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Ecotoxicology Assessment of Waste Water Emitted From Radomir Metal Industries (Bulgaria)

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Abstract. The purpose of this article is to evaluate the toxicological effect of wastewater emitted from „Radomir Metal Industries". It has been registered that the range of 50% mortality (LC_{50}) of great water flea (*Daphnia magna* St.) is limited between 75% and 80% effluent. The data mortality rate-effluent dilution for *Pseudorasbora parva* well correlated with linear regression, R^2=0.86. LC_{50} is reported from exposure to raw sewage (100%). Results indicate that even when individual concentrations of toxic metals are within the permissible limits the effluent remains toxic for the hydrobiota perhaps due to the combine effect of the contaminants.

Keywords: ecotoxicology, heavy metals, effluent, *D. magna* St., *P. parva* Temminck & Schlegels.

Introduction

Metals are perceived as pollutants but they are also naturally existing substances in the environment. Elements like Cd, Hg, Pb, Ni are accepted as priority pollutants for aquatic environment. Most metals do not undergo microbial or chemical degradation and are toxic and their total concentration in soils persists for a long time after their introduction (ADRIANO, 2003; KIRPICHTCH-IKOVA *et al.*, 2006). Their content in aquatic environment is very divers, and it was well known that neither total nor dissolved total metal concentrations are good predictors for their bioavailability and toxicity (NEWMAN, 2008). Many chemical mixtures, with concentrations of individual chemicals considered as nontoxic, are often presented in aquatic systems. However, it is reckoned that such chemical mixtures where individual constituents are present at low, non-toxic concentrations may trigger toxicity due to additive or synergistic effects among the constituents (RAJAPAKSE *et al.*, 2002; MONTVYDIENE & MARCIULIONIENE, 2004).

The surface water environment is under the influence of continuous industrial pressure. It has long been recognized that the formation of organic and inorganic metal complexes and metal sorption to particulate material reduces metal bioavailability and toxicity in the water compartment (LUCK *et al.*, 2008; SAEEDI *et al.*, 2004). Bio-assessment can be used as a tool to detect the presence of hazardous chemicals in the environment evaluating the effects of mixtures with the

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combined effects that can be expressed as synergism, additivity and antagonism demonstrating bioavailability of contaminants to different species (LOUREIRO *et al*., 2006; LANDIS *et al*., 2011; LYUBENOVA & KALCHEV, 2011; LYUBENOVA *et al*., 2012). However assessment of water quality in the presence of mixture of pollutants by using single–species biotest may be insufficient for a more biologically complex system; therefore, organisms of different phylogenetic levels and ontogenesis have to be involved in these investigations (MONTVYDIENE & MARCIULIONIENE, 2004).

The purpose of article is to evaluate the toxicological effect of wastewater emanated from Radomir Metal Industries".

Material and Methods

Study area. Bulgarian metal casting plant Radomir Metals is based the southwestern town of Radomir, Pernik district. The plant has been constructing on 1 800 000 m^2 area and situated at about 50 км south-west of the capital of the Republic of Bulgaria - Sofia, on the road E79 (Coordinates: 42°31'10"N 22°59'12"E). The purification facilities fully meet the requirements of the European Standards for the quality of the air and water (http://radomirmetal industries.com/en/application/index).

„Radomir Metal Industries" has a complex permit № 145-NO/2008 on within the scope of paragraph 2.2. Annex 4 of the Law on Environmental Protection. Under the complex permit "Radomir Metal Industries has established annual emission standards presented in Table 1.

It was reported (PASKALEV, 2001), that there are some strongly polluted sections in Struma River – after the towns of Pernik, Kjustendil, and Dupnitza. Sources of pollution are the industrial wastewater of the towns of Radomir and Pernik, as well as domestic sewage of some of the bigger settlements (the town of Blagoevgrad and others). In the area of enterprise "Radomir Metal Industries" soil and water monitoring at several different points are conducted. According to the data of Basin Directorate - West Region for wastewater, even those emitted from the production are in the emission rate.

