Meligy *Egyptian Journal of Biological Pest Control* (2018) 28:1
DOI 10.1186/s41938-017-0002-3

Egyptian Journal of Biological Pest Control

RESEARCH Open Access

Comparative study of element contents in seven isolates of entomopathogenic nematodes

A. M. A. Meligy[1,2]

Abstract

Little is known about element contents in the entomopathogenic nematodes despite their vital role in the growth and development of all organisms. Ten element contents of seven nematode isolates were determined. No significant differences were observed in concentrations of aluminum (Al), chromium (Cr), and lead (Pb) among all the studied nematode isolates, while significant differences of cadmium (Cd), selenium (Se), zinc (Zn), and manganese (Mn) concentrations among isolates were found. All isolates related to *Heterorhabditidae* family contained significantly higher copper (Cu), iron (Fe), and cobalt (Co) concentrations than isolates related to *Steinernematidae* family of entomopathogenic nematode (EPN). In all of tested isolates, the content of Cu was the highest and that of Se was the lowest. The concentrations of the studied elements were in the following descending order: Cu > Fe > Co > Mn > Zn > Pb > Cr > Cd > Al > Se. It is suggested that *Photorhabdus* bacteria associated with *Heterorhabditis* spp. of EPN accumulate Cu, Fe, and Co for activation of their metalloenzymes to enhance their virulence potential.

Keywords: Entomopathogenic nematodes, *Heterorhabditis* spp., *Steinernema* spp., Egyptian isolates, Elements

Background

Egyptian orchards are inhabited by diverse and abundant communities of native entomopathogenic nematodes (EPNs) of the families *Steinernematidae* and *Heterorhabditidae*, which have excellent potential as biological control agents against numerous insect pests. Advantages of EPNs as one biocontrol agents include the wide range of their host insects, ability to seek a host actively, fast insect killing, fast breeding, and no hazard for the environment or higher animals, as well as a possibility of their formulation, storing, and simple application (Jaworska and Gorczyca, 2002). The original vitality of the infective stage of nematode is one of the important factors that affects the field activity of EPNs. Many elements such as copper (Cu), iron (Fe), cobalt (Co), selenium (Se), and zinc (Zn) occur in trace amounts in living organisms and are essential for their growth, development, health, and vitality. Due to their importance to nematode vitality, elements have long been the subject of nematological research. Most of this research has focused on the effect of external exposure to elements on nematode. In vitro tests carried out in a laboratory revealed that vitality and infectivity of the EPNs are affected in the presence of non-toxic metal ions (Jaworska et al., 1997b). Manganese (Mn) and magnesium (Mg) reduced mortality of *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* infective juveniles (IJs) intoxicated with lead (Pb) ions and increased their infectivity against *Galleria mellonella* (Jaworska et al., 1997b). In contrast, cadmium (Cd), chromium (Cr), zinc (Zn), lead (Pb), and copper (Cu) decreased reproduction of *Steinernema feltiae*, *S. carpocapsae*, and *H. bacteriophora* and lowered their virulence against the wax moth, *G. mellonella* (Jaworska and Gorczyca, 2002 and 2009 and Sun et al., 2016). However, much less is known about the natural levels of elements in IJs or their roles in EPN biology. In vivo studies on the level of metal and elements in IJs are very important as the quantitative differences between essential amounts and biological excesses of such

Correspondence: amelegi@kfu.edu.sa; melegi_a@hotmail.com
[1]Physiology Department, Plant Protection Research Institute PPRI, Agricultural Research Center ARC, Giza, Egypt
[2]Department of Clinical studies, College of Veterinary Medicine, King Faisal University, Hofuf, Saudi Arabia

elements are very small and determined the physiological state and vitality of IJs of EPN.

