0.01	14.29±0.78	+0.56	16.89±1.24	-13.03	111.00±0.37	-3.89	210.30±0.56	-21.26
$CuSO_4$ 0.1	15.00±0.85	+5.56	13.40±1.03	-30.60	106.70±0.67	-7.62	235.60±0.97	-9.42
$CuSO_4$ 1.0	15.80±1.03	+11.20	13.07±1.06	-32.69	110.20±0.43	-4.59	250.20±0.85	-3.81
$CuSO_4$ 10	16.48±0.92	+15.97	13.88±0.98	-28.53	116.98±0.58	+ 1.38	288.87±0.89	+11.06
$CuSO_4$ 100	17.22±0.84	+21.18	10.7±0.95	-44.90	118.15±0.41	+ 2.29	294.61±0.55	+ 3.26
$CuSO_4$ 500	20.18±0.89	+42.02	10.1±0.95	-92.85	72.96±0.74	-36.80	318.31±0.89	+22.38
$CuSO_4$ 1000	60.19 ±0.74	+323.57	8.2±1.12	-136.2	59.31±0.49	-94.00	450.30±0.35	+43.00

Differences are valid for P≥0.95.

Superoxide dismutase activity in spring wheat experimental examples in roots and sprouts also differs both in size and character of changes. For cuprum nano-powder the ferment activity in roots very insignificantly increases within concentrations 0.01 - 10 g/h.n.v. from 0.43 % to 1.86 % and decreases in a case of cuprum content increase from 100 g/h.n.v. to 1000 g/h.n.v. per 6.2 % and 33.4 % correspondingly. In a case with cuprum sulfate superoxide dismutase activity in spring wheat roots increases up to 2.3 % at sulfate concentration 100 g/h.n.v., and at 1000 g/h.n.v. decreases per 94 %.

Superoxide dismutase activity in spring wheat sprouts for cuprum nano-powder increases per 20.47 % with the increase of its content from 0.01 g/h.n.v. to 10 g/h.n.v., and then goes down per 2.0 % at 100 g/h.n.v. and per – 21.3 % at 1000 g/h.n.v. Cuprum sulfate decreases superoxide dismutase activity in relation to the control per 21.2 % (0.01 g/h.n.v.), with the increase of $CuSO_4$ the ferment activity increases remaining lower than the control (for 0.1 g/h.n.v.. – 9.4 %, for 1.0 g/h.n.v. – 3.8 %). The ferment activity increases up to 22.4 % for higher concentrations of cuprum sulfate (more than 10.00 g/h.n.v.) in a case of salt content in soil 500 g/h.n.v. and per 43 % when 1000 g/h.n.v.

Thus, cuprum sulfate concentration 500 g/h.n.v. is destructive for wheat seeds. At the same time sprouts growth and development oppression when treated with cuprum nano-particles begins only from concentration 1000 g/h.n.v.

Further investigations referred to the study of chemical composition and nano-particles size influence on their biological activity.

2.4 Effect of Cuprum, Cobalt Nano-Particles and Humic Acids in Ultra-Dispersed State on Spring Wheat Seedlings

The character of plantlets growth stimulation has been the same while using both cuprum and cobalt nano-particles, i.e. technogenic components, that is why to compare their effect we have chosen natural biologically active substances humic acids but in an ultra-dispersed state and added a new nano-material type (cuprum oxide, 25-30 nm). The most optimal concentrations have been from 0.1 g/h.n.v. to 10.0 g/h.n.v.

The use of cobalt, cuprum, cuprum oxide nano-particles and humic acids in ultra-dispersed state has promoted the increase of wheat seeds germinating energy. They have seen the maximum germinating energy increase in variants at NP concentration 0.5-1.0 g/h.n.v. where the positive difference with the control has been up to 10 %, for humic acids lower – 6 %. At humic acids concentration 0.5 g/h.n.v. spring wheat seeds germinating energy has been authentically higher the control one per 5.5 %, when cuprum oxide nano-particles per 6.6 %, when cuprum per 7.8 %, when cobalt per 7.2 %. Thus, nano-particles presence in medium on the whole has promoted strengthening the processes connected with seeds germination independent of nano-particles chemical composition and the optimal concentration of nano-particles 0.5-1.0 g/h.n.v.