Table 1. Direct annual pollutant emission standards

Pollutants	**Mixed stream wastewater (industrial and domestic-faecal) kg.year^{-1}**
Total nitrogen	709.40
Total phosphorous	77.45
Arsenic and its compounds	1.54
Cadmium and its compounds	0.15
Chromium and its compounds	1.54
Copper and its compounds	8.52
Mercury and its compounds	0.15
Nickel and its compounds	0.42
Lead and its compounds	1.54
Zinc and its compounds	15.02
Phenols	4.64
Total organic carbon	6954.5
Chlorides	-
Cyanides	0.57

"Radomir Metal Industries" has owns water treatment plant. Purification is a mechanical, biological, as is done drying and stabilization of sludge. Receiver of purified water was the Struma River (II - Second category receiving water). Along the outlet wastewater to the water treatment point, canal water is intensively used for irrigation. Industrial wastewater is discharged to the water purification plant through a channel running through arable land. In this regard, research in this work is done to establish whether there is compliance with established standards.

Characteristics of the test – objects. The biological tests evaluated the toxicity of wastewater, polluted air, soil, sediment, etc. or a particular pollutant using standard test organisms. The latter are exposed to different concentrations of the substance and report mortality or change in morphology and physiology of organisms. In determining the toxicity we followed standard protocols in order to have comparability of results.

For the purpose of study are conducted tests with test-objects: *Daphnia magna* (ISO 6341/1996, 10); *Pseudorasbora parva* (ISO 7346/1:1996, 10). The aims of bioassays are to determine the substance concentration or dilution of water waste, in which occurred 50% mortality (LC_{50}) or change in the appropriate indicator in the test-organisms for determined time.

In assessing the toxicity of the effluent of "Radomir Metal Industries" are using change in mortality *of D. magna* (ISO 6341/1996) and *P. parva* (ISO 7346/ 1:1996) respectively, at a constant temperature 20±2°C.

By pretesting a series of concentrations (in %) for the final testing are defined: 100, 95, 90, 85, 75 и 70 where the *D. magna* mortality percentage is rendered in account. Used for testing fish of species *P. parva* are from one and the same generation and with approximately the same dimensions (length ± 5 mm). The sample has been acclimatized in aquaria (1 l aerated water for a fish) and normally fed two weeks until the test beginning. The fish is not fed and the water is not aerated during the test. For the final testing the following dilutions are used: 20%, 40%, 60%, 80%, 100% included smallest lethal and highest not lethal dilution, defined by the pre-testing, for 48 h in a two repetitions. The tests are conducted in aquariums with an individual volume of 10 l (with the appropriate dilution of the effluent and the control with a boiled tap water) and with 10 fishes in every aquaria. The dead fishes have been removed two times a day. In 24 h during the test the wastewaters at different dilution have been renewed and the alive fishes have been moved to the new aquariums. LD_{50} is calculated by graphical interpolation (ISO 6341/1996, ISO 7346/1:1996).

The toxicity of Zn ($Zn_2SO_4.7H_2O$) on *P. parva* is also been tested using concentrations: 0, 0.001, 0.010, 0.030, 0.040, 0.050 μg.l^{-1}.

Sample analysis

Reagents. All solutions were prepared with analytical reagent grade chemicals and ultra-pure water (18 MΩ cm) generated by purifying distilled water with the Milli-QTM PLUS system

Nitric acid: Suprapur HNO_3 (67% v/v) was purchased from Fluka.

The stock standard solutions of Cd, Cu, Cr, Fe, Hg, Ni, Pb and Zn 1000 mg.l^{-1} were Titrisol, Merck in 2% v/v HNO_3 and were used to prepare calibration standards.

Sampling. Fish samples (whole fish body) are thoroughly washed with MQ water. The fish specimens were dissected and samples of fish gills are quickly removed and washed again with MQ water. Fish gills were analyzed as obtained without further homogenization. Fish gills (sample amount between 0.1 and 0.3 g) were digested with nitric acid in MW oven (step 1 - 250 W for 3 min; step 2 - 400 W for 3 min, step 3 - 600 W for 3 min), solutions cooled, transferred in 5 ml volumetric flask and diluted up to the mark with Milli-Q water.

