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Eco-toxic effects of lead and nickel on survivability, reproduction and growth of earthworm (*Eudrilus eugeniae*)

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Abstract
The present study was carried out to determine the toxic effects of lead (Pb) and nickel (Ni) on the survivability, reproduction and growth of earthworm, *Eudrilius eugeniae*. Adult *E. eugeniae* were exposed to the varying concentrations of Pb and Ni. Mortality, growth and cocoon production were measured at every 30 days interval for 90 days. The results suggest clear dose-dependent negative effects of Pb and Ni on survivability, reproduction and growth of earthworms. A maximum reduction (43.35%) in survivability was observed in the case of Pb (0.06 ppm) followed by Ni+Pb (0.03+0.03 ppm) in which there is 31.65% decrement in survivability was observed. A similar trend was observed in growth and reproduction studies. It was concluded that the heavy metal Pb was more noxious to *E. eugeniae* as compared to Ni alone as well as in combinations. These results can be used for environmental monitoring and estimation of heavy metals contamination in soil.

Keywords: Cocoon, *Eudrilus eugeniae*, growth, lead, nickel, survivability

Introduction

Earthworms constitute an important group of soil organisms, which play a vital role in enhancing physicochemical and biological properties of soil as they assist in releasing and recycling of nutrients by breaking down a large variety of organic matter by their feeding and burrowing activities (Singh *et al.*, 2012) [15]. They also aid in improving the fertility of the soil. If the earthworms in any ecosystem are in danger then the development of that particular ecosystem is very difficult (Dutta and Dutta, 2016) [6]. Nowadays soil pollution from heavy metal contamination has become a serious concern as it causes ground water contamination and biomagnification of heavy metals through food webs and therefore, considered as a major environmental issue (Loureiro *et al.*, 2005; Kaur and Hundal, 2016) [12, 9]. Earthworms act as an important link in the food webs by transferring energy from dead and decaying organic matter to higher trophic levels by acting as a prey for birds and terrestrial vertebrates (Parihar *et al.*, 2019) [13]. The biomagnification of heavy metals within the food chain causes serious harm at higher trophic levels (Sivakumar, 2015) [16]. Although heavy metals exist lithologically in the ground, their concentration in the soil is increasing because of different sources, such as intensive use of biocides and fertilizers in agriculture, industrial activities, mining and waste deposition (Ali and Naaz, 2013) [2]. Earthworms are significantly affected by heavy metal contamination due to their strong interactions with the soil system. Among other soil invertebrates, they have got more attention because of their ecological importance (Vasseur and Bonnard, 2014) [21]. They are exposed to heavy metals through their skin and digestive system. Elevated concentration of heavy metals in soil can result in several destructive effects on earthworms such as reduced density, viability, cocoon production, growth and sexual development (Kaur and Hundal, 2016) [9] and thus indirectly affect the soil properties (Wang *et al.*, 2018) [22]. Earthworms have therefore been used as biomarkers to spot contaminated soils and are used as a modal organism for terrestrial ecotoxicology studies (Tang *et al.*, 2016) [18]. In eco-toxicological studies, standardized acute and chronic toxicity tests on earthworms have proven to be useful in evaluating the possible risks of soil contaminants and in risk estimation procedures (Zaltauskaite and Sodiene, 2010) [24]. Acute toxicity tests measure lethality and detect qualitative effects. On the other hand, chronic toxicity tests are used for detecting more subtle effects such as reduced fertility, retarded development, and teratogenic effects, based on inhibition of earthworm reproduction (OECD 2004; Alves *et al.*, 2013) [3].

These tests give a more ecologically pertinent sub-lethal endpoint than lethality. In eco-toxicological estimations reproduction is of pivotal importance because it influences population dynamics (Kaur and Hundal, 2016) [9]. Hence, the present study is set out to explore the effects of lead and nickel on survivability, growth and reproduction of *E. eugeniae*.

Materials and Methods

Experimental animals

African nightcrawler (*E. eugeniae*) procured from the vermicomposting unit situated at the Department of Zoology & Aquaculture, CCS HAU Hisar was used in conducting the experiment. All were healthy adults with well-developed clitellum region and belongs to the third generation to avoid any chances of pre-exposure to heavy metals.