The mass of spring wheat plantlets at cuprum and cobalt nano-particles concentration 0.5 g/h.n.v. has exceeded the control one per 44 %-45 %, and the mass of the underground portion per 58 %-40 %. The plantlets have visually developed normally. The color was characteristic for this type of plants. Humic acids and cuprum oxide (per 40 %-45 %) have stimulated roots development in a less degree. The underground mass excess at concentration of humic acids equal to 0.5 g/h.n.v. has been 36 % relatively to control (Table 4).

Table 4. Spring Wheat Seedlings Mass Indexes when Treated with Biologically Active Nano-Particles

Variant	Seedling Aerial Portion Mass, g	Variant	Seedling Aerial Portion Mass, g	Variant	Seedling Aerial Portion Mass, g
Control	5.23±0.84	Control	5.23±0.84	Control	5.23±0.84
NP-Co 0.1	6.98±0.91	HA - 0.1	6.94±0.77	NP-Cu0.1	5.97±0.61
NP-Co 0.5	7.31±0.74	HA - 0.5	7.31±0.51	NP-Cu 0.5	7.52±0.84
NP-Co 1.0	7.58±0.61	HA - 1.0	7.45±0.71	NP-Cu 1.0	7.46±0.89
NP-Co 5.0	7.31±0.75	HA - 5.0	7.43±0.75	NP-Cu 5.0	6.83±0.72
NP-Co 10.0	6.91±0.81	HA - 10.0	7.31±0.81	NP-Cu 10.0	6.96±0.78
Variant	Seedling Underground Portion, g	Variant	Seedling Underground Portion, g	Variant	Seedling Underground Portion, g
Control	1.43±0.21	Control	1.43±0.21	Control	1.43±0.21
NP-Co 0.1	2.04±0.13	HA — 0.1	1.86±0.42	NP-Cu0.1	1.64±0.13
NP-Co 0.5	2.26±0.15	HA - 0.5	1.95±0.33	NP-Cu 0.5	2.03±0.16
NP-Co 1.0	1.97±0.26	HA - 1.0	1.84±0.34	NP-Cu 1.0	1.94±0.12

NP-Co 5.0	1.95±0.19	HA - 5.0	1.83±0.16	NP-Cu 5.0	1.99±0.15
NP-Co 10.0	1.86±0.12	HA - 10.0	1.93±0.14	NP-Cu 10.0	1.78±0.11
Variant	Seedling Aerial Portion Mass, g		Variant	Seedling Underground Portion, g	
Control	5.23±0.84		Control	1.43±0.21	
NP-CuO 0.1	5.89±0.67		NP-CuO 0.1	1.55±0.11	
NP-CuO 0.5	6.03±0.52		NP- CuO 0.5	1.48±0.24	
NP-CuO 1.0	6.23±0.77		NP- CuO 1.0	1.41±0.13	
NP-CuO 5.0	5.78±0.71		NP- CuO 5.0	1.51±0.23	
NP- CuO10.0	5.35±0.57		NP- CuO 10.0	1.31±0.11	

Differences are valid for P≥0.95.

The lab investigations have proved the high biological activity of cobalt, cuprum, cuprum oxide nano-particles and humic acids in ultra-dispersed state on the processes connected with plants growth and development. But the effect of using metals nano-particles has been 8-12 % higher than that of the humic acids at the same concentrations in liquid cultivation environment. Thus, nano-particles possess higher energy potential to activate

2.5 Effect of Nano-Particles Optimal Concentration on Reductive-Oxidative Ferments Activity of Spring Wheat Plantlets

Peroxydase activity in spring wheat roots (Table 5) in all variants of concentration has not exceeded the critical difference with the control (±30 %). Therefore, the plant body has not caught a stress that could have disturbed the processes of electrons transfer and free radicals neutralizing but the peroxydase content in roots of all experimental variants has exceeded control. This testifies te nano-particles biological activity. When using cobalt nano-particles at concentration 5.0 g/h.n.v. The peroxydase activity in roots has been 13.7 % higher than the control one, when cuprum nano-particles 20.7 % higher, when cuprum oxide 13.9 % higher, when humic acids 26.1 % higher. The peroxydase activity in leaves of all experimental variants has been lower than the control one. This can depend on a selection action of nano-particles that had realized their energy potential in root system. Indeed, we have noticed while having the field tests that plants treated before planting with nano-particles have had a more developed root system (Kutskir, Nazarove, Polishchuk, 2012).