In the present study, ten element levels in nematode IJs of five geographically different Egyptian isolates of *Steinernematidae* and *Heterorhabditidae* families were determined. Also, element contents of IJs from two commercially relevant nematode strains were determined for comparison. This will provide detailed information on the degree of variation in element levels of these nematodes which would mean important progress on the road of improvement of EPN as an effective biocontrol agent and leading to a better understanding of the possible roles of metals in their physiology.

Materials and methods

Nematode populations

Five EPN isolates collected randomly from several locations of five Egyptian governorates that maintained at the Pest Physiology Department, Plant Protection Research Institute, Giza, Egypt. Also, two imported commercial nematode species were analyzed for their element composition (Table 1). Species of the isolated populations were identified using morphological and molecular tools (Nguyen, 2007). Element composition of *S. carpocapsae* isolates was compared to *S. carpocapsae* (all strain) extracted from the commercial product Ecomask (BioLogic, Inc., PA, USA). Due to unavailability of commercial products of *H. indica*, element composition of its isolates was compared to that of the closely related species, *H. bacteriophora* (HP88 strain), extracted from the commercial product Heteromask (BioLogic, Inc., PA, USA).

Nematode culture and preparation

All populations were cultured several times at 1- to 2-month intervals using the last instar larvae of *G. mellonella* L. larvae at 25 °C following isolation to ensure that their contents of elements are not due to field-adapted traits. Freshly harvested IJs were rinsed in deionized water, three times, and the number of IJs in each sample was determined by counting the number of nematodes in five droplets (5 μl each). Harvested IJs were concentrated and vacuum filtered, and their wet weight was measured. Three replicates from each nematode isolate were prepared for metal analysis. Prior to sampling for element analysis, nematode viability and virulence were checked according to the method described by Kaya and Stock (1997) and only viable and highly virulent IJ stocks were used. Nematode IJs were considered viable and highly virulence when both their survival rate and virulence percent against *G. mellonella* larvae were > 95% (Kaya and Stock, 1997).

Metal analysis

Ten elements: cadmium (Cd), aluminum (Al), zinc (Zn), iron (Fe), selenium (Se), chromium (Cr), manganese (Mn), cobalt (Co), copper (Cu), and lead (Pb), were analyzed in IJ samples according to the procedure described by El-Bahr and Abdelghany (2015).

Digestion samples

Mars Xpress (CEM-MARS Express®, Matthews, NC, USA) Microwave Digestion System was used. All the digestion procedures were using Teflon reaction vessels 5 ml accordance to USEPA method 3051(El-Bahr and Abdelghany, 2015). A weight of 0.25 g of each nematode sample was placed into separate digestion vessels with 3 ml of concentrated nitric acid (HNO_3 65%) and 2 ml of hydrogen peroxide (H_2O_2 30%). Double-distilled water were used to clean all samples and remove any contaminate particles. The samples were allowed to the method of digestion with ramp at 200 °C for 15 min and cooled down for 5 min. The digested nematode samples were diluted by 25 ml Millipore deionized water as a total volume.

Instruments

The filtrates of digested nematode samples were analyzed by atomic absorption spectrometry (AAS), according to the method mentioned by Pakshirajan et al. (2013). The determination of Al, Cu, Zn, Fe, and Mn was carried out by using flame atomic absorption spectrophometer, Shimadzu AA-6800 model. The absorbance was obtained by adjusting the

Table 1 Locality, crop, and source of entomopathogenic nematode populations used in the present study

Species	Population	Geographic location	Crop	Source[a]
Heterorhabditis bacteriophora	HP88	USA	Commercial product	BioLogic, Inc., USA
Heterorhabditis indica	EGAZ1	Suez, Egypt	Nectarines	Soil
Heterorhabditis indica	EGAZ2	El-kasasein, Ismailia, Egypt	Mango	Soil
Heterorhabditis indica	EGAZ3	El-kasasein, Ismailia, Egypt	Palm	Soil
Steinernema carpocapase	All	USA	Commercial product	BioLogic, Inc., USA
Steinernema carpocapase	EGAZ9	Belbeis, Sharkia, Egypt	Mango	Soil
Steinernema carpocapase	EGAZ10	Qaha, Qalyubiya, Egypt	Lettuce	Soil