Preparation of substrate

The culture of earthworms was maintained in tubs filled with cow dung which was obtained from the Biogas Plant of Department of Microbiology, CCS HAU, Hisar. Cow dung exempted unwanted debris was macerated with hands and lay open to pre-decomposition for 15 days before using as a substrate. After 15 days adult worms were released in the tubs for their culture. The experimental tubs were covered with gunny bags to avoid direct sunlight. Watering and monitoring of the tubs were continued thereafter.

Experimental setup

The experiment was conducted for a period of 90 days to determine the toxic effect of Pb and Ni on earthworms. Tubs containing twenty adult earthworms were sprayed with different concentrations of Lead nitrate [$Pb(NO_3)_2$] and nickel chloride ($NiCl_2$). The details of the treatments given are mentioned in Table 1. Triplicates of each treatment were maintained. Proper temperature, moisture and aeration were maintained throughout the experiment.

Table 1: Description of treatments given to earthworms along with control

Sr. No.	Treatment	Concentration (ppm)
1.	Control	No treatment of heavy metals
2.	Lead	0.02, 0.04, 0.06
3.	Nickel	0.02, 0.04, 0.06
4.	Lead + Nickel	0.01+0.01,0.02+0.02and 0.03+0.03

Experimental procedure

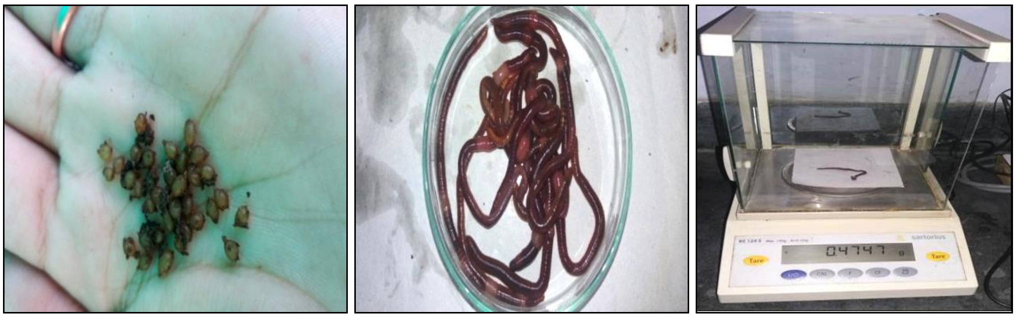
After the interval of 30 days mortality was measured by counting the surviving worms in each tub and growth was measured by measuring the length and weight of earthworms. The worms were considered alive if they were able to respond to a mechanical stimulus.

Cocoons were collected by sorting through the cow dung. The number of cocoons was compared to the survivability data to get a cocoon production rate, i.e. cocoons/worm/month.

Statistical analysis

Completely randomized block design was used for the screen house study. The data collected were subjected to ANOVA at 0.05% level of significance for computing the degree of variance among the different treatments given in the experiment.

Data were analyzed using Software ‘OPSTAT’, created at the Computer Center, College of Basic Sciences and Humanities, CCS Haryana Agricultural University, Hisar.



A B C

Fig 1: (A) Cocoons of *Eudrilus eugeniae* (B) Adult *Eudrilus eugeniae* (C) Weighing of Earthworm

Results and Discussion

This section summarizes and discusses the main findings of the work. The study has shown that exposure of lead (Pb) and nickel (Ni) has a negative impact on the survivability, cocoon production and growth of earthworms at a varied rate.