The superoxide dismutase activity in spring wheat roots (Table 5) has not practically differed from that of control, but the ferment content in leaves has exceeded the control one maximum per 15.4 % at cobalt NP concentration 5.0 g/h.n.v.

Table 5. Peroxydase Activity (optical density unit / fresh weight(g)•sec) and Superoxide Dismutase Activity (activity conventional unit/ fresh weight(g) in Spring Wheat Seedlings under the Influence of Different Nano-Particles

Variant	Peroxydase				Superoxide Dismutase			
	Aerial Portion		Underground Portion		Aerial Portion		Underground Portion	
	Absolute Value	% to Control	Absolute Value	% to Control	Absolute Value	% to Control	Absolute Value	% to Cont-rol
Control	14.21±0.46	-	19.42±0.38	-	115.50±2.48	-	260.1±3.45	-
NP-Co 0,5	14.80±0.76	+4.2	17.3±0.92	-10.9	116.00±3.15	+0.4	280.1±2.87	+7.7
NP-Co 1.0	15.86±0.59	+11.6	16.2±0.86	-16.6	116.50±1.02	+0.9	294.4±3.72	+13.2
NP-Co 5.0	16.16±0.94	+ 13.7	14.6±1.03	-24.8	117.2±1.95	+1.4	300.1±4.01	+15.4
NP-Cu 0.5	16.46±1.70	+15.9	15.89±0.89	-18	114.5±1.65	-0.8	275.6±2.64	+5.9
NP-Cu 1.0	16.86±0.76	+17.8	16.04±1.14	-17.4	121.4±1.88	+5.1	286.7±2.94	+10.2
NP-Cu 5.0	17.15±0.73	+20.7	19.62±0.69	+1.0	126.7±1.21	+9.7	301.7±3.94	+15.9
NP-CuO 0.5	14.29±0.53	+0.6	16.89±0.72	-13.03	111.00±0.09	-3.89	210.3±2.65	-21.26
NP-CuO 1.0	15.00±0.61	+5.6	13.4±0.66	-30.60	106.70±1.12	-7.62	235.6±2.88	-9.42
NP-CuO 5.0	16.19±0.59	+13.9	15.07±0.78	-22.40	117.65±1.04	+ 1.86	331.6±2.16	+27.47
HA – 0.5	14.75±0.53	+3.8	18.94±1.27	-2.5	115.9±1.91	+0.3	270.8±3.98	+4.1
HA – 1.0	15.49±0.46	+9.01	19.36±1.06	-0.3	116.4±1.71	+0.7	298.6±2.78	+14.8
HA – 5.0	17.93±0.74	+26.1	17.82±1.03	-8.2	118.6±3.52	+2.6	295.6±3.61	+13.6

Differences are valid for P≥0.95.

It is necessary to point out that different structure of cuprum and cuprum oxide nano-particles differ in their activity. Cuprum possesses higher biological activity.

To determine the dependence of nano-particles activity on nano-particles size we have studied 200 nm cuprum nano-particles effect.

2.6 200 nm Cuprum Nano-Particles Effect and Spring Wheat Seeds Development and Activity of Reductive-Oxidative Ferments

Particles of this size less influence the plants growth. Probably because of big size they penetrate into the seeds meagerly or do not interact with cells organelles. But when increasing their concentration from 0.01 g/h.n.v. to 1000 g/h.n.v. the wheat seedlings aerial portion growth increases per 12 % as compared with control and the underground portion per 30 %. Linear growth of plants is an important indicator that indirectly characterizing the division or expansion of the cells. This indicator is closely correlated the volume of the bodies of plants and their weight. We observe the mass of the aerial parts of seedlings increased by 8%, and the growth of the root system was 44% (Table 6).