[a]Nematodes were isolated from soil using the last instar larvae of *Galleria mellonella* as bait

cathode lamps at the operation conditions shown in Table 2 as previously described by El-Bahr and Abdelghany (2015). Graphite furnace (GFA-EX7) atomic absorption spectrophometer (Shimadzu, Koyoto, Japan) was used for the determination of Pb, Se, Cd, Cr, and Co. The graphite furnace program for determination of Se, Co, Al, Pb, and Cd by GFA-EX7 and the instrumental setting were similar to that described by El-Bahr and Abdelghany (2015) (Table 3). Standard stock solutions of the studied elements were prepared with deionized water. To avoid error, a slight instrumental drift monitored by analyzing calibration standards at regular intervals during analysis alongside samples was taken into account. Full quantitative analysis mode was applied for all measurements. Calibration curves were obtained for different concentrations of standard solutions prepared from 1000 mg/l stock solution (Merck, Germany).

Analytical quality control

To appropriate quality assurance and the efficiency of procedures such as recovery of the elements was determined. Three samples of nematodes were spiked with the amount of standard solution containing 3 μg/g of each element considered in the present study. Similar nematode samples spiked with the same amount of deionized water without elements were used as control. The samples were then subjected to the digestion instrument procedure and analyzed for metal concentrations to establish confidence in the accuracy and reliability of data generated (Table 4).The accuracy of certified elements varied between 87 and 93%, which can be considered a reliable analysis (El-Bahr and Abdelghany, 2015).

Statistical analysis

The element contents of the studied nematode isolates were expressed as picograms per infective juvenile (pg/IJ). All data were analyzed for analysis of variance (ANOVA). The means were separated by Duncan's multiple-range test for significance at $P < 0.05$ using the computer software package of CoStat for windows (Costat, 2008). Results were recorded as mean ± standard deviation (SD).

Results and discussion

The ANOVA analysis of the concentrations of elements among the studied nematode isolates revealed insignificant differences in their contents of Al, Cr, and Pb (Table 5). For the remaining seven elements, patterns of bioaccumulation in IJs differed depending on the isolate, the species, and the family. Co was significantly higher in *H. bacteriophora* (HP88) and *H. indica* EGAZ3 than the other five isolates (Table 5). The results showed that IJs of *S. carpocapsae* (EGAZ9) contained significantly lower amounts of Se (0.1 ± 0.035 pg/IJ) than *H. indica* (EGAZ1), *H. bacteriophora* (HP88), *H. indica* (EGAZ2), and *S. carpocapsae* (all) (0.22 ± 0.06, 0.19 ± 0.05, 0.14 ± 0.05, and 0.16 ± 0.045 pg/IJ, respectively). IJs of *H. bacteriophora* (HP88) isolates contained significantly higher amounts of Zn than all other isolates (Table 5), while the highest concentration of Mn was detected in *H. indica* (EGAZ2) (27.5 ± 6.0, (Table 5). Also, Mn concentration was significantly higher in *H. bacteriophora* (HP88) than both *H. indica* (EGAZ2) and *H. indica* (EGAZ2) (15.1 ± 5.0 vs 6.1 ± 3.0 and 4.0 ± 2.0 pg/IJ, respectively). In addition, the imported strain of *S. carpocapsae* (all) contained significantly lower concentration of Mn than local *S. carpocapsae* (EGAZ9 and 10) (6.5 ± 2.5 vs 15.1 ± 3.8 and 16.3 ± 3.2 pg/IJ, respectively). Table 5 showed that the four tested heterorhabditid isolates contained comparable concentrations of Co, Fe, and Cu ($P > 0.05$). Also, comparable concentrations of Co, Fe, and Cu ($P > 0.05$) were detected in IJs of the three tested steinernematid isolates. The differences in Co, Fe, and Cu concentrations between heterorhabditid and steinernematid isolates were significant ($P > 0.05$). In all of the tested isolates, the content of Cu was the highest and that of Se was the lowest. The concentrations of the studied elements decreased in the following order: Cu > Fe > Co > Mn > Zn > Pb > Cr > Cd > Al > Se. Comparing to the main concentrations of each element of the four tested heterorhabditid isolates with those of the three tested steinernematid isolates indicated that concentrations of Cu, Fe, and Co were significantly higher in *Heterorhabditidae* family than that in *Steinernematidae* family, whereas the concentrations of the remaining seven elements were comparable with a lack of statistical significances (Figs. 1 and 2). The previously mentioned data were also calculated as pg/g nematode wet body weight