Effect of heavy metals on the survivability of *E. eugeniae*

The earthworm's mortality is often used as a reliable indicator of environmental pollution. Data on the survivability of earthworms exposed to different concentrations of Pb and Ni is presented in table 2. Throughout the experimental period, no earthworms died in control. However, compared to the control, there was a significant reduction in the number of earthworms exposed to heavy metals on the final day (90th) of the study. It was found that survivability of earthworms' decreased as the concentration of heavy metals increased. The maximum decrement (43.35%) in survivability was observed in worms exposed to Pb (0.06 ppm) followed by worms exposed to Ni+Pb (0.03+0.03 ppm) in which there is 31.65% decrement in survivability was observed. While 26.65%, 18.35% and 11.65% decrement in survivability have been seen at Ni (0.06 ppm), Ni (0.04 ppm) and Ni (0.02 ppm), respectively. The toxicity of a chemical to earthworms relies upon the feeding strategies followed by the species of earthworms and on the supply of the pollutant (Schon *et al.*, 2017) [14]. *E. eugeniae* response was more pronounced to Pb than to Ni. The higher toxicity of Pb may be due to the reason that earthworms store Pb in a non-toxic form and die when the stored lead attains a critical concentration (Morgan and Morgan, 1988) [11]. Our results are in agreement with the findings of Zhang *et al.* (2013) [26] who observed that the survival rate of *E. fetida* was 80% in 14 days and 60% in 28 days in the presence of 300 mg kg^{-1} of Al. The observations are also in line as reported by Garg *et al.* (2009) [8] who observed 100% mortality in *Eisenia fetida* and *Allolobophora parva* at all concentrations of Cu (500, 1500 and 2500 mg kg^{-1}). Similarly, Annapoorani (2014) [1] reported that reproduction and survival rate was higher in the control than Al treatments.

Table 2: Effect of different concentrations of heavy metals on the survivability of the earthworm, *E. eugeniae*

Sr. No.	Treatments	Number of Adult Earthworms		
		30 DAT	60 DAT	90 DAT
1	Control	21.00±0.00^{e}	23.00±0.57	24.67±0.33
2	Ni (0.02 ppm)	19.33±0.67^{d,e}	18.33±0.33^{c}	17.67±0.67^{b}
3	Ni (0.04 ppm)	18.00±0.58^{c,d}	17.00±0.58^{c}	16.33±1.20^{b}
4	Ni (0.06 ppm)	16.67±0.67^{b,c}	15.67±0.67^{b}	14.67±0.88^{a}
5	Pb (0.02 ppm)	18.67±0.33^{d}	17.67±0.33^{c}	16.33±0.88^{b}
6	Pb (0.04 ppm)	16.67±0.33^{b,c}	16.33±0.67^{b}	15.00±1.15^{a}
7	Pb (0.06 ppm)	14.67±0.88^{a}	13.33±0.33^{a}	11.33±0.33
8	Ni+Pb (0.01+0.01 ppm)	19.00±0.58^{d}	18.00±0.57^{c}	17.00±0.58^{b}
9	Ni+Pb (0.02+0.02 ppm)	18.00±0.58^{c,d}	16.67±0.67^{b,c}	15.67±0.67^{a,b}
10	Ni+Pb (0.03+0.03 ppm)	15.33±0.67^{a,b}	15.00±1.15^{a,b}	13.67±0.33^{a}
	SE(m) ± CD (P=0.05)	0.58 1.71	0.63 1.88	0.77 2.28

Mean ± S.E
*DAT: Days after treatment.
Values with the same superscript in the same column do not differ significantly

Effect of heavy metals on the cocoon production of *E. eugeniae*

The analysis of earthworm reproduction is a useful method for the estimation of contaminants present in the soil (Hirano and Tamae, 2011) [7]. Table 3 summarizes the results of reproduction studies carried out to check the effect of various concentrations of Pb and Ni on the cocoon production. From the table, it can be inferred that there was a significant reduction in the cocoon production in earthworms exposed to heavy metals in comparison to control. Pb (0.06 ppm) showed the maximum decline (41.72%) in cocoon production. Ni also had an adverse effect on the production of cocoons as the number of cocoons decreased in a dose-dependent manner. But Pb found to be more toxic than Ni. The negative impact of heavy metals on cocoon production maybe because of the detrimental effects of these metals on the sperm morphology, due to which the viable sperm counts reduce and result in a low reproductive rate (Basha and Latha, 2016) [4]. One more reason may be the energy requirements as energy is required for reproductive processes, differentiation to reach maturity and egg production (Kooijman, 1986) [10]. If energy requirement is increased for metal sequestration and elimination, this will limit the energy available for sexual maturation and ultimately results in slow maturation in animals exposed to the metal contaminated soils. The results of the present study are in agreement with the work of Zaltauskaite and Sodiene (2010) [24] who observed that different Pb concentrations significantly inhibited the cocoon production rate (29-65%), and no production was observed in soil having the highest level of Pb (2500 µg g^{-1}). Detrimental effects of heavy metals on cocoon production have been previously reported by Yadav *et al.* (2017) [23] and Urmila *et al.* (2019) [20]. A similar response to cadmium exposure, i.e. absent or delayed maturation and reduced cocoon production, was observed in juveniles of *E. fetida* (Zaltauskaite and Sodiene, 2014) [25].