Table 6. Morphologic-Physiological Indexes of Wheat Seedlings while Interacting with Cuprum Nano-Powder (Size 200 nm) in Gel-Like Cultivation Environment

Variant	Spring Wheat (7 days) Seedling Aerial Portion Mass, g	Spring Wheat (7 days) Seedling Underground Portion Mass, g	Spring Wheat (7 days) Seedling Aerial Portion Length, mm	Spring Wheat (7 days) Seedling Underground Portion Length, mm
Control	0.0359±0.001	0.0301±0.001	21.7±0.34	18.6±0.09
NP-Cu 0.01	0.0346±0.002	0.0327±0.002	21.9±0.22	18.0±0.17
NP-Cu 0.1	0.0388±0.001	0.0335±0.005	21.0±0.21	19.6±0.27
NP-Cu 1.0	0.0342±0.005	0.0422±0.002	20.7±0.31	20.1±0.21
NP-Cu 10	0.0350±0.002	0.0415±0.007	21.3±0.24	19.8±0.08
NP-Cu 100	0.0354±0.003	0.0398±0.003	22.6±0.25	20.5±0.26
NP-Cu 1000	0.0387±0.003	0.0432±0.002	24.3±0.23	24.4±0.23

Differences are valid for P≥0.95.

At that we have estimated that when treating wheat seeds with a suspension of cuprum nano-powder having 200 nm particles the ferments activity has not practically differed from the control one (Table 7).

Table 7. Peroxydase Activity (optical density unit / fresh weight(g)•sec) and Superoxide Dismutase Activity (activity conventional unit/ fresh weight(g) in Wheat Roots and Seedlings under the Influence of Cuprum Nano-Powders with Particles Sized 200 nm

Variant	PEROXYDASE Roots Absolute Value	PEROXYDASE Roots % to Control	PEROXYDASE Seedlings Absolute Value	PEROXYDASE Seedlings % to Control	SUPEROXIDE DISMUTASE Roots Absolute Value	SUPEROXIDE DISMUTASE Roots % to Control	SUPEROXIDE DISMUTASE Seedlings Absolute Value	SUPEROXIDE DISMUTASE Seedlings % to Control
Control	14.21±0.12	-	19.42±0.9	-	115.50±1.12	-	260.10±1,23	-
NP-Cu 0.01	14.50±0.14	+2.04	17.22±0.8	-11.33	115.00±1.10	-0.43	270.10±1,34	+3.84
NP-Cu 0.10	14.80±0.09	+4.15	18.20±0.4	-6.28	116.00±1.14	+0.43	274.40±1,56	+5.50
NP-Cu 1.00	15.00±0.01	+5.56	18.60±0.2	-4.22	116.20±1.14	+0.61	278.10±1,76	+6.92
NP-Cu 10.0	14.90±0.06	+4.86	19.07±0.5	-1.80	115.55±1.09	+0.04	270.40±1,49	+3.96
NP-Cu 100.0	14.78±0.13	+4.01	19.30±0.7	-0.62	114.85±1.08	-0.56	269.30±1,89	+3.54
NP-Cu 1000.0	14.90±0.16	+4.86	18.75±0.9	-3.45	114.05±1.05	-1.26	254.80±1,92	-2.35

Differences are valid for P≥0.95.

The changes occurring in plants cells in a case of metabolism disturbance capture all centers of metabolic activity including mitochondria, plastids, Paladesses granules and core mechanism. As in organelles so in cytoplasm the most considerable changes happen to ferments, in particular peroxydase activity and specter of which change under the influence of biological and non-biological agents. Practically all more or less essential outer impacts and internal plants metabolism disturbance are accompanied with activity or peroxydase isoenzyme specter changes. One can explain the ferments activity stability by the absence of 200 nm nano-particles interaction with cells organelles.

2.7 Effect of Cuprum and Cuprum Oxide Nano-Particles on Activity of Spring Wheat Seedlings Plant Hormones

On different stages of ontogenesis under the influence of different environmental conditions the correlation of plant hormones changes and it is this changes the speed and direction of plants growth and morphogenesis. One of the factors to decrease physiological processes of spring wheat development could have been nano-particles concentration. One can not take into account that hormones can affect the ferments catalyzing other hormones synthesis or collapse and therefore change their content. We have studied the influence of cuprum and cuprum oxide nano-powders having concentrations 0.1 g/h.n.v. - 100 gr/h.n.v. on the activity of such plant hormones as cytokinin (CK), gibberellin (GL), abscisic acid (AA) and indoleacetic acids (IAA) in wheat plantlets grown in a sandy medium for a month.

Hormones content changes in spring wheat when treated with cuprum and cuprum oxide nano-powders differ considerably (Table 8).