Instrumentation. Determination of Fe and Zn: Flame atomic absorption spectrometric measurements were carried out on a Perkin Elmer Zeeman 1100 B spectrometer with an air/acetylene flame. The instrumental parameters were optimized in order to obtain maximum signal-to-noise ratio.

Determination of Cd and Pb in fish gills: Electrothermal atomic absorption spectrometric measurements were carried out on a Perkin Elmer Zeeman 3030 spectrometer with an HGA-600 atomizer. Pyrolytic graphite-coated graphite tubes with integrated platforms were used as atomizers. Pd as $(NH_4)_2PdCl_4$ was used as modifier for ETAAS measurements of Cd. Pretreatment temperatures used were 500 °C for Cd and Pb and atomization temperatures 1300 °C for Cd and 1900 °C for Pb.

Determination of Cd, Cu, Cr, Ni, Pb in water samples: Samples measured by ETAAS under optimized instrumental parameters.

Determination of Hg in water samples: Water samples were previously digested with 1 ml HNO_3, Mercury content was measured by cold vapour AAS (Varian AA 240 atomic absorption spectrometer equipped with a continuous flow VGA-77 Vapor Generation Accessory) under optimal instrumental parameters.

Assessment of water contamination. Biochemical oxygen demand (BOD) and chemical oxygen demand (COD) have been determined for the sewage (ISO 6060:1989). In environmental chemistry, the chemical oxygen demand (COD) test is commonly used to indirectly measure the amount of organic compounds in water. Most applications of COD determine the amount of organic pollutants found in surface water (e.g. lakes and rivers), making COD a useful measure of water quality. It is expressed in milligrams per litre ($mg.l^{-1}$), which indicates the mass of oxygen consumed per litre of solution. Older references may express the units as parts per million (ppm).

Statistics. All obtained results were statistically processed. It has been shown that the resulting averages are representative of performance using t-test. The statistical significance level in this study was defined at $p < 0.05$.

Results

Results of samples from wastewater

The table below presents the results of some additional analysis of samples of wastewater and limit concentrations of metals under Regulation №6 (Table 2). The marginal limit rate are defined in the particular circumstances of production taken into account for the manufacture of iron and steel, production of iron and steel castings, cast cars and other non-ferrous metals.

The results show that the resulting concentrations are within the limits of regulation.

Table 2. Average values with standard deviation of the analyzed parameters of samples effluent compared to the Marginal limit concentrations

Elements	Marginal concentrations*, $\mu g.l^{-1}$	Acidified sample, unfiltered, $\mu g.l^{-1}$	Not acidified sample, unfiltered, $\mu g.l^{-1}$
Cd	500	3.2±0.2	2.3±0.2
Cu	500	12.0±2.0	8±1
Cr	500	2.8±0.2	2.1±0.1
Fe	5000	189±14	156±12
Ni	500	4±1	3±1
Pb	200	3.8±0.1	2.1±0.1
Zn	2000	230±15	190±12
Hg	10	<1	<1
Other indicators	pH – 7.92 Chemical oxygen demand (COD) (BBM 0208:2001; t = 20^0±3) - 17.2±1.63 COD (oximetric) 20.48 $mg.l^{-1}$ – filtered sample; 73.10 $mg.l^{-1}$ – unfiltered sample	NH_4^+ - 0.2 $mg.l^{-1}$ NO_3^- - 5.7 $mg.l^{-1}$	SO_4^{2-} - 19.5 $mg.l^{-1}$ Cl – 10 $mg.l^{-1}$ PO_4^{3-} - < 0.1 $mg.l^{-1}$l

Results from the test with D. magna and P. parva

Daphnia magna

The experiments were carried out with *D. magna*, the rate of mortality (LC_{50}) have been determined. The dose-response curve plotted as a cumulative number of dead organisms by each dose using is represented (Fig. 1). The dose-effect of the effluent on the mortality rate of *D. magna* correlated well as a linear regression (R^2=0.956).

With the increasing of dilution the toxicity is reduced. Test results showed that the range of 50% mortality appears approximately at 86% effluent concentration (undiluted effluent is 100% in the scale, Fig. 2).