Table 2 Flame atomic absorption spectrophotometer (FAAS) operating method conditions

Conditions	Aluminum (Al)	Zinc (Zn)	Iron (Fe)	Manganese (Mn)	Copper (Cu)
Wavelength (nm)	309	213.9	239.6	257.6	324.8
Slit width (nm)	0.2	0.5	0.5	0.2	0.2
Sensitivity (μg/ml)	0.05	0.008	0.07	0.05	0.014
Flame type	Air-acetylene				

Table 3 Programs of heating method for chromium (Cr), cadmium (Cd), cobalt (Co), lead (Pb), and selenium (Se) in graphite furnace atomic absorption spectrophotometer (GFAAS)

Steps	Temperature (°C)					Ramp(s)	Hold(s)	Argon flow rate (ml min^{-1})
	Cd	Cr	Pb	Co	Se			
Drying 1	150	150	150	150	150	5	20	250
Drying 2	200	200	200	200	200	5	15	250
Pyrolysis	500	1600	800	1000	1200	10	20	250
Atomization	1800	2300	2000	2300	2000	0	5	0
Clean-out	2200	2500	2200	2600	2450	1	3	250

and showed the same trend of significance of pg/IJ; thus, the data were not shown to not duplicate the results.

Many elements occur in very small amounts in living matter. While at minute concentrations, elements, particularly the first row transition metals such as manganese, iron, copper, cobalt, and zinc, were essentially for the growth and development of organisms, they can be toxic at higher concentrations. As only a few literature is available about the trace element profile in EPN, and as the IJs analyzed in the present study were viable and have an excellent virulence, data recorded here may not only serve as baseline value and reference data for natural level of elements in nematode but also give some indication of the nematode safe levels of such elements. To this end, values of the measured element values measured in the IJs were compared not just among species and isolates but also as a comparison between the two main EPN families (*Steinernematidae* and *Heterorhabditidae*).

Table 4 The recovery of elements from digested nematode samples

Elements	Concentrations of metal added (mg/kg)	Concentrations of metal recovered (mg/kg)	Recovery (%)
Selenium (Se)	3	2.8	93.3
Cobalt (Co)	3	2.8	93.3
Iron (Fe)	3	2.7	90
Manganese (Mn)	3	2.7	90
Aluminum (Al)	3	2.6	86.7
Cadmium (Cd)	3	2.8	93.3
Copper (Cu)	3	2.6	86.7
Zinc (Zn)	3	2.8	93.3
Lead (Pb)	3	2.8	93.3
Chromium (Cr)	3	2.7	90

Al, Cr, and Pb have mainly been considered as pollutant and were presented in all of the studied nematode isolates in low concentrations that could reflect their level in nematode environment in the laboratory (such as water and food sources used in their cultures). Cd, Se, and Mn are part of several macromolecules and considered as essential elements for many organisms (Nachev et al., 2013). While Mn has proved to possess many positive effects on IJs of *S. carpocapsae* and *H. bacteriophora*, the roles of Cd and Se in EPN biology are yet unknown (Jaworska and Gorczyca, 1993, 2002, 2009).