Table 8. Plants Hormones Content in Experimental Examples of Spring Wheat

	PLANTS HORMONES							
Variants	IAA ngr/g wet weight	% to Control	CK ngr/g wet weight	% to Control	GL ngr/g wet weight	% to Control	AA ngr/g wet weight	% to Control
Control	10.12±0.47	_	552.14±3.60	_	231.15±0.06	_	81.54±0.57	_
NP-Cu 0.5	11.20±0.58	10.67	633.0±4.55*	14.68	38.80±0.17	24.59	82.40±0.73*	1.05
NP-Cu 100	11.23±0.37	10.97	624.65±3.37*	13.13	36.38±0.26	16.79	82.25±0.69*	0.87
NP-Cu0 0.5	11.37±0.83	12.35	624.5±5.38*	13.10	240.34±0.47	29.50	89.78±0.72*	10.11
NP- Cu0 100	13.51±0.62	33.50	381.00±6.90*	-31.00	356.80±0.09	82.34	110.34±0.9*	35.32

Differences are valid for $P \geq 0.05$.

At cuprum nano-powder concentration 0.1 and 100 g/h.n.v. the content of CK, GL and IAA increases. The quantity of AA has not practically changed as compared with control.

If to consider that AA accumulation retards growth processes induced by IAA, cytokinin and gibberellins and leads to decrease of photosynthetic phosphorylation and photosynthesis intensity then cuprum nano-powders must stimulate the plant development. Consequently, cuprum nano-powder concentration increase in 100 times has not caused the decrease of wheat physiologic processes. The activity increase of gibberellin that is considered as a growth hormone strengthens the stem extension, carbohydrates accumulate that has been proved by further field tests. Cuprum nano-powders at all concentrations increase IAA content and it is known that under the influence of the latter the oxidation contingence, phosphorylation (coefficient R/O) and ATP content in cells increase. It gives ground to suppose that IAA increases the energetic efficiency of plants breath and even small shifts in cell's energetic potential lead to noticeable changes in the speed of different fermentative reactions. Consequently, cuprum nano-powders must strengthen nutrients and water movements that is one of the reasons to strengthen plants' growth.

When seeds contact cuprum oxide nano-powder the quantity of IAA increases relative to the control: at 0.1 g/h.n.v. per 10.1 %, and at 100 g/h.n.v. per 35.32 %. The rest indexes at 100 g/h.n.v. change their values from 22 to 33 %. Therefore, it is inadvisable to use cuprum oxide at concentrations higher than 100 g/h.n.v.

2.8 Structural Analysis of Spring Wheat Yield when Seeds Pre-Plant Treatment with Cuprum, Cobalt, Cuprum Oxide Nano-Particles and Humic Acids in Ultra-Dispersed State

The lab investigations have been proved by field tests and the table provides the spring wheat yield structure.

From the data from Table 9 it follows that the use of biologically active drugs based on metals nano-particles promotes the increase of spring wheat yield exponents. We have achieved this by means of increasing the productive haulm stand. So the use of humic acids nano-particles has promoted the increase of this exponent per 7.2 %, the use of cuprum nano-particles per 17.0 %, the use of cobalt nano-particles per 14 % and the use of cuprum oxide per 8.4 % in comparison with the control data. What is more, the experimental plants have had more heads the average length of which has also been substantially bigger than those of the control group.