Table 3: Effect of different concentrations of heavy metals on the cocoon production of the earthworm, *E. eugeniae*

Sr. No.	Treatments	Number of Cocoons per twenty worms		
		30 DAT	60 DAT	90 DAT
1	Control	82.33±2.03	91.00±1.15	101.67±4.26
2	Ni (0.02 ppm)	72.67±2.19^{d}	64.67±1.45^{c}	61.67±2.33^{d}
3	Ni (0.04 ppm)	65.67±1.20^{b,c}	56.33±2.73^{b}	46.33±3.18^{b,c}
4	Ni (0.06 ppm)	53.00±1.73^{a}	40.00±1.53^{a}	39.00±1.73^{a}
5	Pb (0.02 ppm)	69.00±1.73^{c,d}	55.00±2.31^{b}	49.00±1.53^{c}
6	Pb (0.04 ppm)	58.00±1.15	46.33±2.40^{a}	40.33±0.88^{a,b}
7	Pb (0.06 ppm)	42.33±1.45	31.33±2.40	24.67±1.45
8	Ni+Pb (0.01+0.01 ppm)	71.00±1.53^{d}	66.33±2.60^{c}	56.00±1.73^{d}
9	Ni+Pb (0.02+0.02 ppm)	63.33±0.88^{b}	57.33±2.33^{b}	47.33±2.60^{c}
10	Ni+Pb (0.03+0.03 ppm)	50.67±1.45^{a}	43.00±2.08^{a}	38.00±1.73^{a}
	SE(m) ± CD (P=0.05)	1.58 4.70	2.16 6.42	2.34 6.95.

Mean ± S.E
*DAT: Days after treatment.
Values with the same superscript in the same column do not differ significantly

Effect of heavy metals on the growth of *E. eugeniae*

The growth was measured in terms of weight and length of earthworms. The results demonstrate the significant effect of Pb and Ni on the growth of earthworms in a time and dose-dependent manner (Table 4 & 5). Growth was found to be the most prominent in the control (0.84g and 7.93cm) and lowest (0.43g and 4.47cm) when exposed to the highest Pb concentration i.e. Pb (0.06 ppm). Ni+Pb (0.03+0.03 ppm) was the second most toxic concentration. Non-significant reduction of growth in worms exposed to various treatments of nickel was also worth noticing. Due to the elevated concentration of a given pollutant growth can be affected by direct and indirect effects. Growth can be affected by direct toxic effects on the physiology of exposed worms, or indirectly by changes in the energy requirements because the exposed organisms have to use their energy in metabolism, detoxification or sequestration, and excretion of the contaminants. This increased requirement for maintenance energy will reduce the energy available for the growth of exposed worms (Donker *et al.*, 1993) [5]. The results are in line with the findings of Tejada *et al.* (2010) [19] who observed that earthworm weights decrease with time and with an increase in the Al concentration in polluted soil. Shefali *et al.* (2018) [17] reported the significant difference in body weight of worms treated with arsenic and chromium after the 28th day of treatment as compared to control. The weight of worms was significantly affected by high levels of heavy metals in the study of Kaur and Hundal (2016) [9]. Reduction in body length of earthworms due to Pb and Ni exposure has also been reported by Urmila *et al.* (2019) [20].

Table 4: Effect of different concentrations of heavy metals on the body length of earthworm, *E. eugeniae*