Significant differences were observed in this study in concentrations of Cd, Se, and Mn not only among different species of the same family or among the imported and the local populations of the same species but also among isolates of the same species from adjacent Egyptian locality. There was a significant difference in Mn concentration between *H. bacteriophora* (HP88) and *H. indica* (EGAZ2) (belong to the same family), between the imported S. *carpocapsae* (all) and the local S. *carpocapsae* (EGAZ9 and 10), and between *H. indica* (EGAZ2) and *H. indica* (EGAZ3) (both isolated from Ismailia governorate). The variations in element concentrations could be due to active uptake of Cd, Se, and Mn by nematodes and a genetic component but not due to different environmental conditions as all the studied isolates were cultured several times in the laboratory under the same conditions.

In contrast to our results, Cu was reported to be considerably toxic for IJs of EPN (Jaworska and Gorczyca, 2002; Sun et al., 2016). Sambongi et al. (1999) reported that nematodes have elaborated sensorial equipment, including receptors for copper ions, which enables them to avoid their intake. Therefore, it was surprising to discover that Cu was the most abundant element in all nematode isolates in our study and its concentration was the highest among all the other studied elements. However,

Table 5 Concentration of elements in infective juveniles (IJ) of the studied nematode species and/or isolates (concentration reported as mean picograms per IJ ± standard deviation)

	H. bacteriophora (HP88)	*H. indica* (EGAZ1)	*H. indica* (EGAZ2)	*H. indica* (EGAZ3)	*S. carpocapsae* (all)	*S. carpocapsae* (EGAZ9)	*S. carpocapsae* (EGAZ10)
Al	0.18 ± 0.07a	0.26 ± 0.12a	0.19 ± 0.05a	0.22 ± 0.1a	0.18 ± 0.05a	0.18 ± 0.09a	0.16 ± 0.05a
Cr	1.88 ± 0.55a	1.85 ± 0.50a	1.45 ± 0.50a	1.27 ± 0.40a	1.21 ± 0.60a	1.32 ± 0.50a	1.58 ± 0.30a
Pb	1.37 ± 0.40a	2.03 ± 0.80a	1.48 ± 0.45a	1.7 ± 0.5a	1.31 ± 0.35a	1.1 ± 0.25a	1.64 ± 0.75a
Cd	1.27 ± 0.41a	0.49 ± 0.15b	0.32 ± 0.15b	1.34 ± 0.40a	0.45 ± 0.17b	0.46 ± 0.20b	0.61 ± 0.21b
Se	0.19 ± 0.07a	0.22 ± 0.06a	0.14 ± 0.05abc	0.20 ± 0.05a	0.16 ± 0.04 ac	0.10 ± 0.04bc	0.17 ± 0.05 ac
Zn	8.00 ± 1.20a	4.12 ± 0.80b	4.63 ± 0.90b	4.63 ± 0.90b	5.08 ± 1.30b	3.4 ± 0.60b	3.25 ± 0.50b
Mn	15.1 ± 5.0a	6.10 ± 3.5b	27.4 ± 6.0c	4.10 ± 2.0b	6.50 ± 2.5b	15.1 ± 3.8a	16.3 ± 3.2a
Co	16.6 ± 3.5a	19.01 ± 4.0a	19.9 ± 5.8a	18 ± 5.0a	10.7 ± 2.5b	9.68 ± 2.0b	12.6 ± 1.0b
Fe	43.7 ± 7.5a	31.2 ± 5.0a	29.4 ± 6.2a	38.4 ± 6.5a	20.3 ± 3.9b	12.5 ± 8.0b	16.6 ± 7.0b
Cu	62.5 ± 12.5a	44.2 ± 8.5a	40.5 ± 6.0a	49.5 ± 8.0a	31.8 ± 4.4b	23.9 ± 3.8b	28.6 ± 4.5b

Values followed by different lowercase letters in the same row are significantly different ($P < 0.05$)