Pseudorasbora parva (Temminck & Schlegel, 1846) Stone moroko

The dependence of mortality of *P. parva* from effluent with different dilutions is showed on Fig. 3.

The dependence of mortality of P. *parva* from different concentration of Zn in the water is showed on Fig. 4.

Conducting the tests with selected dilutions showed that surviving is superior to that of *Daphnia magna*, LC_{50} is reported from exposure to raw sewage (100%).

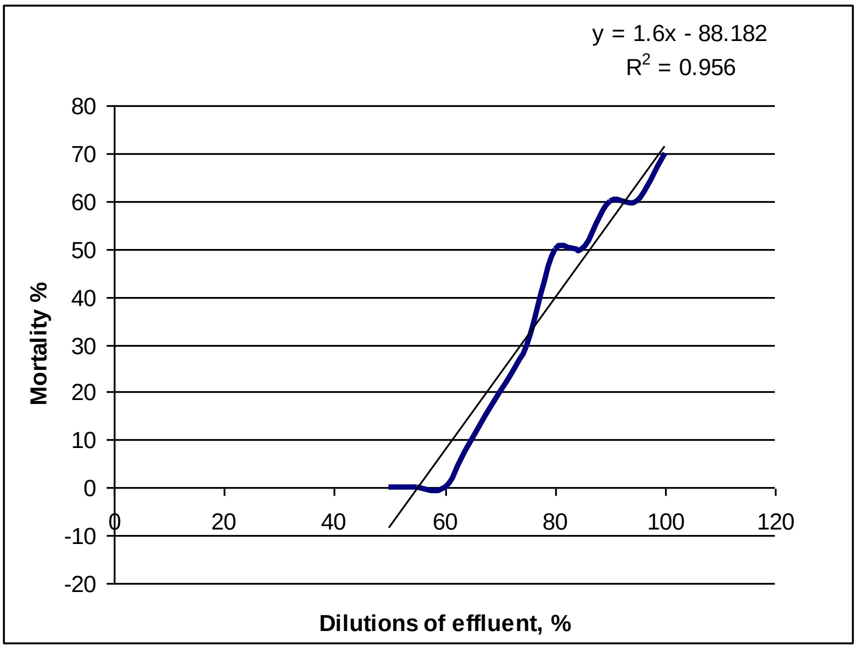

Fig. 1. Dependence of mortality of *D. magna* by diluting the effluent

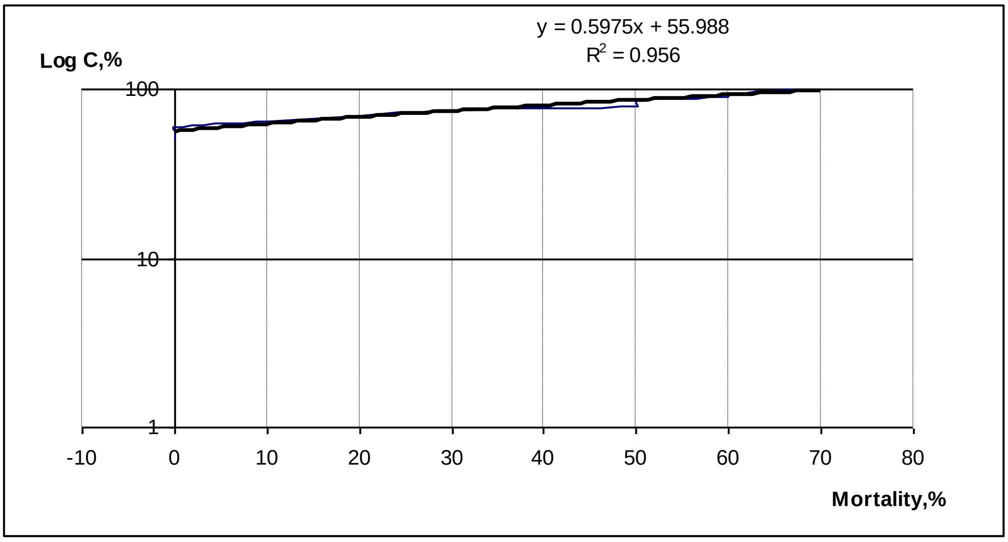

Fig. 2. LC $_{50}$ for *D. magna* under different dilutions of effluent

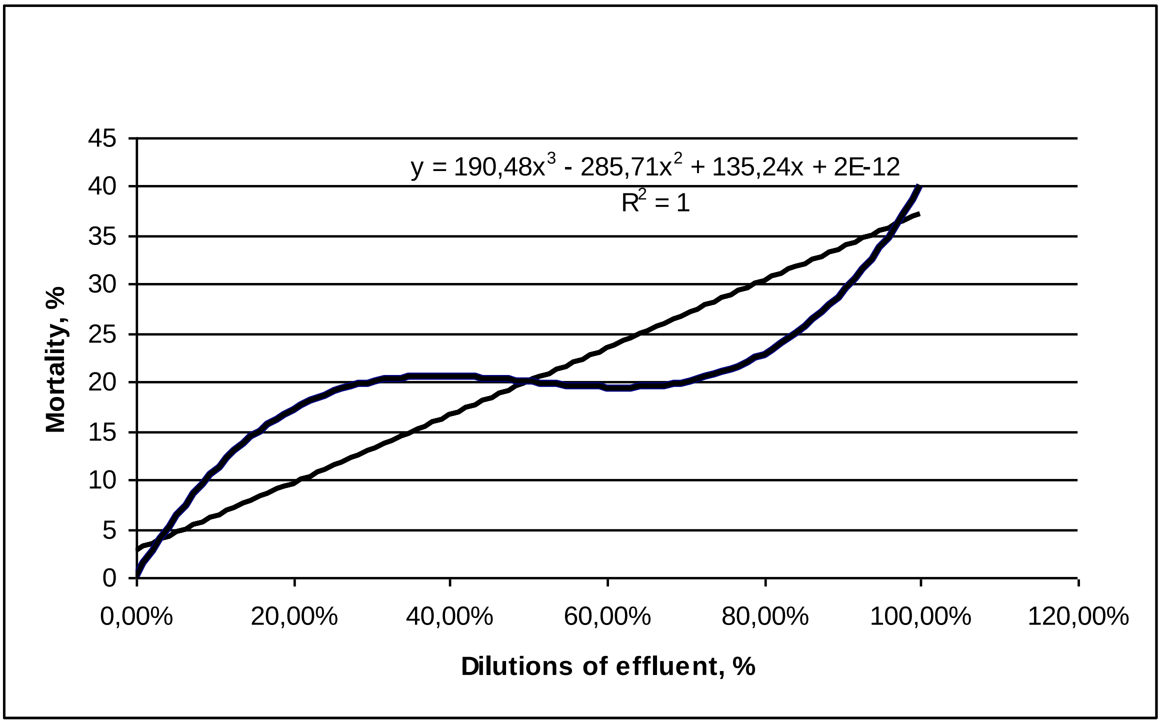

Fig. 3. Dependence of mortality of *P. parva* from effluent with different dilutions