Table 9. Spring Wheat "Agatha" Yield Structure

Variant	Productive Haulm Stand per 1 m^2, items	Productive Stooling	Number of Spikelets in a Head, items	Number of Seeds in a Head, items	Mass of 1000 Seeds, g	Yield, dt/he
Control	480±2.32	2.4±0.10	14,4±0,9	32.6±1.13	30.6±1.32	40.50
HA – 0.5	515±2.12	2.5±0.31	14,9±0,8	32.9±1,12	32.5±1.43	44.79 (+10.6%)
HA – 1.0	510±2.31	2.5±0.43	14,6±0,2	33.1±1.14	33.5±1.30	43.60 (+7.65%)
HA – 5.0	490±2.09	2.6±0.11	14,5±0,1	32.4±0.98	32.9±1.27	45.01 (+11.1%)
NP-Cu 0.5	510±2.51	2.4±0.25	15,2±0,7	33.2±1.15	33.4±1.54	46.41 (+14.6%)
NP-Cu 1.0	562±1.19	2.6±0.31	15,5±0,6	33.2±1.74	34.8±1.86	47.59 (+17.5%)
NP-Cu 5.0	528±2.76	2.7±0.12	15,6±0,4	34.7±1.86	33.8±1.32	46.31 (+14.3%)
NP-Co 0.5	530±2.61	2.6±0.21	15,0±0,6	34.1±1.96	34.5±1.42	45.72 (+12.9%)
NP-Co 1.0	546±2.76	2.6±0.02	15,2±0,3	33.5±1.54	33.8±0.98	46.33 (+14.4%)
NP-Co 5.0	538±1.89	2.5±0.40	15,1±0,2	33.1±1.43	33.9±1.13	46.01 (+13.06)
NP-CuO 0.5	514±1.89	2.3±0.13	14,7±0,6	33.4±1.32	32.5±0.83	45.90 (+13.3%)
NP-CuO 1.0	524±2.03	2.4±0.15	14,5±0,3	35.7±1.24	33.6±0.45	46.05 (+13.7%)
NP-CuO 5.0	516±1.92	2.5±0.16	15,2±0,5	34.4±1.23	34.1±0.64	45.48(+12.3%)

P 0.05, HCP=0.87.

Besides the increase of seeds number in a head per 2 % as compared with the control on the average in variants, the mass of 1000 seeds has also been 9.9 % higher than the control one in a case with ultra-dispersed humic acids usage, 5.6 % higher with cuprum nano-particles, 9.1 % higher with cuprum oxide and 2.6 % higher with cobalt nano-particles. The increase of productive exponents has led to the spring wheat yield increase. The mass of the seeds from 1 hectare in all experimental variants has exceeded the control one per 15.2 % on the average. The maximum yield has been noticed in a variant with cuprum nano-particles and comprised 47.59 dt/he that has been 17.5 % higher than that of the control one. In a case with the preliminary treatment of seeds before planting with cobalt nano-particles solution the yield has increased per 14.4 %, and in a case with cuprum oxide per 14.7 %.

3. Conclusion

1. We have determined in the lab the maximum residue limit (MRL) of nano-materials: for cobalt NP it is 400 g/h.n.v., Cu NP – 500 g/h.n.v., inorganic salt of $CuSO4$, $CoCl2$ the MRL is 8-10 times lower.

2. The vstudied nano-materials possess high biological activity in a range of optimal concentrations 0.1-5.0 g/h.n.v. and therefore their use at low concentrations will not pollute the environment. When using the nano-particles the germinating energy has increased up to 6 %, the viability up to 10 %, the seedlings length up to 25 %, and their mass up to 30 % as compared with the control ones. For the humic acids these indexes have been 4-10 % lower correspondingly. The biological activity of nano-particles depends on particles size. The maximum size of cuprum nano-particles (200 nm) does not change their morphologic-physiologic indexes and the ferments activity as compared with the control.

3. The activity of peroxudase and superoxide dismutase depends on particles content (cuprum and cuprum oxide). The activity also varies according to the location: roots, seedlings at the same nano-particles concentration. The changes of ferments activity in a case with cuprum nano-powder at concentration up to 500 g/h.n.v. as compared with the control has not exceeded deviations of more than 30 % that shows that biochemical processes go on within the norm.

4. The changes of cuprum nano-particles content from 0.1 to 100 g/h.n.v. has not caused the decrease of spring wheat physiologic processes development. Cuprum nano-powder at concentration up to 100 g/h.n.v. has caused the increase of cytokinins, gibberellin acid and indoleacetic acid. The amount of abscisic acid has lowered per 9.1 %. But cuprum oxide at concentration 100 g/h.n.v. changes these data from 22 to 33 % it is not recommended to use it at concentrations higher than 100 g/h.n.v.

5. We have presupposed the influence of metals nano-particles on plants that differs from the influence of salts and natural sources of biologically active compounds, that is humic acids. The metals nano-particles as distinct from microelements and natural suppliers of biologically active compounds, i.e. humic acids possess the energetic potential and are biological accelerators.

6. These conclusions have been proved by field tests. In a case of preliminary treatment of seeds before planting with a solution of cobalt nano-particles the yield has increased per 14.4 %, with cuprum oxide per 14.7 % and cuprum per 17.5 %. The yield in a case with humic acids has lowered up to 5 %.