Jaworska et al. (1997a) mentioned that Fe and Cu ions vitalized IJs of *Heterorhabditis bacteriophora* and increased their mobility and pathogenic abilities which could support our findings. One explanation for the high level of Cu concentration in all the studied EPN isolates is that nematodes during their early stages inside the insect body collect and accumulate Cu from the surrounding Cu-rich insect hemolymph (Malik and Malik, 2009). However this explanation does not elucidate the significant difference in Cu concentration between the two nematode families. Another plausible explanation is that Cu accumulation in IJs could be due to bacteria associated with EPN, for which Cu is known to be an essential trace element (Kuzuya and Inouye, 2001; Massaoud et al., 2011). The nematode bacteria of *Photorhabdus* for *Heterorhabditis* spp. and *Xenorhabdus* for the *Steinernema* spp. produce a range of activities, including hydrolytic enzymes, that converting the insects' internal organs and tissues into bacterial biomass (Watson et al., 2010). *Photorhabdus* secreted more than four proteases such as PrtA peptidase, PhpC (*Photorhabdus* protease C), thermolysin-like enzymes, and other enzymes that may be used in the suppression of the immune

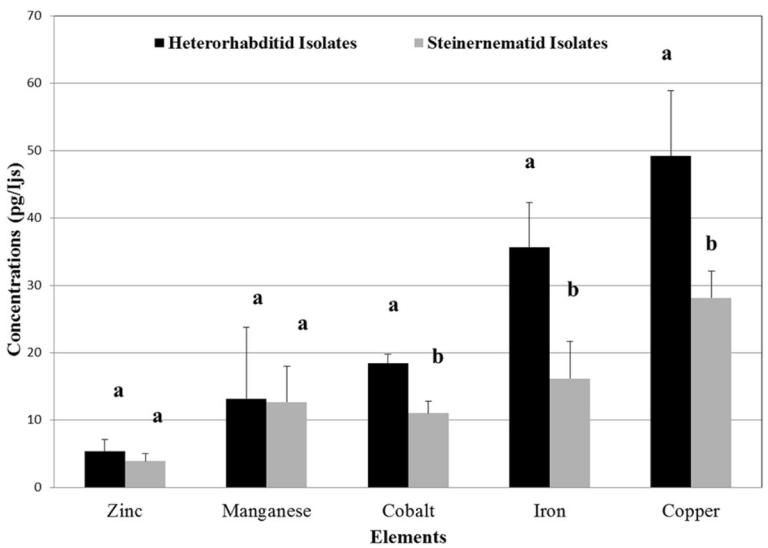

Fig. 1 Variations of cobalt, manganese, zinc, iron, and copper contents (mean ± SD) between the infective juveniles of heterorhabditid and steinernematid isolates (adjacent bars with different letters are significantly different, $P < 0.05$)

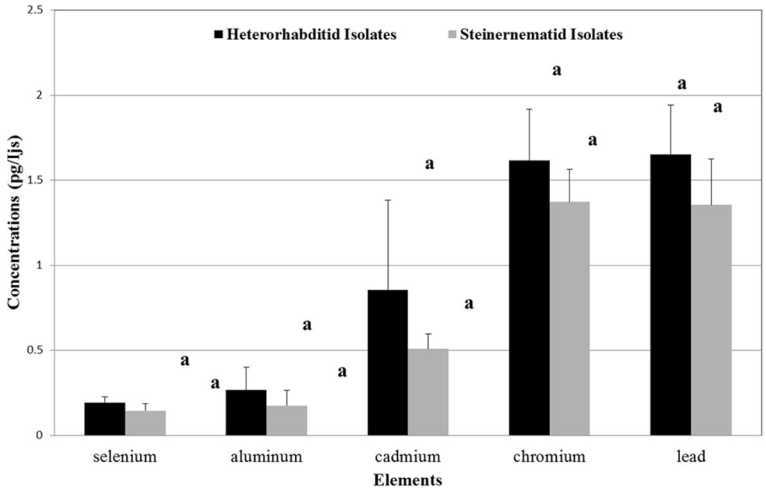

Fig. 2 Variations of lead, chromium, cadmium, aluminum, and selenium contents (mean ± SD) between the infective juveniles of heterorhabditid and steinernematid isolates (adjacent bars with the same letter are not significantly different, $P > 0.05$)