Sr. No.	Treatments	Body length of earthworms (cm)		
		30 DAT	60 DAT	90 DAT
1	Control	7.33±0.09	7.63±0.22	7.93±0.20
2	Ni (0.02 ppm)	6.66±0.15^{c}	6.77±0.20^{c}	6.47±0.12^{c}
3	Ni (0.04 ppm)	6.27±0.20^{b}	6.40±0.06^{b,c}	6.13±0.03^{b,c}
4	Ni (0.06 ppm)	5.87±0.14^{a}	5.97±0.19^{a}	5.70±0.26^{a,b}
5	Pb (0.02 ppm)	6.47±0.14^{b}	6.60±0.21^{c}	6.23±0.09^{c}
6	Pb (0.04 ppm)	5.87±0.30^{a}	6.07±0.12^{a,b}	5.70±0.15^{a,b}
7	Pb (0.06 ppm)	4.70±0.15	4.97±0.18	4.47±0.20
8	Ni+Pb (0.01+0.01 ppm)	6.57±0.24^{b,c}	6.67±0.09^{c}	6.37±0.09^{c}
9	Ni+Pb (0.02+0.02 ppm)	6.00±0.25^{a,b}	6.20±0.23^{b}	5.87±0.18^{b}
10	Ni+Pb (0.03+0.03 ppm)	5.43±0.19^{a}	5.57±0.18^{a}	5.30±0.15^{a}
	SE(m) ± CD (P=0.05)	0.19 0.58	0.17 0.52	0.16 0.48

Mean ± S.E
*DAT: Days after treatment.
Values with the same superscript in the same column do not differ significantly

Table 5: Effect of different concentrations of heavy metals on the body weight of earthworm, *E. eugeniae*

Sr. No.	Treatments	Body weight of earthworms (g)		
		30 DAT	60 DAT	90 DAT
1	Control	0.76±0.01^{c}	0.80±0.03	0.84±0.01
2	Ni (0.02 ppm)	0.73±0.01^{b,c}	0.64±0.02^{c}	0.57±0.02^{c}
3	Ni (0.04 ppm)	0.69±0.03^{b}	0.57±0.03^{b,c}	0.53±0.01^{b,c}
4	Ni (0.06 ppm)	0.64±0.02^{a}	0.53±0.03^{b}	0.47±0.03^{a,b}
5	Pb (0.02 ppm)	0.72±0.02^{b}	0.63±0.02^{c}	0.55±0.02^{c}
6	Pb (0.04 ppm)	0.65±0.02^{a}	0.54±0.02^{b}	0.52±0.01^{b}
7	Pb (0.06 ppm)	0.52±0.04	0.45±0.03^{a}	0.43±0.02^{a}
8	Ni+Pb (0.01+0.01 ppm)	0.73±0.02^{b,c}	0.64±0.02^{c}	0.55±0.02^{c}
9	Ni+Pb (0.02+0.02 ppm)	0.66±0.01^{a,b}	0.56±0.02^{b}	0.53±0.02^{b,c}
10	Ni+Pb (0.03+0.03 ppm)	0.60±0.03^{a}	0.50±0.03^{a,b}	0.46±0.02^{a}
	SE(m) ± CD (P=0.05)	0.02 0.07	0.02 0.07	0.02 0.06

Mean ± S.E
*DAT: Days after treatment.
Values with the same superscript in the same column do not differ significantly

Conclusion

This study has demonstrated the detrimental effects of Pb and Ni on the survivability, reproduction and growth of *E. eugeniae*. These effects were found to be mediated by dose and duration of heavy metals exposure. Heavy metal Pb was more noxious to *E. eugeniae* as compared to Ni alone as well as in combinations. The information provided by results is particularly useful for environmental monitoring and assessment of heavy metals contamination in soil.