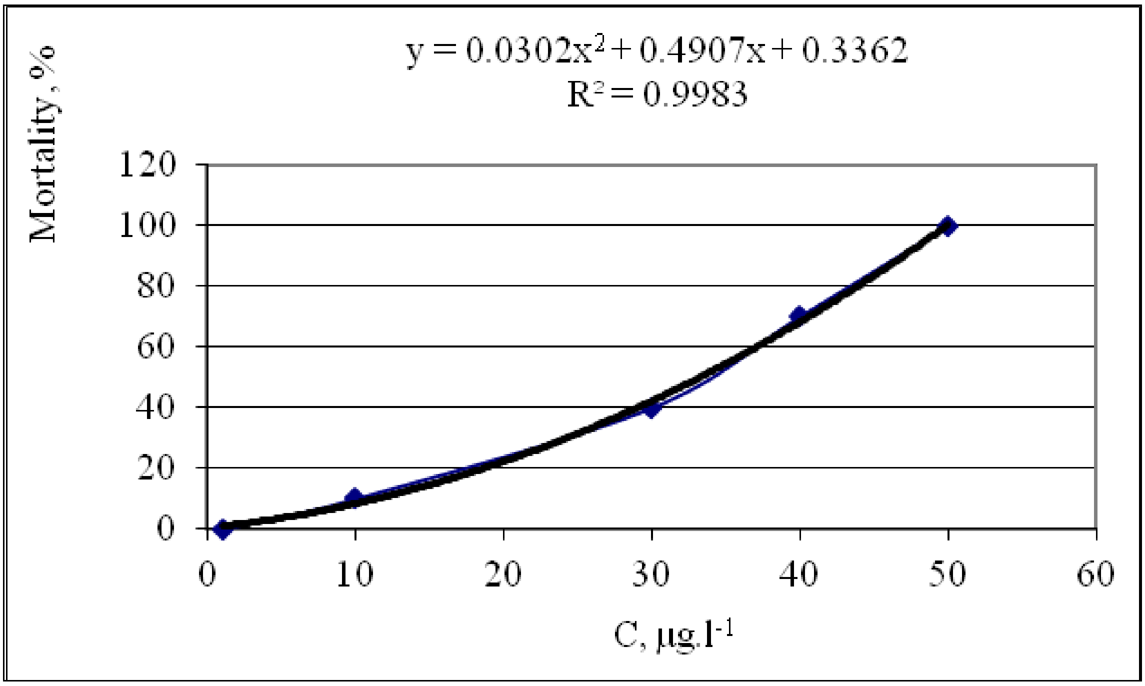

Fig. 4. LC $_{50}$ for *P. parva* under different concentration of Ni in the water (mg.l^{-1})

In all samples of gill Cd is <0.01 ppm, and Pb - <0.1 ppm. For untreated fish, found in the gills content of Fe and Zn is respectively 2.8 and 3.9 ppm. The contents of only two of the studied heavy metals in the gills of *P. parva* increases - low for Zn (1.12 times) and almost double for Fe (1.7 times) compared to untreated specimens.

Discussion

Daphnia magna

D. magna is a Crustacean in the order of *Cladocera*. This aquatic animal extensively used as a test organism in aquatic toxicology due to their small size, short life cycle and amenability to lab culture. *D. magna* is the most sensitive (PETERS & BERNARDI, 1987). *D. magna* are used as a model organism to simulate response of wide ranges of aquatic invertebrates to toxicants (LANDIS *at al*., 2011; PETERS & BERNARDI, 1987; VEDLEGG, 2004; VILLAVICENCIO, 2005). The great water flea (*D. magna*) has been reported as the most sensitive test-object in relation of different pollutants (organophosphates, organochlorines, heavy metals, pyrethroids etc.) among all known biological objects including experimental animals (PETERS & BERNARDI, 1987; Vedlegg for Cyprodinil Kilde: Svensk undersøkelse, 2004). The test of mortality has been also reported as the most effective and reliable (HERMENS *et al*., 1984) with high degree of predictive performance (VILLAVICENCIO, 2005).

The dose-effect of the effluent on the mortality rate of *D. magna* correlated well as a linear regression with correlation coefficient R^2=0.956 (fig.1). The observed tendency is a typical pattern for the dependence of cumulative mortality versus environmental concentration or dose of the toxicant (LANDIS *et al*., 2011). The comparable dose-response curve has been reported when the effect of different concentrations of four compounds on *D. magna* mortality was investigated (KUNGOLOS *et al*., 2001).