responses, while only one proteolytic enzyme (protease B) has been partially characterized in *Xenorhabdus* strains (Massaoud et al., 2010, 2011). Although thermolysin-like enzymes are related to zinc metalloenzymes, it was found that Cu and Co ions increased their activities to 200% of their original one, while Zn alone is insufficient (Holmquist and Vallee, 1974; Kuzuya and Inouye, 2001 and Massaoud et al., 2011). Thus, it could be concluded that *Photorhabdus* bacteria *of Heterorhabditis* spp. accumulate Cu and Co for activation of their metalloenzymes to enhance their virulence. This conclusion could be supported by the finding that concentrations of Cu and Co ions in nematode isolates related to heterorhabditid EPN family were significantly higher than the ones related to *Steinernematidae* family lacking of *Photorhabdus* bacteria. The uptake of *Photorhabdus* bacteria associated with *Heterorhabditidae* EPN to Fe could also explain the detected high concentrations of Fe ions in IJs and also explains their significant higher concentration in nematode isolates related to *Heterorhabditidae* family than concentration in isolates related to *Steinernematidae* family in the present study. Iron is an essential nutrient for bacteria that have different mechanisms for obtaining both the ferrous (Fe2+) and ferric (Fe3+) forms of this metal from their environments. Watson et al. (2010) constructed *P. luminescens* mutant bacteria that was predicted to be crippled in its ability to obtain Fe3+ from surrounding. They found that this mutant does not grow well in iron-limited media and lost their ability to kill insects and concluded that Fe++ was essential for *P. luminescens* bacteria of *Heterorhabditidae*-related nematodes. However, further experiments might determine whether Cu, Fe, and Co are concentrated in IJs or in bacteria associated with the EPNs.

Conclusions

In all of tested isolates of Entomopathogenic Nematodes, the content of Cu was the highest and that of Se was the lowest. The concentrations of the studied elements were in the following descending order: Cu >Fe > Co > Mn > Zn > Pb > Cr > Cd > Al > Se. It is suggested that Photorhabdus bacteria associated with Heterorhabditis spp of EPN accumulate Cu, Fe and Co for activation of their metalloenzymes to enhance their virulence potential. However, further experiments might determine whether Cu, Fe, and Co are concentrated in IJs or in bacteria associated with the EPNs.

Acknowledgements

I would like to thank the Deanship of Scientific Research, King Faisal University, Saudi Arabia, for their support (project no. 130025). And also, I would like to thank Prof. Dr. Monzer Abd-Elrahman and Prof. Dr. Ahmed Azazy, Plant Protection Research Institute PPRI, Physiology Department, Agricultural Research Center ARC, Egypt, for their kind cooperation in conducting this valuable study.

Competing interests

The author declares that they have no competing interests.

Publisher's Note

Received: 12 September 2017 Accepted: 6 December 2017
Published online: 30 January 2018

References

Costat (2008) Statistical software package. CoHort Software Inc., Berkeley version 6.45. www.cohort.com

El-Bahr SM, Abdelghany AM (2015) Heavy metal and trace element contents in edible muscle of three commercial fish species, and assessment of possible risks associated with their human consumption in Saudi Arabia. J Adv Vet Anim Res 2(3):1–8

Holmquist B, Vallee BL (1974) Metal substitutions and inhibition of thermolysin: spectra of the cobalt enzyme. Biol Chem 249:4601–4607