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References

1. Annapoorani CA. Toxic effect of Aluminium on reproduction and survival of *Eudrilus eugeniae (Kinberg)* on leaf litter. International Journal of Current Microbiology and Applied Sciences. 2014; 3(5):493-500.
2. Ali AS, Naaz I. Earthworm biomarkers: The new tools of environmental impact assessment. Bioscience Biotechnology Research Communications. 2013; 6(2):163-169.
3. Alves PRL, Cardoso EJBN, Martines AM, Sousa JP, Pasini Amarildo P. Earthworm ecotoxicological assessments of pesticides used to treat seeds under tropical conditions. Chemosphere. 2013; 90:2674-2682.
4. Basha PM, Latha V. Evaluation of sublethal toxicity of zinc and chromium in *Eudrilus eugeniae* using biochemical and reproductive parameters. Ecotoxicology. 2016; 25:802-813.
5. Donker MH, Zonneveld C, Van Straalen NM. Early reproduction and reproductive allocation in metal adapted populations of the terrestrial isopod *Porcellio scaber*. Oecologia. 1993; 96:316-323.
6. Dutta A, Dutta H. Some Insights on the Effect of Pesticides on Earthworm. International Research Journal of Environment Sciences. 2016; 5(4):61-66.
7. Hirano T, Tamae K. Earthworms and Soil Pollutants. Sensors. 2011; 11:11157-11167.
8. Garg P, Satya S, Sharma S. Effect of heavy metal supplementation on local (*Allolobophora parva*) and exotic (*Eisenia fetida*) earthworm species: A comparative study. Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering. 2009; 44:1025-1032.
9. Kaur G, Hundal SS. Effect of heavy metals on the survival, growth and development of earthworm *Eisenia fetida*. Journal of Applied and Natural Science. 2016; 8(1):208-212.
10. Kooijman SALM. Energy budget models can explain body size relations. Journal of Theoretical Biology. 1986; 121:268-282.
11. Morgan JE, Morgan AJ. Earthworms as biological monitors of cadmium, copper, lead and zinc in metalliferous soils. Environmental Pollution. 1988; 54:123-138.
12. Loureiro S, Soares AMVM, Nogueira AJA. Terrestrial avoidance behaviour tests as screening tool to assess soil contamination. Environmental Pollution. 2005; 138:121-31.
13. Parihar K, Kumar R, Sankhla MS, Shefali. Impact of Heavy Metals on Survivability of Earthworms. International medico-legal reporter journal. 2019; 2(3).
14. Schon N, Mackay A, Gray R, Van Koten C, Dodd M. Influence of earthworm abundance and diversity on soil structure and the implications for soil services throughout

the season. Pedobiologia. 2017; 62:41-47.
15. Singh A, Saha P, Kumari S, Sinha MP. Impact of different litters on growth and production of a megascolecid earthworm (*Perionyx sansibaricus*) in experimental condition. African Journal of Agricultural Research. 2012; 7(39):5381-5386.
16. Sivakumar S. Effects of metals on earthworm life cycles: a review. Environmental Monitoring and Assessment. 2015; 187:530.
17. Shefali, Yadav J, Gupta RK. Reproductive parameters as assessment tools for arsenic and chromium induced toxicity in *Eudrilus eugeniae.* Indian Journal of Agricultural Research. 2018; 52(6):676-680.
18. Tang H, Yan Q, Wang X, Ai X, RobinP, Matthew C *et al*. Earthworm (*Eisenia fetida*) behavioural and respiration responses to sublethal mercury concentrations in an artificial soil substrate. Applied Soil Ecology. 2016; 104:48-53.
19. Tejada M, Gomez I, Hernandez T, Garcia C. Response of *Eisenia fetida* to the application of different organic wastes in an aluminium contaminated soil. Ecotoxicology and Environmental Safety. 2010; 73:1944-1949.
20. Urmila, Gupta RK, Gill P, Sandeep. Effect of lead and Nickel on growth and fecundity of earthworm, *Eisenia fetida*. Journal of Entomology and Zoology Studies. 2019; 7(5):305-309.
21. Vasseur P, Bonnard M. Ecogenotoxicology in earthworms: A review. Current Zoology. 2014; 60(2):255-272.
22. Wang Y, Wu Y, Cavanagh J, Yiming A, Wang X, Gao W *et al*. Toxicity of arsenite to earthworms and subsequent effects on soil properties. Soil Biology and Biochemistry. 2018; 117:36-47.
23. Yadav J, Gupta RK, Kumar D. Heavy metals toxicity on growth and reproduction of *Eisenia fetida*. Research in Environment and Life Sciences. 2017; 6:565-568.
24. Zaltauskaite J, Sodiene I. Effects of total cadmium and lead concentrations in soil on growth, reproduction and survival of earthworm *Eisenia fetida*. Ekologija. 2010; 56:10-16.
25. Zaltauskaite J, Sodiene I. Effects of cadmium and lead on the life-cycle parameters of juvenile earthworm *Eisenia fetida*. Ecotoxicology and Environmental Safety. 2014; 103:9-16.
26. Zhang J, Yu J, Ouyang Y, Xu H. Responses of earthworm to aluminum toxicity in latosol. Environmental Science and Pollution Research. 2013; 20:1135-11.