Relationship between the concentration of dissolved copper in the overlying water and the mortality of exposed D. *magna*, yielding an estimated LC_{50} of 26 mg.l^{-1} dissolved copper (GILLIS *at al*., 2006). Water analyses of effluent from the plant of heavy industry "Radomir Metal Industries", given in table 2, showed that the amount of cooper in the waste water is approximately 12±2 mg.l^{-1}. The acute toxicity has been studied of heavy metal chromium as well as tannery effluent (chromium containing) water has been evaluated by laboratory bioassay experiments for 48 h duration in water flea, *D. magna* (TALAPATRA & BANERJEE, 2005). The median lethal concentration (LC_{50}) value of chromium metal for 48 h duration was determined to be 0.4027 ppm. Whole effluent toxicity (WET) test confirmed median lethal concentration (LC_{50}) values of tannery effluent water for 48 h duration was 6.540% dilutions respectively. Chromium content in tannery effluent water was found 3.47 ppm or it is approximately 0.225 ppm (TALAPATRA & BANERJEE, 2005). The data in our experiments is showed that the content of Zn and of Cr in wastewater is relatively 0.00189± 0.0014 and 0.0028 ± 0.0002 ppm (table 2). The survival rate of great water flea (*D. magna*) in our study showed that the effluent from the plant of heavy industry "Radomir Metal Industries" is toxic to the test hydro biota, although the concentrations of individual heavy metals are within the allowed limits, accepted from the Regulation № 6. The effluent remains toxic perhaps the combine affect that also occur. It has been reported that the joint toxicity of the mixtures of pollutants remains much higher than that of the individual chemicals (HERMENS *et.al*., 1984).

Pseudorasbora parva (Temminck & Schlegel, 1846) Stone moroko

Stone moroko (also known as topmouth gudgeon), *P. parva*, is a fish belonging to the Cyprinid family, native to Asia, but introduced and now considered an invasive species in Europe (WITKOWSKI, 2006). A review of literature on the ichthyofauna of lowland rivers of Central Europe (WITKOWSKI *et al.* 2000, 2004) indicates that the species is now a constant component of the Central European fauna, and is often a dominant. *Pseudorasbora parva* is a small planktivore and feeds mainly on aquatic insects, algae, and zooplankton (ZHANG *et* *al*., 2000; XIE *et al*., 2000; YANG *et al*., 2004). In both its original distribution range and in secondarily invaded areas the stone moroko inhabits shallow lakes, carp ponds, irrigation canals, ditches, slow sections of lowland rivers and their oxbows. It prefers much-vegetated places (BERG, 1949; KOZLOV, 1974; MUCHACEVA, 1950; WITKOWSKI, 2000).

The data mortality rate-effluent dilution well correlated with polynomial equation $190,48x^3 - 285,71x^2 + 135,24x + 2E\text{-}12$, $R^2=1$ (Fig.3). Conducting the tests with selected dilutions showed that surviving is superior compare to that of daphnia. LC_{50} is reported from exposure to raw sewage (100%). *P. parva* is generally regarded as a pest due to its very high reproductive rate, which gives rise to dense populations of fish (WELCOMME, 1988). This species is more resistant than many European fish species to a moderate degree of pollution, elevated temperatures, and low water levels. There is evidence that it also can move a limited distance through polluted water (BANARESCU, 1999). High success in population growth and the colonization rate of P. *parva* could be explained by its wider ecological physiological tolerance (ROSECCHI *et al*., 1993; 2001). Our results confirm that this species has high tolerance to the water pollution and that wastewater has slight toxic effect on populations of hydro biota. Nevertheless the tested toxicity of Zn is expressed by ascending polynomial curve (fig. 4), where $LC_{50\%}$ for 48 h treatment is 33, 44 $mg.l^{-1}$. Perhaps the combine effect between the toxicants in effluent that occurs is expressed by inverse antagonism (NEWMAN, 2008).

Conclusions

The concentrations of the toxicants in the waste waters are under limits. Nevertheless the toxic effect of metal plant sewage on the tested hydro biota has been registered. For the great water flea (*D. magna*) 48 h LC_{50} appears approximately at 86% effluent concentration. The combined toxic effect of the effluent on the two tested species is different. The surviving of *P. parva* is superior to that of *D. magna*. The 48 h LC_{50} is reported from exposure to raw sewage (100%). Even the concentrations of individual heavy metals are within the allowed limits the effluent remains toxic due to the combine effect of toxicants in waste waters that occurs. For the *P. parva* the inverse antagonism is observed. Also the gills content of Fe and Zn increases in treated fishes.

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