Jaworska M, Gorczyca A (1993) Effect of manganese ions on entomopathogenic nematodes. Entomon 2(4):518–519
Jaworska M, Gorczyca A (2002) The effect of metal ions on mortality, pathogenicity and reproduction of entomopathogenic nematodes *Steinernema feltiae* Filipjev (Rhabditida, Steinernematidae). Pol J Environ Stud 11(5):517–519
Jaworska M, Gorczyca A (2009) Effect of manganese ions on beneficial organisms. J Elem 14(2):257–263
Jaworska M, Gorczyca A, Sepiol J, Szeliga E, Tomsik P (1997a) Effect of metal ions on the entomopathogenic nematode *Heterorhabditis bacteriophora* Poinar (Nematoda: Heterorhabditidae) under laboratory condtions. Water Air Soil Pollut 93:157–166
Jaworska, M.; A. Gorczyca; J. Sepiol, E. Szeliga and P. Tomsik. 1997b. Metal-metal interaction in biological systems. Part V. *Steinernema carpocapsae* (Stinernematidae) and *Heterorhabditis bacteriophora* (Heterorhabditidae) entomopathogenic nematodes. Water Air Soil Pollut, 93:213-223, 1997
Kaya HK, Stock SP (1997) Techniques in insect nematology. In: Lacey LA (ed) Manual of techniques in insect pathology. Academic Press, London, pp 281–324
Kuzuya K, Inouye K (2001) Effects of cobalt-substitution of the active zinc ion in thermolysin on its activity and active-site microenvironment. J Biochem 130(6):783–788
Malik A, Malik MA (2009) Variation in metal ion concentrations in the haemolymph of the silkworm, Bombyx mori during development. Acad J Entomol (AJE) 2(1):10–15
Massaoud MK, Marokházi J, Fodor A, Venekei I (2010) Proteolytic enzyme production by strains of the insect pathogen *Xenorhabdus* and characterization of an early-log-phase-secreted protease as a potential virulence factor. Appl Environ Microbiol 76(20):6901–6909
Massaoud MK, Marokházi J, Venekei I (2011) Enzymatic characterization of a serralysin-like metalloprotease from the entomopathogen bacterium, *Xenorhabdus*. Biochim Biophys Acta 1814(10):1333–1339
Nachev M, Schertzinger G, Sures B (2013) Comparison of the metal accumulation capacity between the acanthocephalan Pomphorhynchus laevis and larval nematodes of the genus Eustrongylides sp. infecting barbel (Barbus barbus). Parasit. Vectors 36:21–29
Nguyen KB (2007) Methodology, morphology and identification. In: Nguyen KB, Hunt DJ (eds) Entomopathogenic nematodes: systematics, phylogeny and bacterial symbionts. Nematology monographs and perspectives, vol Vol. 5. Brill, Leiden-Boston, pp 59–119
Pakshirajan K, Worku AN, Acheampong MA, Lubberding HJ, Lens PN (2013) Cr (III) and Cr (VI) removal from aqueous solution by cheaply available fruit waste and algal biomass. Biotechnol Appl Biochem 170:498–513
Sambongi Y, Nagae T, Liu Y, Yoshimizu T, Takeda K, Wada Y, Futai M (1999) Sensing of cadmium and copper ions by externally exposed ADL, ASE, and ASH neurons elicits avoidance response in Caenorhabditis elegans. Neuroreport 10:753–757
Sun Y, Bai G, Wang Y, Zhang Y, Pan J, Cheng W, Feng X, Li H, Ma C, Ruan W, Shapiro D (2016) The impact of Cu, Zn and Cr salts on the relationship between insect and plant parasitic nematodes: a reduction in biocontrol efficacy. Appl Soil Ecol 107:108–115
Watson RJ, Millichap P, Joyce SA, Reynolds S, Clarke DJ (2010) The role of iron uptake in pathogenicity and symbiosis in Photorhabdus luminescens TT01. BMC Microbiol 22(10):177–200