References

Churilov, G. I. (2010). Nano-Crystal Metals Effect on Ecologic-Biological State of Soil and Biologically Active Compounds Accumulation in Plants. Herald of Russian University of Nations Friendship. *Series Ecology and Life Safety, 1*, 18-23.

Churilov, G. I., Fomina, M. A., & Ivanycheva, Y. N. (2012). Cuprum and Cuprum Oxide Nano-Powders Influence on Morphological Indexes, Chemical Constitution and State Antioxidative Protection of Winter Vetch. *Herald of Russian University of Nations Friendship, 3*, 67-72.

Churilov, G. I., Nazarova, A. A., Ampleyeva L. E., Polishchuk, S. D., & Cherkasov, O. V. (2010). Biological Effect of Nano-Dimensioned Metals Influence on Different Groups of Plants. Monograph. Ryazan, 148 p.

Churilov, G. I., Polishchuk, S. D., & Chau, N. H. (2012). Ecological and Biological Effects of Nanocrystalline Metals, 6th International Workshop on Advanced Materials Science and Nanotechnology and 6th Korea-Vietnam Joint Symposium on Photonics and Applications. Programme and Abstracts. Vietnam, s. 175.

Churilov, G. I., Polishchuk, S. D., & Nazarova, A. A. (2013 B). Cuprum and Cobalt Nano-Particles Influence on Bull-Calves, Growth and Development. Journal of Materials Science and Engineering, 3, S. 379-385.

Eduardo, C., Pilar, S., Testillano, M. José., Coronado, P., González, M., Rodrigo, F. P., Clara, M. M., Ricardo, I., Jesús, M. de la Fuente, D. R., Alejandro, P. de-L., & María, C. (2009). RisueñoNanoparticle penetration and transport in living pumpkin plants: in situ subcellular identification. *BMC Plant Biology, 9*, 45. http://dx.doi.org/10.1186/1471-2229-9-45

Folmanis, G. E., & Kovalenko, L. V. (1999). Ultradisperse Metals in Agricultural Production. M.: IMET RAS. P. 80.

Foster, L. (2008). Nano-Technologies. Science, Innovations, Possibilities. Moscow. *TechnoSphere,* 352 p.

Gogos, A., Knauer, K., & Bucheli, T. (2012). Nanomaterials in plant protection and fertilization: Current state, foreseen applications, and research priorities. *Journal of Agricultural and Food Chemistry, 60*(39), 9781-9792. ISSN: 0021-8561. http://dx.doi.org/10.1021/jf302154y

Ilyichev, E. I., Nazarova, A. A., Polishchuk, S. D., & Inozemtsev, V. V. (2011). Diet Digestibility and Nutrients Balance when Feeding Calves with Cobalt and Cuprum Nano-Powders. *Diary and Meat Cattle Breeding, 5*, 27-29.

Kutskir, M. V., Nazarova, A. A., & Polishchuk, S. D. (2012). Different Cuprum Micro-Fertilizers Influence on Physiological, Biochemical and Productive Indexes of Spring Wheat. *Ecology and Natural Management.* Selectas of International Symposium on Fundamental and Applied Problems of Science, 135-152.

Kutskir, M. V., Nazarova, A. A., & Polishchuk, S. D. (2012). Cuprum and Cuprum Sulfate Nano-Powders Toxic Effect when Treating Spring Wheat. Materials of the VIIth International Symposium. *Fundamental and Applied Problems of Science,* 126-135.

Nazarova, A. A., Polishchuk, S. D., Stepanova, I. A., Churilov, G. I., & Nguyen, H. C. (2013). Biosafety of the application of biogenic nanometal powders in husbandry. The main 4th International Workshop on Nanotechnology and Application. Vung Tau, Vietnam, S. 291-295.

Polishchuk, S. D., Nazarova, A. A., Stepanova, I. A., Kutskir, M. V., & Churilov, D. G. (2014) Biologically Active Drugs on the Basis of Metals Nano-Dispersed Particles in Agribusiness. *Nano-Technique, 1*(37), 72-81.

Schwab, F., Bucheli, T., Lukhele, L. P., Magrez, A., Nowack, B., Sigg, L., & Knauer, K. (2011). Are carbon nanotube effects on green algae caused by shading and agglomeration? *Environmental Science & Technology, 45*(14), 6136-6144. http://dx.doi.org/10.1021./es200506b

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